CHE 301: BIOCHEMISTRY



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CHE 301: Biochemistry

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Licensing

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CHAPTER OVERVIEW

1: Intro to Biochem

1.1: Basic Biology

1.2: Basic Chemistry

1.3: Water and Buffers

1.4: Prebiotic Earth and the origin of life

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1.1: Basic Biology

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The Bio of Biochemistry

Biochemistry is the science that explains life at a molecular level. All living things are made of **molecules,** which in turn, are made of **atoms**. Molecules can exist in different sizes: they can be small (for example, O_2, CO_2), medium (for example, $C_6H_{12}O_6$, glucose); or large (for example proteins, which are made of millions of atoms) Molecules are the building blocks to all structures found in living organisms. We can identify four types of **biomolecules,** that is, molecules common to all cells. These biomolecules are:

- **Carbohydrates**
- **Lipids**
- **Proteins**
- **Nucleic acids**

One common characteristic of these biomolecules is that they are formed by the association of smaller molecules. Except in the case of lipids, carbohydrates, proteins, and nucleic acids are **polymers**, that is, large molecules made of smaller units called monomers, linked by **covalent bonds**. In the case of lipids, monomers do not associate thorough covalent bonds but rather by non-polar intermolecular forces (London dispersion forces).

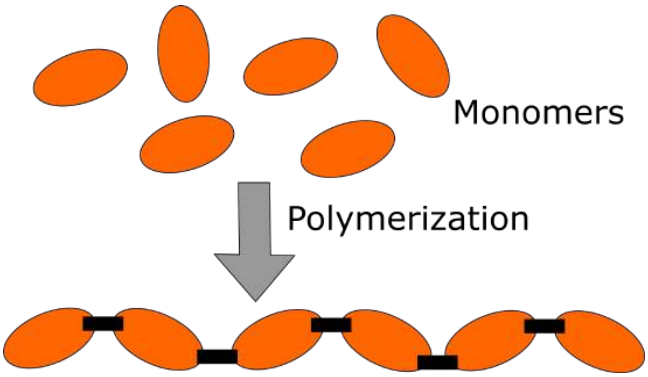


Figure 1.1. Formation of Polymers

Cells

All cells, no matter what kind, have a plasma membrane that serves as a boundary for the cell, separating it from its surroundings. They also possess a genome made up of DNA that encodes the information for making the proteins required by the cell. To translate the information in the DNA and make the proteins it encodes, all cells have the machinery for protein synthesis, namely, ribosomes and tRNAs. DNA is also the repository of information that gets copied and transmitted to the next generation, allowing living cells to reproduce.

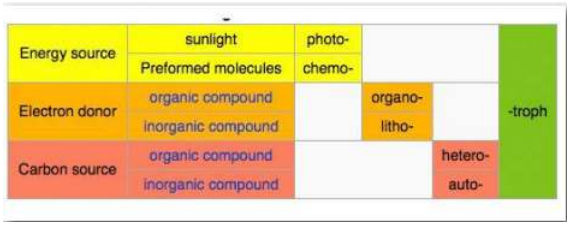


Figure 1.2 - Organization of organisms by metabolic type Wikipedia

All cells also need to be able to obtain and use energy. The source of this energy is different in different organisms (Figure 1.4). Phototrophs are organisms that obtain metabolic energy from light, while chemotrophs get their energy from the oxidation of chemical fuels. Organisms that can capture energy from light or from chemical sources are termed autotrophs (auto=self, troph=nourishing). Others are heterotrophs, which use, as their energy source, the organic compounds made by other organisms. Plants, and other photosynthetic organisms are autotrophs, while animals are heterotrophs.

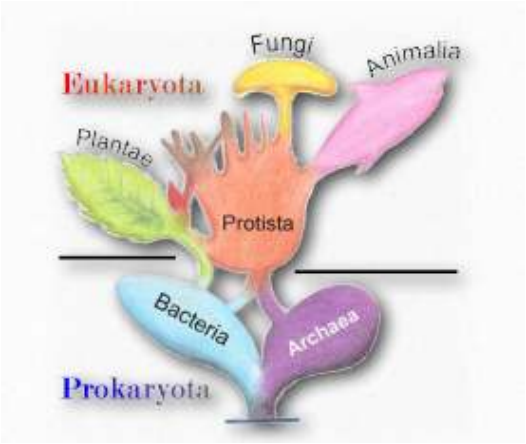


Figure 1.3 - Tree of life Wikipedia

Cells may be **aerobic** (i.e., use oxygen) or **anaerobic** (able to live without oxygen). Some anaerobic cells are obligate anaerobes, that is, they require an environment free of oxygen. Others are facultative anaerobes, cells that can live with, or without, oxygen.

Prokaryotic and eukaryotic cells

Organisms may be divided into two major groups, the **prokaryotes** and the **eukaryotes**. The cells of the former lack a nucleus and other organelles, while those of the latter are characterized by numerous internal, membrane-bounded compartments, including a nucleus.

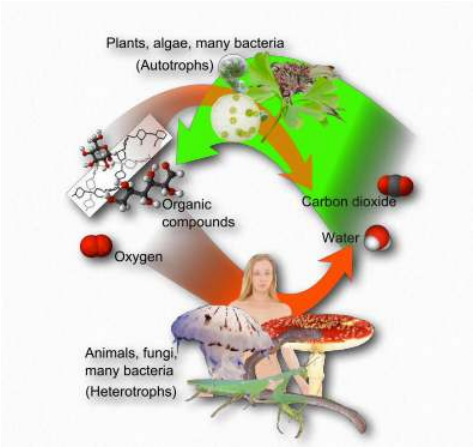


Figure 1.4 - Interplay between autotrophs and heterotrophs Wikipedia

Prokaryotes are unicellular and generally considerably smaller than their eukaryotic cousins, with sizes ranging from 0.5 to 5 μm in diameter. Prokaryotes typically have circular chromosomes, and may sometimes contain extra-chromosomal DNA elements (also Image by usually circular) called plasmids. Although the DNA in prokaryotes is not wrapped around histones, as is the case for eukaryotes, prokaryotes have proteins associated with their DNA. The DNA-protein complexes in prokaryotes create a structure called a nucleoid, which is different from the eukaryotic nucleus in not being enclosed by a nuclear envelope (Figure 1.7). The proteins associated with the DNA in Archaea resemble eukaryotic histones, while those in bacteria are different from both eukaryotic and archaeal DNA packaging proteins.

Cellular Differences between Prokaryotes and Eukaryotes		
Prokaryotes	Organelle	Eukaryotes
No definite nucleus	Nucleus	Present
Present	Cell Membrane	Present
None	Mitochondria	Present
None	Endoplasmic Reticulum	Present
None	Chloroplasts	Present in Plants

Aleia Kim Table 1.1

Prokaryotes may be divided into two broad categories, bacteria and archaea. These single-celled organisms are both ancient and widespread. Archaea were once thought to be a subgroup of bacteria, but have subsequently been shown to be a completely different group of organisms that are so distinct from both bacteria and eukaryotes that they now are classified in a domain of their own.

Bacteria

Like eukaryotic cells, bacterial cells have a plasma membrane surrounding them, but in addition, they also contain an exterior cell wall, comprised of an interlocked peptidoglycan network. On their exterior surfaces, bacteria have hair-like appendages called pili that allow them to adhere to other cells. Pili play an important role in bacterial conjugation, a process in which DNA is transferred between bacterial cells. In addition, bacterial cells may have flagella that enable them to move through their surroundings.

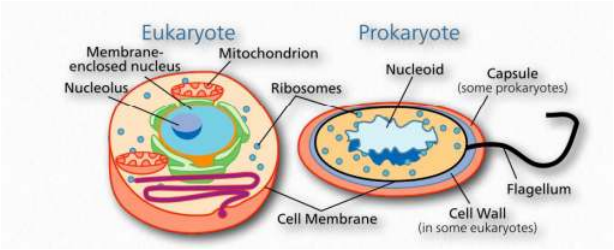


Figure 1.5 - Prokaryotic vs. eukaryotic cell structures (not to scale)

Interestingly, bacteria can communicate, not only with members of their own species, but also with other bacterial species, using chemical signals, in a process called quorum sensing. These signaling mechanisms enable bacteria to assess conditions around them (such as the size of their population). Quorum sensing plays a role in the process of infection by bacterial pathogens as well as the formation of biofilms (mats of cells that adhere to each other tightly and protect the bacteria against environmental hazards or other harmful agents).

Archaea

The first archaeans to be studied were all found in harsh environments such as salt flats and hot springs. Because of this, they were initially believed to live only in extreme environments and were described as extremophiles (Figure 1.8). We now know that archaeans can be found in every environment, moderate or extreme. Archaea have been found in the human gut, and in such huge numbers in marine plankton that it has been suggested that they may be the most abundant organisms on earth.

While they are unicellular, and superficially resemble bacteria, archaea are in some respects more similar to eukaryotes. Their transcriptional machinery, promoter sequences and ribosomes are much more like those of eukaryotes than of prokaryotes. Archaea are also unique among living organisms in their use of ether linkages to join the lipids used in their plasma membranes to glycerol. Not only are the ether linkages different from the ester linkages in all other forms of life, but the lipids themselves are different. In place of the fatty acids used in both bacterial and eukaryotic membranes, archaea use long isoprene-derived chains (Figure 1.9) This difference in membrane composition and structure makes archaeal membranes highly stable and may be advantageous in extreme conditions.



Figure 1.6 - Archaeans growing in acid mine waste

Archaea, like bacteria, also have a cell wall, but the cell walls do not contain peptidoglycans. Some archaea have peptidoglycan-like molecules in their cell walls, while others build their cell walls entirely of glycoproteins and polysaccharides.

Eukaryotes

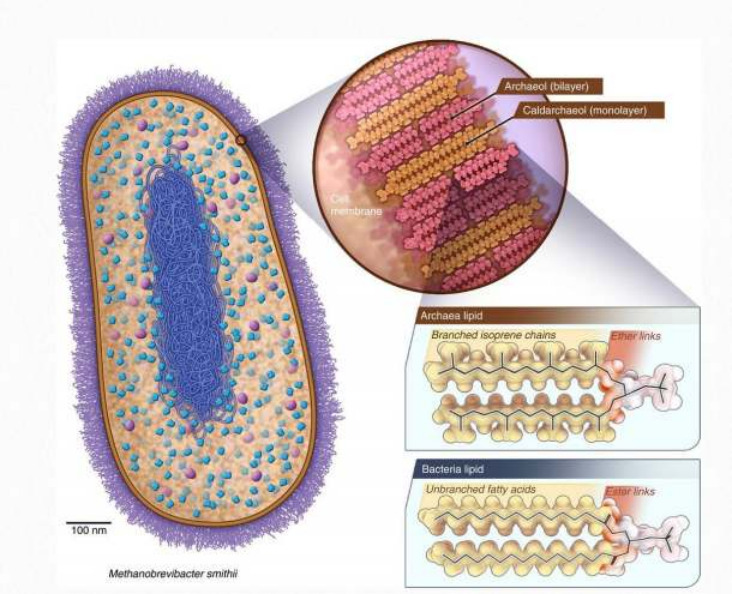


Figure 1.7. Archaeal membrane, top, showing unusual ether linkages and isoprene chains and bacterial membrane, below. Wikipedia

Eukaryotic cells are found in both unicellular and multicellular organizational schemes. Unicellular forms include yeast and many protists, familiar to students from introductory biology labs, like Paramecium and Amoeba. Multicellular eukaryotes include plants, animals, and fungi. Eukaryotic cells are surrounded by a plasma membrane. Animal cells have no cell walls, whereas plant cells use cellulose, hemicellulose, and pectins to build cell walls outside their plasma membranes. Fungal cells have cell walls that are unusual in containing the polymer, chitin, which is also found in the exoskeletons of arthropods.

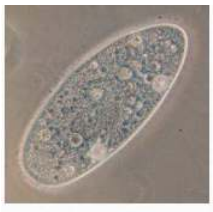


Figure 1.8 Paramecium Wikipedia

Eukaryotic cells are typically much larger (typically 10-100 µm) and contain considerably more DNA than prokaryotic cells. The most distinctive feature of eukaryotic cells, however, is the presence of a variety of internal membrane-bounded structures, called organelles.

Organelles

Eukaryotic cells are characterized by internal membrane-bounded compartments or organelles. These compartments divide up the interior of the cell into discrete parts that have specialized functions. Organelles found in eukaryotic cells include the nucleus (houses DNA), mitochondria (electron transport system/ oxidative phosphorylation for ATP synthesis), nucleolus (ribosome synthesis and assembly), endoplasmic reticulum (lipid metabolism and targeted protein synthesis and folding), the Golgi apparatus (protein modification and secretion), peroxisomes (oxidation of very long-chain fatty acids), chloroplasts (plants - photosynthesis), plastids (synthesis and storage of compounds in plants), lysosomes (animals - hydrolytic enzymes), endosomes (contain endocytosed material), and vacuoles.

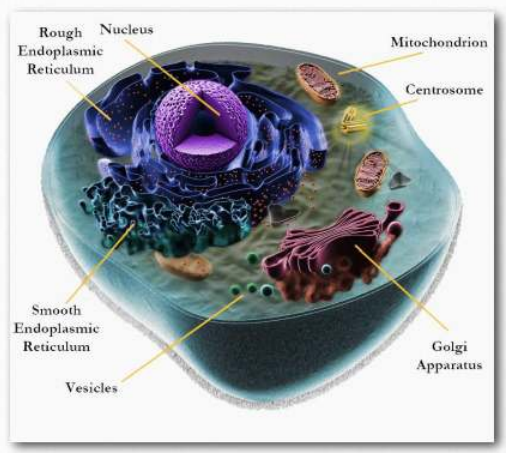


Figure 1.9 Animal cell structure Wikipedia

The presence of multiple compartments within the cell permits reactions requiring specific conditions to be carried out in isolation from the rest of the cell. For example, the formation of disulfide bonds in proteins is possible in the conditions within the endoplasmic reticulum, but would not readily occur in the different environment of the cytosol. The presence of membrane-bounded compartments also allows reactants to be more concentrated because of the smaller volume of the organelle.

Eukaryotic DNA in a cell is divided into several linear bundles called chromosomes. Chromosomes contain the genomic DNA wrapped around cores of positively charged proteins called histones. The ends of linear eukaryotic chromosomes have telomeres, short (less than 10 bp) sequences repeated thousands of times. The role of telomeres in preventing loss of information when linear chromosomes are replicated is discussed later.

The chromosomes in eukaryotic cells are surrounded by the nuclear envelope, a double membrane structure that encloses the nucleus (Figure 1.12). Within the nucleus, there are enzymes required for the replication and transcription of genetic information. The presence of the nuclear envelope also regulates which proteins can enter the nucleus at any given time. This, as you will see later, is an important way to control gene expression.

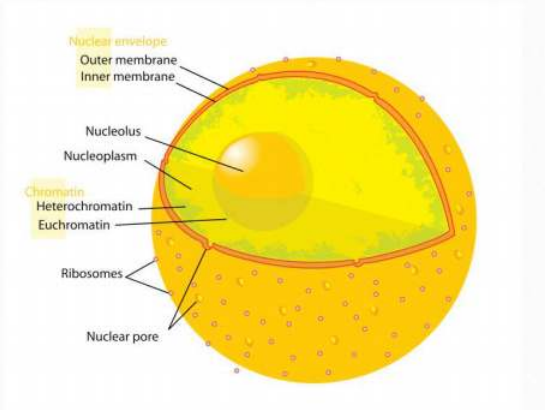


Figure 1.10 Cell nucleus

Mitochondria and chloroplasts have their own DNA, separate from and in addition to the nuclear DNA. This DNA is small and circular and resembles a prokaryotic chromosome. Mitochondria and chloroplasts also have their own ribosomes and tRNAs and can carry out their own protein synthesis. This is not as odd as it seems, because these organelles are likely derived from prokaryotes that once lived as endosymbionts within ancient eukaryotic cells and eventually became integrated into their host cells.

Level of Organization of Living Organisms

Level 1: Cells

The first and most basic level of organization is the cellular level. A cell is the basic unit of life and the smallest unit capable of reproduction. While cells vary greatly in their structure and function based on the type of organism, all cells have a few things in common. Cells are made up of organic molecules, contain nucleic acids (such as DNA and RNA), are filled with fluid called cytoplasm, and have a membrane made out of lipids. Cells also contain many structures within the cytoplasm called organelles, which perform various cellular functions.

Cells may be prokaryotic (without a nucleus) in bacteria and archaea (single-celled organisms), or eukaryotic (with nucleus-enclosing DNA) in plants, animals, protists, and fungi. In humans, most cells combine to form tissues, but some cells are found independent of solid tissues and have their own functions. A red blood cell found circulating in the bloodstream carrying oxygen throughout the human body is an example of an independent cell.

Level 2: Tissues

Tissues are a group of similar cells of the same origin that carry out a specific function together. Humans have four different types of basic tissues. Connective tissues such as bone tissue are made up of fibrous cells and give shape and structure to organs. Muscle tissue is made up of cells that can contract together and allow animals to move. Epithelial tissues make up the outer layers of organs, such as the skin or the outer layer of the stomach. Nervous tissue is made of specialized cells that transmit information through electrochemical impulses, such as the tissue of nerves, the spinal cord, and the brain.

Level 3: Organs

An organ is a structure made up of different tissues that perform specific bodily functions. Most organs contain tissues such as parenchyma (used to perform the organ functions), stroma (connective tissue specific to organs) and epithelial. Organs may be solid or hollow, and vary considerably in size and complexity. The heart, lungs, and brain are all examples of organs.

Level 4: Organ Systems

An organ system is a collection of organs that work together to perform a similar function. There are eleven different organ systems in the human body, each with its own specific functions. One example is the digestive system, which is made up of many organs that work together to digest and absorb nutrients from food. While most organ systems control a few specific physiological

processes, some processes are more complex and require multiple organ systems to work together. For example, blood pressure is controlled by a combination of the renal system (kidneys), the circulatory system, and the nervous system.



Figure 1.11. Levels of organization in living organisms. Image by LadyofHats, CC0, via Wikimedia Commons

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1.2: Basic Chemistry

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> *"Organic chemistry is the chemistry of carbon compounds. Biochemistry is the chemistry of carbon compounds that crawl" -Michael Adams.*

To understand biochemistry, one must possess at least a basic understanding of organic and general chemistry. In this brief section, we will provide a rapid review of the simple concepts necessary to understand cellular chemistry. Chemistry is chemistry, whether in a cell or outside it, but biological chemistry is a particular subset of organic chemistry that often involves enormous macromolecules, and that happens in the aqueous environment of the cell.

Chemical bonds: ionic vs covalent bonds

Atoms consist of a nucleus (formed by neutrons and protons) plus electrons moving around the nucleus. In a neutral atom (element), the number of electrons and protons are equal (an atom has zero net charge). The **octet rule** stipulates that atoms react to form compounds trying to emulate the number of electrons of the noble gas (group 18) closer to their location on the period table; that is, atoms like to be surrounded by 8 electrons (helium being the exception with only two electrons). In order to fulfill the octet rule, atoms have two options: they can either transfer electrons or share electrons.

An **ionic bond** happens when an metallic element (to the left on the period table) transfers an electron to a non-metallic element (to the right on the period table) forming a **cation** and an **anion**, respectively. This **ions** have opposite charges, so they attract mutually resulting in the formation of an ionic compound. Because of the electrostatic attraction between the ionic net charges, ionic compounds have very high melting and boiling points. and they form crystals at room temperature. The formation of sodium chloride is a typical example of an ionic compound.



Figure 1.2.1. Formation of an ionic compound. Image by BruceBlaus, CC BY-SA 4.0, via Wikimedia Commons

Another important characteristic of ionic compounds is that many are soluble in water, due to an intermolecular interaction called **ion-dipole** (see below). When the ions in an ionic compound get in contact with a polar solvent like water, the ions can interact with this polar solvent, and as the consequence the ionic compound desintegrates forming ions in solutions. This process is called **dissociation**. Because the ions in solution are free to move, solutions of ionic compounds can conduct the electricity, so they are called **electrolytes.** Highly polarized covalent compounds (such as HCl) can also undergo dissociation in water. In this case, the process is called **ionization**. In ionization, a hydrogen ion (H^+) leaves behind its electron as it exits (leaving behind a negative charge on the Cl^-).

A **covalent compound** happens when a nonmetallic element reacts with another nonmetallic element, resulting in an electron sharing to fulfill the octet rule. For example, two atoms of Fluorine can share one pair of electrons resulting in the formation of a F_2 molecule. Covalent compounds tend to have lower melting points and lower points than ionic compounds and their physical properties (melting point, boiling point, solubility, etc) depend on the strength of the forces with which different molecules interact with each other. These forces are called **intermolecular interactions,** which in turn, are related to the **electronegativity difference** between atoms in a covalent bond (and molecular geometry)



Figure 1.2.2. Formation of covalent bond in fluorine molecule. Image by Jacek FH, CC BY-SA 3.0, via Wikimedia Commons.

Electronegativity

Electronegativity is a measure of the affinity a nucleus has for outer shell electrons (Table 1.2). High electronegativity corresponds to high affinity for electrons. Electrons in a covalent bond are held closer to the nucleus with a greater electronegativity compared to a nucleus with lower electronegativity.

Table 1.2. Electronegativity of various atoms

Oxygen	3.5
Nitrogen	3.0
Fluorine	3.9
Chlorine	3.1
Hydrogen	2.1
Carbon	2.5

For example, in a molecule of HCl, with hydrogen covalently bonded to chlorine, the electrons are “pulled” toward the chlorine, which is more electronegative. Because of this, there is a slightly greater negative charge near the chlorine atom, compared to the hydrogen (which, correspondingly has a slightly higher positive charge). This unequal charge distribution sets up a **permanent dipole**, with one side being somewhat negative and the other somewhat positive. Because of this, the molecule is described as **polar.**

Intermolecular Forces

As mentioned above, covalent molecules can interact with other molecules through intermolecular forces. The nature of these intermolecular interactions determines many of the properties of covalent compounds. Intermolecular forces are always weaker than chemical bonds.

For example, the permanent dipoles in H-Cl can interact through **dipole-dipole interactions**. This interaction is present between all polar covalent molecules, and it is due to the electrostatic attraction between the partial charges in the permanent dipoles.



Figure 1.2.3. Dipole-Dipole interaction in hydrogen chloride. Image by P.wormer, CC BY-SA 3.0, via Wikimedia Commons

For the most electronegative elements on the period table, that is fluorine, oxygen, and nitrogen (FON), when they are directly attached to hydrogen, the intensity of the electrostatic attraction is so intense that reserves its unique denomination. This intermolecular interaction is known as **hydrogen bond**. Hydrogen bond is the main type of intermoelcular forces present in HF, NH_3, and H_2O. Hydrogen bonds between water molecules are the result of the attraction of the partial positive and partial negative charges on different water molecules (Figure 1.2.4). Hydrogen bonds can also form between hydrogens with a partial positive charge and other strongly electronegative atoms, like nitrogen, with a partial negative charge. It is important to remember that hydrogen bonds are interactions between molecules (or parts of molecules) and are not bonds between atoms, like covalent or ionic bonds.

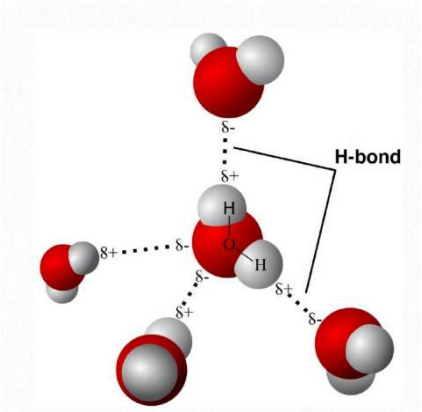


Figure 1.2.4. Hydrogen bonds (dotted lines) between water molecules. Image by Qwerter at Czech wikipedia: Qwerter. Transferred from cs.wikipedia to Commons by sevela.p. Translated to english by by Michal Maňas (User:snek01). Vectorized by Magasjukur2, Public domain, via Wikimedia Commons

The presence of permanent dipoles in polar molecules like water explains the solubility of ionic compounds in this solvent. The net charges present in ionic compounds (cations with positive charge and anions with a negative charge) can interact very effectively with the partial charges present in the dipole molecules through an interaction known as **ion-dipole**. This interaction is strong enough that many ionic compounds are soluble in water, although sometimes the interaction between teh ions in teh crystal are stronger than the ion-dipoles forces, so not every ionic compound is soluble in water.

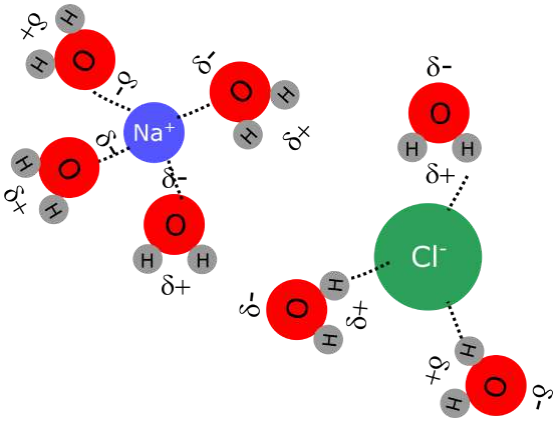


Figure 1.2.5. Ion dipole interactions between ions in sodium chloride and water molecules.

Bonds between hydrogen and carbon do not form significant partial charges because the electronegativities of the two atoms are similar. Consequently, molecules containing many carbon-hydrogen bonds will not form hydrogen bonds and therefore, do not mix well with polar solvents water. These compounds for which the difference of electronegativity between atoms is zero (or almost zero) are known as **non-polar covalent compounds**. Examples are F_2, O_2, CO_2 (consider the linear molecular geometry here), or CH_4. In non-polar compounds, the only possible intermolecular forces result from the mobility of electrons that might generate an uneven distribution of charge, with one atom becoming temporarily more negatively charged and another atom becoming more positively charged. This type of dipoles is very short-lived, and the attraction that results from these dipoles is very weak. This interaction is called **London Dispersion Forces** (LDF). Although London dispersion forces are weak, they increase with the number of atoms, so large molecules can exhibit quite strong LDF.

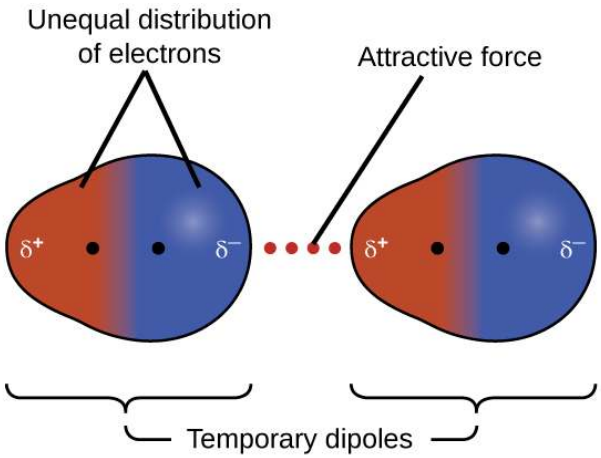


Figure 1.2.6. Temporary dipoles and London Dispersion forces. Image by OpenStax, CC BY 4.0, via Wikimedia Commons

Non-polar molecules with LDF are called **hydrophobic** because they do not mix well with water. Other polar compounds with the ability to dissolve in water are called **hydrophilic**. Molecules possessing both characteristics are called **amphiphilic.**

In compounds with similar molecular mass, the strength of these intermolecular forces decree in the following order:

ion-dipole > hydrogen bond > dipole-dipole > London Dispersion Forces

Functional groups

Organic chemistry os the study of carbon-containing compounds. These compounds are classified according to particular structural features called **functional groups**. Figure 1.2.7 shows the various organic functional groups common in biochemistry. You will encounter these functional groups as you study the biosynthetic and breakdown pathways that build and recycle the chemical compounds of which cells are made. In addition to knowing the names and structures of these groups, students need a basic understanding of covalent and ionic bonds.

Class	General Structure	Name	Functional Group
Alkenes	RCH═CHR	Double Bond	C═C
Alcohols	ROH	Hydroxyl	—OH
Ethers	ROR	Ether	—O
Amines	RNH_2		
	R_2NH		
	R_3N	Amino	—N<
Thiols	RSH	Sulfhydryl	—SH
Aldehydes	R—C(=O)—H	Carbonyl	—C(=O)—
Ketones	R—C(=O)—R	Carbonyl	—C(=O)—
Carboxylic Acids	R—C(=O)—OH	Carboxyl	—C(=O)—OH
Amides	R—C(=O)—NR_2	Amide	—C(=O)—N<
Esters	R—C(=O)—OR'	Ester	—C(=O)—OR
Phosphoric Acid Esters	R—O—P(=O)(OH)—OH	Phosphoric Ester	—O—P(=O)(OH)—OH

Figure 1.2.7. Important functional groups in biochemistry Image by Aleia Kim

Covalent bonds, as you know, are the result of sharing of electrons between two atoms. Ionic bonds, by contrast, are formed when one atom donates an electron to another, such as in the formation of sodium chloride. Single covalent bonds can rotate freely, but double bonds cannot. Single bonds around a carbon atom are arranged in a tetrahedron with bond angles of 109.5° relative to each other, with the carbon at the center (Figure 1.19). Double bonded carbons create a planar structure with bond angles typically of about 120°

Oxidation/reduction in organic molecules.

Oxidation involves loss of electrons (oxidation number increases) and reduction results in the gain of electrons (oxidation number decreases). Oxidation reactions tend to release energy and are a source off energy. Every oxidation is always accompanied by a reduction, so these reactions are called **redox reactions.** This definition is very useful when dealing with inorganic compounds. However, most molecules encountered in biological systems are organic. When dealing with organic molecules, we need an easier to identify oxidation and reduction rather than counting oxidation numbers. For organic molecules, oxidation reactions can be understood as the gain of oxygen or the loss of hydrogen atoms. On the other hand, reduction reactions can be understood as the gain of hydrogen atoms, or the loss of oxygen.

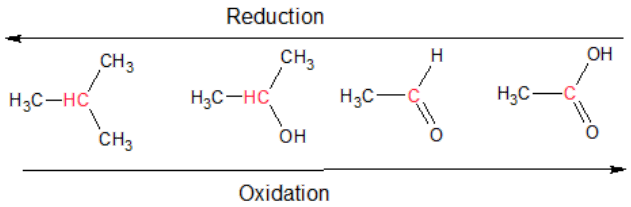


Figure 1.2.8. Redox reactions in organic chemistry. The carbon indicated in red is oxidized from left to right, and reduced from right to left.

Stereochemistry

A carbon has the ability to make four single bonds (forming a tetrahedral structure) and if it bonds to four different chemical groups, their atoms can be arranged around the carbon in two different ways, giving rise to **enantiomers** (Figure 1.2.9). Each carbon with such a property is referred to as an stereogenic center or chiral carbon. The property of handedness only occurs when a carbon has four different groups bonded to it. Enantiomers are stereoisomers that constitute mirror images from each other. Enantiomers have the same chemical and physical properties, but they differ in their biological properties. Enzymes have very specific 3-D structures, so they can differentiate between stereoisomers or enantiomers. By contrast, molecules made chemically (not using enzymes) end up with equal amounts of both enantiomers, called a **racemic mix**.

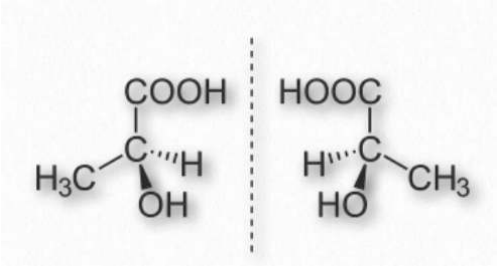


Figure 1.2.9. Enantiomers of lactic acid

Gibbs free energy

Chemical reactions are accompanied by energy changes. That energy can represent changes in heat (enthalpy, H) or disorder (entropy, S). In terms of enthalpy, a chemical reaction can be accompanied by a release of heat ($\Delta H<0$) or absorption of heat ($\Delta H>0$). The former is an exothermic reaction, and the latter is an endothermic reaction. In terms of entropy, a reaction can be accompanied by an increase ($\Delta S>0$) or decrease of disorder ($\Delta S<0$)

The total energy change in a chemical reaction can be expressed in terms of its free energy change, also known as Gibbs energy (G).

$$\Delta G = \Delta H - T\Delta S \tag{1.2.1}$$

The Gibbs free energy calculation allows us to determine whether a reaction will be spontaneous, by taking into consideration two factors, change in enthalpy (ΔH) and change in entropy (ΔS). The free energy change ΔG for a reaction equal to the enthalpy change (ΔH) minus the absolute temperature (T) times the entropy change (ΔS):

$$\Delta G = \Delta H - T\Delta S \tag{1.2.2}$$

A negative ΔG corresponds to release of free energy. Reactions that release energy are **exergonic**, whereas those that absorb energy are called **endergonic**. Exergonic reactions are **spontaneous**, while endergonic reactions are **non-spontaneous.**

The biological standard Gibbs free energy change (ΔG°') corresponds to the ΔG for a process under standard conditions of temperature, pressure, and at pH = 7. For a reaction

$$aA + bB \rightleftharpoons cC + dD, \tag{1.2.3}$$

If a process has a $\Delta G = Z$ and a second process has a $\Delta G = Y$, then if the two processes are linked, ΔG and $\Delta G^{o\prime}$ values for the overall reaction will be the sum of the individual ΔG and ΔG°' values.

$$\Delta G_{total} = \Delta G_1 + \Delta G_2 = Z + Y \tag{1.2.4}$$

$$\Delta G^{o\prime}_{total} = \Delta G^{o\prime}_1 + \Delta G^{o\prime}_2 \tag{1.2.5}$$

Reversible and irreversible reactions

There two types of chemical reactions. **Irreversible reactions** are those for which the conversion of reactants into products is close to 100%, meaning that after teh reaction has taken place, almost no reactants remain and all we find in the reaction container are products. These reactions are indicated by a single reaction arrow:

$$aA + bB \rightarrow cC + dD, \tag{1.2.6}$$

Irreversible reactions are difficult to undo. The same reactioncannot be used to convert product into reactants.

The other type of chemical reactions are reversible. For a **reversible reaction**, we use a double arrow to indicate that reactants react forming products, but at the same time, products can also react regenerating the reactants:

$$aA + bB \rightleftharpoons cC + dD, \tag{1.2.7}$$

After a certain time, a container in which a reversible reaction takes place reaches a situation in which the concentration of reactants and product remain constant, since the rate of the forward reactions the rate of the reverse reactions. When this happens, we say that the system has reached equilibrium, and we can define the equilibrium constant, K_{eq}, which is equal to:

$$K_{eq} = \frac{[C]^c_{eq}[D]^d_{eq}}{[A]^a_{eq}[B]^b_{eq}} \tag{1.2.8}$$

where a, b, c, and d are integers in the balanced equation. Large values of K_{eq} correspond to favorable reactions (more C and D produced than A and B) and small values of K_{eq} mean the opposite. At equilibrium,

$$\Delta G^{o\prime} = -RT \ln K_{eq} \tag{1.2.9}$$

Le Chatelier's principle establishes that a reaction at equilibrium can be shifted towards reactants or products by changing the reaction conditions, such as adding products or reactants, or removing products or reactants. In other words, reversible reactions can favor the formation of reactants or products, depending on the situation.

Both types of reactions, reversible and irreversible, can be found in biological systems, and therefore, they play a crucial role in maintaining and regulating the metabolic balance.

Reaction rates and catalysis

The rate of a chemical reaction depends on factors such as the temperature and the concentration of reactants. Increasing the temperature or the concentration of reactants can increase the reaction rate. The main factor regulating the rate of a chemical reaction is its **activation energy**. Reactions with high activation energy are slower than reactions with low activation energy, independently if the reaction is very spontaneous or not. Therefore, reducing the activation energy is another way by which we can increase reaction rate. A substance capable of reducing the activation energy for a reaction is called a **catalyst**. Because catalysts remain unchanged at the end of a reaction, a single catalyst molecule can be reused for many reaction cycles. Proteins that catalyze reactions in cells are called **enzymes**, while ribozymes are RNA molecules that act as catalysts.

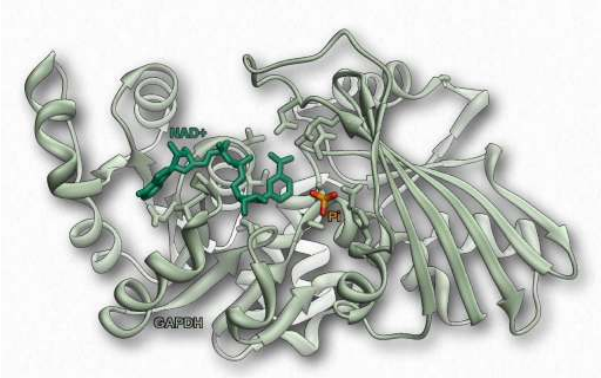


Figure 1.2.10. Glyceraldehyde-3-phosphate dehydrogenase in the midst of catalysis. Image by Vossman, CC BY-SA 3.0, via Wikimedia Commons

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1.3: Water and Buffers

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When it comes to water, we're literally drowning in it, as water is by far the most abundant component of every cell. To understand life, we begin the discussion with the basics of water, because everything that happens in cells, even reactions buried deep inside enzymes, away from water, is influenced by water's chemistry.

The water molecule has wide 'V' shape (the HO-H angle is slightly smaller than 109°) with uneven sharing of electrons between the oxygen and the hydrogen atoms (Figure 1.3.1). Oxygen, with its higher electronegativity, holds electrons closer to itself than the hydrogens do. The hydrogens, as a result, are described as having a partial positive charge (typically designated as δ+) and the oxygen has a partial negative charge (written as δ-). Thus, **water is a polar molecule** because charges are distributed around it unevenly, not symmetrically.

Water as a solvent

Water (Figure 1.3.1.) is described as a solvent because of its ability to solvate (dissolve) many, but not all, molecules. Molecules that are ionic or polar dissolve readily in water, but non-polar substances dissolve poorly in water, if at all. Oil, for example, which is non-polar, separates from water when mixed with it. On the other hand, sodium chloride, which dissociate, forms ion-dipole interaction, while ethanol, which is polar, forms hydrogen bonds with water. Both compounds dissolve in water. Ethanol's solubility in water is crucial for brewers, winemakers, and distillers – but for this property, there would be no wine, beer or spirits. As explained in an earlier section, we use the term **hydrophilic** to describe substances that interact well with water and dissolve in it, and the term **hydrophobic** to refer to materials that are non-polar and do not dissolve in water. Table 1.3 illustrates some polar and non-polar substances. A third term, **amphiphilic**, refers to compounds that have both properties. Soaps, for example are amphiphilic, containing a long, non-polar aliphatic tail and a head that ionizes.

Hydrophilic vs Hydrophobic Compounds

Hydrophobic	Hydrophilic
Nonpolar hydrocarbons (hexane)	Ionic compounds (NaCl)
Lipids (fats and cholesterol)	Polar organic compounds (alcohols, ketones or carbonyls)
	Weak acids (phosphates, amino acids)
	Sugars/carbohydrates

Table 1.3 Image by Aleia Kim

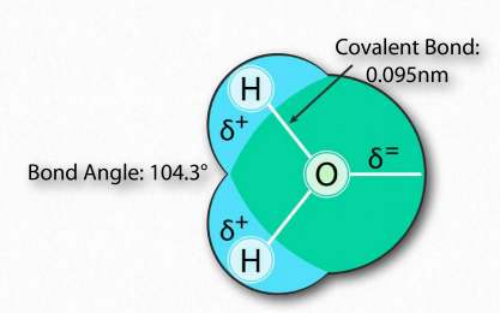


Figure 1.3.1 .Arrangement of atoms in water Image by Aleia Kim

The solubility of materials in water is based in free energy changes, as measured by ΔG. Remember, from chemistry, that H is the enthalpy (heat at constant pressure) and S is entropy. Given this,

$$\Delta G = \Delta H - T\Delta S \tag{1.3.1}$$

where T is the temperature in Kelvin. For a process to be favorable, the ΔG for it must be less than zero.

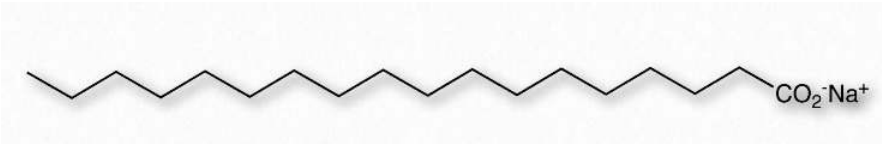


Figure 1.3.2. Structure of a Soap

From the equation, lowered ΔG values will be favored with decreases in enthalpy and/or increases in entropy. Let us first consider why non-polar materials do not dissolve in water. We could imagine a situation where the process of dissolving involves the "surrounding" of each molecule of the nonpolar solute in water, just like each sodium and each chloride ion gets surrounded by water molecules as salt dissolves.

Hydrogen bonds in biological systems

The importance of hydrogen bonds in biochemistry (Figure 1.3.4) is hard to overstate. Linus Pauling himself said,

" . . . . I believe that as the methods of structural chemistry are further applied to physiological problems it will be found that the significance of the hydrogen bond for physiology is greater than that of any other single structural feature."

According to IUPAC, hydrogen bond is an attractive interaction between a hydrogen atom from a molecule or a molecular fragment X–H in which X is more electronegative than H (X=F, O, N), and an atom or a group of atoms in the same or a different molecule".

The role of hydrogen bonds does not apply to solubility (Figure 1.3.4) of polar substances in water, but it is crucial to understand protein structure and DNA double-helix formation (Figure 1.3.5). Hydrogen bonds also play roles in binding of substrates to enzymes, catalysis, and protein-protein interaction, as well as other kinds of binding, such as protein-DNA, or antibody-antigen.

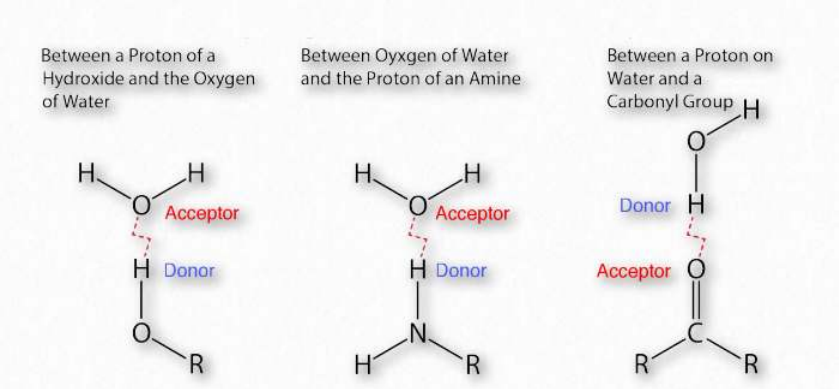


Figure 1.3.4 - Hydrogen bonds between water molecules Image by Pehr Jacobson

Bond Energies

	Type of Bond	Bond Energy (kJ/mol)
Covalent Bonds	C—H	413
	O—H	460
Noncovalent Bonds	Hydrophobic Interaction	4-12
	Hydrogen Bond	20
	Ion-dipole Interaction	20

Table 1.3.2 Image by Aleia Kim

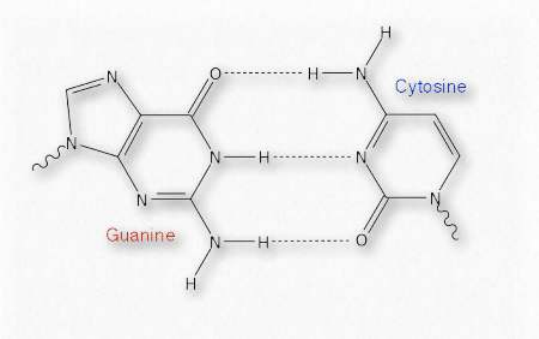


Figure 1.3.5 - Hydrogen bonds in a base pair of DNA Image by Aleia Kim

As noted, hydrogen bonds are weaker than covalent bonds (Table 1.4) and their strength varies form very weak (1-2 kJ/mol) to fairly strong (29 kJ/mol). Hydrogen bonds only occur over relatively short distances (2.2 to 4.0 Å). The farther apart the hydrogen bond distance is, the weaker the bond is.

The strength of the bond in kJ/mol represents the amount of heat that must be put into the system to break the bond - the larger the number, the greater the strength of the bond. Hydrogen bonds are readily broken using heat. The boiling of water, for example, requires breaking of H-bonds. When a biological structure, such as a protein or a DNA molecule, is stabilized by hydrogen bonds, breaking those bonds destabilizes the structure and can result in denaturation of the substance - loss of structure. It is partly for this reason that most proteins and all DNAs lose their native, or folded, structures when heated to boiling.

Weak Acid pKa Values

Name	Chemical Structure of Acid	Chemical Structure of Salt	pKa
Acetic Acid	CH_3COOH	CH_3COO^-	4.76
Formic Acid	$HCOOH$	$HCOO^-$	3.75
Lactic Acid	$CH_3CHOHCOOH$	$CH_3CH—HCOO^-$	3.86
Pyruvic Acid	$CH_3COCOOH$	$CH_3C—COO^-$	2.50
Oxalic Acid (1)	$HOOC—COOH$	$HOOC—COO^-$	1.23
Oxalic Acid (2)	$HOOC—COO^-$	$OOC—COO^-$	4.19
Carbonic Acid (1)	H_2CO_3	HCO_3^-	6.37
Carbonic Acid (2)	HCO_3^-	CO_3^{2-}	10.20
Malic Acid (1)	$HOOC—CH_2—CHOH—COOH$	$HOOC—CH_2—CHOH—COO^-$	3.40
Malic Acid (2)	$HOOC—CH_2—CHOH—COO^-$	$^-OOC—CH_2—CHOH—COO^-$	5.26
Malonic Acid (1)	$HOOC—CH_2—COOH$	$HOOC—CH_2—COO^-$	2.83
Malonic Acid (2)	$HOOC—CH_2—COO^-$	$^-OOC—CH_2—COO^-$	5.69
Phosphoric Acid (1)	H_3PO_4	$H_2PO_4^-$	2.14
Phosphoric Acid (2)	$H_2PO_4^-$	HPO_4^{2-}	7.20
Phosphoric Acid (3)	HPO_4^{2-}	PO_4^{3-}	12.40
Succinic Acid (1)	$HOOC—CH_2—CH_2—COOH$	$HOOC—CH_2—CH_2—COO^-$	4.21
Succinic Acid (2)	$HOOC—CH_2—CH_2—COO^-$	$^-OOC—CH_2—CH_2—COO^-$	5.63

Image by Aleia Kim Table 1.5

For DNA molecules, denaturation results in complete separation of the strands from each other. For most proteins, this means loss of their characteristic three-dimensional structure and with it, loss of the function they performed. Though a few proteins can readily reassume their original structure when the solution they are in is cooled, most can't. This is one of the reasons that we cook our food. Proteins are essential for life, so denaturation of bacterial proteins results in death of any microorganisms contaminating the food.

Acids vs bases

Water can ionize to a slight extent (10^{-7} M) to form H^+ (proton) and OH^- (hydroxide). We measure the proton concentration of a solution with pH, which is the negative log of the proton concentration.

$$pH = -\log[H+]$$

In pure water, the proton concentration is $[H+]= 10^{-7}$ M, then the pH is 7. We could just as easily measure the hydroxide concentration with the pOH by the parallel equation,

$$pOH = -\log[OH^-]$$

In pure water, dissociation of a proton simultaneously creates a hydroxide, so the pOH of pure water is 7, as well. This also means that

$$pH + pOH = 14$$

Now, because protons and hydroxides can combine to form water, a large amount of one will cause there to be a small amount of the other. Why is this the case? In simple terms, if I dump 0.1 moles of H^+ into a pure water solution, the high proton concentration will react with the relatively small amount of hydroxides to create water, thus reducing hydroxide concentration. Similarly, if I dump excess hydroxide (as NaOH, for example) into pure water, the proton concentration falls for the same reason.

Chemists use the term "acid" to refer to a substance which has protons that can dissociate (come off) when dissolved in water. They use the term "base" to refer to a substance that can absorb protons when dissolved in water. Both acids and bases come in strong and weak forms. (Examples of weak acids are shown in Table 1.5.) Strong acids, such as HCl, dissociate completely in water. If we add 0.1 moles (6.02×10^{22} molecules) of HCl to a solution to make a liter, it will have 0.1 moles of H^+ and 0.1 moles of Cl^- or

$6.02x10^{22}$ molecules of each . There will be no remaining HCl when this happens. A strong base like NaOH also dissociates completely into Na^+ and OH^- .

Weak Acids

Weak acids and bases differ from their strong counterparts. When you put one mole of acetic acid (HAc) into pure water, only a tiny percentage of the HAc molecules dissociate into H^+ and Ac^-. Clearly, weak acids are very different from strong acids. Weak bases behave similarly, except that they accept protons, rather than donate them. Since we can view everything as a form of a weak acid, we will not use the term weak base here.

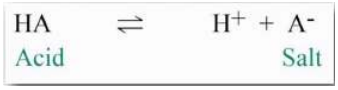


Figure 1.3.6 - Dissociation of a weak acid. Image by Aleia Kim

Students are often puzzled and expect that $[H^+] = [A^-]$ because the dissociation equation shows one of each from HA. This is, in fact, true ONLY when HA is allowed to dissociate in pure water. Usually the HA is placed into solution that has protons and hydroxides to affect things. Those protons and /or hydroxides change the H^+ and A concentration unequally, since A^- can absorb some of the protons and/or HA can release H^+ when influenced by the OH- in the solution. Therefore, one must calculate the proton concentration from the pH using the **Henderson Hasselbalch equation**.

$$pH = pKa + \log([Ac^-]/[HAc])$$

Salt/Acid Ratio as a Function of pK_as

pH	[Salt]/[Acid]
$pK_a + 3$	1000
$pK_a + 2$	100
$pK_a + 1$	10
pK_a	1
$pK_a - 1$	1/10
$pK_a - 2$	1/100
$pK_a - 3$	1/1000

Image by Aleia Kim Table 1.6

You may wonder why we care about weak acids. You may never have thought much of weak acids when you were in General Chemistry. Your instructor described them as buffers and you probably dutifully memorized the fact that “buffers are substances that resist change in pH” without really learning what Clearing Confusion - this meant. Buffers are much too important to be thought of in this way.

Weak acids are critical for life because their affinity for protons causes them to behave like a buffer in certain pH ranges, providing (or absorbing) protons as needed. Weak acids thus help to keep the H^+ concentration (and thus the pH) of the solution they are in relatively constant.

The importance of buffers: **Buffered vs non-buffered solutions**

To understand how well a buffer protects against changes in pH, consider the effect of adding .01 moles of HCl to 1.0 liter of pure water (no volume change) at pH 7, compared to adding it to 1.0 liter of a 1M acetate buffer at pH 4.76. Since HCl completely dissociates, in 0.01M (10^{-2} M) HCl you will have 0.01M H^+. For the pure water, the pH drops from 7.0 down to 2.0 (pH = -log(0.01M)).

By contrast, the acetate buffer’s pH after adding the same amount of HCl is 4.74. Thus, the pure water solution sees its pH fall from 7 to 2 (5 pH units), whereas the buffered solution saw its pH drop from 4.76 to 4.74 (0.02 pH units). Clearly, the buffer minimizes the impact of the added protons compared to the pure water.

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1.4: Prebiotic Earth and the origin of life

Thinking about Life's Origins- A Short Summary of a Long History

By all accounts, the earth must have been a very unpleasant place soon after its formation! Volcanic eruptions, meteorites, thunder, lighting, dust, fires, etc. For that reason, the period from 4.8 to 4.0 billion years ago is called the Hadean Eon, after Hades, the hell of the ancient Greeks! Until recently, geological, geochemical and fossil evidence suggested that life arose between 3.8 and 4.1 billion years ago. In fact, questions about life's origins are probably as old as the ancient Greeks! We only have records of human notions of life's origins dating from biblical accounts and, just a bit later, from Aristotle's musings. While Aristotle did not suggest that life began in hell, he and other ancient Greeks did speculate about life's origins by *spontaneous generation*, in the sense of *abiogenesis* (*life* originating from *non-life*).

Later, the dominant theological accounts of creation in Europe in the middle ages muted any notions of origins and evolution. While a few medieval voices ran counter to strict biblical readings of the creation stories, it was not until the Renaissance in the 14th -17th century that an appreciation of ancient Greek *humanism* was reawakened, and with it, scientific curiosity and the ability to engage in rational questioning and research.

Charles Darwin suggested in 1859 that life might have begun in a "*warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, &c., present, that a protein compound was chemically formed ready to undergo still more complex changes.*" He even realized that these chemical constituents would not have survived in the atmosphere and waters of his day, but must have done so in a **prebiotic world.** In *On the Origin of Species*, he referred to life having been 'created'. There, Darwin was not referring to a biblical basis of creation; he clearly meant that life originated "*by some wholly unknown process*" at a time before which there was no life.

Among Darwin's friends and contemporaries were Charles Lyell and Roderick Murchison, both geologists who understood much about the slow geological changes that shaped the earth. Darwin was therefore familiar with the concept of extended periods of geological time, amounts of time he believed was necessary for the natural selection of traits leading to species divergence.

Fast-forward to the 1920s when J.H.B.S. Haldane and A. Oparin offered an hypothesis about the life's origins based on notions of the chemistry and physical conditions that might have existed on a **prebiotic earth**. Their proposal assumed that the earth's atmosphere was hot, hellish and reducing (i.e., filled with inorganic molecules able to give up electrons and hydrogens). There are more than a few hypotheses for which chemicals were already present on earth, or that formed when the planet formed about 4.8 billion years ago. We'll start our exploration with Oparin and Haldane's *reducing atmosphere*. Then we look at possibility that life began under *non-reducing conditions* .

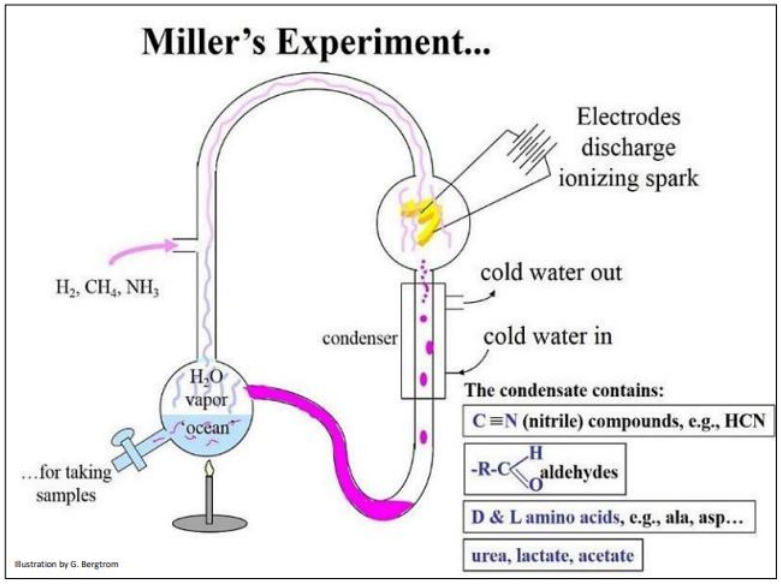
From Inorganic to Organic molecules, and to Life

A prerequisite to the prebiotic chemical experimentation is a source of organic molecules. Just as life requires energy (to do anything and everything!), converting inorganic molecules into organic molecules requires an input of *free energy*. Today, most living things get free energy by oxidizing nutrients or directly from the sun by photosynthesis. Recall that in fact all the chemical energy sustaining life today ultimately comes from the sun. But, before there were cells, how did organic molecules form from inorganic precursors? Oparin and Haldane hypothesized **a reducing atmosphere on the prebiotic earth** (there was no oxygen before photosynthesis was possible), rich in inorganic molecules with *reducing power* (like H_2, NH_3, CH_4, and H_2S) as well as CO_2 to serve as a carbon source. The predicted physical conditions on this prebiotic earth were:

- lots of water (oceans).
- hot (no free O_2).
- lots ionizing (e.g., X, γ) radiation from space, (no protective ozone layer).
- frequent ionizing (electrical) storms generated in an unstable atmosphere.
- volcanic and thermal vent activity.

Origins of Organic Molecules and a Primordial Soup

Oparin suggested that abundant sources of free energy fueled the reductive synthesis of the first organic molecules to create what he called a "**primeval soup**". No doubt, he called this primeval concoction a "soup" because it would have been rich in chemical (nutrient) free energy. The Oparin/Haldane proposal received strong support from the experiments of **Stanley Miller and Harold Urey** (Urey had already won the 1934 Nobel Prize in Chemistry for discovering deuterium). Miller and Urey tested the prediction that, under Haldane and Oparin's prebiotic earth conditions, inorganic molecules could produce the organic molecules in what came known as the *primordial soup*. Their famous experiment, in which they provided energy to a mixture of inorganic molecules with reducing power, is illustrated below.



Miller's earliest published data indicated the presence of several organic molecules in their *ocean* flask, including a few familiar metabolic organic acids (lactate, acetate, several amino acids…) as well as several highly reactive *aldehydes* and *nitriles*. The latter can interact in spontaneous chemical reactions to form organic compounds. Later analyses further revealed purines, carbohydrates and fatty acids in the flask. Later still, 50 years after Miller's experiments (and a few years after his death), some un-analyzed sample collection tubes from those early experiments were discovered.

When the contents of these tubes were analyzed with newer, more sensitive detection techniques, they were shown to contain additional organic molecules not originally reported, including 23 amino acids.

Clearly, the thermodynamic and chemical conditions proposed by Oparin and Haldane could support the *reductive synthesis* of organic molecules. At some point, Oparin and Haldane's evolving chemistries would have to have been internalized inside of semipermeable aggregates (or boundaries) destined to become cells. Examples of such structures are discussed below. A nutrient-rich primordial soup would likely have favored the genesis of *heterotrophic* cells that could use environmental nutrients for energy and growth, implying an early evolution of fermentative pathways similar to glycolysis. But, these first cells would quickly consume the nutrients in the soup, quickly ending the earth's new vitality!

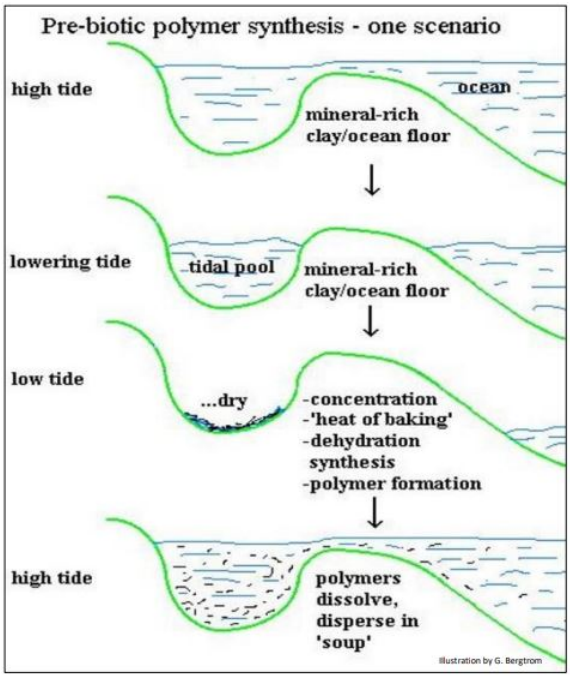
So, one must propose an early evolution of least small populations of cells that could capture free energy from inorganic molecules (**chemoautotrophs**) or even sunlight (**photoautotrophs**). As energy-rich organic nutrients in the 'soup' declined, autotrophs (notably photoautotrophs that could split water using solar energy) would be selected. Photoautotrophs would *fix* CO_2, reducing it with H^- ions from water. Photoautotrophy (**photosynthesis**) would thus replenish carbohydrates and other nutrients in the oceans and add O_2 to the atmosphere.

Oxygen would have been toxic to most cells, but a few already had the ability to survive oxygen. Presumably these spread, evolving into cells that could *respire*, i.e., use oxygen to *burn* environmental nutrients. Respiratory metabolism must have followed hard on the heels of the spread of photosynthesis.

Photosynthesis began between 3.5 and 2.5 billion years ago (the Archaean Eon). Eventually, photosynthetic and aerobic cells and organisms achieved a natural balance to become the dominant species in our oxygen-rich world.

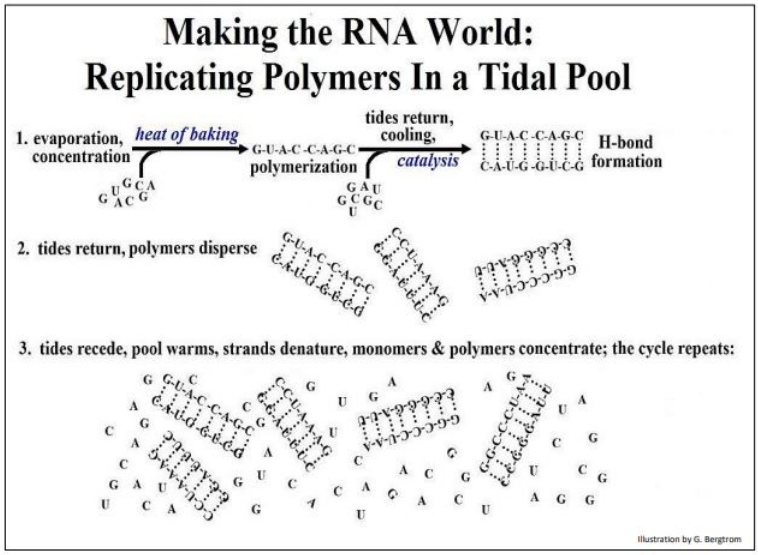
The Tidal Pool Scenario for an Origin of Polymers and Replicating Chemistries

In this scenario, prebiotic organic monomers would concentrate in tidal pools in the heat of a primordial day, followed by polymerization by dehydration synthesis. The formation of polymer linkages is an 'uphill' reaction requiring free energy. Very high temperatures (the **heat of baking**) can link monomers by dehydration synthesis in the laboratory, and may have done so in tidal pool sediments to form random polymers. This scenario further assumes that the dispersal of these polymers from the tidal pools with the ebb and flow of high tides. The tidal pool scenario is illustrated below.



The concentration of putative organic monomers at the bottom of tidal pools may have offered opportunities to catalyze polymerization, even in the absence of very high heat. Many metals (nickel, platinum, silver, even hydrogen) are inorganic catalysts, able to speed up many chemical reactions. The heavier metals were likely to exist in the earth's crust as well as in the sediments of primordial oceans, as they do today. Such mineral aggregates in soils and clays have been shown to possess catalytic properties. Furthermore, metals (e.g., magnesium, manganese…) are now an integral part of many enzymes, consistent with an origin of biological catalysts in simpler aggregated mineral catalysts in ocean sediments.

Before life, the micro-surfaces of mineral-enriched sediment, if undisturbed, could have been able to catalyze the same or at least similar reactions repeatedly, leading to related sets of polymers. Consider the possibilities for RNA monomers and polymers, based on the assumption that life began in an RNA world. The possibilities are illustrated below.



The result predicted here is the formation not only of RNA polymers (perhaps only short ones at first), but of H-bonded double-stranded RNA molecules that might effectively replicate at each cycle of concentration, polymerization and dispersal. Heat and the free energy released by these same reactions could have supported polymerization, while catalysis would have enhanced the fidelity of RNA replication.

Of course, in the tidal pool scenario, repeated high heat or other physical or chemical attack might also degrade newly formed polymers. But what if some RNA double strands were more resistant to destruction. Such early RNA duplexes would accumulate at the expense of the weaker, more susceptible ones. Only the *fittest* replicated molecules would be selected and persist in the environment! The environmental accumulation of structurally related, replicable and stable polymers reflects a prebiotic chemical **homeostasis** (one of those properties of life!)

Overall, this scenario hangs together nicely, and has done so for many decades. However, there are now challenging questions about the premise of a prebiotic reducing environment. Newer evidence points to an earth atmosphere that was not at all reducing, casting doubt on the idea that the first cells on the planet were heterotrophs. Recent proposals posit alternative sources of prebiotic free energy and organic molecules that look quite different from those assumed by Oparin, Haldane, Urey and Miller.

Citations

Gerald Bergtrom. Formation of Organic Molecules in an Earthly Reducing Atmosphere. (2021, January 3). Retrieved April 21, 2021, from https://bio.libretexts.org/@go/page/16543

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CHAPTER OVERVIEW

2: Carbohydrates

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2.2: Classification of Carbohydrates
2.3: Fischer Projections
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2.5: Physical properties of monosaccharides
2.6: Cyclic Structures of Monosaccharides
2.7: Reactions of Monosaccharides
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2.1: Introduction

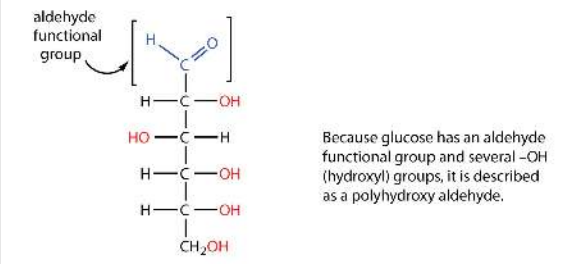
Objectives

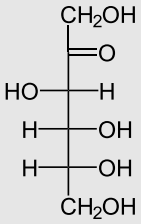
After completing this section, you should be able to

1. identify carbohydrates (sugars) as being polyhydroxylated aldehydes and ketones.

Introduction

All carbohydrates consist of carbon, hydrogen, and oxygen atoms and are **polyhydroxy aldehydes** or **polyhydroxyketones** or are compounds that can be broken down to form such compounds. Examples of carbohydrates include glucose, ribose, fructose, table sugar, starch, and structural materials such as cellulose. The term *carbohydrate* had its origin in a misinterpretation of the molecular formulas of many of these substances. The molecular formula for a carbohydrate can be considered as $C_n(H_2O)n$, where n is the number of carbon atoms. or example, because its formula is $C_6H_{12}O_6$, glucose was once thought to be a "carbon hydrate" with the structure $C_6(H_2O)_6$.

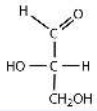




Fructose has a ketone group, so it is considered a polyhydroxy ketone. Image by Ayacop, Public domain, via Wikimedia Commons

Example 1

Which compounds would be classified as carbohydrates?

a. 

b. 

c. 

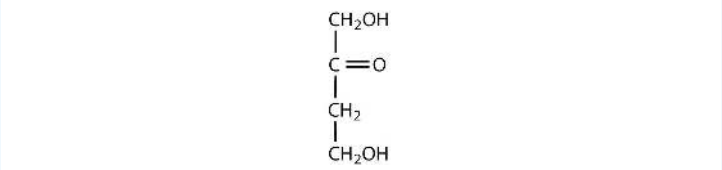
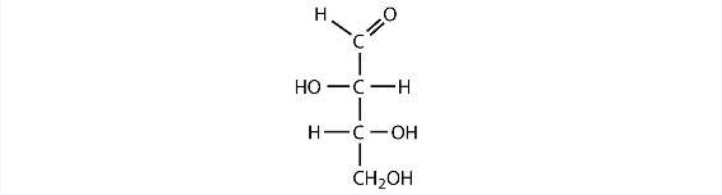
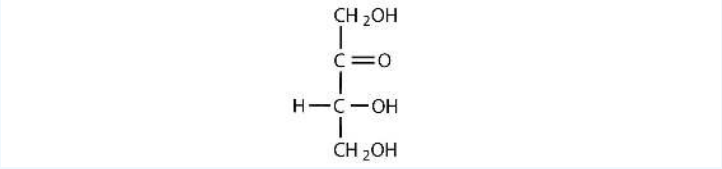
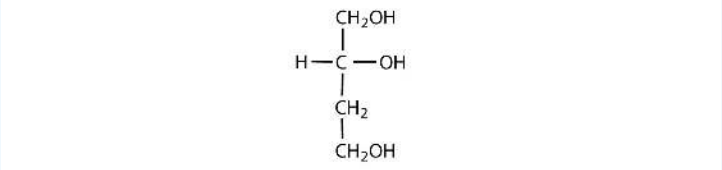
d. 

Solution

a. This is a carbohydrate because the molecule contains an aldehyde functional group with OH groups on the other two carbon atoms.
b. This is not a carbohydrate because the molecule does not contain an aldehyde or a ketone functional group.
c. This is a carbohydrate because the molecule contains a ketone functional group with OH groups on the other two carbon atoms.
d. This is not a carbohydrate; although it has a ketone functional group, one of the other carbons atoms does not have an OH group attached.

Exercise 1

Which compounds would be classified as carbohydrates?

1. 
2. 
3. 
4. 

Green plants are capable of synthesizing glucose ($C_6H_{12}O_6$) from carbon dioxide (CO_2) and water (H_2O) by using solar energy in the process known as photosynthesis:

$$6CO_2 + 6H_2O + 2870\ \text{kJ} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (1)$$

(The 2870 kJ comes from solar energy.) Plants can use the glucose for energy or convert it to larger carbohydrates, such as starch or cellulose. Starch provides energy for later use, perhaps as nourishment for a plant's seeds, while cellulose is the structural material of plants. We can gather and eat the parts of a plant that store energy—seeds, roots, tubers, and fruits—and use some of that energy ourselves. Carbohydrates are also needed for the synthesis of nucleic acids and many proteins and lipids.

Animals, including humans, cannot synthesize carbohydrates from carbon dioxide and water and are therefore dependent on the plant kingdom to provide these vital compounds. We use carbohydrates not only for food (about 60%–65% by mass of the average diet) but also for clothing (cotton, linen, rayon), shelter (wood), fuel (wood), and paper (wood).

Contributors and Attributions

- Dr. Dietmar Kennepohl FCIC (Professor of Chemistry, Athabasca University)
- *The Basics of General, Organic, and Biological Chemistry* by David W. Ball, John W. Hill, and Rhonda J. Scott.

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2.2: Classification of Carbohydrates

Objectives

After completing this section, you should be able to

1. classify a specific carbohydrate as being a monosaccharide, disaccharide, trisaccharide, etc., given the structure of the carbohydrate or sufficient information about its structure.
2. classify a monosaccharide according to the number of carbon atoms present and whether it contains an aldehyde or ketone group.

Key Terms

Make certain that you can define, and use in context, the key terms below.

- aldose
- disaccharide
- ketose
- monosaccharide (simple sugar)
- polysaccharide

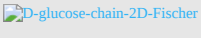
Role of carbohydrates

Carbohydrates are a major source of metabolic energy and energy storage, both for plants and for animals. Aside from the sugars and starches that meet this vital nutritional role, carbohydrates also serve as a structural material (cellulose in plants, tissues, and cells in animals), and as a carbon source for the synthesis of other molecules.

Carbohydrates are called **saccharides** or, if they are relatively small, **sugars**. Several classifications of carbohydrates have proven useful, and are outlined in the following table.

Classification according to Molecular size or Complexity	**Simple Carbohydrates** monosaccharides (1 unit)	**Complex Carbohydrates** disaccharides (2 units) oligosaccharides (3-10 units) polysaccharides (hundreds or thousands of units)
Classification according to Number of carbon atoms	**Triose** C_3 sugars	**Tetrose** C_4 sugars
Classification according to Functional group	**Aldose** sugars having an aldehyde functional group R-HC=O **Ketose** sugars having a ketone functional group R_2-C=O	
Classification according to Reactivity in Redox Reactions	**Reducing** sugars oxidized by Tollens' reagent (or Benedict's or Fehling's reagents). **Non-reducing** sugars not oxidized by Tollens' or other reagents.	

We will first center on monosaccharides, that is, carbohydrates consisting of a single polyhydroxy ketone or polyhydroxy aldehyde unit. Monosaccharides can be classified based on the number of carbon atoms and the functional groups, for example, glucose is an aldose (it contains an aldehyde functional group) and glucose is also a hexose (it contains six carbon atoms. Therefore, glucose is considered as an **aldohexose**.



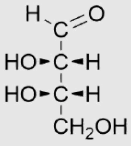
Glucose is classified as an aldohexose, because it contains six carbon atoms and an aldehyde functional group. Image by Ben; Yikrazuul, Public domain, via Wikimedia Commons

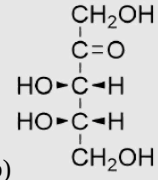
Exercises

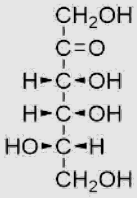
Questions

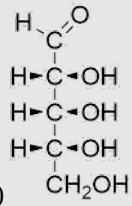
Q25.1.1

Classify each of the following sugars.

(a) 

(b) 

(c) 

(d) 

Solutions

S25.1.1

(a) Aldoterose

(b) Ketopentose

(c) Ketohexose

(d) Aldopentose

Contributors and Attributions

- Dr. Dietmar Kennepohl FCIC (Professor of Chemistry, Athabasca University)
- Prof. Steven Farmer (Sonoma State University)
- William Reusch, Professor Emeritus (Michigan State U.), Virtual Textbook of Organic Chemistry

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2.3: Fischer Projections

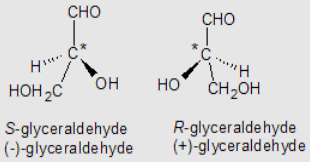
Objectives

After completing this section, you should be able to

1. draw the Fischer projection of a monosaccharide, given its wedge-and-broken-line structure or a molecular model.
2. draw the wedge-and-broken-line structure of a monosaccharide, given its Fischer projection or a molecular model.

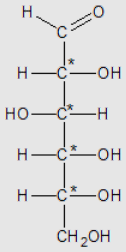
Chirality of carbohydrates

Carbohydrates contain several stereogenic (or chiral) centers, that is, a carbon atom bonded to four different groups. For each stereogenic center, there are two possible arrangements in space for those four groups. Glyceraldehyde is the simplest carbohydrate, and it contains one chiral center (indicated with an *):



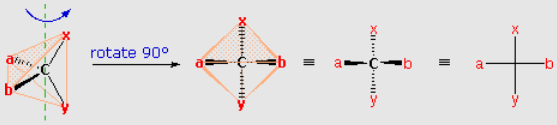
S-glyceraldehyde (-)-glyceraldehyde | *R*-glyceraldehyde (+)-glyceraldehyde

If we have n chiral centers, the maximum number of possible stereoisomers is 2^n. For example in an aldohexose (like glucose), there are four stereogenic centers, and therefore 16 possible stereoisomers, each one with a different three-dimensional configuration.



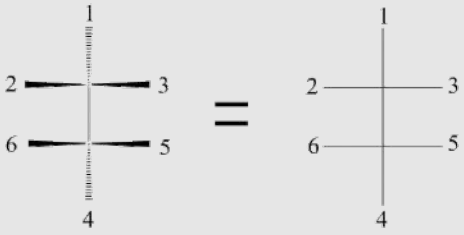
In this case, using the traditional wedge and hatched line representation around each chiral carbon to draw the stereochemistry of each particle stereoisomer becomes nonpractical.

The problem of drawing three-dimensional configurations on a two-dimensional surface, such as a piece of paper, has been a long-standing concern of chemists. The wedge and hatched line notations we have been using are effective but can be troublesome when applied to compounds having many chiral centers. As part of his Nobel Prize-winning research on carbohydrates, the great German chemist Emil Fischer, devised a simple notation that is still widely used. In a Fischer projection drawing, the four bonds to a chiral carbon make a cross with the carbon atom at the intersection of the horizontal and vertical lines. The two horizontal bonds are directed toward the viewer (forward of the stereogenic carbon). The two vertical bonds are directed behind the central carbon (away from the viewer). Since this is not the usual way in which we have viewed such structures, the following diagram shows how a stereogenic carbon positioned in the common two-bonds-in-a-plane orientation (x–C–y define the reference plane) is rotated into the Fischer projection orientation (the far-right formula). When writing Fischer projection formulas it is important to remember these conventions. Since the vertical bonds extend away from the viewer and the horizontal bonds toward the viewer, a Fischer structure may only be turned by 180º within the plane, thus maintaining this relationship. **The structure must not be flipped over or rotated by 90º**.



In the above diagram, if x = COH, y = CH_2OH, a = H & b = OH, the resulting formula describes (*R*)-(–)-glyceraldehyde. The mirror-image formula, where x = COH, y = CH_2OH, a = OH & b = H, would, of course, represent (*S*)-(+)-glyceraldehyde.

The Fischer Projection consists of both horizontal and vertical lines, where the **horizontal lines represent the atoms that are pointed toward the viewer while the vertical line represents atoms that are pointed away from the viewer. The point of intersection between the horizontal and vertical lines represents the central carbon. The main carbon chain (longest) is set as the vertical line.**



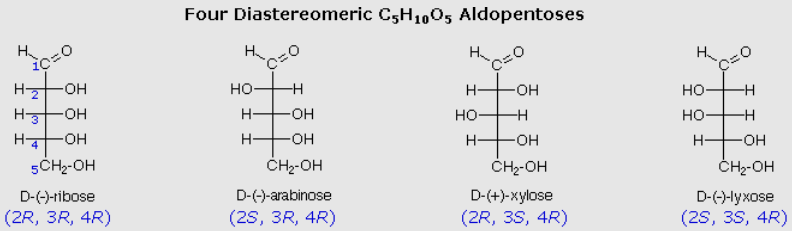
Using the Fischer projection notation, the stereoisomers of glyceraldehyde are drawn in the following manner:

D-Glyceraldehyde 2D Fischer	L-Glyceraldehyde 2D Fischer
(*R*)-glyceraldehyde	(*s*)-glyceraldehyde
D-glyceraldehyde	**L-glyceraldehyde**

Images by Master Uegly, Public domain, via Wikimedia Commons

The structure in which the OH groups is on the **right** is designed as **D**, while the the structure in which the OH groups is on the left is designed as **L**. This nomenclature system is known as the D,L-relative configuration.

The usefulness of this notation to Fischer and D,L-relative configuration, in carbohydrate studies, is evident in the following diagram. Since there are three chiral centers in 2,3,4,5-tetrahydroxypentanal (an aldopentose) we should expect a maximum of 2^3 = 8 stereoisomers. These eight stereoisomers consist of four sets of enantiomers. If the configuration at C-4 is kept constant (*R* in the examples shown here), the four stereoisomers that result will be **diastereomers**. Fischer formulas for these isomers, which Fischer designated as the "D"-family, are shown in the diagram. Each of these compounds has an enantiomer, which is a member of the "L"-family so, as expected, there are eight stereoisomers in all.



The aldopentose structures drawn above are all diastereomers. A more selective term, **epimer**, is used to designate diastereomers that differ in configuration at only one chiral center. Thus, ribose and arabinose are epimers at C-2, and arabinose and lyxose are epimers at C-3. However, arabinose and xylose are not epimers, since their configurations differ at both C-2 and C-3.

How to make Fischer Projections

To make a Fischer Projection, it is easier to show through examples than through words. Let's start with the first example, turning a 3D structure of ethane into a 2D Fischer Projection.

Example 25.2.1

Start by mentally converting a 3D structure into a Dashed-Wedged Line Structure. Remember, the atoms that are pointed toward the viewer would be designated with a wedged lines and the ones pointed away from the viewer are designated with dashed lines.

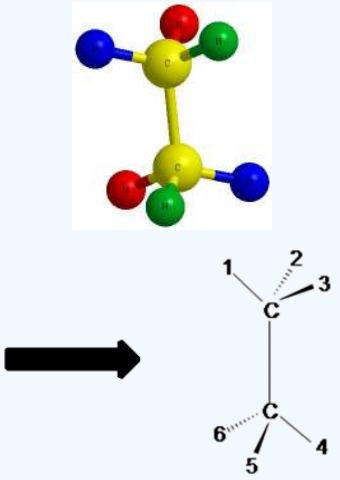


Figure B

Notice the red balls (atoms) in Figure A above are pointed away from the screen. These atoms will be designated with dashed lines like those in Figure B by number 2 and 6. The green balls (atoms) are pointed toward the screen. These atoms will be designated with wedged lines like those in Figure B by number 3 and 5. The blue atoms are in the plane of the screen so they are designated with straight lines.

Now that we have our Dashed- Wedged Line Structure, we can convert it to a Fischer Projection. However, before we can convert this Dashed-Wedged Line Structure into a Fischer Projection, we must first convert it to a “flat” Dashed-Wedged Line Structure. Then from there we can draw our Fischer Projection. Lets start with a more simpler example. Instead of using the ethane shown in Figure A and B, we will start with a methane. The reason being is that it allows us to only focus on one central carbon, which make things a little bit easier.

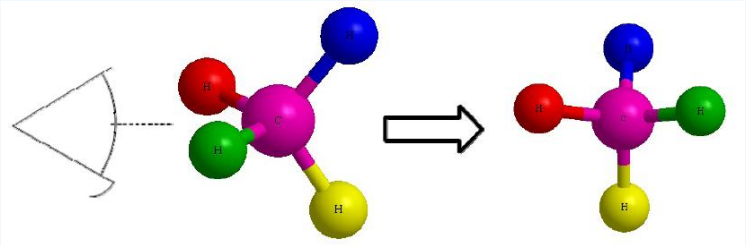
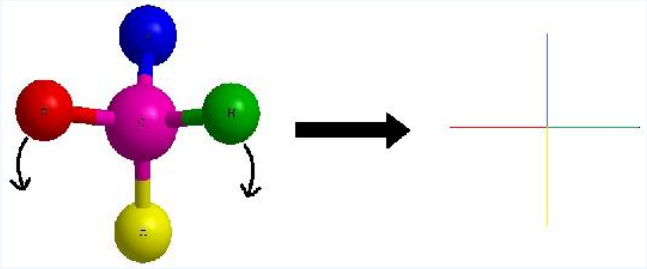


Figure D

Lets start with this 3D image and work our way to a dashed-wedged image. Start by imagining yourself looking directly at the central carbon from the left side as shown in Figure C. It should look something like Figure D. Now take this Figure D and flatten it out on the surface of the paper and you should get an image of a cross.



> As a reminder, the horizontal line represents atoms that are coming out of the paper and the vertical line represents atoms that are going into the paper. The cross image to the right of the arrow is a Fischer projection.

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2.4: D and L Monosaccharides

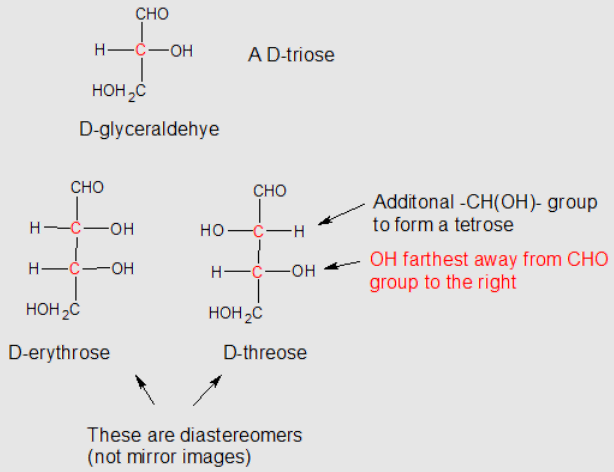
Objectives

After completing this section, you should be able to

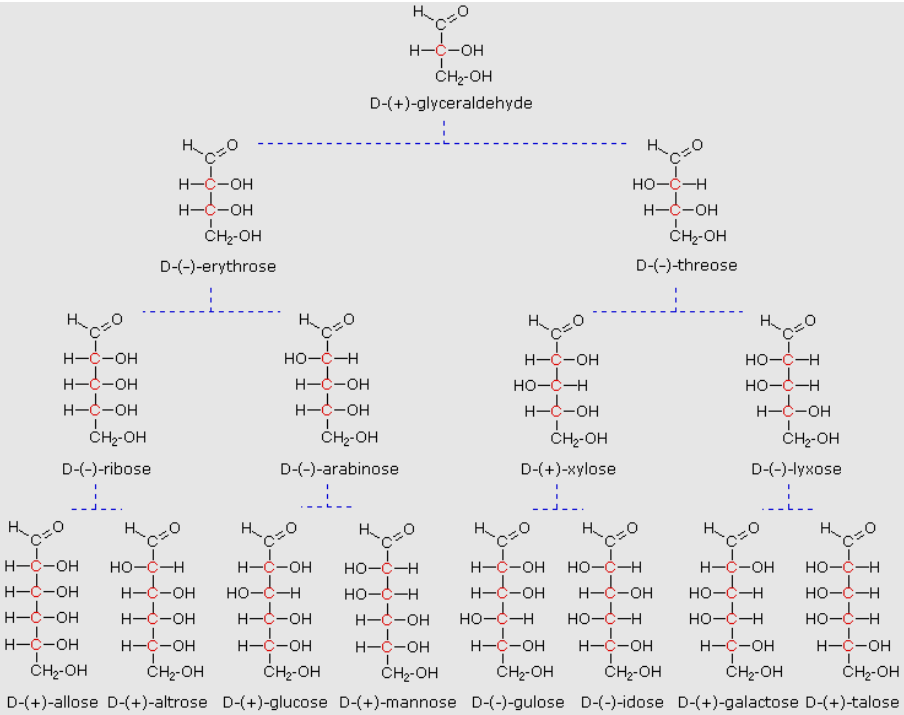
1. identify a specific enantioner of a monosaccharide as being D or L, given its Fischer projection.
2. identify the limitations of the D, L system of nomenclature for carbohydrates.
3. Draw the Fischer projection formula for a monosaccharide, given its systematic name, complete with the configuration of each chiral carbon atom.

The D,L-Configuration of Monosaccharides

Using D-glyceraldehyde as the starting compound, the complete series of D-monosaccharides can de be developed. Being an aldotriose, glyceraldehyde has only one chiral center. Starting with the isomer in which the OH is on the right, we can another -CH(OH)- group to build a tetrose. The OH group can now be on the right or on the left, generating D-erythrose and D-threose, respectively. By successively adding more carbons, we can build the Fisher structures for all D-monosaccharides, from aldotriose to aldohexose.



To illustrate this concept, Fischer projection formulas and names for the D-aldose family (three to six-carbon atoms) are shown below, with the asymmetric carbon atoms (chiral centers) colored red. **The last chiral center in an aldose chain (farthest from the aldehyde group) was chosen by Fischer as the D / L designator site**. If the hydroxyl group in the projection formula pointed to the right, it was defined as a member of the D-family. A left directed hydroxyl group (the mirror image) then represented the L-family. *The mirror images of these configurations were then designated the **L**-family of aldoses*. **It is important to notice that monosaccharides found in nature belong to the D-series.**



It is also important to recognize that the sign of a compound's specific rotation (an experimental number) does not correlate with its configuration (D or L). It is a simple matter to measure an optical rotation with a polarimeter.

What is the Fisher projection for an L-monosaccharide?

For every D-aldose on the figure above, there is a L-aldose. These two compounds are enantiomers from each ether. For example, the structural formulas for D-Glucose and L-Glucose are shown below. Notice that every chiral center must be reversed, not only the one farthest from the aldehyde group.

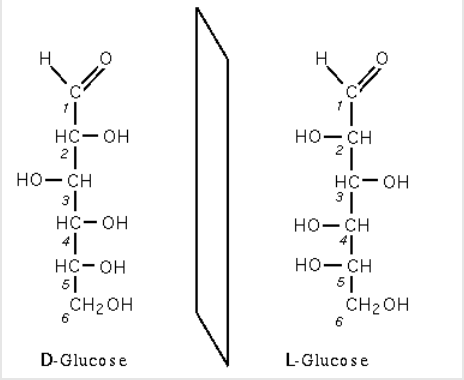


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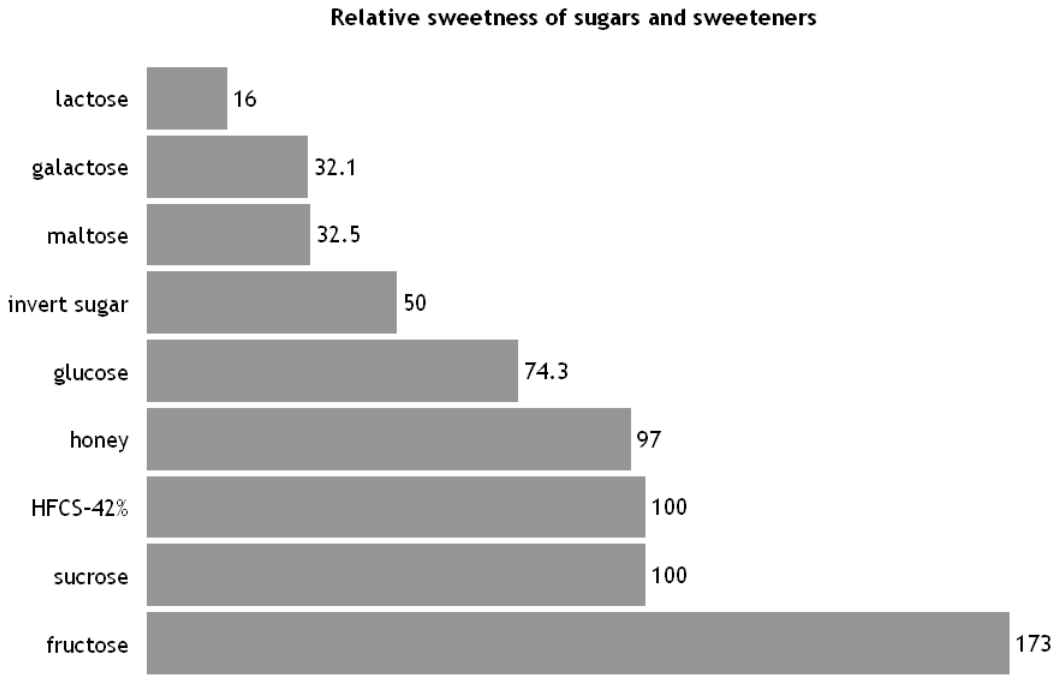
- Wikipedia contributors. (2021, April 26). Absolute configuration. In *Wikipedia, The Free Encyclopedia*. Retrieved 22:46, May 5, 2021, from https://en.wikipedia.org/w/index.php?title=Absolute_configuration&oldid=1020043094

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2.5: Physical properties of monosaccharides

Physical Properties

Monosaccharides such as glucose and fructose are crystalline solids at room temperature, but they are quite soluble in water, each molecule having several OH groups that readily engage in hydrogen bonding. Monosaccharides have a characteristic sweet taste. The sweetness of different carbohydrates can be compared using sucrose as the "100 sweetness parameter". Fructose is the sweetest of all sugars, approximately 73% more sweet than table sugar (sucrose)



Sources

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2.6: Cyclic Structures of Monosaccharides

Objectives

After completing this section, you should be able to

1. determine whether a given monosaccharide will exist as a pyranose or furanose.
2. draw the cyclic pyranose form of a monosaccharide, given its Fischer projection.
3. draw the Fischer projection of a monosaccharide, given its cyclic pyranose form.
4. draw, from memory, the cyclic pyranose form of D-glucose.
5. determine whether a given cyclic pyranose form represents the D or L form of the monosaccharide concerned.
6. describe the phenomenon known as mutarotation.
7. explain, through the use of chemical equations, exactly what happens at the molecular level during the mutarotation process.

Key Terms

Make certain that you can define, and use in context, the key terms below.

- alpha anomer
- anomer
- anomeric centre
- beta anomer
- furanose
- mutarotation
- pyranose

Study Notes

If necessary, before you attempt to study this section, review the formation of hemiacetals discussed in Section 19.10.

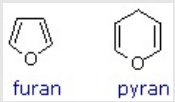
Cyclic hemiacetal formation

In organic chemistry, we described the reaction between carbonyl compounds and alcohol to form hemiacetal and hemiketal respectively,



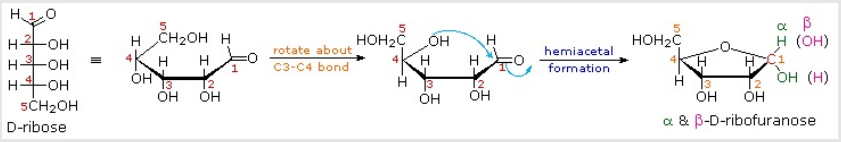
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In a monosaccharide, the carbonyl (C=O) and alcohol group (OH) exist within the same molecule, so they can react forming a cyclic hemiacetal (or hemiketal, in the case of ketoses). The resulting structure will be an intramolecular **cyclic hemiacetal.** The carbonyl carbon (C1) becomes sp3 hybridized, with four different groups attached to it. This carbon is now chiral, and it is called the **anomeric carbon**. Five and six-membered rings are favored over other ring sizes because of their low angle and eclipsing strain. This means that the aldehyde group in aldohexoses react with carbon 5 to format a six-membered ring, while in the case of aldopentoses, the aldehyde group in aldohexoses react with carbon 4 to format a five-membered ring. These are the most important examples of cyclic hemiacetal formation in monosaccharides. Cyclic structures of this kind are termed furanose (five-membered) or pyranose (six-membered), reflecting the ring size relationship to the common heterocyclic compounds furan and pyran shown on the right.



For example, ribose, an important aldopentose, commonly adopts a furanose structure, as shown in the following illustration. By convention for the D-family, the five-membered furanose ring is drawn in an edgewise projection with the ring oxygen positioned away from the viewer. The anomeric carbon atom (colored orange here) is placed on the right.

When a straight-chain monosaccharide, such as any of the structures shown in ribose, forms a cyclic structure, the carbonyl oxygen atom may be pushed either up or down, giving rise to two stereoisomers. The structure shown on the left side of Figure 2, with the OH group on the first carbon atom projected downward, represent what is called the *alpha (α) form*. The structures on the right side, with the OH group on the first carbon atom pointed upward, is the *beta (β) form*. These two stereoisomers of a cyclic monosaccharide are known as **anomers**; they differ in structure around the anomeric carbon—that is, the carbon atom that was the carbonyl carbon atom in the straight-chain form.



Commonly, the cyclic forms of sugars are depicted using a convention first suggested by Walter N. Haworth, an English chemist. In a Haworth projection or formula, the molecules are drawn as planar hexagons with a darkened edge representing the side facing toward the viewer. The anomeric carbon is placed on the right with the ring oxygen to the back of the edgewise view. In the D-family, the -CH_2OH always points up.
The structure is simplified to show only the functional groups attached to the carbon atoms. Any group written to the right in a Fischer projection appears below the plane of the ring in a Haworth projection, and any group written to the left in a Fischer projection appears above the plane in a Haworth projection.

A similar intramolecular reaction occurs with the glucose molecule, resulting in two possible anomers: *α*-D-glucopyranose and *β*-D-glucopyranose:

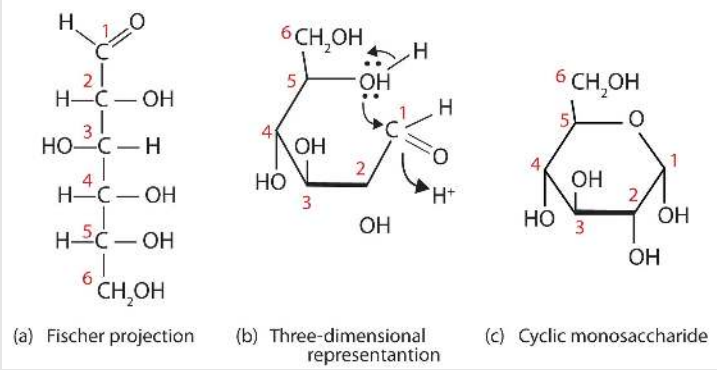
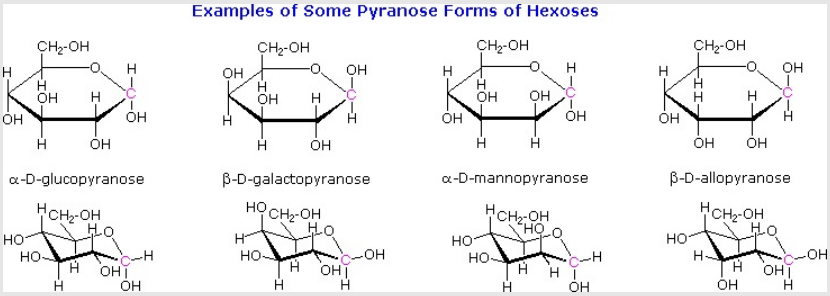


Figure 1: Cyclization of D-Glucose. D-Glucose can be represented with a Fischer projection (a) or three dimensionally (b). By reacting the OH group on the fifth carbon atom with the aldehyde group, the cyclic monosaccharide *α*-D-glucopyranose (c) is produced. In *β*-D-glucopyranose, the OH group on the anomeric carbon points up.

These Haworth formulas are convenient for displaying stereochemical relationships, but do not represent the true shape of the molecules. We know that these molecules are actually puckered in a fashion we call a **chair conformation**. Examples of four typical pyranose structures are shown below, both as Haworth projections and as the more representative chair conformers. The anomeric carbons are colored red.



In order to interconvert between Fisher projection (for open-chain structures), Haworth and Chair conformations (for cyclic structures), this table can be very useful:

Fischer Projection	Haworth Projection	Chair conformation	Anomer
Right	Down	Axial	**α**
Left	Up	Equatorial	**β**

Anomers of Simple Sugars: Mutarotation of Glucose

It is possible to obtain a sample of crystalline glucose in which all the molecules have the α structure or all have the β structure. The α form melts at 146°C and has a specific rotation of +112°, while the β form melts at 150°C and has a specific rotation of +18.7°. When the sample is dissolved in water, however, a mixture is soon produced containing both anomers as well as the straight-chain form, in dynamic equilibrium (part (a) of Figure 2). You can start with a pure crystalline sample of glucose consisting entirely of either anomer, but as soon as the molecules dissolve in water, they open to form the carbonyl group and then reclose to form either the α or the β anomer. The opening and closing repeats continuously in an ongoing interconversion between anomeric forms and is referred to as **mutarotation** (Latin *mutare*, meaning "to change"). At equilibrium, the mixture consists of about 36% α-D-glucose, 64% β-D-glucose, and less than 0.02% of the open-chain aldehyde form. The observed rotation of this solution is +52.7°.

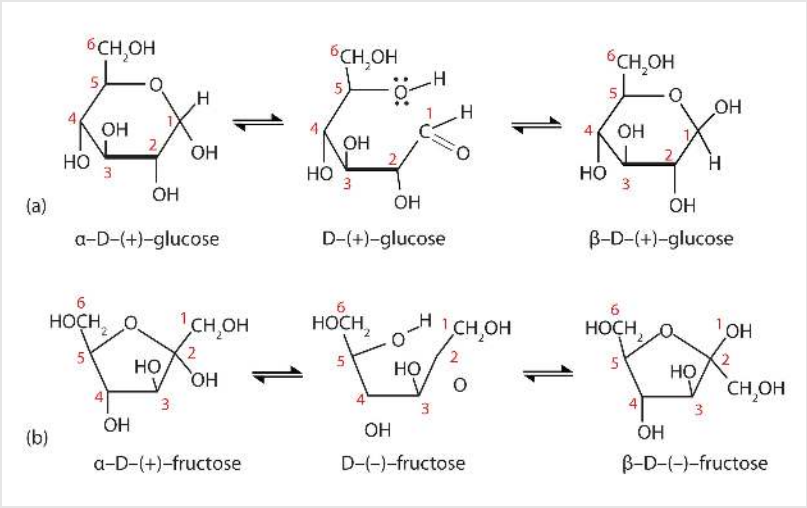


Figure 2: Monosaccharides. In an aqueous solution, monosaccharides exist as an equilibrium mixture of three forms. The interconversion between the forms is known as *mutarotation*, which is shown for D-glucose (a) and D-fructose (b).

Even though only a small percentage of the molecules are in the open-chain aldehyde form at any time, the solution will nevertheless exhibit the characteristic reactions of an aldehyde. As the small amount of free aldehyde is used up in a reaction, there is a shift in the equilibrium to yield more aldehyde. Thus, *all* the molecules may eventually react, even though very little free aldehyde is present at a time. This is because the mutarotation reaction is an equilibrium reaction, so removing any of the molecules involved in this equilibrium would make the equilibrium shift in that direction (le Chatelier's princriple)

The difference between the α and the β forms of sugars may seem trivial, but such structural differences are often crucial in biochemical reactions. This explains why we can get energy from the starch in potatoes and other plants but not from cellulose, even though both starch and cellulose are polysaccharides composed of glucose molecules linked together.

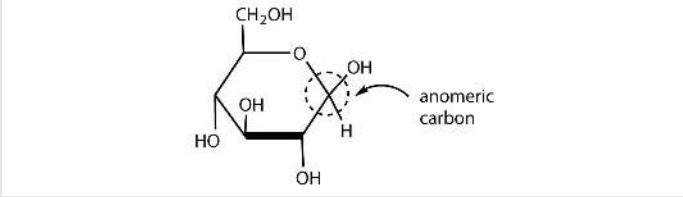
Summary

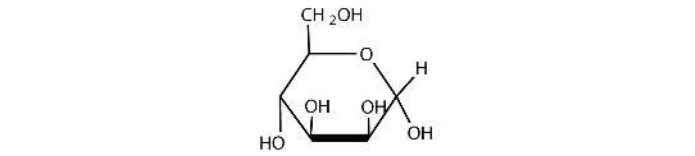
Monosaccharides that contain five or more carbons atoms form cyclic structures in aqueous solution. Two cyclic stereoisomers can form from each straight-chain monosaccharide; these are known as anomers. In an aqueous solution, an equilibrium mixture forms between the two anomers and the straight-chain structure of a monosaccharide in a process known as mutarotation.

Exercises

1. Draw the cyclic structure for β-D-glucose. Identify the anomeric carbon.
2. Given that the aldohexose D-mannose differs from D-glucose only in the configuration at the second carbon atom, draw the cyclic structure for α-D-mannose.

Answers

1. 

2. 

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- William Reusch, Professor Emeritus (Michigan State U.), Virtual Textbook of Organic Chemistry
- *The Basics of General, Organic, and Biological Chemistry* by David W. Ball, John W. Hill, and Rhonda J. Scott.

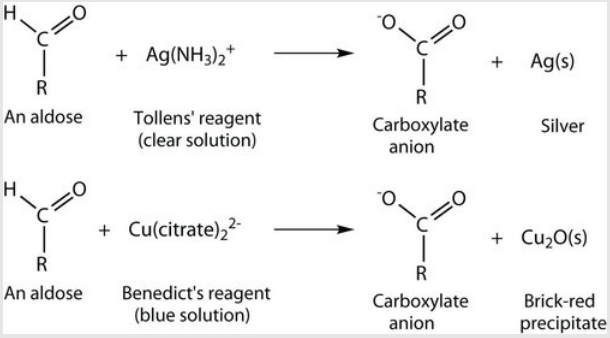
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2.7: Reactions of Monosaccharides

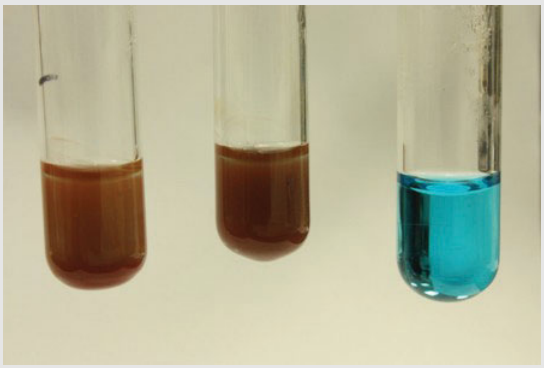
Objectives

Oxidation

An important reaction of monosaccharides is the oxidation of the aldehyde group, one of the most easily oxidized organic functional groups. Aldehyde oxidation can be accomplished with any mild oxidizing agent, such as Tollens' reagent or Benedict's reagent. With the latter, complexed copper(II) ions are reduced to copper(I) ions that form a brick-red precipitate [copper(I) oxide;



Any carbohydrate capable of reducing either Tollens' or Benedict's reagents without first undergoing hydrolysis is said to be a **reducing sugar**. Because both the Tollens' and Benedict's reagents are basic solutions, ketoses (such as fructose) also give positive tests due to an equilibrium that exists between ketoses and aldoses in a reaction known as *tautomerism*.



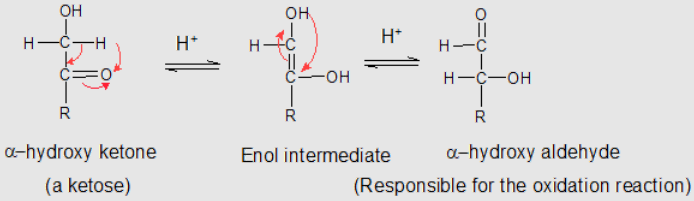
Benedict's Test. Benedict's test was performed on three carbohydrates, depicted from left to right: fructose, glucose, and sucrose. The solution containing sucrose remains blue because sucrose is a nonreducing sugar



Tollen's test: left side positive (silver mirror, reducing sugar), right side negative (clear solution, non-reducing sugar). Image by FK1954, Public domain, via Wikimedia Commons

These reactions have been used as simple and rapid diagnostic tests for the presence of glucose in blood or urine. For example, Clinitest tablets, which are used to test for sugar in the urine, contain copper(II) ions and are based on Benedict's test. A green color indicates very little sugar, whereas a brick-red color indicates sugar in excess of 2 g/100 mL of urine.

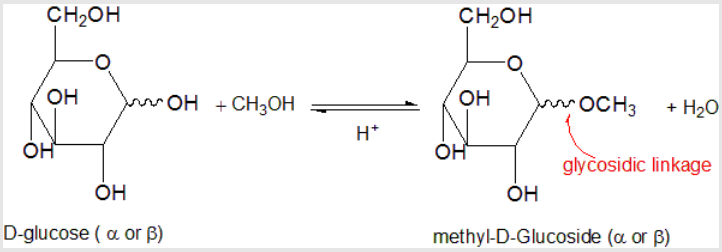
Ketoses are also reducing sugars due to an enol isomerism equilibrium. In this equilibrium, the ketone form is in equilibrium with the aldehyde form, which is responsible for the observed oxidation reaction:



Glycoside Formation

Being hemicacetal, monosaccharides in their cyclic form can react with a second alcohol molecule, forming an acetal. In this case, the creation can't be intramolecular due to steric hindrance. Acetal derivatives formed when a monosaccharide reacts with an alcohol in the presence of an acid catalyst are called **glycosides.**

This reaction is illustrated for glucose and methanol in the diagram below. In naming of glycosides, the "ose" suffix of the sugar name is replaced by "oside", and the alcohol group name is placed first. As is generally true for most acetals, glycoside formation involves the loss of an equivalent of water. The acetak product is stable to base and alkaline oxidants such as Tollen's reagent. Since acid-catalyzed formation of acetals is reversible, glycosides may be hydrolyzed back to their alcohol and sugar components by aqueous acid.

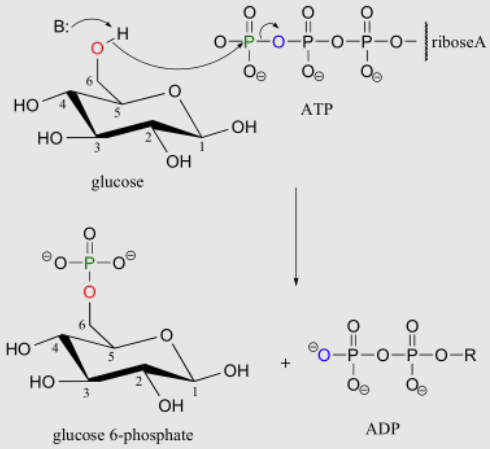


Glycosides abound in biological systems. By attaching a sugar moiety to a lipid or benzenoid structure, the solubility and other properties of the compound may be changed substantially. Because of the important modifying influence of such derivatization, numerous enzyme systems, known as glycosidases, have evolved for the attachment and removal of sugars from alcohols, phenols and amines. Chemists refer to the sugar component of natural glycosides as the glycon and the alcohol component as the aglycon.

Biological Ester Formation: Phosphorylation

Recall that almost all biomolecules are charged species, which 1) keeps them water soluble, and 2) prevents them from diffusing across lipid bilayer membranes. Although many biomolecules are ionized by virtue of negatively charged carboxylate and positively charged amino groups, the most common ionic group in biologically important organic compounds is phosphate - thus the phosphorylation of alcohol groups is a critical metabolic step. In alcohol phosphorylations, ATP is

almost always the phosphate donor, and the mechanism is very consistent: the alcohol oxygen acts as a nucleophile, attacking the gamma-phosphorus of ATP and expelling ADP



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2.8: Important Hexoses

Learning Objectives

- To identify the structures of D-glucose, D-galactose, and D-fructose and describe how they differ from each other.

Although a variety of monosaccharides are found in living organisms, three hexoses are particularly abundant: D-glucose, D-galactose, and D-fructose (Figure **2.8.1**). Glucose and galactose are both aldohexoses, while fructose is a ketohexose.

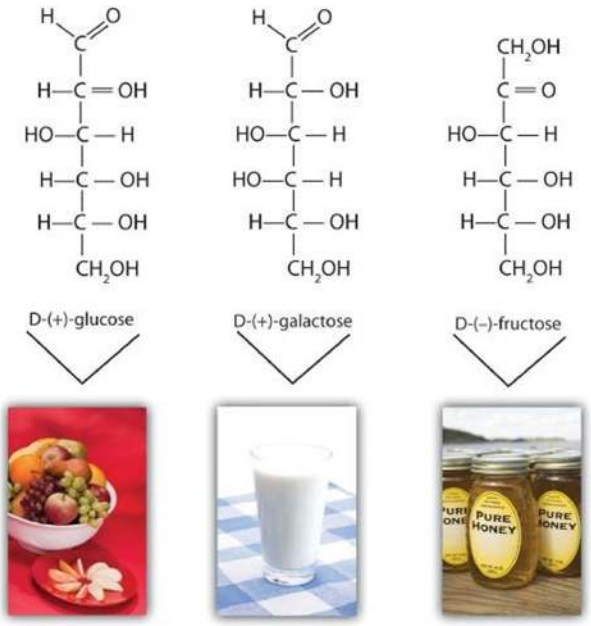


Figure **2.8.1**: Structures of Three Important Hexoses. Each hexose is pictured with a food source in which it is commonly found. Source: Photos © Thinkstock.

Glucose

D-Glucose, generally referred to as simply glucose, is the most abundant sugar found in nature; most of the carbohydrates we eat are eventually converted to it in a series of biochemical reactions that produce energy for our cells. It is also known by three other names: *dextrose*, from the fact that it rotates plane-polarized light in a clockwise (dextrorotatory) direction; *corn sugar* because in the United States cornstarch is used in the commercial process that produces glucose from the hydrolysis of starch; and *blood sugar* because it is the carbohydrate found in the circulatory system of animals. Normal blood sugar values range from 70 to 105 mg glucose/dL plasma, and normal urine may contain anywhere from a trace to 20 mg glucose/dL urine.

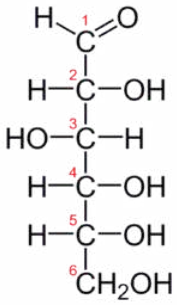


Figure **2.8.2**: Fischer projection of D-glucose

The Fischer projection of D-glucose is given in Figure **2.8.2**. Glucose is a D sugar because the OH group on the fifth carbon atom (the chiral center farthest from the carbonyl group) is on the right. In fact, all the OH groups except the one on the third carbon atom are to the right.

Galactose

D-Galactose does not occur in nature in the uncombined state. It is released when lactose, a disaccharide found in milk, is hydrolyzed. The galactose needed by the human body for the synthesis of lactose is obtained by the metabolic conversion of D-glucose to D-galactose. Galactose is also an important constituent of the glycolipids that occur in the brain and the myelin sheath of nerve cells. For this reason it is also known as *brain sugar*. The structure of D-galactose is shown in Figure **2.8.1**. Notice that the configuration differs from that of glucose only at the fourth carbon atom.

Fructose

D-Fructose, also shown in Figure **2.8.1**, is the most abundant ketohexose. Note that from the third through the sixth carbon atoms, its structure is the same as that of glucose. It occurs, along with glucose and sucrose, in honey (which is 40% fructose) and sweet fruits. Fructose (from the Latin *fructus*, meaning "fruit") is also referred to as *levulose* because it has a specific rotation that is strongly levorotatory (−92.4°). It is the sweetest sugar, being 1.7 times sweeter than sucrose, although many nonsugars are several hundred or several thousand times as sweet (Table **2.8.1**).

Table **2.8.1**: The Relative Sweetness of Some Compounds (Sucrose = 100)

Compound	Relative Sweetness
lactose	16
maltose	32
glucose	74
sucrose	100
fructose	173
aspartame	18,000
acesulfame K	20,000
saccharin	30,000
sucralose	60,000

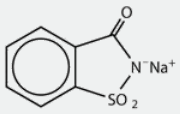
Looking Closer: Artificial Sweeteners

Although sweetness is commonly associated with mono- and disaccharides, it is not a property found only in sugars. Several other kinds of organic compounds have been synthesized that are far superior as sweetening agents. These so-called high-intensity or artificial sweeteners are useful for people with diabetes or other medical conditions that require them to control their carbohydrate intake. The synthetic compounds are noncaloric or used in such small quantities that they do not add significantly to the caloric value of food.

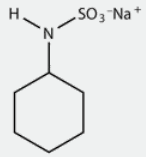
The first artificial sweetener—saccharin—was discovered by accident in 1879. It is 300 times sweeter than sucrose, but it passes through the body unchanged and thus adds no calories to the diet. After its discovery, saccharin was used until it was banned in the early 1900s. However, during the sugar-short years of World War I, the ban was lifted and was not reinstated at the war's end. One drawback to the use of saccharin is its bitter, metallic aftertaste. The initial solution to this problem was to combine saccharin with cyclamate, a second artificial sweetener discovered in 1937.

In the 1960s and 1970s, several clinical tests with laboratory animals implicated both cyclamate and saccharin as carcinogenic (cancer-causing) substances. The results from the cyclamate tests were completed first, and cyclamate was banned in the United States in 1969. Then a major study was released in Canada in 1977 indicating that saccharin increased the incidence of

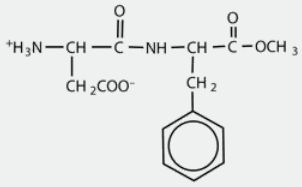
bladder cancer in rats. The US Food and Drug Administration (FDA) proposed a ban on saccharin that raised immediate public opposition because saccharin was the only artificial sweetener still available. In response, Congress passed the Saccharin Study and Labeling Act in 1977, permitting the use of saccharin as long as any product containing it was labeled with a consumer warning regarding the possible elevation of the risk of bladder cancer. Today this warning is no longer required; moreover, the FDA is currently reviewing the ban on cyclamate, as 75 additional studies and years of usage in other countries, such as Canada, have failed to show that it has any carcinogenic effect.



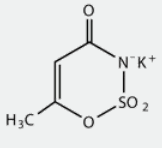
Saccharin



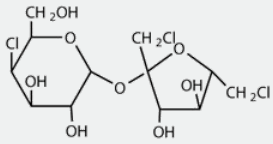
Cyclamate



Aspartame



Acesulfame K



Sucralose

A third artificial sweetener, aspartame, was discovered in 1965. This white crystalline compound is about 180 times sweeter than sucrose and has no aftertaste. It was approved for use in 1981 and is used to sweeten a wide variety of foods because it blends well with other food flavors. Aspartame is not used in baked goods, however, because it is not heat stable.

In the body (or when heated), aspartame is initially hydrolyzed to three molecules: the amino acids aspartic acid and phenylalanine and an alcohol methanol. Repeated controversy regarding the safety of aspartame arises partly from the fact that the body metabolizes the released methanol to formaldehyde. It should be noted, though, that a glass of tomato juice has six times as much methanol as a similar amount of a diet soda containing aspartame. The only documented risk connected to aspartame use is for individuals with the genetic disease *phenylketonuria* (PKU); these individuals lack the enzyme needed to metabolize the phenylalanine released when aspartame is broken down by the body. Because of the danger to people with PKU, all products containing aspartame must carry a warning label.

Acesulfame K, discovered just two years after aspartame (1967), was approved for use in the United States in 1988. It is 200 times sweeter than sugar and, unlike aspartame, is heat stable. It has no lingering aftertaste.

One of the newest artificial sweeteners to gain FDA approval (April 1998) for use in the United States is sucralose, a white crystalline solid approximately 600 times sweeter than sucrose. Sucralose is synthesized from sucrose and has three chlorine atoms substituted for three OH groups. It is noncaloric because it passes through the body unchanged. It can be used in baking because it is heat stable.

All of the extensive clinical studies completed to date have indicated that these artificial sweeteners approved for use in the United States are safe for consumption by healthy individuals in moderate amounts.

Summary

Three abundant hexoses in living organisms are the aldohexoses D-glucose and D-galactose and the ketohexose D-fructose.

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2.9: Disaccharides and Glycosidic Bonds

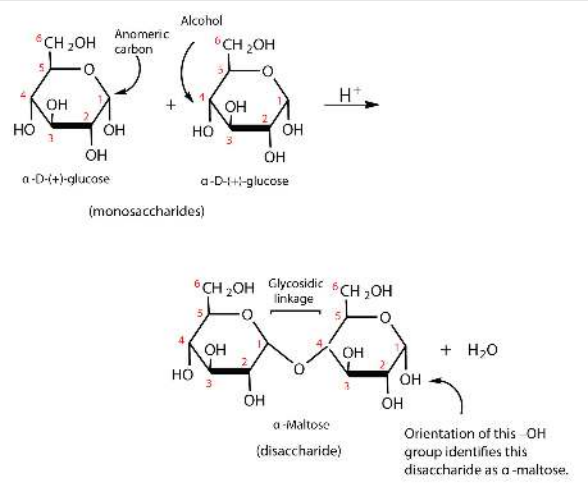
Objectives

After completing this section, you should be able to

1. identify disaccharides as compounds consisting of two monosaccharide units joined by a glycoside link between the C1 of one sugar and one of the hydroxyl groups of a second sugar.
2. identify the two monosaccharide units in a given disaccharide.
3. identify the type of glycoside link (e.g., 1,4′-β) present in a given disaccharide structure.
4. draw the structure of a specific disaccharide, given the structure of the monosaccharide units and the type of glycoside link involved.
5. identify the structural feature that determines whether or not a given disaccharide behaves as a reducing sugar and undergoes mutarotation, and write equations to illustrate these phenomena.
6. identify the products formed from the hydrolysis of a given disaccharide.

Disaccharides

Previously, you learned that monosaccharides can form cyclic structures by the reaction of the carbonyl group with an OH group, resulting in an cyclic hemiacetal (or hemiketal for ketoses). In the same way that hemiacetals and ketals can react with an additional alcohol forming acetals and ketals, these cyclic monosaccahrides can in turn react with another alcohol molecule. In the case of disaccharides, one monosaccharide acts a the hemiacetal while the other monosaccharides acts as the alcohol. The formation of an acetal (or ketal) bond between two monosaccharides is called a **glycosidic bond or glycosidic linkage**. Therefore, disaccharides are sugars composed of two monosaccharide units that are joined by a carbon–oxygen-carbon linkage known as a **glycosidic linkage**. This linkage is formed from the reaction of the anomeric carbon of one cyclic monosaccharide with the OH group of a second monosaccharide.



The disaccharides differ from one another in their monosaccharide constituents and in the specific type of glycosidic linkage connecting them. There are three common disaccharides: maltose, lactose, and sucrose. All three are white crystalline solids at room temperature and are soluble in water. We'll consider each sugar in more detail.

Disaccharides can be reducing or non-reducing, depending on the specific groups involved in the formation of the glycosidic bond. Disaccharides can only be reducing when there is a free anomeric carbon, not involved in the formation of the glycosidic bond, that can undergo the oxidation reduction with Tollen's or Benedict's reagents. When the anomeric carbon on both monosaccharides is involved in the formation of the glycosidic bond, the disaccharides is non-reducing.

Maltose

Maltose occurs to a limited extent in sprouting grain. It is formed most often by the partial hydrolysis of starch and glycogen. In the manufacture of beer, maltose is liberated by the action of malt (germinating barley) on starch; for this reason, it is often

referred to as *malt sugar*. Maltose is about 30% as sweet as sucrose. The human body is unable to metabolize maltose or any other disaccharide directly from the diet because the molecules are too large to pass through the cell membranes of the intestinal wall. Therefore, an ingested disaccharide must first be broken down by hydrolysis into its two constituent monosaccharide units. In the body, such hydrolysis reactions are catalyzed by enzymes such as *maltase*. The same reactions can be carried out in the laboratory with dilute acid as a catalyst, although in that case the rate is much slower, and high temperatures are required. Whether it occurs in the body or a glass beaker, the hydrolysis of maltose produces two molecules of D-glucose.

$$\text{maltose} \xrightarrow{H^+ \text{ or maltase}} 2 \text{ D-glucose} \tag{2.9.1}$$

Maltose is a reducing sugar. Thus, its two glucose molecules must be linked in such a way as to leave one anomeric carbon that can open to form an aldehyde group. The glucose units in maltose are joined in a *head-to-tail* fashion through an α-linkage from the first carbon atom of one glucose molecule to the fourth carbon atom of the second glucose molecule (that is, an α-1,4-glycosidic linkage; see Figure 1). The bond from the anomeric carbon of the first monosaccharide unit is directed downward, which is why this is known as an α-glycosidic linkage. The OH group on the anomeric carbon of the second glucose can be in either the α or the β position, as shown in Figure 1.

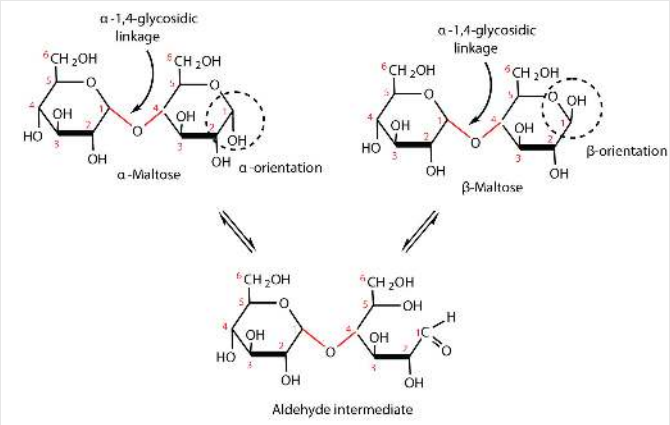
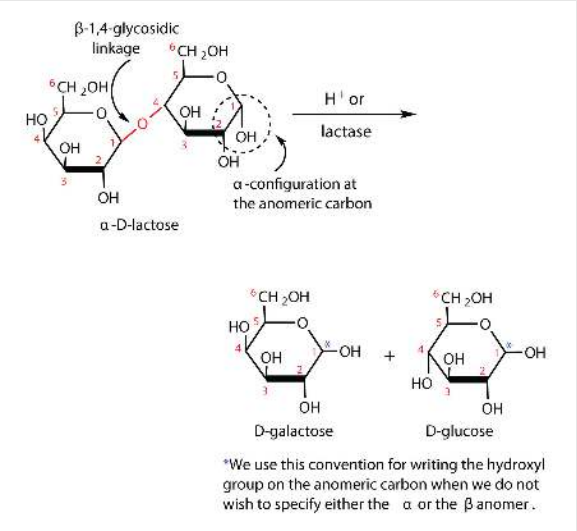


Figure 1 An Equilibrium Mixture of Maltose Isomers

- ## Lactose

  Lactose is known as *milk sugar* because it occurs in the milk of humans, cows, and other mammals. In fact, the natural synthesis of lactose occurs only in mammary tissue, whereas most other carbohydrates are plant products. Human milk contains about 7.5% lactose, and cow's milk contains about 4.5%. This sugar is one of the lowest ranking in terms of sweetness, being about one-sixth as sweet as sucrose. Lactose is produced commercially from whey, a by-product in the manufacture of cheese. It is important as an infant food and in the production of penicillin.

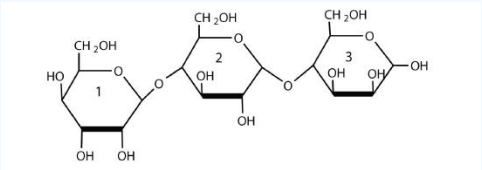
  Lactose is a reducing sugar composed of one molecule of D-galactose and one molecule of D-glucose joined by a β-1,4-glycosidic bond (the bond from the anomeric carbon of the first monosaccharide unit being directed upward). The two monosaccharides are obtained from lactose by acid hydrolysis or the catalytic action of the enzyme *lactase*:



Many adults and some children suffer from a deficiency of lactase. These individuals are said to be **lactose intolerant** because they cannot digest the lactose found in milk. A more serious problem is the genetic disease **galactosemia**, which results from the absence of an enzyme needed to convert galactose to glucose. Certain bacteria can metabolize lactose, forming lactic acid as one of the products. This reaction is responsible for the "souring" of milk.

Example 1

For this trisaccharide, indicate whether each glycosidic linkage is α or β.



Solution

The glycosidic linkage between sugars 1 and 2 is β because the bond is directed up from the anomeric carbon. The glycosidic linkage between sugars 2 and 3 is α because the bond is directed down from the anomeric carbon.

To Your Health: Lactose Intolerance and Galactosemia

Lactose makes up about 40% of an infant's diet during the first year of life. Infants and small children have one form of the enzyme lactase in their small intestines and can digest the sugar easily; however, adults usually have a less active form of the enzyme, and about 70% of the world's adult population has some deficiency in its production. As a result, many adults experience a reduction in the ability to hydrolyze lactose to galactose and glucose in their small intestine. For some people the inability to synthesize sufficient enzyme increases with age. Up to 20% of the US population suffers some degree of lactose intolerance.

In people with lactose intolerance, some of the unhydrolyzed lactose passes into the colon, where it tends to draw water from the interstitial fluid into the intestinal lumen by osmosis. At the same time, intestinal bacteria may act on the lactose to produce organic acids and gases. The buildup of water and bacterial decay products leads to abdominal distention, cramps, and diarrhea, which are symptoms of the condition.

The symptoms disappear if milk or other sources of lactose are excluded from the diet or consumed only sparingly. Alternatively, many food stores now carry special brands of milk that have been pretreated with lactase to hydrolyze the lactose. Cooking or fermenting milk causes at least partial hydrolysis of the lactose, so some people with lactose intolerance are still able to enjoy cheese, yogurt, or cooked foods containing milk. The most common treatment for lactose intolerance, however, is the use of lactase preparations (e.g., Lactaid), which are available in liquid and tablet

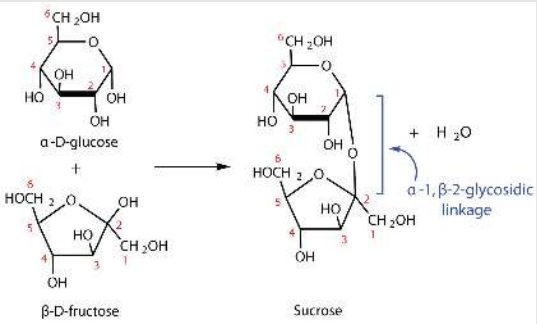
form at drugstores and grocery stores. These are taken orally with dairy foods—or may be added to them directly—to assist in their digestion.

Galactosemia is a condition in which one of the enzymes needed to convert galactose to glucose is missing. Consequently, the blood galactose level is markedly elevated, and galactose is found in the urine. An infant with galactosemia experiences a lack of appetite, weight loss, diarrhea, and jaundice. The disease may result in impaired liver function, cataracts, mental retardation, and even death. If galactosemia is recognized in early infancy, its effects can be prevented by the exclusion of milk and all other sources of galactose from the diet. As a child with galactosemia grows older, he or she usually develops an alternate pathway for metabolizing galactose, so the need to restrict milk is not permanent. The incidence of galactosemia in the United States is 1 in every 65,000 newborn babies.

Sucrose

Sucrose, probably the largest-selling pure organic compound in the world, is known as *beet sugar*, *cane sugar*, *table sugar*, or simply *sugar*. Most of the sucrose sold commercially is obtained from sugar cane and sugar beets (whose juices are 14%–20% sucrose) by evaporation of the water and recrystallization. The dark brown liquid that remains after the recrystallization of sugar is sold as molasses.

The sucrose molecule is unique among the common disaccharides in having an α-1,β-2-glycosidic (head-to-head) linkage. Because this glycosidic linkage is formed by the OH group on the anomeric carbon of α-D-glucose and the OH group on the anomeric carbon of β-D-fructose, it ties up the anomeric carbons of both glucose and fructose.



This linkage gives sucrose certain properties that are quite different from those of maltose and lactose. As long as the sucrose molecule remains intact, neither monosaccharide “uncyclizes” to form an open-chain structure. Thus, sucrose is incapable of mutarotation and exists in only one form both in the solid state and in solution. In addition, sucrose does not undergo reactions that are typical of aldehydes and ketones. Therefore, sucrose is a nonreducing sugar.

The hydrolysis of sucrose in dilute acid or through the action of the enzyme *sucrase* (also known as invertase) gives an equimolar mixture of glucose and fructose. This 1:1 mixture is referred to as *invert sugar* because it rotates plane-polarized light in the opposite direction than sucrose. The hydrolysis reaction has several practical applications. Sucrose readily recrystallizes from a solution, but invert sugar has a much greater tendency to remain in solution. In the manufacture of jelly and candy and in the canning of fruit, the recrystallization of sugar is undesirable. Therefore, conditions leading to the hydrolysis of sucrose are employed in these processes. Moreover, because fructose is sweeter than sucrose, the hydrolysis adds to the sweetening effect. Bees carry out this reaction when they make honey.

The average American consumes more than 100 lb of sucrose every year. About two-thirds of this amount is ingested in soft drinks, presweetened cereals, and other highly processed foods. The widespread use of sucrose is a contributing factor to obesity and tooth decay. Carbohydrates such as sucrose, are converted to fat when the caloric intake exceeds the body’s requirements, and sucrose causes tooth decay by promoting the formation of plaque that sticks to teeth.

Summary

Maltose is composed of two molecules of glucose joined by an α-1,4-glycosidic linkage. It is a reducing sugar that is found in sprouting grain. Lactose is composed of a molecule of galactose joined to a molecule of glucose by a β-1,4-glycosidic

linkage. It is a reducing sugar that is found in milk. Sucrose is composed of a molecule of glucose joined to a molecule of fructose by an α-1,β-2-glycosidic linkage. It is a nonreducing sugar that is found in sugar cane and sugar beets.

Concept Review Exercise

1. What monosaccharides are obtained by the hydrolysis of each disaccharide?

    a. sucrose
    b. maltose
    c. lactose

Answer

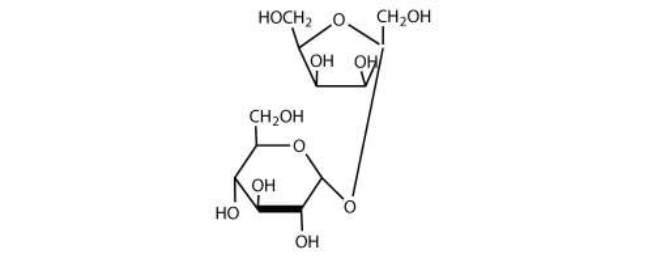
1. a. D-glucose and D-fructose
    b. two molecules of D-glucose
    c. D-glucose and D-galactose

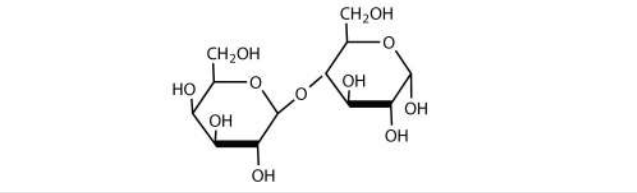
Exercises

1. Identify each sugar by its common chemical name.

    a. milk sugar
    b. table sugar

2. For each disaccharide, indicate whether the glycosidic linkage is α or β.

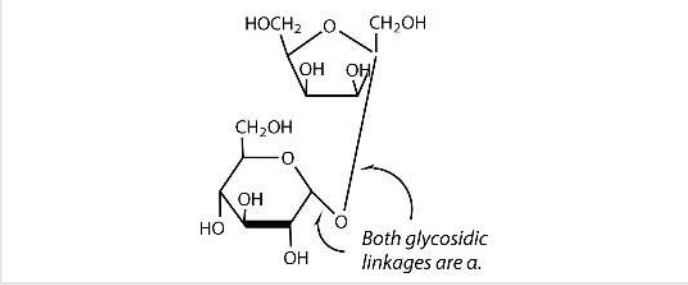
a. 

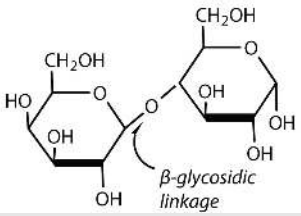
b. 

3. Identify each disaccharide in Exercise 2 as a reducing or nonreducing sugar. If it is a reducing sugar, draw its structure and circle the anomeric carbon. State if the OH group at the anomeric carbon is in the α or the β position

Answers

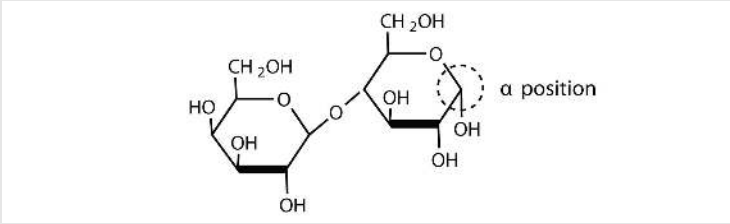
1. a. lactose
    b. sucrose

2. a. 

2. b. 

3. a. nonreducing

3. b. reducing



Contributors and Attributions

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2.10: Polysaccharides

Learning Objectives

After completing this section, you should be able to

1. identify the structural difference between cellulose and the cold-water-insoluble fraction of starch (amylose), and identify both of these substances as containing many glucose molecules joined by 1,4′-glycoside links.
2. identify the cold-water-soluble fraction of starch (amylopectin) as having a more complex structure than amylose because of the existence of 1,6′-glycoside links in addition to the 1,4′-links.
3. compare and contrast the structures and uses of starch, glycogen and cellulose.

Key Terms

Make certain that you can define, and use in context, the key terms below.

- amylopectin
- amylose
- polysaccharide

Learning Objectives

- To compare and contrast the structures and uses of starch, glycogen, and cellulose.

The polysaccharides are the most abundant carbohydrates in nature and serve a variety of functions, such as energy storage or as components of plant cell walls. Polysaccharides are very large polymers composed of tens to thousands of monosaccharides joined together by glycosidic linkages. The three most abundant polysaccharides are starch, glycogen, and cellulose. These three are referred to as *homopolymers* because each yields only one type of monosaccharide (glucose) after complete hydrolysis. *Heteropolymers* may contain sugar acids, amino sugars, or noncarbohydrate substances in addition to monosaccharides. Heteropolymers are common in nature (gums, pectins, and other substances) but will not be discussed further in this textbook. The polysaccharides are nonreducing carbohydrates, are not sweet tasting, and do not undergo mutarotation.

Starch

Starch is the most important source of carbohydrates in the human diet and accounts for more than 50% of our carbohydrate intake. It occurs in plants in the form of granules, and these are particularly abundant in seeds (especially the cereal grains) and tubers, where they serve as a storage form of carbohydrates. The breakdown of starch to glucose nourishes the plant during periods of reduced photosynthetic activity. We often think of potatoes as a “starchy” food, yet other plants contain a much greater percentage of starch (potatoes 15%, wheat 55%, corn 65%, and rice 75%). Commercial starch is a white powder.

Starch is a mixture of two polymers: amylose and amylopectin. Natural starches consist of about 10%–30% amylose and 70%–90% amylopectin. Amylose is a linear polysaccharide composed entirely of D-glucose units joined by the α-1,4-glycosidic linkages we saw in maltose (part (a) of Figure **2.10.1**). Experimental evidence indicates that amylose is not a straight chain of glucose units but instead is coiled like a spring, with six glucose monomers per turn (part (b) of Figure **2.10.1**). When coiled in this fashion, amylose has just enough room in its core to accommodate an iodine molecule. The characteristic blue-violet color that appears when starch is treated with iodine is due to the formation of the amylose-iodine complex. This color test is sensitive enough to detect even minute amounts of starch in solution.

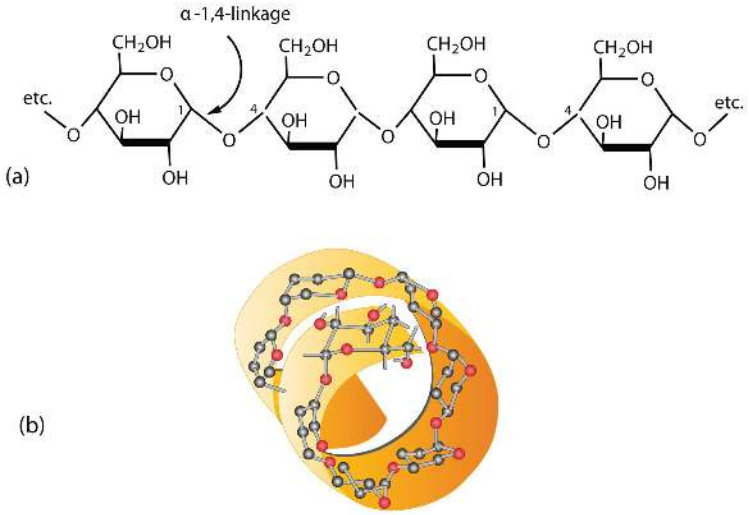


Figure **2.10.1**: Amylose. (a) Amylose is a linear chain of α-D-glucose units joined together by α-1,4-glycosidic bonds. (b) Because of hydrogen bonding, amylose acquires a spiral structure that contains six glucose units per turn.

Amylopectin is a branched-chain polysaccharide composed of glucose units linked primarily by α-1,4-glycosidic bonds but with occasional α-1,6-glycosidic bonds, which are responsible for the branching. A molecule of amylopectin may contain many thousands of glucose units with branch points occurring about every 25–30 units (Figure **2.10.2**). The helical structure of amylopectin is disrupted by the branching of the chain, so instead of the deep blue-violet color amylose gives with iodine, amylopectin produces a less intense reddish brown.

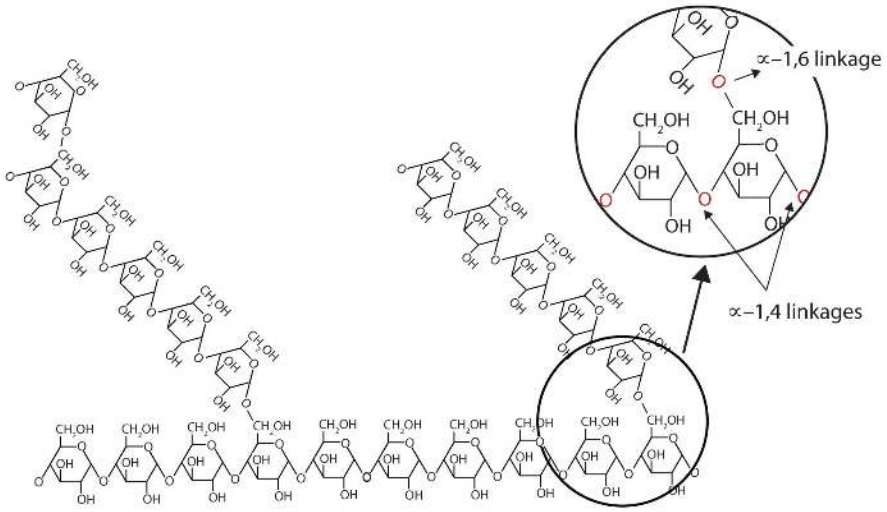


Figure **2.10.2**: Representation of the Branching in Amylopectin and Glycogen. Both amylopectin and glycogen contain branch points that are linked through α-1,6-linkages. These branch points occur more often in glycogen.

Dextrins are glucose polysaccharides of intermediate size. The shine and stiffness imparted to clothing by starch are due to the presence of dextrins formed when clothing is ironed. Because of their characteristic stickiness with wetting, dextrins are used as adhesives on stamps, envelopes, and labels; as binders to hold pills and tablets together; and as pastes. Dextrins are more easily digested than starch and are therefore used extensively in the commercial preparation of infant foods.

The complete hydrolysis of starch yields, in successive stages, glucose:

$$\text{starch} \rightarrow \text{dextrins} \rightarrow \text{maltose} \rightarrow \text{glucose}$$

In the human body, several enzymes known collectively as amylases degrade starch sequentially into usable glucose units.

Glycogen

Glycogen is the energy reserve carbohydrate of animals. Practically all mammalian cells contain some stored carbohydrates in the form of glycogen, but it is especially abundant in the liver (4%–8% by weight of tissue) and in skeletal muscle cells (0.5%–1.0%). Like starch in plants, glycogen is found as granules in liver and muscle cells. When fasting, animals draw on these glycogen reserves during the first day without food to obtain the glucose needed to maintain metabolic balance.

Glycogen is structurally quite similar to amylopectin, although glycogen is more highly branched (8–12 glucose units between branches) and the branches are shorter. When treated with iodine, glycogen gives a reddish brown color. Glycogen can be broken down into its D-glucose subunits by acid hydrolysis or by the same enzymes that catalyze the breakdown of starch. In animals, the enzyme phosphorylase catalyzes the breakdown of glycogen to phosphate esters of glucose.

> *About 70% of the total glycogen in the body is stored in muscle cells. Although the percentage of glycogen (by weight) is higher in the liver, the much greater mass of skeletal muscle stores a greater total amount of glycogen.*

Cellulose

Cellulose, a fibrous carbohydrate found in all plants, is the structural component of plant cell walls. Because the earth is covered with vegetation, cellulose is the most abundant of all carbohydrates, accounting for over 50% of all the carbon found in the vegetable kingdom. Cotton fibrils and filter paper are almost entirely cellulose (about 95%), wood is about 50% cellulose, and the dry weight of leaves is about 10%–20% cellulose. The largest use of cellulose is in the manufacture of paper and paper products. Although the use of noncellulose synthetic fibers is increasing, rayon (made from cellulose) and cotton still account for over 70% of textile production.

Like amylose, cellulose is a linear polymer of glucose. It differs, however, in that the glucose units are joined by β-1,4-glycosidic linkages, producing a more extended structure than amylose (part (a) of Figure **2.10.3**). This extreme linearity allows a great deal of hydrogen bonding between OH groups on adjacent chains, causing them to pack closely into fibers (part (b) of Figure **2.10.3**). As a result, cellulose exhibits little interaction with water or any other solvent. Cotton and wood, for example, are completely insoluble in water and have considerable mechanical strength. Because cellulose does not have a helical structure, it does not bind to iodine to form a colored product.

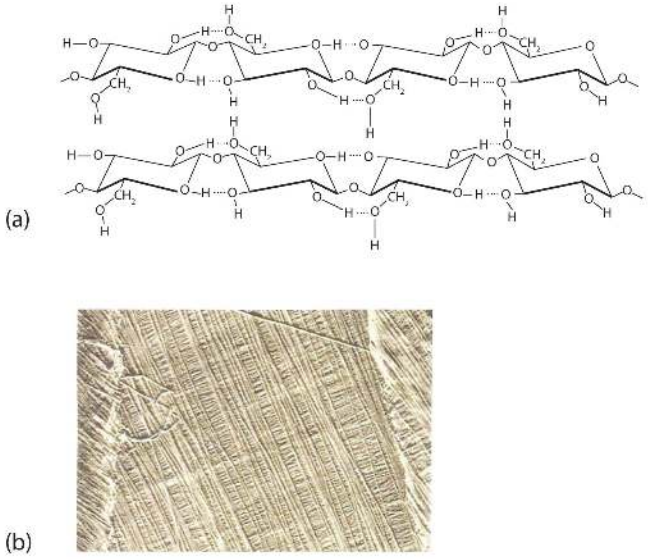


Figure **2.10.3**: Cellulose. (a) There is extensive hydrogen bonding in the structure of cellulose. (b) In this electron micrograph of the cell wall of an alga, the wall consists of successive layers of cellulose fibers in parallel arrangement.

Cellulose yields D-glucose after complete acid hydrolysis, yet humans are unable to metabolize cellulose as a source of glucose. Our digestive juices lack enzymes that can hydrolyze the β-glycosidic linkages found in cellulose, so although we can eat potatoes, we cannot eat grass. However, certain microorganisms can digest cellulose because they make the enzyme cellulase, which

catalyzes the hydrolysis of cellulose. The presence of these microorganisms in the digestive tracts of herbivorous animals (such as cows, horses, and sheep) allows these animals to degrade the cellulose from plant material into glucose for energy. Termites also contain cellulase-secreting microorganisms and thus can subsist on a wood diet. This example once again demonstrates the extreme stereospecificity of biochemical processes.

Career Focus: Certified Diabetes Educator

Certified diabetes educators come from a variety of health professions, such as nursing and dietetics, and specialize in the education and treatment of patients with diabetes. A diabetes educator will work with patients to manage their diabetes. This involves teaching the patient to monitor blood sugar levels, make good food choices, develop and maintain an exercise program, and take medication, if required.



A certified diabetes educator at Naval Medical Center Portsmouth (left) and a registered dietician at the medical center (center), provide nutritional information to a diabetes patient and her mother at the Diabetes Boot Camp.

Diabetes educators also work with hospital or nursing home staff to improve the care of diabetic patients. Educators must be willing to spend time attending meetings and reading the current literature to maintain their knowledge of diabetes medications, nutrition, and blood monitoring devices so that they can pass this information to their patients.

Summary

Starch is a storage form of energy in plants. It contains two polymers composed of glucose units: amylose (linear) and amylopectin (branched). Glycogen is a storage form of energy in animals. It is a branched polymer composed of glucose units. It is more highly branched than amylopectin. Cellulose is a structural polymer of glucose units found in plants. It is a linear polymer with the glucose units linked through β-1,4-glycosidic bonds.

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2.11: Other Important Carbohydrates

Objectives

After completing this section, you should be able to identify deoxy and amino sugars, given their structures.

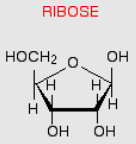
Key Terms

Make certain that you can define, and use in context, the key terms below.

- amino sugar
- deoxy sugar

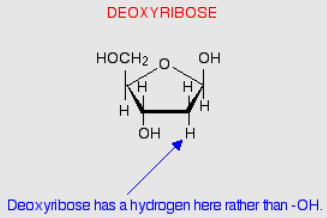
The sugars in the backbone of DNA

The backbone of DNA is based on a repeated pattern of a sugar group and a phosphate group. The full name of DNA, deoxyribonucleic acid, gives you the name of the sugar present - deoxyribose. Deoxyribose is a modified form of another sugar called ribose. I'm going to give you the structure of that first, because you will need it later anyway. Ribose is the sugar in the backbone of RNA, ribonucleic acid.

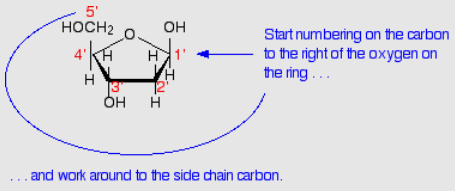


This diagram misses out the carbon atoms in the ring for clarity. Each of the four corners where there isn't an atom shown has a carbon atom. The heavier lines are coming out of the screen or paper towards you. In other words, you are looking at the molecule from a bit above the plane of the ring.

So that's ribose. Deoxyribose, as the name might suggest, is ribose which has lost an oxygen atom - **"de-oxy"**.



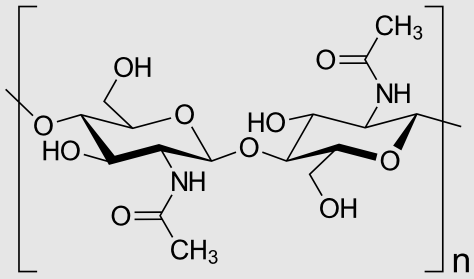
The only other thing you need to know about deoxyribose (or ribose, for that matter) is how the carbon atoms in the ring are numbered. The carbon atom to the right of the oxygen as we have drawn the ring is given the number 1, and then you work around to the carbon on the CH_2OH side group which is number 5.



You will notice that each of the numbers has a small dash by it - 3' or 5', for example. If you just had ribose or deoxyribose on its own, that wouldn't be necessary, but in DNA and RNA these sugars are attached to other ring compounds. The carbons in the sugars are given the little dashes so that they can be distinguished from any numbers given to atoms in the other rings. You read 3' or 5' as "3-prime" or "5-prime".

Amino sugar

An amino sugar (or more technically a 2-amino-2-deoxysugar) is a sugar molecule in which a hydroxyl group has been replaced with an amine group. More than 60 amino sugars are known, with one of the most abundant being *N*-acetylglucosamine, which is the main component of chitin.



Structure of the chitin molecule, showing two of the *N*-acetylglucosamine units that repeat to form long chains in β-(1→4)-linkage. Image by Dschanz / Public domain. Wikimedia Commons

Chitin is is a polymer of *N*-acetylglucosamine and is found in many places throughout the natural world. It is a characteristic component of the cell walls of fungi, the exoskeletons of arthropods such as crustaceans (e.g., crabs, lobsters and shrimps) and insects, the radulae of molluscs, and the beaks and internal shells of cephalopods, including squid and octopuses and on the scales and other soft tissues of fish and lissamphibians.

Contributors and Attributions

- Dr. Dietmar Kennepohl FCIC (Professor of Chemistry, Athabasca University)
- Prof. Steven Farmer (Sonoma State University)
- Jim Clark (Chemguide.co.uk)
- Wikipedia

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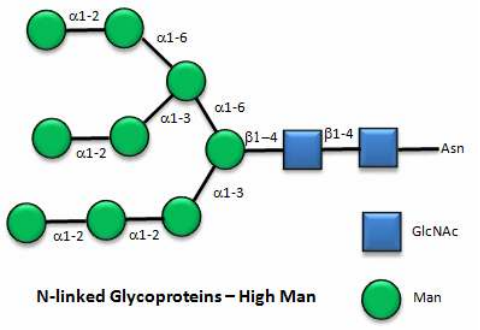
2.12: Cell Surface Carbohydrates and Influenza Viruses

Objectives

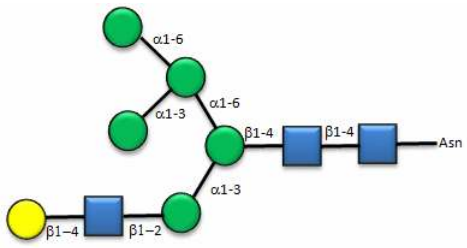
You may omit Section 25.11.

Carbohydrates are covalently attached to many different biomolecules, including lipids, to form glycolipids, and proteins, to form glycoproteins. Glycoproteins and glycolipids are often found in biological membranes, to which they are anchored by through nonpolar interactions. A special kind of glycoprotein, a proteoglycan, actually has more carbohydrate mass than protein. What is the function of these carbohydrates? Two are apparent. First, glycosylation of proteins helps protect the protein from degradation by enzyme catalysts within the body. However, there main functions arises from the fact that covalently attached carbohydrates that "decorate" the surface of glycoproteins or glycolipids provide new binding site interactions that allow interactions with other biomolecules. Hence glycosylation allows for cell:cell, cell:protein, or protein:protein interactions. Unfortunately, bacteria and viruses often recognize glycosylated molecules on cell membranes, allowing for their import into the cell.

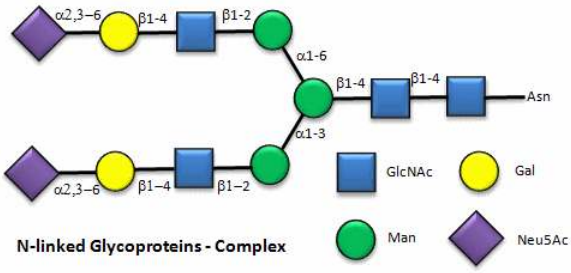
Here are some "cartoon" examples of carbohydrates covalently linked to the amino acid asparagine (Asn) on a glycoprotein.



N-linked Glycoproteins – High Man



N-linked Glycoproteins – Mixed (Hybrid)

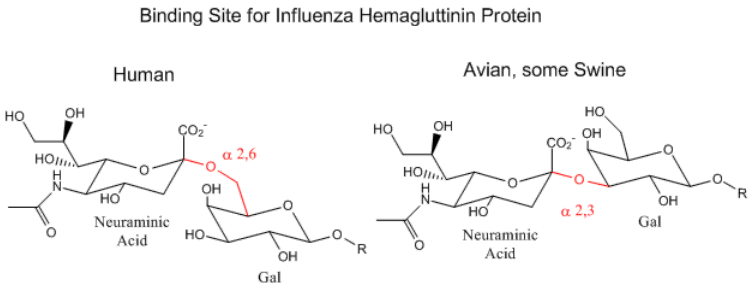


N-linked Glycoproteins - Complex

Here are some examples of biomolecular interactions promoted by IMFs involving carbohydrates.

Influenza Virus binding to Cell Surface Glycoproteins with Neu5Ac - A protein on the surface of influenza virus, hemagluttinin, bind to sialic acid (Sia), which is covalently attached to many cell membrane glycoproteins on host cells. The sialic acid is usually connected through an alpha (2,3) or alpha (2,6) link to galactose on N-linked glycoproteins. The subtypes found in avian (and equine) influenza isolates bind preferentially to Sia (alpha 2,3) Gal which predominates in avian GI tract where viruses replicate. Human virus of H1, H2, and H3 subtype (cause of the 1918, 1957, and 1968 pandemics) recognize Sia (alpha 2,6) Gal, the major

form in human respiratory tract. The swine influenza HA bind to Sia (alpha 2,6) Gal and some Sia (alpha 2,3)both of which found in swine.



- Jmol model of viral hemagluttinin bound to antiviral drugs and sialic (neuraminic acid) from Proteopedia

Leukocyte: Cell Wall binding - During inflammation, circulating leukocytes (a type of white blood cell) tether and roll on the walls of blood vessels where they become active. E-, L- and P-selectin proteins are the primary proteins responsible for the tethering and rolling of these leukocytes. P-selectin binds, in part, to a tetrasaccharide, sialyl-Lewisx (SLEX) on the cell surface.. The interaction between P-selectin and the cell mediates the initial binding/rolling of the leukocyte on the vessel wall.

- Jmol model of P-selectin binding to tetrasaccharide

Contributors and Attributions

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CHAPTER OVERVIEW

3: Lipids

The lipids are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents (e.g., ether, chloroform, acetone and benzene) and general insolubility in water. There is great structural variety among the lipids, as will be demonstrated in the following sections.

3.1: Prelude to Lipids
3.2: Fatty Acids
3.3: Fats and Oils
3.4: Waxes
3.5: Membrane Lipids- Phosphoglycerides and Spingholipids
3.6: Saponification of Fats and Oils; Soaps and Detergents
3.7: Steroids
3.8: Prostaglandins and other Eicosanoids

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3.1: Prelude to Lipids

Lipids

Fats and oils, found in many of the foods we eat, belong to a class of biomolecules known as lipids. Gram for gram, they pack more than twice the caloric content of carbohydrates: the oxidation of fats and oils supplies about 9 kcal of energy for every gram oxidized, whereas the oxidation of carbohydrates supplies only 4 kcal/g. Although the high caloric content of fats may be bad news for the dieter, it says something about the efficiency of nature's designs. Our bodies use carbohydrates, primarily in the form of glucose, for our *immediate* energy needs. Our capacity for storing carbohydrates for later use is limited to tucking away a bit of glycogen in the liver or in muscle tissue. We store our *reserve* energy in lipid form, which requires far less space than the same amount of energy stored in carbohydrate form. Lipids have other biological functions besides energy storage. They are a major component of the membranes of the 10 trillion cells in our bodies. They serve as protective padding and insulation for vital organs. Furthermore, without lipids in our diets, we would be deficient in the fat-soluble vitamins A, D, E, and K.

Lipids are not defined by the presence of specific functional groups, as carbohydrates are, but by a physical property—solubility. Compounds isolated from body tissues are classified as lipids if they are more soluble in organic solvents, such as dichloromethane, than in water. By this criterion, the lipid category includes not only fats and oils, which are esters of the trihydroxy alcohol glycerol and fatty acids, but also compounds that incorporate functional groups derived from phosphoric acid, carbohydrates, or amino alcohols, as well as steroid compounds such as cholesterol (Figure **3.1.1** presents one scheme for classifying the various kinds of lipids). We will discuss the various kinds of lipids by considering one subclass at a time and pointing out structural similarities and differences as we go.

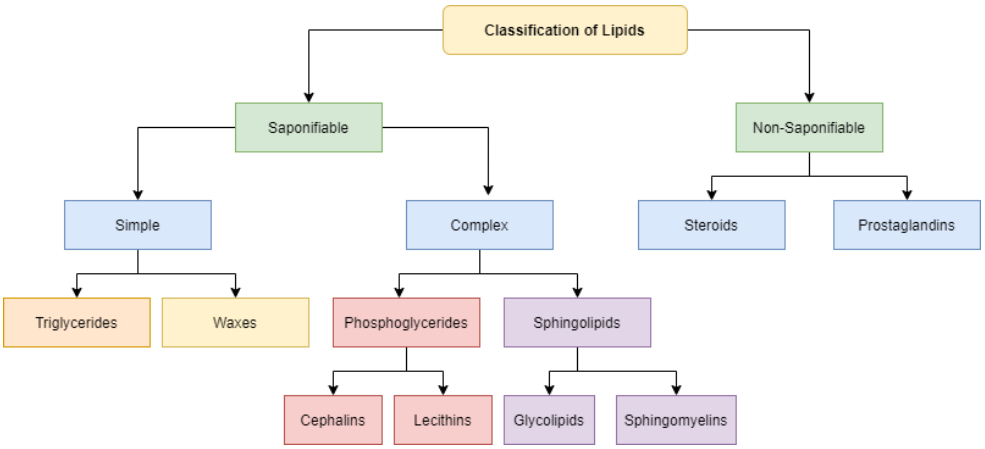


Figure **3.1.1**: Lipid Organization Based on Structural Relationships

Sources

- Ball et al. The Basics of GOB Chemistry. LibreTexts adapted under CC BY-NC-SA 3.0 license.
- Seager, S. L., Hensen, M. S., & Slabaugh, M. R. (2018). *Chemistry for today: General, organic, and biochemistry* (9th ed.). Boston, MA, USA: Brooks/Cole, Cengage Learning.

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3.2: Fatty Acids

Learning Objectives

- To recognize the structures of common fatty acids and classify them as saturated, monounsaturated, or polyunsaturated.

Fatty acids are carboxylic acids that are structural components of fats, oils, and all other categories of lipids, except steroids. More than 70 have been identified in nature. They usually contain an even number of carbon atoms (typically 12–20), are generally unbranched, and can be classified by the presence and number of carbon-to-carbon double bonds. Thus, saturated fatty acids contain no carbon-to-carbon double bonds, monounsaturated fatty acids contain one carbon-to-carbon double bond, and polyunsaturated fatty acids contain two or more carbon-to-carbon double bonds.

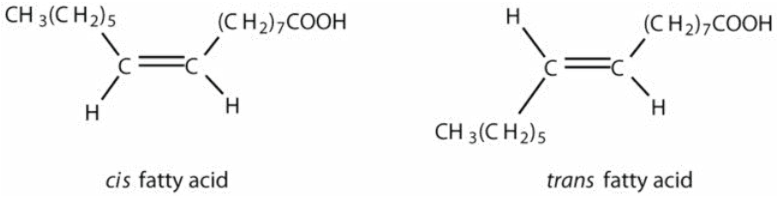


Table **3.2.1** lists some common fatty acids and one important source for each. The atoms or groups around the double bonds in unsaturated fatty acids can be arranged in either the cis or trans isomeric form. Naturally occurring fatty acids are generally in the cis configuration.

Table **3.2.1**: Some Common Fatty Acids Found in Natural Fats

Name	Abbreviated Structural Formula	Condensed Structural Formula	Melting Point (°C)	Source
lauric acid	$C_{11}H_{23}COOH$	$CH_3(CH_2)_{10}COOH$	44	palm kernel oil
myristic acid	$C_{13}H_{27}COOH$	$CH_3(CH_2)_{12}COOH$	58	oil of nutmeg
palmitic acid	$C_{15}H_{31}COOH$	$CH_3(CH_2)_{14}COOH$	63	palm oil
palmitoleic acid	$C_{15}H_{29}COOH$	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	0.5	macadamia oil
stearic acid	$C_{17}H_{35}COOH$	$CH_3(CH_2)_{16}COOH$	70	cocoa butter
oleic acid	$C_{17}H_{33}COOH$	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	16	olive oil
linoleic acid	$C_{17}H_{31}COOH$	$CH_3(CH_2)_3(CH_2CH=CH)_2(CH_2)_7COOH$	−5	canola oil
α-linolenic acid	$C_{17}H_{29}COOH$	$CH_3(CH_2CH=CH)_3(CH_2)_7COOH$	−11	flaxseed
arachidonic acid	$C_{19}H_{31}COOH$	$CH_3(CH_2)_4(CH_2CH=CH)_4(CH_2)_2COOH$	−50	liver

Two polyunsaturated fatty acids—linoleic and α-linolenic acids—are termed essential fatty acids because humans must obtain them from their diets. Both substances are required for normal growth and development, but the human body does not synthesize them. The body uses linoleic acid to synthesize many of the other unsaturated fatty acids, such as arachidonic acid, a precursor for the synthesis of prostaglandins. In addition, the essential fatty acids are necessary for the efficient transport and metabolism of cholesterol. The average daily diet should contain about 4–6 g of the essential fatty acids.

Although we often draw the carbon atoms in a straight line, they actually have more of a zigzag configuration (Figure **3.2.2*a***). Viewed as a whole, however, the saturated fatty acid molecule is relatively straight (Figure **3.2.2*b***). Such molecules pack closely

together into a crystal lattice, maximizing the strength of dispersion forces and causing fatty acids and the fats derived from them to have relatively high melting points. In contrast, each *cis* carbon-to-carbon double bond in an unsaturated fatty acid produces a pronounced bend in the molecule, so that these molecules do not stack neatly. As a result, the intermolecular attractions of unsaturated fatty acids (and unsaturated fats) are weaker, causing these substances to have lower melting points. Most are liquids at room temperature.

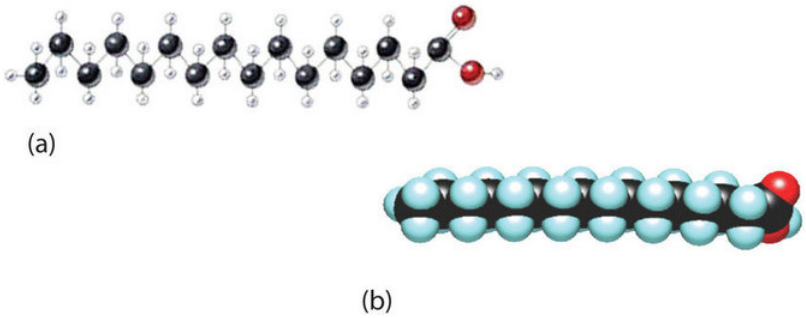


Figure **3.2.2**: The Structure of Saturated Fatty Acids. (a) There is a zigzag pattern formed by the carbon-to-carbon single bonds in the ball-and-stick model of a palmitic acid molecule. (b) A space-filling model of palmitic acid shows the overall straightness of a saturated fatty acid molecule.

Summary

Fatty acids are carboxylic acids that are the structural components of many lipids. They may be saturated or unsaturated. Most fatty acids are unbranched and contain an even number of carbon atoms. Unsaturated fatty acids have lower melting points than saturated fatty acids containing the same number of carbon atoms.

Concept Review Exercises

1. Give an example of each compound.
    a. saturated fatty acid
    b. polyunsaturated fatty acid
    c. monounsaturated fatty acid
2. Why do unsaturated fatty acids have lower melting points than saturated fatty acids?

Answers

1. a. stearic acid (answers will vary)
    b. linoleic acid (answers will vary)
    c. palmitoleic acid (answers will vary)
2. Unsaturated fatty acids cannot pack as tightly together as saturated fatty acids due to the presence of the cis double bond that puts a “kink” or bend in the hydrocarbon chain.

Exercises

1. Classify each fatty acid as saturated or unsaturated and indicate the number of carbon atoms in each molecule.
    a. palmitoleic acid
    b. myristic acid
    c. linoleic acid
2. Classify each fatty acid as saturated or unsaturated and indicate the number of carbon atoms in each molecule.
    a. stearic acid
    b. oleic acid
    c. palmitic acid

3. Write the condensed structural formula for each fatty acid.

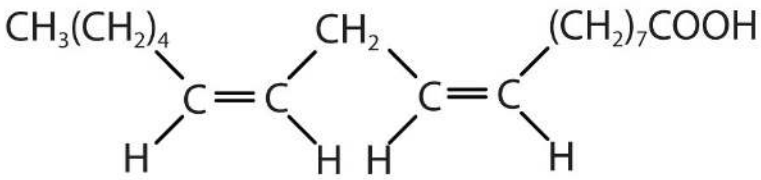
   a. lauric acid
   b. palmitoleic acid
   c. linoleic acid

4. Write the condensed structural formulas for each fatty acid.

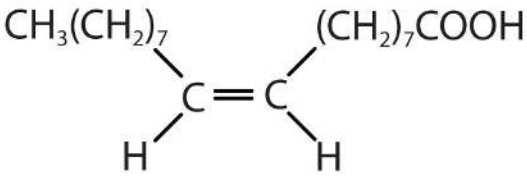
   a. oleic acid
   b. α-linolenic acid
   c. palmitic acid

5. Arrange these fatty acids (all contain 18 carbon atoms) in order of increasing melting point. Justify your arrangement.

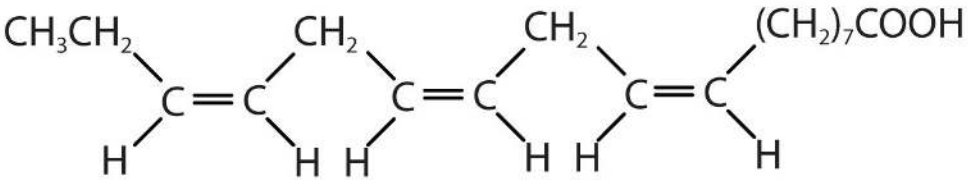
   a.



   b.



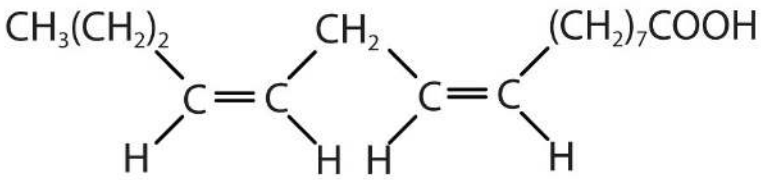
   c.



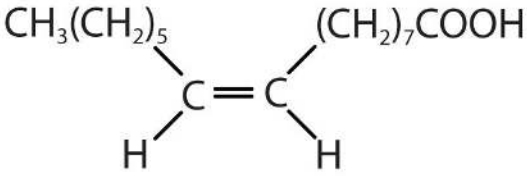
6. Arrange these fatty acids (all contain 16 carbon atoms) in order of increasing melting point. Justify your arrangement.

   a. $CH_3(CH_2)_{14}COOH$

   b.



   c.



Answers

1. a. unsaturated; 16 carbon atoms
   b. saturated; 14 carbon atoms

c. unsaturated; 18 carbon atoms

3. a. $CH_3(CH_2)_{10}COOH$
   b. $CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
   c. $CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7COOH$

5. c < a < b; an increase in the number of double bonds will lower the melting point because it is more difficult to closely pack the fatty acids together.

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3.3: Fats and Oils

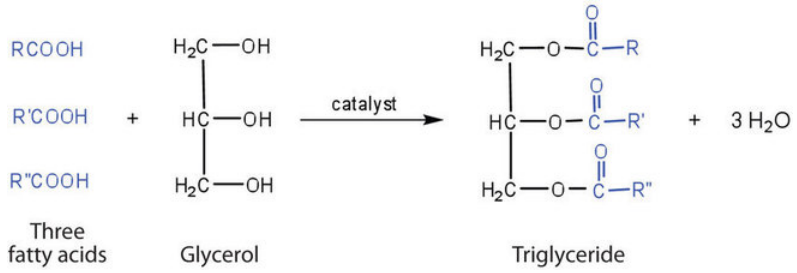
Learning Objectives

- Explain why fats and oils are referred to as triglycerides.
- Explain how the fatty acid composition of the triglycerides determines whether a substance is a fat or oil.
- Describe the importance of key reactions of triglycerides, such as hydrolysis, hydrogenation, and oxidation.

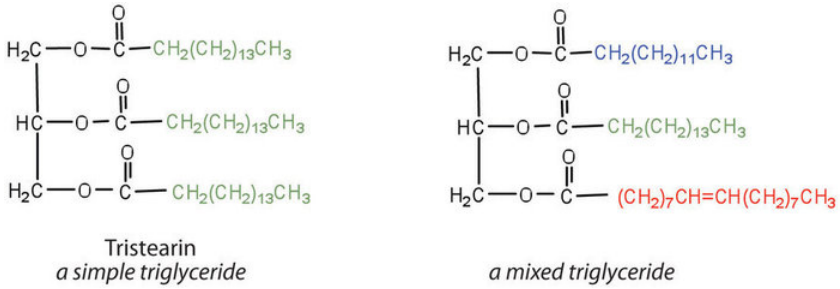
Fats and oils are the most abundant lipids in nature. They provide energy for living organisms, insulate body organs, and transport fat-soluble vitamins through the blood.

Structures of Fats and Oils

Fats and oils are called triglycerides (or *triacylcylgerols*) because they are esters composed of three fatty acid units joined to *glycerol*, a trihydroxy alcohol:



If all three OH groups on the glycerol molecule are esterified with the same fatty acid, the resulting ester is called a *simple triglyceride*. Although simple triglycerides have been synthesized in the laboratory, they rarely occur in nature. Instead, a typical triglyceride obtained from naturally occurring fats and oils contains two or three different fatty acid components and is thus termed a *mixed triglyceride*.



A triglyceride is called a fat if it is a solid at 25°C; it is called an oil if it is a liquid at that temperature. These differences in melting points reflect differences in the degree of unsaturation and number of carbon atoms in the constituent fatty acids. Triglycerides obtained from animal sources are usually solids, while those of plant origin are generally oils. Therefore, we commonly speak of animal fats and vegetable oils.

No single formula can be written to represent the naturally occurring fats and oils because they are highly complex mixtures of triglycerides in which many different fatty acids are represented. Table **3.3.1** shows the fatty acid compositions of some common fats and oils. The composition of any given fat or oil can vary depending on the plant or animal species it comes from as well as on dietetic and climatic factors. To cite just one example, lard from corn-fed hogs is more highly saturated than lard from peanut-fed hogs. Palmitic acid is the most abundant of the saturated fatty acids, while oleic acid is the most abundant unsaturated fatty acid.

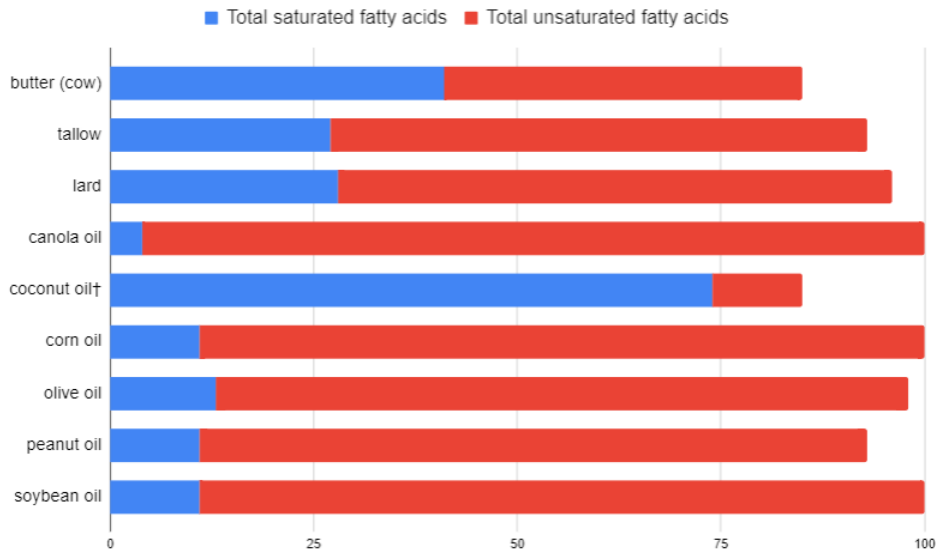
Table **3.3.1**: Average Fatty Acid Composition of Some Common Fats and Oils (%)*

	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Fats							
butter (cow)	3	11	27	12	29	2	1
tallow		3	24	19	43	3	1
lard		2	26	14	44	10	
Oils							
canola oil			4	2	62	22	10
coconut oil†	47	18	9	3	6	2	
corn oil			11	2	28	58	1
olive oil			13	3	71	10	1
peanut oil			11	2	48	32	
soybean oil			11	4	24	54	7

*Totals less than 100% indicate the presence of fatty acids with fewer than 12 carbon atoms or more than 18 carbon atoms.

†Coconut oil is highly saturated. It contains an unusually high percentage of the low-melting C_8, C_{10}, and C_{12} saturated fatty acids.

The figure below shows the information on the table in terms of saturated or unsaturated fatty acid content:

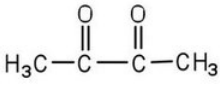


Fats contain a high proportion of saturated fatty acids, while oils contain a high proportion of unsaturated fatty acids (coconut oil is an exception). The high consumption of saturated fats is a factor, along with the high consumption of cholesterol, in increased risks of heart disease.

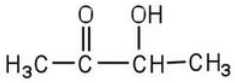
- ## Physical Properties of Fats and Oils

  Contrary to what you might expect, *pure* fats and oils are colorless, odorless, and tasteless. The characteristic colors, odors, and flavors that we associate with some of them are imparted by foreign substances that are lipid soluble and have been absorbed by these lipids. For example, the yellow color of butter is due to the presence of the pigment carotene; the taste of butter comes

from two compounds—diacetyl and 3-hydroxy-2-butanone—produced by bacteria in the ripening cream from which the butter is made.



Diacetyl

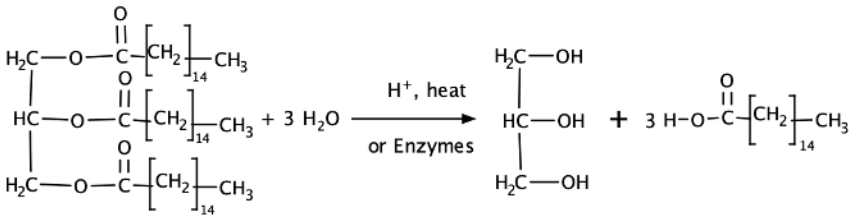


3-hydroxy-2-butanone

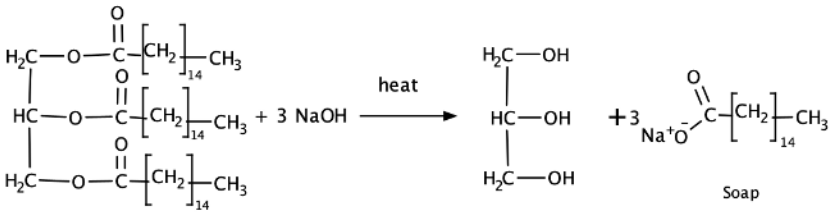
Fats and oils are lighter than water, having densities of about 0.8 g/cm^3. They are poor conductors of heat and electricity and therefore serve as excellent insulators for the body, slowing the loss of heat through the skin.

Chemical Reactions of Fats and Oils

Fats and oils can participate in a variety of chemical reactions—for example, because triglycerides are esters, they can be hydrolyzed in the presence of an acid, a base, or specific enzymes known as lipases. The hydrolysis of fats and oils in the presence of a base is used to make soap and is called saponification. Today most soaps are prepared through the hydrolysis of triglycerides (often from tallow, coconut oil, or both) using water under high pressure and temperature [700 lb/in^2 (~50 atm or 5,000 kPa) and 200°C]. Sodium carbonate or sodium hydroxide is then used to convert the fatty acids to their sodium salts (soap molecules):



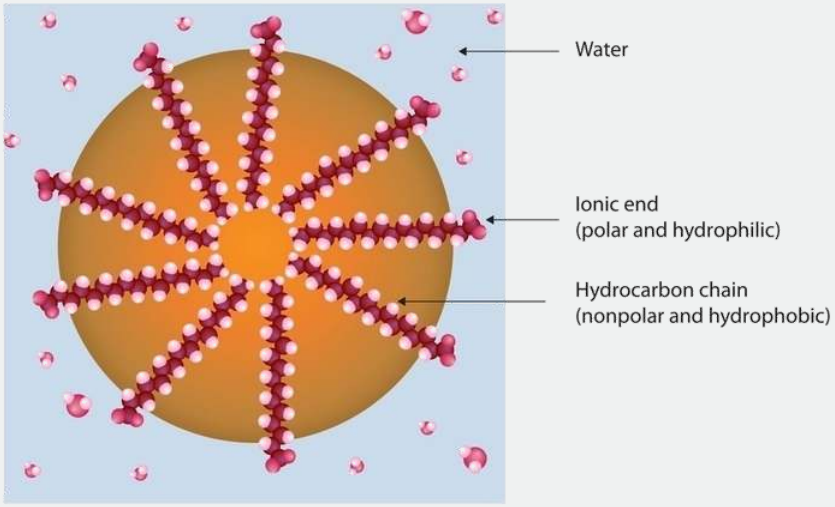
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- Looking Closer: Soaps

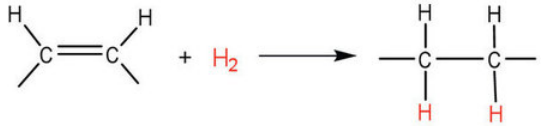
  Ordinary soap is a mixture of the sodium salts of various fatty acids, produced in one of the oldest organic syntheses practiced by humans (second only to the fermentation of sugars to produce ethyl alcohol). Both the Phoenicians (600 BCE) and the Romans made soap from animal fat and wood ash. Even so, the widespread production of soap did not begin until the 1700s. Soap was traditionally made by treating molten lard or tallow with a slight excess of alkali in large open vats. The mixture was heated, and steam was bubbled through it. After saponification was completed, the soap was precipitated from the mixture by the addition of sodium chloride (NaCl), removed by filtration, and washed several times with water. It was then dissolved in water and reprecipitated by the addition of more NaCl. The glycerol produced in the reaction was also recovered from the aqueous wash solutions.

Pumice or sand is added to produce scouring soap, while ingredients such as perfumes or dyes are added to produce fragrant, colored soaps. Blowing air through molten soap produces a floating soap. Soft soaps, made with potassium salts, are more expensive but produce a finer lather and are more soluble. They are used in liquid soaps, shampoos, and shaving creams.



Dirt and grime usually adhere to skin, clothing, and other surfaces by combining with body oils, cooking fats, lubricating greases, and similar substances that act like glues. Because these substances are not miscible in water, washing with water alone does little to remove them. Soap removes them, however, because soap molecules have a dual nature. One end, called the *head*, carries an ionic charge (a carboxylate anion) and therefore dissolves in water; the other end, the *tail*, has a hydrocarbon structure and dissolves in oils. The hydrocarbon tails dissolve in the soil; the ionic heads remain in the aqueous phase, and the soap breaks the oil into tiny soap-enclosed droplets called *micelles*, which disperse throughout the solution. The droplets repel each other because of their charged surfaces and do not coalesce. With the oil no longer "gluing" the dirt to the soiled surface (skin, cloth, dish), the soap-enclosed dirt can easily be rinsed away.

The double bonds in fats and oils can undergo hydrogenation and also oxidation. The hydrogenation of vegetable oils to produce semisolid fats is an important process in the food industry. Chemically, it is essentially identical to the catalytic hydrogenation reaction described for alkenes.



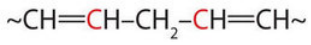
In commercial processes, the number of double bonds that are hydrogenated is carefully controlled to produce fats with the desired consistency (soft and pliable). Inexpensive and abundant vegetable oils (canola, corn, soybean) are thus transformed into margarine and cooking fats. In the preparation of margarine, for example, partially hydrogenated oils are mixed with water, salt, and nonfat dry milk, along with flavoring agents, coloring agents, and vitamins A and D, which are added to approximate the look, taste, and nutrition of butter. (Preservatives and antioxidants are also added.) In most commercial peanut butter, the peanut oil has been partially hydrogenated to prevent it from separating out. Consumers could decrease the amount of saturated fat in their diet by using the original unprocessed oils on their foods, but most people would rather spread margarine on their toast than pour oil on it.

Many people have switched from butter to margarine or vegetable shortening because of concerns that saturated animal fats can raise blood cholesterol levels and result in clogged arteries. However, during the hydrogenation of vegetable oils, an isomerization reaction occurs that produces the *trans* fatty acids mentioned in the opening essay. However, studies have shown that *trans* fatty acids also raise cholesterol levels and increase the incidence of heart disease. *Trans* fatty acids do not have the bend in their structures, which occurs in *cis* fatty acids and thus pack closely together in the same way that the saturated fatty

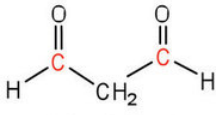
acids do. Consumers are now being advised to use polyunsaturated oils and soft or liquid margarine and reduce their total fat consumption to less than 30% of their total calorie intake each day.

Fats and oils that are in contact with moist air at room temperature eventually undergo oxidation and hydrolysis reactions that cause them to turn rancid, acquiring a characteristic disagreeable odor. One cause of the odor is the release of volatile fatty acids by hydrolysis of the ester bonds. Butter, for example, releases foul-smelling butyric, caprylic, and capric acids. Microorganisms present in the air furnish lipases that catalyze this process. Hydrolytic rancidity can easily be prevented by covering the fat or oil and keeping it in a refrigerator.

Another cause of volatile, odorous compounds is the oxidation of the unsaturated fatty acid components, particularly the readily oxidized structural unit



in polyunsaturated fatty acids, such as linoleic and linolenic acids. One particularly offensive product, formed by the oxidative cleavage of both double bonds in this unit, is a compound called *malonaldehyde*.



Malonaldehyde

Rancidity is a major concern of the food industry, which is why food chemists are always seeking new and better antioxidants, substances added in very small amounts (0.001%–0.01%) to prevent oxidation and thus suppress rancidity. Antioxidants are compounds whose affinity for oxygen is greater than that of the lipids in the food; thus they function by preferentially depleting the supply of oxygen absorbed into the product. Because vitamin E has antioxidant properties, it helps reduce damage to lipids in the body, particularly to unsaturated fatty acids found in cell membrane lipids.

Summary

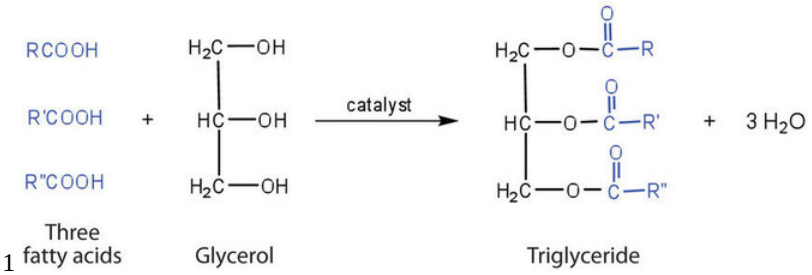
Fats and oils are composed of molecules known as triglycerides, which are esters composed of three fatty acid units linked to glycerol. An increase in the percentage of shorter-chain fatty acids and/or unsaturated fatty acids lowers the melting point of a fat or oil. The hydrolysis of fats and oils in the presence of a base makes soap and is known as saponification. Double bonds present in unsaturated triglycerides can be hydrogenated to convert oils (liquid) into margarine (solid). The oxidation of fatty acids can form compounds with disagreeable odors. This oxidation can be minimized by the addition of antioxidants.

Concept Review Exercises

Answers

Exercises

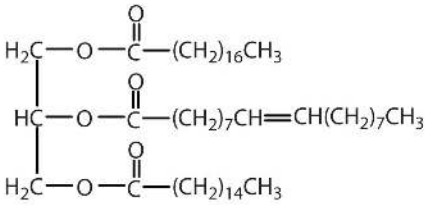
Answers



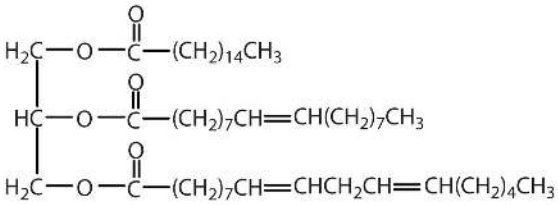
1

1. What functions does fat serve in the body?

2. Which of these triglycerides would you expect to find in higher amounts in oils? In fats? Justify your choice.

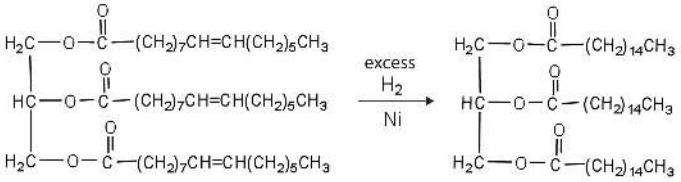


(a)

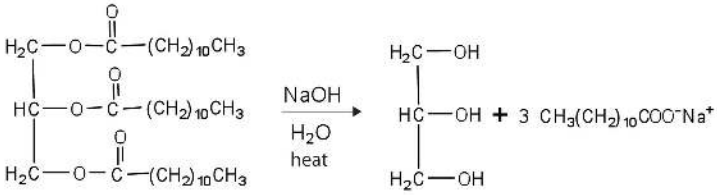


(b)

3. Fats provide energy for living organisms. They also provide insulation for body organs and transport fat-soluble vitamins.
4. The triglyceride on the left is expected to be present in higher amounts in fats because it is composed of a greater number of saturated fatty acids. The triglyceride on the right is expected to be present in higher amounts in oils because it is composed of a greater number of unsaturated fatty acids.
5. Draw the structure for each compound.
   a. glyceryl trimyristin
   b. a triglyceride likely to be found in peanut oil
6. Draw the structure for each compound.
   a. glyceryl tripalmitin
   b. a triglyceride likely to be found in butter
7. Draw structures to write the reaction for the complete hydrogenation of glyceryl tripalmitolein (Table **3.3.1** for the condensed structure of palmitoleic acid). Name the product formed.
8. Draw structures to write the reaction for the complete hydrogenation of glyceryl trilinolein (Table **3.3.1** for the condensed structure of linoleic acid). Name the product formed.
9. Draw structures to write the reaction for the hydrolysis of glyceryl trilaurin in a basic solution (Table **3.3.1** for the condensed structure of lauric acid).
10. Draw structures to write the reaction for the hydrolysis of glyceryl tristearin in a basic solution (Table **3.3.1** for the condensed structure of stearic acid).
11. a. What compounds with a disagreeable odor are formed when butter becomes rancid?
    b. How are these compounds formed?
    c. How can rancidity be prevented?
12. a. What compound with a disagreeable odor is formed when unsaturated fatty acids react with oxygen in the atmosphere?
    b. How can this process be prevented?

13. 

tripalmitin

14. 

15. a. smaller carboxylic acids, such as butyric, caprylic, and capric acids
    b. These compounds are formed by the hydrolysis of the triglycerides found in butter.
    c. Rancidity can be prevented by covering the butter (to keep out moisture) and storing it in a refrigerator. (Cold temperatures slow down hydrolysis reactions.)

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3.4: Waxes

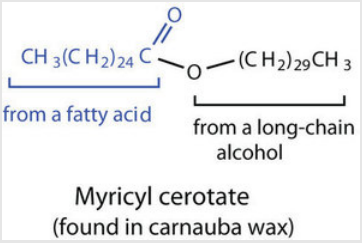
Objectives

After completing this section, you should be able to

1. identify waxes as being mixtures of long-chain esters, and write the general structure for such compounds.

Waxes

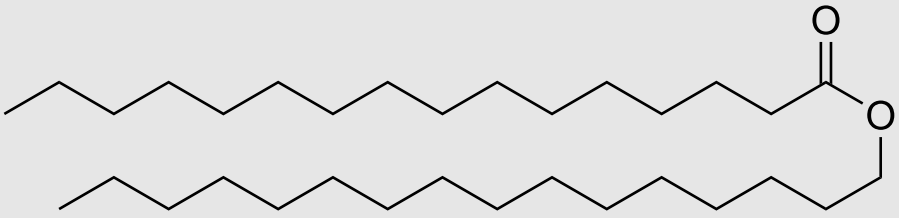
Waxes are esters of fatty acids with long chain monohydric alcohols (one hydroxyl group). Natural waxes are often mixtures of such esters, and may also contain hydrocarbons. Plant waxes on the surfaces of leaves, stems, flowers, and fruits protect the plant from dehydration and invasion by harmful microorganisms. Carnauba wax, used extensively in floor waxes, automobile waxes, and furniture polish, is largely myricyl cerotate, obtained from the leaves of certain Brazilian palm trees. Animals also produce waxes that serve as protective coatings, keeping the surfaces of feathers, skin, and hair pliable and water repellent. In fact, if the waxy coating on the feathers of a water bird is dissolved as a result of the bird swimming in an oil slick, the feathers become wet and heavy, and the bird, unable to maintain its buoyancy, drowns.



Myricyl cerotate
(found in carnauba wax)

The formulas for three well known waxes are given below, with the carboxylic acid moiety colored red and the alcohol colored blue.

Spermaceti		**Beeswax**		**Carnuba wax**
$CH_3(CH_2)_{14}CO_2$-$(CH_2)_{15}CH_3$		$CH_3(CH_2)_{24}CO_2$-$(CH_2)_{29}CH_3$		$CH_3(CH_2)_{30}CO_2$-$(CH_2)_{33}CH_3$



Cetyl palmitate, a typical wax ester.

Waxes are widely distributed in nature. The leaves and fruits of many plants have waxy coatings, which may protect them from dehydration and small predators. The feathers of birds and the fur of some animals have similar coatings which serve as a water repellent. Carnuba wax is valued for its toughness and water resistance.

Contributors and Attributions

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- Charles Ophardt, Professor Emeritus, Elmhurst College; Virtual Chembook
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- Fatty Acids. (2020, August 17). Retrieved May 11, 2021, from https://chem.libretexts.org/@go/page/16123

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3.5: Membrane Lipids- Phosphoglycerides and Spingholipids

Learning Objectives

- Identify the distinguishing characteristics of membrane lipids.
- Describe membrane components and how they are arranged.

All living cells are surrounded by a cell membrane. Plant cells (Figure **3.5.1*A***) and animal cells (Figure **3.5.1*B***) contain a cell nucleus that is also surrounded by a membrane and holds the genetic information for the cell. Everything between the cell membrane and the nuclear membrane—including intracellular fluids and various subcellular components such as the mitochondria and ribosomes—is called the cytoplasm. The membranes of all cells have a fundamentally similar structure, but membrane function varies tremendously from one organism to another and even from one cell to another within a single organism. This diversity arises mainly from the presence of different proteins and lipids in the membrane.

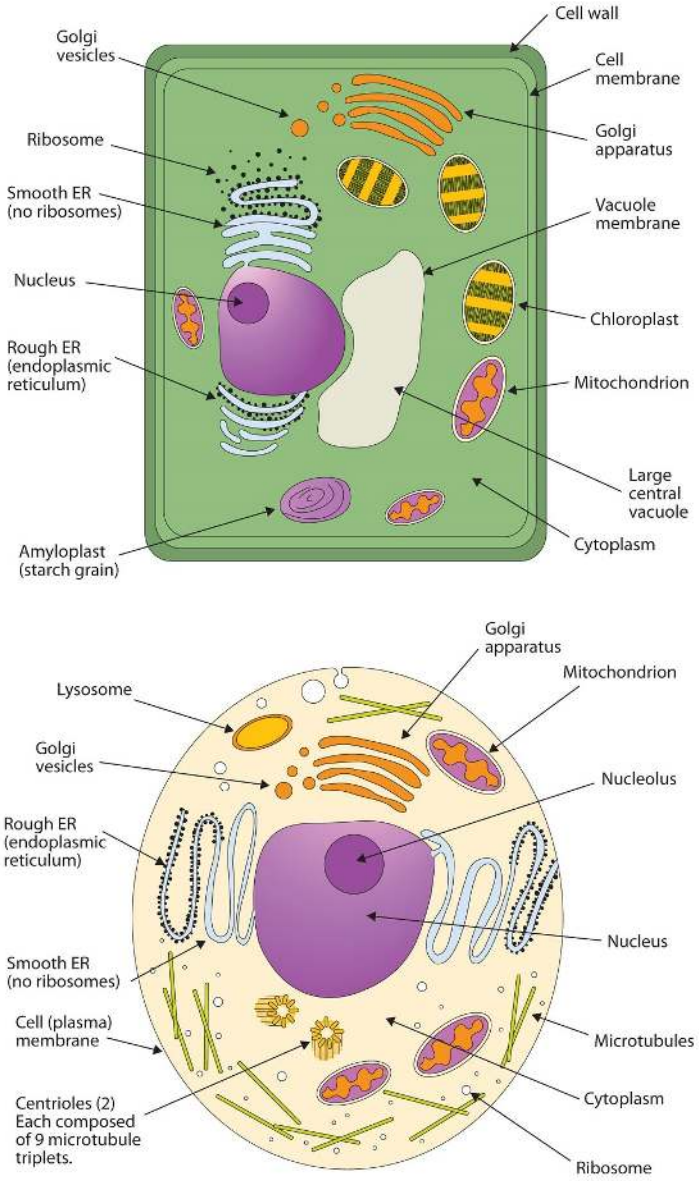


Figure **3.5.1**: (A) An Idealized Plant Cell. Not all the structures shown here occur in every type of plant cell. (B) An Idealized Animal Cell. The structures shown here will seldom all be found in a single animal cell.

The lipids in cell membranes are highly polar but have dual characteristics: part of the lipid is ionic and therefore dissolves in water, whereas the rest has a hydrocarbon structure and therefore dissolves in nonpolar substances. Often, the ionic part is referred to as hydrophilic, meaning “water loving,” and the nonpolar part as hydrophobic, meaning “water fearing” (repelled by water). When allowed to float freely in water, polar lipids spontaneously cluster together in any one of three arrangements: micelles, monolayers, and bilayers (Figure **3.5.2**).

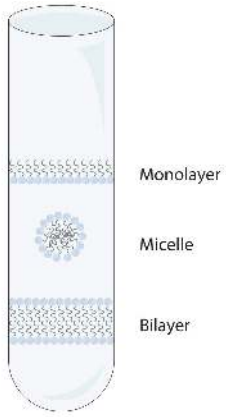


Figure **3.5.2**: Spontaneously Formed Polar Lipid Structures in Water: Monolayer, Micelle, and Bilayer

Micelles are aggregations in which the lipids’ hydrocarbon tails—being hydrophobic—are directed toward the center of the assemblage and away from the surrounding water while the hydrophilic heads are directed outward, in contact with the water. Each micelle may contain thousands of lipid molecules. Polar lipids may also form a monolayer, a layer one molecule thick on the surface of the water. The polar heads face into water, and the nonpolar tails stick up into the air. Bilayers are double layers of lipids arranged so that the hydrophobic tails are sandwiched between an inner surface and an outer surface consisting of hydrophilic heads. The hydrophilic heads are in contact with water on either side of the bilayer, whereas the tails, sequestered inside the bilayer, are prevented from having contact with the water. Bilayers like this make up every cell membrane (Figure **3.5.3**).

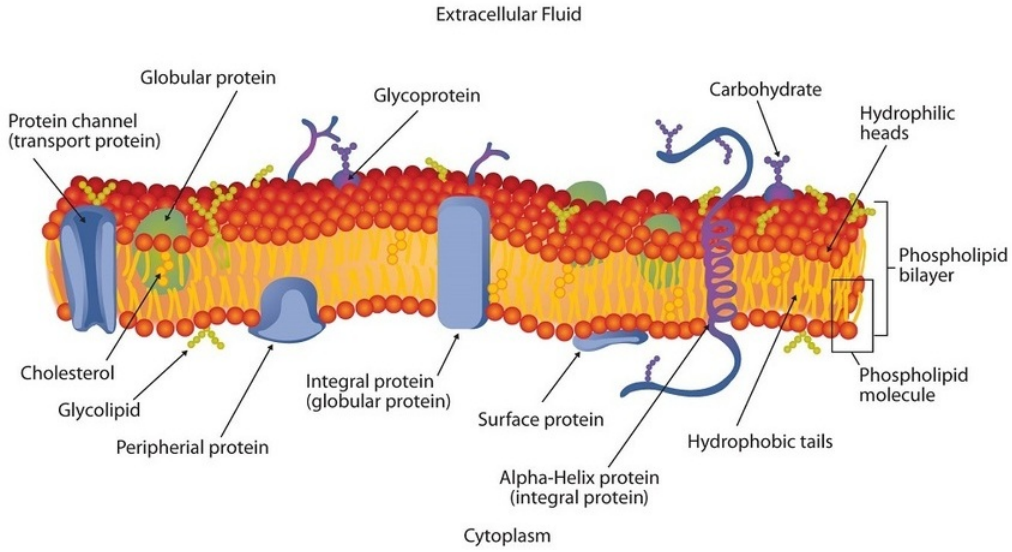


Figure **3.5.3**: Schematic Diagram of a Cell Membrane. The membrane enclosing a typical animal cell is a phospholipid bilayer with embedded cholesterol and protein molecules. Short oligosaccharide chains are attached to the outer surface.

In the bilayer interior, the hydrophobic tails (that is, the fatty acid portions of lipid molecules) interact by means of dispersion forces. The interactions are weakened by the presence of unsaturated fatty acids. As a result, the membrane components are free to mill about to some extent, and the membrane is described as fluid.

The lipids found in cell membranes can be categorized in various ways. Phospholipids are lipids containing phosphorus. Glycolipids are sugar-containing lipids. The latter are found exclusively on the outer surface of the cell membrane, acting as distinguishing surface markers for the cell and thus serving in cellular recognition and cell-to-cell communication. Sphingolipids

are phospholipids or glycolipids that contain the unsaturated amino alcohol sphingosine rather than glycerol. Diagrammatic structures of representative membrane lipids are presented in Figure **3.5.4**.

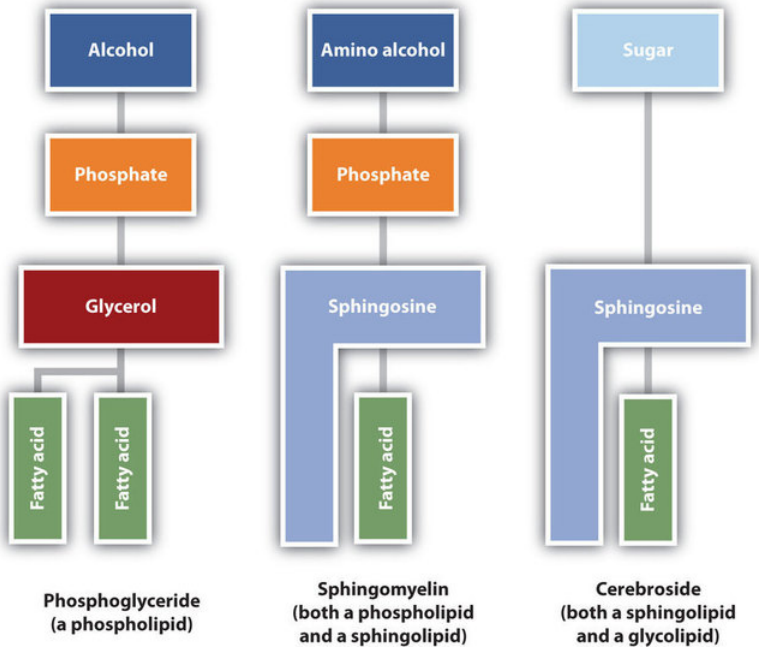


Figure **3.5.4**: Component Structures of Some Important Membrane Lipids

Phosphoglyceride are composed of alcohol, phosphate, glycerol and 2 fatty acids. A sphingomyelin contains phosphate, sphingosine, and a fatty acid. A cerbroside contains sugar, sphingosine and a fatty acid.

Phosphoglycerides (also known as glycerophospholipids) are the most abundant phospholipids in cell membranes. They consist of a glycerol unit with fatty acids attached to the first two carbon atoms, while a phosphoric acid unit, esterified with an alcohol molecule (usually an amino alcohol, as in part (a) of Figure **3.5.5**) is attached to the third carbon atom of glycerol (part (b) of Figure **3.5.5**). Notice that the phosphoglyceride molecule is identical to a triglyceride up to the phosphoric acid unit (part (b) of Figure **3.5.5**).

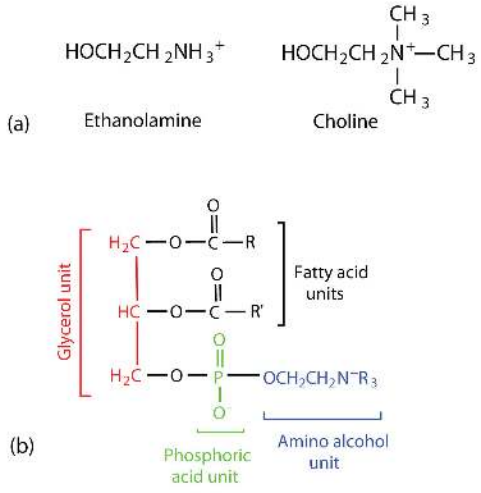
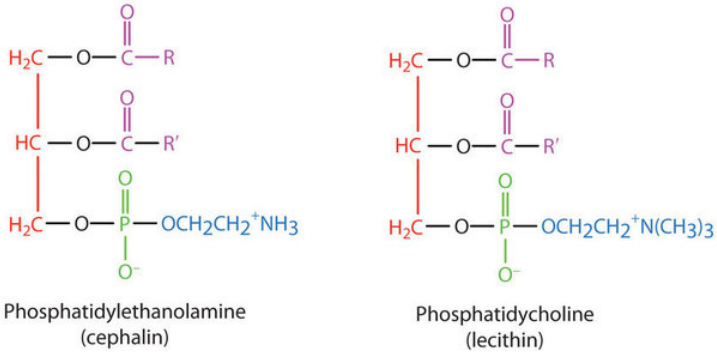


Figure **3.5.5**: Phosphoglycerides. (a) Amino alcohols are commonly found in phosphoglycerides, which are evident in its structural formula (b).

Structural formula of ethanolamine and choline are shown. The structural formula of a phosphoglyceride is shown with the glycerol unit, phosphoric unit, and amino alcohol unit highlighted in different colors.

There are two common types of phosphoglycerides. Phosphoglycerides containing ethanolamine as the amino alcohol are called *phosphatidylethanolamines* or *cephalins*. Cephalins are found in brain tissue and nerves and also have a role in blood clotting. Phosphoglycerides containing choline as the amino alcohol unit are called *phosphatidylcholines* or *lecithins*. Lecithins occur in all

living organisms. Like cephalins, they are important constituents of nerve and brain tissue. Egg yolks are especially rich in lecithins. Commercial-grade lecithins isolated from soybeans are widely used in foods as emulsifying agents. An emulsifying agent is used to stabilize an emulsion—a dispersion of two liquids that do not normally mix, such as oil and water. Many foods are emulsions. Milk is an emulsion of butterfat in water. The emulsifying agent in milk is a protein called *casein*. Mayonnaise is an emulsion of salad oil in water, stabilized by lecithins present in egg yolk.



Sphingomyelins, the simplest sphingolipids, each contain a fatty acid, a phosphoric acid, sphingosine, and choline (Figure **3.5.6**). Because they contain phosphoric acid, they are also classified as phospholipids. Sphingomyelins are important constituents of the myelin sheath surrounding the axon of a nerve cell. Multiple sclerosis is one of several diseases resulting from damage to the myelin sheath.

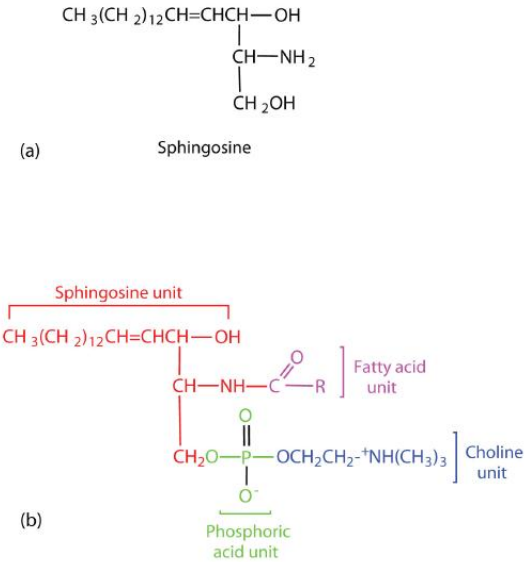


Figure **3.5.6**: Sphingolipids. (a) Sphingosine, an amino alcohol, is found in all sphingolipids. (b) A sphingomyelin is also known as a phospholipid, as evidenced by the phosphoric acid unit in its structure.

Structural formula of sphingosine is shown. The general structure of a sphingolipid is shown with the sphingosine unit, fatty acid unit, phosphoric acid unit, and choline unit highlighted in different colors.

Most animal cells contain sphingolipids called cerebrosides (Figure **3.5.7**). Cerebrosides are composed of sphingosine, a fatty acid, and galactose or glucose. They therefore resemble sphingomyelins but have a sugar unit in place of the choline phosphate group. Cerebrosides are important constituents of the membranes of nerve and brain cells.

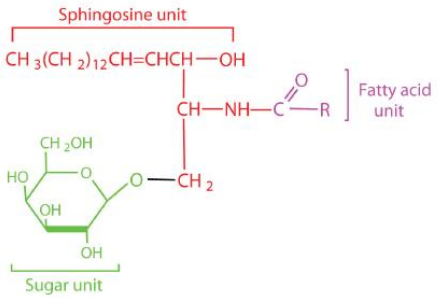


Figure 3.5.7: Cerebrosides. Cerebrosides are sphingolipids that contain a sugar unit.

General structure of a cerebroside with its sugar unit, sphingosine unit, and fatty acid unit highlighted in different colors.

The sphingolipids called gangliosides are more complex, usually containing a branched chain of three to eight monosaccharides and/or substituted sugars. Because of considerable variation in their sugar components, about 130 varieties of gangliosides have been identified. Most cell-to-cell recognition and communication processes (e.g., blood group antigens) depend on differences in the sequences of sugars in these compounds. Gangliosides are most prevalent in the outer membranes of nerve cells, although they also occur in smaller quantities in the outer membranes of most other cells. Because cerebrosides and gangliosides contain sugar groups, they are also classified as glycolipids.

Membrane Proteins

If membranes were composed only of lipids, very few ions or polar molecules could pass through their hydrophobic "sandwich filling" to enter or leave any cell. However, certain charged and polar species do cross the membrane, aided by proteins that move about in the lipid bilayer. The two major classes of proteins in the cell membrane are integral proteins, which span the hydrophobic interior of the bilayer, and peripheral proteins, which are more loosely associated with the surface of the lipid bilayer (Figure 3.5.3). Peripheral proteins may be attached to integral proteins, to the polar head groups of phospholipids, or to both by hydrogen bonding and electrostatic forces.

Small ions and molecules soluble in water enter and leave the cell by way of channels through the integral proteins. Some proteins, called *carrier proteins*, facilitate the passage of certain molecules, such as hormones and neurotransmitters, by specific interactions between the protein and the molecule being transported.

Summary

Lipids are important components of biological membranes. These lipids have dual characteristics: part of the molecule is hydrophilic, and part of the molecule is hydrophobic. Membrane lipids may be classified as phospholipids, glycolipids, and/or sphingolipids. Proteins are another important component of biological membranes. Integral proteins span the lipid bilayer, while peripheral proteins are more loosely associated with the surface of the membrane.

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3.6: Saponification of Fats and Oils; Soaps and Detergents

Learning Objectives

After completing this section, you should be able to

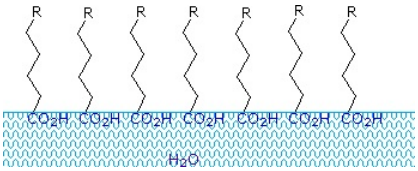
1. a. write an equation to represent the formation of a soap.
   b. identify the structure of the fat required to produce a given soap.
   c. identify the structure of a soap, given the structure of the fat from which it is produced.
2. describe the mechanism by which soaps exert their cleansing action.
3. give a chemical explanation of the problems encountered when carboxylate soaps are used in hard-water areas, and explain how they may be overcome by the use of sulphonate detergents.

Key Terms

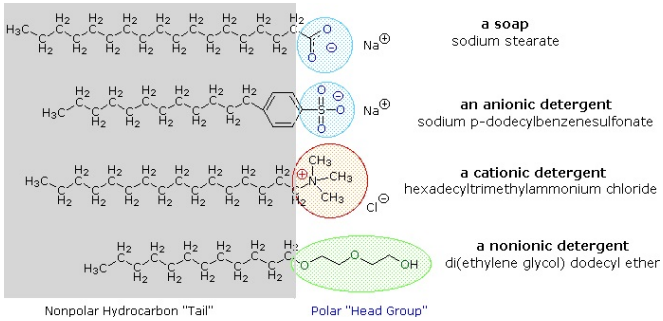
Make certain that you can define, and use in context, the key terms below.

- hydrophilic
- lipophilic (hydrophobic)
- amphiphilic
- micelles

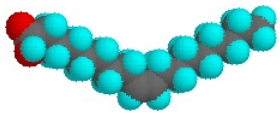
Carboxylic acids and salts having alkyl chains longer than eight carbons exhibit unusual behavior in water due to the presence of both hydrophilic (CO_2) and hydrophobic (alkyl) regions in the same molecule. Such molecules are termed **amphiphilic** (Gk. amphi = both) or **amphipathic**. Fatty acids made up of ten or more carbon atoms are nearly insoluble in water, and because of their lower density, float on the surface when mixed with water. Unlike paraffin or other alkanes, which tend to puddle on the waters surface, these fatty acids spread evenly over an extended water surface, eventually forming a monomolecular layer in which the polar carboxyl groups are hydrogen bonded at the water interface, and the hydrocarbon chains are aligned together away from the water. This behavior is illustrated in the diagram on the right. Substances that accumulate at water surfaces and change the surface properties are called **surfactants**.



Alkali metal salts of fatty acids are more soluble in water than the acids themselves, and the amphiphilic character of these substances also make them strong surfactants. The most common examples of such compounds are soaps and detergents, four of which are shown below. Note that each of these molecules has a nonpolar hydrocarbon chain, the "tail", and a polar (often ionic) "head group". The use of such compounds as cleaning agents is facilitated by their surfactant character, which lowers the surface tension of water, allowing it to penetrate and wet a variety of materials.



Very small amounts of these surfactants dissolve in water to give a random dispersion of solute molecules. However, when the concentration is increased an interesting change occurs. The surfactant molecules reversibly assemble into polymolecular aggregates called micelles. By gathering the hydrophobic chains together in the center of the micelle, disruption of the hydrogen bonded structure of liquid water is minimized, and the polar head groups extend into the surrounding water where they participate in hydrogen bonding. These micelles are often spherical in shape, but may also assume cylindrical and branched forms, as illustrated on the right. Here the polar head group is designated by a blue circle, and the nonpolar tail is a zig-zag black line.



The oldest amphiphilic cleaning agent known to humans is soap. Soap is manufactured by the base-catalyzed hydrolysis (saponification) of animal fat (see below). Before sodium hydroxide was commercially available, a boiling solution of potassium carbonate leached from wood ashes was used. Soft potassium soaps were then converted to the harder sodium soaps by washing with salt solution. The importance of soap to human civilization is documented by history, but some problems associated with its use have been recognized. One of these is caused by the weak acidity (pKa ca. 4.9) of the fatty acids. Solutions of alkali metal soaps are slightly alkaline (pH 8 to 9) due to hydrolysis. If the pH of a soap solution is lowered by acidic contaminants, insoluble fatty acids precipitate and form a scum. A second problem is caused by the presence of calcium and magnesium salts in the water supply (hard water). These divalent cations cause aggregation of the micelles, which then deposit as a dirty scum.

These problems have been alleviated by the development of synthetic amphiphiles called detergents (or syndets). By using a much stronger acid for the polar head group, water solutions of the amphiphile are less sensitive to pH changes. Also the sulfonate functions used for virtually all anionic detergents confer greater solubility on micelles incorporating the alkaline earth cations found in hard water. Variations on the amphiphile theme have led to the development of other classes, such as the cationic and nonionic detergents shown above. Cationic detergents often exhibit germicidal properties, and their ability to change surface pH has made them useful as fabric softeners and hair conditioners. These versatile chemical "tools" have dramatically transformed the household and personal care cleaning product markets over the past fifty years

Contributors and Attributions

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- *The Basics of General, Organic, and Biological Chemistry* by David W. Ball, John W. Hill, and Rhonda J. Scott.

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3.7: Steroids

Learning Objectives

- To identify the functions of steroids produced in mammals.

All the lipids discussed so far are *saponifiable*, reacting with aqueous alkali to yield simpler components, such as glycerol, fatty acids, amino alcohols, and sugars. Lipid samples extracted from cellular material, however, also contain a small but important fraction that does not react with alkali. The most important nonsaponifiable lipids are the steroids. These compounds include the *bile salts*, *cholesterol* and related compounds, and certain *hormones* (such as cortisone and the sex hormones).

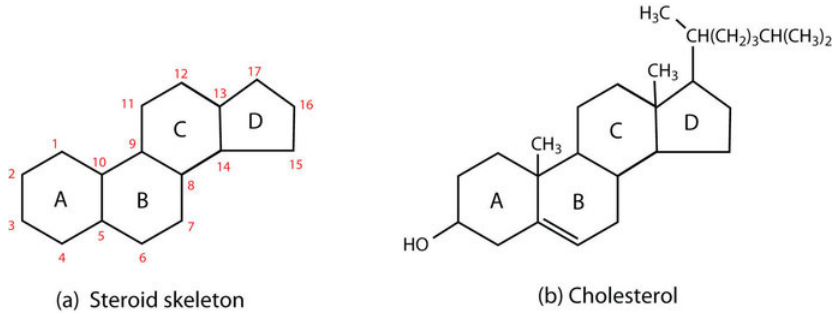


Figure 3.7.1 Steroids. (a) The four-fused-ring steroid skeleton uses letter designations for each ring and the numbering of the carbon atoms. (b) The cholesterol molecule follows this pattern.

Steroids occur in plants, animals, yeasts, and molds but not in bacteria. They may exist in free form or combined with fatty acids or carbohydrates. All steroids have a characteristic structural component consisting of four fused rings. Chemists identify the rings by capital letters and number the carbon atoms as shown in Figure 3.7.1*a*. Slight variations in this structure or in the atoms or groups attached to it produce profound differences in biological activity.

Cholesterol

Cholesterol (Figure 3.7.1*b*) does not occur in plants, but it is the most abundant steroid in the human body (240 g is a typical amount). Excess cholesterol is believed to be a primary factor in the development of atherosclerosis and heart disease, which are major health problems in the United States today. About half of the body's cholesterol is interspersed in the lipid bilayer of cell membranes. Much of the rest is converted to cholic acid, which is used in the formation of bile salts. Cholesterol is also a precursor in the synthesis of sex hormones, adrenal hormones, and vitamin D.

Excess cholesterol not metabolized by the body is released from the liver and transported by the blood to the gallbladder. Normally, it stays in solution there until being secreted into the intestine (as a component of bile) to be eliminated. Sometimes, however, cholesterol in the gallbladder precipitates in the form of gallstones (Figure 3.7.2). Indeed, the name *cholesterol* is derived from the Greek *chole*, meaning "bile," and *stereos*, meaning "solid."



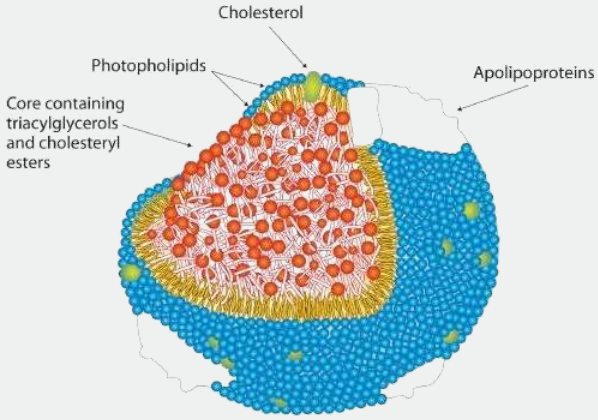
Figure 3.7.2: Numerous small gallstones made up largely of cholesterol, all removed in one patient. Grid scale 1 mm

To Your Health: Cholesterol and Heart Disease

Heart disease is the leading cause of death in the United States for both men and women. The Centers for Disease Control and Prevention reported that heart disease claimed 631,636 lives in the United States (26% of all reported deaths) in 2006.

Scientists agree that elevated cholesterol levels in the blood, as well as high blood pressure, obesity, diabetes, and cigarette smoking, are associated with an increased risk of heart disease. A long-term investigation by the National Institutes of Health showed that among men ages 30 to 49, the incidence of heart disease was five times greater for those whose cholesterol levels were above 260 mg/100 mL of serum than for those with cholesterol levels of 200 mg/100 mL or less. The cholesterol content of blood varies considerably with age, diet, and sex. Young adults average about 170 mg of cholesterol per 100 mL of blood, whereas males at age 55 may have cholesterol levels at 250 mg/100 mL or higher because the rate of cholesterol breakdown decreases with age. Females tend to have lower blood cholesterol levels than males.

To understand the link between heart disease and cholesterol levels, it is important to understand how cholesterol and other lipids are transported in the body. Lipids, such as cholesterol, are not soluble in water and therefore cannot be transported in the blood (an aqueous medium) unless they are complexed with proteins that are soluble in water, forming assemblages called *lipoproteins*. Lipoproteins are classified according to their density, which is dependent on the relative amounts of protein and lipid they contain. Lipids are less dense than proteins, so lipoproteins containing a greater proportion of lipid are less dense than those containing a greater proportion of protein.



Research on cholesterol and its role in heart disease has focused on serum levels of low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs). One of the most fascinating discoveries is that high levels of HDLs reduce a person's risk of developing heart disease, whereas high levels of LDLs increase that risk. Thus the serum LDL:HDL ratio is a better predictor of heart disease risk than the overall level of serum cholesterol. Persons who, because of hereditary or dietary factors, have high LDL:HDL ratios in their blood have a higher incidence of heart disease.

How do HDLs reduce the risk of developing heart disease? No one knows for sure, but one role of HDLs appears to be the transport of excess cholesterol to the liver, where it can be metabolized. Therefore, HDLs aid in removing cholesterol from blood and from the smooth muscle cells of the arterial wall.

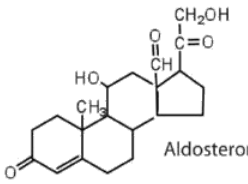
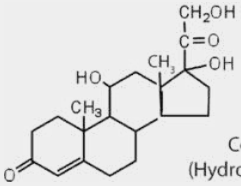
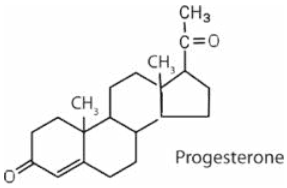
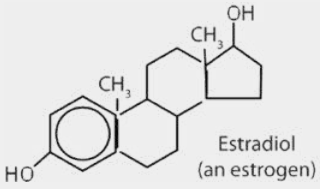
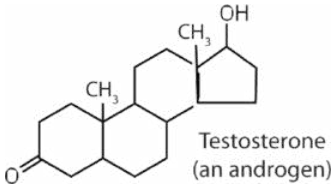
Dietary modifications and increased physical activity can help lower total cholesterol and improve the LDL:HDL ratio. The average American consumes about 600 mg of cholesterol from animal products each day and also synthesizes approximately 1 g of cholesterol each day, mostly in the liver. The amount of cholesterol synthesized is controlled by the cholesterol level in the blood; when the blood cholesterol level exceeds 150 mg/100 mL, the rate of cholesterol biosynthesis is halved. Hence, if cholesterol is present in the diet, a feedback mechanism suppresses its synthesis in the liver. However, the ratio of suppression is not a 1:1 ratio; the reduction in biosynthesis does not equal the amount of cholesterol ingested. Thus, dietary substitutions of unsaturated fat for saturated fat, as well as a reduction in consumption of *trans* fatty acids, is recommended to help lower serum cholesterol and the risk of heart disease.

Steroid Hormones

Hormones are chemical messengers that are released in one tissue and transported through the circulatory system to one or more other tissues. One group of hormones is known as steroid hormones because these hormones are synthesized from cholesterol, which is also a steroid. There are two main groups of steroid hormones: adrenocortical hormones and sex hormones.

The adrenocortical hormones, such as aldosterone and cortisol (Table **3.7.1**), are produced by the adrenal gland, which is located adjacent to each kidney. Aldosterone acts on most cells in the body, but it is particularly effective at enhancing the rate of reabsorption of sodium ions in the kidney tubules and increasing the secretion of potassium ions and/or hydrogen ions by the tubules. Because the concentration of sodium ions is the major factor influencing water retention in tissues, aldosterone promotes water retention and reduces urine output. Cortisol regulates several key metabolic reactions (for example, increasing glucose production and mobilizing fatty acids and amino acids). It also inhibits the inflammatory response of tissue to injury or stress. Cortisol and its analogs are therefore used pharmacologically as immunosuppressants after transplant operations and in the treatment of severe skin allergies and autoimmune diseases, such as rheumatoid arthritis.

Table **3.7.1**: Representative Steroid Hormones and Their Physiological Effects

Hormone	Effect
 Aldosterone	regulates salt metabolism; stimulates kidneys to retain sodium and excrete potassium
 Cortisol (Hydrocortisone)	stimulates the conversion of proteins to carbohydrates
 Progesterone	regulates the menstrual cycle; maintains pregnancy
 Estradiol (an estrogen)	stimulates female sex characteristics; regulates changes during the menstrual cycle
 Testosterone (an androgen)	stimulates and maintains male sex characteristics

The sex hormones are a class of steroid hormones secreted by the gonads (ovaries or testes), the placenta, and the adrenal glands. Testosterone and androstenedione are the primary male sex hormones, or *androgens*, controlling the primary sexual characteristics of males, or the development of the male genital organs and the continuous production of sperm. Androgens are also responsible for the development of secondary male characteristics, such as facial hair, deep voice, and muscle strength. Two kinds of sex

hormones are of particular importance in females: progesterone, which prepares the uterus for pregnancy and prevents the further release of eggs from the ovaries during pregnancy, and the estrogens, which are mainly responsible for the development of female secondary sexual characteristics, such as breast development and increased deposition of fat tissue in the breasts, the buttocks, and the thighs. Both males and females produce androgens and estrogens, differing in the amounts of secreted hormones rather than in the presence or absence of one or the other.

Sex hormones, both natural and synthetic, are sometimes used therapeutically. For example, a woman who has had her ovaries removed may be given female hormones to compensate. Some of the earliest chemical compounds employed in cancer chemotherapy were sex hormones. For example, estrogens are one treatment option for prostate cancer because they block the release and activity of testosterone. Testosterone enhances prostate cancer growth. Sex hormones are also administered in preparation for sex-change operations, to promote the development of the proper secondary sexual characteristics. Oral contraceptives are synthetic derivatives of the female sex hormones; they work by preventing ovulation.

Bile Salts

Bile is a yellowish green liquid (pH 7.8–8.6) produced in the liver. The most important constituents of bile are bile salts, which are sodium salts of amidelike combinations of bile acids, such as cholic acid (part (a) of Figure **3.7.3**) and an amine such as the amino acid glycine (part (b) of Figure **3.7.3**). They are synthesized from cholesterol in the liver, stored in the gallbladder, and then secreted in bile into the small intestine. In the gallbladder, the composition of bile gradually changes as water is absorbed and the other components become more concentrated.

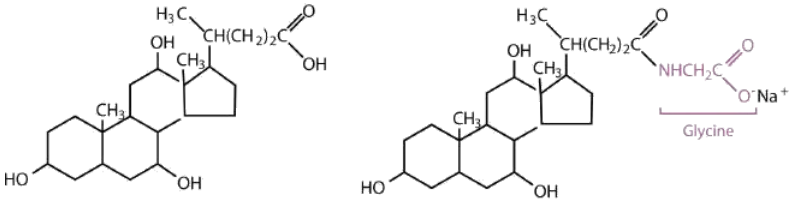


Figure **3.7.3** Bile Acids. (a) Cholic acid is an example of a bile acid. (b) Sodium glycocholate is a bile salt synthesized from cholic acid and glycine.

Because they contain both hydrophobic and hydrophilic groups, bile salts are highly effective detergents and emulsifying agents; they break down large fat globules into smaller ones and keep those smaller globules suspended in the aqueous digestive environment. Enzymes can then hydrolyze fat molecules more efficiently. Thus, the major function of bile salts is to aid in the digestion of dietary lipids.

> Surgical removal is often advised for a gallbladder that becomes infected, inflamed, or perforated. This surgery does not seriously affect digestion because bile is still produced by the liver, but the liver's bile is more dilute and its secretion into the small intestine is not as closely tied to the arrival of food.

Summary

Steroids have a four-fused-ring structure and have a variety of functions. Cholesterol is a steroid found in mammals that is needed for the formation of cell membranes, bile acids, and several hormones. Bile salts are secreted into the small intestine to aid in the digestion of fats.

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3.8: Prostaglandins and other Eicosanoids

Learning Objectives

After completing this section, you should be able to

1. describe the general structure of the prostaglandins, and identify a prostaglandin from a given list of organic structures.
2. identify at least two important biological functions of prostaglandins.

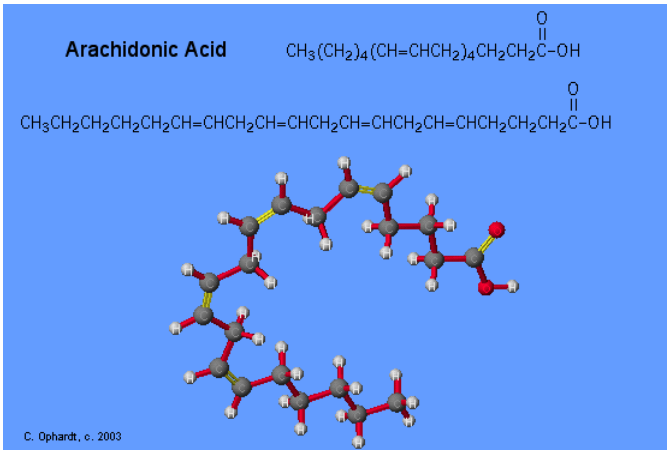
Key Terms

Make certain that you can define, and use in context, the key term below.

- prostaglandin

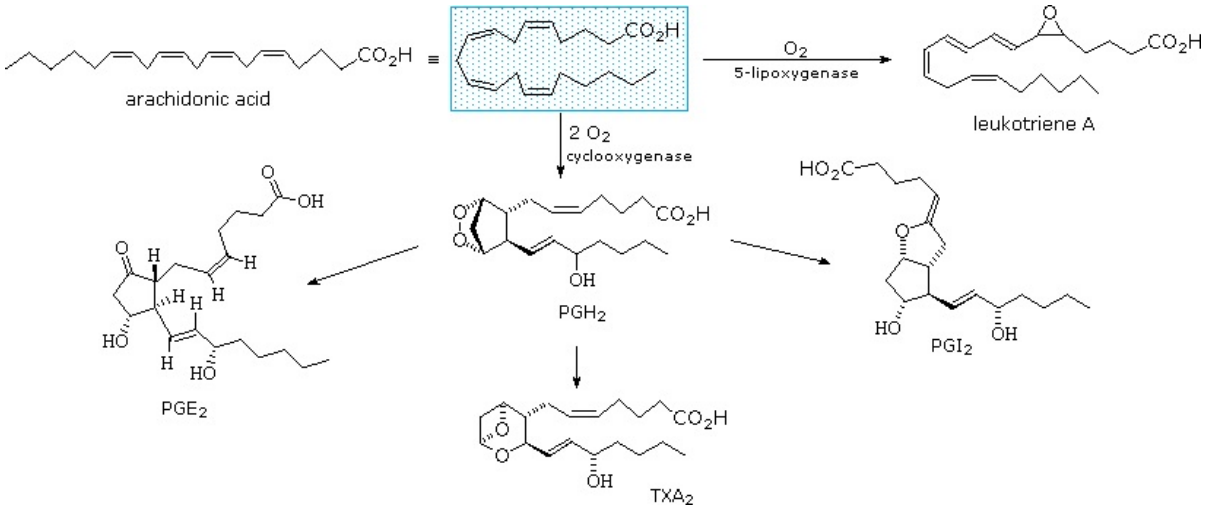
Prostaglandin Structure

Prostaglandins are chemical messengers synthesized in the cells in which their physiological activity is expressed. They are unsaturated fatty acids containing 20 carbon atoms and are synthesized from arachidonic acid—a polyunsaturated fatty acid—when needed by a particular cell. They are called *prostaglandins* because they were originally isolated from semen found in the prostate gland. The unique shape of the arachidonic acid caused by a series of cis double bonds helps to put it into position to make the five member ring of the prostaglandin.



Prostaglandins are one example of biologically important class of fatty acids called eicosanoids. Derived primarily from arachidonic acid (5,8,11,14-eicosatetraenoic acid), eicosanoids include prostaglandins, leukotrienes, and thromboxanes. It is now known that they are synthesized in nearly all mammalian tissues and affect almost all organs in the body. The five major classes of prostaglandins are designated as PGA, PGB, PGE, PGF, and PGI. Subscripts are attached at the end of these abbreviations to denote the number of double bonds outside the five-carbon ring in a given prostaglandin.

The metabolic pathways by which arachidonic acid is converted to the various prostaglandins and eicosanoids are complex and will not be discussed here. A rough outline of some of the transformations that take place is provided below. It is helpful to view arachadonic acid in the coiled conformation shown in the shaded box.



Functions of Prostaglandins

Prostaglandins are chemical messengers (hormones) synthesized in the cells in which their physiological activity is expressed. The prostaglandins are among the most potent biological substances known. Slight structural differences give them highly distinct biological effects; however, all prostaglandins exhibit some ability to induce smooth muscle contraction, lower blood pressure, and contribute to the inflammatory response. Aspirin and other nonsteroidal anti-inflammatory agents, such as ibuprofen, obstruct the synthesis of prostaglandins by inhibiting cyclooxygenase, the enzyme needed for the initial step in the conversion of arachidonic acid to prostaglandins.

There are a variety of physiological effects for prostaglandins, including:

1. Activation of the inflammatory response, production of pain, and fever. When tissues are damaged, white blood cells flood to the site to try to minimize tissue destruction. Prostaglandins are produced as a result.
2. Blood clots form when a blood vessel is damaged. A type of prostaglandin called thromboxane stimulates constriction and clotting of platelets. Conversely, PGI2, is produced to have the opposite effect on the walls of blood vessels where clots should not be forming.
3. Certain prostaglandins are involved with the induction of labor and other reproductive processes. PGE2 causes uterine contractions and has been used to induce labor.
4. Prostaglandins are involved in several other organs such as the gastrointestinal tract (inhibit acid synthesis and increase secretion of protective mucus), increase blood flow in kidneys, and leukotriens promote constriction of bronchi associated with asthma.

Contributors and Attributions

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CHAPTER OVERVIEW

4: Amino Acids and Proteins

4.1: Properties of Amino Acids
4.2: Reactions of Amino Acids
4.3: Peptides
4.4: Proteins
4.5: Classification of Proteins
4.6: Hydrolysis of Proteins
4.7: Protein Purification
4.8: Electrophoresis

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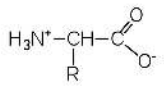
4.1: Properties of Amino Acids

Learning Objectives

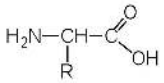
- To recognize amino acids and classify them based on the characteristics of their side chains.

The proteins in all living species, from bacteria to humans, are constructed from the same set of 20 amino acids, so called because each contains an amino group attached to a carboxylic acid. The amino acids in proteins are α-amino acids, which means the amino group is attached to the α-carbon of the carboxylic acid. Humans can synthesize only about half of the needed amino acids; the remainder must be obtained from the diet and are known as essential amino acids. However, two additional amino acids have been found in limited quantities in proteins: Selenocysteine was discovered in 1986, while pyrrolysine was discovered in 2002.

The amino acids are colorless, nonvolatile, crystalline solids, melting and decomposing at temperatures above 200°C. These melting temperatures are more like those of inorganic salts than those of amines or organic acids and indicate that the structures of the amino acids in the solid state and in neutral solution are best represented as having both a negatively charged group and a positively charged group. Such a species is known as a zwitterion.



α-Amino acid drawn as a zwitterion

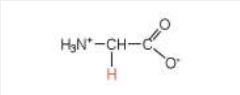
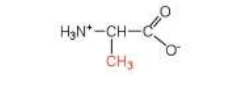
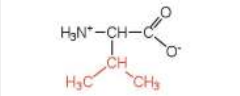
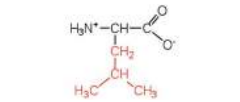
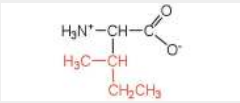
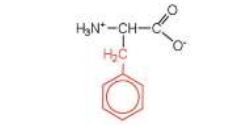
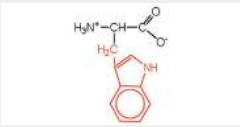
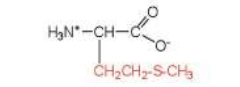
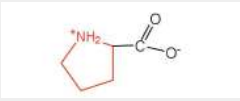
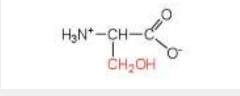
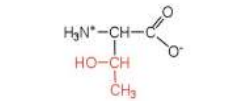
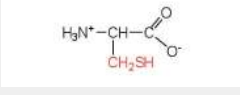


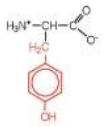
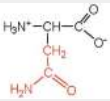
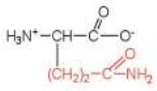
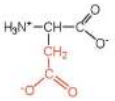
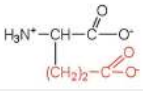
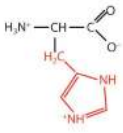
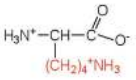
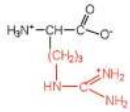
α-Amino acid drawn as an uncharged molecule; not an accurate respresentation of amino acid structure

Classification

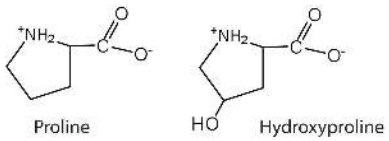
In addition to the amino and carboxyl groups, amino acids have a side chain or R group attached to the α-carbon. Each amino acid has unique characteristics arising from the size, shape, solubility, and ionization properties of its R group. As a result, the side chains of amino acids exert a profound effect on the structure and biological activity of proteins. Although amino acids can be classified in various ways, one common approach is to classify them according to whether the functional group on the side chain at neutral pH is nonpolar, polar but uncharged, negatively charged, or positively charged. The structures and names of the 20 amino acids, their one- and three-letter abbreviations, and some of their distinctive features are given in Table **4.1.1**.

Table **4.1.1**: Common Amino Acids Found in Proteins

Common Name	Abbreviation	Structural Formula (at pH 6)	Molar Mass	Distinctive Feature
Amino acids with a nonpolar R group				
glycine	gly (G)		75	the only amino acid lacking a chiral carbon
alanine	ala (A)		89	—
valine	val (V)		117	a branched-chain amino acid
leucine	leu (L)		131	a branched-chain amino acid
isoleucine	ile (I)		131	an essential amino acid because most animals cannot synthesize branched-chain amino acids
phenylalanine	phe (F)		165	also classified as an aromatic amino acid
tryptophan	trp (W)		204	also classified as an aromatic amino acid
methionine	met (M)		149	side chain functions as a methyl group donor
proline	pro (P)		115	contains a secondary amine group; referred to as an *α-imino acid*
Amino acids with a polar but neutral R group				
serine	ser (S)		105	found at the active site of many enzymes
threonine	thr (T)		119	named for its similarity to the sugar threose
cysteine	cys (C)		121	oxidation of two cysteine molecules yields *cystine*

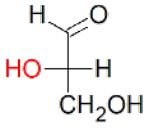
Common Name	Abbreviation	Structural Formula (at pH 6)	Molar Mass	Distinctive Feature
tyrosine	tyr (Y)		181	also classified as an aromatic amino acid
asparagine	asn (N)		132	the amide of aspartic acid
glutamine	gln (Q)		146	the amide of glutamic acid
Amino acids with a negatively charged R group				
aspartic acid	asp (D)		132	carboxyl groups are ionized at physiological pH; also known as aspartate
glutamic acid	glu (E)		146	carboxyl groups are ionized at physiological pH; also known as glutamate
Amino acids with a positively charged R group				
histidine	his (H)		155	the only amino acid whose R group has a pK_a (6.0) near physiological pH
lysine	lys (K)		147	—
arginine	arg (R)		175	almost as strong a base as sodium hydroxide

The first amino acid to be isolated was asparagine in 1806. It was obtained from protein found in asparagus juice (hence the name). Glycine, the major amino acid found in gelatin, was named for its sweet taste (Greek *glykys*, meaning “sweet”). In some cases an amino acid found in a protein is actually a derivative of one of the common 20 amino acids (one such derivative is hydroxyproline). The modification occurs *after* the amino acid has been assembled into a protein.

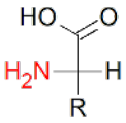


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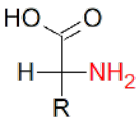
Notice in Table **4.1.1** that glycine is the only amino acid whose α-carbon is *not* chiral. Therefore, with the exception of glycine, the amino acids could theoretically exist in either the D- or the L-enantiomeric form and rotate plane-polarized light. As with sugars, chemists used L-glyceraldehyde as the reference compound for the assignment of absolute configuration to amino acids. Its structure closely resembles an amino acid structure except that in the latter, an amino group takes the place of the OH group on the chiral carbon of the L-glyceraldehyde and a carboxylic acid replaces the aldehyde. Modern stereochemistry assignments using the Cahn-Ingold-Prelog priority rules used ubiquitously in chemistry show that all of the naturally occurring chiral amino acids are S except Cys which is R.



L-Glyceraldehyde



L-Amino acid



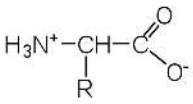
D-Amino acid

We learned that all naturally occurring sugars belong to the D series. It is interesting, therefore, that nearly all known plant and animal proteins are composed entirely of L-amino acids. However, certain bacteria contain D-amino acids in their cell walls, and several antibiotics (e.g., actinomycin D and the gramicidins) contain varying amounts of D-leucine, D-phenylalanine, and D-valine.

Summary

Amino acids can be classified based on the characteristics of their distinctive side chains as nonpolar, polar but uncharged, negatively charged, or positively charged. The amino acids found in proteins are L-amino acids.

Concept Review Exercises

1. What is the general structure of an α-amino acid?
2. Identify the amino acid that fits each description.
    a. also known as aspartate
    b. almost as strong a base as sodium hydroxide
    c. does not have a chiral carbon
3. 

4. a. aspartic acid
    b. arginine
    c. glycine
5. Write the side chain of each amino acid.
    a. serine
    b. arginine
    c. phenylalanine
6. Write the side chain of each amino acid.
    a. aspartic acid
    b. methionine
    c. valine
7. Draw the structure for each amino acid.

a. alanine
b. cysteine
c. histidine

8. Draw the structure for each amino acid.

a. threonine
b. glutamic acid
c. leucine

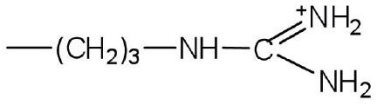
9. Identify an amino acid whose side chain contains a(n)

a. amide functional group.
b. aromatic ring.
c. carboxyl group.

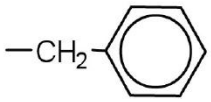
10. Identify an amino acid whose side chain contains a(n)

a. OH group
b. branched chain
c. amino group

11. a. CH_2OH^-
b.

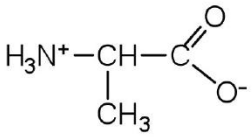


c.

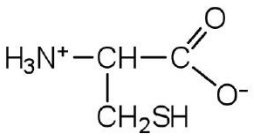


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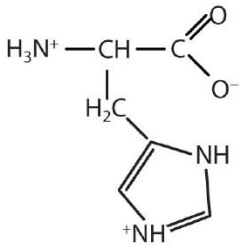
13. a.



b.



c.



14.

15. a. asparagine or glutamine
    b. phenylalanine, tyrosine, or tryptophan
    c. aspartic acid or glutamic acid

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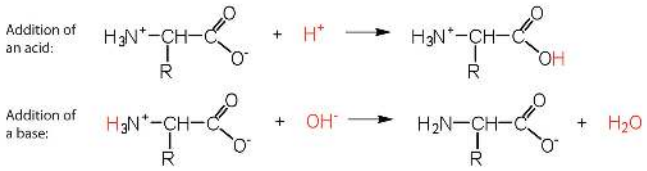
4.2: Reactions of Amino Acids

Learning Objectives

- To explain how an amino acid can act as both an acid and a base.

Acid-Base properties

The structure of an amino acid allows it to act as both an acid and a base. An amino acid has this ability because at a certain pH value (different for each amino acid) nearly all the amino acid molecules exist as zwitterions. If acid is added to a solution containing the zwitterion, the carboxylate group captures a hydrogen (H^+) ion, and the amino acid becomes positively charged. If base is added, ion removal of the H^+ ion from the amino group of the zwitterion produces a negatively charged amino acid. In both circumstances, the amino acid acts to maintain the pH of the system—that is, to remove the added acid (H^+) or base (OH^-) from solution.

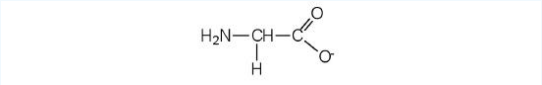


Example 4.2.1

a. Draw the structure for the anion formed when glycine (at neutral pH) reacts with a base.
b. Draw the structure for the cation formed when glycine (at neutral pH) reacts with an acid.

Solution

a. The base removes H^+ from the protonated amine group.



b. The acid adds H^+ to the carboxylate group.



Exercise 4.2.1

a. Draw the structure for the cation formed when valine (at neutral pH) reacts with an acid.
b. Draw the structure for the anion formed when valine (at neutral pH) reacts with a base.

The particular pH at which a given amino acid exists in solution as a zwitterion is called the **isoelectric point** (pI). At its pI, the positive and negative charges on the amino acid balance, and the molecule as a whole is electrically neutral. The amino acids whose side chains are always neutral have isoelectric points ranging from 5.0 to 6.5. The basic amino acids (which have positively charged side chains at neutral pH) have relatively high pI. Acidic amino acids (which have negatively charged side chains at neutral pH) have quite low pI (Table **4.2.1**).

Table **4.2.1**: ExampIes of Some Representative Amino Acids

Amino Acid	Classification	pI
alanine	nonpolar	6.0
valine	nonpolar	6.0
serine	polar, uncharged	5.7
threonine	polar, uncharged	6.5
arginine	positively charged (basic)	10.8
histidine	positively charged (basic)	7.6
lysine	positively charged (basic)	9.8
aspartic acid	negatively charged (acidic)	3.0
glutamic acid	negatively charged (acidic)	3.2

Peptide formation

Amino acids undergo reactions characteristic of carboxylic acids and amines. When the carboxyl group of one amino acid molecule reacts with the amino group of the other amino acid molecule, an amide bond is formed, causing the release of a molecule of water (H_2O). When the amide bond involves two amino acids, it is called a **peptide bond or peptide linkage**.



Reaction between amino acids forming a peptide bond. Image by V8rik at English Wikipedia, Public domain, via Wikimedia Commons.

The formation of peptide bonds s is particularly important in linking amino acids together to form peptides and proteins, as you will see later in this chapter.

Simple chemical tests that are used to detect amino acids take advantage of the reactivity of these functional groups present in amino acids. An example is the ninhydrin test in which the amine functional group of α-amino acids reacts with ninhydrin to form purple-colored compounds. Ninhydrin is used to detect fingerprints because it reacts with amino acids from the proteins in skin cells transferred to the surface by the individual leaving the fingerprint.



Ninhydrin

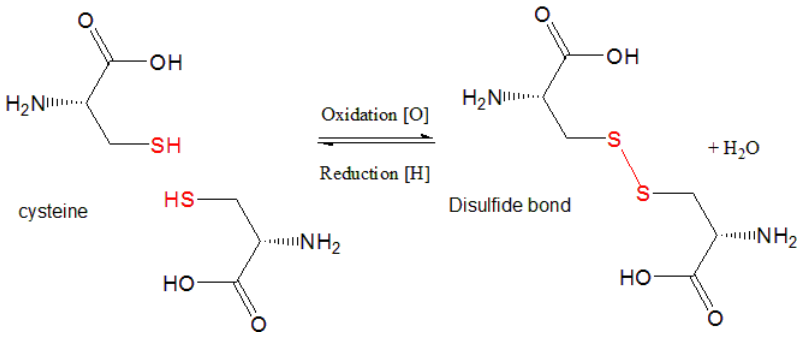




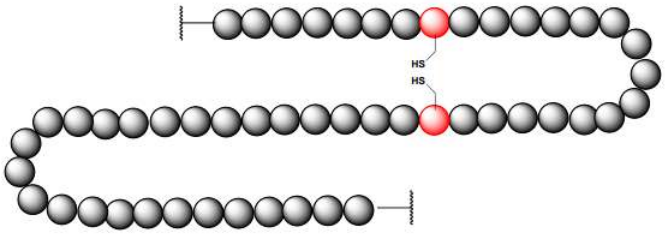
Reaction between ninhydrin and amino acids to generate the ninhydrin chromophore. Image by !Original: Puppy8800Vector: Puppy8800, Public domain, via Wikimedia Commons

Oxidation of Cysteine

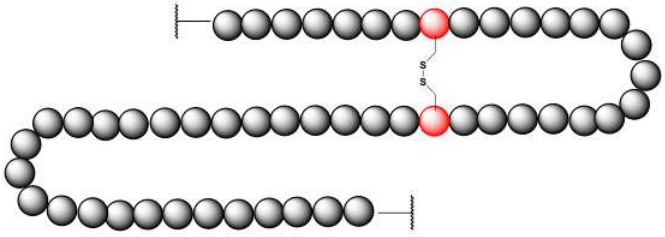
Oxidation of the side chain of cysteine, which contains a sulfhydryl group (-SH), forms a disulfide (-S-S-) bridge



This reaction is very important in determining the structure of many proteins and peptides.



reduced protein (free cysteines)



oxidized protein (disulfide)

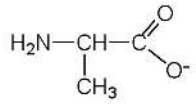
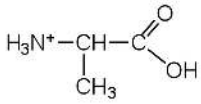
Summary

Amino acids can act as both an acid and a base due to the presence of the amino and carboxyl functional groups. The pH at which a given amino acid exists in solution as a zwitterion is called the *isoelectric point* (pI). The oxidation of cysteine to form disulfide bonds is very important in structural biology.

Concept Review Exercises

1. Define each term.
    a. zwitterion
    b. isoelectric point
2. Draw the structure for the anion formed when alanine (at neutral pH) reacts with a base.
3. Draw the structure for the cation formed when alanine (at neutral pH) reacts with an acid.

Answers

1. a. an electrically neutral compound that contains both negatively and positively charged groups
   b. the pH at which a given amino acid exists in solution as a zwitterion
2. 
3. 

Exercises

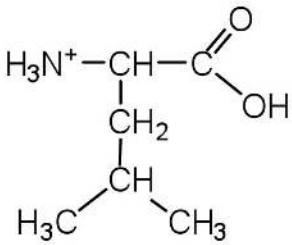
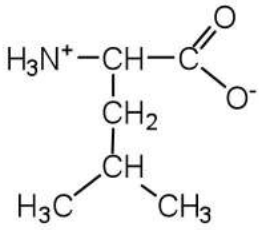
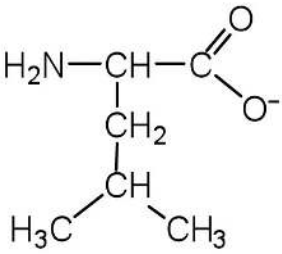
1. Draw the structure of leucine and determine the charge on the molecule in a(n)

   a. acidic solution (pH = 1).
   b. neutral solution (pH = 7).
   c. a basic solution (pH = 11)

2. Draw the structure of isoleucine and determine the charge on the molecule in a(n)

   a. acidic solution (pH = 1).
   b. neutral solution (pH = 7).
   c. basic solution (pH = 11).

Answer

1. a. 
   b. 
   c. 

Contributors

Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

Wikipedia contributors. (2021, January 11). Peptide bond. In *Wikipedia, The Free Encyclopedia*. Retrieved 16:47, May 11, 2021, from https://en.wikipedia.org/w/index.php?title=Peptide_bond&oldid=999704521

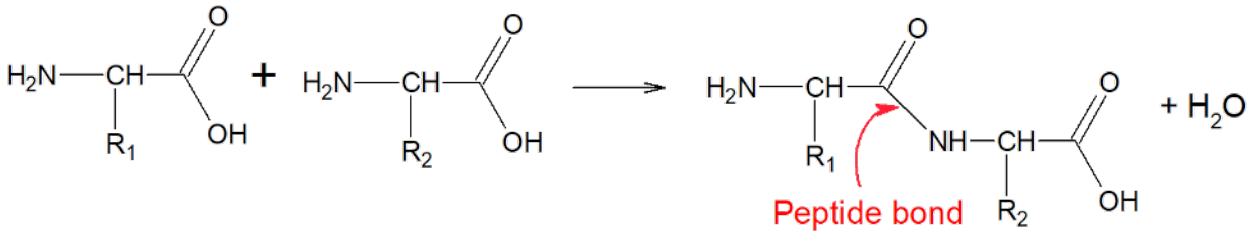
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4.3: Peptides

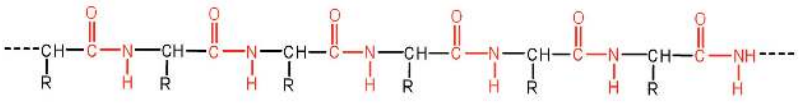
Learning Objectives

- Explain how a peptide is formed from individual amino acids.
- Explain why the sequence of amino acids in a protein is important.

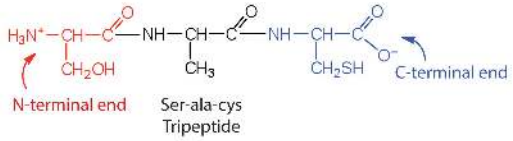
Two or more amino acids can join together into chains called peptides. Previously, we discussed the reaction between ammonia and a carboxylic acid to form an amide. In a similar reaction, the amino group on one amino acid molecule reacts with the carboxyl group on another, releasing a molecule of water and forming an amide linkage:



An amide bond joining two amino acid units is called a peptide bond. Note that the product molecule still has a reactive amino group on the left and a reactive carboxyl group on the right. These can react with additional amino acids to lengthen the peptide. The process can continue until thousands of units have joined, resulting in large proteins.



A chain consisting of only two amino acid units is called a *dipeptide*; a chain consisting of three is a *tripeptide*. By convention, peptide and protein structures are depicted with the amino acid whose amino group is free (the N-terminal end) on the left and the amino acid with a free carboxyl group (the C-terminal end) to the right. Notice that a proton transfer also can occur between the C-terminal and the N-terminal resulting in the corresponding $-NH_3^+$ and $-COO^-$ charged groups:



The general term *peptide* refers to an amino acid chain of unspecified length. However, chains of about 50 amino acids or more are usually called proteins or polypeptides. In its physiologically active form, a protein may be composed of one or more polypeptide chains.

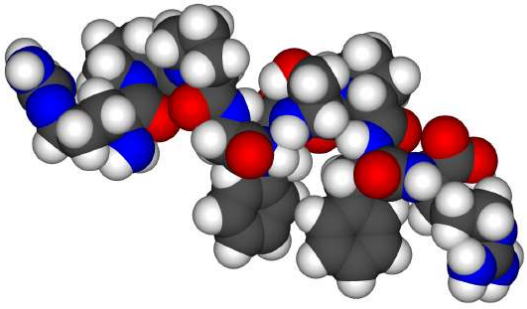


Figure **4.3.1**: Space-filling model of bradykinin. (Public Domain; Fvasconcellos)

For peptides and proteins to be physiologically active, it is not enough that they incorporate certain amounts of specific amino acids. The order, or *sequence*, in which the amino acids are connected is also of critical importance. Bradykinin is a nine-amino acid peptide (Figure **4.3.1**) produced in the blood that has the following amino acid sequence:

arg-pro-pro-gly-phe-ser-pro-phe-arg

This peptide lowers blood pressure, stimulates smooth muscle tissue, increases capillary permeability, and causes pain. When the order of amino acids in bradykinin is reversed,

arg-phe-pro-ser-phe-gly-pro-pro-arg

the peptide resulting from this synthesis shows none of the activity of bradykinin.

Just as millions of different words are spelled with our 26-letter English alphabet, millions of different proteins are made with the 20 common amino acids. However, just as the English alphabet can be used to write gibberish, amino acids can be put together in the *wrong sequence* to produce nonfunctional proteins. Although the correct sequence is ordinarily of utmost importance, it is not always absolutely required. Just as you can sometimes make sense of incorrectly spelled English words, a protein with a small percentage of “incorrect” amino acids may continue to function. However, it rarely functions as well as a protein having the correct sequence. There are also instances in which seemingly minor errors of sequence have disastrous effects. For example, in some people, every molecule of hemoglobin (a protein in the blood that transports oxygen) has a single incorrect amino acid unit out of about 300 (a single valine replaces a glutamic acid). That “minor” error is responsible for sickle cell anemia, an inherited condition that usually is fatal.

Important peptides

Vasopressin

Vasopressin is released from the pituitary gland and causes the kidneys to reabsorb solute-free water, thus regulating the tonicity of the body fluids. Vasopressin may also raise blood pressure by inducing moderate vasoconstriction.



Vasopressin peptide. Notice the disulfide bond. Image by Edgar181, Public domain, via Wikimedia Commons

Oxytocin

Oxytocin is a peptide hormone and neuropeptide produced in the hypothalamus and released by the posterior pituitary. It plays a role in social bonding, reproduction, childbirth, and lactation. Oxytocin is released into the bloodstream as a hormone in response to love and during labor. This helps with uterus contraction during childbirth and milk production by stimulating the mammary glands. Oxytocin is used to induce labor.



Oxytocin. Notice the disulfide bond. Image by Edgar181, Public domain, via Wikimedia Commons

Summary

The amino group of one amino acid can react with the carboxyl group on another amino acid to form a peptide bond that links the two amino acids together. Additional amino acids can be added on through the formation of addition peptide (amide) bonds. A sequence of amino acids in a peptide or protein is written with the N-terminal amino acid first and the C-terminal amino acid at the end (writing left to right).

Concept Review Exercises

1. Distinguish between the N-terminal amino acid and the C-terminal amino acid of a peptide or protein.
2. Describe the difference between an amino acid and a peptide.

3. Amino acid units in a protein are connected by peptide bonds. What is another name for the functional group linking the amino acids?

Answers

1. The N-terminal end is the end of a peptide or protein whose amino group is free (not involved in the formation of a peptide bond), while the C-terminal end has a free carboxyl group.
2. A peptide is composed of two or more amino acids. Amino acids are the building blocks of peptides.
3. amide bond

Exercises

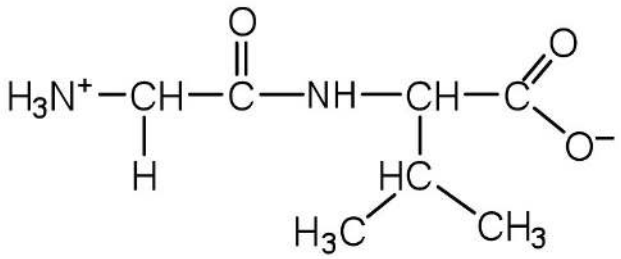
1. Draw the structure for each peptide.

    a. gly-val
    b. val-gly

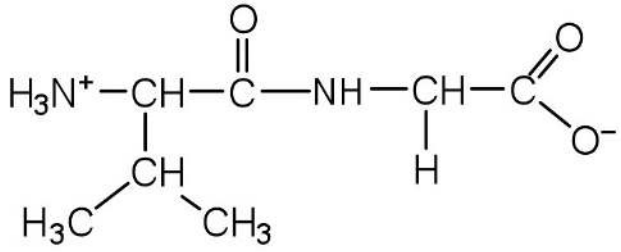
2. Draw the structure for cys-val-ala.
3. Identify the C- and N-terminal amino acids for the peptide lys-val-phe-gly-arg-cys.
4. Identify the C- and N-terminal amino acids for the peptide asp-arg-val-tyr-ile-his-pro-phe.

Answers

1. a.



    b.



3. C-terminal amino acid: cys; N-terminal amino acid: lys

Citations

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4.4: Proteins

Learning Objectives

- Describe the four levels of protein structure.
- Identify the types of attractive interactions that hold proteins in their most stable three-dimensional structure.
- Explain what happens when proteins are denatured.
- Identify how a protein can be denatured.

Each of the thousands of naturally occurring proteins has its own characteristic amino acid composition and sequence that result in a unique three-dimensional shape. Since the 1950s, scientists have determined the amino acid sequences and three-dimensional conformation of numerous proteins and thus obtained important clues on how each protein performs its specific function in the body.

Proteins are compounds of high molar mass consisting largely or entirely of chains of amino acids. Because of their great complexity, protein molecules cannot be classified on the basis of specific structural similarities, as carbohydrates and lipids are categorized. The two major structural classifications of proteins are based on far more general qualities: whether the protein is (1) fiberlike and insoluble or (2) globular and soluble. Some proteins, such as those that compose hair, skin, muscles, and connective tissue, are fiberlike. These fibrous proteins are insoluble in water and usually serve structural, connective, and protective functions. Examples of fibrous proteins are keratins, collagens, myosins, and elastins. Hair and the outer layer of skin are composed of keratin. Connective tissues contain collagen. Myosins are muscle proteins and are capable of contraction and extension. Elastins are found in ligaments and the elastic tissue of artery walls.

Globular proteins, the other major class, are soluble in aqueous media. In these proteins, the chains are folded so that the molecule as a whole is roughly spherical. Familiar examples include egg albumin from egg whites and serum albumin in blood. Serum albumin plays a major role in transporting fatty acids and maintaining a proper balance of osmotic pressures in the body. Hemoglobin and myoglobin, which are important for binding oxygen, are also globular proteins.

Levels of Protein Structure

The structure of proteins is generally described as having four organizational levels. The first of these is the primary structure, which is the number and sequence of amino acids in a protein's polypeptide chain or chains, beginning with the free amino group and maintained by the peptide bonds connecting each amino acid to the next. The primary structure of insulin, composed of 51 amino acids, is shown in Figure **4.4.1**.

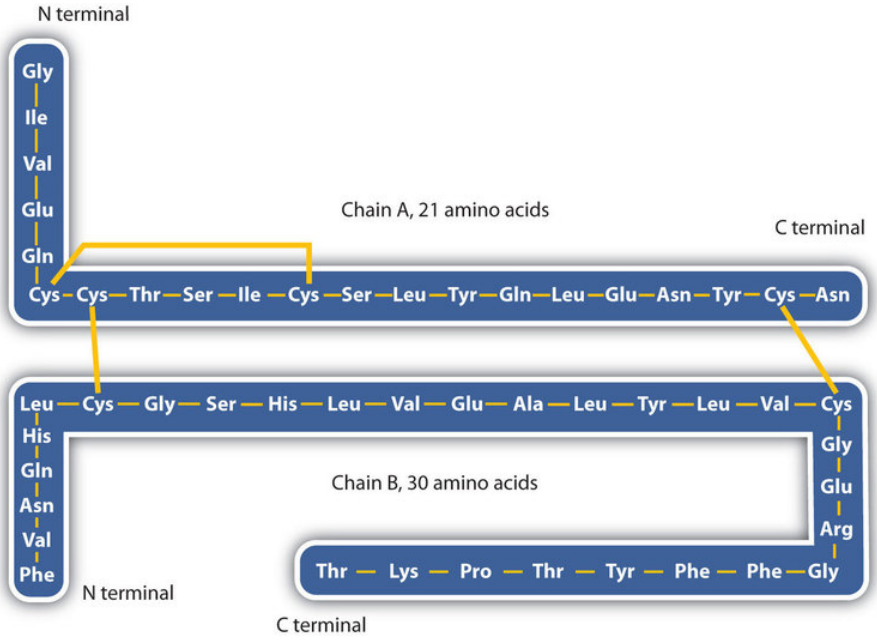


Figure **4.4.1**: Primary Structure of Human Insulin. Human insulin, whose amino acid sequence is shown here, is a hormone that is required for the proper metabolism of glucose.

A protein molecule is not a random tangle of polypeptide chains. Instead, the chains are arranged in unique but specific conformations. The term secondary structure refers to the fixed arrangement of the polypeptide backbone. On the basis of X ray studies, Linus Pauling and Robert Corey postulated that certain proteins or portions of proteins twist into a spiral or a helix. This helix is stabilized by *intrachain* hydrogen bonding between the carbonyl oxygen atom of one amino acid and the amide hydrogen atom four amino acids up the chain (located on the next turn of the helix) and is known as a right-handed α-helix. X ray data indicate that this helix makes one turn for every 3.6 amino acids, and the side chains of these amino acids project outward from the coiled backbone (Figure **4.4.2**). The α-keratins, found in hair and wool, are exclusively α-helical in conformation. Some proteins, such as gamma globulin, chymotrypsin, and cytochrome c, have little or no helical structure. Others, such as hemoglobin and myoglobin, are helical in certain regions but not in others.

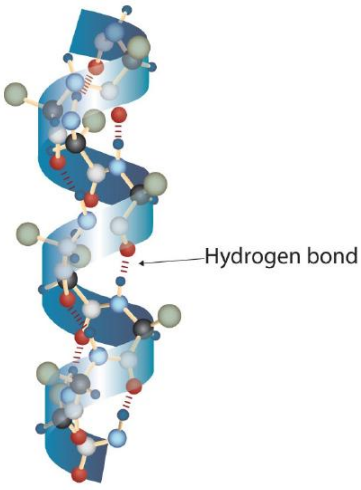


Figure **4.4.2** A Ball-and-Stick Model of an α-Helix. This ball-and-stick model shows the intrachain hydrogen bonding between carbonyl oxygen atoms and amide hydrogen atoms. Each turn of the helix spans 3.6 amino acids. Note that the side chains (represented as green spheres) point out from the helix.

Another common type of secondary structure, called the *β-pleated sheet conformation*, is a sheetlike arrangement in which two or more extended polypeptide chains (or separate regions on the same chain) are aligned side by side. The aligned segments can run either parallel or antiparallel—that is, the N-terminals can face in the same direction on adjacent chains or in different directions—

and are connected by *interchain* hydrogen bonding (Figure **4.4.3**). The β-pleated sheet is particularly important in structural proteins, such as silk fibroin. It is also seen in portions of many enzymes, such as carboxypeptidase A and lysozyme.

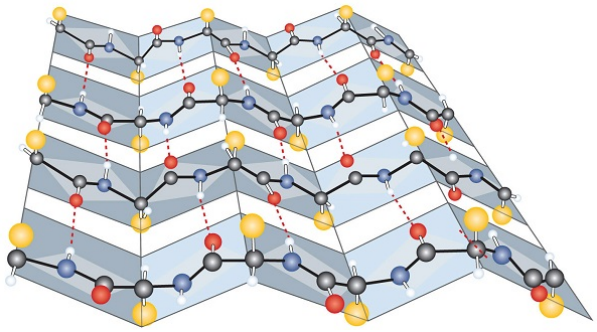


Figure **4.4.3**: A Ball-and-Stick Model of the β-Pleated Sheet Structure in Proteins. The side chains extend above or below the sheet and alternate along the chain. The protein chains are held together by interchain hydrogen bonding.

Tertiary structure refers to the unique three-dimensional shape of the protein as a whole, which results from the folding and bending of the protein backbone. The tertiary structure is intimately tied to the proper biochemical functioning of the protein. Figure **4.4.4** shows a depiction of the three-dimensional structure of insulin.

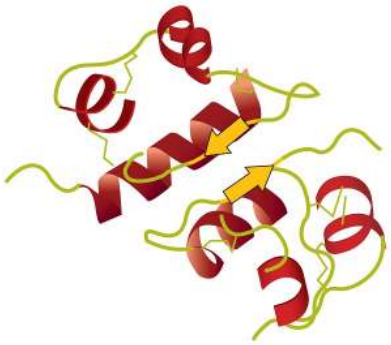
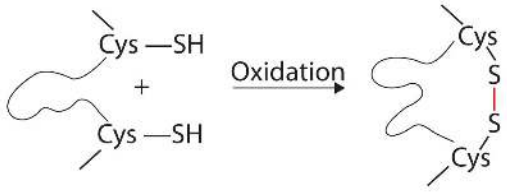


Figure **4.4.4**: A Ribbon Model of the Three-Dimensional Structure of Insulin. The spiral regions represent sections of the polypeptide chain that have an α-helical structure, while the broad arrows represent β-pleated sheet structures.

Four major types of attractive interactions determine the shape and stability of the tertiary structure of proteins. You studied several of them previously.

1. Ionic bonding. Ionic bonds result from electrostatic attractions between positively and negatively charged side chains of amino acids. For example, the mutual attraction between an aspartic acid carboxylate ion and a lysine ammonium ion helps to maintain a particular folded area of a protein (part (a) of Figure **4.4.5**).
2. Hydrogen bonding. Hydrogen bonding forms between a highly electronegative oxygen atom or a nitrogen atom and a hydrogen atom attached to another oxygen atom or a nitrogen atom, such as those found in polar amino acid side chains. Hydrogen bonding (as well as ionic attractions) is extremely important in both the intra- and intermolecular interactions of proteins (part (b) of Figure **4.4.5**).
3. Disulfide linkages. Two cysteine amino acid units may be brought close together as the protein molecule folds. Subsequent oxidation and linkage of the sulfur atoms in the highly reactive sulfhydryl (SH) groups leads to the formation of cystine (part (c) of Figure **4.4.5**). Intrachain disulfide linkages are found in many proteins, including insulin (yellow bars in Figure **4.4.1**) and have a strong stabilizing effect on the tertiary structure.



4. Dispersion forces. Dispersion forces arise when a normally nonpolar atom becomes momentarily polar due to an uneven distribution of electrons, leading to an instantaneous dipole that induces a shift of electrons in a neighboring nonpolar atom. Dispersion forces are weak but can be important when other types of interactions are either missing or minimal (part (d) of Figure **4.4.5**). This is the case with fibroin, the major protein in silk, in which a high proportion of amino acids in the protein have nonpolar side chains. The term *hydrophobic interaction* is often misused as a synonym for dispersion forces. Hydrophobic interactions arise because water molecules engage in hydrogen bonding with other water molecules (or groups in proteins capable of hydrogen bonding). Because nonpolar groups cannot engage in hydrogen bonding, the protein folds in such a way that these groups are buried in the interior part of the protein structure, minimizing their contact with water.

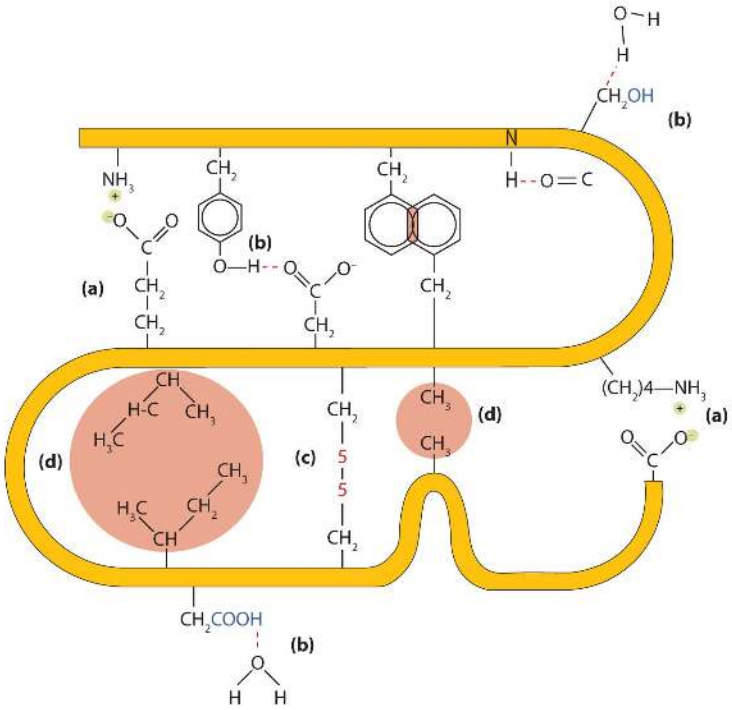


Figure **4.4.5**: Tertiary Protein Structure Interactions. Four interactions stabilize the tertiary structure of a protein: (a) ionic bonding, (b) hydrogen bonding, (c) disulfide linkages, and (d) dispersion forces.

When a protein contains more than one polypeptide chain, each chain is called a *subunit*. The arrangement of multiple subunits represents a fourth level of structure, the quaternary structure of a protein. Hemoglobin, with four polypeptide chains or subunits, is the most frequently cited example of a protein having quaternary structure (Figure **4.4.6**). The quaternary structure of a protein is produced and stabilized by the same kinds of interactions that produce and maintain the tertiary structure. A schematic representation of the four levels of protein structure is in Figure **4.4.7**.

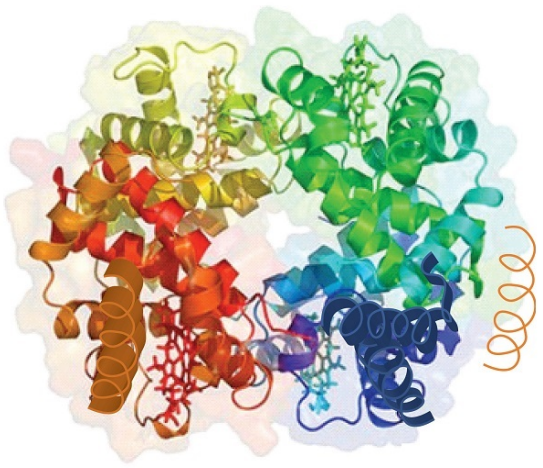


Figure 4.4.6 The Quaternary Structure of Hemoglobin. Hemoglobin is a protein that transports oxygen throughout the body.

Source: Image from the RCSB PDB (www.pdb.org) of PDB ID 1I3D (R.D. Kidd, H.M. Baker, A.J. Mathews, T. Brittain, E.N. Baker (2001) Oligomerization and ligand binding in a homotetrameric hemoglobin: two high-resolution crystal structures of hemoglobin Bart's (gamma(4)), a marker for alpha-thalassemia. Protein Sci. 1739–1749).

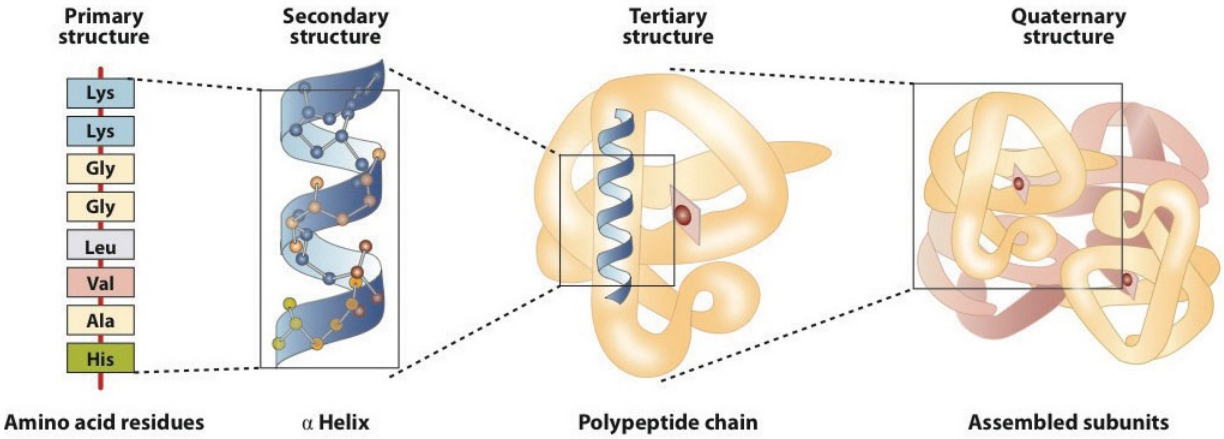


Figure 4.4.7: Levels of Structure in Proteins

The *primary structure* consists of the specific amino acid sequence. The resulting peptide chain can twist into an α-helix, which is one type of *secondary structure*. This helical segment is incorporated into the *tertiary structure* of the folded polypeptide chain. The single polypeptide chain is a subunit that constitutes the *quaternary structure* of a protein, such as hemoglobin that has four polypeptide chains.

Denaturation of Proteins

The highly organized structures of proteins are truly masterworks of chemical architecture. But highly organized structures tend to have a certain delicacy, and this is true of proteins. Denaturation is the term used for any change in the three-dimensional structure of a protein that renders it incapable of performing its assigned function. A denatured protein cannot do its job. (Sometimes denaturation is equated with the precipitation or coagulation of a protein; our definition is a bit broader.) A wide variety of reagents and conditions, such as heat, organic compounds, pH changes, and heavy metal ions can cause protein denaturation (Figure 4.4.1).

Figure 4.4.1: Protein Denaturation Methods

Method	Effect on Protein Structure
Heat above 50°C or ultraviolet (UV) radiation	Heat or UV radiation supplies kinetic energy to protein molecules, causing their atoms to vibrate more rapidly and disrupting relatively weak hydrogen bonding and dispersion forces.
Use of organic compounds, such as ethyl alcohol	These compounds are capable of engaging in intermolecular hydrogen bonding with protein molecules, disrupting intramolecular hydrogen bonding within the protein.
Salts of heavy metal ions, such as mercury, silver, and lead	These ions form strong bonds with the carboxylate anions of the acidic amino acids or SH groups of cysteine, disrupting ionic bonds and disulfide linkages.
pH changes	pH changes affect the protonation state of amino acid residues, disrupting ionic bonds.

Anyone who has fried an egg has observed denaturation. The clear egg white turns opaque as the albumin denatures and coagulates. No one has yet reversed that process. However, given the proper circumstances and enough time, a protein that has unfolded under sufficiently gentle conditions can refold and may again exhibit biological activity (Figure 4.4.8). Such evidence suggests that, at least for these proteins, the primary structure determines the secondary and tertiary structure. A given sequence of amino acids seems to adopt its particular three-dimensional arrangement naturally if conditions are right.

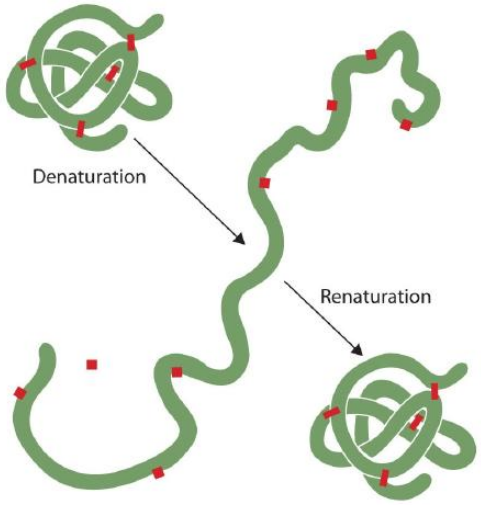


Figure 4.4.8: Denaturation and Renaturation of a Protein. The denaturation (unfolding) and renaturation (refolding) of a protein is depicted. The red boxes represent stabilizing interactions, such as disulfide linkages, hydrogen bonding, and/or ionic bonds.

The primary structures of proteins are quite sturdy. In general, fairly vigorous conditions are needed to hydrolyze peptide bonds. At the secondary through quaternary levels, however, proteins are quite vulnerable to attack, though they vary in their vulnerability to denaturation. The delicately folded globular proteins are much easier to denature than are the tough, fibrous proteins of hair and skin.

Summary

Proteins can be divided into two categories: fibrous, which tend to be insoluble in water, and globular, which are more soluble in water. A protein may have up to four levels of structure. The primary structure consists of the specific amino acid sequence. The resulting peptide chain can form an α-helix or β-pleated sheet (or local structures not as easily categorized), which is known as secondary structure. These segments of secondary structure are incorporated into the tertiary structure of the folded polypeptide chain. The quaternary structure describes the arrangements of subunits in a protein that contains more than one subunit. Four major types of attractive interactions determine the shape and stability of the folded protein: ionic bonding, hydrogen bonding, disulfide linkages, and dispersion forces. A wide variety of reagents and conditions can cause a protein to unfold or denature.

Concept Review Exercises

1. What is the predominant attractive force that stabilizes the formation of secondary structure in proteins?
2. Distinguish between the tertiary and quaternary levels of protein structure.
3. Briefly describe four ways in which a protein could be denatured.

Answers

1. hydrogen bonding
2. Tertiary structure refers to the unique three-dimensional shape of a single polypeptide chain, while quaternary structure describes the interaction between multiple polypeptide chains for proteins that have more than one polypeptide chain.
3. (1) heat a protein above 50°C or expose it to UV radiation; (2) add organic solvents, such as ethyl alcohol, to a protein solution; (3) add salts of heavy metal ions, such as mercury, silver, or lead; and (4) add alkaloid reagents such as tannic acid

Exercises

1. Classify each protein as fibrous or globular.

    a. albumin
    b. myosin
    c. fibroin

2. Classify each protein as fibrous or globular.

    a. hemoglobin
    b. keratin
    c. myoglobin

3. What name is given to the predominant secondary structure found in silk?
4. What name is given to the predominant secondary structure found in wool protein?
5. A protein has a tertiary structure formed by interactions between the side chains of the following pairs of amino acids. For each pair, identify the strongest type of interaction between these amino acids.

    a. aspartic acid and lysine
    b. phenylalanine and alanine
    c. serine and lysine
    d. two cysteines

6. A protein has a tertiary structure formed by interactions between the side chains of the following pairs of amino acids. For each pair, identify the strongest type of interaction between these amino acids.

    a. valine and isoleucine
    b. asparagine and serine
    c. glutamic acid and arginine
    d. tryptophan and methionine

7. What level(s) of protein structure is(are) ordinarily disrupted in denaturation? What level(s) is(are) not?
8. Which class of proteins is more easily denatured—fibrous or globular?

Answers

1. a. globular
    b. fibrous
    c. fibrous

3. β-pleated sheet

5. a. ionic bonding
   b. dispersion forces
   c. dispersion forces
   d. disulfide linkage

7. Protein denaturation disrupts the secondary, tertiary, and quaternary levels of structure. Only primary structure is unaffected by denaturation.

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4.5: Classification of Proteins

The ability to serve a variety of functions is characteristic of most biomolecules. Nowhere is this versatility better exemplified than by the proteins. Perhaps because of their many functions, proteins are the most abundant organic molecules in living cells, constituting more than 50 percent of the mass once water is removed. It is estimated that the human body contains well over a million different kinds of protein, and even a single-cell organism contains thousands. Each of these is a polymer of amino acids which has a highly specific composition, a unique molecular weight (usually in the range from 6000 to 1 000 000) and its own sequence of different amino acids along the polymer chain.

Classification based on composition

Proteins may be divided into two major classes on the basis of their behavior when reacted with water. The products obtained upon hydrolysis of **simple proteins** are all amino acids. In the case of **conjugated proteins** other organic and/or inorganic substances are obtained. The non-amino acid portions of conjugated proteins may consist of metals, lipids, sugars, phosphate, or other types of molecules. These components are referred to as **prosthetic groups**.

Classification based on structural shape

Proteins may also be subdivided on the basis of their molecular shape or conformation. In the **fibrous proteins** long polymer chains are arranged parallel or nearly parallel to one another to give long fibers or sheets. This arrangement results in physically tough materials which do not dissolve in water. The fibrous proteins are fundamental components of structural tissues such as tendons, bone, hair, horn, leather, claws, and feathers.



Collagen, a fibrous, simple protein. Image by © Nevit Dilmen, CC BY-SA 3.0, via Wikimedia Commons

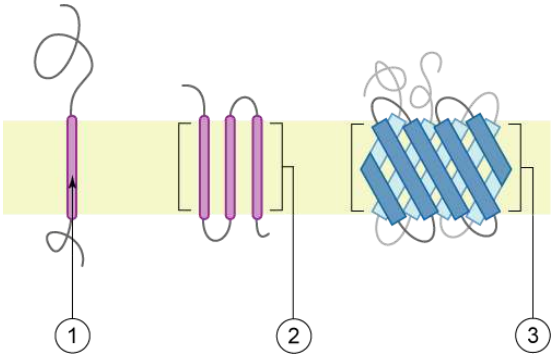
By contrast, polymer chains of the **globular proteins** fold back on themselves to produce compact, nearly spherical shapes. Most globular proteins are water-soluble and hence are relatively mobile within a cell. Some examples are enzymes, antibodies, hormones, toxins, and substances such as hemoglobin whose function is to transport simple molecules or even electrons from one place to another. The enzyme trypsin, is a typical globular protein.



Hemoglobin, a globular, conjugated protein. Note the prosthetic group (heme) in green. Image by Zephyris at the English-language Wikipedia, CC BY-SA 3.0, via Wikimedia Commons

Membrane proteins

Another class of proteins are the **membrane proteins**, which, as the name would suggest, reside in a cell's lipid bilayer membrane. Such proteins can act as channels for ions or other molecules unable to pass through the lipid bilayer; as signal transducers, able to respond to signal molecules on one side of a membrane to begin a molecular response on the other side of the membrane; or as anchors of other molecules to the cell membrane, to name a few exemplars of membrane protein function. Because these proteins interface with non-polar portions of the lipid bilayer, they do no maintain function and structure in an aqueous solution, making them far more difficult to study than globular proteins or fibrous proteins.



Schematic representation of transmembrane proteins: 1. a single transmembrane. 2. a polytopic transmembrane α-helical protein 3. a polytopic transmembrane β-sheet protein
The membrane is represented in light-brown. Image by Foobar, CC BY-SA 3.0, via Wikimedia Commons

Contributors and citations

- Ed Vitz (Kutztown University), John W. Moore (UW-Madison), Justin Shorb (Hope College), Xavier Prat-Resina (University of Minnesota Rochester), Tim Wendorff, and Adam Hahn.
- Wikipedia contributors. (2021, April 30). Membrane protein. In *Wikipedia*, *The Free Encyclopedia*. Retrieved 18:36, May 13, 2021, from https://en.wikipedia.org/w/index.php?title=Membrane_protein&oldid=1020617813

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4.6: Hydrolysis of Proteins

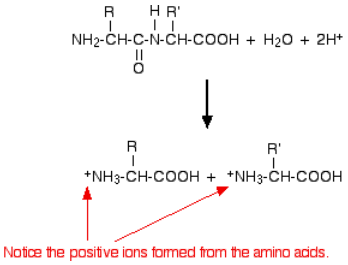
This page looks briefly at the hydrolysis of proteins into their constituent amino acids using hydrochloric acid.

The chemistry of the reaction

If you have already studied the hydrolysis of amides under acidic conditions, you will find that this is basically the same reaction. That's not surprising because what biologists and biochemists call a peptide link (in proteins, for example) is what chemists call an amide link. With an amide like ethanamide, the carbon-nitrogen bond in the amide group is broken and you get a carboxylic acid formed:

$$CH_3CONH_2 + H_2O + H^+ \rightarrow CH_3COOH + NH_4^+ \tag{4.6.1}$$

Now imagine doing the same thing with a simple dipeptide made of any two amino acids.



Instead of ammonium ions, you get positive ions made from the -NH_2 groups reacting with hydrogen ions.

You need the extra hydrogen ion in the equation (compared with the amide equation) to react with the -NH_2 group on the left-hand end of the dipeptide - the one not involved in the peptide link.

If you scale this up to a polypeptide (a protein chain), each of the peptide links will be broken in exactly the same way. That means that you will end up with a mixture of the amino acids that made up the protein - although in the form of their positive ions because of the presence of the hydrogen ions from the hydrochloric acid.

Doing the reaction

There are two ways of carrying out this reaction - an old, slow method, and a new, fast one.

The old slow way

The protein is heated with 6 M hydrochloric acid for about 24 hours at 110°C. (6M hydrochloric acid is slightly more than semi-concentrated.)

The new fast way

Protein samples are placed in tubes in a sealed container containing 6 M hydrochloric acid in an atmosphere of nitrogen. The whole container is then placed in a microwave oven for about 5 - 30 minutes (depending on the protein) with temperatures up to 200°C. The hydrochloric acid vaporizes, comes into contact with the protein samples and hydrolyses them. This method is used to hydrolyse small samples of protein during protein analysis.

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4.7: Protein Purification

Assays, Specific Activity, Initial Fractionation

A successful protein purification procedure can be nothing short of amazing. Whether you are starting off with a recombinant protein which is produced in *E. coli*, or trying to isolate a protein from some mammalian tissue, you are typically starting with gram quantities of a complex mixture of protein, nucleic acids, polysaccharide, etc. from which you may have to extract milligram (or microgram!) quantities of desired protein at high purity, and hopefully with high yield.

The first step in any purification is the development of a *specific assay* for the protein of interest. The specific assay can be based upon some *unique characteristic* of the protein of interest

- Enzymatic activity
- Immunological activity
- Physical characteristics (e.g. molecular mass, spectroscopic properties, etc.)
- Biological activity
- Ideally, an assay should be
  - *Specific* (you don't want a false positive)
  - *rapid* (you don't want to wait a week for the results)
  - *sensitive* (you don't want to consume all your sample in order to assay it)
  - *quantitative* (you need an accurate way to measure the quantity of your protein at each step in the purification)

Western Blotting

Antibodies can be used in a method called Western blotting, which is useful for determining levels of protein expression and for assaying proteins during purification. This method usually involves the following steps:

1. A protein sample is subjected to polyacrylamide gel electrophoresis.
2. After this the gel is placed over a sheet of nitrocellulose and the protein in the gel is electrophoretically transfered to the nitrocellulose.
3. The nitrocellulose is then soaked in gelatin to "block" its ability to non-specifically bind proteins.
4. The nitrocellulose is then incubated with the specific antibody for the protein of interest.
5. The nitrocellulose is then incubated with a second antibody which is specific for the first antibody. For example, if the first antibody was raised in rabbits, the second antibody might be termed "goat anti-rabbit immunoglobulin". What this means is that rabbit immunoglobulins were used to elicit an antibody response in goats. The goat antibodies (polyclonal) will include those which recognize the conserved region in the rabbit antibodies. Since the Fc region is conserved, it will bind to any and all rabbit antibodies, including those on the nitrocellulose paper.
6. The second antibody will typically have a covalently attached enzyme which, when provided with a chromogenic substrate, will cause a color reaction.
7. Thus the molecular weight and amount of the desired protein can be characterized from a complex mixture (e.g. crude cell extract) of other proteins.

In a variation of the above, the protein sample may be blotted directly on a nitrocellulose paper (called a *dot blot*) without first running a gel. This may be desirable if, for example, the antibody is monoclonal and recognizes an epitope which is dependent upon native structure (which would be destroyed upon running an SDS PAGE).

In addition to their varied uses, antibodies can also be used to purify proteins.

- If relatively large amounts of an antibody can be obtained, they can be covalently attached to a chromatography resin (e.g. sephadex beads).
- If a crude cell extract is run over such a column, only the protein of interest should bind, and everything else will flow through.
- The bound protein can then be eluted. This is typically achieved by moderately low pH conditions (using acetic acid). As long as the protein of interest is not irreversibly denatured by such conditions, the method will work quite well.
- One potential pitfall involves that of monoclonal antibodies being utilized to purify mutant proteins. The regions of the protein comprising the epitope cannot be modified without destroying the ability of the antibody to bind. Thus, the use of monoclonal antibodies in a purification scheme may preclude its use in purifying certain mutants.

Protein purification can be thought of as a series of *fractionation steps* designed so that:

- The protein of interest is found almost exclusively in one fraction (and with good yield)
- A significant amount of the contaminants can be found in a different fraction

During purification you will need to monitor several parameters, including:

1. *Total sample volume*
2. *Total sample protein* (can be estimated by A_{280}; 1.4 ~ 1.0 mg/ml)
3. *Units of activity of desired protein* (based on specific assay)

This basic information will allow you to keep track of the following information during each step of purification:

1. *% yield* for each purification step
2. *Specific activity* of the desired protein (units/mg total protein)
3. *Purification enhancement* of each step (e.g. "3.5x purification)

In designing a purification scheme you typically have to balance *purification* with *yield*.

- For example, it may be relatively straightforward to obtain 90% pure material with good yield.
- However, it may be difficult to improve that purity an additional few percentile with good yield.
- *The planned application of the purified protein determines the target purity*.
- If the protein is to be used to determine amino acid sequence information, maybe 90% is acceptable. However, if the material is to be used in clinical trials, 99.99+% may be the target purity.

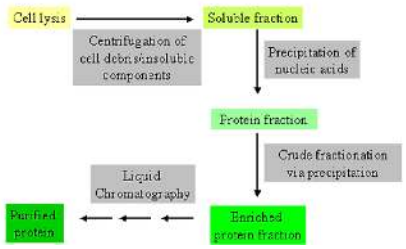


Figure 4.1.1: *Purification steps*

- It is extremely helpful to have some information not only on the general physical and chemical characteristics of the protein you are trying to purify, but also on the *contaminating* components.
- For example, many *E. coli* proteins are generally *low molecular weight* (<50,000 Da) and somewhat *acidic* in isoelectric point

Usually the initial steps in purification make use of general physical and/or chemical differences between soluble proteins and other cell components.

- For example, soluble proteins can be separated from general cellular debris, and intact cells, by *centrifugation*.
- Thus, cells are physically disrupted (via homogenization or a cell press) to allow release of cell contents. This is then followed by centrifugation to separate generally soluble components from those which are insoluble.
- It is at this point that data collection begins in order to monitor the purification.

Nucleic acids can sometimes be readily removed from the sample by the addition of large cationic compounds such as *polyethylene imine*, or *streptomycin sulfate*.

- The nucleic acids bind to these compounds via electrostatic interactions and the complex precipitates and can be removed via centrifugation.
- The same general result can be obtained by mixing in ion exchange resins which are *anion exchangers* (i.e. the resins contain cationic groups) and then filtering or centrifuging to remove. As with either method, it should be confirmed that the desired protein is *not* bound as well.

Crude fractionations of proteins can be achieved by adding various quantitites of precipitants such as *ammonium sulfate*, or *polyethylene glycol* (PEG).

- For this type of purification step an initial experiment is performed to monitor the fraction of overall protein, as well as desired protein, remaining in solution (and pellet) as a function of precipitant concentration.

Ammonium Sulfate (% saturated)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**
Sample A_{280}	1000	900	600	200	100	75	50	40	25	20
Activity assay(units)	200	200	200	190	170	100	30	5	0	0

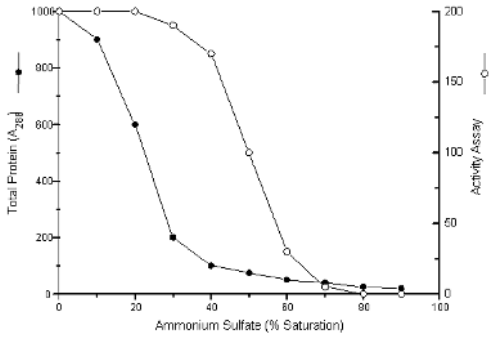


Figure 4.1.2: *Protein activity as a function of precipitant concentration*

- In this particular example we are in luck: at around 30% ammonium sulfate we can precipitate about 80% of the total protein concentration in our sample, yet our activity assay for our desired protein indicates that about 95% of our desired protein is still soluble.
- At 80% ammonium sulfate all of our desired protein has precipitated. Thus, from these results we would do the following:

1. Add ammonium sulfate to our sample to a concentration of 30% saturation
2. Centrifuge and discard the pellet
3. Add ammonium sulfate to 80% saturation
4. Centrifuge and keep the pellet. Resuspend the pellet in buffer to solubilize the protein.

- We would expect about a 5-fold purification with about 95% yield.

Column Chromatography - Ion exchange; Dialysis and Concentration

Column chromatography

After initial fractionation steps the typical procedure is to move to *column chromatography*.

- In column chromatography we have a glass tube (column) which is filled with a material ("resin") which has certain physical/chemical characteristics.
- These characteristics allow it to interact in various ways with different proteins.
- Some common types of chromatographic resins include:

1. Ion exchange
2. Hydrophobic
3. Gel filtration
4. Affinity

Ion exchange

Ion exchange resins contain *charged groups*.

- These may be *acidic* in nature (in which case the resin is a *cation* exchanger)
- or *basic* (in which case it is an *anion* exchanger).
- Cation and anion exchangers may be broken down further into *weak* and *strong* exchangers (reflecting binding affinity).

Type of exchanger	Functional group	Common name
Weak cation exchanger	carboxymethyl	CM cellulose/sephadex
Strong cation exchanger	sulfopropyl	SP sephadex
Weak anion exchanger	diethylaminoethyl	DE cellulose/sephadex
Strong anion exchanger	quaternary amine	QAE sephadex

- Usually, samples are loaded under *low ionic strength* conditions and bound material is eluted using either a step or gradient elution of buffer with *higher ionic strength*.
- Generally speaking, a protein will bind to a cation exchange resin if the buffer pH is *lower than the isoelectric point (pI)* of the protein, and will bind to an anion exchange resin if the pH is *higher than the pI*.

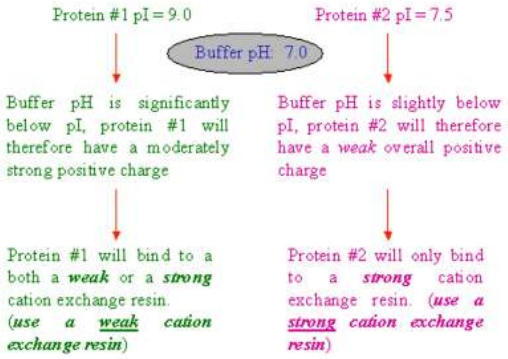


Figure 4.1.3: *Protein binding to resins*

- Knowledge of the pI of the protein is therefore helpful in designing a purification protocol using ion exchange resins (however, you can always simply try different resins to see which works best).

Generally speaking, ion exchange columns are short and fat in dimensions.

Elution of proteins from ion exchange resins

- Proteins bound to ion exchange resins are bound via non-covalent ionic (salt-bridge) interactions. We can compete for these ionic binding sites on the resin with other ionic groups, namely, ***salts***
- There are two general types of methods when eluting with a salt solution: 1. **Gradient** elution and 2. **Step** elution
- A gradient elution refers to a smooth transition of salt concentration (from low to high) in the elution buffer. Weakly binding proteins elute first, and stronger binding proteins elute last (i.e. they require higher salt concentrations in the buffer to compete them off the column)
- A gradient salt concentration can be made using a **gradient maker**. In its simplest form, this consists of two containers (***must be the same shape***) connected by a siphon (or tube at the bottom). One container contains the low salt buffer, and the other contains high salt buffer. The buffer is withdrawn from the low salt container:

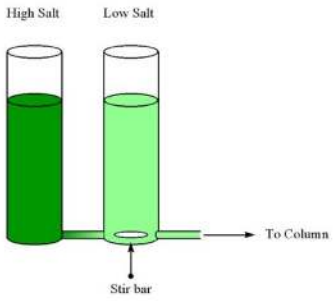


Figure 4.1.4: *Gradient maker*

- This will produce a linear gradient from low to high salt concentrations over the total volume of the gradient

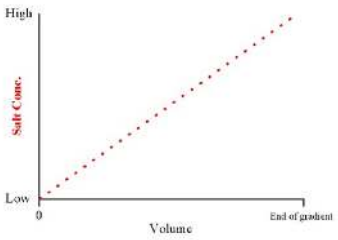


Figure 4.1.5: *Salt concentration and volume*

- If we know the concentration range of salt over which a protein of interest will elute we can simply elute with a buffer containing that concentration of salt. This is known as a **step elution**.
- Step elutions are generally faster to run, and elute the protein in a smaller overall volume than with gradient elutions. They generally work best when contaminants elute at a significantly different salt concentration than the protein of interest

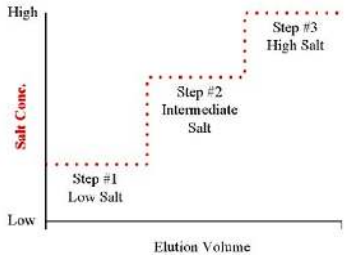


Figure 4.1.6: *Step elution*

Note that after ion exchange chromatography the protein of interest will be in a buffer with a potentially high salt concentration. This must be taken into account before proceeding with the next step in the purification scheme

Dialysis

- After an ammonium sulfate precipitation step, or an ion exchange chromatography step, the protein of interest may be in a high salt buffer. This may be undesirable for several reasons. How do we get rid of salt in our sample?
- One of the most common methods is that of **dialysis**
- The method of dialysis makes use of **semi-permeable membranes**. In the simplest example, this membrane is manufactured in the form of tubing (looking much like a sausage casing)
- The main feature of this membrane is that it is porous. However, the pore size is such that while small salt ions can freely pass through the membrane, larger protein molecules cannot (i.e. they are retained). Thus, dialysis membranes are characterized by the molecular mass of the smallest typical globular protein which it will retain.
- This is commonly referred to as the **cutoff** of the tubing (e.g. Spectrapore #6 dialysis tubing has a cutoff of 1,000 Daltons, meaning that a 1,000 Dalton protein will be retained by the tubing but that smaller molecular mass solutes will pass through the tubing)
- Dialysis proceeds by placing a high salt sample in dialysis tubing (i.e. the dialysis "bag") and putting it into the desired low salt buffer:

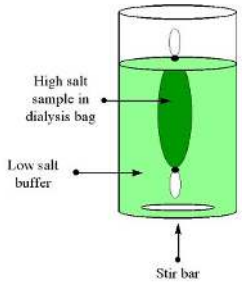


Figure 4.1.7: *Dialysis*

- Over time the concentration of low molecular mass solutes within the bag, and in the low salt buffer, will come achieve equilibrium. In practical terms (for the above case) salt molecules will diffuse out of the bag into the low salt buffer:

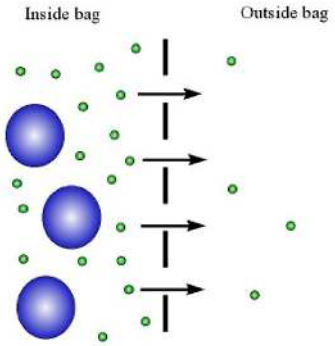


Figure 4.1.8: *Salt diffusion*

- At equilibrium the salt concentration of the sample can be calculated as follows:

$$\frac{(\textit{sample volume}) \times (\textit{sample salt concentration}) + (\textit{buffer volume}) \times (\textit{buffer salt concentration})}{\textit{total volume}} = \textit{final salt concentration} \quad (4.7.1)$$

Note

Often the buffer salt concentration is 0 M

- The buffer volume for the dialysis is a function of the required final concentration of salt in the sample

Example 4.1.1:

Dialysis example

We have a 10ml protein sample from an ion exchange column elution pool which contains 1.0M NaCl. For our next step in the purification we can have no more than 1mM NaCl in the sample.

$$\frac{(\text{sample vol})*(\text{sample salt conc}) + (\text{buffer vol})*(\text{buffer salt conc})}{\text{total vol}} = \text{final salt conc}$$

$$\frac{(0.01\text{L})*(1.0\text{M}) + (\text{buffer vol})*(0)}{\text{total vol}} = 0.001\text{M}$$

$$\frac{(0.01\text{L})*(1.0\text{M})}{0.001\text{ M}} = \text{total vol}$$

$$10\text{ L} = \text{total vol}$$

Therefore, the required buffer volume would be (total vol - sample vol) = 9.990 L (or ~ 10 L)

- Thus, if we dialyzed 10mls of sample (with 1.0M NaCl conc) in 10 L of water after equilibrium the NaCl concentration in the sample would be 1.0 mM.

- Note that in the above example this would commonly be referred to as a "1:1,000" dialysis.
- Suppose that we don't want to make up 10 L of buffer? We can actually achieve the same results with two sequential "1:32" dialyses (i.e. the square root of the 1:1,000 dialysis - in other words, two sequential 1:32 dialyses is equivalent to a single 1:1,000 dialysis):

First dialysis versus 310 ml of buffer: sample NaCl conc will be (10*1.0)/(320) = 31 mM

Second dialysis versus 310 ml of buffer: sample NaCl conc will be (10*0.031)/(320) = 0.97 mM

Thus, instead of making 10 L of buffer, we could make only 620 ml and achieve the same results with two dialysis steps

- In this case, removing the salt would take twice as long, i.e. we need to perform two dialysis steps. How long does dialysis take?

A useful rule of thumb is that for most types of dialysis tubing the dialysis is 80% compete after four hours

- One consequence of dialysis to watch out for is that while salt ions are moving out of the bag, water molecules are moving into the bag. Thus the volume of sample may actually increase (the bag will swell) and, therefore, the protein concentration will decrease
- In the extreme case, the bag may actually swell to the point of rupture. Therefore, *it is a good idea not to fill the bag completely, but leave a void to allow for potential swelling*.

Concentration

- What if our protein sample is too dilute for our needs? How can we concentrate our samples?
- One common method is, again, to use a semi-permeable membrane for this purpose.
- A very simple method is to place our sample in a dialysis bag and coat it with a high molecular weight solute which can readily be dissolved by the buffer.
- For example, polyethylene glycols and polyvinyl pyrolidones can have very large molecular masses (i.e. 20,000 Da). These compounds are also readily dissolved in water. If our sample in a dialysis bag is coated with dry forms of the above polymers, water will leave the dialysis bag (it can go through the pores) and hydrate the polymers. The result is a decrease in volume of buffer in the dialysis bag (the protein will be concentrated).
- In another variation, the semi-permeable membrane is manufactured into a flat disk and placed at the bottom of a container which holds our sample. In one method the container is pressurized and forces buffer out of the container (protein is retained and is concentrated). In another method, the vessel is centrifuged and the centripetal force achieves the same goal as pressure in the previous example.

For both dialysis and concentration, it is essential that the membrane does not interact with the protein (i.e. has no affinity for, and will not bind, the protein)

- With the pressure type concentrators, ***dialysis and concentration can be achieved in tandem***. For example, the sample can be concentrated and then buffer added to the sample. The sample is then concentrated again. Every time buffer is added the salt concentration is reduced. After repeated cycles of this, the salt concentration is at the desired level and the sample is concentrated to the desired final volume.

Gel Filtration, Affinity and Hydrophobic resins; Preparation of Resin, Plumbing

Gel filtration

Gel filtration does not rely on any chemical interaction with the protein, rather it is based on a **physical** property of the protein - that being the *effective molecular radius* (which relates to mass for most typical globular proteins).

- Gel filtration resin can be thought of as beads which contain pores of a defined size range.
- Large proteins which cannot enter these pores pass around the *outside* of the beads.
- Smaller proteins which can enter the pores of the beads have a longer, tortuous path before they exit the bead.
- Thus, a sample of proteins passing through a gel filtration column will *separate based on molecular size*: the big ones will elute first and the smallest ones will elute last (and "middle" sized proteins will elute in the middle).

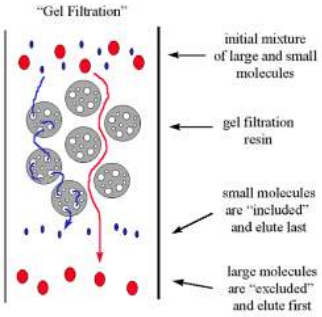


Figure 4.1.9: *Gel filtration*

- If your protein is unusually "small" or "large"*in comparison to contaminating proteins* then gel filtration may work quite well.

Where will a protein elute in a gel filtration experiment?

- There are two extremes in the separation profile of a gel filtration column.
- There is a critical molecular mass (large mass) which will be ***completely excluded*** from the gel filtration beads. All solutes in the sample which are equal to, or larger, than this critical size will behave identically: they will all eluted in the excluded volume of the column
- There is a critical molecular mass (small mass) which will be ***completely included*** within the pores of the gel filtration beads. All solutes in the sample which are equal to, or smaller, than this critical size will behave identically: they will all eluted in the included volume of the column
- Solutes between these two ranges of molecular mass will elute between the excluded and included volumes

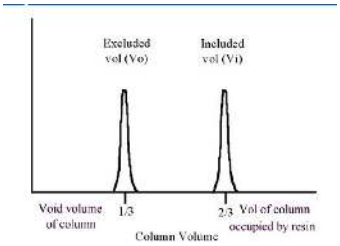


Figure 4.1.10: *Excluded vs. included volume*

As a general rule of thumb, the excluded volume (Vo) is approximately equal to one third of the column volume, the included volume is approximately equal to two thirds of the column volume

- In gel filtration the resolution is a function of column length (the longer the better)
- However, one drawback is related to the maximum sample volume which can be loaded. The larger the volume of sample loaded, the more the overlap between separated peaks. Generally speaking, the sample size one can load is limited to about *3-5% of the total column volume*.
- Thus, gel filtration is best saved for *the end stages of a purification* ,when the sample can be readily concentrated to a small volume.
- Gel filtration can also be used to remove salts from the sample, due to its ability to separate "small" from "large" components.
- Finally, gel filtration can be among the most "gentle" purification methods due to the lack of chemical interaction with the resin.

Affinity chromatography

Affinity chromatography is a general term which applies to a wide range of chromatographic media. It can be basically thought of as some inert resin to which has been attached some compound which has a *specific affinity* for your protein of interest.

- Thus, a specific antibody attached to an inert resin would be a type of affinity chromatography.
- Other examples might include: a protease inhibitor attached to some matrix, designed to bind a specific protease
- a cofactor bound to some matrix, designed to bind to a particular enzyme
- a metal ion bound to a matrix, designed to chelate a protein with a metal binding site, and so on.

In each case, the type of resins used and the method of attachment may vary, as will the method of elution. One generalization regarding method of elution is that the bound ligand can be competed off of the column's functional group by including in the elution buffer a high concentration of the free functional group. For example, if the functional group of the column is a cofactor, then the bound protein can be competed off the column by passing a buffer containing a high concentration of cofactor (or cofactor analog) through the column.

Other methods of elution include changing the buffer conditions such that the protein is no longer in the native state (since it is the native state which confers the structure required for the specific binding interaction). This can be achieved by changing pH or by adding denaturing agents such as urea or guanidine.

With affinity chromatography, typically the purification achieved in a single step can be dramatic - on the order of several thousand fold. Single step purifications with specific affinity columns are not unheard - in fact it is an ideal goal of purification - *a matrix which recognizes only the protein of interest and none other*.

Hydrophobic resins

Hydrophobic resins contain a non-polar functional group, such as an alkane or aromatic group.

- Many proteins are able to sequester such groups on their surface and this exclusion from solvent provides the basis of the binding energy (i.e. the "hydrophobic effect").
- This interaction is enhanced by increasing ionic strength, such that proteins may *bind under high salt conditions* and *elute under low salt conditions*.
- As such these columns may be used to not only provide purification, but to *desalt* samples (for example after an initial ammonium sulfate precipitation).
- It is usually not possible to predict in advance which particular resin will bind a given protein, this is usually determined empirically. However, the longer the alkane, or the larger the aromatic compound, the stronger the binding typically will be.

Due to the nature of hydrophobic interactions and ionic strength, hydrophobic chromatography and ion exchange chromatography can be conveniently used sequentially. For example, after ion exchange the protein is in high salt conditions, thus it can be loaded directly onto a hydrophobic column. Conversely, a hydrophobic column is eluted in low salt, which is a requirement for binding to an ion exchange resin.

A distinction should be noted between hydrophobic interaction chromatorgraphy and reverse phase chromatography

- Hydrophobic interaction chromatography is performed in aqueous solvent conditions and changes in ionic strength are used to elute the column. The protein typically binds in the native state via hydrophobic groups located on the surface of the protein. The native state is retained during the elution conditions
- Reverse phase chromatography utilizes a hydrophobic solvent (typically acetonitrile) and the binding of a ligand is a function of the phase partition between the hydrophobic nature of the solvent and column functional group. Proteins are typically denatured in such solvents and bind due to the hydrophobic nature of the entire polypeptide sequence. Since the majority of hydrophobic groups are located in the core of globular proteins, the binding is related to the denaturation of the protein and the accessibility of these groups to the column functional groups. Proteins can be purified using reverse phase chromatography, but usually must be refolded in some way to regain functionality (i.e. the native state)

Preparation of resins

The steps in preparing a chromatographic resin typically involve:

1. Hydration of resin
2. Decanting fines
3. Equilibrating the resin and preparing a slurry
4. Degassing the slurry

- Resins come either dry or preswollen. If they are dry they need to be hydrated. This is usually accomplished by mixing the dry resin with buffer and letting it hydrate slowly overnight (or faster at higher temperatures
- After the resin has hydrated and settled, very fine particles will settle at the top. These "fines" slow the flow rate of the packed resin. The settled resin is therefore carefully decanted to discard these fines.
- The resin is then equilibrated in the buffer to be used for the analysis. Equilibration usually involves pH'ing the resin, or buffer exchanges. Never use a stir bar when pH'ing the resin (it can mechanically shear the resin and produce fines), rather stir the resin slurry with a stir rod.
- After the equilibrated resin has settled, an equal volume of buffer is added to produce a 50% *slurry* of resin. This is usually "thin" enough to allow air bubbles to escape when packing the column.
- Finally, the slurry is degassed prior to packing the column. This will help minimize the formation of air bubbles.

Packing the column

Low pressure columns are typically packed using gravity.

- Add a small amount of buffer to the bottom of the column.
- Place a *packing reservoir* on the top of the column. Since we will be using a 50% slurry we will have a volume which is 2x the column volume and its best to pour the resin in all at one time. Thus, the packing reservoir should have a volume equal to, or greater, than the column volume.
- Carefully pour the resin slurry into the packing reservoir/column, avoiding the introduction of air bubbles as much as possible
- Let the column sit for about 5 minutes to allow large air bubbles to escape
- Open the column valve at the bottom and allow the column to pack under gravity
- Note the top of the resin bed. It will move down as the column packs. When the column is packed the top of the resin bed will no longer move down.

Plumbing

Chromatography systems may be run using only gravity and a beaker to collect the appropriate fraction. Most common systems, however, will include the following:

- A pump. Usually a peristaltic pump with variable flow rate and a communications port for a controller. *The pump is usually set up to push buffer through the column, rather than sucking buffer out of the column (which can cause a low pressure condition with production of air bubbles)*
- A detector. This is typically a UV (A_{280}) detector. Most detectors are of the two-cell type - meaning that you can have plain buffer as a blank in the detector while analyzing your column fractions. The detector sends the absorbance information to a chart recorder to be displayed (printed)
- A fraction collector. This allows you to collect fractions either by number of drops (~30 per ml) or by time. In conjunction with a controllable pump, time collection translates to volume. The fraction collector will typically have an communications port to output a signal when it changes fractions and to receive commands from the detector/controller on some sophisticated systems.
- A chart recorder. This will print a continuous trace of the detector output and the fraction collector event marker (signalling when a fraction changes). Fractions can also be read individually on a UV spectrophotometer if a chart recorder is unavailable.

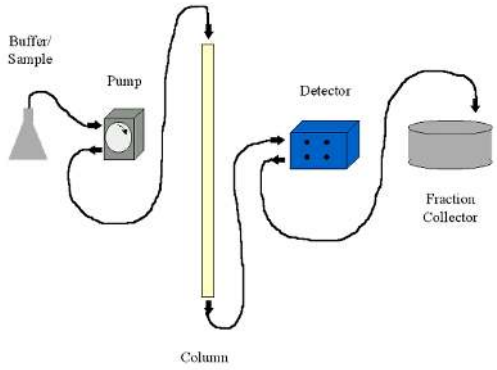


Figure 4.1.11: *Chromatography setup*

- If a gravity system is used, a safety loop should be installed to prevent the column from drying up if the buffer is used up when the column is unattended

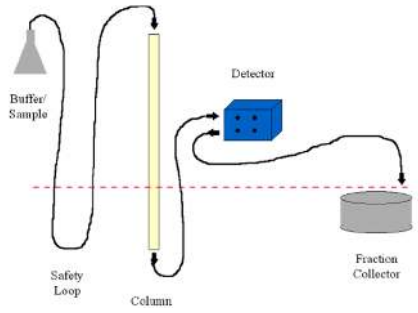


Figure 4.1.12: *Safety loop*

Note that the bottom of the safety loop is lower than the outlet to the fraction collector.

Running the Experiment, Resolving Peaks

The following represents an example of a low pressure liquid chromatography (ion exchange resin) experiment.

Sample:

- Volume = 90 mls
- A_{280} = 1.8
- Total A_{280} = 162

Column:

- DE-52 (diethylaminoethyl cellulose; anion exchange)
- Size = 1.0 x 12.7 cm
- Volume = $\pi \times (radius)^2 \times height$ = 40 mL

Fraction Collector:

- 10 mls / fraction (~300 drops/fraction)

The chromatogram for this experiment looked like this:

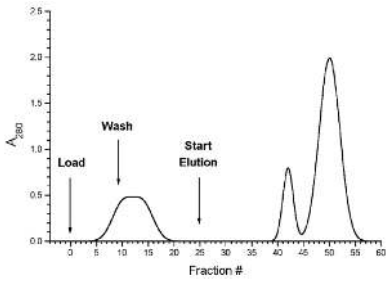


Figure 4.1.13: *Chromatogram*

The following events took place during this chromatography run:

1. Note the tick marks on the chromatogram.

- The "event" marker from the fraction collector notifies the chart recorder when a tube change takes place.
- The experiment begins with the tick next to the '0' on the x-axis ("*tick 0*"); this indicates the start of fraction (tube) number 1.

- The next tick mark ("tick 1") indicates the *end* of fraction number 1, and the *start* of fraction number 2.
- Thus, *fractions span the gap between the tick marks*.

2. The sample loading is begun at tick 0.

3. Sometime during fraction 5 we begin to notice the absorbence of the column effluent increasing

- It has taken about (5 fractions x 10mls per fraction) or *50 mls* from the start of loading until the detector notes any absorbance.
- This compares well with the fact that the column volume is about 40 mls and there is some volume associated with the tubing going in and out of the column.
- Thus, this 'delay' from sample load to sample detection is the *dead volume* of the system

4. Obviously, some material is not binding to the resin during the loading step. This is the *flow-through*. Is this some component of the sample which does not have affinity for the resin, or, does it represent that we have exceeded the capacity of the resin?

- If we have exceeded the capacity of the resin, then the flow-through will have an A_{280} similar to the sample being loaded
- Also, prior to exceeding the capacity, the flow-through will have some characteristic A_{280} which will then transition to another A_{280} (that of the loaded sample), resulting in a *double-plateau* chromatogram.
- In the above experiment the flow-through plateaus around A_{280}=0.5 or about 25% of the absorbance of the load. This would seem to indicate that a component, or component(s), representing one quarter of our sample, does not have affinity for the resin in the column

5. Around fraction 9 we begin to wash the column

- This makes sense because 9 fractions x 10mls per fraction = 90 mls have loaded and this is equivalent to our original sample volume (i.e. all the sample has loaded)
- The column is typically washed using the same buffer conditions in the protein sample

6. Around fraction 14 we note the A_{280} begins to decrease

- This makes sense given that we determined the dead volume of the system to be approximately 50 mls or 5 fractions. Thus, a wash which was begun at fraction 9 is observed to decrease the absorbance around fraction 14
- We continue washing the column until the A_{280} approaches 0 (*baseline*). In other words, all of the non-binding material in the sample has been washed away

7. After the A_{280} comes back down to baseline we begin our *elution* protocol. In this particular experiment we will use a linear gradient of increasing salt (NaCl) concentration (in wash buffer) to compete off the material bound to the ion exchange resin.

8. Our elution has produced two peaks: a small peak centered around fraction 42 and a larger peak centered around fraction 50

- We will have to assay each peak (and the flow through) to find out where our protein of interest has gone
- The two *elution peaks* are fairly well resolved. We could combine fractions 40-44 and call that "peak 1", and combine fractions 46-55 and call that "peak 2".

9. *Is there any material left on the column?* The integrated areas (i.e. summing the A_{280}'s of each fraction in a pool) of the flow-through, peak 1 and peak 2 are as follows

- Flow through: 4
- Peak 1: 2
- Peak 2: 10
- This gives a total integrated area of 16. Each fraction is 10 mls, so this gives a total A_{280} = 16 x 10 = 160 which is quite close to the total A_{280} of our loaded sample.
- In other words, it looks like our chromatogram is accounting for all the components in our original sample.

10. If our protein of interest was actually peak 1 (and if our yield was 100%), then this column has provided an eight fold purification (2 x 10 / 162).

Resolving peaks

- Contaminating peaks will not necessarily be completely separated from the peak which contains our protein of interest
- In the following picture there are two components being resolved, and they are present in equimolar amounts (thus, the starting purity is 50%). The yield and purity are listed for the situation where we were to pool each peak by splitting at the midpoint between them (in this particular example the yield and purity are identical in each case)

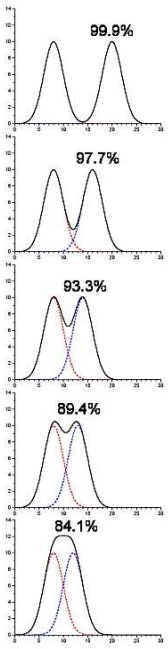


Figure 4.1.14: *Contaminating peaks*

- This gives you some idea of the amount of cross-contamination in each peak as a function of their separation from one another.
- Software to fit gaussians to a chromatogram can provide this type of information

Pooling for purity verses yield

Do we try to pool to maximize yield or to maximize purity?

Usually, you will probably be pooling fractions in such a way as to maximize the recovery of your protein of interest. However, you always have the option of pooling to increase purity, and if you have lots of protein to work with this may allow you to achieve the desired purity with fewer steps. Here's an example of how it's done:

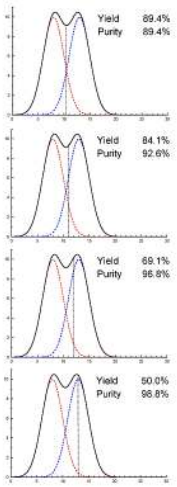


Figure 4.1.15: *Yield vs. purity*

- These are all the same chromatogram, however, we can pool them differently to get better purity (at the expense of yield
- The blue peak is the peak of interest and it is not resolved from a contaminating peak (in red).
- The vertical line represents the left-most fraction we use to pool the peak (we pool all fractions to the right of the vertical line to get our protein of interest)
- In the last panel we see that we can achieve about 98.8% purity if we are willing to part with half our protein!

Monitoring the purification

How do you know when you are finished purifying a protein?

There are several criteria. One criteria is that we cannot improve upon the *specific activity* of our sample. This value refers to the *functional activity of our sample in relationship to the total protein concentration of the sample*.

- In the initial stages of purification this value will be low (not much activity in relationship to the total amount of protein).
- This value will increase after each purification step as we *remove* other proteins from the sample.
- At some point the specific activity will *plateau*, and by definition, *if it is pure we cannot increase the specific activity*.

- There may be a published value for the specific activity which we can compare ours to.

Also, each step of the purification should be monitored by gel electrophoresis.

- In the initial stages of purification we will probably see a variety of bands, of various molecular weights, on our gel.
- After the different purification steps, we should see the disappearance of certain bands concomitant with the increasing concentration of a certain band (or bands) representing our protein.
- If we have successfully purified our protein (and if it is a single polypeptide) we should arrive at a constant specific activity and *a single band on a gel*.
- Analytical methods like HPLC or densitometer scanning of a stained gel can give us a quantitative idea of the purity of our final sample.

The following chart represents the typical data one would monitor during a purification:

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification	% Yield
Crude cell lysate	5500	6600	1.2		
30-70% Ammonium sulfate cut	1020	5910	5.8	4.8	89.5
DEAE Sephadex pool	187	5070	27.1	4.7	85.8
CM Sephadex pool	102	4420	43.3	1.6	87.2
Phenyl Sepharose pool	56	3930	70.2	1.6	88.9
Gel Filtration pool	32	2970	92.8	1.3	75.6
Affinity resin type #1 pool	5.8	2520	434.5	4.7	84.8
Affinity resin type #2 pool	5.3	2390	450.9	1.0	94.8
Total purification	**376**				
Total yield (%)	**36**				

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4.8: Electrophoresis

Electrophoresis is a method of separating charged molecules from one another. In order to do so, a voltage is applied across the sample. Positively charges molecules are attracted to the negative electrode and negatively charged molecules are attracted to the positive electrode.

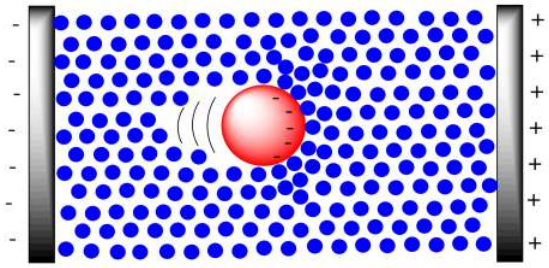


Figure **4.8.1**: The electrical basis of electrophoresis.

Typically, this method is applied for the purification or analysis of very large molecules, such as proteins and DNA. These molecules are charged to begin with, so they make good candidates for movement via electric field. DNA is negatively charged because of the phosphate groups along the backbone. Of course the DNA is accompanied by counterions, but in a buffer solution the DNA molecule itself will act as a large anion, and it will move toward the positive electrode.

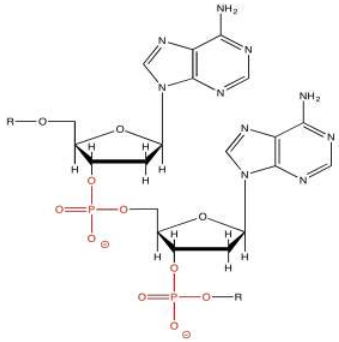


Figure **4.8.2**: The phosphate group on DNA makes it anionic.

Proteins are also charged because they contain charged end groups and side chains. A buffer is chosen to give the protein net negative charge, with more anionic sites than cationic ones. That way, it will also move toward the positive electrode.

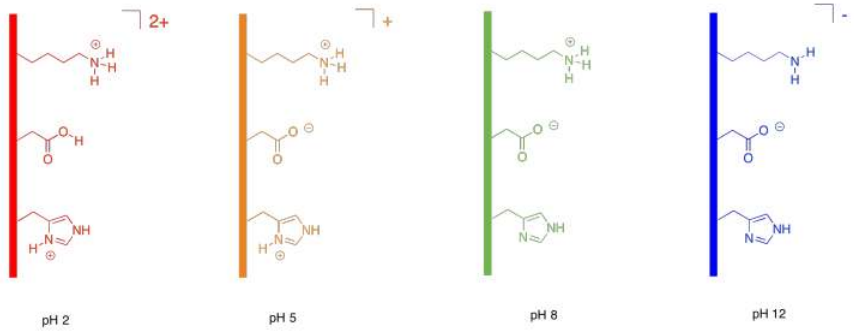


Figure **4.8.3**: The effect of buffer on polar side chains in a protein.

One practical aspect of electrophoresis is that molecules must move through a stationary gel medium. A gel is composed of a solid matrix swollen with liquid. For example, the medium may be based on agar or, in a common method, a polyacrylamide gel (used in PAGE gel electrophoresis), and it is swollen with a buffer solution. The medium is not designed to bind the molecules, but it does exert drag on macromolecules as they move through it. That's true of a large molecule moving through any surroundings; think of all the water molecules that will have to get pushed out of the way to make room for a huge protein as it migrates through. That drag will slow the progress of the sample through the electric field.

The overall result is that the electric field keeps all of these anions moving in the same direction, but the drag from the medium exerts a slowing effect. The drag is really the key part of separation. The larger the molecule, the greater the drag, and the more slowly the molecule moves. In gel electrophoresis, small molecules move the fastest, and large molecules move the slowest.

Setting up electrophoresis is basically similar to chromatography, although a little more complicated. The idea is still like a race between the molecules. We start with all of the molecules at the same starting line and see who is fastest. After a gel is run through electrophoresis, the gel is usually stained with a dye (such as bromothymol blue) so that we can see how far each protein has moved.

Usually a gel is calibrated so that you get some idea of how big the proteins are in your mixture. A mixture with known components of known molecular weights is run alongside the sample that you are analysing. By comparing how far proteins in our mixture moved compared to proteins in the standard mixture, we can estimate the molecular weights of proteins in our own mixture.

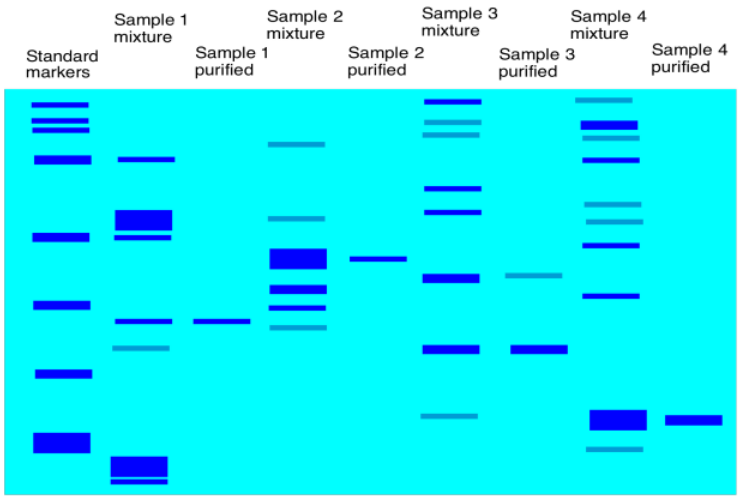


Figure 4.8.4: A cartoon showing a developed gel.

There may be different steps in electrophoresis that are designed to make things go more smoothly. For example, a protein mixture is complicated by the fact that the proteins have different secondary and tertiary structures; they have different shapes. A compact, spherical protein might move more efficiently through a gel than a more open, floppy one. As a result, a heavier protein may move farther on the gel than a lighter one simply because of its shape. To get around this problem, a surfactant (kind of like soap) may be added to the mixture; the surfactant with help to unwind the proteins so that they are all in open chains. The proteins are denatured now, but they are all more similar to each other, and it is easier to compare them based solely on molecular weight or chain length.

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CHAPTER OVERVIEW

5: Enzymes

5.1: Enzymes
5.2: Enzyme Cofactors
5.3: Mechanism of Enzymatic Catalysis
5.4: The Kinetics of Enzymatic Catalysis
5.5: Factos Affecting Enzyme Activity
5.6: Enzyme Inhibition
5.7: Regulation of Enzymatic activity
5.8: Enzymes Used in Industry

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5.1: Enzymes

Learning Objectives

- Explain the functions of enzymes.
- Explain how enzymes are classified and named.

A catalyst is any substance that increases the *rate* or speed of a chemical reaction without being changed or consumed in the reaction. **Enzymes are biological catalysts, and nearly all of them are proteins**. The reaction rates attained by enzymes are truly amazing. In their presence, reactions occur at rates that are a million (10^6) or more times faster than would be attainable in their absence. What is even more amazing is that enzymes perform this function at body temperature (~37°C) and physiological pH (pH ~7), rather than at the conditions that are typically necessary to increase reaction rates (high temperature or pressure, the use of strong oxidizing or reducing agents or strong acids or bases, or a combination of any of these). In addition, enzymes are highly specific in their action; that is, each enzyme catalyzes only one type of reaction in only one compound or a group of structurally related compounds. The compound or compounds on which an enzyme acts are known as its substrates.

Hundreds of enzymes have been purified and studied in an effort to understand how they work so effectively and with such specificity. The resulting knowledge has been used to design drugs that inhibit or activate particular enzymes. An example is the intensive research to improve the treatment of or find a cure for acquired immunodeficiency syndrome (AIDS). AIDS is caused by the human immunodeficiency virus (HIV). Researchers are studying the enzymes produced by this virus and are developing drugs intended to block the action of those enzymes without interfering with enzymes produced by the human body. Several of these drugs have now been approved for use by AIDS patients.

Table 5.1.1: Classes of Enzymes

Class	Type of Reaction Catalyzed	Examples
oxidoreductases	oxidation-reduction reactions	Dehydrogenases catalyze oxidation-reduction reactions involving hydrogen and reductases catalyze reactions in which a substrate is reduced.
transferases	transfer reactions of groups, such as methyl, amino, and acetyl	Transaminases catalyze the transfer of amino group, and kinases catalyze the transfer of a phosphate group.
hydrolases	hydrolysis reactions	Lipases catalyze the hydrolysis of lipids, and proteases catalyze the hydrolysis of proteins
lyases	reactions in which groups are removed without hydrolysis or addition of groups to a double bond	Decarboxylases catalyze the removal of carboxyl groups.
isomerases	reactions in which a compound is converted to its isomer	Isomerases may catalyze the conversion of an aldose to a ketose, and mutases catalyze reactions in which a functional group is transferred from one atom in a substrate to another.
ligases	reactions in which new bonds are formed between carbon and another atom; energy is required	Synthetases catalyze reactions in which two smaller molecules are linked to form a larger one.

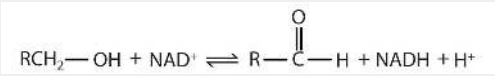
The first enzymes to be discovered were named according to their source or method of discovery. The enzyme *pepsin*, which aids in the hydrolysis of proteins, is found in the digestive juices of the stomach (Greek *pepsis*, meaning “digestion”). *Papain*, another

enzyme that hydrolyzes protein (in fact, it is used in meat tenderizers), is isolated from papayas. As more enzymes were discovered, chemists recognized the need for a more systematic and chemically informative identification scheme. In the current numbering and naming scheme, under the oversight of the Nomenclature Commission of the International Union of Biochemistry, enzymes are arranged into six groups according to the general type of reaction they catalyze (Table **5.1.1**), with subgroups and secondary subgroups that specify the reaction more precisely.

Figure* 5.1.1*: *Structure of the alcohol dehydrogenase protein (E.C.1.1.1.1) (EE ISOZYME) complexed wtih nicotinamide adenini dinulceotide (NAD) and zinc (PDB: 1CDO).*

Each enzyme is assigned a four-digit number, preceded by the prefix EC—for enzyme classification—that indicates its group, subgroup, and so forth. This is demonstrated in Table **5.1.2** for alcohol dehydrogenase. Each enzyme is also given a name consisting of the root of the name of its substrate or substrates and the *-ase* suffix. Thus urease is the enzyme that catalyzes the hydrolysis of urea.

Table **5.1.2**: Assignment of an Enzyme Classification Number

Alcohol Dehydrogenase: EC 1.1.1.1	
The first digit indicates that this enzyme is an oxidoreductase; that is, an enzyme that catalyzes an oxidation-reduction reaction. The second digit indicates that this oxidoreductase catalyzes a reaction involving a primary or secondary alcohol. The third digit indicates that either the coenzyme NAD^+ or $NADP^+$ is required for this reaction.	
The fourth digit indicates that this was the first enzyme isolated, characterized, and named using this system of nomenclature.	
The systematic name for this enzyme is *alcohol:NAD^+ oxidoreductase*, while the recommended or common name is alcohol dehydrogenase.	
Reaction catalyzed:	

Common Nomenclature

The systematic names are informative but not very practical. Therefore, most chemists still use the common nomenclature system. In this system, enzyme names consists of either one of these two options:

- name of the substrate + “ase”. Example: alcohol dehydrogenase.
- substrate name + functional group action on + type of reaction + “ase”. Example: Urea amidohydrolase.

Quantifying Enzyme concentration

The quantity or concentration of an enzyme in a solution can be expressed in molarity amounts, as with any other chemical. However, this is not the most practical option. Being proteins, enzymes have very large molar masses so their concentration in terms of molarity is extremely low. Instead, it is more useful to measure enzyme concentration in terms of the enzymatic activity exhibited by the enzyme in a certain volume of solution. This second option is preferred as the amount of enzyme is usually very low in terms of moles or grams, but its activity is very high and easier to determine.

Enzyme international unit (symbol U, sometimes also IU) is a unit of enzyme's catalytic activity. This unit is defined as the **amount of the enzyme that catalyzes the conversion of one micromole of substrate per minute** under the specified conditions. The specified conditions will usually be the enzyme optimum conditions, which including temperature, pH, and substrate concentration, that yield the maximal substrate conversion rate for that particular enzyme.

$$1\ U = 1\ \mu mol\ substrate/min$$

Characteristics of enzymes

Chemically, enzymes are generally globular proteins. Some RNA molecules called ribozymes can also be enzymes. These are usually found in the nuclear region of cells and catalyze the splitting of RNA molecules. Enzymes are catalysts that breakdown or synthesize more complex chemical compounds. Enzymes are characterized by the following factors:

1-Efficiency. Enzymes allow chemical reactions to occur fast enough to support life. They speed up the rate of chemical reactions because they lower the energy of activation, the energy that must be supplied in order for molecules to react with one another. An enzyme generally can typically catalyze between 1 and 10,000 molecules of substrate per second. In contrast to traditional chemical catalysts, enzymes are able to perform their action at body temperature and physiological pH.

2-Specificity. Enzymes are highly specific for their substrate. Generally there is one specific enzyme for each specific chemical reaction.

3-Regulation. The activity of enzymes is highly regulated in the cell to adapt to different metabolic and environmental conditions. The activity of an enzyme can be altered by either changing the total amount of enzyme (enzyme concentration in the cell) or by changing the rate at which an enzyme can catalyze a chemical reaction (enzymatic activity).

Summary

An enzyme is a biological catalyst, a substance that increases the rate of a chemical reaction without being changed or consumed in the reaction. A systematic process is used to name and classify enzymes.

Concept Review Exercise

In the small intestine, sucrose is hydrolyzed to form glucose and fructose in a reaction catalyzed by sucrase.

1. Identify the substrate in this reaction.
2. Name the enzyme.

Answers

1. sucrose
2. sucrase

Exercises

1. Identify the substrate catalyzed by each enzyme.
    a. lactase
    b. cellulase
    c. peptidase
2. Identify the substrate catalyzed by each enzyme.
    a. lipase
    b. amylase
    c. maltase
3. Identify each type of enzyme.
    a. decarboxylase
    b. protease
    c. transaminase
4. Identify each type of enzyme.
    a. dehydrogenase
    b. isomerase
    c. lipase

Answers

1. a. lactose
   b. cellulose
   c. peptides

3. a. lyase
   b. hydrolase
   c. transferase

Citations

- Wikipedia contributors. (2020, November 28). Enzyme unit. In *Wikipedia, The Free Encyclopedia*. Retrieved 17:34, May 17, 2021, from https://en.wikipedia.org/w/index.php?title=Enzyme_unit&oldid=991101591
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5.2: Enzyme Cofactors

Learning Objectives

- To explain the role of cofactors in enzymatic function

Many enzymes are simple proteins consisting entirely of one or more amino acid chains. Other enzymes contain a nonprotein component called a **cofactor** that is necessary for the enzyme's proper functioning. This cofactor is usually *weakly bonded* to the polypeptide chains through intermolecular interactions. There are two types of cofactors: inorganic ions [e.g., zinc or Cu(I) ions, also known as **minerals**] and organic molecules known as coenzymes. Most **coenzymes** are vitamins or are derived from vitamins. When the cofactor is *tightly bonded* to the polypeptide chain through a covalent bond is called a **prosthetic group**.

Vitamins are organic compounds that are essential in very small (trace) amounts for the maintenance of normal metabolism. They generally cannot be synthesized at adequate levels by the body and must be obtained from the diet. The absence or shortage of a vitamin may result in a vitamin-deficiency disease. In the first half of the 20th century, a major focus of biochemistry was the identification, isolation, and characterization of vitamins. Despite accumulating evidence that people needed more than just carbohydrates, fats, and proteins in their diets for normal growth and health, it was not until the early 1900s that research established the need for trace nutrients in the diet.

Table **5.2.1**: Fat-Soluble Vitamins and Physiological Functions

Vitamin	Physiological Function	Effect of Deficiency
vitamin A (retinol)	formation of vision pigments; differentiation of epithelial cells	night blindness; continued deficiency leads to total blindness
vitamin D (cholecalciferol)	increases the body's ability to absorb calcium and phosphorus	osteomalacia (softening of the bones); known as rickets in children
vitamin E (tocopherol)	fat-soluble antioxidant	damage to cell membranes
vitamin K (phylloquinone)	formation of prothrombin, a key enzyme in the blood-clotting process	increases the time required for blood to clot

Because organisms differ in their synthetic abilities, a substance that is a vitamin for one species may not be so for another. Over the past 100 years, scientists have identified and isolated 13 vitamins required in the human diet and have divided them into two broad categories: the *fat-soluble vitamins*, which include vitamins A, D, E, and K, and the *water-soluble vitamins*, which are the B complex vitamins and vitamin C. All fat-soluble vitamins contain a high proportion of hydrocarbon structural components. There are one or two oxygen atoms present, but the compounds as a whole are nonpolar. In contrast, water-soluble vitamins contain large numbers of electronegative oxygen and nitrogen atoms, which can engage in hydrogen bonding with water. Most water-soluble vitamins act as coenzymes or are required for the synthesis of coenzymes. The fat-soluble vitamins are important for a variety of physiological functions. The key vitamins and their functions are found in Tables **5.2.1** and **5.2.2**.

Table **5.2.2**: Water-Soluble Vitamins and Physiological Functions

Vitamin	Coenzyme	Coenzyme Function	Deficiency Disease
vitamin B_1 (thiamine)	thiamine pyrophosphate	decarboxylation reactions	beri-beri
vitamin B_2 (riboflavin)	flavin mononucleotide or flavin adenine dinucleotide	oxidation-reduction reactions involving two hydrogen atoms	—
vitamin B_3 (niacin)	nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate	oxidation-reduction reactions involving the hydride ion (H^-)	pellagra
vitamin B_6 (pyridoxine)	pyridoxal phosphate	variety of reactions including the transfer of amino groups	—
vitamin B_{12} (cyanocobalamin)	methylcobalamin or deoxyadenoxylcobalamin	intramolecular rearrangement reactions	pernicious anemia
biotin	biotin	carboxylation reactions	—
folic acid	tetrahydrofolate	carrier of one-carbon units such as the formyl group	anemia
pantothenic Acid	coenzyme A	carrier of acyl groups	—
vitamin C (ascorbic acid)	none	antioxidant; formation of collagen, a protein found in tendons, ligaments, and bone	scurvy

Vitamins C and E, as well as the provitamin β-carotene can act as antioxidants in the body. Antioxidants prevent damage from free radicals, which are molecules that are highly reactive because they have unpaired electrons. Free radicals are formed not only through metabolic reactions involving oxygen but also by such environmental factors as radiation and pollution.

> *β-carotene is known as a provitamin because it can be converted to vitamin A in the body.*

Free radicals react most commonly react with lipoproteins and unsaturated fatty acids in cell membranes, removing an electron from those molecules and thus generating a new free radical. The process becomes a chain reaction that finally leads to the oxidative degradation of the affected compounds. Antioxidants react with free radicals to stop these chain reactions by forming a more stable molecule or, in the case of vitamin E, a free radical that is much less reactive (vitamin E is converted back to its original form through interaction with vitamin C).

Summary

Vitamins are organic compounds that are essential in very small amounts for the maintenance of normal metabolism. Vitamins are divided into two broad categories: fat-soluble vitamins and water-soluble vitamins. Most water-soluble vitamins are needed for the formation of coenzymes, which are organic molecules needed by some enzymes for catalytic activity.

Concept Review Exercises

1. What is the difference between a cofactor and a coenzyme?
2. How are vitamins related to coenzymes?

Answers

1. A coenzyme is one type of cofactor. Coenzymes are organic molecules required by some enzymes for activity. A cofactor can be either a coenzyme or an inorganic ion.
2. Coenzymes are synthesized from vitamins.

Exercises

1. Identify each vitamin as water soluble or fat soluble.
   a. vitamin D
   b. vitamin C
   c. vitamin B_{12}
2. Identify each vitamin as water soluble or fat soluble.
   a. niacin
   b. cholecalciferol
   c. biotin
3. What vitamin is needed to form each coenzyme?
   a. pyridoxal phosphate
   b. flavin adenine dinucleotide
   c. coenzyme A
   d. nicotinamide adenine dinucleotide
4. What coenzyme is formed from each vitamin?
   a. niacin
   b. thiamine
   c. cyanocobalamin
   d. pantothenic acid
5. What is the function of each vitamin or coenzyme?
   a. flavin adenine dinucleotide
   b. vitamin A
   c. biotin
6. What is the function of each vitamin or coenzyme?
   a. vitamin K
   b. pyridoxal phosphate
   c. tetrahydrofolate

Answers

1. a. fat soluble
   b. water soluble
   c. water soluble

3. a. vitamin B_6 or pyridoxine
   b. vitamin B_2 or riboflavin
   c. pantothenic acid
   d. vitamin B_3 or niacin

5. a. needed by enzymes that catalyze oxidation-reduction reactions in which two hydrogen atoms are transferred
   b. needed for the formation of vision pigments
   c. needed by enzymes that catalyze carboxylation reactions

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5.3: Mechanism of Enzymatic Catalysis

Learning Objectives

- To describe the interaction between an enzyme and its substrate.

Enzyme-catalyzed reactions occur in at least two steps. In the first step, an enzyme molecule (E) and the substrate molecule or molecules (S) collide and react to form an intermediate compound called the *enzyme-substrate* (E–S) *complex*. (This step is reversible because the complex can break apart into the original substrate or substrates and the free enzyme.) Once the E–S complex forms, the enzyme is able to catalyze the formation of product (P), which is then released from the enzyme surface:

$$S + E \rightarrow E\text{–}S \tag{5.3.1}$$

$$E\text{–}S \rightarrow P + E \tag{5.3.2}$$

Hydrogen bonding and other electrostatic interactions hold the enzyme and substrate together in the complex. The structural features or functional groups on the enzyme that participate in these interactions are located in a cleft or pocket on the enzyme surface. This pocket, where the enzyme combines with the substrate and transforms the substrate to product is called the active site of the enzyme (Figure 5.3.1).

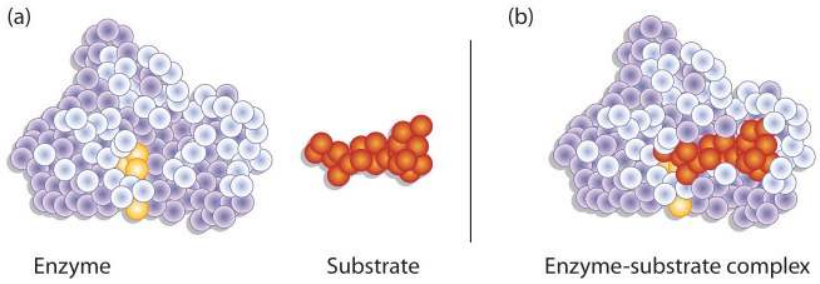


Figure 5.3.1: Substrate Binding to the Active Site of an Enzyme. The enzyme dihydrofolate reductase is shown with one of its substrates: $NADP^+$ (a) unbound and (b) bound. The $NADP^+$ (shown in red) binds to a pocket that is complementary to it in shape and ionic properties.

The active site of an enzyme possesses a unique conformation (including correctly positioned bonding groups) that is complementary to the structure of the substrate, so that the enzyme and substrate molecules fit together in much the same manner as a key fits into a tumbler lock. In fact, an early model describing the formation of the enzyme-substrate complex was called the lock-and-key model (Figure 5.3.2). This model portrayed the enzyme as conformationally rigid and able to bond only to substrates that exactly fit the active site.

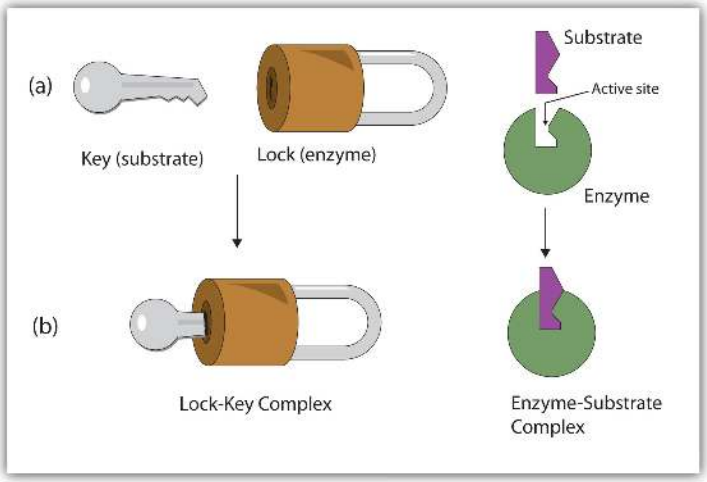


Figure **5.3.2**: The Lock-and-Key Model of Enzyme Action. (a) Because the substrate and the active site of the enzyme have complementary structures and bonding groups, they fit together as a key fits a lock. (b) The catalytic reaction occurs while the two are bonded together in the enzyme-substrate complex.

Working out the precise three-dimensional structures of numerous enzymes has enabled chemists to refine the original lock-and-key model of enzyme actions. They discovered that the binding of a substrate often leads to a large conformational change in the enzyme, as well as to changes in the structure of the substrate or substrates. The current theory, known as the induced-fit model, says that enzymes can undergo a change in conformation when they bind substrate molecules, and the active site has a shape complementary to that of the substrate only *after* the substrate is bound, as shown for hexokinase in Figure **5.3.3**. After catalysis, the enzyme resumes its original structure.

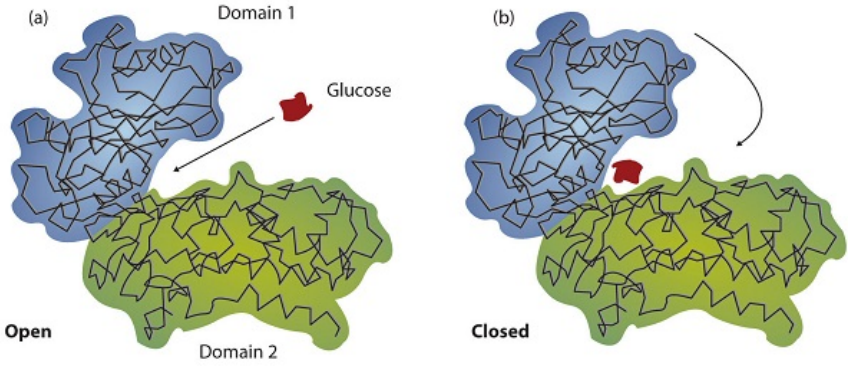


Figure **5.3.3**: The Induced-Fit Model of Enzyme Action. (a) The enzyme hexokinase without its substrate (glucose, shown in red) is bound to the active site. (b) The enzyme conformation changes dramatically when the substrate binds to it, resulting in additional interactions between hexokinase and glucose.

The structural changes that occur when an enzyme and a substrate join together bring specific parts of a substrate into alignment with specific parts of the enzyme's active site. Amino acid side chains in or near the binding site can then act as acid or base catalysts, provide binding sites for the transfer of functional groups from one substrate to another or aid in the rearrangement of a substrate. The participating amino acids, which are usually widely separated in the primary sequence of the protein, are brought close together in the active site as a result of the folding and bending of the polypeptide chain or chains when the protein acquires its tertiary and quaternary structure. Binding to enzymes brings reactants close to each other and aligns them properly, which has the same effect as increasing the concentration of the reacting compounds.

✔ Example **5.3.1**

a. What type of interaction would occur between an OH group present on a substrate molecule and a functional group in the active site of an enzyme?

b. Suggest an amino acid whose side chain might be in the active site of an enzyme and form the type of interaction you just identified.

Solution

a. An OH group would most likely engage in hydrogen bonding with an appropriate functional group present in the active site of an enzyme.

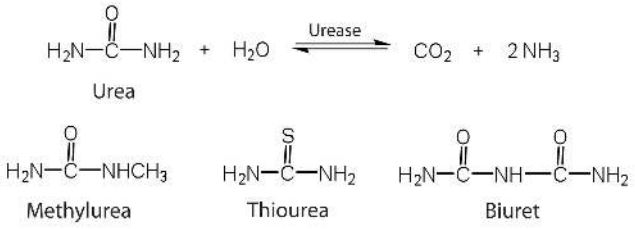
b. Several amino acid side chains would be able to engage in hydrogen bonding with an OH group. One example would be asparagine, which has an amide functional group.

? Exercise 5.3.1

a. What type of interaction would occur between an COO^- group present on a substrate molecule and a functional group in the active site of an enzyme?

b. Suggest an amino acid whose side chain might be in the active site of an enzyme and form the type of interaction you just identified.

One characteristic that distinguishes an enzyme from all other types of catalysts is its *substrate specificity*. An inorganic acid such as sulfuric acid can be used to increase the reaction rates of many different reactions, such as the hydrolysis of disaccharides, polysaccharides, lipids, and proteins, with complete impartiality. In contrast, enzymes are much more specific. Some enzymes act on a single substrate, while other enzymes act on any of a group of related molecules containing a similar functional group or chemical bond. Some enzymes even distinguish between D- and L-stereoisomers, binding one stereoisomer but not the other. Urease, for example, is an enzyme that catalyzes the hydrolysis of a single substrate—urea—but not the closely related compounds methyl urea, thiourea, or biuret. The enzyme carboxypeptidase, on the other hand, is far less specific. It catalyzes the removal of nearly any amino acid from the carboxyl end of any peptide or protein.



Enzyme specificity results from the uniqueness of the active site in each different enzyme because of the identity, charge, and spatial orientation of the functional groups located there. It regulates cell chemistry so that the proper reactions occur in the proper place at the proper time. Clearly, it is crucial to the proper functioning of the living cell.

Summary

A substrate binds to a specific region on an enzyme known as the active site, where the substrate can be converted to product. The substrate binds to the enzyme primarily through hydrogen bonding and other electrostatic interactions. The induced-fit model says that an enzyme can undergo a conformational change when binding a substrate. Enzymes exhibit varying degrees of substrate specificity.

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5.4: The Kinetics of Enzymatic Catalysis

Michaelis-Menten Enzyme Kinetics

Enzymes are highly specific catalysts for biochemical reactions, with each enzyme showing a selectivity for a single reactant, or **substrate**. Many enzyme–substrate reactions follow a simple mechanism that consists of the initial formation of an enzyme–substrate complex, ES, which subsequently decomposes to form product, releasing the enzyme to react again.

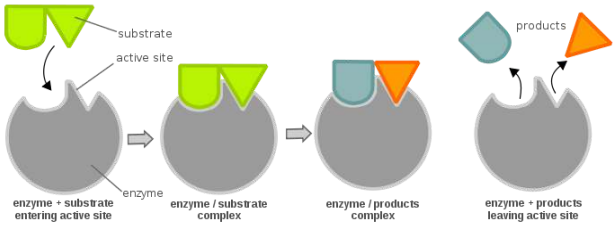


Figure **5.4.1**: An enzyme catalyzes the reaction of two substrates and to form one product. from Wikipedia.

This is described within the following multi-step mechanism for a reversible reaction catalyzed by an enzyme

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P \tag{5.4.1}$$

where k_1, k_{-1}, k_2, and k_{-2} are rate constants for the forward and backward reactions. The reaction's rate law for generating the product $[P]$ is

$$reactionrate = \frac{d[P]}{dt} = k_2[ES] - k_{-2}[E][P] \tag{5.4.2}$$

However, if we make measurement early in the reaction, the concentration of products is negligible, i.e.,

$$[P] \approx 0 \tag{5.4.3}$$

and we can ignore the back reaction (second term in right side of Equation 5.4.2). Then under these conditions, the reaction's rate is

$$reactionrate = \frac{d[P]}{dt} = k_2[ES] \tag{5.4.4}$$

To be analytically useful we need to write Equation 5.4.4 in terms of the reactants, e.g., the concentrations of enzyme and substrate, which are the independent variables we can control when setting up an experiment. To do this we use the **steady-state approximation**, in which we assume that the concentration of ES remains essentially constant. Following an initial period, during which the enzyme–substrate complex first forms, the rate at which ES forms

$$\frac{d[ES]}{dt} = k_1[E][S] = k_1([E]_0 - [ES])[S] \tag{5.4.5}$$

is equal to the rate at which it disappears

$$-\frac{d[ES]}{dt} = k_{-1}[ES] + k_2[ES] \tag{5.4.6}$$

where $[E]_0$ is the enzyme's original concentration. Combining Equations 5.4.5 and 5.4.6 gives

$$k_1([E]_0 - [ES])[S] = k_{-1}[ES] + k_2[ES] \tag{5.4.7}$$

which we solve for the concentration of the enzyme–substrate complex

$$[ES] = \frac{[E]_0[S]}{\frac{k_{-1} + k_2}{k_1} + [S]} = \frac{[E]_0[S]}{K_m + [S]} \tag{5.4.8}$$

where K_m is the **Michaelis constant**. Substituting Equation 5.4.8 into Equation 5.4.4 leaves us with the rate equation.

$$\frac{d[P]}{dt} = reactionrate = \frac{k_2[E]_0[S]}{K_m + [S]} \tag{5.4.9}$$

The factor $k_2[E]_0$ equals the maximum possible rate at a given initial concentration of enzyme, that is $k_2[E]_0 = V_{max}$. Therefore Equation 5.4.9 simplifies to

$$\frac{d[P]}{dt} = reactionrate = \frac{V_{max}[S]}{K_m + [S]} \tag{5.4.10}$$

Equation 5.4.10 is known as **Michaelis-Menten equation,** and it represents how the reaction rate depends on the concentration of substrate, under normal conditions in which the concentration of substrate is always much higher than the concentration of enzyme (the catalyst). A plot of Equation 5.4.10 is shown in Figure 5.4.1



Figure 5.4.1 : Plot of Equation 5.4.10 showing the relationship between reaction rate and substrate concentration in an enzyme-catalyzed reaction. Image by Thomas Shafee, CC BY 4.0, via Wikimedia Commons

From the plot, we can see that **The Michaelis constant K_m is the substrate concentration at which the reaction rate is at half-maximum**, and is an inverse measure of the substrate's affinity for the enzyme—as a small K_m indicates high affinity, meaning that the rate will approach V_{max} more quickly.

At lower substrate concentrations, where $[S] \ll K_m$, Equation 5.4.10 becomes

$$\frac{d[P]}{dt} = \frac{V_{max}[S]}{K_m + [S]} \approx \frac{V_{max}[S]}{K_m} \tag{5.4.11}$$

so the reaction rate increases linearly when increasing the concentration of substrate.

For high substrate concentrations, where $[S] \gg K_m$, Equation 5.4.10 simplifies to

$$\frac{d[P]}{dt} = \frac{V_{max}[S]}{K_m + [S]} \approx V_{max} \tag{5.4.12}$$

so under these conditions the reaction rate is independent of the concentration of substrate, and equals the V_{max}. This is because all the enzyme is forming an ES complex and there is no free enzyme to bind to the free substrate. Therefore each cycle of catalysis must conclude before there is free enzyme to catalyze the conversation of more free substrate (the enzyme is saturated with the substrate when the concentration of substrate is too high) (see figure below)



Figure5.4.2when the substrate concentration is too high, the available enzyme binding sites become completely filled. The enzyme is saturated with substrate and the reaction rate ceases to increase beyond {V_{max} Image by GYassineMrabetTalk✉ This W3C-unspecified vector image was created with Inkscape ., Public domain, via Wikimedia Commons

Turnover number

From the last two terms in Equation 5.4.11, we can express V_{max} in terms of a **turnover number** (k_{cat}):

$$V_{max} = k_{cat}[E]_o \tag{5.4.13}$$

where $[E]_0$ is the enzyme concentration and k_{cat} is the **turnover number,** defined as the maximum number of substrate molecules converted to product per enzyme molecule per second. Hence, the turnover number is defined as the maximum number of chemical conversions of substrate molecules per second that a single catalytic site will execute for a given enzyme concentration $[E]_o$.

Example 5.4.1: Turnover number of acetylcholinesterase

Acetylcholinesterase (AChE) may be one of the fastest enzymes. It hydrolyzes acetylcholine to choline and an acetate group. One of the earliest values of the turnover number was 3×10^7 (molecules of acetylcholine) per minute per molecule of enzyme. A more recent value at 25°C, pH = 7.0, acetylcholine concentration of $2.5 \times 10^{-3}\ M$, was found to be $7.4 \times 10^5\ min^{-1}$ (*J Biol Chem. 236 (8): 2292–5.*).

There may be some 30 active centers per molecule. AChE is a serine hydrolase that reacts with acetylcholine at close to **the diffusion-controlled rate**. A diffusion-controlled reaction occurs so quickly that the reaction rate is the rate of transport of the reactants through the solution; as quickly as the reactants encounter each other, they react.

The Significance of K_M and V_{max}

The Michaelis-Menten model is used in a variety of biochemical situations other than enzyme-substrate interaction, including antigen-antibody binding, DNA-DNA hybridization, and protein-protein interaction. It can be used to characterize a generic biochemical reaction. The experimentally determined parameters values vary wildly between enzymes (Table 5.4.1):

Table 5.4.1: Enzyme Kinetic parameters

Enzyme	K_m (M)	k_{cat} (1/s)	k_{cat}/K_m (1/M.s)
Chymotrypsin	1.5×10^{-2}	0.14	9.3
Pepsin	3.0×10^{-4}	0.50	1.7×10^3
Tyrosyl-tRNA synthetase	9.0×10^{-4}	7.6	8.4×10^3
Ribonuclease	7.9×10^{-3}	7.9×10^2	1.0×10^5
Carbonic anhydrase	2.6×10^{-2}	4.0×10^5	1.5×10^7
Fumarase	5.0×10^{-6}	8.0×10^2	1.6×10^8

While K_m is equal to the substrate concentration at which the enzyme converts substrates into products at half its maximal rate and hence is related to the affinity of the substrate for the enzyme. The catalytic rate k_{cat} is the rate of product formation when the enzyme is saturated with substrate and therefore reflects the enzyme's maximum rate. The rate of product formation is dependent on both how well the enzyme binds substrate and how fast the enzyme converts substrate into product once substrate is bound. For a kinetically perfect enzyme, every encounter between enzyme and substrate leads to product and hence the reaction velocity is only limited by the rate the enzyme encounters substrate in solution. From Equation 5.4.8, the catalytic efficiency of a protein can be evaluated.

$$\frac{k_{cat}}{K_m} = \frac{k_2}{K_m} = \frac{k_1 k_2}{k_{-1} + k_2} \tag{5.4.14}$$

This k_{cat}/K_m ratio is called the specificity constant measure of how efficiently an enzyme converts a substrate into product. It has a theoretical upper limit of 10^8 – 10^{10} /M.s; enzymes working close to this, such as fumarase, are termed superefficient (Table 5.4.1).

Determining V_m and K_m from experimental data can be difficult and the most common way is to determine initial rates, v_0, from experimental values of $[P]$ or $[S]$ as a function of time. Hyperbolic graphs of v_0 vs. $[S]$ can be fit or transformed as we explored with the different mathematical transformations of the hyperbolic binding equation to determine K_d. These included:

- nonlinear hyperbolic fit (e.g., Figure 5.4.1)
- double reciprocal plot (e.g., Lineweaver–Burk plot discussed below

Lineweaver–Burk plot

Another commonly-used plot in examining enzyme kinetics is the **Lineweaver-Burk plot**, in with the inverse of the reaction rate, $1/r$, is plotted against the inverse of the substrate concentration $1/[\mathrm{S}]$. Rearranging Equation 5.4.10,

$$\frac{1}{r} = \frac{K_M + [\mathrm{S}]}{k_2 [\mathrm{E}]_0 [\mathrm{S}]} = \frac{K_M}{k_2 [\mathrm{E}]_0} \frac{1}{[\mathrm{S}]} + \frac{1}{k_2 [\mathrm{E}]_0} \tag{5.4.15}$$

The Lineweaver–Burk plot (or double reciprocal plot) is a graphical representation of the Lineweaver–Burk equation of enzyme kinetics, described by Hans Lineweaver and Dean Burk in 1934 (Figure 5.4.2). The Lineweaver-Burk plot results in a straight line with the slope equal to $K_M/k_2 [\mathrm{E}]_0$ and y-intercept equal to $1/k_2 [\mathrm{E}]_0$ which is $1/V_{max}$ via Equation 5.4.10.

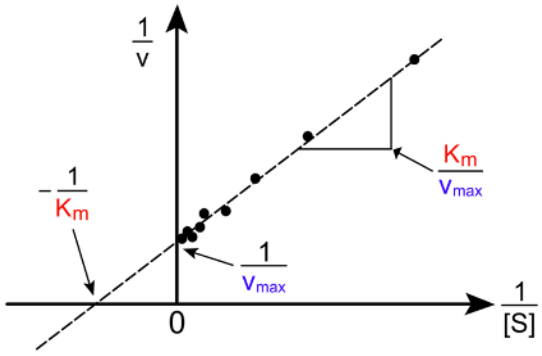


Figure 5.4.3 : Lineweaver–Burk plot of Michaelis–Menten kineitcs.

The plot provides a useful graphical method for analysis of the Michaelis–Menten equation:

$$V = \frac{V_{\max}[S]}{K_m + [S]} \tag{5.4.16}$$

Taking the reciprocal gives

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max}[S]} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \tag{5.4.17}$$

where

- V is the reaction velocity (the reaction rate),
- K_m is the Michaelis–Menten constant,
- V_{max} is the maximum reaction velocity, and
- $[S]$ is the substrate concentration.

The Lineweaver–Burk plot was widely used to determine important terms in enzyme kinetics, such as K_m and V_{max}, before the wide availability of powerful computers and non-linear regression software. The y-intercept of such a graph is equivalent to the inverse of V_{max}; the x-intercept of the graph represents $-1/K_m$. It also gives a quick, visual impression of the different forms of enzyme inhibition.

Example 5.4.2

The reaction between nicotineamide mononucleotide and ATP to form nicotineamide–adenine dinucleotide and pyrophosphate is catalyzed by the enzyme nicotinamide mononucleotide adenylyltransferase. The following table provides typical data obtained at a pH of 4.95. The substrate, S, is nicotinamide mononucleotide and the initial rate, *v*, is the μmol of nicotinamide–adenine dinucleotide formed in a 3-min reaction period.

[S] (mM)	v (µmol)	[S] (mM)	v (µmol)
0.138	0.148	0.560	0.324
0.220	0.171	0.766	0.390
0.291	0.234	1.460	0.493

Determine values for V_{max} and K_m.

Solution

Figure 13.12 shows the Lineweaver–Burk plot for this data and the resulting regression equation. Using the *y*-intercept, we calculate V_{max} as

$$V_{max} = 1 / y\text{−intercept} = 1 / 1.708 \text{ mol} = 0.585 \text{ mol}$$

and using the slope we find that K_m is

$$K_m = \text{slope} \times V_{max} = 0.7528 \text{ molimM} \times 0.585 \text{ mol} = 0.440 \text{ mM}$$

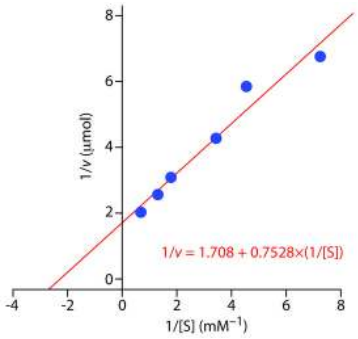
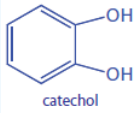


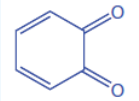
Figure 13.12: Linweaver–Burk plot and regression equation for the data in Example 13.6.

-diphenyl oxidase

The following data are for the oxidation of catechol (the substrate) to *o*-quinone by the enzyme *o*-diphenyl oxidase. The reaction is followed by monitoring the change in absorbance at 540 nm. The data in this exercise are adapted from jkimball.



catechol



o-quinone

[catechol] (mM)	0.3	0.6	1.2	4.8
rate (ΔAU/min)	0.020	0.035	0.048	0.056

Determine values for V_{max} and K_m.

Problems with the Method

The Lineweaver–Burk plot is classically used in older texts, but is prone to error, as the *y*-axis takes the reciprocal of the rate of reaction – in turn increasing any small errors in measurement. Also, most points on the plot are found far to the right of the *y*-axis (due to limiting solubility not allowing for large values of $[S]$ and hence no small values for $1/[S]$), calling for a large extrapolation back to obtain *x*- and *y*-intercepts.

When used for determining the type of enzyme inhibition, the Lineweaver–Burk plot can distinguish competitive and non-competitive. Competitive inhibitors have the same y-intercept as uninhibited enzyme (since V_{max} is unaffected by competitive inhibitors the inverse of V_{max} also doesn't change) but there are different slopes and x-intercepts between the two data sets. Non-competitive inhibition produces plots with the same x-intercept as uninhibited enzyme (K_m is unaffected) but different slopes and y-intercepts.

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5.5: Factos Affecting Enzyme Activity

Learning Objectives

- To describe how pH, temperature, and the concentration of an enzyme and its substrate influence enzyme activity.

The single most important property of enzymes is the ability to increase the rates of reactions occurring in living organisms, a property known as *catalytic activity*. Because most enzymes are proteins, their activity is affected by factors that disrupt protein structure, as well as by factors that affect catalysts in general. Factors that disrupt protein structure include temperature and pH; factors that affect catalysts in general include reactant or substrate concentration and catalyst or enzyme concentration. The activity of an enzyme can be measured by monitoring either the rate at which a substrate disappears or the rate at which a product forms.

Concentration of Substrate

In the presence of a given amount of enzyme, the rate of an enzymatic reaction increases as the substrate concentration increases until a limiting rate is reached, after which further increase in the substrate concentration produces no significant change in the reaction rate (part (a) of Figure **5.5.1**). At this point, so much substrate is present that essentially all of the enzyme active sites have substrate bound to them. In other words, the enzyme molecules are saturated with substrate. The excess substrate molecules cannot react until the substrate already bound to the enzymes has reacted and been released (or been released without reacting).

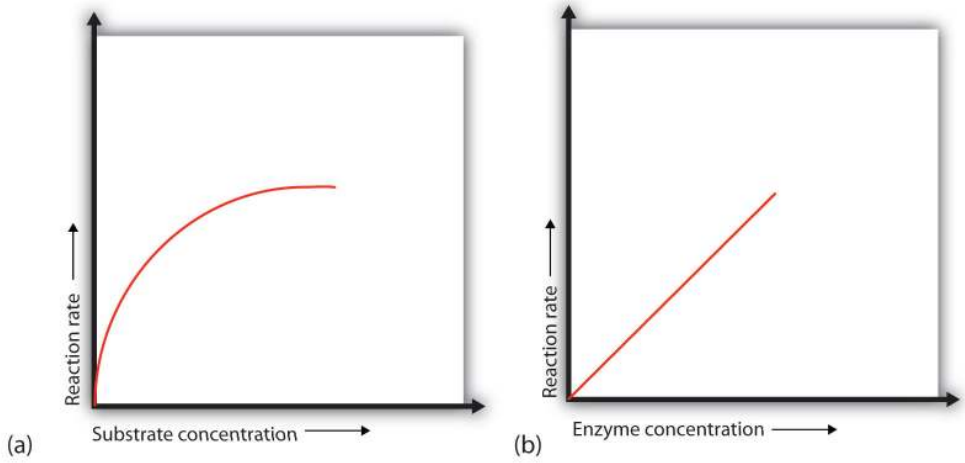


Figure **5.5.1**: Concentration versus Reaction Rate. (a) This graph shows the effect of substrate concentration on the rate of a reaction that is catalyzed by a fixed amount of enzyme. (b) This graph shows the effect of enzyme concentration on the reaction rate at a constant level of substrate.

Let's consider an analogy. Ten taxis (enzyme molecules) are waiting at a taxi stand to take people (substrate) on a 10-minute trip to a concert hall, one passenger at a time. If only 5 people are present at the stand, the rate of their arrival at the concert hall is 5 people in 10 minutes. If the number of people at the stand is increased to 10, the rate increases to 10 arrivals in 10 minutes. With 20 people at the stand, the rate would still be 10 arrivals in 10 minutes. The taxis have been "saturated." If the taxis could carry 2 or 3 passengers each, the same principle would apply. The rate would simply be higher (20 or 30 people in 10 minutes) before it leveled off.

Concentration of Enzyme

When the concentration of the enzyme is significantly lower than the concentration of the substrate (as when the number of taxis is far lower than the number of waiting passengers), the rate of an enzyme-catalyzed reaction is directly dependent on the enzyme concentration (part (b) of Figure **5.5.1**). This is true for any catalyst; the reaction rate increases as the concentration of the catalyst is increased.

Temperature

A general rule of thumb for most chemical reactions is that a temperature rise of 10°C approximately doubles the reaction rate. To some extent, this rule holds for all enzymatic reactions. After a certain point, however, an increase in temperature causes a decrease in the reaction rate, due to denaturation of the protein structure and disruption of the active site (part (a) of Figure **5.5.2**). For many proteins, denaturation occurs between 45°C and 55°C. Furthermore, even though an enzyme may appear to have a maximum reaction rate between 40°C and 50°C, most biochemical reactions are carried out at lower temperatures because enzymes are not stable at these higher temperatures and will denature after a few minutes.

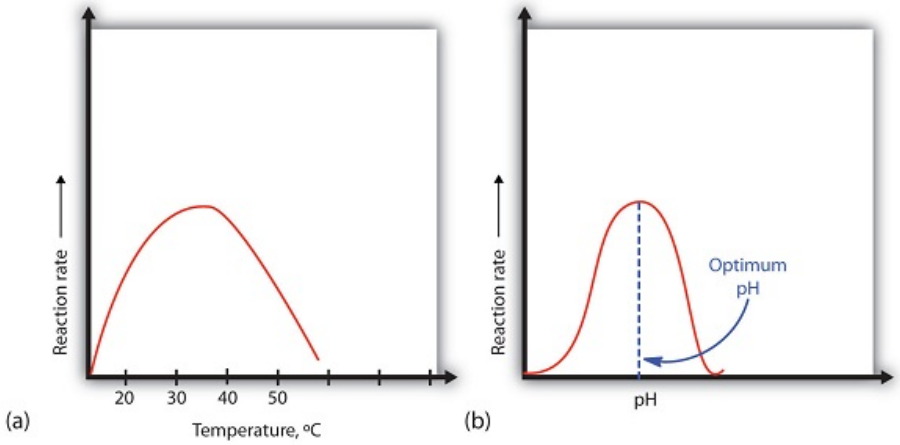


Figure **5.5.2**: Temperature and pH versus Concentration. (a) This graph depicts the effect of temperature on the rate of a reaction that is catalyzed by a fixed amount of enzyme. (b) This graph depicts the effect of pH on the rate of a reaction that is catalyzed by a fixed amount of enzyme.

At 0°C and 100°C, the rate of enzyme-catalyzed reactions is nearly zero. This fact has several practical applications. We sterilize objects by placing them in boiling water, which denatures the enzymes of any bacteria that may be in or on them. We preserve our food by refrigerating or freezing it, which slows enzyme activity. When animals go into hibernation in winter, their body temperature drops, decreasing the rates of their metabolic processes to levels that can be maintained by the amount of energy stored in the fat reserves in the animals' tissues.

Hydrogen Ion Concentration (pH)

Because most enzymes are proteins, they are sensitive to changes in the hydrogen ion concentration or pH. Enzymes may be denatured by extreme levels of hydrogen ions (whether high or low); *any* change in pH, even a small one, alters the degree of ionization of an enzyme's acidic and basic side groups and the substrate components as well. Ionizable side groups located in the active site must have a certain charge for the enzyme to bind its substrate. Neutralization of even one of these charges alters an enzyme's catalytic activity.

An enzyme exhibits maximum activity over the narrow pH range in which a molecule exists in its properly charged form. The median value of this pH range is called the optimum pH of the enzyme (part (b) of Figure **5.5.2**). With the notable exception of gastric juice (the fluids secreted in the stomach), most body fluids have pH values between 6 and 8. Not surprisingly, most enzymes exhibit optimal activity in this pH range. However, a few enzymes have optimum pH values outside this range. For example, the optimum pH for pepsin, an enzyme that is active in the stomach, is 2.0.

Summary

Initially, an increase in substrate concentration leads to an increase in the rate of an enzyme-catalyzed reaction. As the enzyme molecules become saturated with substrate, this increase in reaction rate levels off. The rate of an enzyme-catalyzed reaction increases with an increase in the concentration of an enzyme. At low temperatures, an increase in temperature increases the rate of an enzyme-catalyzed reaction. At higher temperatures, the protein is denatured, and the rate of the reaction dramatically decreases. An enzyme has an optimum pH range in which it exhibits maximum activity.

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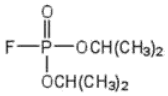
5.6: Enzyme Inhibition

Learning Objectives

- Explain what an enzyme inhibitor is.
- Distinguish between reversible and irreversible inhibitors.
- Distinguish between competitive and noncompetitive inhibitors.

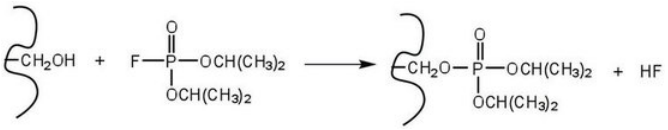
Previously, we noted that enzymes are inactivated at high temperatures and by changes in pH. These are nonspecific factors that would inactivate any enzyme. The activity of enzymes can also be regulated by more specific inhibitors. Many compounds are poisons because they bind covalently to particular enzymes or kinds of enzymes and inactivate them (Table **5.6.1**).

Table **5.6.1**: Poisons as Enzyme Inhibitors

Poison	Formula	Example of Enzyme Inhibited	Action
arsenate	AsO_4^{3-}	glyceraldehyde 3-phosphate dehydrogenase	substitutes for phosphate
iodoacetate	ICH_2COO^-	triose phosphate dehydrogenase	binds to cysteine **SH** group
diisopropylfluoro-phosphate (DIFP; a nerve poison)		acetylcholinesterase	binds to serine **OH** group

Irreversible Inhibition: Poisons

An irreversible inhibitor inactivates an enzyme by bonding covalently to a particular group at the active site. The inhibitor-enzyme bond is so strong that the inhibition cannot be reversed by the addition of excess substrate. The nerve gases, especially Diisopropyl fluorophosphate (DIFP), irreversibly inhibit biological systems by forming an enzyme-inhibitor complex with a specific OH group of serine situated at the active sites of certain enzymes. The peptidases trypsin and chymotrypsin contain serine groups at the active site and are inhibited by DIFP.



Reversible Inhibition

A reversible inhibitor inactivates an enzyme through noncovalent, more easily reversed, interactions. Unlike an irreversible inhibitor, a reversible inhibitor can dissociate from the enzyme. Reversible inhibitors include competitive **inhibitors** and **noncompetitive inhibitors**. (There are additional types of reversible inhibitors.) A competitive inhibitor is any compound that bears a structural resemblance to a particular substrate and thus competes with that substrate for binding at the active site of an enzyme. The inhibitor is not acted on by the enzyme but does prevent the substrate from approaching the active site.



Competitive vs Non-competitive inhibition. Image by CNX OpenStax, CC BY 4.0, via Wikimedia Commons

The degree to which a competitive inhibitor interferes with an enzyme's activity depends on the relative concentrations of the substrate and the inhibitor. If the inhibitor is present in relatively large quantities, it will initially block most of the active sites. But because the binding is reversible, some substrate molecules will eventually bind to the active site and be converted to product.

Increasing the substrate concentration promotes displacement of the inhibitor from the active site. **Competitive inhibition can be completely reversed by adding substrate** so that it reaches a much higher concentration than that of the inhibitor.

Studies of competitive inhibition have provided helpful information about certain enzyme-substrate complexes and the interactions of specific groups at the active sites. As a result, pharmaceutical companies have synthesized drugs that competitively inhibit metabolic processes in bacteria and certain cancer cells. Many drugs are competitive inhibitors of specific enzymes.

A classic example of competitive inhibition is the effect of malonate on the enzyme activity of succinate dehydrogenase (Figure 5.6.1). Malonate and succinate are the anions of dicarboxylic acids and contain three and four carbon atoms, respectively. The malonate molecule binds to the active site because the spacing of its carboxyl groups is not greatly different from that of succinate. However, no catalytic reaction occurs because malonate does not have a CH_2CH_2 group to convert to CH=CH. This reaction will also be discussed in connection with the Krebs cycle and energy production.

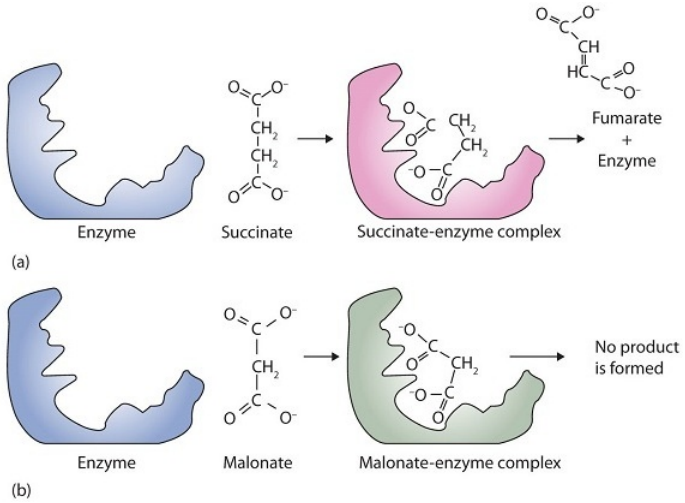


Figure 5.6.1: Competitive Inhibition. (a) Succinate binds to the enzyme succinate dehydrogenase. A dehydrogenation reaction occurs, and the product—fumarate—is released from the enzyme. (b) Malonate also binds to the active site of succinate dehydrogenase. In this case, however, no subsequent reaction occurs while malonate remains bound to the enzyme.

Some reversible inhibitors are noncompetitive. A **noncompetitive inhibitor** can combine with either the free enzyme or the enzyme-substrate complex because its **binding site on the enzyme is distinct from the active site**. This binging site is called the **allosteric site** (different from the active site) Binding of this kind of inhibitor alters the three-dimensional conformation of the enzyme, changing the configuration of the active site with one of two results. Either the enzyme-substrate complex does not form at its normal rate, or, once formed, it does not yield products at the normal rate. Because the inhibitor does not structurally resemble the substrate, **the addition of excess substrate does *not* reverse the inhibitory effect.**

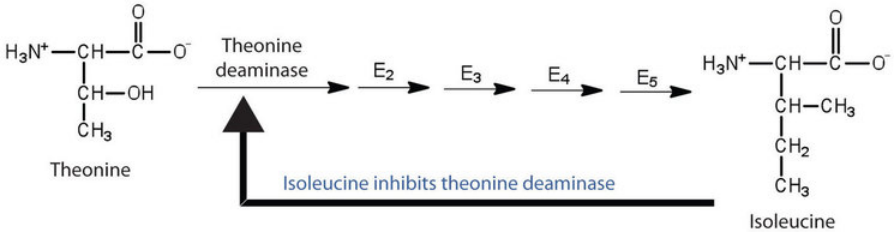


Figure 5.6.2: Feedback Inhibition of Threonine Deaminase by Isoleucine. Threonine deaminase is the first enzyme in the conversion of threonine to isoleucine. Isoleucine inhibits threonine deaminase through feedback inhibition.

Feedback inhibition is a normal biochemical process that makes use of noncompetitive inhibitors to control some enzymatic activity. In this process, the final product inhibits the enzyme that catalyzes the first step in a series of reactions. Feedback inhibition is used to regulate the synthesis of many amino acids. For example, bacteria synthesize isoleucine from threonine in a series of five enzyme-catalyzed steps. As the concentration of isoleucine increases, some of it binds as a noncompetitive inhibitor to the first enzyme of the series (threonine deaminase), thus bringing about a decrease in the amount of isoleucine being formed (Figure 5.6.2).

Summary

An irreversible inhibitor inactivates an enzyme by bonding covalently to a particular group at the active site. A reversible inhibitor inactivates an enzyme through noncovalent, reversible interactions. A competitive inhibitor competes with the substrate for binding at the active site of the enzyme. A noncompetitive inhibitor binds at a allosteric site distinct from the active site.

Concept Review Exercises

1. What are the characteristics of an irreversible inhibitor?
2. In what ways does a competitive inhibitor differ from a noncompetitive inhibitor?

Answers

1. It inactivates an enzyme by bonding covalently to a particular group at the active site.
2. A competitive inhibitor structurally resembles the substrate for a given enzyme and competes with the substrate for binding at the active site of the enzyme. A noncompetitive inhibitor binds at a site distinct from the active site and can bind to either the free enzyme or the enzyme-substrate complex.

Exercises

1. What amino acid is present in the active site of all enzymes that are irreversibly inhibited by nerve gases such as DIFP?
2. Oxaloacetate (OOCCH2COCOO) inhibits succinate dehydrogenase. Would you expect oxaloacetate to be a competitive or noncompetitive inhibitor? Explain.

Answer

1. serine

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5.7: Regulation of Enzymatic activity

Enzymes can be regulated by changing the activity of a preexisting enzyme or changing the amount of an enzyme.

A. Changing the activity of a pre-existing enzyme

The quickest way to modulate the activity of an enzyme is to alter the activity of an enzyme that already exists in the cell. The list below, illustrated in the following figure, gives common ways to regulate enzyme activity

1. **Substrate availability:** Substrates (reactants) bind to enzymes with a characteristic affinity, characterized by a kinetic parameter Km. If the actual concentration of a substrate in a cell is much less than the Km, the activity of the enzyme is very low. If the substrate concentration is much greater than Km, the enzyme active site is saturated with substrate and the enzyme is maximally active.
2. **Product inhibition**: A product of an enzyme-catalyzed reaction often resembles a starting reactant, so it should be clear that the product should also bind to the active site, albeit probably with lower affinity (competitive inhibitor). Under conditions in which the product of a reaction is present in high concentration, it would be energetically advantageous to the cell if no more product was synthesized. Product inhibition is hence commonly observed. Likewise it be energetically advantageous to a cell if the end product of an entire pathway could likewise bind to the initial enzyme in the pathways and inhibit it, allowing the whole pathway to be inhibited. This type of feedback inhibition is commonly observed.
3. **Allosteric regulation:** Molecules that bind to sites on target enzymes other than the active site (allosteric sites) can regulate the activity of the target enzyme (in the same way as noncompetitive inhibitor). These molecules can be structurally dissimilar to those that bind at the active site. They do so my conformational changes which can either activate or inhibit the target enzyme's activity. One type of very common allosteric regulation is **feedback inhibition**. In this type of allosteric regulation, the end product of a metabolic pathways inhibits the first enzyme in the pathway by binding to an allosteric site.



Figure: Feedback inhibition vs product inhibition.

3. **Covalent modification:** Many if not most proteins are subjected to post-translational modifications which can affect enzyme activity through local or global shape changes, by promoting or inhibiting binding interaction of substrates and allosteric regulators, and even by changing the location of the protein within the cell. Proteins may be phosphorylated, acetylated, methylated, sulfated, glycosylated, hydroxylated,etc., often in a reversible fashion. Some of these modifications are reversible. Regulation by phosphorylation through the action of kinases, and dephosphorylation by phosphates is extremely common. Control of phosphorylation state is mediated through signal transduction process starting at the cell membrane, leading to the activation or inhibition of protein kinases and phosphatases within the cell.

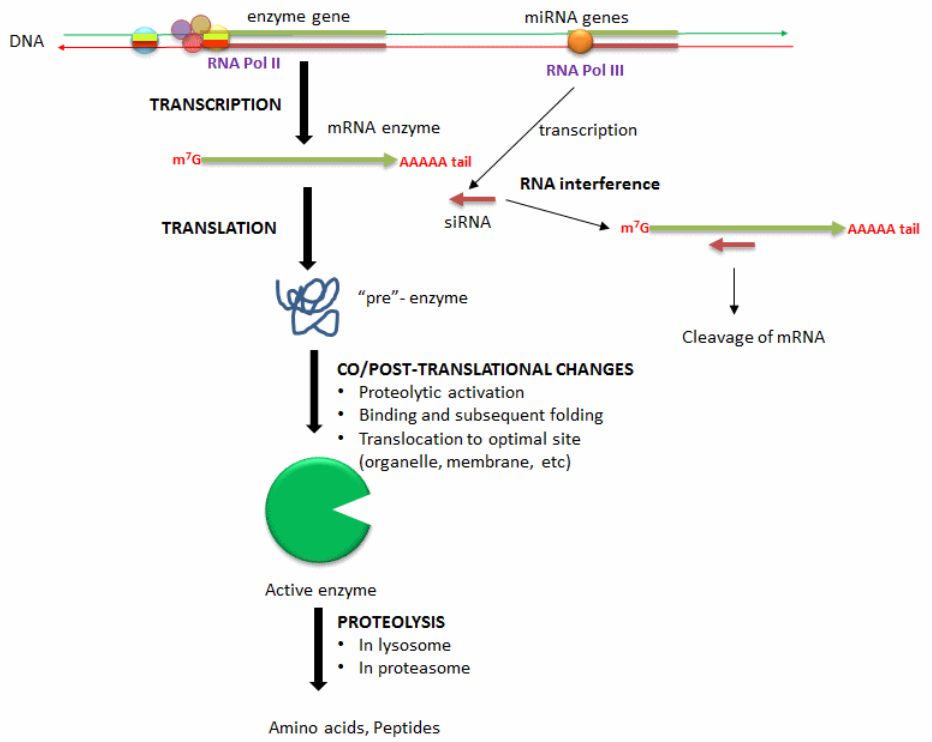
B. Changing the amount of an enzyme (genetic control)

Another and less immediate but longer duration method to modulate the activity of an enzyme is to alter the activity of an enzyme that already exists in the cell. The list below, illustrated in the following figure, shows way in which enzyme concentration is regulated.

1. **Alternation in the transcription of enzyme's gene:** Extracellular signal (hormones, neurotransmitters, etc) can lead to signal transductions responses and ultimate activation or inhibition of the transcription of the gene for a protein enzyme. These changes result from recruitment of transcription factors (proteins) to DNA sequences that regulate transcription of the enzyme gene.
2. **Degradation of messenger RNA for the enzyme:** The levels of messenger RNA for a protein will directly determine the amount of that protein synthesized. Small inhibitor RNAs, derived from microRNA molecules transcribed from cellular DNA,

can bind to specific sequences in the mRNA of a target enzyme. The resulting double-stranded RNA complex recruits an enzyme (Dicer) that cleaves the complex with the effect of decreasing translation of the protein enzyme from its mRNA.

3. **Post-translational changes:** Once a protein enzymes is translated from its mRNA, it can undergo a changes to affect enzyme levels. Some proteins are synthesized in a "pre-form which must be cleaved in a targeted and limited fashion by proteases to active the protein enzyme (proteolytic activation). This type of enzymes are called **zymogens.** The precursor is often called a **proenyzme**. Examples of zymogens are enzymes involved in protein degradation (proteases) and programmed cell death (apoptosis). Some proteins are not fully folded and must bind to other factors in the cell to adopt a catalytically active form. Finally, fully active protein can be fully proteolyzed by the proteasome, a complex within cells, or in lysosomes, which are organelles within cells containing proteolytic enzymes.



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5.8: Enzymes Used in Industry

Enzymes are biological molecules that catalyze (increase the rates of) chemical reactions.

Learning Objectives

- Describe the use of enzymes in industry

Key Points

- Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.
- Synthetic molecules called artificial enzymes also display enzyme-like catalysis.
- Enzymes are used in the chemical industry and other industrial applications when extremely specific catalysts are required.

Enzymes are used in the chemical industry and other industrial applications when extremely specific catalysts are required. However, enzymes in general are limited in the number of reactions they have evolved to catalyze, and by their lack of stability in organic solvents and at high temperatures. As a consequence, protein engineering is an active area of research and involves attempts to create new enzymes with novel properties, either through rational design or in vitro evolution. These efforts have begun to be successful, and a few enzymes have now been designed “from scratch” to catalyze reactions that do not occur in nature.

In food processing, the enzymes used include amylases from fungi and plants. These enzymes are used in the production of sugars from starch, such as in making high-fructose corn syrup. In baking, they catalyze the breakdown of starch in the flour to sugar. Yeast fermentation of sugar produces the carbon dioxide that raises the dough. Proteases are used by biscuit manufacturers to lower the protein level of flour. Trypsin is used to predigest baby foods. For the processing of fruit juices, cellulases and pectinases are used to clarify fruit juices. Papain is used to tenderize meat for cooking.

In the dairy industry, rennin, derived from the stomachs of young ruminant animals (like calves and lambs) is used to manufacture of cheese, used to hydrolyze protein. Lipases are implemented during the production of Roquefort cheese to enhance the ripening of the blue-mold cheese. Lactases are used to break down lactose to glucose and galactose.

In the brewing industry, enzymes from barley are released during the mashing stage of beer production. They degrade starch and proteins to produce simple sugar, amino acids, and peptides that are used by yeast for fermentation. Industrially-produced barley enzymes are widely used in the brewing process to substitute for the natural enzymes found in barley. Amylase, glucanases, and proteases are used to split polysaccharides and proteins in the malt. Betaglucanases and arabinoxylanases are used to improve the wort and beer filtration characteristics. Amyloglucosidase and pullulanases are used for low-calorie beer and adjustment of fermentability. Proteases are used to remove cloudiness produced during storage of beers.

In the starch industry, amylases, amyloglucosideases, and glucoamylases convert starch into glucose and various syrups. Glucose isomerase converts glucose into fructose in production of high-fructose syrups from starchy materials.

In the paper industry, amylases, xylanases, cellulases, and ligninases are used to degrade starch to lower viscosity, aiding sizing and coating paper.

In the biofuel industry, cellulases used to break down cellulose into sugars that can be fermented (see cellulosic ethanol).

In the production of biological detergents, proteases, produced in an extracellular form from bacteria, are used in pre-soak conditions and direct liquid applications, helping with the removal of protein stains from clothes.

In molecular biology, restriction enzymes, DNA ligase, and polymerases are used to manipulate DNA in genetic engineering, important in pharmacology, agriculture and medicine, and are essential for restriction digestion and the polymerase chain reaction. Molecular biology is also important in forensic science.

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CHAPTER OVERVIEW

6: Nucleic Acids

The blueprint for the reproduction and the maintenance of each organism is found in the nuclei of its cells, concentrated in elongated, threadlike structures called chromosomes. These complex structures, consisting of DNA and proteins, contain the basic units of heredity, called genes. The number of chromosomes (and genes) varies with each species. Human body cells have 23 pairs of chromosomes having 20,000–40,000 different genes.

6.1: Prelude to Nucleic Acids
6.2: Nucleotides
6.3: Nucleic Acid Structure
6.4: Genomic DNA in Prokaryotes and Eukaryotes
6.5: Eukaryotic Chromosomal Structure and Compaction
6.6: DNA Replication in Prokaryotes
6.7: DNA Replication in Eukaryotes
6.8: DNA Repair
6.9: The Polymerase Chain Reaction (PCR)
6.10: Structure and Function of RNA
6.11: Transcription
6.12: Translation
6.13: Mutations and Genetic Diseases
6.14: Viruses
6.15: DNA Sequencing

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6.1: Prelude to Nucleic Acids

Following the initial isolation of insulin in 1921, diabetic patients could be treated with insulin obtained from the pancreases of cattle and pigs. Unfortunately, some patients developed an allergic reaction to this insulin because its amino acid sequence was not identical to that of human insulin. In the 1970s, an intense research effort began that eventually led to the production of genetically engineered human insulin—the first genetically engineered product to be approved for medical use. To accomplish this feat, researchers first had to determine how insulin is made in the body and then find a way of causing the same process to occur in nonhuman organisms, such as bacteria or yeast cells. Many aspects of these discoveries are presented in this chapter on nucleic acids.



Figure **6.1.1**: A vial of insulin. It has been given a trade name, Actrapid, by the manufacturer. (Public Domain; Mr Hyde).

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6.2: Nucleotides

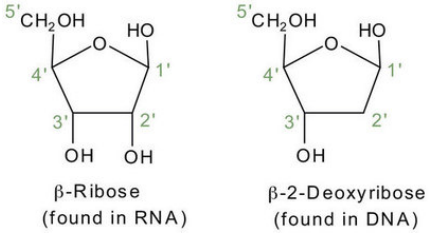
Learning Objectives

- To identify the different molecules that combine to form nucleotides.

The repeating, or monomer, units that are linked together to form nucleic acids are known as nucleotides. The deoxyribonucleic acid (DNA) of a typical mammalian cell contains about 3×10^9 nucleotides. Nucleotides can be further broken down to phosphoric acid (H_3PO_4), a pentose sugar (a sugar with five carbon atoms), and a nitrogenous base (a base containing nitrogen atoms).

$$\text{nucleic acids} \xrightarrow[\text{down into}]{\text{can be broken}} \text{nucleotides} \xrightarrow[\text{down into}]{\text{can be broken}} H_3PO_4 + \text{nitrogen base} + \text{pentose sugar}$$

If the pentose sugar is ribose, the nucleotide is more specifically referred to as a *ribonucleotide*, and the resulting nucleic acid is ribonucleic acid (RNA). If the sugar is 2-deoxyribose, the nucleotide is a *deoxyribonucleotide*, and the nucleic acid is DNA.



The nitrogenous bases found in nucleotides are classified as pyrimidines or purines. Pyrimidines are heterocyclic amines with two nitrogen atoms in a six-member ring and include uracil, thymine, and cytosine. Purines are heterocyclic amines consisting of a pyrimidine ring fused to a five-member ring with two nitrogen atoms. Adenine and guanine are the major purines found in nucleic acids (Figure **6.2.1**).

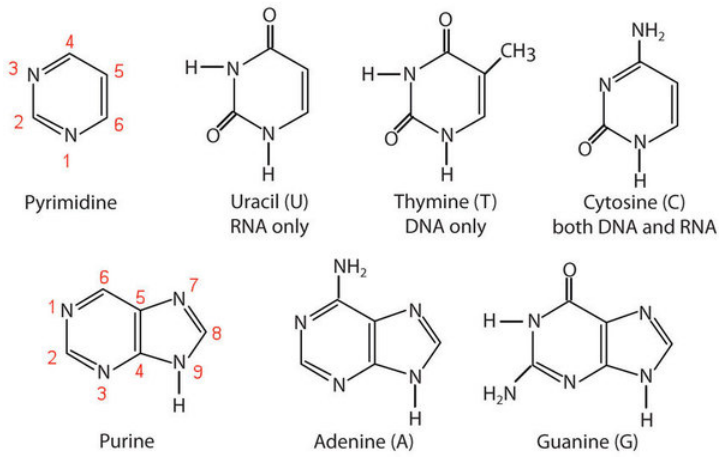


Figure **6.2.1**: The Nitrogenous Bases Found in DNA and RNA

The formation of a bond between C1′ of the pentose sugar and N1 of the pyrimidine base or N9 of the purine base joins the pentose sugar to the nitrogenous base. In the formation of this bond, a molecule of water is removed. Table **6.2.1** summarizes the similarities and differences in the composition of nucleotides in DNA and RNA.

> The numbering convention is that primed numbers designate the atoms of the pentose ring, and unprimed numbers designate the atoms of the purine or pyrimidine ring.

Table **6.2.1**: Composition of Nucleotides in DNA and RNA

Composition	DNA	RNA
purine bases	adenine and guanine	adenine and guanine
pyrimidine bases	cytosine and thymine	cytosine and uracil
pentose sugar	2-deoxyribose	ribose
inorganic acid	phosphoric acid (H_3PO_4)	H_3PO_4

The names and structures of the major ribonucleotides and one of the deoxyribonucleotides are given in Figure **6.2.2**.

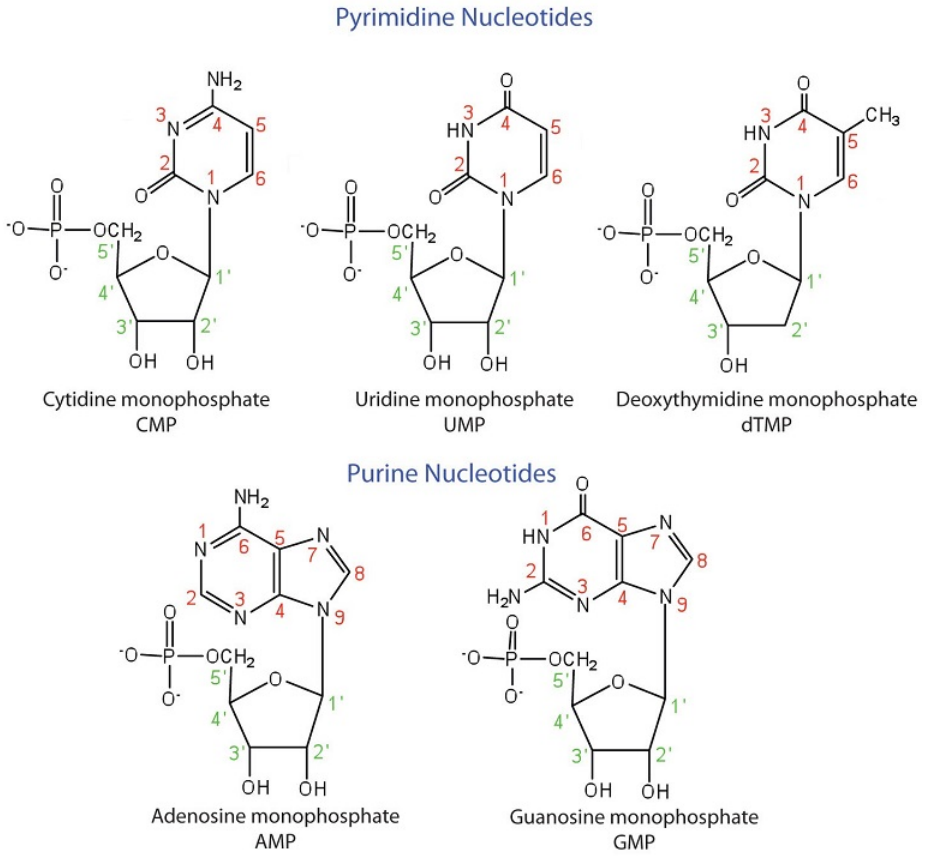


Figure **6.2.2**: The Pyrimidine and Purine Nucleotides

Apart from being the monomer units of DNA and RNA, the nucleotides and some of their derivatives have other functions as well. Adenosine diphosphate (ADP) and adenosine triphosphate (ATP), shown in Figure **6.2.3**, have a role in cell metabolism. Moreover, a number of coenzymes, including flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD^+), and coenzyme A, contain adenine nucleotides as structural components.

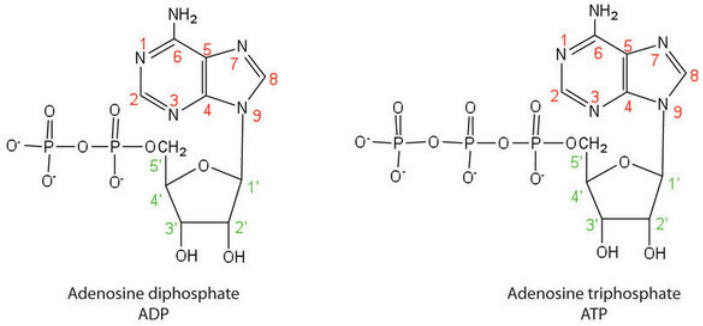


Figure **6.2.3**: Structures of Two Important Adenine-Containing Nucleotides

Summary

Nucleotides are composed of phosphoric acid, a pentose sugar (ribose or deoxyribose), and a nitrogen-containing base (adenine, cytosine, guanine, thymine, or uracil). Ribonucleotides contain ribose, while deoxyribonucleotides contain deoxyribose.

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6.3: Nucleic Acid Structure

Skills to Develop

- Identify the two types of nucleic acids and the function of each type.
- Describe how nucleotides are linked together to form nucleic acids.
- Describe the secondary structure of DNA and the importance of complementary base pairing.

Nucleic acids are large polymers formed by linking nucleotides together and are found in every cell. Deoxyribonucleic acid (DNA) is the nucleic acid that stores genetic information. If all the DNA in a typical mammalian cell were stretched out end to end, it would extend more than 2 m. Ribonucleic acid (RNA) is the nucleic acid responsible for using the genetic information encoded in DNA to produce the thousands of proteins found in living organisms.

Primary Structure of Nucleic Acids

Nucleotides are joined together through the phosphate group of one nucleotide connecting in an ester linkage to the OH group on the third carbon atom of the sugar unit of a second nucleotide. This unit joins to a third nucleotide, and the process is repeated to produce a long nucleic acid chain (Figure **6.3.1**). The backbone of the chain consists of alternating phosphate and sugar units (2-deoxyribose in DNA and ribose in RNA). The purine and pyrimidine bases branch off this backbone.

> *Each phosphate group has one acidic hydrogen atom that is ionized at physiological pH. This is why these compounds are known as nucleic acids.*

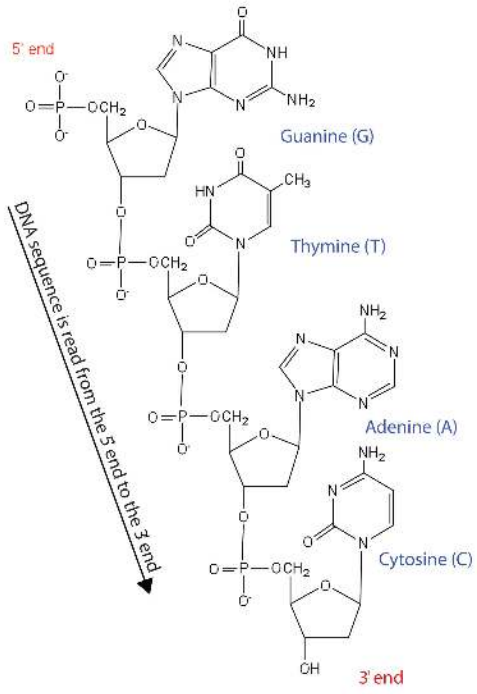


***Figure* 6.3.1** *Structure of a Segment of DNA. A similar segment of RNA would have OH groups on each C2', and uracil would replace thymine.*

Like proteins, nucleic acids have a primary structure that is defined as the sequence of their nucleotides. Unlike proteins, which have 20 different kinds of amino acids, there are only 4 different kinds of nucleotides in nucleic acids. For amino acid sequences in proteins, the convention is to write the amino acids in order starting with the N-terminal amino acid. In writing nucleotide sequences for nucleic acids, the convention is to write the nucleotides (usually using the one-letter abbreviations for the bases, shown in Figure **6.3.1**) starting with the nucleotide having a free phosphate group, which is known as the 5′ end, and indicate the nucleotides in order. For DNA, a lowercase *d* is often written in front of the sequence to indicate that the monomers are

deoxyribonucleotides. The final nucleotide has a free OH group on the 3′ carbon atom and is called the *3′ end*. The sequence of nucleotides in the DNA segment shown in Figure **6.3.1** would be written 5′-dG-dT-dA-dC-3′, which is often further abbreviated to dGTAC or just GTAC.

Secondary Structure of DNA

The three-dimensional structure of DNA was the subject of an intensive research effort in the late 1940s to early 1950s. Initial work revealed that the polymer had a regular repeating structure. In 1950, Erwin Chargaff of Columbia University showed that the molar amount of adenine (A) in DNA was always equal to that of thymine (T). Similarly, he showed that the molar amount of guanine (G) was the same as that of cytosine (C). Chargaff drew no conclusions from his work, but others soon did.

At Cambridge University in 1953, James D. Watson and Francis Crick announced that they had a model for the secondary structure of DNA. Using the information from Chargaff's experiments (as well as other experiments) and data from the X ray studies of Rosalind Franklin (which involved sophisticated chemistry, physics, and mathematics), Watson and Crick worked with models that were not unlike a child's construction set and finally concluded that DNA is composed of two nucleic acid chains running antiparallel to one another—that is, side-by-side with the 5′ end of one chain next to the 3′ end of the other. Moreover, as their model showed, the two chains are twisted to form a double helix—a structure that can be compared to a spiral staircase, with the phosphate and sugar groups (the backbone of the nucleic acid polymer) representing the outside edges of the staircase. The purine and pyrimidine bases face the inside of the helix, with guanine always opposite cytosine and adenine always opposite thymine. These specific base pairs, referred to as complementary bases, are the steps, or treads, in our staircase analogy (Figure **6.3.2**).

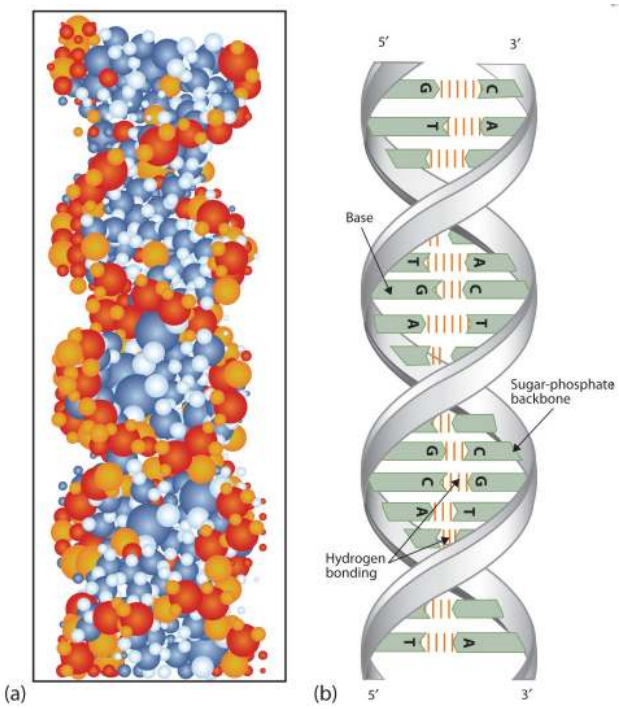


***Figure* 6.3.2** *DNA Double Helix. (a) This represents a computer-generated model of the DNA double helix. (b) This represents a schematic representation of the double helix, showing the complementary bases.*

The structure proposed by Watson and Crick provided clues to the mechanisms by which cells are able to divide into two identical, functioning daughter cells; how genetic data are passed to new generations; and even how proteins are built to required specifications. All these abilities depend on the pairing of complementary bases. Figure **6.3.3** shows the two sets of base pairs and illustrates two things. First, a pyrimidine is paired with a purine in each case, so that the long dimensions of both pairs are identical (1.08 nm).

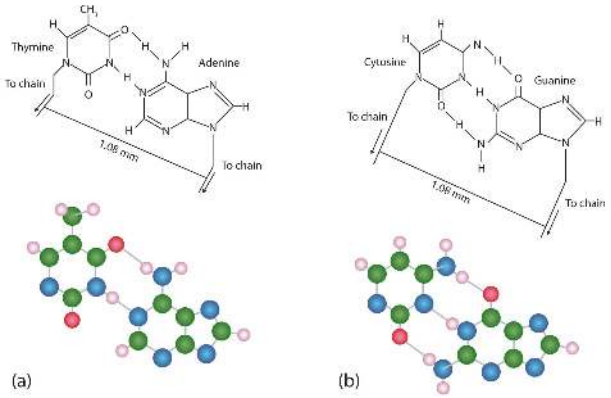


***Figure* 6.3.3** *Complementary Base Pairing. Complementary bases engage in hydrogen bonding with one another: (a) thymine and adenine; (b) cytosine and guanine.*

If two pyrimidines were paired or two purines were paired, the two pyrimidines would take up less space than a purine and a pyrimidine, and the two purines would take up more space, as illustrated in Figure **6.3.4**. If these pairings were ever to occur, the structure of DNA would be like a staircase made with stairs of different widths. For the two strands of the double helix to fit neatly, a pyrimidine must always be paired with a purine. The second thing you should notice in Figure **6.3.3** is that the correct pairing enables formation of three instances of hydrogen bonding between guanine and cytosine and two between adenine and thymine. The additive contribution of this hydrogen bonding imparts great stability to the DNA double helix.

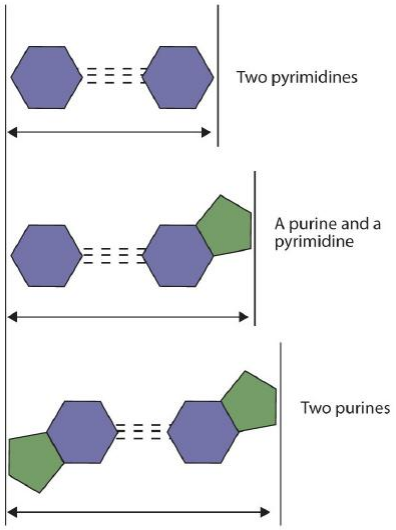


***Figure* 6.3.4** *Difference in Widths of Possible Base Pairs*

Summary

- DNA is the nucleic acid that stores genetic information. RNA is the nucleic acid responsible for using the genetic information in DNA to produce proteins.
- Nucleotides are joined together to form nucleic acids through the phosphate group of one nucleotide connecting in an ester linkage to the OH group on the third carbon atom of the sugar unit of a second nucleotide.
- Nucleic acid sequences are written starting with the nucleotide having a free phosphate group (the 5′ end).
- Two DNA strands link together in an antiparallel direction and are twisted to form a double helix. The nitrogenous bases face the inside of the helix. Guanine is always opposite cytosine, and adenine is always opposite thymine.

Concept Review Exercises

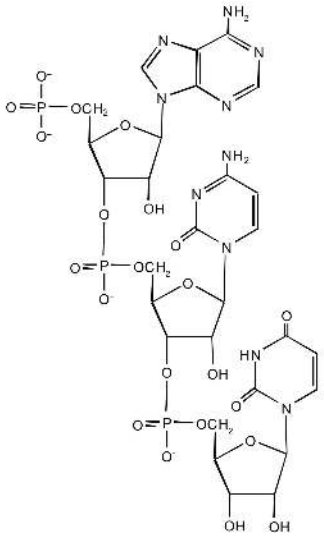
1.
   a. Name the two kinds of nucleic acids.
   b. Which type of nucleic acid stores genetic information in the cell?
2. What are complementary bases?
3. Why is it structurally important that a purine base always pair with a pyrimidine base in the DNA double helix?

Answers

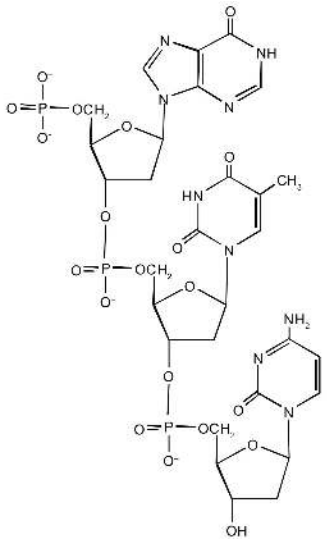
1.
   a. deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)
   b. DNA
2. the specific base pairings in the DNA double helix in which guanine is paired with cytosine and adenine is paired with thymine
3. The width of the DNA double helix is kept at a constant width, rather than narrowing (if two pyrimidines were across from each other) or widening (if two purines were across from each other).

Exercises

1. For this short RNA segment,
   a. identify the 5′ end and the 3′ end of the molecule.
   b. circle the atoms that comprise the backbone of the nucleic acid chain.
   c. write the nucleotide sequence of this RNA segment.



2. For this short DNA segment,
   a. identify the 5′ end and the 3′ end of the molecule.
   b. circle the atoms that comprise the backbone of the nucleic acid chain.
   c. write the nucleotide sequence of this DNA segment.



3. Which nitrogenous base in DNA pairs with each nitrogenous base?

   a. cytosine
   b. adenine
   c. guanine
   d. thymine

4. Which nitrogenous base in RNA pairs with each nitrogenous base?

   a. cytosine
   b. adenine
   c. guanine
   d. thymine

5. How many hydrogen bonds can form between the two strands in the short DNA segment shown below?

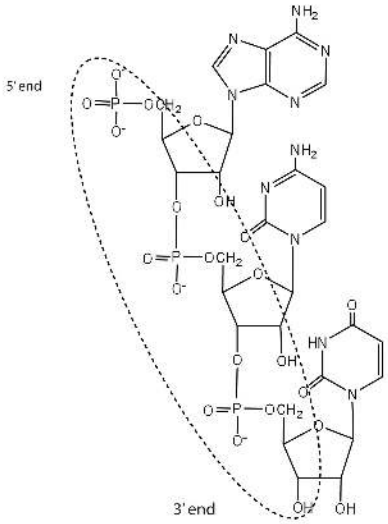
   5′ ATGCGACTA 3′ 3′ TACGCTGAT 5′

6. How many hydrogen bonds can form between the two strands in the short DNA segment shown below?

   5′ CGATGAGCC 3′ 3′ GCTACTCGG 5′

Answers

1.



c. ACU

3.

a. guanine
b. thymine
c. cytosine
d. adenine

5. 22 (2 between each AT base pair and 3 between each GC base pair)

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6.4: Genomic DNA in Prokaryotes and Eukaryotes

The genome of an organism consists of its entire complement of DNA, which encodes the genes that control the organism's characteristics.

Learning Objectives

- Explain the importance of a genome to an organism

Genomic DNA

Before discussing the steps a cell must undertake to replicate, a deeper understanding of the structure and function of a cell's genetic information is necessary. A cell's DNA, packaged as a double-stranded DNA molecule, is called its genome. In prokaryotes, the genome is composed of a single, double-stranded DNA molecule in the form of a loop or circle. The region in the cell containing this genetic material is called a nucleoid. Some prokaryotes also have smaller loops of DNA called plasmids that are not essential for normal growth. Bacteria can exchange these plasmids with other bacteria, sometimes receiving beneficial new genes that the recipient can add to their chromosomal DNA. Antibiotic resistance is one trait that often spreads through a bacterial colony through plasmid exchange.

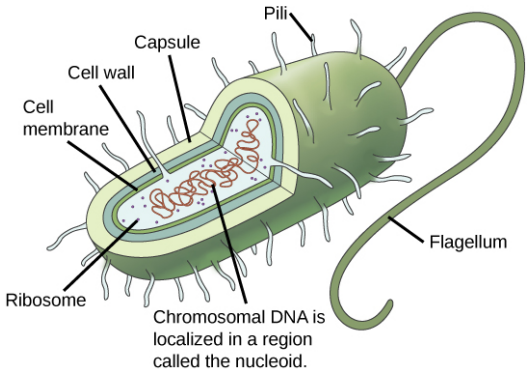


Figure **6.4.1**: **Prokaryotic Genome**: Prokaryotes, including bacteria and archaea, have a single, circular chromosome located in a central region called the nucleoid.

In eukaryotes, the genome consists of several double-stranded linear DNA molecules packaged into chromosomes. Each species of eukaryotes has a characteristic number of chromosomes in the nuclei of its cells. Human body cells have 46 chromosomes, while human gametes (sperm or eggs) have 23 chromosomes each. A typical body cell, or somatic cell, contains two matched sets of chromosomes, a configuration known as diploid. The letter n is used to represent a single set of chromosomes; therefore, a diploid organism is designated 2n. Human cells that contain one set of chromosomes are called gametes, or sex cells; these are eggs and sperm, and are designated 1n, or haploid.

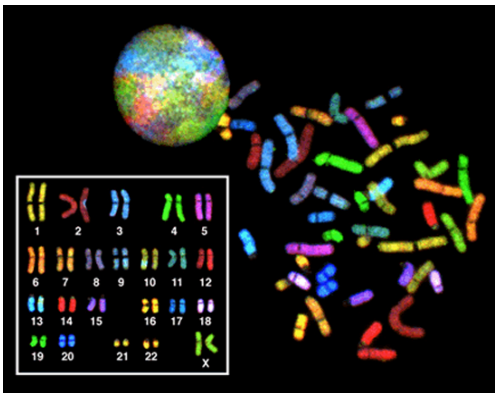


Figure 6.4.1: **Eukaryotic Genome**: There are 23 pairs of homologous chromosomes in a female human somatic cell. The condensed chromosomes are viewed within the nucleus (top), removed from a cell in mitosis and spread out on a slide (right), and artificially arranged according to length (left); an arrangement like this is called a karyotype. In this image, the chromosomes were exposed to fluorescent stains for differentiation of the different chromosomes. A method of staining called "chromosome painting" employs fluorescent dyes that highlight chromosomes in different colors.

Matched pairs of chromosomes in a diploid organism are called homologous ("same knowledge") chromosomes. Homologous chromosomes are the same length and have specific nucleotide segments called genes in exactly the same location, or locus. Genes, the functional units of chromosomes, determine specific characteristics, or traits, by coding for specific proteins. For example, hair color is a trait that can be blonde, brown, or black.

Each copy of a homologous pair of chromosomes originates from a different parent; therefore, the genes themselves are not identical. The variation of individuals within a species is due to the specific combination of the genes inherited from both parents. Even a slightly altered sequence of nucleotides within a gene can result in an alternative trait. For example, there are three possible gene sequences on the human chromosome that code for blood type: sequence A, sequence B, and sequence O. Because all diploid human cells have two copies of the chromosome that determines blood type, the blood type (the trait) is determined by which two versions of the marker gene are inherited. It is possible to have two copies of the same gene sequence on both homologous chromosomes, with one on each (for example, AA, BB, or OO), or two different sequences, such as AB, AO, or BO.

Minor variations of traits, such as blood type, eye color, and handedness, contribute to the natural variation found within a species. However, if the entire DNA sequence from any pair of human homologous chromosomes is compared, the difference is less than one percent. The sex chromosomes, X and Y, are the single exception to the rule of homologous chromosome uniformity. Other than a small amount of homology that is necessary to accurately produce gametes, the genes found on the X and Y chromosomes are different.

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6.5: Eukaryotic Chromosomal Structure and Compaction

Chromosomes must coil to pack DNA into the cell during cell division, a process involving 3 levels of compaction.

Learning Objectives

- Describe the levels of chromosomal structure and compaction

Eukaryotic Chromosomal Structure and Compaction

If the DNA from all 46 chromosomes in a human cell nucleus was laid out end to end, it would measure approximately two meters. However, the diameter would be only 2 nm. Considering that the size of a typical human cell is about 10 µm (100,000 cells lined up to equal one meter), DNA must be tightly packaged to fit in the cell's nucleus. At the same time, it must also be readily accessible for the genes to be expressed. During some stages of the cell cycle, the long strands of DNA are condensed into compact chromosomes. There are a number of ways that chromosomes are compacted to fit in the cell's nucleus and be accessible for gene expression.

In the first level of compaction, short stretches of the DNA double helix wrap around a core of eight histone proteins at regular intervals along the entire length of the chromosome. The DNA-histone complex is called chromatin. The beadlike, histone DNA complex is called a nucleosome. DNA connecting the nucleosomes is called linker DNA. A DNA molecule in this form is about seven times shorter than the double helix without the histones. The beads are about 10 nm in diameter, in contrast with the 2-nm diameter of a DNA double helix. The next level of compaction occurs as the nucleosomes and the linker DNA between them are coiled into a 30-nm chromatin fiber. This coiling further shortens the chromosome so that it is now about 50 times shorter than the extended form. In the third level of packing, a variety of fibrous proteins is used to pack the chromatin. These fibrous proteins also ensure that each chromosome in a non-dividing cell occupies a particular area of the nucleus that does not overlap with that of any other chromosome.

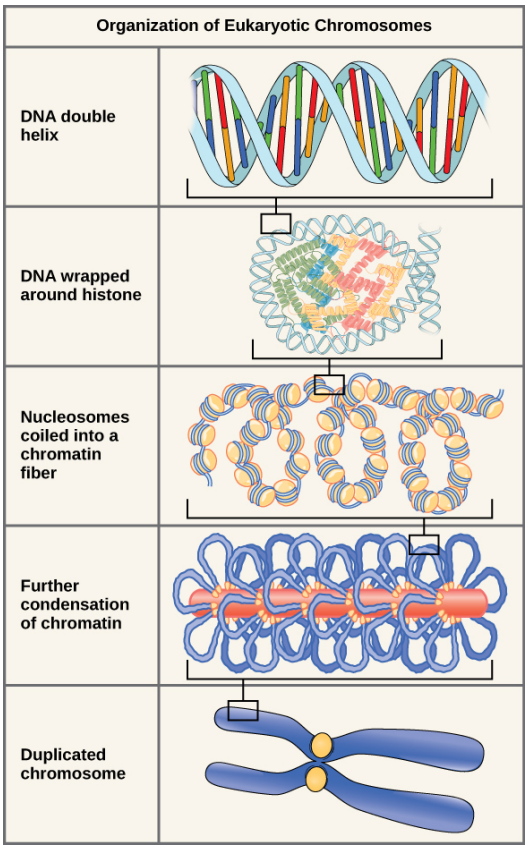


Figure **6.5.1**: **Levels of DNA Compaction**: Double-stranded DNA wraps around histone proteins to form nucleosomes that have the appearance of "beads on a string." The nucleosomes are coiled into a 30-nm chromatin fiber. When a cell undergoes mitosis, the chromosomes condense even further.

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6.6: DNA Replication in Prokaryotes

Skills to Develop

- Explain the process of DNA replication in prokaryotes
- Discuss the role of different enzymes and proteins in supporting this process

DNA replication has been extremely well studied in prokaryotes primarily because of the small size of the genome and the mutants that are available. *E. coli* has 4.6 million base pairs in a single circular chromosome and all of it gets replicated in approximately 42 minutes, starting from a single origin of replication and proceeding around the circle in both directions. This means that approximately 1000 nucleotides are added per second. The process is quite rapid and occurs without many mistakes.

DNA replication employs a large number of proteins and enzymes, each of which plays a critical role during the process. One of the key players is the enzyme DNA polymerase, also known as DNA pol, which adds nucleotides one by one to the growing DNA chain that are complementary to the template strand. The addition of nucleotides requires energy; this energy is obtained from the nucleotides that have three phosphates attached to them, similar to ATP which has three phosphate groups attached. When the bond between the phosphates is broken, the energy released is used to form the phosphodiester bond between the incoming nucleotide and the growing chain. In prokaryotes, three main types of polymerases are known: DNA pol I, DNA pol II, and DNA pol III. It is now known that DNA pol III is the enzyme required for DNA synthesis; DNA pol I and DNA pol II are primarily required for repair.

DNA replication can be divided into three stages: initiation, elongation, and termination.

Stage I- Initiation

How does the replication machinery know where to begin? It turns out that there are specific nucleotide sequences called origins of replication where replication begins. In *E. coli*, which has a single origin of replication on its one chromosome (as do most prokaryotes), it is approximately 245 base pairs long and is rich in AT sequences. The origin of replication is recognized by certain proteins that bind to this site. An enzyme called helicase unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process. As the DNA opens up, Y-shaped structures called **replication forks** are formed. Two replication forks are formed at the origin of replication and these get extended bi- directionally as replication proceeds. Single-strand binding proteins coat the single strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix.

Stage II-Elongation

DNA polymerase is able to add nucleotides only in the 5' to 3' direction (a new DNA strand can be only extended in this direction). It also requires a free 3'-OH group to which it can add nucleotides by forming a phosphodiester bond between the 3'-OH end and the 5' phosphate of the next nucleotide. This essentially means that it cannot add nucleotides if a free 3'-OH group is not available. Then how does it add the first nucleotide? The problem is solved with the help of a primer that provides the free 3'-OH end. Another enzyme, RNA primase, synthesizes an RNA primer that is about five to ten nucleotides long and complementary to the DNA. Because this sequence primes the DNA synthesis, it is appropriately called the primer. DNA polymerase can now extend this RNA primer, adding nucleotides one by one that are complementary to the template strand (Figure **6.6.1**).

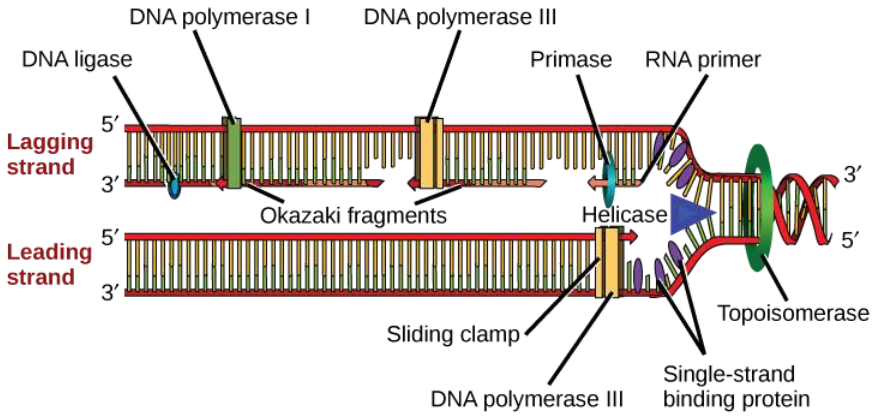


Figure **6.6.1**: A replication fork is formed when helicase separates the DNA strands at the origin of replication. The DNA tends to become more highly coiled ahead of the replication fork. Topoisomerase breaks and reforms DNA's phosphate backbone ahead of the replication fork, thereby relieving the pressure that results from this supercoiling. Single-strand binding proteins bind to the single-stranded DNA to prevent the helix from re-forming. Primase synthesizes an RNA primer. DNA polymerase III uses this primer to synthesize the daughter DNA strand. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches called Okazaki fragments. DNA polymerase I replaces the RNA primer with DNA. DNA ligase seals the gaps between the Okazaki fragments, joining the fragments into a single DNA molecule. (credit: modification of work by Mariana Ruiz Villareal)

Exercise 6.6.1

You isolate a cell strain in which the joining together of Okazaki fragments is impaired and suspect that a mutation has occurred in an enzyme found at the replication fork. Which enzyme is most likely to be mutated?

Answer

DNA ligase, as this enzyme joins together Okazaki fragments.

The replication fork moves at the rate of 1000 nucleotides per second. DNA polymerase can only extend in the 5' to 3' direction, which poses a slight problem at the replication fork. As we know, the DNA double helix is anti-parallel; that is, one strand is in the 5' to 3' direction and the other is oriented in the 3' to 5' direction. One strand, which is complementary to the 3' to 5' parental DNA strand, is synthesized continuously towards the replication fork because the polymerase can add nucleotides in this direction. This continuously synthesized strand is known as the leading strand. The other strand, complementary to the 5' to 3' parental DNA, is extended away from the replication fork, in small fragments known as Okazaki fragments, each requiring a primer to start the synthesis. Okazaki fragments are named after the Japanese scientist who first discovered them. The strand with the Okazaki fragments is known as the lagging strand.

The leading strand can be extended by one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3' to 5', and that of the leading strand 5' to 3'. A protein called the sliding clamp holds the DNA polymerase in place as it continues to add nucleotides. The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place. Topoisomerase prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it.

Stage III-Termination

As synthesis proceeds, the RNA primers are replaced by DNA. The primers are removed by the exonuclease activity of DNA pol I, and the gaps are filled in by deoxyribonucleotides. The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme DNA ligase that catalyzes the formation of phosphodiester linkage between the 3'-OH end of one nucleotide and the 5' phosphate end of the other fragment.

Once the chromosome has been completely replicated, the two DNA copies move into two different cells during cell division. The process of DNA replication can be summarized as follows.

DNA replication steps

1. DNA unwinds at the origin of replication.
2. Helicase opens up the DNA-forming replication forks; these are extended bidirectionally.
3. Single-strand binding proteins coat the DNA around the replication fork to prevent rewinding of the DNA.
4. Topoisomerase binds at the region ahead of the replication fork to prevent supercoiling.
5. Primase synthesizes RNA primers complementary to the DNA strand.
6. DNA polymerase starts adding nucleotides to the 3'-OH end of the primer.
7. Elongation of both the lagging and the leading strand continues.
8. RNA primers are removed by exonuclease activity.
9. Gaps are filled by DNA pol by adding dNTPs.
10. The gap between the two DNA fragments is sealed by DNA ligase, which helps in the formation of phosphodiester bonds.

Table **6.6.1** summarizes the enzymes involved in prokaryotic DNA replication and the functions of each.

Table 6.6.1: Prokaryotic DNA Replication: Enzymes and Their Function

Enzyme/protein	Specific Function
DNA pol I	Exonuclease activity removes RNA primer and replaces with newly synthesized DNA
DNA pol II	Repair function
DNA pol III	Main enzyme that adds nucleotides in the 5'-3' direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Sliding Clamp	Helps to hold the DNA polymerase in place when nucleotides are being added
Topoisomerase	Helps relieve the stress on DNA when unwinding by causing breaks and then resealing the DNA
Single-strand binding proteins (SSB)	Binds to single-stranded DNA to avoid DNA rewinding back.

Link to Learning: Review the full process of DNA replication here.

Summary

Replication in prokaryotes starts from a sequence found on the chromosome called the origin of replication—the point at which the DNA opens up. Helicase opens up the DNA double helix, resulting in the formation of the replication fork. Single-strand binding proteins bind to the single-stranded DNA near the replication fork to keep the fork open. Primase synthesizes an RNA primer to initiate synthesis by DNA polymerase, which can add nucleotides only in the 5' to 3' direction. One strand is synthesized continuously in the direction of the replication fork; this is called the leading strand. The other strand is synthesized in a direction away from the replication fork, in short stretches of DNA known as Okazaki fragments. This strand is known as the lagging strand. Once replication is completed, the RNA primers are replaced by DNA nucleotides and the DNA is sealed with DNA ligase, which creates phosphodiester bonds between the 3'-OH of one end and the 5' phosphate of the other strand.

Glossary

helicase

during replication, this enzyme helps to open up the DNA helix by breaking the hydrogen bonds

lagging strand

during replication, the strand that is replicated in short fragments and away from the replication fork

leading strand

strand that is synthesized continuously in the 5'-3' direction which is synthesized in the direction of the replication fork

ligase

enzyme that catalyzes the formation of a phosphodiester linkage between the 3' OH and 5' phosphate ends of the DNA

Okazaki fragment

DNA fragment that is synthesized in short stretches on the lagging strand

primase

enzyme that synthesizes the RNA primer; the primer is needed for DNA pol to start synthesis of a new DNA strand

primer

short stretch of nucleotides that is required to initiate replication; in the case of replication, the primer has RNA nucleotides

replication fork

Y-shaped structure formed during initiation of replication

single-strand binding protein

during replication, protein that binds to the single-stranded DNA; this helps in keeping the two strands of DNA apart so that they may serve as templates

sliding clamp

ring-shaped protein that holds the DNA pol on the DNA strand

topoisomerase

enzyme that causes underwinding or overwinding of DNA when DNA replication is taking place

Contributors and Attributions

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6.7: DNA Replication in Eukaryotes

Skills to Develop

- Discuss the similarities and differences between DNA replication in eukaryotes and prokaryotes
- State the role of telomerase in DNA replication

Eukaryotic genomes are much more complex and larger in size than prokaryotic genomes. The human genome has three billion base pairs per haploid set of chromosomes, and 6 billion base pairs are replicated during the S phase of the cell cycle. There are multiple origins of replication on the eukaryotic chromosome; humans can have up to 100,000 origins of replication. The rate of replication is approximately 100 nucleotides per second, much slower than prokaryotic replication. In yeast, which is a eukaryote, special sequences known as Autonomously Replicating Sequences (ARS) are found on the chromosomes. These are equivalent to the origin of replication in *E. coli*.

The number of DNA polymerases in eukaryotes is much more than prokaryotes: 14 are known, of which five are known to have major roles during replication and have been well studied. They are known as pol α, pol β, pol γ, pol δ, and pol ε.

The essential steps of replication are the same as in prokaryotes. Before replication can start, the DNA has to be made available as template. Eukaryotic DNA is bound to basic proteins known as histones to form structures called nucleosomes. The chromatin (the complex between DNA and proteins) may undergo some chemical modifications, so that the DNA may be able to slide off the proteins or be accessible to the enzymes of the DNA replication machinery. At the origin of replication, a pre-replication complex is made with other initiator proteins. Other proteins are then recruited to start the replication process (Table **6.7.1**).

A helicase using the energy from ATP hydrolysis opens up the DNA helix. Replication forks are formed at each replication origin as the DNA unwinds. The opening of the double helix causes over-winding, or supercoiling, in the DNA ahead of the replication fork. These are resolved with the action of topoisomerases. Primers are formed by the enzyme primase, and using the primer, DNA pol can start synthesis. While the leading strand is continuously synthesized by the enzyme pol δ, the lagging strand is synthesized by pol ε. A sliding clamp protein known as PCNA (Proliferating Cell Nuclear Antigen) holds the DNA pol in place so that it does not slide off the DNA. RNase H removes the RNA primer, which is then replaced with DNA nucleotides. The Okazaki fragments in the lagging strand are joined together after the replacement of the RNA primers with DNA. The gaps that remain are sealed by DNA ligase, which forms the phosphodiester bond.

Telomere replication

Unlike prokaryotic chromosomes, eukaryotic chromosomes are linear. As you've learned, the enzyme DNA pol can add nucleotides only in the 5' to 3' direction. In the leading strand, synthesis continues until the end of the chromosome is reached. On the lagging strand, DNA is synthesized in short stretches, each of which is initiated by a separate primer. When the replication fork reaches the end of the linear chromosome, there is no place for a primer to be made for the DNA fragment to be copied at the end of the chromosome. These ends thus remain unpaired, and over time these ends may get progressively shorter as cells continue to divide.

The ends of the linear chromosomes are known as telomeres, which have repetitive sequences that code for no particular gene. In a way, these telomeres protect the genes from getting deleted as cells continue to divide. In humans, a six base pair sequence, TTAGGG, is repeated 100 to 1000 times. The discovery of the enzyme telomerase (Figure **6.7.1**) helped in the understanding of how chromosome ends are maintained. The telomerase enzyme contains a catalytic part and a built-in RNA template. It attaches to the end of the chromosome, and complementary bases to the RNA template are added on the 3' end of the DNA strand. Once the 3' end of the lagging strand template is sufficiently elongated, DNA polymerase can add the nucleotides complementary to the ends of the chromosomes. Thus, the ends of the chromosomes are replicated.

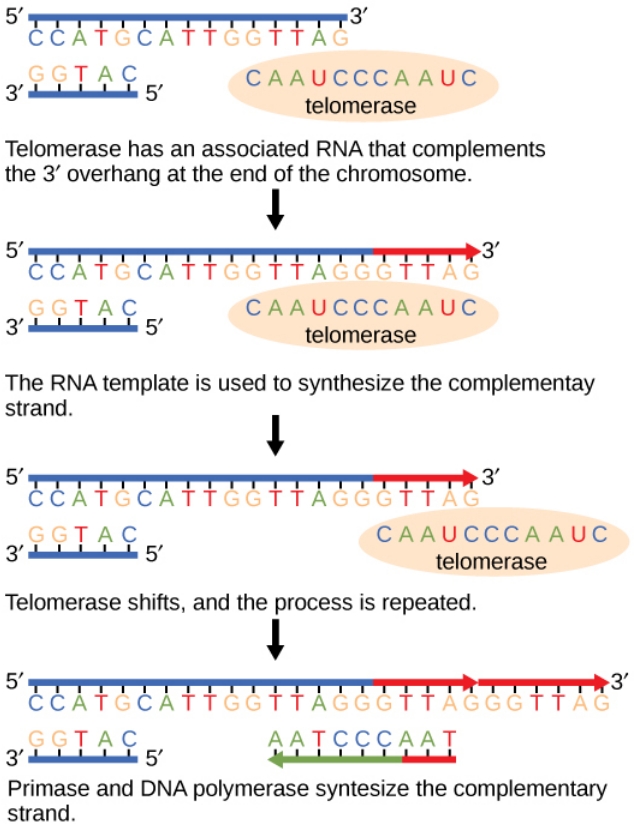


Figure **6.7.1**: The ends of linear chromosomes are maintained by the action of the telomerase enzyme.

Telomerase is typically active in germ cells and adult stem cells. It is not active in adult somatic cells. For her discovery of telomerase and its action, Elizabeth Blackburn (Figure **6.7.2**) received the Nobel Prize for Medicine and Physiology in 2009.



Figure **6.7.2**: Elizabeth Blackburn, 2009 Nobel Laureate, is the scientist who discovered how telomerase works. (credit: US Embassy Sweden)

Telomerase and Aging

Cells that undergo cell division continue to have their telomeres shortened because most somatic cells do not make telomerase. This essentially means that telomere shortening is associated with aging. With the advent of modern medicine, preventative health care, and healthier lifestyles, the human life span has increased, and there is an increasing demand for people to look younger and have a better quality of life as they grow older.

In 2010, scientists found that telomerase can reverse some age-related conditions in mice. This may have potential in regenerative medicine.[1] Telomerase-deficient mice were used in these studies; these mice have tissue atrophy, stem cell depletion, organ system failure, and impaired tissue injury responses. Telomerase reactivation in these mice caused extension of telomeres, reduced DNA damage, reversed neurodegeneration, and improved the function of the testes, spleen, and intestines. Thus, telomere reactivation may have potential for treating age-related diseases in humans.

Cancer is characterized by uncontrolled cell division of abnormal cells. The cells accumulate mutations, proliferate uncontrollably, and can migrate to different parts of the body through a process called metastasis. Scientists have observed that cancerous cells have considerably shortened telomeres and that telomerase is active in these cells. Interestingly, only after the telomeres were

shortened in the cancer cells did the telomerase become active. If the action of telomerase in these cells can be inhibited by drugs during cancer therapy, then the cancerous cells could potentially be stopped from further division.

Table **6.7.1**: Difference between Prokaryotic and Eukaryotic Replication

Property	Prokaryotes	Eukaryotes
Origin of replication	Single	Multiple
Rate of replication	1000 nucleotides/s	50 to 100 nucleotides/s
DNA polymerase types	5	14
Telomerase	Not present	Present
RNA primer removal	DNA pol I	RNase H
Strand elongation	DNA pol III	Pol δ, pol ε
Sliding clamp	Sliding clamp	PCNA

Summary

Replication in eukaryotes starts at multiple origins of replication. The mechanism is quite similar to prokaryotes. A primer is required to initiate synthesis, which is then extended by DNA polymerase as it adds nucleotides one by one to the growing chain. The leading strand is synthesized continuously, whereas the lagging strand is synthesized in short stretches called Okazaki fragments. The RNA primers are replaced with DNA nucleotides; the DNA remains one continuous strand by linking the DNA fragments with DNA ligase. The ends of the chromosomes pose a problem as polymerase is unable to extend them without a primer. Telomerase, an enzyme with an inbuilt RNA template, extends the ends by copying the RNA template and extending one end of the chromosome. DNA polymerase can then extend the DNA using the primer. In this way, the ends of the chromosomes are protected.

Footnotes

1. 1 Jaskelioff et al., “Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice,” *Nature* 469 (2011): 102-7.

Glossary

telomerase

enzyme that contains a catalytic part and an inbuilt RNA template; it functions to maintain telomeres at chromosome ends

telomere

DNA at the end of linear chromosomes

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6.8: DNA Repair

Skills to Develop

- Discuss the different types of mutations in DNA
- Explain DNA repair mechanisms

DNA replication is a highly accurate process, but mistakes can occasionally occur, such as a DNA polymerase inserting a wrong base. Uncorrected mistakes may sometimes lead to serious consequences, such as cancer. Repair mechanisms correct the mistakes. In rare cases, mistakes are not corrected, leading to mutations; in other cases, repair enzymes are themselves mutated or defective.

Most of the mistakes during DNA replication are promptly corrected by DNA polymerase by proofreading the base that has been just added (Figure **6.8.1**). In proofreading, the DNA pol reads the newly added base before adding the next one, so a correction can be made. The polymerase checks whether the newly added base has paired correctly with the base in the template strand. If it is the right base, the next nucleotide is added. If an incorrect base has been added, the enzyme makes a cut at the phosphodiester bond and releases the wrong nucleotide. This is performed by the exonuclease action of DNA pol III. Once the incorrect nucleotide has been removed, a new one will be added again.

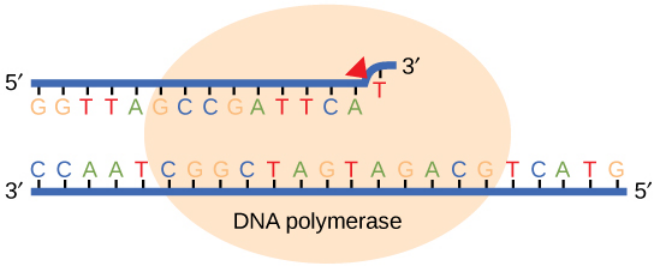


Figure **6.8.1**: Proofreading by DNA polymerase corrects errors during replication.

Some errors are not corrected during replication, but are instead corrected after replication is completed; this type of repair is known as mismatch repair (Figure **6.8.2**). The enzymes recognize the incorrectly added nucleotide and excise it; this is then replaced by the correct base. If this remains uncorrected, it may lead to more permanent damage. How do mismatch repair enzymes recognize which of the two bases is the incorrect one? In *E. coli*, after replication, the nitrogenous base adenine acquires a methyl group; the parental DNA strand will have methyl groups, whereas the newly synthesized strand lacks them. Thus, DNA polymerase is able to remove the wrongly incorporated bases from the newly synthesized, non-methylated strand. In eukaryotes, the mechanism is not very well understood, but it is believed to involve recognition of unsealed nicks in the new strand, as well as a short-term continuing association of some of the replication proteins with the new daughter strand after replication has completed.

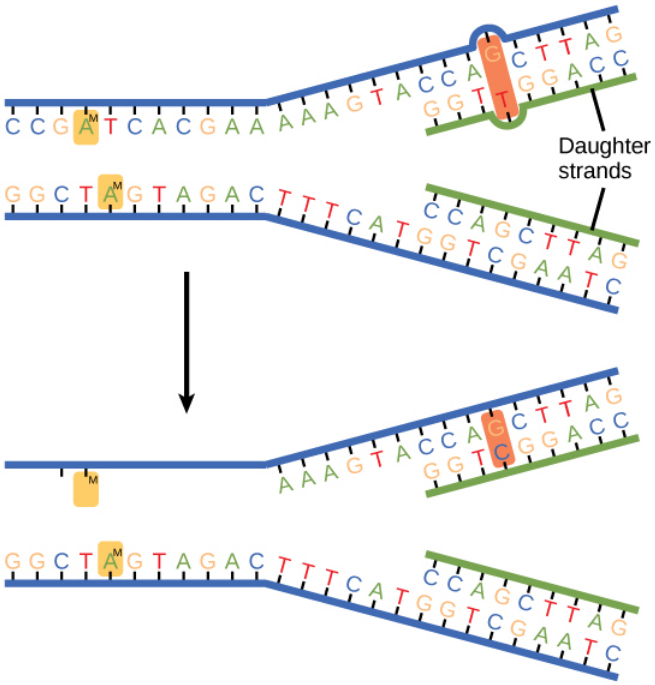


Figure 6.8.2: In mismatch repair, the incorrectly added base is detected after replication. The mismatch repair proteins detect this base and remove it from the newly synthesized strand by nuclease action. The gap is now filled with the correctly paired base.

In another type of repair mechanism, nucleotide excision repair, enzymes replace incorrect bases by making a cut on both the 3' and 5' ends of the incorrect base (Figure 6.8.3). The segment of DNA is removed and replaced with the correctly paired nucleotides by the action of DNA pol. Once the bases are filled in, the remaining gap is sealed with a phosphodiester linkage catalyzed by DNA ligase. This repair mechanism is often employed when UV exposure causes the formation of pyrimidine dimers.

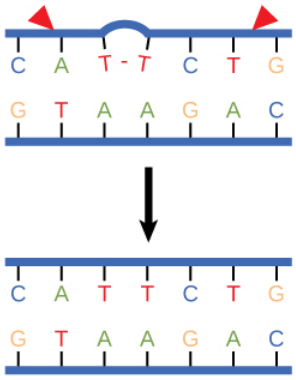


Figure 6.8.3: Nucleotide excision repairs thymine dimers. When exposed to UV, thymines lying adjacent to each other can form thymine dimers. In normal cells, they are excised and replaced.

A well-studied example of mistakes not being corrected is seen in people suffering from xeroderma pigmentosa (Figure 6.8.4). Affected individuals have skin that is highly sensitive to UV rays from the sun. When individuals are exposed to UV, pyrimidine dimers, especially those of thymine, are formed; people with xeroderma pigmentosa are not able to repair the damage. These are not repaired because of a defect in the nucleotide excision repair enzymes, whereas in normal individuals, the thymine dimers are excised and the defect is corrected. The thymine dimers distort the structure of the DNA double helix, and this may cause problems during DNA replication. People with xeroderma pigmentosa may have a higher risk of contracting skin cancer than those who dont have the condition.


Figure 6.8.4: Xeroderma pigmentosa is a condition in which thymine dimerization from exposure to UV is not repaired. Exposure to sunlight results in skin lesions. (credit: James Halpern et al.)

Errors during DNA replication are not the only reason why mutations arise in DNA. Mutations, variations in the nucleotide sequence of a genome, can also occur because of damage to DNA. Such mutations may be of two types: induced or spontaneous. Induced mutations are those that result from an exposure to chemicals, UV rays, x-rays, or some other environmental agent. Spontaneous mutations occur without any exposure to any environmental agent; they are a result of natural reactions taking place within the body.

Mutations may have a wide range of effects. Some mutations are not expressed; these are known as silent mutations. Point mutations are those mutations that affect a single base pair. The most common nucleotide mutations are substitutions, in which one base is replaced by another. These can be of two types, either transitions or transversions. Transition substitution refers to a purine or pyrimidine being replaced by a base of the same kind; for example, a purine such as adenine may be replaced by the purine guanine. Transversion substitution refers to a purine being replaced by a pyrimidine, or vice versa; for example, cytosine, a pyrimidine, is replaced by adenine, a purine. Mutations can also be the result of the addition of a base, known as an insertion, or the removal of a base, also known as deletion. Sometimes a piece of DNA from one chromosome may get translocated to another chromosome or to another region of the same chromosome; this is also known as translocation. These mutation types are shown in Figure 6.8.5.

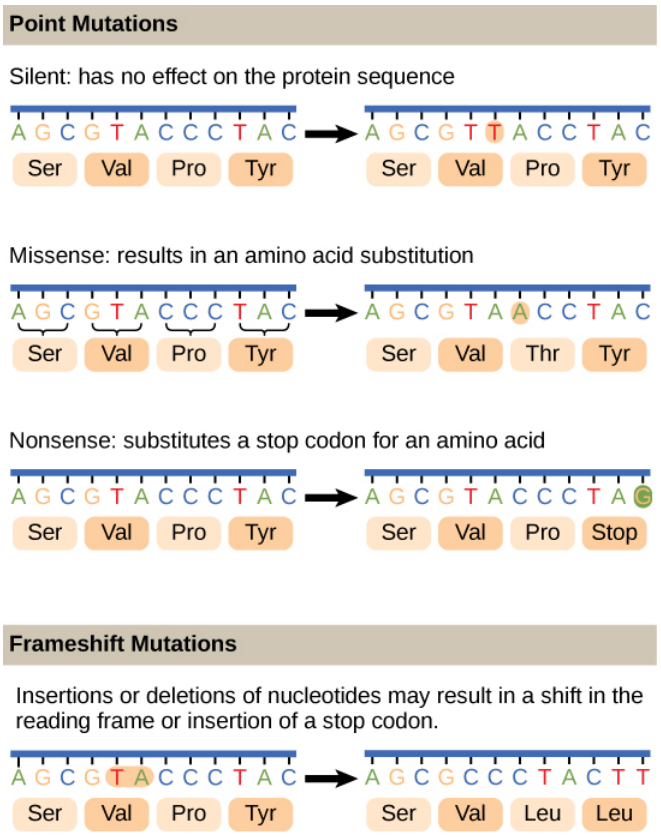


Figure 6.8.5: Mutations can lead to changes in the protein sequence encoded by the DNA.

Exercise 6.8.1

A frameshift mutation that results in the insertion of three nucleotides is often less deleterious than a mutation that results in the insertion of one nucleotide. Why?

Answer

If three nucleotides are added, one additional amino acid will be incorporated into the protein chain, but the reading frame won't shift.

Mutations in repair genes have been known to cause cancer. Many mutated repair genes have been implicated in certain forms of pancreatic cancer, colon cancer, and colorectal cancer. Mutations can affect either somatic cells or germ cells. If many mutations accumulate in a somatic cell, they may lead to problems such as the uncontrolled cell division observed in cancer. If a mutation takes place in germ cells, the mutation will be passed on to the next generation, as in the case of hemophilia and xeroderma pigmentosa.

Summary

DNA polymerase can make mistakes while adding nucleotides. It edits the DNA by proofreading every newly added base. Incorrect bases are removed and replaced by the correct base, and then a new base is added. Most mistakes are corrected during replication, although when this does not happen, the mismatch repair mechanism is employed. Mismatch repair enzymes recognize the wrongly incorporated base and excise it from the DNA, replacing it with the correct base. In yet another type of repair, nucleotide excision repair, the incorrect base is removed along with a few bases on the 5' and 3' end, and these are replaced by copying the template with the help of DNA polymerase. The ends of the newly synthesized fragment are attached to the rest of the DNA using DNA ligase, which creates a phosphodiester bond.

Most mistakes are corrected, and if they are not, they may result in a mutation defined as a permanent change in the DNA sequence. Mutations can be of many types, such as substitution, deletion, insertion, and translocation. Mutations in repair genes

may lead to serious consequences such as cancer. Mutations can be induced or may occur spontaneously.

Glossary

induced mutation
mutation that results from exposure to chemicals or environmental agents

mutation
variation in the nucleotide sequence of a genome

mismatch repair
type of repair mechanism in which mismatched bases are removed after replication

nucleotide excision repair
type of DNA repair mechanism in which the wrong base, along with a few nucleotides upstream or downstream, are removed

proofreading
function of DNA pol in which it reads the newly added base before adding the next one

point mutation
mutation that affects a single base

silent mutation
mutation that is not expressed

spontaneous mutation
mutation that takes place in the cells as a result of chemical reactions taking place naturally without exposure to any external agent

transition substitution
when a purine is replaced with a purine or a pyrimidine is replaced with another pyrimidine

transversion substitution
when a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine

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6.9: The Polymerase Chain Reaction (PCR)

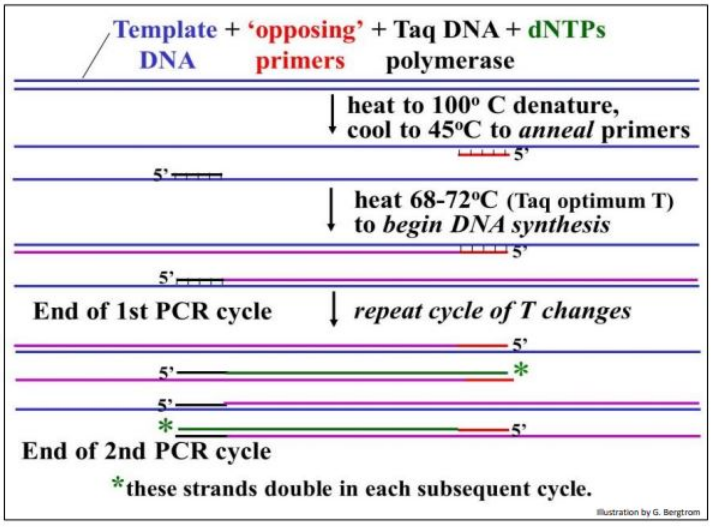
The polymerase chain reaction (PCR) can *amplify* a region of DNA from any source, even from a single cell's worth of DNA or from fragments of DNA obtained from a fossil. This amplification usually takes just a few hours, generating millions of copies of the desired *target* DNA sequence. The effect is to purify the DNA from surrounding sequences in a single reaction! Kary B. Mullis was awarded a Nobel Prize in 1993 for his development of PCR, which is now the basis of innumerable research studies of gene structure, function and evolution as well as applications in criminal forensics, medical diagnostics and other commercial uses. PCR is described in detail below.

A. PCR - the Basic Process

Typical PCR relies on knowing two bits of DNA sequence that will be used to design and synthesize short oligonucleotide sequences (*oligomers*) in the laboratory. The two oligomers are chosen to be complementary to sequences opposite strands of double-stranded DNA containing the gene to be studied. We say that the two oligomers face, or **oppose** each other. That just means that the 3' end of one oligomer faces the 3' end of the opposing oligomer. This way the two oligomers can serve as *primers* for the elongation replication of both strands of a double stranded target DNA sequence. Check out the link below for further explanation.

272 PCR: Design and Synthesize Opposing Oligonucleotide Primers

The first step in PCR is to add oligomer primers to the target DNA from which a gene (or other genomic sequence) is to be amplified. The mixture is then heated to denature the target DNA. The mixture is cooled to allow the primers to H-bond to complementary target DNA strands. Next, the four deoxynucleotide precursors to DNA (dATP, dCTP, dTTP and dGTP) are added along with a small amount of a DNA polymerase. New DNA strands will now lengthen from the oligonucleotide primers on the template DNAs. To make lots of the PCR product, this reaction cycle must be repeated many times. Therefore, after allowing elongation, the mixture is heated to denature (separate) all the DNA strands. When the mixture is again cooled, the oligomers again find complementary sequences with which to H-bond. Early versions of PCR originally relied on an *E. coli* DNA polymerase, which is inactivated by heating, and so had to be re-added to the PCR mixture for each elongation cycle. Just as with DNA sequencing, researchers very quickly switched to the heat-stable **Taq polymerase**, of *Thermus aquaticus*. The enzymes of *T. aquaticus* remain active at the very high temperatures at which these organisms live. Since heating does not destroy the Taq polymerase *in vitro*, PCR, like DNA sequencing reactions, could be automated with programmable *thermocylers* that raised and lowered temperature required by the PCR reactions. There was no longer a need to replenish a DNA polymerase once the reaction cycles were begun. Thermocyling in a typical PCR amplification is illustrated below for the first two PCR cycles, the second of which, produces the first strands of DNA that will actually be amplified exponentially.

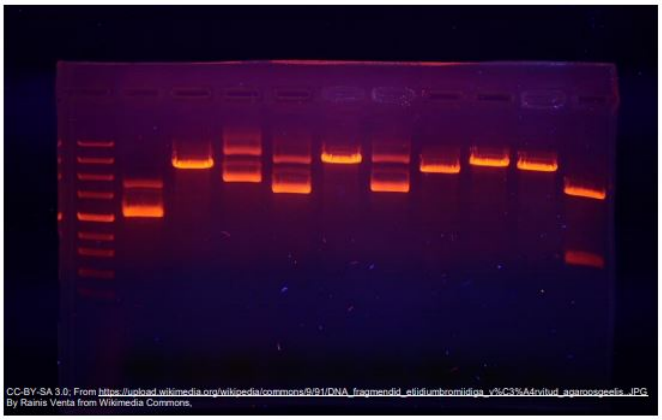


You can see from the illustration that the second cycle of PCR has generated the two DNA strands that will be templates for doubling and re-doubling the desired product after each subsequent cycle. A typical PCR reaction might involve 30 PCR cycles, resulting in a nearly exponential amplification of the desired sequence.

Challenge:

Starting with a pair of complementary target DNA molecules (after the 3rd PCR cycle), how many double stranded PCR products should you theoretically have at the end of all 30 PCR cycles?

The amplified products of PCR amplification products are in such abundance that they can easily be seen under fluorescent illumination on an ethidium bromide-stained agarose gel (below).



In this gel, the first lane (on the left) contains a *DNA ladder*, a mixture of DNAs of known lengths that can be used to estimate the size of the PCR fragments in the 3 rd and 4th lanes (the gel lane next to the ladder is empty). The two bright bands in lanes 3 and 4 are PCR products generated with two different oligomer primer pairs. PCRamplified DNAs can be sequenced and used in many subsequent studies.

B. The Many Uses of PCR

PCR-amplified products can be labeled with radioactive or fluorescent tags to serve as hybridization probes for

- screening cDNA or genomic libraries and isolation of clones.
- determining migration position on a Southern blot.
- determining migration position on a northern blot (a fanciful name for RNAs that are separated by size on gels and blotted to filter).
- and more!

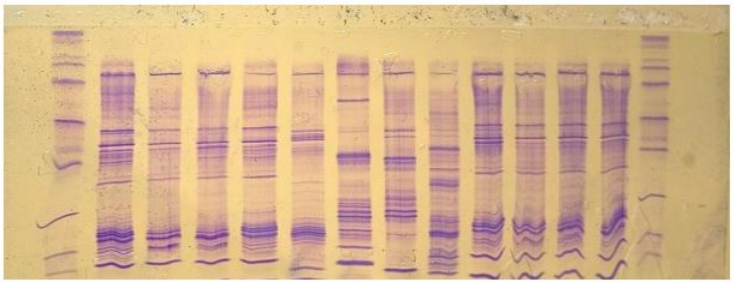
1. Quantitative PCR

We noted above that PCR has wide applications to research, medicine and other practical applications. A major advance was *Quantitative PCR*, applied to studies of differential gene expression and gene regulation. In Quantitative PCR, initial cDNAs are amplified to detect not only the presence, but also the relative amounts of specific transcripts being made in cells.

2. Forensics

Another application of PCR is in forensic science, to identify a person or organism by comparing its DNA to some standard, or control DNA. An example of one of these acrylamide gel DNA *fingerprints* is shown below.

DNA Fingerprint



Using this technology, it is now possible to detect genetic relationships between near and distant relatives (as well as to exclude such relationships), determine paternity, demonstrate evolutionary relationships between organisms, and on many occasions, solve recent and even 'cold-case' crimes. Click Sir Alec Jeffries to learn about the origins of DNA fingerprinting in real life …and on all those TV CSI programs! Check out here for a brief history of the birth of DNA fingerprinting, and to see how analysis of changes in gene activity that occur after death may even help ID criminals. For a video on DNA fingerprinting, click Alu and DNA fingerprinting. *Alu* is a highly repeated ~300bp DNA sequence found throughout the human genome. *Alu* sequences are *short interspersed elements*, or **SINES**, a retrotransposon we saw earlier. DNA fingerprinting is possible in part because each of us has a unique number and distribution of *Alu* SINEs in our genome. To read more about Alu sequences and human diversity, click Alu Sequences and Human Diversity.

Intriguing examples of the use of PCR for identification include establishing the identities of Egyptian mummies, the Russian Tsar deposed and killed during the Russian revolution (along with his family members), and the recently unearthed body of King Richard the 3rd of England. Variant PCR protocols and applications are manifold and often quite inventive! For a list, click Variations on Basic PCR.

274 The Power of PCR: Some Examples

3. Who are your Ancestors?

Tracing your ethnic, racial and regional ancestry is related to DNA fingerprinting, in that it relies on PCR amplification of genes and other DNA regions and comparison of these your sequences to distinguishing DNA markers in large sequence databases. The price of these services have come down, and as a result, their popularity has gone up in recent years. Typically, you provide spit or a salivary (buccal) swab to the service and they amplify and sequence the DNA in your samples. The analysis compares your DNA sequences to database sequences looking for patterns of ethnic and regional markers that you might share with the database(s). Based on these comparisons, you are provided with a (more…, or less) accurate map of your DNA-based ancestry. Folks who are spending around $100.00 (less when on sale!) often ask just how accurate are these analyses, and what do they actually mean. For example, what does it mean if your DNA says you are 5% native American? In fact, different services can sometimes give you different results! You can get some answers and explanations DNA Ancestry Testing.

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6.10: Structure and Function of RNA

Learning Objectives

- Describe the biochemical structure of ribonucleotides
- Describe the similarities and differences between RNA and DNA
- Describe the functions of the three main types of RNA used in protein synthesis
- Explain how RNA can serve as hereditary information

Structurally speaking, ribonucleic acid (RNA), is quite similar to DNA. However, whereas DNA molecules are typically long and double stranded, RNA molecules are much shorter and are typically single stranded. RNA molecules perform a variety of roles in the cell but are mainly involved in the process of protein synthesis (translation) and its regulation.

RNA Structure

RNA is typically single stranded and is made of ribonucleotides that are linked by phosphodiester bonds. A ribonucleotide in the RNA chain contains ribose (the pentose sugar), one of the four nitrogenous bases (A, U, G, and C), and a phosphate group. The subtle structural difference between the sugars gives DNA added stability, making DNA more suitable for storage of genetic information, whereas the relative instability of RNA makes it more suitable for its more short-term functions.

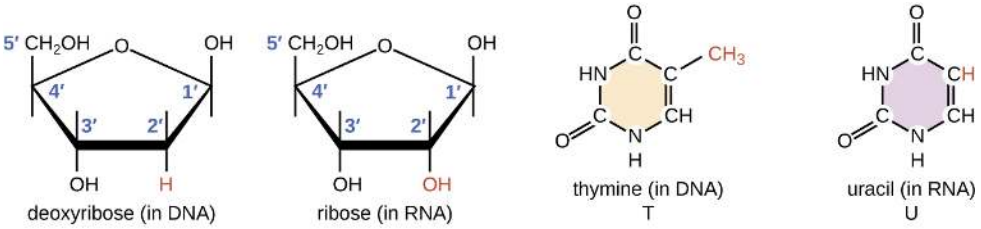


Figure **6.10.1**: (a) Ribonucleotides contain the pentose sugar ribose instead of the deoxyribose found in deoxyribonucleotides. (b) RNA contains the pyrimidine uracil in place of thymine found in DNA.

The RNA-specific pyrimidine uracil forms a complementary base pair with adenine and is used instead of the thymine used in DNA. Even though RNA is single stranded, most types of RNA molecules show extensive intramolecular base pairing between complementary sequences within the RNA strand, creating a predictable three-dimensional structure essential for their function (Figure **6.10.1** and Figure **6.10.2**).

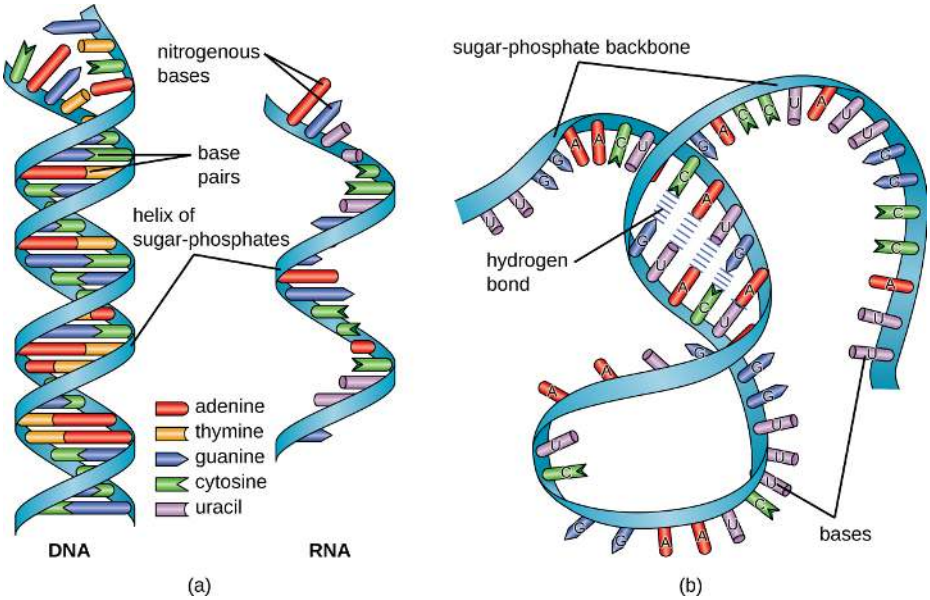


Figure **6.10.2**: (a) DNA is typically double stranded, whereas RNA is typically single stranded. (b) Although it is single stranded, RNA can fold upon itself, with the

folds stabilized by short areas of complementary base pairing within the molecule, forming a three-dimensional structure.

? Exercise 6.10.1

How does the structure of RNA differ from the structure of DNA?

Functions of RNA in Protein Synthesis

Cells access the information stored in DNA by creating RNA to direct the synthesis of proteins through the process of translation. Proteins within a cell have many functions, including building cellular structures and serving as enzyme catalysts for cellular chemical reactions that give cells their specific characteristics. The three main types of RNA directly involved in protein synthesis are messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA).

In 1961, French scientists François Jacob and Jacques Monod hypothesized the existence of an intermediary between DNA and its protein products, which they called messenger RNA.[1] Evidence supporting their hypothesis was gathered soon afterwards showing that information from DNA is transmitted to the ribosome for protein synthesis using mRNA. If DNA serves as the complete library of cellular information, mRNA serves as a photocopy of specific information needed at a particular point in time that serves as the instructions to make a protein.

The mRNA carries the message from the DNA, which controls all of the cellular activities in a cell. If a cell requires a certain protein to be synthesized, the gene for this product is "turned on" and the mRNA is synthesized through the process of transcription (see RNA Transcription). The mRNA then interacts with ribosomes and other cellular machinery (Figure **6.10.3**) to direct the synthesis of the protein it encodes during the process of translation (see Protein Synthesis). mRNA is relatively unstable and short-lived in the cell, especially in prokaryotic cells, ensuring that proteins are only made when needed.

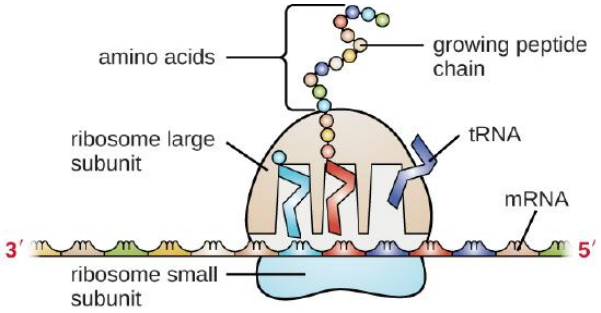


Figure **6.10.3**: A generalized illustration of how mRNA and tRNA are used in protein synthesis within a cell.

rRNA and tRNA are stable types of RNA. In prokaryotes and eukaryotes, tRNA and rRNA are encoded in the DNA, then copied into long RNA molecules that are cut to release smaller fragments containing the individual mature RNA species. In eukaryotes, synthesis, cutting, and assembly of rRNA into ribosomes takes place in the nucleolus region of the nucleus, but these activities occur in the cytoplasm of prokaryotes. Neither of these types of RNA carries instructions to direct the synthesis of a polypeptide, but they play other important roles in protein synthesis.

Ribosomes are composed of rRNA and protein. As its name suggests, rRNA is a major constituent of ribosomes, composing up to about 60% of the ribosome by mass and providing the location where the mRNA binds. The rRNA ensures the proper alignment of the mRNA, tRNA, and the ribosomes; the rRNA of the ribosome also has an enzymatic activity (peptidyl transferase) and catalyzes the formation of the peptide bonds between two aligned amino acids during protein synthesis. Although rRNA had long been thought to serve primarily a structural role, its catalytic role within the ribosome was proven in 2000.[2] Scientists in the laboratories of Thomas Steitz (1940–) and Peter Moore(1939–) at Yale University were able to crystallize the ribosome structure from *Haloarcula marismortui*, a halophilic archaeon isolated from the Dead Sea. Because of the importance of this work, Steitz shared the 2009 Nobel Prize in Chemistry with other scientists who made significant contributions to the understanding of ribosome structure.

Transfer RNA is the third main type of RNA and one of the smallest, usually only 70–90 nucleotides long. It carries the correct amino acid to the site of protein synthesis in the ribosome. It is the base pairing between the tRNA and mRNA that allows for the

correct amino acid to be inserted in the polypeptide chain being synthesized (Figure **6.10.4**). Any mutations in the tRNA or rRNA can result in global problems for the cell because both are necessary for proper protein synthesis (Table **6.10.1**).

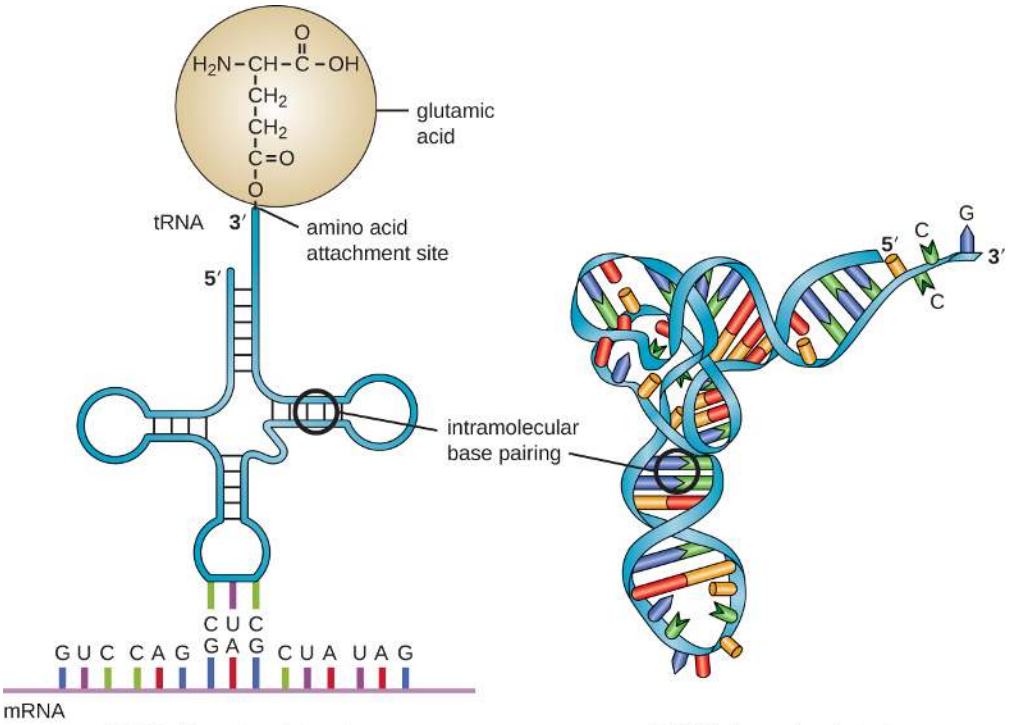


Figure **6.10.4**: A tRNA molecule is a single-stranded molecule that exhibits significant intracellular base pairing, giving it its characteristic three-dimensional shape.

Table **6.10.1**: Structure and Function of RNA

	mRNA	rRNA	tRNA
Structure	Short, unstable, single-stranded RNAcorresponding to a gene encoded within DNA	Longer, stable RNA molecules composing 60% of ribosome's mass	Short (70-90 nucleotides), stable RNA with extensive intramolecular base pairing; contains an amino acid binding site and an mRNA binding site
Function	Serves as intermediary between DNA and protein; used by ribosome to direct synthesis of protein it encodes	Ensures the proper alignment of mRNA, tRNA, and ribosome during protein synthesis; catalyzes peptide bond formation between amino acids	Carries the correct amino acid to the site of protein synthesis in the ribosome

? Exercise 6.10.1

What are the functions of the three major types of RNA molecules involved in protein synthesis?

RNA as Hereditary Information

Although RNA does not serve as the hereditary information in most cells, RNA does hold this function for many viruses that do not contain DNA. Thus, RNA clearly does have the additional capacity to serve as genetic information. Although RNA is typically single stranded within cells, there is significant diversity in viruses. Rhinoviruses, which cause the common cold; influenza viruses; and the Ebola virus are single-stranded RNA viruses. Rotaviruses, which cause severe gastroenteritis in children and other immunocompromised individuals, are examples of double-stranded RNA viruses. Because double-stranded RNA is uncommon in eukaryotic cells, its presence serves as an indicator of viral infection. The implications for a virus having an RNA genome instead of a DNA genome are discussed in more detail in Viruses.

Key Concepts and Summary

- **Ribonucleic acid (RNA)** is typically single stranded and contains ribose as its pentose sugar and the pyrimidine uracil instead of thymine. An RNA strand can undergo significant intramolecular base pairing to take on a three-dimensional structure.
- There are three main types of RNA, all involved in protein synthesis.
- Messenger RNA (**mRNA**) serves as the intermediary between DNA and the synthesis of protein products during translation.
- Ribosomal RNA (**rRNA**) is a type of stable RNA that is a major constituent of ribosomes. It ensures the proper alignment of the mRNA and the ribosomes during protein synthesis and catalyzes the formation of the peptide bonds between two aligned amino acids during protein synthesis.
- Transfer RNA (**tRNA**) is a small type of stable RNA that carries an amino acid to the corresponding site of protein synthesis in the ribosome. It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to be inserted in the polypeptide chain being synthesized.
- Although RNA is not used for long-term genetic information in cells, many viruses do use RNA as their genetic material.

Footnotes

1. 1 A. Rich. “The Era of RNA Awakening: Structural Biology of RNA in the Early Years.” *Quarterly Reviews of Biophysics* 42 no. 2 (2009):117–137.
2. 2 P. Nissen et al. “The Structural Basis of Ribosome Activity in Peptide Bond Synthesis.” *Science* 289 no. 5481 (2000):920–930.

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6.11: Transcription

In both prokaryotes and eukaryotes, the second function of DNA (the first was replication) is to provide the information needed to construct the proteins necessary so that the cell can perform all of its functions. To do this, the DNA is "read" or transcribed into an mRNA molecule. The mRNA then provides the code to form a protein by a process called translation. Through the processes of transcription and translation, a protein is built with a specific sequence of amino acids that was originally encoded in the DNA. This module discusses the details of transcription.

The Central Dogma: DNA Encodes RNA; RNA Encodes Protein

The flow of genetic information in cells from DNA to mRNA to protein is described by the central dogma (Figure 6.11.1), which states that genes specify the sequences of mRNAs, which in turn specify the sequences of proteins.

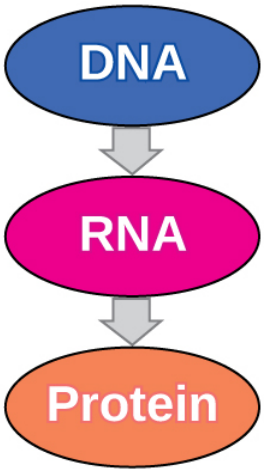


Figure 6.11.1: The central dogma states that DNA encodes RNA, which in turn encodes protein.

The copying of DNA to mRNA is relatively straightforward, with one nucleotide being added to the mRNA strand for every complementary nucleotide read in the DNA strand. The translation to protein is more complex because groups of three mRNA nucleotides correspond to one amino acid of the protein sequence. However, as we shall see in the next module, the translation to protein is still systematic, such that nucleotides 1 to 3 correspond to amino acid 1, nucleotides 4 to 6 correspond to amino acid 2, and so on.

Transcription: from DNA to mRNA

Both prokaryotes and eukaryotes perform fundamentally the same process of transcription, with the important difference of the membrane-bound nucleus in eukaryotes. With the genes bound in the nucleus, transcription occurs in the nucleus of the cell and the mRNA transcript must be transported to the cytoplasm. The prokaryotes, which include bacteria and archaea, lack membrane-bound nuclei and other organelles, and transcription occurs in the cytoplasm of the cell. In both prokaryotes and eukaryotes, transcription occurs in three main stages: initiation, elongation, and termination.

Initiation

Transcription requires the DNA double helix to partially unwind in the region of mRNA synthesis. The region of unwinding is called a transcription bubble. The DNA sequence onto which the proteins and enzymes involved in transcription bind to initiate the process is called a promoter. In most cases, promoters exist upstream of the genes they regulate. The specific sequence of a promoter is very important because it determines whether the corresponding gene is transcribed all of the time, some of the time, or hardly at all (Figure 6.11.2).

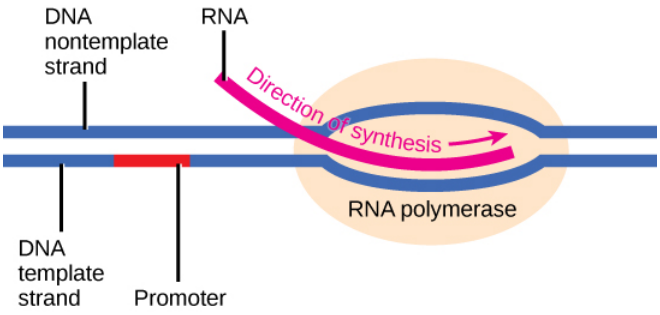


Figure 6.11.2: The initiation of transcription begins when DNA is unwound, forming a transcription bubble. Enzymes and other proteins involved in transcription bind at the promoter.

Elongation

Transcription always proceeds from one of the two DNA strands, which is called the template strand. The mRNA product is complementary to the template strand and is almost identical to the other DNA strand, called the nontemplate strand, with the exception that RNA contains a uracil (U) in place of the thymine (T) found in DNA. During elongation, an enzyme called RNA polymerase proceeds along the DNA template adding nucleotides by base pairing with the DNA template in a manner similar to DNA replication, with the difference that an RNA strand is being synthesized that does not remain bound to the DNA template. As elongation proceeds, the DNA is continuously unwound ahead of the core enzyme and rewound behind it (Figure **6.11.3**).

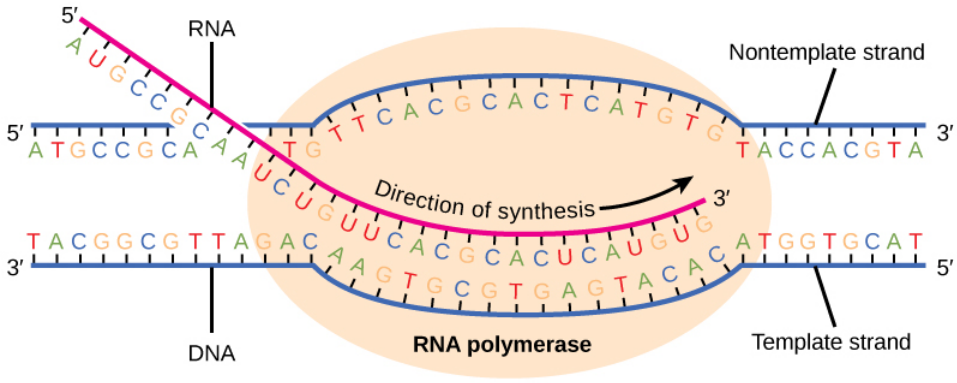


Figure 6.11.3: During elongation, RNA polymerase tracks along the DNA template, synthesizes mRNA in the 5' to 3' direction, and unwinds then rewinds the DNA as it is read.

Termination

Once a gene is transcribed, the prokaryotic polymerase needs to be instructed to dissociate from the DNA template and liberate the newly made mRNA. Depending on the gene being transcribed, there are two kinds of termination signals, but both involve repeated nucleotide sequences in the DNA template that result in RNA polymerase stalling, leaving the DNA template, and freeing the mRNA transcript.

On termination, the process of transcription is complete. In a prokaryotic cell, by the time termination occurs, the transcript would already have been used to partially synthesize numerous copies of the encoded protein because these processes can occur concurrently using multiple ribosomes (polyribosomes) (Figure **6.11.4**). In contrast, the presence of a nucleus in eukaryotic cells precludes simultaneous transcription and translation.

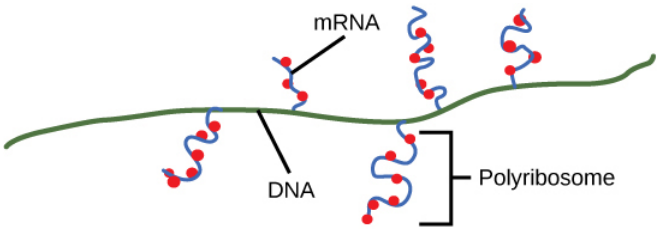


Figure 6.11.4: Multiple polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

Eukaryotic RNA Processing

The newly transcribed eukaryotic mRNAs must undergo several processing steps before they can be transferred from the nucleus to the cytoplasm and translated into a protein. The additional steps involved in eukaryotic mRNA maturation create a molecule that is much more stable than a prokaryotic mRNA. For example, eukaryotic mRNAs last for several hours, whereas the typical prokaryotic mRNA lasts no more than five seconds.

The mRNA transcript is first coated in RNA-stabilizing proteins to prevent it from degrading while it is processed and exported out of the nucleus. This occurs while the pre-mRNA still is being synthesized by adding a special nucleotide "cap" to the 5' end of the growing transcript. In addition to preventing degradation, factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes.

Once elongation is complete, an enzyme then adds a string of approximately 200 adenine residues to the 3' end, called the poly-A tail. This modification further protects the pre-mRNA from degradation and signals to cellular factors that the transcript needs to be exported to the cytoplasm.

Eukaryotic genes are composed of protein-coding sequences called exons (*ex*-on signifies that they are *ex*pressed) and *int*ervening sequences called introns (*int*-ron denotes their *int*ervening role). Introns are removed from the pre-mRNA during processing. Intron sequences in mRNA do not encode functional proteins. It is essential that all of a pre-mRNA's introns be completely and precisely removed before protein synthesis so that the exons join together to code for the correct amino acids. If the process errs by even a single nucleotide, the sequence of the rejoined exons would be shifted, and the resulting protein would be nonfunctional. The process of removing introns and reconnecting exons is called splicing (Figure 6.11.5). Introns are removed and degraded while the pre-mRNA is still in the nucleus.

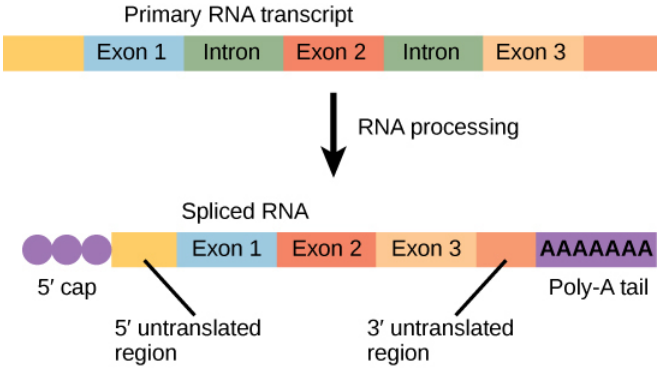


Figure 6.11.5: Eukaryotic mRNA contains introns that must be spliced out. A 5' cap and 3' tail are also added.

Summary

In prokaryotes, mRNA synthesis is initiated at a promoter sequence on the DNA template. Elongation synthesizes new mRNA. Termination liberates the mRNA and occurs by mechanisms that stall the RNA polymerase and cause it to fall off the DNA template. Newly transcribed eukaryotic mRNAs are modified with a cap and a poly-A tail. These structures protect the mature mRNA from degradation and help export it from the nucleus. Eukaryotic mRNAs also undergo splicing, in which introns are removed and exons are reconnected with single-nucleotide accuracy. Only finished mRNAs are exported from the nucleus to the cytoplasm.

Glossary

exon

a sequence present in protein-coding mRNA after completion of pre-mRNA splicing

intron

non–protein-coding intervening sequences that are spliced from mRNA during processing

mRNA

messenger RNA; a form of RNA that carries the nucleotide sequence code for a protein sequence that is translated into a polypeptide sequence

nontemplate strand
the strand of DNA that is not used to transcribe mRNA; this strand is identical to the mRNA except that T nucleotides in the DNA are replaced by U nucleotides in the mRNA

promoter
a sequence on DNA to which RNA polymerase and associated factors bind and initiate transcription

RNA polymerase
an enzyme that synthesizes an RNA strand from a DNA template strand

splicing
the process of removing introns and reconnecting exons in a pre-mRNA

template strand
the strand of DNA that specifies the complementary mRNA molecule

transcription bubble
the region of locally unwound DNA that allows for transcription of mRNA

Contributors and Attributions

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6.12: Translation

The synthesis of proteins is one of a cell's most energy-consuming metabolic processes. In turn, proteins account for more mass than any other component of living organisms (with the exception of water), and proteins perform a wide variety of the functions of a cell. The process of translation, or protein synthesis, involves decoding an mRNA message into a polypeptide product. Amino acids are covalently strung together in lengths ranging from approximately 50 amino acids to more than 1,000.

The Protein Synthesis Machinery

In addition to the mRNA template, many other molecules contribute to the process of translation. The composition of each component may vary across species; for instance, ribosomes may consist of different numbers of ribosomal RNAs (rRNA) and polypeptides depending on the organism. However, the general structures and functions of the protein synthesis machinery are comparable from bacteria to human cells. Translation requires the input of an mRNA template, ribosomes, tRNAs, and various enzymatic factors (Figure 9.4.1).

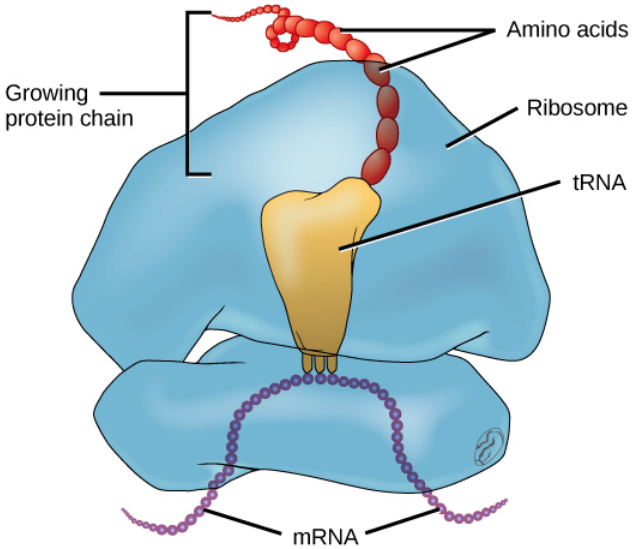


Figure 9.4.1: The protein synthesis machinery includes the large and small subunits of the ribosome, mRNA, and tRNA. (credit: modification of work by NIGMS, NIH)

In *E. coli*, there are 200,000 ribosomes present in every cell at any given time. A ribosome is a complex macromolecule composed of structural and catalytic rRNAs, and many distinct polypeptides. In eukaryotes, the nucleolus is completely specialized for the synthesis and assembly of rRNAs.

Ribosomes are located in the cytoplasm in prokaryotes and in the cytoplasm and endoplasmic reticulum of eukaryotes. Ribosomes are made up of a large and a small subunit that come together for translation. The small subunit is responsible for binding the mRNA template, whereas the large subunit sequentially binds tRNAs, a type of RNA molecule that brings amino acids to the growing chain of the polypeptide. Each mRNA molecule is simultaneously translated by many ribosomes, all synthesizing protein in the same direction.

Depending on the species, 40 to 60 types of tRNA exist in the cytoplasm. Serving as adaptors, specific tRNAs bind to sequences on the mRNA template and add the corresponding amino acid to the polypeptide chain. Therefore, tRNAs are the molecules that actually "translate" the language of RNA into the language of proteins. For each tRNA to function, it must have its specific amino acid bonded to it. In the process of tRNA "charging," each tRNA molecule is bonded to its correct amino acid.

The Genetic Code

To summarize what we know to this point, the cellular process of transcription generates messenger RNA (mRNA), a mobile molecular copy of one or more genes with an alphabet of A, C, G, and uracil (U). Translation of the mRNA template converts nucleotide-based genetic information into a protein product. Protein sequences consist of 20 commonly occurring amino acids;

therefore, it can be said that the protein alphabet consists of 20 letters. Each amino acid is defined by a three-nucleotide sequence called the triplet codon. The relationship between a nucleotide codon and its corresponding amino acid is called the genetic code.

Given the different numbers of “letters” in the mRNA and protein “alphabets,” combinations of nucleotides corresponded to single amino acids. Using a three-nucleotide code means that there are a total of 64 (4 × 4 × 4) possible combinations; therefore, a given amino acid is encoded by more than one nucleotide triplet (Figure 9.4.2).

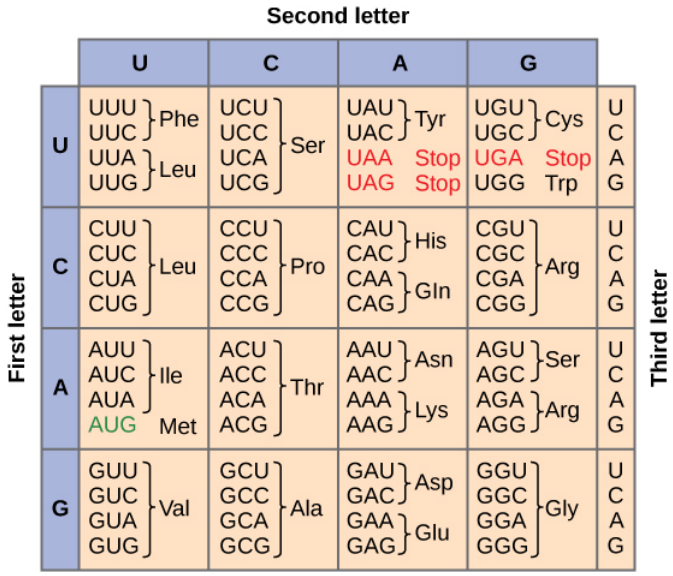


Figure 9.4.2: This figure shows the genetic code for translating each nucleotide triplet, or codon, in mRNA into an amino acid or a termination signal in a nascent protein. (credit: modification of work by NIH)

Three of the 64 codons terminate protein synthesis and release the polypeptide from the translation machinery. These triplets are called stop codons. Another codon, AUG, also has a special function. In addition to specifying the amino acid methionine, it also serves as the start codon to initiate translation. The reading frame for translation is set by the AUG start codon near the 5' end of the mRNA. The genetic code is universal. With a few exceptions, virtually all species use the same genetic code for protein synthesis, which is powerful evidence that all life on Earth shares a common origin.



Figure 9.4.3: Alternative figure showing the genetic code for translating each nucleotide triplet, or codon, in mRNA into an amino acid or a termination signal in a nascent protein.Image by Mouagip, Public domain, via Wikimedia Commons.

The Mechanism of Protein Synthesis

Just as with mRNA synthesis, protein synthesis can be divided into three phases: initiation, elongation, and termination. The process of translation is similar in prokaryotes and eukaryotes. Here we will explore how translation occurs in *E. coli*, a representative prokaryote, and specify any differences between prokaryotic and eukaryotic translation.

Protein synthesis begins with the formation of an initiation complex. In *E. coli*, this complex involves the small ribosome subunit, the mRNA template, three initiation factors, and a special initiator tRNA. The initiator tRNA interacts with the AUG start codon, and links to a special form of the amino acid methionine that is typically removed from the polypeptide after translation is complete.

In prokaryotes and eukaryotes, the basics of polypeptide elongation are the same, so we will review elongation from the perspective of *E. coli*. The large ribosomal subunit of *E. coli* consists of three compartments: the A site binds incoming charged

tRNAs (tRNAs with their attached specific amino acids). The P site binds charged tRNAs carrying amino acids that have formed bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA. The E site releases dissociated tRNAs so they can be recharged with free amino acids. The ribosome shifts one codon at a time, catalyzing each process that occurs in the three sites. With each step, a charged tRNA enters the complex, the polypeptide becomes one amino acid longer, and an uncharged tRNA departs. The energy for each bond between amino acids is derived from GTP, a molecule similar to ATP (Figure 9.4.4). Amazingly, the *E. coli* translation apparatus takes only 0.05 seconds to add each amino acid, meaning that a 200-amino acid polypeptide could be translated in just 10 seconds.

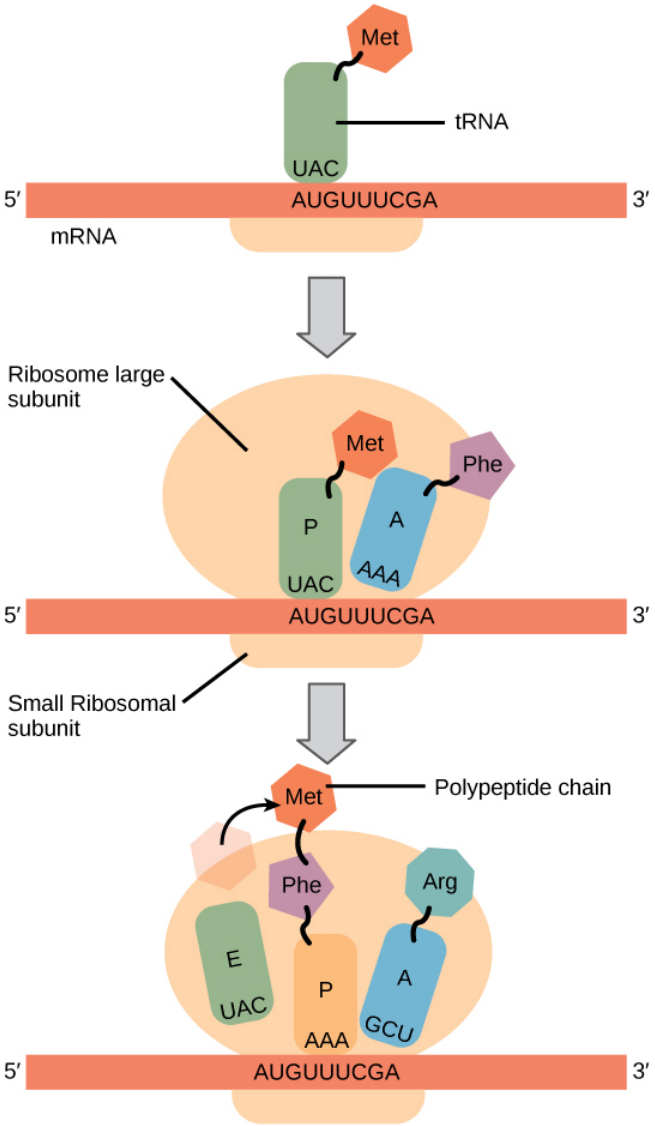


Figure 9.4.4: Translation begins when a tRNA anticodon recognizes a codon on the mRNA. The large ribosomal subunit joins the small subunit, and a second tRNA is recruited. As the mRNA moves relative to the ribosome, the polypeptide chain is formed. Entry of a release factor into the A site terminates translation and the components dissociate.

Termination of translation occurs when a stop codon (UAA, UAG, or UGA) is encountered. When the ribosome encounters the stop codon, the growing polypeptide is released and the ribosome subunits dissociate and leave the mRNA. After many ribosomes have completed translation, the mRNA is degraded so the nucleotides can be reused in another transcription reaction.

CONCEPT IN ACTION

Transcribe a gene and translate it to protein using complementary pairing and the genetic code at this site.

Summary

The central dogma describes the flow of genetic information in the cell from genes to mRNA to proteins. Genes are used to make mRNA by the process of transcription; mRNA is used to synthesize proteins by the process of translation. The genetic code is the correspondence between the three-nucleotide mRNA codon and an amino acid. The genetic code is "translated" by the tRNA molecules, which associate a specific codon with a specific amino acid. The genetic code is degenerate because 64 triplet codons in mRNA specify only 20 amino acids and three stop codons. This means that more than one codon corresponds to an amino acid. Almost every species on the planet uses the same genetic code.

The players in translation include the mRNA template, ribosomes, tRNAs, and various enzymatic factors. The small ribosomal subunit binds to the mRNA template. Translation begins at the initiating AUG on the mRNA. The formation of bonds occurs between sequential amino acids specified by the mRNA template according to the genetic code. The ribosome accepts charged tRNAs, and as it steps along the mRNA, it catalyzes bonding between the new amino acid and the end of the growing polypeptide. The entire mRNA is translated in three-nucleotide "steps" of the ribosome. When a stop codon is encountered, a release factor binds and dissociates the components and frees the new protein.

Glossary

codon
three consecutive nucleotides in mRNA that specify the addition of a specific amino acid or the release of a polypeptide chain during translation

genetic code
the amino acids that correspond to three-nucleotide codons of mRNA

rRNA
ribosomal RNA; molecules of RNA that combine to form part of the ribosome

stop codon
one of the three mRNA codons that specifies termination of translation

start codon
the AUG (or, rarely GUG) on an mRNA from which translation begins; always specifies methionine

tRNA
transfer RNA; an RNA molecule that contains a specific three-nucleotide anticodon sequence to pair with the mRNA codon and also binds to a specific amino acid

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6.13: Mutations and Genetic Diseases

Learning Objectives

- To describe the causes of genetic mutations and how they lead to genetic diseases.

We have seen that the sequence of nucleotides in a cell's deoxyribonucleic acid (DNA) is what ultimately determines the sequence of amino acids in proteins made by the cell and thus is critical for the proper functioning of the cell. On rare occasions, however, the nucleotide sequence in DNA may be modified either spontaneously (by errors during replication, occurring approximately once for every 10 billion nucleotides) or from exposure to heat, radiation, or certain chemicals. Any chemical or physical change that alters the nucleotide sequence in DNA is called a mutation. When a mutation occurs in an egg or sperm cell that then produces a living organism, it will be inherited by all the offspring of that organism.

Common types of mutations include **substitution** (a different nucleotide is substituted), **insertion** (the addition of a new nucleotide), and **deletion** (the loss of a nucleotide). These changes within DNA are called point mutations because only one nucleotide is substituted, added, or deleted (Figure **6.13.1**). Because an insertion or deletion results in a frame-shift that changes the reading of subsequent codons and, therefore, alters the entire amino acid sequence that follows the mutation, insertions and deletions are usually more harmful than a substitution in which only a single amino acid is altered.

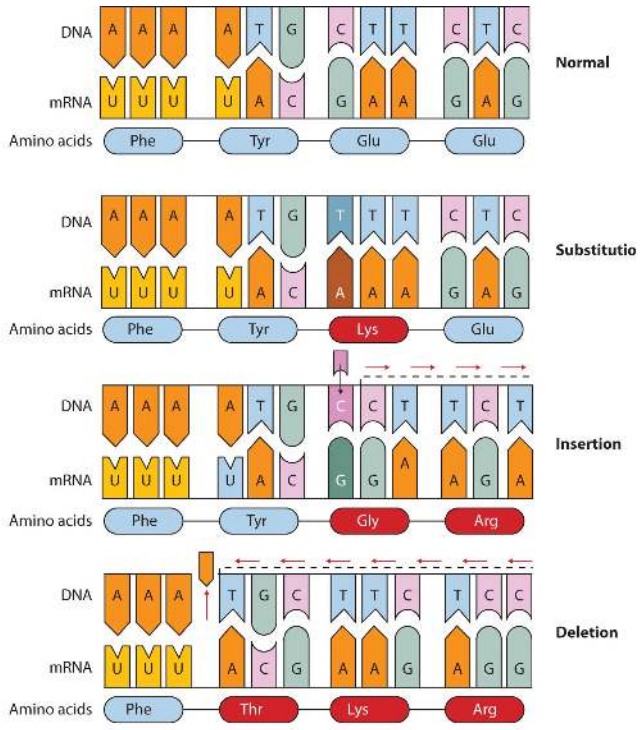


Figure **6.13.1**: Three Types of Point Mutations

The chemical or physical agents that cause mutations are called mutagens. Examples of physical mutagens are ultraviolet (UV) and gamma radiation. Radiation exerts its mutagenic effect either directly or by creating free radicals that in turn have mutagenic effects. Radiation and free radicals can lead to the formation of bonds between nitrogenous bases in DNA. For example, exposure to UV light can result in the formation of a covalent bond between two adjacent thymines on a DNA strand, producing a thymine dimer (Figure **6.13.2**). If not repaired, the dimer prevents the formation of the double helix at the point where it occurs. The genetic disease *xeroderma pigmentosum* is caused by a lack of the enzyme that cuts out the thymine dimers in damaged DNA. Individuals affected by this condition are abnormally sensitive to light and are more prone to skin cancer than normal individuals.

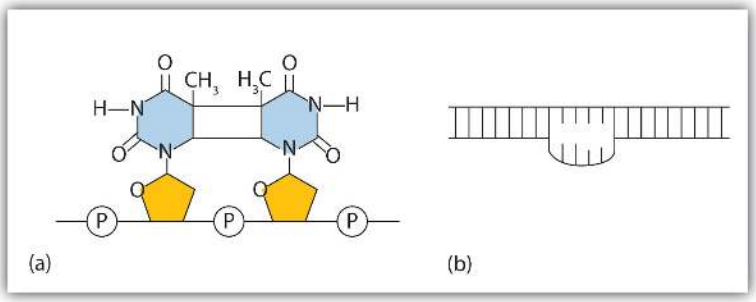


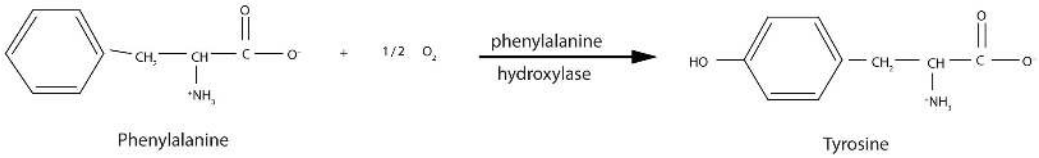
Figure **6.13.2**: An Example of Radiation Damage to DNA. (a) The thymine dimer is formed by the action of UV light. (b) When a defect in the double strand is produced by the thymine dimer, this defect temporarily stops DNA replication, but the dimer can be removed, and the region can be repaired by an enzyme repair system.

Sometimes gene mutations are beneficial, but most of them are detrimental. For example, if a point mutation occurs at a crucial position in a DNA sequence, the affected protein will lack biological activity, perhaps resulting in the death of a cell. In such cases the altered DNA sequence is lost and will not be copied into daughter cells. Nonlethal mutations in an egg or sperm cell may lead to metabolic abnormalities or hereditary diseases. Such diseases are called *inborn errors of metabolism* or genetic diseases. A partial listing of genetic diseases is presented in Figure **6.13.1**, and two specific diseases are discussed in the following sections. In most cases, the defective gene results in a failure to synthesize a particular enzyme.

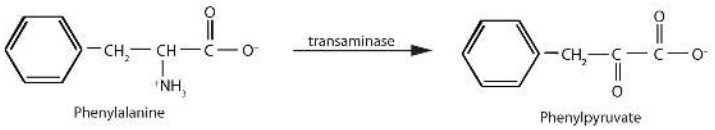
Figure **6.13.1**: Some Representative Genetic Diseases in Humans and the Protein or Enzyme Responsible

Disease	Responsible Protein or Enzyme
alkaptonuria	homogentisic acid oxidase
galactosemia	galactose 1-phosphate uridyl transferase, galactokinase, or UDP galactose epimerase
Gaucher disease	glucocerebrosidase
gout and Lesch-Nyhan syndrome	hypoxanthine-guanine phosphoribosyl transferase
hemophilia	antihemophilic factor (factor VIII) or Christmas factor (factor IX)
homocystinuria	cystathionine synthetase
maple syrup urine disease	branched chain α-keto acid dehydrogenase complex
McArdle syndrome	muscle phosphorylase
Niemann-Pick disease	sphingomyelinase
phenylketonuria (PKU)	phenylalanine hydroxylase
sickle cell anemia	hemoglobin
Tay-Sachs disease	hexosaminidase A
tyrosinemia	fumarylacetoacetate hydrolase or tyrosine aminotransferase
von Gierke disease	glucose 6-phosphatase
Wilson disease	Wilson disease protein

PKU results from the absence of the enzyme phenylalanine hydroxylase. Without this enzyme, a person cannot convert phenylalanine to tyrosine, which is the precursor of the neurotransmitters dopamine and norepinephrine as well as the skin pigment melanin.



When this reaction cannot occur, phenylalanine accumulates and is then converted to higher than normal quantities of phenylpyruvate. The disease acquired its name from the high levels of phenylpyruvate (a phenyl ketone) in urine. Excessive amounts of phenylpyruvate impair normal brain development, which causes severe mental retardation.



PKU may be diagnosed by assaying a sample of blood or urine for phenylalanine or one of its metabolites. Medical authorities recommend testing every newborn's blood for phenylalanine within 24 h to 3 weeks after birth. If the condition is detected, mental retardation can be prevented by immediately placing the infant on a diet containing little or no phenylalanine. Because phenylalanine is plentiful in naturally produced proteins, the low-phenylalanine diet depends on a synthetic protein substitute plus very small measured amounts of naturally produced foods. Before dietary treatment was introduced in the early 1960s, severe mental retardation was a common outcome for children with PKU. Prior to the 1960s, 85% of patients with PKU had an intelligence quotient (IQ) less than 40, and 37% had IQ scores below 10. Since the introduction of dietary treatments, however, over 95% of children with PKU have developed normal or near-normal intelligence. The incidence of PKU in newborns is about 1 in 12,000 in North America.

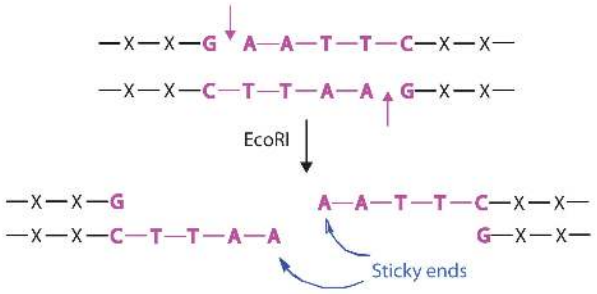
> *Every state in the United States has mandated that screening for PKU be provided to all newborns.*

Several genetic diseases are collectively categorized as *lipid-storage diseases*. Lipids are constantly being synthesized and broken down in the body, so if the enzymes that catalyze lipid degradation are missing, the lipids tend to accumulate and cause a variety of medical problems. When a genetic mutation occurs in the gene for the enzyme hexosaminidase A, for example, gangliosides cannot be degraded but accumulate in brain tissue, causing the ganglion cells of the brain to become greatly enlarged and nonfunctional. This genetic disease, known as *Tay-Sachs disease*, leads to a regression in development, dementia, paralysis, and blindness, with death usually occurring before the age of three. There is currently no treatment, but Tay-Sachs disease can be diagnosed in a fetus by assaying the amniotic fluid (amniocentesis) for hexosaminidase A. A blood test can identify Tay-Sachs carriers—people who inherit a defective gene from only one rather than both parents—because they produce only half the normal amount of hexosaminidase A, although they do not exhibit symptoms of the disease.

Looking Closer: Recombinant DNA Technology

More than 3,000 human diseases have been shown to have a genetic component, caused or in some way modulated by the person's genetic composition. Moreover, in the last decade or so, researchers have succeeded in identifying many of the genes and even mutations that are responsible for specific genetic diseases. Now scientists have found ways of identifying and isolating genes that have specific biological functions and placing those genes in another organism, such as a bacterium, which can be easily grown in culture. With these techniques, known as *recombinant DNA technology*, the ability to cure many serious genetic diseases appears to be within our grasp.

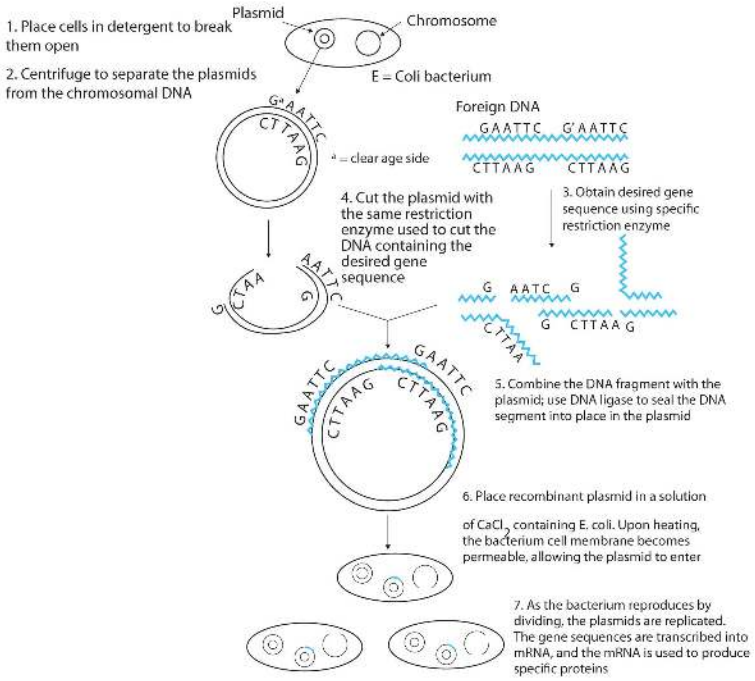
Isolating the specific gene or genes that cause a particular genetic disease is a monumental task. One reason for the difficulty is the enormous amount of a cell's DNA, only a minute portion of which contains the gene sequence. Thus, the first task is to obtain smaller pieces of DNA that can be more easily handled. Fortunately, researchers are able to use *restriction enzymes* (also known as restriction endonucleases), discovered in 1970, which are enzymes that cut DNA at specific, known nucleotide sequences, yielding DNA fragments of shorter length. For example, the restriction enzyme *EcoRI* recognizes the nucleotide sequence shown here and cuts both DNA strands as indicated:



Once a DNA strand has been fragmented, it must be cloned; that is, multiple identical copies of each DNA fragment are produced to make sure there are sufficient amounts of each to detect and manipulate in the laboratory. Cloning is accomplished by inserting the individual DNA fragments into phages (bacterial viruses) that can enter bacterial cells and be replicated. When a bacterial cell infected by the modified phage is placed in an appropriate culture medium, it forms a colony of cells, all containing copies of the original DNA fragment. This technique is used to produce many bacterial colonies, each containing a different DNA fragment. The result is a *DNA library*, a collection of bacterial colonies that together contain the entire genome of a particular organism.

The next task is to screen the DNA library to determine which bacterial colony (or colonies) has incorporated the DNA fragment containing the desired gene. A short piece of DNA, known as a *hybridization probe*, which has a nucleotide sequence complementary to a known sequence in the gene, is synthesized, and a radioactive phosphate group is added to it as a "tag." You might be wondering how researchers are able to prepare such a probe if the gene has not yet been isolated. One way is to use a segment of the desired gene isolated from another organism. An alternative method depends on knowing all or part of the amino acid sequence of the protein produced by the gene of interest: the amino acid sequence is used to produce an approximate genetic code for the gene, and this nucleotide sequence is then produced synthetically. (The amino acid sequence used is carefully chosen to include, if possible, many amino acids such as methionine and tryptophan, which have only a single codon each.)

After a probe identifies a colony containing the desired gene, the DNA fragment is clipped out, again using restriction enzymes, and spliced into another replicating entity, usually a plasmid. *Plasmids* are tiny mini-chromosomes found in many bacteria, such as *Escherichia coli* (*E. coli*). A recombined plasmid would then be inserted into the host organism (usually the bacterium *E. coli*), where it would go to work to produce the desired protein.



Proponents of recombinant DNA research are excited about its great potential benefits. An example is the production of human growth hormone, which is used to treat children who fail to grow properly. Formerly, human growth hormone was available only in tiny amounts obtained from cadavers. Now it is readily available through recombinant DNA technology. Another gene that has been cloned is the gene for epidermal growth factor, which stimulates the growth of skin cells and can be used to speed the healing of burns and other skin wounds. Recombinant techniques are also a powerful research tool, providing enormous aid to scientists as they map and sequence genes and determine the functions of different segments of an organism's DNA.

In addition to advancements in the ongoing treatment of genetic diseases, recombinant DNA technology may actually lead to cures. When appropriate genes are successfully inserted into *E. coli*, the bacteria can become miniature pharmaceutical factories, producing great quantities of insulin for people with diabetes, clotting factor for people with hemophilia, missing enzymes, hormones, vitamins, antibodies, vaccines, and so on. Recent accomplishments include the production in *E. coli* of recombinant DNA molecules containing synthetic genes for tissue plasminogen activator, a clot-dissolving enzyme that can rescue heart attack victims, as well as the production of vaccines against hepatitis B (humans) and hoof-and-mouth disease (cattle).

Scientists have used other bacteria besides *E. coli* in gene-splicing experiments and also yeast and fungi. Plant molecular biologists use a bacterial plasmid to introduce genes for several foreign proteins (including animal proteins) into plants. The bacterium is *Agrobacterium tumefaciens*, which can cause tumors in many plants, but which can be treated so that its tumor-causing ability is eliminated. One practical application of its plasmids would be to enhance a plant's nutritional value by transferring into it the gene necessary for the synthesis of an amino acid in which the plant is normally deficient (for example, transferring the gene for methionine synthesis into pinto beans, which normally do not synthesize high levels of methionine).

> Restriction enzymes have been isolated from a number of bacteria and are named after the bacterium of origin. *EcoRI* is a restriction enzyme obtained from the R strain of *E. coli*. The roman numeral I indicates that it was the first restriction enzyme obtained from this strain of bacteria.

Summary

- The nucleotide sequence in DNA may be modified either spontaneously or from exposure to heat, radiation, or certain chemicals and can lead to mutations.
- Mutagens are the chemical or physical agents that cause mutations.
- Genetic diseases are hereditary diseases that occur because of a mutation in a critical gene.

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6.14: Viruses

Learning Objectives

- To explain how viruses reproduce in cells.

Viruses are visible only under an electron microscope. They come in a variety of shapes, ranging from spherical to rod shaped. The fact that they contain either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)—*but never both*—allows them to be divided into two major classes: DNA viruses and RNA viruses (Figure **6.14.1**).

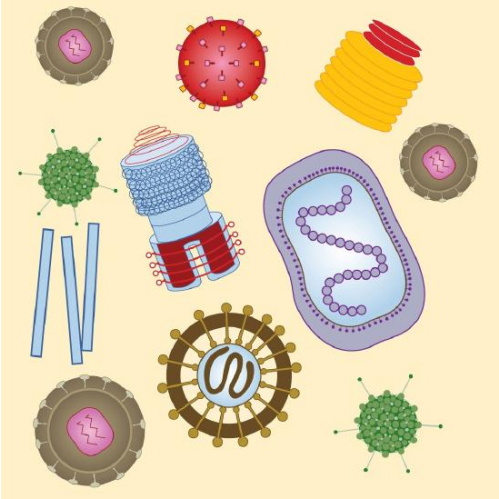


Figure **6.14.1**: Viruses. Viruses come in a variety of shapes that are determined by their protein coats.

Most *RNA viruses* use their nucleic acids in much the same way as the DNA viruses, penetrating a host cell and inducing it to replicate the viral RNA and synthesize viral proteins. The new RNA strands and viral proteins are then assembled into new viruses. Some RNA viruses, however, called retroviruses (Figure **6.14.2**), synthesize DNA in the host cell, in a process that is the reverse of the DNA-to-RNA transcription that normally occurs in cells. The synthesis of DNA from an RNA template is catalyzed by the enzyme reverse transcriptase.

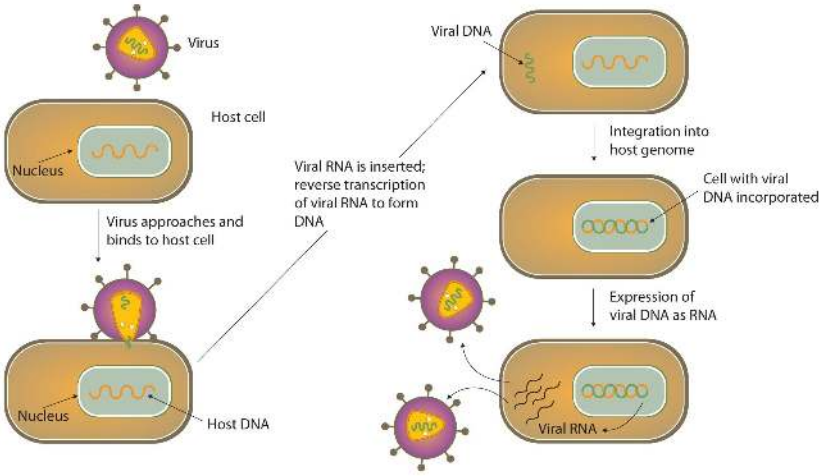
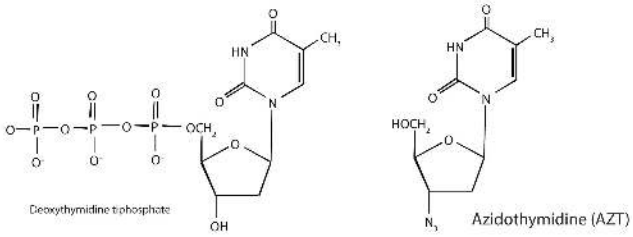
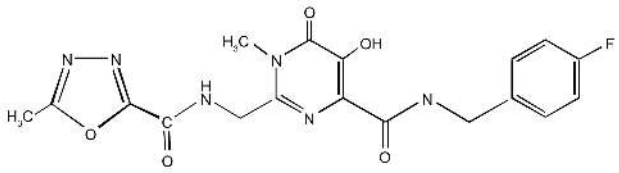


Figure **6.14.2**: Life Cycle of a Retrovirus

In 1987, **azidothymidine** (AZT, also known as zidovudine or the brand name Retrovir) became the first drug approved for the treatment of AIDS. It works by binding to reverse transcriptase in place of deoxythymidine triphosphate, after which, because AZT does not have a 3′OH group, further replication is blocked. In the past 10 years, several other drugs have been approved that also act by inhibiting the viral reverse transcriptase.



Raltegravir (Isentress) is a newer anti-AIDS drug that was approved by the FDA in October 2007. This drug inhibits the integrase enzyme that is needed to integrate the HIV DNA into cellular DNA, an essential step in the production of more HIV particles.



A major problem in treating HIV infections is that the virus can become resistant to any of these drugs. One way to combat the problem has been to administer a “cocktail” of drugs, typically a combination of two reverse transcriptase inhibitors along with a protease inhibitor. These treatments can significantly reduce the amount of HIV in an infected person.

Career Focus: Genetics Counselor

A genetics counselor works with individuals and families who have birth defects or genetic disorders or a family history of a disease, such as cancer, with a genetic link. A genetics counselor may work in a variety of health-care settings (such as a hospital) to obtain family medical and reproductive histories; explain how genetic conditions are inherited; explain the causes, diagnosis, and care of these conditions; interpret the results of genetic tests; and aid the individual or family in making decisions regarding genetic diseases or conditions. A certified genetics counselor must obtain a master’s degree from an accredited program. Applicants to these graduate programs usually have an undergraduate degree in biology, psychology, or genetics.



Photo courtesy of the United States National Institutes for Health, commons.wikimedia.org/wiki/File:Geneticcounseling.jpg.

Summary

Viruses are very small infectious agents that contain either DNA or RNA as their genetic material. The human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS).

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6.15: DNA Sequencing

Objectives

To briefly describe one method for DNA sequencing

We will discuss one method of reading the sequence of DNA. This method, developed by Sanger won him a second Nobel prize. To sequence a single stranded piece of DNA, the complementary strand is synthesized. Four different reaction mixtures are set up. Each contain all 4 radioactive deoxynucleotides (dATP, dCTP, dGTP, dTTP) required for the reaction and DNA polymerase. In addition, dideoxyATP (ddATP) is added to one reaction tube The dATP and ddATP attach randomly to the growing 3' end of the complementary stranded. If ddATP is added no further nucleotides can be added after since its 3' end has an H and not a OH. That's why they call it dideoxy. The new chain is terminated.. If dATP is added, the chain will continue to grow until another A needs to be added. Hence a whole series of discreet fragments of DNA chains will be made, all terminated when ddATP was added. The same scenario occurs for the other 3 tubes, which contain dCTP and ddCTP, dTTP and ddTTP, and dGTP and ddGTP respectively. All the fragments made in each tube will be placed in separate lanes for electrophoresis, where the fragments will separate by size.

Didexoynucleotides

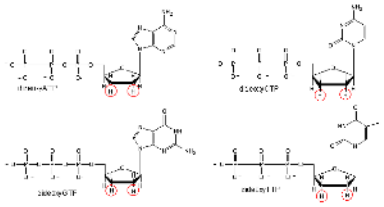


Figure: Didexoynucleotides

$$t = \frac{0.9\lambda}{\sqrt{B_M^2 - B_S^2}\cos\theta}$$

Example 28.6.1

You will pretend to sequence a single stranded piece of DNA as shown below. The new nucleotides are added by the enzyme DNA polymerase to the primer, GACT, in the 5' to 3' direction. You will set up 4 reaction tubes, Each tube contains all the dXTP's. In addition, add ddATP to tube 1, ddTTP to tube 2, ddCTP to tube 3, and ddGTP to tube 4. For each separate reaction mixture, determine all the possible sequences made by writing the possible sequences on one of the unfinished complementary sequences below. Cut the completed sequences from the page, determine the size of the polynucleotide sequences made, and place them as they would migrate (based on size) in the appropriate lane of a imaginary gel which you have drawn on a piece of paper. Lane 1 will contain the nucleotides made in tube 1, etc. Then draw lines under the positions of the cutout nucleotides to represent DNA bands in the gel. Read the sequence of the complementary DNA synthesized. Then write the sequence of the ssDNA that was to be sequenced.

- 5' T C A A C G A T C T G A 3' (STAND TO SEQUENCE)
- 3' G A C T 5' (primer)
- 3' G A C T 5' (primer)
- 3' G A C T 5' (primer)
- 3' G A C T 5' (primer)
- 3' G A C T 5' (primer)
- 3' G A C T 5' (primer)
- 3' G A C T 5' (primer)
- 3' G A C T 5' (primer)

Since the DNA fragments have no detectable color, they can not be directly visualized in the gel. Alternative methods are used. In the one described above, radiolabeled ddXTP's where used. Once the sequencing gel is run, it can be dried and the bands visualized by radioautography (also called autoradiography). A place of x-ray film is placed over the dried gel in a dark environment. The radiolabeled bands will emit radiation which will expose the x-ray film directly over the bands. The film can be developed to detect the bands. In a newer technique, the primer can be labeled with a flourescent dye. If a different dye is used for each reaction mixture, all the reaction mixtures can be run in one lane of a gel. (Actually only one reaction mix containing all the ddXTP's together need be performed.) The gel can then be scanned by a laser, which detects fluorescence from the dyes, each at a different wavelength.

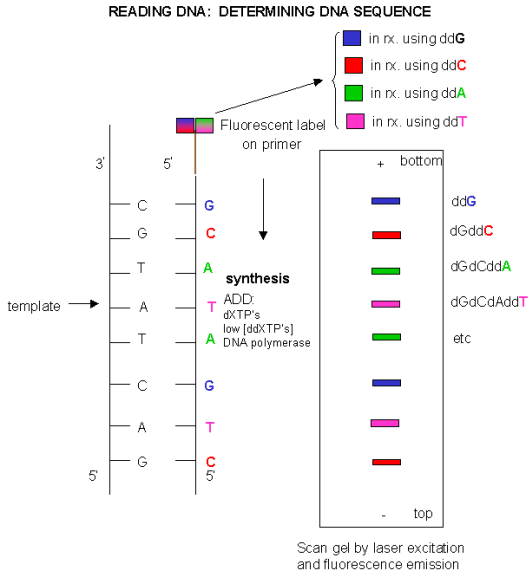
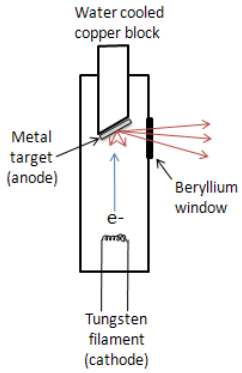


***Figure**: DNA sequencing using different fluorescent primers for each ddXTP reaction*

One recent advance in sequencing allows for real-time determination of a sequence. The four deoxynucleotides are each labeled with a different fluorphore on the 5' phosphate (not the base as above). A tethered DNA polymerase elongates the DNA on a template, releasing the fluorophore into solution (i.e. the fluorophore is not incorporated into the DNA chain). The reaction takes place in a visualization chamber called a zero mode waveguide which is a cylindrical metallic chamber with a width of 70 nm and a volume of 20 zeptoliters (20 x 10-21 L). It sits on a glass support through which laser illumination of the sample is achieved. Given the small volume, non-incorporated fluorescently tagged deoxynucleotides diffuse in and out in the microsecond timescale. When a deoxynucleotide is incorporated into the DNA, its residence time is in the millisecond time scale. This allows for prolonged detection of fluorescence which give a high signal to noise ratio. This method might bring the cost of sequencing the human genome down from the initial billion dollar range to $100.



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CHAPTER OVERVIEW

7: Nutrition

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7.2: Minerals and Vitamins- a closer look
7.3: Calories - Quantity and Quality
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7.1: NUTRIENTS

WHAT ARE NUTRIENTS?

Nutrients are substances the body needs for energy, building materials, and control of body processes. There are six major classes of nutrients based on biochemical properties: carbohydrates, proteins, lipids, water, vitamins, and minerals. Fiber, which consists largely of nondigestible carbohydrates, is sometimes added as the seventh class of nutrients.

Besides the biochemical classification of nutrients, nutrients are also categorized as either essential or nonessential nutrients. **Essential nutrients** cannot be synthesized by the human body, at least not in sufficient amounts for normal functioning, so these nutrients must be obtained from food. **Nonessential nutrients,** in contrast, can be synthesized in the body in sufficient quantities for normal functioning, although they are generally obtained from food as well. Except for dietary fiber, all dietary carbohydrates are considered nonessential. Every other major class of nutrients contains multiple essential compounds. For example, there are nine essential amino acids, at least two essential fatty acids, and many essential vitamins and minerals. Water and fiber are also essential nutrients.

The major classes of nutrients are also categorized as macronutrients or micronutrients depending on how much of them the body needs.

1. MACRONUTRIENTS

Macronutrients are nutrients that the body needs in relatively large amounts. They include carbohydrates, proteins, lipids, and water. All macronutrients except water are used by the body for energy, although this is not their sole physiological function. The energy provided by macronutrients in food is measured in kilocalories, commonly called **Calories,** where 1 Calorie is the amount of energy needed to raise 1 kilogram of water by 1 degree Celsius.

1.1. CARBOHYDRATES



Figure **7.1.2**: This cotton candy may look like a big cotton ball made of real cotton, which consists mostly of cellulose, but it actually consists almost entirely of simple sugars. (CC BY 2.0; college.library via Wikimedia Commons)

Carbohydrates are organic compounds made up of simple sugars (as in the cotton candy pictured below). Dietary carbohydrates come mainly from grains, fruits, and vegetables. All digestible carbohydrates in the diet are used by the body for energy. One gram of carbohydrates provides around 4 Calories energy.

Carbohydrates are classified by the number of sugars they contain as monosaccharides (one sugar), such as glucose and fructose; disaccharides (two sugars), such as sucrose and lactose; and polysaccharides (three or more sugars), including starch, glycogen, and cellulose. Monosaccharides and disaccharide are considered **simple carbohydrates**, while digestible polysaccharides (starch, glycogen) are considered **complex carbohydrates**.

1.2. FIBER

Fiber are carbohydrates, such as the cellulose in plant foods, that cannot be digested by the human digestive system, so most of it just passes through the digestive tract. Although it does not provide energy as other carbohydrates do, it is nonetheless considered an essential nutrient for its physiological roles. There are two types of fiber in many plant foods: soluble fiber and insoluble fiber.

- **Soluble fiber** consists of nondigestible complex plant **oligosaccharides** that dissolve in water, forming a gel. This type of dietary fiber thickens and slows the movement of chyme through the small intestine and thereby slows the absorption of glucose into the blood. The consistency of food after it has been mechanically digested in the stomach is referred to as chyme. This may lessen insulin spikes and the risk of type 2 diabetes. Soluble fiber can also help lower blood cholesterol. Good dietary sources of soluble fiber include oats, apples, and beans.

- **Insoluble fiber** consists mainly of **cellulose polysaccharide** and does not dissolve in water. As insoluble fiber moves through the large intestine, it stimulates peristalsis. Peristalsis is the involuntary constriction of the smooth muscle of the GI tract that pushes the food content in the tract. This keeps food wastes moving and helps prevent constipation. The insoluble fiber in the diet may also lessen the risk of colon cancer. Good dietary sources of insoluble fiber include cabbage, bell peppers, and grapes.

1.3. PROTEINS

Proteins are organic compounds made up of amino acids. You may think of meat and fish as major sources of dietary proteins — and they are — but there are many good plant sources as well, including soybeans (see the figure below) and other legumes. Proteins in food are broken down during digestion to provide the amino acids needed for protein synthesis. Proteins in the human body are the basis of many body structures, including muscles and skin. Proteins also function as enzymes that catalyze biochemical reactions, hormones that regulate body functions in other ways, and antibodies that help fight pathogens. Any amino acids from food that are not needed for these purposes are excreted in the urine, and under certain extreme conditions, converted to glucose for energy.

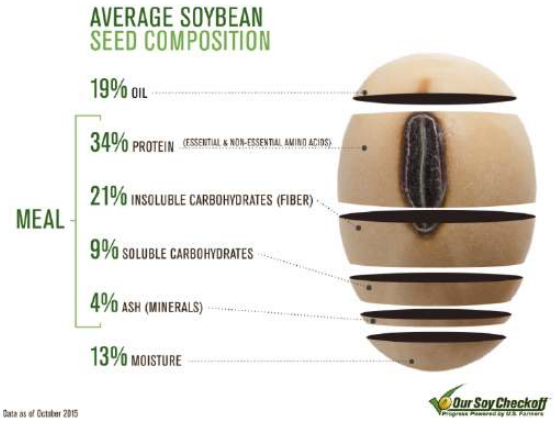


Figure **7.1.3**: As shown in this diagram, soybeans are an excellent source of protein, providing both essential and nonessential amino acids. They are also a very good source of fiber and lipids. (CC BY 2.0; United Soybean Board via Wikimedia Commons)

The most important aspect of protein structure from a nutritional standpoint is amino acid composition. About 20 amino acids are commonly found in the human body, of which about 11 are nonessential because they can be synthesized internally. The other 9 amino acids are essential amino acids that must be obtained from dietary sources. Essential amino acids are phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine, and histidine. Animal proteins such as meat and fish are concentrated sources of all 9 essential amino acids, whereas plant proteins may have only trace amounts of one or more essential amino acids.



Figure **7.1.4**: A variety of forms of fat are commonly used in food preparation. Fatty food sources shown here include butter, mayonnaise, vegetable oil, and salad dressing. (Public domain; Bill Branson via Wikimedia Commons)

1.4. LIPIDS

Lipids, commonly called fats, are organic compounds made up mainly of fatty acids. Fats in foods (Figure **7.1.4**), as well as fats in the human body, are typically triglycerides (three fatty acids attached to a molecule of glycerol). Fats provide the body with energy and serve other vital functions, including helping to make and maintain cell membranes and functioning as hormones. When used for energy, one gram of fat provides 9 Calories of energy.

ESSENTIAL FATTY ACIDS

Most fatty acids are not essential. The body can make them as needed, generally from other fatty acids, although this takes energy. Only two fatty acids are known to be essential, called omega-3 and omega-6 fatty acids. They cannot be synthesized in the body, so they must be obtained from food. The most commonly used cooking oils in processed foods are rich in omega-6 fatty acids, so most people get plenty of these fatty acids in their diet. Omega-3 fatty acids are not as prevalent in foods, and most people do not get enough of them in food. Good food sources of omega-3 fatty acids include oily fish such as salmon, walnuts, and flax seeds.

SATURATED VS UNSATURATED FATS

Fats are classified as either saturated or unsaturated depending on the type of bonds in their fatty acids.

- In saturated fats, carbon atoms share only single bonds, so each carbon atom is bonded to as many hydrogen atoms as possible. Saturated fats tend to be solids at room temperature. Most saturated fat in the diet comes from animal foods, such as meat and butter.
- In unsaturated fats, at least one pair of carbon atoms share a double bond, so these carbon atoms are not bonded to as many hydrogen atoms as possible. Unsaturated fats with just one double bond are called monounsaturated fats. Those with multiple double bonds are called polyunsaturated fats. Unsaturated fats tend to be liquids at room temperature. Unsaturated fats in the diet come mainly from certain fish such as salmon and from plant foods such as seeds and nuts. Unsaturated fats can be *cis* or *trans*, depending on the configuration of the C=C double bonds. Most fats in nature are

cis. **Trans fats** are unsaturated fats that contain types of bonds that are rare in nature. Trans fats are typically created in an industrial process called partial hydrogenation. They may be used in a variety of processed foods (such as those shown in the photo below) because they tend to have a longer shelf life without going rancid. Trans fats are known to be detrimental to human health.



Figure **7.1.5**: All of the foods pictured here contain harmful trans fats. (Public domain; U.S. Food and Drug Administration via Wikimedia Commons)

1.6. WATER

Water is essential to life because biochemical reactions take place in water. Water is continuously lost from the body in multiple ways, including in urine and feces, during sweating, and as water vapor in exhaled breath. This constant loss of water makes water an essential nutrient that must be replenished often.

Too little water is called dehydration. It can cause weakness, dizziness, and heart palpitations. Severe dehydration can lead to death. It is easy to become dehydrated in hot weather, especially when exercising. It is more difficult to consume too much water, but overhydration is also possible. It can result in water intoxication, a serious and potentially fatal condition.

2. MICRONUTRIENTS

Micronutrients are nutrients the body needs in relatively small amounts. Micronutrients do not provide energy. Instead, they are necessary for the biochemical reactions of metabolism, among other vital functions. They include vitamins, minerals, and possibly phytochemicals as well.

2.1. VITAMINS

Vitamins are organic compounds that generally function as coenzymes. A coenzyme is a "helper" molecule that is required for a protein enzyme to work. In this capacity, vitamins play many roles in good health, ranging from maintaining normal vision (vitamin A) to help the blood to clot (vitamin K). Depending on their chemical structure, vitamins can be classified as **water-soluble** or **fat-soluble**. Some functions of these and several other vitamins are listed in the table below. Most vitamins are essential nutrients and must be obtained from food. Fruits, vegetables, meat, and fish are all high in one or more essential vitamins. There are only a few nonessential vitamins. Vitamins B_7 and K are produced by bacteria in the large intestine, and vitamin D is synthesized in the skin when it is exposed to UV light

Table **7.1.1**: Selected Vitamins and Some of Their Functions

Vitamin	Function
A	normal vision
B_1 (thiamin)	production of cellular energy from food
B_3(niacin)	cardiovascular health
B_7 (biotin)	support of carbohydrate, protein, and fat metabolism
B_9 (folic acid)	fetal health and development
B_{12}	normal nerve function and production of red blood cells
C	making connective tissue
D	healthy bones and teeth
E	normal cell membranes
K	blood clotting

2.2. MINERALS

Minerals are inorganic chemical elements that are necessary for normal body processes and good health. Because they are inorganic and not synthesized biologically, all nutrient minerals are considered essential nutrients.

Several minerals are needed in relatively large quantities (> 150 mg/day), so they are sometimes referred to as **macrominerals** or bulk minerals. They include:

- calcium, which is needed for bone strength, neutralizing acidity in the digestive tract, and nerve and cell membrane functions. Dairy products are good sources of calcium.
- magnesium, which is needed for strong bones, maintaining pH, processing ATP, and other functions. Green leafy vegetables, bran, and almonds are high in magnesium.
- phosphorus, which is needed for bone strength, energy processing, pH regulation, and phospholipids in cell membranes. Milk and meat are good sources of phosphorus.
- sodium, which is needed to regulate blood volume, blood pressure, water balance, and pH. Most processed foods have added sodium. A salt shaker is another common source of sodium.
- chloride, which is needed for the production of hydrochloric acid in the stomach and for cell membrane transport. Chloride in table salt and added to processed foods provides plenty of chloride in most diets.
- potassium, which is needed for the proper functioning of the heart and nerves, water balance, and pH. Many fruits and vegetables are high in potassium.
- sulfur, which is needed for the synthesis of many proteins. Meat and fish are good sources of sulfur.

Other minerals are needed in much smaller quantities (≤150 mg/day), so they are often referred to as **trace minerals**. The table below lists several trace minerals and some of their functions. Good dietary sources of trace minerals include whole grains, seafood, fruits, vegetables, nuts, and legumes.

Table 7.1.2: Selected Trace Minerals and Some of Their Functions

Trace Mineral	Function
Cobalt	synthesis of vitamin B_{12} by gut bacteria
Copper	component of many enzymes
Chromium	metabolism of sugar
Iodine	synthesis of thyroid hormones
Iron	component of hemoglobin and many enzymes
Manganese	processing of oxygen
Molybdenum	component of several enzymes
Selenium	component of oxidases (antioxidants)
Zinc	component of several enzymes

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7.2: Minerals and Vitamins- a closer look

Learning Objectives

- List reasons why vitamins and minerals are critical to a healthy diet.
- Describe the functional role, intake recommendations and sources of vitamins and major minerals.
- Learn about the importance and sources of dietary fiber.
- Learn about the importance of water.

Vitamins and minerals are essential to human health and can be obtained in our diet from different types of food.

Dietary Minerals

Minerals in food are inorganic compounds that work with other nutrients to ensure the body functions properly. Minerals are abundant in our everyday lives. From the soil in your front yard to the jewelry you wear on your body, we interact with minerals constantly. There are 20 essential minerals that must be consumed in our diets to remain healthy. The amount of each mineral found in our bodies vary greatly and therefore, so does consumption of those minerals. When there is a deficiency in an essential mineral, health problems may arise.

Major minerals (Figure **7.2.1**) are classified as minerals that are required in the diet each day in amounts larger than 100 milligrams. These include sodium, potassium, chloride, calcium, phosphorus, magnesium, and sulfur. These major minerals can be found in various foods. Consuming a varied diet significantly improves an individual's ability to meet their nutrient needs. The most common minerals in the body are calcium and phosphorous, both of which are stored in the skeleton and necessary for the hardening of bones. Most minerals are ionized, and their ionic forms are used in physiological processes throughout the body. Sodium and chloride ions are electrolytes in the blood and extracellular tissues, and iron ions are critical to the formation of hemoglobin. There are additional trace minerals that are still important to the body's functions, but their required quantities are much lower.

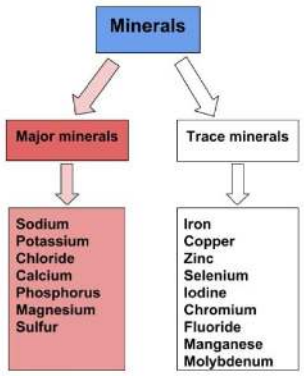


***Figure* 7.2.1** *The major and trace minerals. Image by Allison Calabrese / CC BY 4.0.*

Like vitamins, minerals can be consumed in toxic quantities (although it is rare). A healthy diet includes most of the minerals your body requires, so supplements and processed foods can add potentially toxic levels of minerals. Tables **7.2.1** and **7.2.2** provide a summary of minerals and their function in the body.

***Table* 7.2.1** *Major Minerals and their Function in the Body.*

Major Minerals				
Mineral	Sources	Recommended daily allowance	Function	Problems associated with deficiency
Potassium	Meats, some fish, fruits, vegetables, legumes, dairy products	4700 mg	Nerve and muscle function; acts as an electrolyte	Hypokalemia: weakness, fatigue, muscle cramping, gastrointestinal problems, cardiac problems
Sodium	Table salt, milk, beets, celery, processed foods	2300 mg	Blood pressure, blood volume, muscle and nerve function	Rare
Calcium	Dairy products, dark green leafy vegetables, blackstrap molasses, nuts, brewer's yeast, some fish	1000 mg	Bone structure and health; nerve and muscle functions, especially cardiac function	Slow growth, weak and brittle bones
Phosphorous	Meat, milk	700 mg	Bone formation, metabolism, ATP production	Rare
Magnesium	Whole grains, nuts, leafy green vegetables	310–420 mg	Enzyme activation, production of energy, regulation of other nutrients	Agitation, anxiety, sleep problems, nausea and vomiting, abnormal heart rhythms, low blood pressure, muscular problems
Chloride	Most foods, salt, vegetables, especially seaweed, tomatoes, lettuce, celery, olives	2300 mg	Balance of body fluids, digestion	Loss of appetite, muscle cramps

Table 7.2.2 *Trace Minerals and their Function in the Body.*

Trace Minerals				
Mineral	Sources	Recommended daily allowance	Function	Problems associated with deficiency
Iron	Meat, poultry, fish, shellfish, legumes, nuts, seeds, whole grains, dark leafy green vegetables	8–18 mg	Transport of oxygen in blood, production of ATP	Anemia, weakness, fatigue
Zinc	Meat, fish, poultry, cheese, shellfish	8–11 mg	Immunity, reproduction, growth, blood clotting, insulin and thyroid function	Loss of appetite, poor growth, weight loss, skin problems, hair loss, vision problems, lack of taste or smell
Copper	Seafood, organ meats, nuts, legumes, chocolate, enriched breads and cereals, some fruits and vegetables	900 μg	Red blood cell production, nerve and immune system function, collagen formation, acts as an antioxidant	Anemia, low body temperature, bone fractures, low white blood cell concentration, irregular heartbeat, thyroid problems
Iodine	Fish, shellfish, garlic, lima beans, sesame seeds, soybeans, dark leafy green vegetables	150 μg	Thyroid function	Hypothyroidism: fatigue, weight gain, dry skin, temperature sensitivity
Sulfur	Eggs, meat, poultry, fish, legumes	None	Component of amino acids	Protein deficiency
Fluoride	Fluoridated water	3–4 mg	Maintenance of bone and tooth structure	Increased cavities, weak bones and teeth
Manganese	Nuts, seeds, whole grains, legumes	1.8–2.3 mg	Formation of connective tissue and bones, blood clotting, sex hormone development, metabolism, brain and nerve function	Infertility, bone malformation, weakness, seizures
Cobalt	Fish, nuts, leafy green vegetables, whole grains	None	Component of B_{12}	None
Selenium	Brewer’s yeast, wheat germ, liver, butter, fish, shellfish, whole grains	55 μg	Antioxidant, thyroid function, immune system function	Muscle pain
Chromium	Whole grains, lean meats, cheese, black pepper, thyme, brewer’s yeast	25–35 μg	Insulin function	High blood sugar, triglyceride, and cholesterol levels
Molybdenum	Legumes, whole grains, nuts	45 μg	Cofactor for enzymes	Rare

The Vitamins: Vital, but Not All are Amines

Vitamins are organic compounds found in foods and are a necessary part of the biochemical reactions in the body. They are involved in a number of processes, including mineral and bone metabolism, and cell and tissue growth, and they act as cofactors for energy metabolism. The B vitamins play the largest role of any vitamins in metabolism (Tables **7.2.3** and **7.2.4**)

You get most of your vitamins through your diet, although some can be formed from the precursors absorbed during digestion. For example, the body synthesizes vitamin A from the β-carotene in orange vegetables like carrots and sweet potatoes. Vitamins are either fat-soluble or water-soluble. Fat-soluble vitamins A, D, E, and K, are absorbed through the intestinal tract with lipids in chylomicrons. Vitamin D is also synthesized in the skin through exposure to sunlight. Because they are carried in lipids, fat-soluble vitamins can accumulate in the lipids stored in the body. If excess vitamins are retained in the lipid stores in the body, hypervitaminosis can result.

Water-soluble vitamins, including the eight B vitamins and vitamin C, are absorbed with water in the gastrointestinal tract. These vitamins move easily through bodily fluids, which are water based, so they are not stored in the body. Excess water-soluble vitamins are excreted in the urine. Therefore, hypervitaminosis of water-soluble vitamins rarely occurs, except with an excess of vitamin supplements.

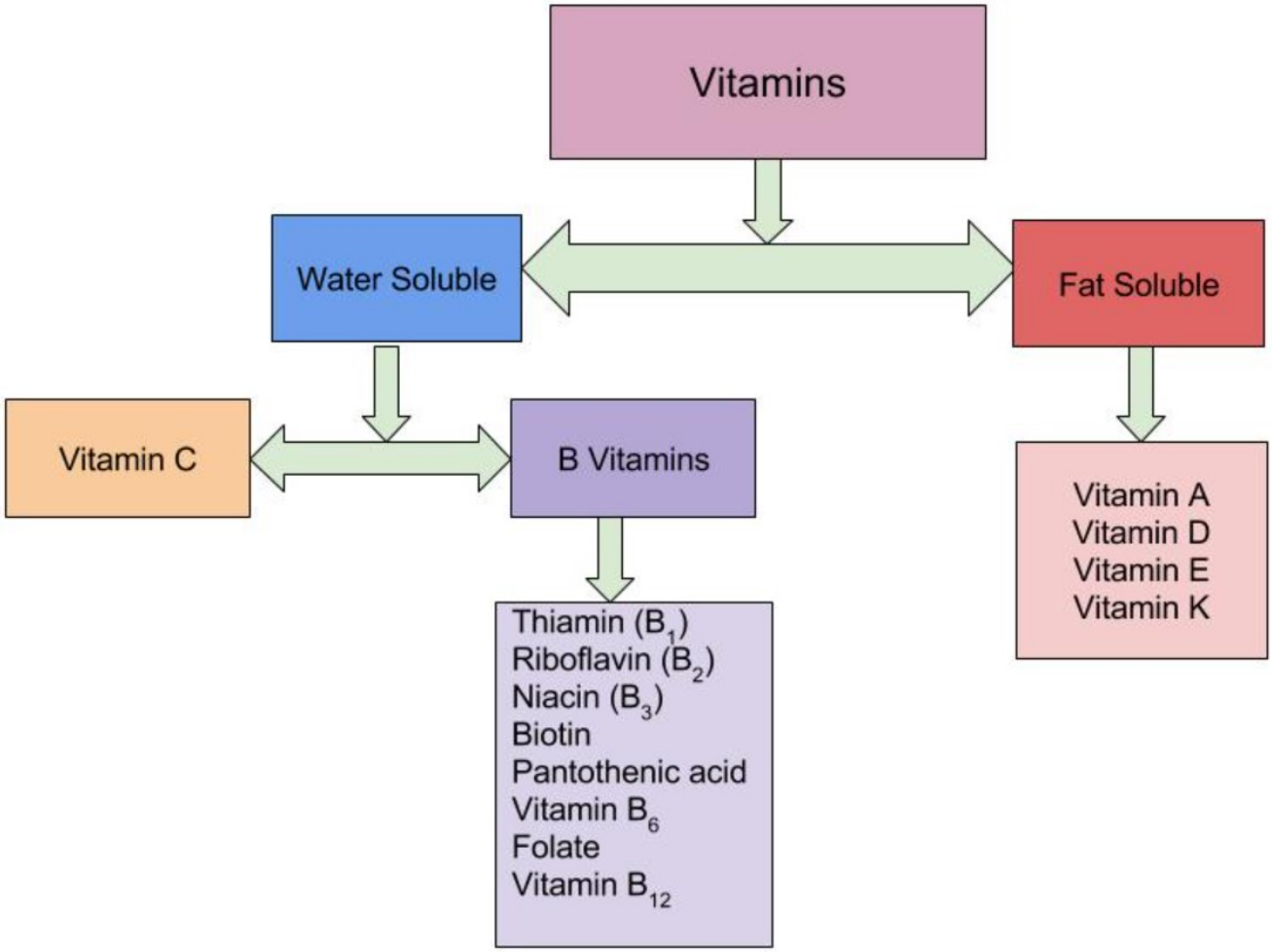


Figure **7.2.3**: The Vitamins. *Image by Allison Calabrese / CC BY 4.0.*

Fat Soluble Vitamins

From the structures shown below, it should be clear that these compounds have more than a solubility connection with lipids. VitaminsA is a terpene, and vitamins E and K have long terpene chains attached to an aromatic moiety. The structure of vitamin D can be described as a steroid in which ring B is cut open and the remaining three rings remain unchanged. The precursors of vitamins A and D have been identified as the tetraterpene beta-carotene and the steroid ergosterol, respectively.

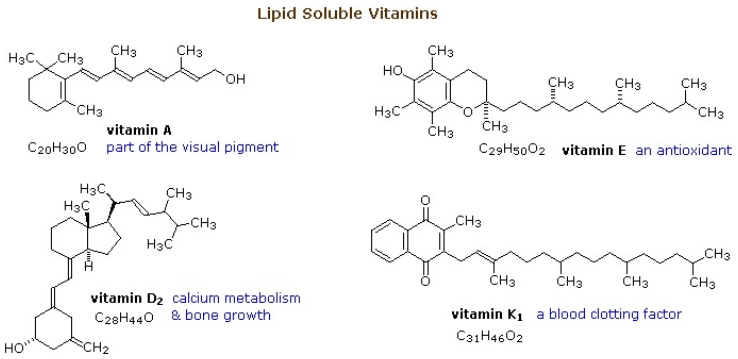


Table **7.2.3** lists the different fat soluble vitamins and its function.

***Table* 7.2.3** *Fat Soluble Vitamins and Their Function.*

Vitamin and alternative name	Sources	Recommended daily allowance	Function	Problems associated with deficiency
A retinal or β-carotene	Yellow and orange fruits and vegetables, dark green leafy vegetables, eggs, milk, liver	700–900 μg	Eye and bone development, immune function	Night blindness, epithelial changes, immune system deficiency
D cholecalciferol	Dairy products, egg yolks; also synthesized in the skin from exposure to sunlight	5–15 μg	Aids in calcium absorption, promoting bone growth	Rickets, bone pain, muscle weakness, increased risk of death from cardiovascular disease, cognitive impairment, asthma in children, cancer
E tocopherols	Seeds, nuts, vegetable oils, avocados, wheat germ	15 mg	Antioxidant	Anemia
K phylloquinone	Dark green leafy vegetables, broccoli, Brussels sprouts, cabbage	90–120 μg	Blood clotting, bone health	Hemorrhagic disease of newborn in infants; uncommon in adults

Web link

More detailed information on the different fat soluble vitamins can be found on the link below.

https://med.libretexts.org/Bookshelves/Nutrition/Book%3A_Human_Nutrition_(University_of_Hawaii)/9%3A_Vitamins/9.2%3A_Fat-Soluble_Vitamins

Water Soluble Vitamins

All water-soluble vitamins (Table **7.2.4**) play a different kind of role in energy metabolism; they are required as functional parts of enzymes involved in energy release and storage. Vitamins and minerals that make up part of enzymes are referred to as coenzymes and cofactors**,** respectively. Coenzymes and cofactors are required by enzymes to catalyze a specific reaction. They assist in converting a substrate to an end-product. Coenzymes and cofactors are essential in catabolic pathways and play a role in many anabolic pathways too. In addition to being essential for metabolism, many vitamins and minerals are required for blood renewal

and function. At insufficient levels in the diet these vitamins and minerals impair the health of blood and consequently the delivery of nutrients in and wastes out, amongst its many other functions.

***Table* 7.2.4** *Water Soluble Vitamins and Their Function.*

Vitamin and alternative name	Sources	Recommended daily allowance	Function	Problems associated with deficiency
B_1 thiamine	Whole grains, enriched bread and cereals, milk, meat	1.1–1.2 mg	Carbohydrate metabolism	Beriberi, Wernicke-Korsikoff syndrome
B_2 riboflavin	Brewer's yeast, almonds, milk, organ meats, legumes, enriched breads and cereals, broccoli, asparagus	1.1–1.3 mg	Synthesis of FAD for metabolism, production of red blood cells	Fatigue, slowed growth, digestive problems, light sensitivity, epithelial problems like cracks in the corners of the mouth
B_3 niacin	Meat, fish, poultry, enriched breads and cereals, peanuts	14–16 mg	Synthesis of NAD, nerve function, cholesterol production	Cracked, scaly skin; dementia; diarrhea; also known as pellagra
B_5 pantothenic acid	Meat, poultry, potatoes, oats, enriched breads and cereals, tomatoes	5 mg	Synthesis of coenzyme A in fatty acid metabolism	Rare: symptoms may include fatigue, insomnia, depression, irritability
B_6 pyridoxine	Potatoes, bananas, beans, seeds, nuts, meat, poultry, fish, eggs, dark green leafy vegetables, soy, organ meats	1.3–1.5 mg	Sodium and potassium balance, red blood cell synthesis, protein metabolism	Confusion, irritability, depression, mouth and tongue sores
B_7 biotin	Liver, fruits, meats	30 μg	Cell growth, metabolism of fatty acids, production of blood cells	Rare in developed countries; symptoms include dermatitis, hair loss, loss of muscular coordination
B_9 folic acid	Liver, legumes, dark green leafy vegetables, enriched breads and cereals, citrus fruits	400 μg	DNA/protein synthesis	Poor growth, gingivitis, appetite loss, shortness of breath, gastrointestinal problems, mental deficits
B_{12} cyanocobalamin	Fish, meat, poultry, dairy products, eggs	2.4 μg	Fatty acid oxidation, nerve cell function, red blood cell production	Pernicious anemia, leading to nerve cell damage
C ascorbic acid	Citrus fruits, red berries, peppers, tomatoes, broccoli, dark green leafy vegetables	75–90 mg	Necessary to produce collagen for formation of connective tissue and teeth, and for wound healing	Dry hair, gingivitis, bleeding gums, dry and scaly skin, slow wound healing, easy bruising, compromised immunity; can lead to scurvy

Web link

More detailed information on the different water soluble vitamins can be found on the link below.

https://med.libretexts.org/Bookshelves/Nutrition/Book%3A_Human_Nutrition_(University_of_Hawaii)/9%3A_Vitamins/9.3%3A_Water-Soluble_Vitamins

Summary

- Vitamins and minerals are essential parts of the diet. They are needed for the proper function of metabolic pathways in the body.
- Vitamins are not stored in the body, so they must be obtained from the diet or synthesized from precursors available in the diet.
- Minerals are also obtained from the diet, but they are also stored, primarily in skeletal tissues.

Contributors

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- Wikipedia
- Marisa Alviar-Agnew (Sacramento City College)
- Minerals, Vitamins, and Other Essentials. (2021, June 19). Retrieved June 22, 2021, from https://chem.libretexts.org/@go/page/152248

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7.3: Calories - Quantity and Quality

Learning Objective

- Know the daily recommended guidelines to achieve a healthy diet.

The U.S. Dietary Guidelines for Americans and the DRI are important scientific reports to educate health professionals about nutrition and to guide government and other health-related organizations to develop evidence-based health policies that improve the health of all Americans. The United States government has also been providing food and nutrition guidance directly to the public for more than a century to help individuals make healthier dietary and lifestyle choices . You may have heard about "the Four Food Groups" or "The Food Guide Pyramid" or most recently, "My Plate." The government food guidance system has evolved over the years as our understanding of nutrition science and the impact of diet and lifestyle on health has grown.

Web Links

To learn more about MyPlate visit: https://www.choosemyplate.gov/MyPlate

MyPlate

MyPlate is the most up-to-date nutrition teaching tool. MyPlate was developed by the United States Department of Agriculture (U.S.D.A.) Center for Nutrition Policy and Promotion as an easy to use visual guide to help all American develop healthy eating patterns. It replaces the former MyPyramid teaching tool and correlates with the 2015 - 2020 U.S. Dietary Guidelines.

MyPlate organizes foods with similar nutritional value into specific food groups and provides recommendations about how to build a healthy diet. The ChooseMyPlate.gov website also provides a wide range of support materials including information about each food group, an individualized meal planner, recipes and professional videos and handouts such as the *MyPlate, MyWins* poster shown below to support learning for people of all ages.

MyPlate Key Messages include:

- *Focus on whole fruits*
- *Vary your veggies*
- *Vary your protein routine*
- *Make half your grains whole grains*
- *Move to low-fat or fat-free milk or yogurt*
- *Drink and eat beverages and food with less sodium saturated fat and added sugars*
- *Start with small changes that you can enjoy, like having an extra piece of fruit today*

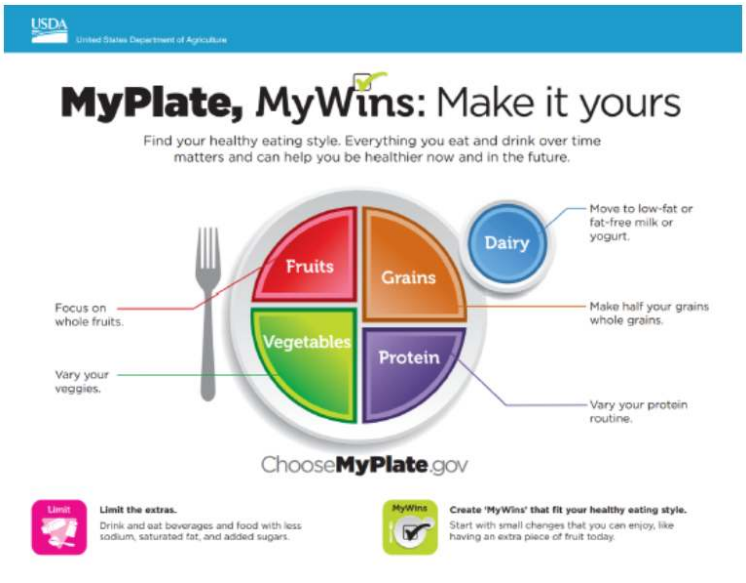


***Figure* 7.3.1** *The ideal healthy plate.*

Acceptable Macronutrient Distribution Range

Young men typically have higher nutrient needs than young women. For ages nineteen to thirty, the energy requirements for women are 1,800 to 2,400 calories, and 2,400 to 3,000 calories for men, depending on activity level. These estimates do not include women who are pregnant or breastfeeding, who require a higher energy intake.

AMDR describes the proportions of daily caloric intake that should be carbohydrates, lipids, and proteins. The range in caloric intake in a daily diet should be:

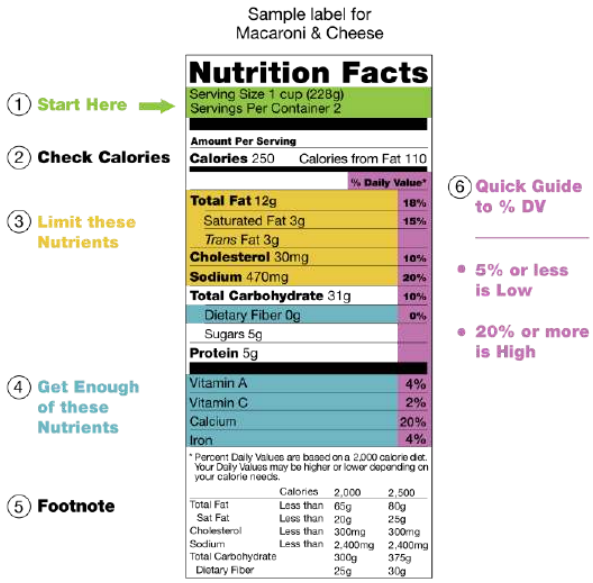
Macronutrient	AMDR	Additional Information
Carbohydrates	45 to 65 % All age groups	All adults, young and old, should eat fewer energy-dense carbohydrates, especially refined, sugar-dense sources, particularly for those who lead a more sedentary lifestyle.
Protein	10-35% (Adults) 10-30% (4-18 years old) 5-20% (1-3 years old)	The diet should include a variety of lean meat and poultry, eggs, beans, peas, nuts, and seeds. The guidelines also recommend that adults eat two 4-ounce servings (or one 8-ounce serving) of seafood per week.
Total Fat	20-35% (Adults) 25-35% (4-18 years old) 30-40% (1-3 years old)	• Keep saturated fatty acids to less than 10 percent of total calories by replacing them with monounsaturated and polyunsaturated fatty acids. • Avoid trans fats by limiting foods that contain synthetic sources, such as partially hydrogenated oils.
Fiber	22 to 28 grams per day for women and 28 to 34 grams per day for men	Soluble fiber may help improve cholesterol and blood sugar levels, while insoluble fiber can help prevent constipation.

Nutrition labels

The **nutrition facts label** (also known as the **nutrition information panel**, and other slight variations) is a label required on most packaged food in many countries, showing what nutrients (to limit and get enough of) are in the food. Nutrition facts labels are one of many types of food labels required by regulation or applied by manufacturers. In the United States, the Nutritional Facts label lists the percentage supplied that is recommended to be met, or to be limited, in one day of human nutrients based on a daily diet of 2,000 calories.

With certain exceptions, such as foods meant for babies, the following **Daily Values** are used. These are called **Reference Daily Intake** (RDI) values and were originally based on the highest 1968 Recommended Dietary Allowances (RDA) for each nutrient in order to assure that the needs of all age and sex combinations were met.

How to understand and use the US Nutritional Fact Label



A sample nutrition facts label, with instructions from the U.S. Food and Drug Administration. Image by Trounce, Public domain, via Wikimedia Commons

Summary

- MyPlate is the most up-to-date nutrition teaching tool developed by the United States Department of Agriculture (U.S.D.A.) Center for Nutrition Policy and Promotion as an easy to use visual guide to help all American develop healthy eating patterns.
- Planning a healthy diet using the MyPlate approach is not difficult. According to the icon, half of your plate should have fruits and vegetables, one-quarter should have whole grains, and one-quarter should have protein. Dairy products should be low-fat or non-fat.
- There are five key factors that make up a healthful diet namely: a. adequacy, b. balance, c. calorie control, d. moderation, and e. variety.
- The total number of calories a person needs each day varies depending on a number of factors, including the person's age, sex, height, weight, and level of physical activity.

Sources

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- Wikipedia contributors. (2021, March 14). Nutrition facts label. In *Wikipedia, The Free Encyclopedia*. Retrieved 20:15, May 17, 2021, from https://en.wikipedia.org/w/index.php?title=Nutrition_facts_label&oldid=1012100208

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7.4: Energy Flow and Metabolism

Skills to Develop

- Explain what metabolic pathways are and describe the two major types of metabolic pathways
- Discuss how chemical reactions play a role in energy transfer

Scientists use the term bioenergetics to discuss the concept of energy flow (Figure **7.4.1**) through living systems, such as cells. Cellular processes such as the building and breaking down of complex molecules occur through stepwise chemical reactions. Some of these chemical reactions are spontaneous and release energy, whereas others require energy to proceed. Just as living things must continually consume food to replenish what has been used, cells must continually produce more energy to replenish that used by the many energy-requiring chemical reactions that constantly take place. All of the chemical reactions that take place inside cells, including those that use energy and those that release energy, are the cell's metabolism.

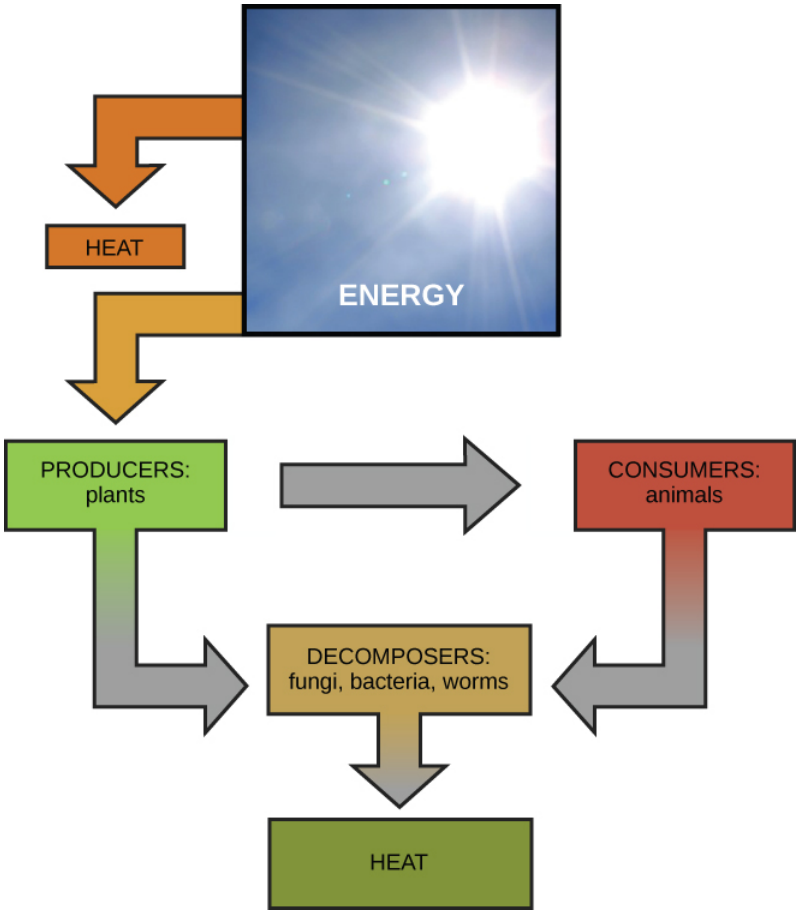


Figure **7.4.1**: The flow of energy in the biosphere. Most life forms on earth get their energy from the sun. Plants use photosynthesis to capture sunlight, and herbivores eat those plants to obtain energy. Carnivores eat the herbivores, and decomposers digest plant and animal matter.

Metabolism of Carbohydrates Overview

The metabolism of sugar (a simple carbohydrate) is a classic example of the many cellular processes that use and produce energy. Living things consume sugar as a major energy source, because sugar molecules have a great deal of energy stored within their bonds. The breakdown of glucose, a simple sugar, is described by the equation:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + (\text{energy}) \tag{6.1.1}$$

Carbohydrates that are consumed have their origins in photosynthesizing organisms like plants (Figure 6.1.2). During photosynthesis, plants use the energy of sunlight to convert carbon dioxide gas (CO_2) into sugar molecules, like glucose

($C_6H_{12}O_6$). Because this process involves synthesizing a larger, energy-storing molecule, it requires an input of energy to proceed. The synthesis of glucose is described by this equation (notice that it is the reverse of the previous equation):

$$6CO_2 + 6H_2O + (\text{energy}) \rightarrow C_6H_{12}O_6 + 6O_2 \tag{6.1.2}$$

During the chemical reactions of photosynthesis, energy is provided in the form of a very high-energy molecule called ATP, or adenosine triphosphate, which is the primary energy currency of all cells. Just as the dollar is used as currency to buy goods, cells use molecules of ATP as energy currency to perform immediate work. The sugar (glucose) is stored as starch or glycogen. Energy-storing polymers like these are broken down into glucose to supply molecules of ATP.

Solar energy is required to synthesize a molecule of glucose during the reactions of photosynthesis. In photosynthesis, light energy from the sun is initially transformed into chemical energy that is temporally stored in the energy carrier molecules ATP and NADPH (nicotinamide adenine dinucleotide phosphate). The stored energy in ATP and NADPH is then used later in photosynthesis to build one molecule of glucose from six molecules of CO_2. This process is analogous to eating breakfast in the morning to acquire energy for your body that can be used later in the day. Under ideal conditions, energy from 18 molecules of ATP is required to synthesize one molecule of glucose during the reactions of photosynthesis. Glucose molecules can also be combined with and converted into other types of sugars. When sugars are consumed, molecules of glucose eventually make their way into each living cell of the organism. Inside the cell, each sugar molecule is broken down through a complex series of chemical reactions. The goal of these reactions is to harvest the energy stored inside the sugar molecules. The harvested energy is used to make high-energy ATP molecules, which can be used to perform work, powering many chemical reactions in the cell. The amount of energy needed to make one molecule of glucose from six molecules of carbon dioxide is 18 molecules of ATP and 12 molecules of NADPH (each one of which is energetically equivalent to three molecules of ATP), or a total of 54 molecule equivalents required for the synthesis of one molecule of glucose. This process is a fundamental and efficient way for cells to generate the molecular energy that they require.



Figure **7.4.2**: Plants, like this oak tree and acorn, use energy from sunlight to make sugar and other organic molecules. Both plants and animals (like this squirrel) use cellular respiration to derive energy from the organic molecules originally produced by plants. (credit “acorn”: modification of work by Noel Reynolds; credit “squirrel”: modification of work by Dawn Huczek)

Metabolic Pathways

The processes of making and breaking down sugar molecules illustrate two types of metabolic pathways. A metabolic pathway is a series of interconnected biochemical reactions that convert a substrate molecule or molecules, step-by-step, through a series of metabolic intermediates, eventually yielding a final product or products. In the case of sugar metabolism, the first metabolic pathway synthesized sugar from smaller molecules, and the other pathway broke sugar down into smaller molecules. These two opposite processes—the first requiring energy and the second producing energy—are referred to as anabolic (building) and catabolic (breaking down) pathways, respectively. Consequently, metabolism is composed of building (anabolism) and degradation (catabolism).

Anabolic and Catabolic Pathways

Anabolic pathways require an input of energy to synthesize complex molecules from simpler ones. Synthesizing sugar from CO_2 is one example. Other examples are the synthesis of large proteins from amino acid building blocks, and the synthesis of new DNA strands from nucleic acid building blocks. These biosynthetic processes are critical to the life of the cell, take place constantly, and demand energy provided by ATP and other high-energy molecules like NADH (nicotinamide adenine dinucleotide) and NADPH (Figure **7.4.4**).

ATP is an important molecule for cells to have in sufficient supply at all times. The breakdown of sugars illustrates how a single molecule of glucose can store enough energy to make a great deal of ATP, 36 to 38 molecules. This is a catabolic pathway. Catabolic pathways involve the degradation (or breakdown) of complex molecules into simpler ones. Molecular energy stored in the bonds of complex molecules is released in catabolic pathways and harvested in such a way that it can be used to produce ATP. Other energy-storing molecules, such as fats, are also broken down through similar catabolic reactions to release energy and make ATP (Figure **7.4.4**).

It is important to know that the chemical reactions of metabolic pathways don't take place spontaneously. Each reaction step is facilitated, or catalyzed, by a protein called an enzyme. Enzymes are important for catalyzing all types of biological reactions—those that require energy as well as those that release energy.

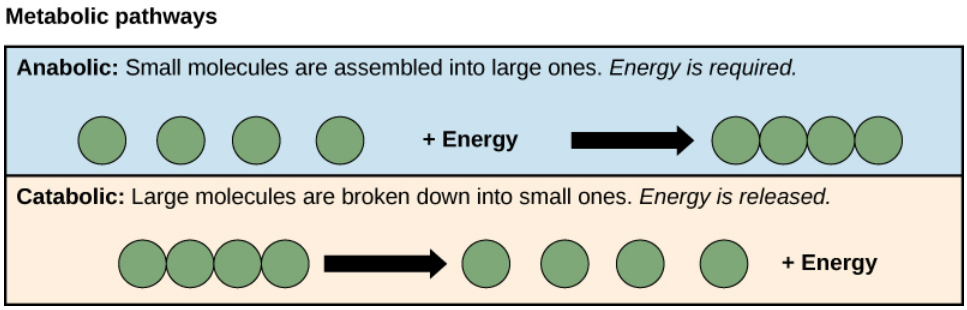


Figure **7.4.4**: Anabolic pathways are those that require energy to synthesize larger molecules. Catabolic pathways are those that generate energy by breaking down larger molecules. Both types of pathways are required for maintaining the cell's energy balance.

Summary

Cells perform the functions of life through various chemical reactions. A cell's metabolism refers to the chemical reactions that take place within it. There are metabolic reactions that involve the breaking down of complex chemicals into simpler ones, such as the breakdown of large macromolecules. This process is referred to as catabolism, and such reactions are associated with a release of energy. On the other end of the spectrum, anabolism refers to metabolic processes that build complex molecules out of simpler ones, such as the synthesis of macromolecules. Anabolic processes require energy. Glucose synthesis and glucose breakdown are examples of anabolic and catabolic pathways, respectively.

Multiple Choice

Energy is stored long-term in the bonds of _____ and used short-term to perform work from a(n) _____ molecule.

1. ATP : glucose
2. an anabolic molecule : catabolic molecule
3. glucose : ATP
4. a catabolic molecule : anabolic molecule

C

DNA replication involves unwinding two strands of parent DNA, copying each strand to synthesize complementary strands, and releasing the parent and daughter DNA. Which of the following accurately describes this process?

1. This is an anabolic process
2. This is a catabolic process

3. This is both anabolic and catabolic
4. This is a metabolic process but is neither anabolic nor catabolic

A

Free Response

Does physical exercise involve anabolic and/or catabolic processes? Give evidence for your answer.

Physical exercise involves both anabolic and catabolic processes. Body cells break down sugars to provide ATP to do the work necessary for exercise, such as muscle contractions. This is catabolism. Muscle cells also must repair muscle tissue damaged by exercise by building new muscle. This is anabolism.

Name two different cellular functions that require energy that parallel human energy-requiring functions.

Energy is required for cellular motion, through beating of cilia or flagella, as well as human motion, produced by muscle contraction. Cells also need energy to perform digestion, as humans require energy to digest food.

Glossary

anabolic
(also, anabolism) pathways that require an input of energy to synthesize complex molecules from simpler ones

bioenergetics
study of energy flowing through living systems

catabolic
(also, catabolism) pathways in which complex molecules are broken down into simpler ones

metabolism
all the chemical reactions that take place inside cells, including anabolism and catabolism

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7.5: Catabolism of food

Learning Objectives

- To describe how carbohydrates, fats, and proteins are broken down during digestion.

We have said that animals obtain chemical energy from the food—carbohydrates, fats, and proteins—they eat through reactions defined collectively as *catabolism*. We can think of catabolism as occurring in three stages (Figure **7.5.1**). In stage I, carbohydrates, fats, and proteins are broken down into their individual monomer units: carbohydrates into simple sugars, fats into fatty acids and glycerol, and proteins into amino acids. One part of stage I of catabolism is the breakdown of food molecules by hydrolysis reactions into the individual monomer units—which occurs in the mouth, stomach, and small intestine—and is referred to as digestion.

In stage II, these monomer units (or building blocks) are further broken down through different reaction pathways, one of which produces ATP, to form a common end product that can then be used in stage III to produce even more ATP. In this chapter, we will look at each stage of catabolism—as an overview and in detail.

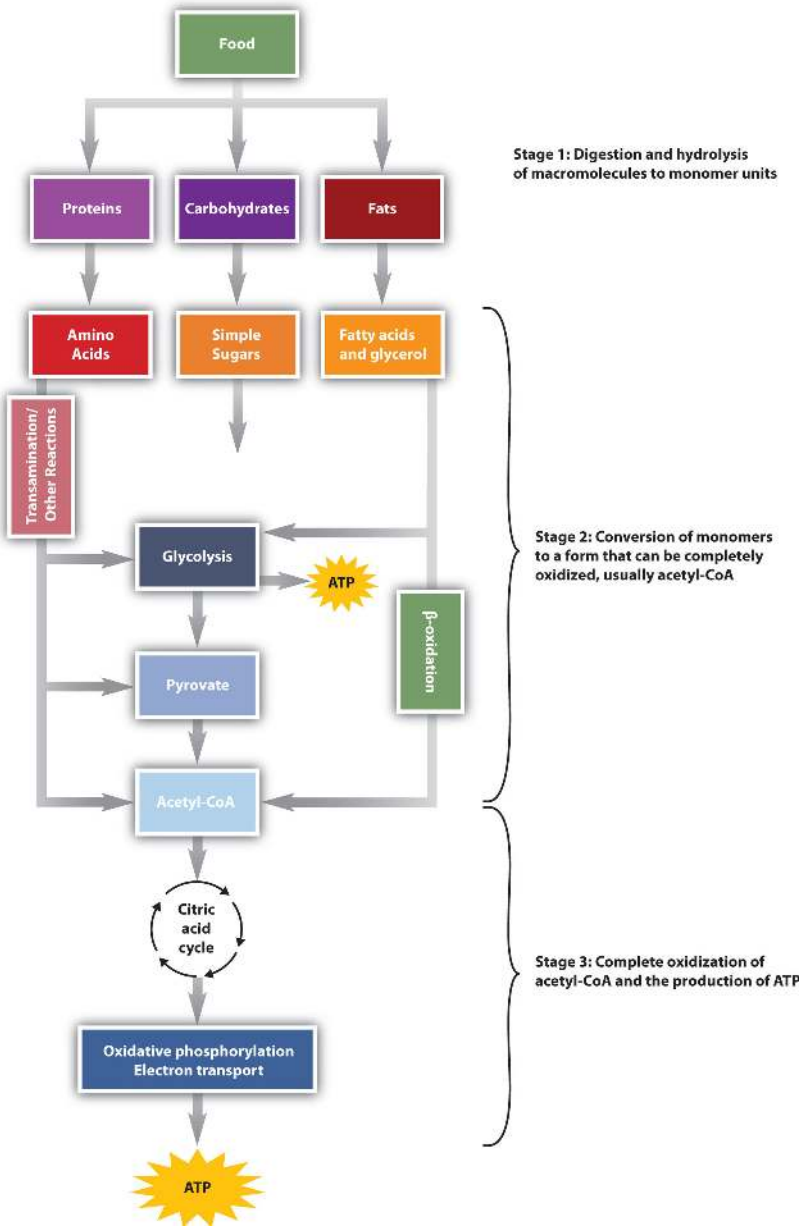


Figure **7.5.1**: Energy Conversions. The conversion of food into cellular energy (as ATP) occurs in three stages.

Summary

During digestion, carbohydrates are broken down into monosaccharides, proteins are broken down into amino acids, and triglycerides are broken down into glycerol and fatty acids. Most of the digestion reactions occur in the small intestine.

Concept Review Exercises

Answers

Exercises

1. Distinguish between each pair of compounds.
    a. pepsin and pepsinogen
    b. chymotrypsin and trypsin
    c. aminopeptidase and carboxypeptidase
2. What are the primary end products of each form of digestion?
    a. carbohydrate digestion
    b. lipid digestion
    c. protein digestion
3. In what section of the digestive tract does most of the carbohydrate, lipid, and protein digestion take place?
4. a. Pepsinogen is an inactive form of pepsin; pepsin is the active form of the enzyme.
    b. Both enzymes catalyze the hydrolysis of peptide bonds. Chymotrypsin catalyzes the hydrolysis of peptide bonds following aromatic amino acids, while trypsin catalyzes the hydrolysis of peptide bonds following lysine and arginine.
    c. Aminopeptidase catalyzes the hydrolysis of amino acids from the N-terminal end of a protein, while carboxypeptidase catalyzes the hydrolysis of amino acids from the C-terminal end of a protein.
5. a. glucose, fructose, and galactose
    b. monoglycerides and fatty acids
    c. amino acids
6. the small intestine
7. What are the products of digestion (or stage I of catabolism)?
8. What is the general type of reaction used in digestion?
9. Give the site of action and the function of each enzyme.
    a. chymotrypsin
    b. lactase
    c. pepsin
    d. maltase
10. Give the site of action and the function of each enzyme.
    a. α-amylase
    b. trypsin
    c. sucrase
    d. aminopeptidase
11. a. What is the meaning of the following statement? “Bile salts act to emulsify lipids in the small intestine.”
    b. Why is emulsification important?
12. Using chemical equations, describe the chemical changes that triglycerides undergo during digestion.
13. What are the expected products from the enzymatic action of chymotrypsin on each amino acid segment?
    a. gly-ala-phe-thr-leu
    b. ala-ile-tyr-ser-arg
    c. val-trp-arg-leu-cys
14. What are the expected products from the enzymatic action of trypsin on each amino acid segment?
    a. leu-thr-glu-lys-ala

b. phe-arg-ala-leu-val
c. ala-arg-glu-trp-lys

15. proteins: amino acids; carbohydrates: monosaccharides; fats: fatty acids and glycerol

16. a. Chymotrypsin is found in the small intestine and catalyzes the hydrolysis of peptide bonds following aromatic amino acids.
b. Lactase is found in the small intestine and catalyzes the hydrolysis of lactose.
c. Pepsin is found in the stomach and catalyzes the hydrolysis of peptide bonds, primarily those that occur after aromatic amino acids.
d. Maltase is found in the small intestine and catalyzes the hydrolysis of maltose.

17. a. Bile salts aid in digestion by dispersing lipids throughout the aqueous solution in the small intestine.
b. Emulsification is important because lipids are not soluble in water; it breaks lipids up into smaller particles that can be more readily hydrolyzed by lipases.

18. a. gly-ala-phe and thr-leu
b. ala-ile-tyr and ser-arg
c. val-trp and arg-leu-cys

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7.6: ATP as Energy carrier

Cells couple the exergonic reaction of ATP hydrolysis with endergonic reactions to harness the energy within the bonds of ATP.

Learning Objectives

- Explain the role of ATP as the currency of cellular energy

ATP: Adenosine Triphosphate

Adenosine triphosphate (ATP) is the energy currency for cellular processes. ATP provides the energy for both energy-consuming endergonic reactions and energy-releasing exergonic reactions, which require a small input of activation energy. When the chemical bonds within ATP are broken, energy is released and can be harnessed for cellular work. The more bonds in a molecule, the more potential energy it contains. Because the bond in ATP is so easily broken and reformed, ATP is like a rechargeable battery that powers cellular process ranging from DNA replication to protein synthesis.

Molecular Structure

Adenosine triphosphate (ATP) is comprised of the molecule adenosine bound to three phosphate groups. Adenosine is a nucleoside consisting of the nitrogenous base adenine and the five-carbon sugar ribose. The three phosphate groups, in order of closest to furthest from the ribose sugar, are labeled alpha, beta, and gamma. Together, these chemical groups constitute an energy powerhouse. The two bonds between the phosphates are equal high-energy bonds (phosphoanhydride bonds) that, when broken, release sufficient energy to power a variety of cellular reactions and processes. The bond between the beta and gamma phosphate is considered “high-energy” because when the bond breaks, the products [adenosine diphosphate (ADP) and one inorganic phosphate group (P_i)] have a lower free energy than the reactants (ATP and a water molecule). ATP breakdown into ADP and P_i is called hydrolysis because it consumes a water molecule (hydro-, meaning “water”, and lysis, meaning “separation”).

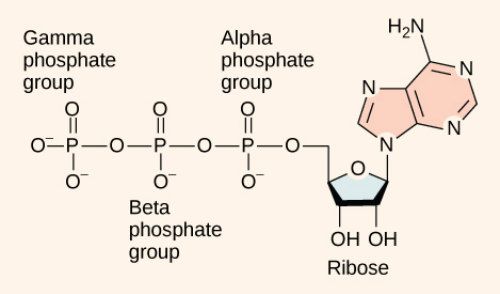


Figure 7.6.1: **Adenosine Triphosphate (ATP)**: ATP is the primary energy currency of the cell. It has an adenosine backbone with three phosphate groups attached.

ATP Hydrolysis and Synthesis

ATP is hydrolyzed into ADP in the following reaction:

$$ATP + H_2O \rightarrow ADP + P_i + \text{free energy}$$

Like most chemical reactions, the hydrolysis of ATP to ADP is reversible. The reverse reaction combines ADP + P_i to regenerate ATP from ADP. Since ATP hydrolysis releases energy, ATP synthesis must require an input of free energy.

ADP is combined with a phosphate to form ATP in the following reaction:

$$ADP + P_i + \text{free energy} \rightarrow ATP + H_2O$$

The **phosphorylation** (or condensation of phosphate groups onto AMP) is an endergonic process. By contrast, the hydrolysis of one or two phosphate groups from ATP, a process called **dephosphorylation**, is exergonic. Why? Let's recall that the terms endergonic and exergonic refer to the sign on the difference in free energy of a reaction between the products and reactants, ΔG. In this case we are explicitly assigning direction to the reaction, either in the direction of phosphorylation or dephosphorylation of the nucleotide. In the phosphorylation reaction the reactants are the nucleotide and an inorganic phosphate while the products are a

phosphorylated nucleotide and WATER. In the dephosphorylation/hydrolysis reaction, the reactants are the phosphorylated nucleotide and WATER while the products are inorganic phosphate and the nucleotide minus one phosphate.

"High-Energy" bonds

What about the term "high-energy bonds" that we so often hear associated with ATP? If there is nothing "special" about the bonds in ATP, why do we always hear the term "high-energy bonds" associated with the molecule? The answer is deceptively simple. In biology the term "high-energy bond" is used to describe an exergonic reaction involving the hydrolysis of the bond in question that results in a "large," negative change in free energy. Remember that this change in free energy does not only have to do with the bond in question but rather the sum of all bond rearrangements in the reaction. What constitutes a large change? It is a rather arbitrary assignment usually associated with an amount of energy associated with the types of anabolic reactions we typically observe in biology. If there is something special about the bonds in ATP, it is not uniquely tied to the free energy of hydrolysis, as there are plenty of other bonds whose hydrolysis results in greater negative differences in free energy.

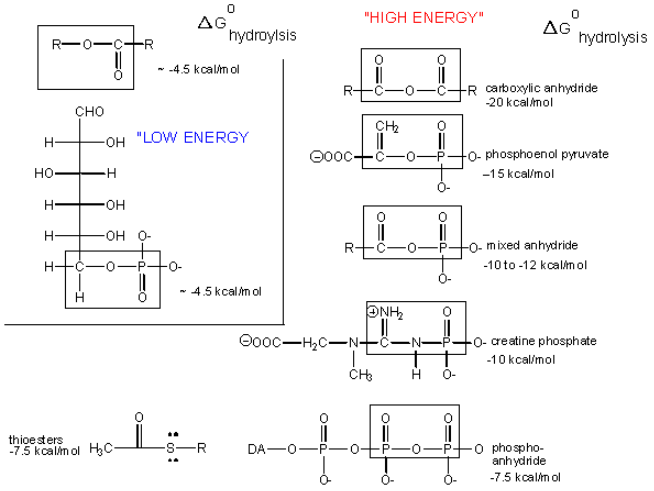


Figure 2. *The free energy of hydrolysis of different types of bonds can be compared to that of the hydrolysis of ATP.*
Source: http://bio.libretexts.org/Core/Biochemistry/Oxidation_and_Phosphorylation/ATP_and_Oxidative_Phosphorylation/Properties_of_ATP

Table 1. *Table of common cellular phosphorylated molecules and their respective free energies of hydrolysis, under physiological conditions.*

Compound	$\Delta G^{0\prime}$ kJ/mol
Phosphoenol pyruvate	-51.6
1,3-bis-Phosphoglycerate	-52.0
Acetylphosphate	-44.8
ATP	-31.8
ADP	-31.8
Acetyl-CoA	-31
AMP	-14.2
Glucose-6-P	-13.8

ATP and Energy Coupling

Exactly how much free energy (ΔG) is released with the hydrolysis of ATP, and how is that free energy used to do cellular work? The calculated ΔG for the hydrolysis of one mole of ATP into ADP and P_i is −7.3 kcal/mole (−30.5 kJ/mol). However, this is only true under standard conditions, and the ΔG for the hydrolysis of one mole of ATP in a living cell is almost double the value at standard conditions: 14 kcal/mol (−57 kJ/mol).

ATP is a highly unstable molecule. Unless quickly used to perform work, ATP spontaneously dissociates into ADP + P_i, and the free energy released during this process is lost as heat. To harness the energy within the bonds of ATP, cells use a strategy called

energy coupling.

Cells couple the exergonic reaction of ATP hydrolysis with the endergonic reactions of cellular processes. For example, during cellular metabolic reactions, or the synthesis and breakdown of nutrients, certain molecules must be altered slightly in their conformation to become substrates for the next step in the reaction series. In the very first steps of cellular respiration, glucose is broken down through the process of glycolysis. ATP is required for the phosphorylation of glucose, creating a high-energy but unstable intermediate. This phosphorylation reaction causes a conformational change that allows enzymes to convert the phosphorylated glucose molecule to the phosphorylated sugar fructose. Fructose is a necessary intermediate for glycolysis to move forward. In this example, the exergonic reaction of ATP hydrolysis is coupled with the endergonic reaction of converting glucose for use in the metabolic pathway.

The cycling of ATP pools

Estimates for the number of ATP molecules in a typical human cell range from ~$3x10^{7}$ (~$5x10^{-17}$ moles ATP/cell) in a white blood cell to $5x10^{9}$ (~$9x10^{-15}$ moles ATP/cell) in an active cancer cell. While these numbers might seem large, and already amazing, consider that it is estimated that this pool of ATP turns over (becomes ADP and then back to ATP) 1.5 x per minute. Extending this analysis yields the estimate that this daily turnover in your body, amounts to roughly the equivalent of one body weight of ATP getting turned over per day. That is, if no turnover/recycling of ATP happened, it would take 1 body weights worth of ATP for the human body to function - hence our previous characterization of ATP as a "short term" energy transfer device for the cell.

While the pool of ATP/ADP may be recycled, some of the energy that is transferred in the many conversions between ATP, ADP and other biomolecules is also transferred to the environment. In order to maintain cellular energy pools (that is, keep the concentration of ATP (vs. ADP) up to the level the cell needs) energy must transfer in from the environment as well. Where does this energy come from? The answer depends a lot on where energy is available and what mechanisms have evolved to transfer energy from the environment to molecular carriers like ATP. In nearly all cases, however, the mechanism of transfer includes some form of redox chemistry. In this and the sections that follow we will be studying some critical examples of energy transfer from the environment, key types of chemistry and biological reactions involved in this process, and some of the key biological reactions and cellular components associated with energy flow between different parts of the living system. We focus first on reactions involved in the (re)generation of ATP in the cell (not those involved in the creation of the nucleotide per se but rather those associated with the transfer of phosphates onto AMP and ADP- in other words, "recharging" of ADP/ATP).

How do cells generate ATP?

A variety of mechanisms have emerged over the 3.25 billion years of evolution to create ATP from ADP and AMP. The majority of these mechanism are modifications on two basic classes of mechanisms known as **Substrate Level Phosphorylation (SLP)** and **oxidative phosphorylation**. These topics are substantive enough that they will be discussed in detail in the next few modules. Both mechanisms rely on biochemical reactions that transfer energy from some energy source to ADP or AMP, to synthesize ATP.

Key Points

- Adenosine triphosphate is composed of the nitrogenous base adenine, the five-carbon sugar ribose, and three phosphate groups.
- ATP is hydrolyzed to ADP in the reaction ATP+H2O→ADP+Pi+ free energy; the calculated ΔG for the hydrolysis of 1 mole of ATP is -57 kJ/mol.
- ADP is combined with a phosphate to form ATP in the reaction ADP+Pi+free energy→ATP+H2O.
- The energy released from the hydrolysis of ATP into ADP is used to perform cellular work, usually by coupling the exergonic reaction of ATP hydrolysis with endergonic reactions.
- Sodium-potassium pumps use the energy derived from exergonic ATP hydrolysis to pump sodium and potassium ions across the cell membrane while phosphorylation drives the endergonic reaction.

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7.7: Coenzyme A

Coenzyme A

Coenzyme A (CoA) is a central compound in metabolism. It is a derivative of Pantothenic acid (vitamin B5) and a component of coenzyme A (CoA).The main functions of CoA is the activation and transfer of acyl groups. This function involves the reactive sulfhydryl group through the formation of thioester linkages with acyl groups.



Structure of coenzyme A: 1: 3′-phosphoadenosine. 2: diphosphate, organophosphate anhydride. 3: pantoic acid. 4: β-alanine. 5: cysteamine. Image by NEUROtiker, Public domain, via Wikimedia Commons.

Structure of acetyl-CoA



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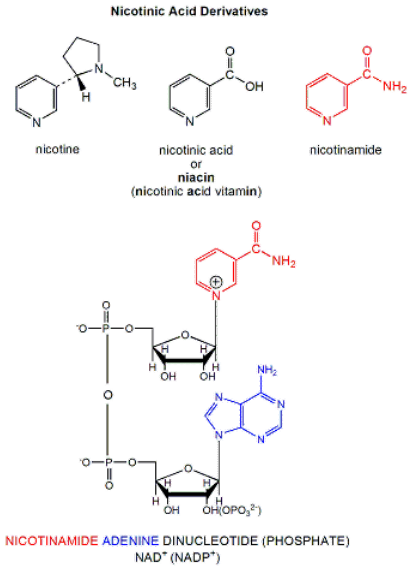
7.8: The Chemistry of NAD+ and FAD

In metabolism, many redox reactions are involved. Redox reactions require that electrons can be transferred or removed to either reduce or oxidize a particular substrate or molecule. Therefore, we need intermediates capable of undergoing electron transfer. These are the coenzymes NAD/NADH and FAD/FADH2. These coenzymes can exist in their oxidized (NAD+ and FAD) or reduced forms (NADH and FADH2). NADPH is a close derivatives of NADH that also acts as a redox couple.

NAD+ and NADP+

NAD+ and NADP+ are derivatives of nicotinic acid or nicotinamide. They intervene in biological redox reactions.

Figure: NAD is a derivative of nicotinic acid or nicotinamide. NADP+ contains an additional phosphate group



Both NAD+ and NADP+ can undergo two electron redox steps, in which a hydride is transferred from an organic molecule to the NAD+ or NADP+, with the electrons flowing to the positively charged nitrogen of NAD+ which serves as an electron sink. All NAD+/NADH reactions in the body involve 2 electron transfers. The products of these reactions is indicated ad NADH or NADPH, respectively.

Figure: All NAD+/NADH reactions in the body involve 2 electron hydride transfers

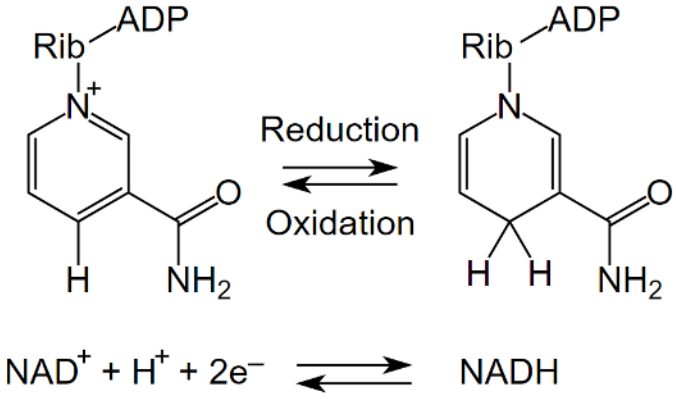


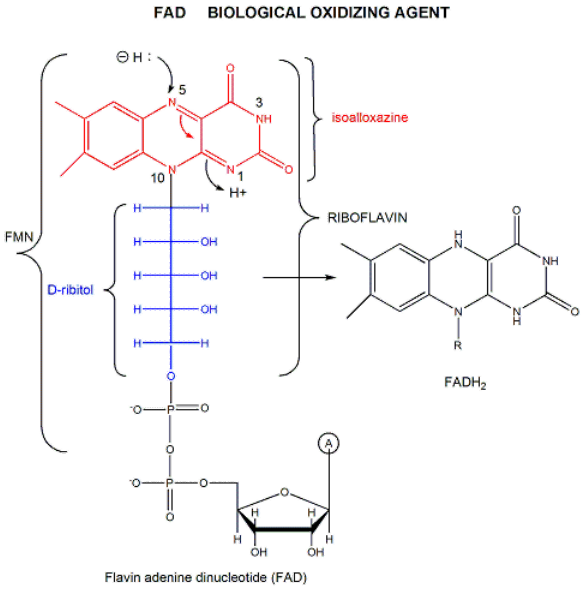
Image by Fvasconcellos 19:44, 9 December 2007 (UTC). w:Image:NAD oxidation reduction.png by Tim Vickers. / Public domain. Wikimedia Commons

The main difference between NADH and NADPH is that NADH is mainly involved in catabolic reactions, such as respiration, whereas NADPH is involved in anabolic reactions, such as photosynthesis.

FAD and FADH2

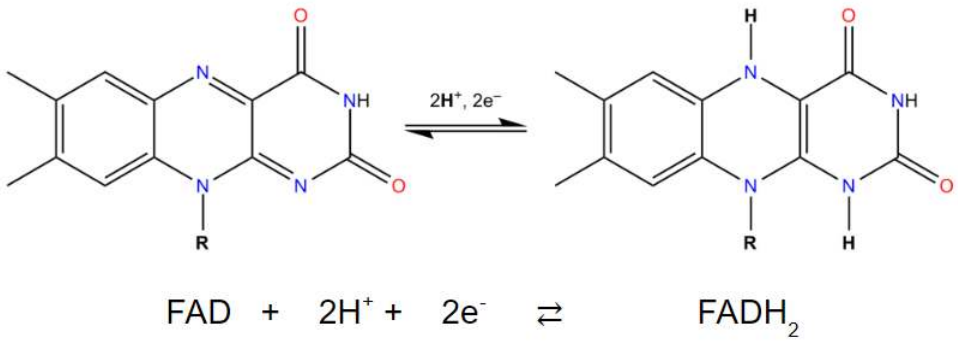
FAD (or flavin mononucleotide-FMN) and its reduction product, FADH2, are derivatives of riboflavin, and can also undergo redox reactions:

Figure: derivatives of riboflavin

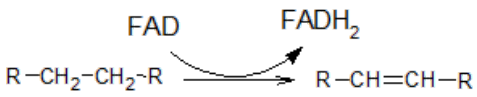


FAD/FADH2 differ from NAD+/NADH since they are bound tightly to enyzmes which use them. This is because FADH2 is susceptible to reaction with dioxygen while NADH is not. FAD/FADH2 is another redox pair that intervene in redox processes in biological systems

Figure: FAD/FADH2 electrons transfers. Image adapted from original by DMacks / Public domain on Wikimedia Commons



FAD/FADH2 are tightly bound to enzymes so as to control the nature of the oxidizing/reducing agent that interacts with them. (i.e. so dioxygen in the cell won't react with them in the cytoplasm.). FAD is usually involved in the oxidation of saturated carbon chains to form double bonds:



Contributors and Attributions

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CHAPTER OVERVIEW

8: Metabolism of carbohydrates

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8.1: Stage I of Carbohydrate Catabolism

Learning Objectives

- Describe the metabolism of carbohydrates.
- Know the source and function of common carbohydrates in the diet.

Dietary carbohydrates are sugars and sugar derivatives whose formulas can be written in the general form: $C_x(H_2O)_y$. (The subscripts *x* and *y* are whole numbers.). Some typical carbohydrates are sucrose (ordinary cane sugar), $C_{12}H_{22}O_{11}$; glucose (dextrose), $C_6H_{12}O_6$; fructose (fruit sugar), $C_6H_{12}O_6$; and ribose, $C_5H_{10}O_5$. Glucose is also the monomer from which the polymers cellulose and starch are built up. In food science and in many informal contexts, the term "carbohydrate" often means any food that is particularly rich in the complex carbohydrate starch (such as cereals, bread and pasta) or simple carbohydrates, such as sugar (found in candy, jams, and desserts). In the strict sense, "sugar" is applied for sweet, soluble carbohydrates, many of which are used in food.

Carbohydrates may be classified according to their degree of polymerization, and may be divided initially into three principal groups, namely sugars, oligosaccharides and polysaccharides[14]as shown in Table 8.1.1.

***Table* 8.1.1** *The Major Dietary Carbohydrates. Source: Wikipedia*

Class (DP*)	Subgroup	Components
Sugars (1–2)	Monosaccharides	Glucose, galactose, fructose, xylose
	Disaccharides	Sucrose, lactose, maltose, trehalose
Oligosaccharides (3–9)	Oligosaccharides	Maltodextrins, raffinose, stachyose, fructo-oligosaccharides
Polysaccharides (>9)	Starch, Glycogen	Amylose, amylopectin, modified starches
	Cellulose	Cellulose, Hemicellulose, Pectins, Hydrocolloids

DP * = Degree of polymerization

Digestion of Carbohydrates

The human body breaks down complex carbohydrates into **glucose and other monosaccharides**. Glucose in the blood (often referred to as “blood sugar”) is the **primary energy source** for the body. Sugars provide calories, or “energy,” for the body. Each gram of sugar provides **4 calories**. Glucose can be used immediately or stored in the liver and muscles for later use.

Carbohydrate digestion begins in the mouth (Figure 8.1.1) where salivary α-amylase attacks the α-glycosidic linkages in starch, the main polycarbohydrate ingested by humans. Cleavage of the glycosidic

linkages produces a mixture of dextrins, maltose, and glucose. The α-amylase mixed into the food remains active as the food passes through the esophagus, but it is rapidly inactivated in the acidic environment of the stomach.

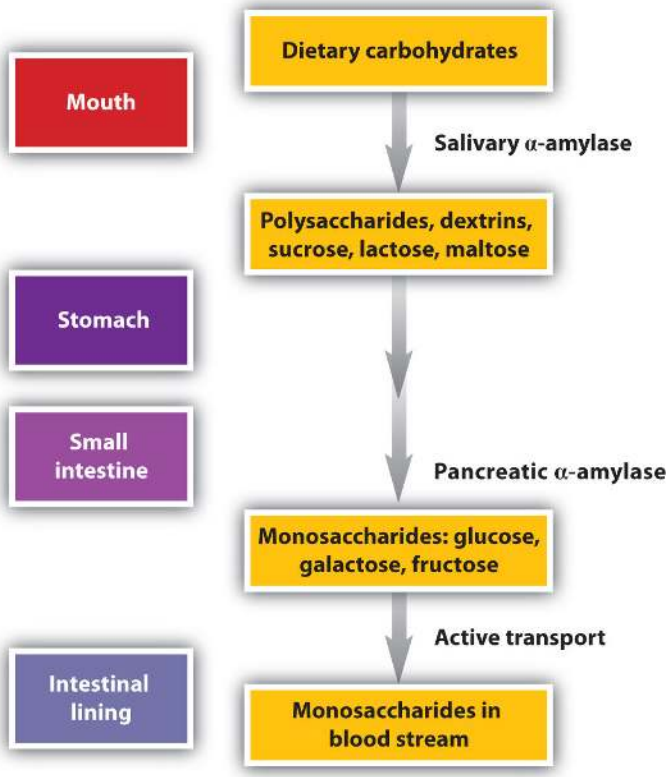


Figure **8.1.1** *The Principal events and sites of carbohydrate digestion.*

The primary site of carbohydrate digestion is the small intestine. The secretion of α-amylase in the small intestine converts any remaining starch molecules. Starch is then cleaved into glucose molecules. Disaccharides such as sucrose and lactose are not digested until they reach the small intestine, where they are acted on by sucrase and lactase, respectively. The major products of the complete hydrolysis of disaccharide and polysaccharides are three monosaccharide units: glucose, fructose, and galactose. These are absorbed through the wall of the small intestine into the bloodstream.

Fuel for the Brain

The brain is a marvelous organ. And it's a hungry one, too. The major fuel for the brain is the carbohydrate glucose. The average adult brain represents about 2% of our body's weight, but uses 25% of the glucose in the body. Moreover, specific areas of the brain use glucose at different rates. If you are concentrating hard (taking a test, for example), certain parts of the brain need a lot of extra glucose while other parts of the brain only use their normal amount. Something to think about.

Some foods that are high in carbohydrates include bread, pasta, and potatoes (Figure 8.1.2) . Because carbohydrates are easily digested, athletes often rely on carbohydrate rich foods to enable a high level of performance.

Figure **8.1.2** *Foods that serve as carbohydrate sources.*



Blood sugar level

The **blood sugar level, blood sugar concentration**, or **blood glucose level** is the concentration of glucose present in the blood of humans and other animals. Normal blood glucose level (tested while fasting) for non-diabetics is between 70 to 110 mg/dL. Glucose levels are usually lowest in the morning, before the first meal of the day, and rise after meals for an hour or two by a few millimoles. Blood sugar levels outside the normal range may be an indicator of a medical condition. A persistently high level is referred to as **hyperglycemia.** Long-term hyperglycemia causes many health problems including heart disease, cancer, eye, kidney, and nerve damage

A persistently low levels of blood glucose are referred to as **hypoglycemia**. . Symptoms may include lethargy, impaired mental functioning; irritability; shaking, twitching, weakness in arm and leg muscles; pale complexion; sweating; loss of consciousness. **Diabetes mellitus** is characterized by persistent hyperglycemia from any of several causes, and is the most prominent disease related to failure of blood sugar regulation.

- ### Summary
- Carbohydrate digestion begins in the mouth (in the presence of salivary α-amylase) and continues in the small intestine. The major products of the complete hydrolysis of disaccharides and polysaccharides are three monosaccharide units: glucose, fructose, and galactose. These are absorbed through the wall of the small intestine into the bloodstream.
- **The blood sugar level, blood sugar concentration, or blood glucose level is the concentration of glucose present in the blood of humans and other animals.**

Sources

Contributors and Attributions

- Wikipedia
- US FDA
- Ed Vitz (Kutztown University), John W. Moore (UW-Madison), Justin Shorb (Hope College), Xavier Prat-Resina (University of Minnesota Rochester), Tim Wendorff, and Adam Hahn.
- Libretext: Basics of GOB Chemistry (Ball, et al.)
- Template:ContribLindshield
-
- Marisa Alviar-Agnew (Sacramento City College)
- Blood sugar level on Wikipedia. Content adapted under Creative Commons Attribution-ShareAlike License. Retrieved Sept 23, 2020.

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8.2: Stage II of Carbohydrate Catabolism

Learning Objectives

- Describe the function of glycolysis and identify its major products.
- Describe how the presence or absence of oxygen determines what happens to the pyruvate and the NADH that are produced in glycolysis.
- Determine the amount of ATP produced by the oxidation of glucose in the presence and absence of oxygen.

In stage II of catabolism, the metabolic pathway known as glycolysis converts glucose into two molecules of pyruvate (a three-carbon compound with three carbon atoms) with the corresponding production of adenosine triphosphate (ATP). The individual reactions in glycolysis were determined during the first part of the 20th century. It was the first metabolic pathway to be elucidated, in part because the participating enzymes are found in soluble form in the cell and are readily isolated and purified. The pathway is structured so that the product of one enzyme-catalyzed reaction becomes the substrate of the next. The transfer of intermediates from one enzyme to the next occurs by diffusion.

Steps in Glycolysis

The 10 reactions of glycolysis, summarized in Figures 8.2.1, can be divided into two phases. In the first 5 reactions—phase I—glucose is broken down into two molecules of glyceraldehyde 3-phosphate. In the last five reactions—phase II—each glyceraldehyde 3-phosphate is converted into pyruvate, and ATP is generated. Notice that all the intermediates in glycolysis are phosphorylated and contain either six or three carbon atoms. Also, some of the glycolysis reactions are reversible (⇔), while some others are irreversible (→)

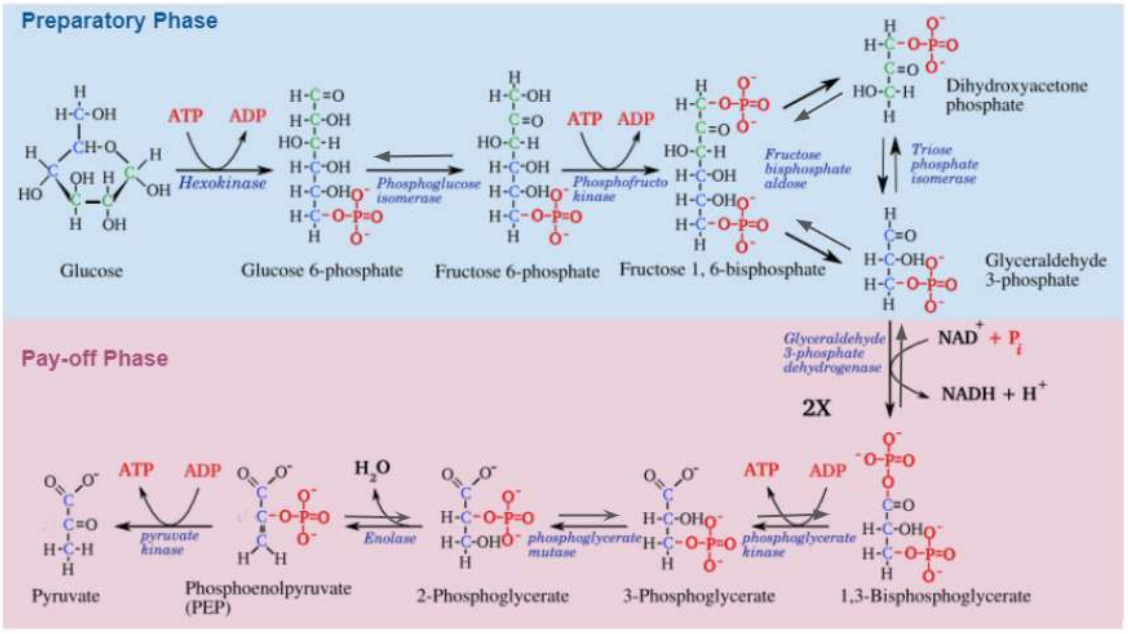


Figure 8.2.1: Reactions in Glycolysis. Image adapted from original image by Rozzychan / Public domain via Wikimedia Commons

- When glucose enters a cell, it is immediately phosphorylated to form glucose 6-phosphate, in the first reaction of phase I. The phosphate donor in this reaction is ATP, and the enzyme—which requires magnesium ions for its activity—is *hexokinase*. In this reaction, ATP is being used rather than being synthesized. The presence of such a reaction in a catabolic pathway that is supposed to *generate* energy may surprise you. However, in addition to activating the glucose molecule, this initial reaction is essentially irreversible, an added benefit that keeps the overall process moving in the right direction.

Furthermore, the addition of the negatively charged phosphate group prevents the intermediates formed in glycolysis from diffusing through the cell membrane, as neutral molecules such as glucose can do.

- In the next reaction, *phosphoglucose isomerase* catalyzes the isomerization of glucose 6-phosphate to fructose 6-phosphate. This reaction is important because it creates a primary alcohol, which can be readily phosphorylated.
- The subsequent phosphorylation of fructose 6-phosphate to form fructose 1,6-bisphosphate is catalyzed by *phosphofructokinase*, which requires magnesium ions for activity. ATP is again the phosphate donor. This irreversible reaction is considered a **committed step in glycolysis,** because once fructose 6-phosphate is converted into fructose 1,6-bisphosphate,all other reactions are reversible and glycolysis will proceed all the way to pyruvate. products formed up to this point can be used for purposes other than glycolysis (i.e. glycogen synthesis). Therefore the enzymatic activity of phosphofructokinase is highly regulated.

> When a molecule contains two phosphate groups on different carbon atoms, the convention is to use the prefix *bis*. When the two phosphate groups are bonded to each other on the same carbon atom (for example, adenosine diphosphate [ADP]), the prefix is *di*.

- Fructose 1,6-bisphosphate is enzymatically cleaved by *aldolase* to form two triose phosphates: dihydroxyacetone phosphate and glyceraldehyde 3-phosphate.
- Isomerization of dihydroxyacetone phosphate into a second molecule of glyceraldehyde 3-phosphate is the final step in phase I. The enzyme catalyzing this reaction is *triose phosphate isomerase*.

> In steps 4 and 5, aldolase and triose phosphate isomerase effectively convert one molecule of fructose 1,6-bisphosphate into *two* molecules of glyceraldehyde 3-phosphate. Thus, phase I of glycolysis requires energy in the form of two molecules of ATP and releases none of the energy stored in glucose.

In the initial step of phase II (Figure **8.2.1**), glyceraldehyde 3-phosphate is both oxidized and phosphorylated in a reaction catalyzed by *glyceraldehyde-3-phosphate dehydrogenase*, an enzyme that requires nicotinamide adenine dinucleotide (NAD^+) as the oxidizing agent and inorganic phosphate as the phosphate donor. In the reaction, NAD^+ is reduced to reduced nicotinamide adenine dinucleotide (NADH), and 1,3-bisphosphoglycerate (BPG) is formed.

- BPG has a high-energy phosphate bond (Table **8.2.1**) joining a phosphate group to C1. This phosphate group is now transferred directly to a molecule of ADP, thus forming ATP and 3-phosphoglycerate. The enzyme that catalyzes the reaction is *phosphoglycerate kinase*, which, like all other kinases, requires magnesium ions to function. This is the first reaction to produce ATP in the pathway. Because the ATP is formed by a direct transfer of a phosphate group from a metabolite to ADP —that is, from one substrate to another—the process is referred to as substrate-level phosphorylation, to distinguish it from the *oxidative phosphorylation* discussed in Section 20.4.
- In the next reaction, the phosphate group on 3-phosphoglycerate is transferred from the OH group of C3 to the OH group of C2, forming 2-phosphoglycerate in a reaction catalyzed by *phosphoglyceromutase*.
- A dehydration reaction, catalyzed by *enolase*, forms phosphoenolpyruvate (PEP), another compound possessing a high-energy phosphate group.
- The final step is irreversible and is the second reaction in which substrate-level phosphorylation occurs. The phosphate group of PEP is transferred to ADP, with one molecule of ATP being produced per molecule of PEP. The reaction is catalyzed by *pyruvate kinase*, which requires both magnesium and potassium ions to be active.

> In phase II, two molecules of glyceraldehyde 3-phosphate are converted to two molecules of pyruvate, along with the production of four molecules of ATP and two molecules of NADH.

To Your Health: Diabetes

Although medical science has made significant progress against diabetes , it continues to be a major health threat. Some of the serious complications of diabetes are as follows:

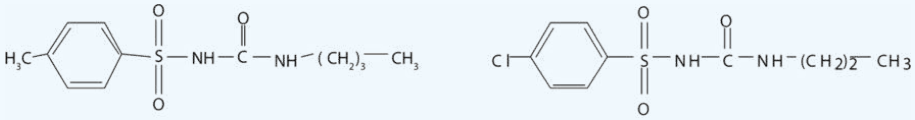
- It is the leading cause of lower limb amputations in the United States.
- It is the leading cause of blindness in adults over age 20.
- It is the leading cause of kidney failure.
- It increases the risk of having a heart attack or stroke by two to four times.

Because a person with diabetes is unable to use glucose properly, excessive quantities accumulate in the blood and the urine. Other characteristic symptoms are constant hunger, weight loss, extreme thirst, and frequent urination because the kidneys excrete large amounts of water in an attempt to remove excess sugar from the blood.

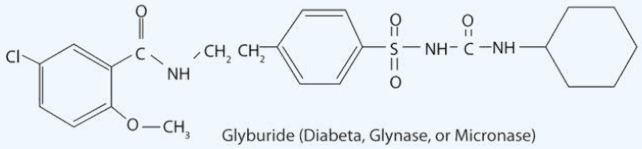
There are two types of diabetes. In immune-mediated diabetes, insufficient amounts of insulin are produced. This type of diabetes develops early in life and is also known as *Type 1 diabetes*, as well as insulin-dependent or juvenile-onset diabetes. Symptoms are rapidly reversed by the administration of insulin, and Type 1 diabetics can lead active lives provided they receive insulin as needed. Because insulin is a protein that is readily digested in the small intestine, it cannot be taken orally and must be injected at least once a day.

In Type 1 diabetes, insulin-producing cells of the pancreas are destroyed by the body's immune system. Researchers are still trying to find out why. Meanwhile, they have developed a simple blood test capable of predicting who will develop Type 1 diabetes several years before the disease becomes apparent. The blood test reveals the presence of antibodies that destroy the body's insulin-producing cells.

Type 2 diabetes, also known as noninsulin-dependent or adult-onset diabetes, is by far the more common, representing about 95% of diagnosed diabetic cases. (This translates to about 16 million Americans.) Type 2 diabetics usually produce sufficient amounts of insulin, but either the insulin-producing cells in the pancreas do not release enough of it, or it is not used properly because of defective insulin receptors or a lack of insulin receptors on the target cells. In many of these people, the disease can be controlled with a combination of diet and exercise alone. For some people who are overweight, losing weight is sufficient to bring their blood sugar level into the normal range, after which medication is not required if they exercise regularly and eat wisely.



First-Generation Antidiabetic Drugs



Second-Generation Antidiabetic Drugs

Those who require medication may use oral antidiabetic drugs that stimulate the islet cells to secrete insulin. First-generation antidiabetic drugs stimulated the release of insulin. Newer second-generation drugs, such as glyburide, do as well, but they also increase the sensitivity of cell receptors to insulin. Some individuals with Type 2 diabetes do not produce enough insulin and thus do not respond to these oral medications; they must use insulin. In both Type 1 and Type 2 diabetes, the blood sugar level must be carefully monitored and adjustments made in diet or medication to keep the level as normal as possible (70–120 mg/dL).

Metabolism of Pyruvate

The presence or absence of oxygen determines the fates of the pyruvate and the NADH produced in glycolysis. When plenty of oxygen is available, pyruvate is completely oxidized to carbon dioxide, previous conversation into acetyl-CoA, with the release of much greater amounts of ATP through the combined actions of the citric acid cycle, the electron transport chain, and

oxidative phosphorylation. However, in the absence of oxygen (that is, under anaerobic conditions), the fate of pyruvate is different in different organisms. In vertebrates, pyruvate is converted to lactate, while other organisms, such as yeast, convert pyruvate to ethanol and carbon dioxide. These possible fates of pyruvate are summarized in Figure **8.2.2**. The conversion to lactate or ethanol under anaerobic conditions allows for the reoxidation of NADH to NAD^+ in the absence of oxygen.

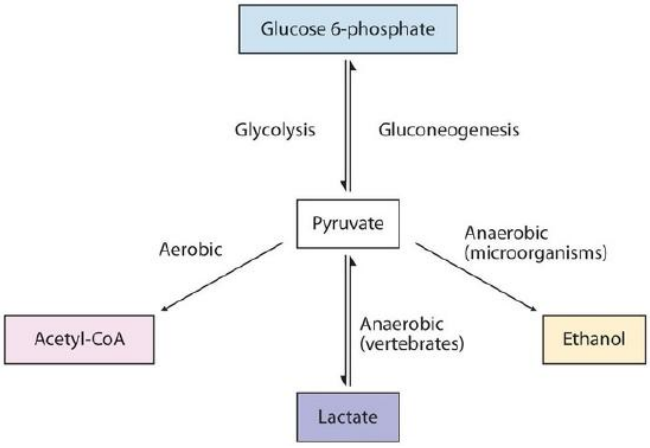


Figure **8.2.2**: Metabolic Fates of Pyruvate

- ## ATP Yield from Glycolysis

The net energy yield from anaerobic glucose metabolism can readily be calculated in moles of ATP. In the initial phosphorylation of glucose (step 1), 1 mol of ATP is expended, along with another in the phosphorylation of fructose 6-phosphate (step 3). In step 7, 2 mol of BPG (recall that 2 mol of 1,3-BPG are formed for each mole of glucose) are converted to 2 mol of 3-phosphoglycerate, and 2 mol of ATP are produced. In step 10, 2 mol of pyruvate and 2 mol of ATP are formed per mole of glucose.

For every mole of glucose degraded, 2 mol of ATP are initially consumed and 4 mol of ATP are ultimately produced. The net production of ATP is thus 2 mol for each mole of glucose converted to lactate or ethanol. If 7.4 kcal of energy is conserved per mole of ATP produced, and the total amount of energy that can theoretically be obtained from the complete oxidation of 1 mol of glucose is 670 kcal (as stated in the chapter introduction), the energy conserved in the anaerobic catabolism of glucose to two molecules of lactate (or ethanol) is as follows:

$$\frac{2 \times 7.4\ \text{kcal}}{670\ \text{kcal}} \times 100 = 2.2\% \tag{8.2.1}$$

Thus anaerobic cells extract only a very small fraction of the total energy of the glucose molecule.

Contrast this result with the amount of energy obtained when glucose is completely oxidized to carbon dioxide and water through glycolysis, the citric acid cycle, the electron transport chain, and oxidative phosphorylation as summarized in Table **8.2.1**. Note the indication in the table that a variable amount of ATP is synthesized, depending on the tissue, from the NADH formed in the cytoplasm during glycolysis. This is because NADH is not transported into the inner mitochondrial membrane where the enzymes for the electron transport chain are located. Instead, brain and muscle cells use a transport mechanism that passes electrons from the cytoplasmic NADH through the membrane to flavin adenine dinucleotide (FAD) molecules inside the mitochondria, forming reduced flavin adenine dinucleotide ($FADH_2$), which then feeds the electrons into the electron transport chain. This route lowers the yield of ATP to 1.5–2 molecules of ATP, rather than the usual 2.5–3 molecules. A more efficient transport system is found in liver, heart, and kidney cells where the formation of one cytoplasmic NADH molecule results in the formation of one mitochondrial NADH molecule, which leads to the formation of 2.5–3 molecules of ATP.The total amount of energy conserved in the aerobic catabolism of glucose in the liver is as follows:

$$\frac{38 \times 7.4\ \text{kcal}}{670\ \text{kcal}} \times 100 = 42\% \tag{8.2.2}$$

Conservation of 42% of the total energy released compares favorably with the efficiency of any machine. In comparison, automobiles are only about 20%–25% efficient in using the energy released by the combustion of gasoline.

As indicated earlier, the 58% of released energy that is not conserved enters the surroundings (that is, the cell) as heat that helps to maintain body temperature. If we are exercising strenuously and our metabolism speeds up to provide the energy needed for muscle contraction, more heat is produced. We begin to perspire to dissipate some of that heat. As the perspiration evaporates, the excess heat is carried away from the body by the departing water vapor.

Summary

- The monosaccharide glucose is broken down through a series of enzyme-catalyzed reactions known as glycolysis.
- For each molecule of glucose that is broken down, two molecules of pyruvate, two molecules of ATP, and two molecules of NADH are produced.
- In the absence of oxygen, pyruvate is converted to lactate, and NADH is reoxidized to NAD+. In the presence of oxygen, pyruvate is converted to acetyl-CoA and then enters the citric acid cycle.
- More ATP can be formed from the breakdown of glucose when oxygen is present.

Concept Review Exercises

1. In glycolysis, how many molecules of pyruvate are produced from one molecule of glucose?
2. In vertebrates, what happens to pyruvate when
   a. plenty of oxygen is available?
   b. oxygen supplies are limited?
3. In anaerobic glycolysis, how many molecules of ATP are produced from one molecule of glucose?

Answers

1. two
2. a. Pyruvate is completely oxidized to carbon dioxide.
   b. Pyruvate is reduced to lactate, allowing for the reoxidation of NADH to NAD^+.
3. There is a net production of two molecules of ATP.

Exercises

1. Replace each question mark with the correct compound.
   a. $\text{fructose 1,6-bisphosphate} \xrightarrow{\text{aldolase}} ? + ?$
   b. $? + \text{ADP} \xrightarrow{\text{pyruvate kinase}} \text{pyruvate} + \text{ATP}$
   c. $\text{dihydroxyacetone phosphate} \xrightarrow{?} \text{glyceraldehyde 3-phosphate}$
   d. $\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} ? + \text{ADP}$
2. Replace each question mark with the correct compound.
   a. $\text{fructose 6-phosphate} + \text{ATP} \xrightarrow{?} \text{fructose 1,6-bisphosphate} + \text{ADP}$
   b. $? \xrightarrow{\text{phosphoglucose isomerase}} \text{fructose 6-phosphate}$
   c. $\text{glyceraldehyde 3-phosphate} + \text{NAD}^+ + \text{P}_\text{i} \xrightarrow{?} \text{1,3-bisphosphoglycerate} + \text{NADH}$
   d. $\text{3-phosphoglycerate} \xrightarrow{\text{phosphoglyceromutase}} ?$
3. From the reactions in Exercises 1 and 2, select the equation(s) by number and letter in which each type of reaction occurs.
   a. hydrolysis of a high-energy phosphate compound
   b. synthesis of ATP
4. From the reactions in Exercises 1 and 2, select the equation(s) by number and letter in which each type of reaction occurs.
   a. isomerization
   b. oxidation
5. What coenzyme is needed as an oxidizing agent in glycolysis?

6. Calculate

   a. the *total* number of molecules of ATP produced for each molecule of glucose converted to pyruvate in glycolysis.
   b. the number of molecules of ATP hydrolyzed in phase I of glycolysis.
   c. the *net* ATP production from glycolysis alone.

7. How is the NADH produced in glycolysis reoxidized when oxygen supplies are limited in

   a. muscle cells?
   b. yeast?

Answers

1. a. glyceraldehyde 3-phosphate + dihydroxyacetone phosphate
   b. phosphoenolpyruvate
   c. triose phosphate isomerase
   d. glucose 6-phosphate

3. a. reactions 1b, 1d, and 2a
   b. reaction 1b

5. NAD^+

7. a. Pyruvate is reduced to lactate, and NADH is reoxidized to NAD^+.
   b. Pyruvate is converted to ethanol and carbon dioxide, and NADH is reoxidized to NAD^+.

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8.3: Glycolysis Regulation

Regulation

Control of glycolysis occurs at three enzymatic points:

$$\underbrace{\text{Glucose} \rightleftharpoons \text{G}_6\text{P}}_{\text{hexokinase}}$$

$$\underbrace{\text{F}_6\text{P} \rightleftharpoons \text{F}_1,6\,\text{BP}}_{\text{phosphofructokinase (PFK)}}$$

$$\underbrace{\text{PEP} \rightleftharpoons \text{pyruvate}.}_{\text{pyruvate kinase}}$$

Consider the regulation of **Hexokinase**. This enzyme is allosterically inhibited by glucose-6-phosphate (G6P). The enzyme **phosphofructokinase** (PFK) is inhibited by ATP and activated by AMP and fructose-2,6- bisphosphate (F2,6BP, a molecule that regulates glycolysis and gluconeogenesis). **Pyruvate kinase** is allosterically inhibited by ATP and activated by fructose-1,6-bisphosphate (F1,6BP) and AMP.

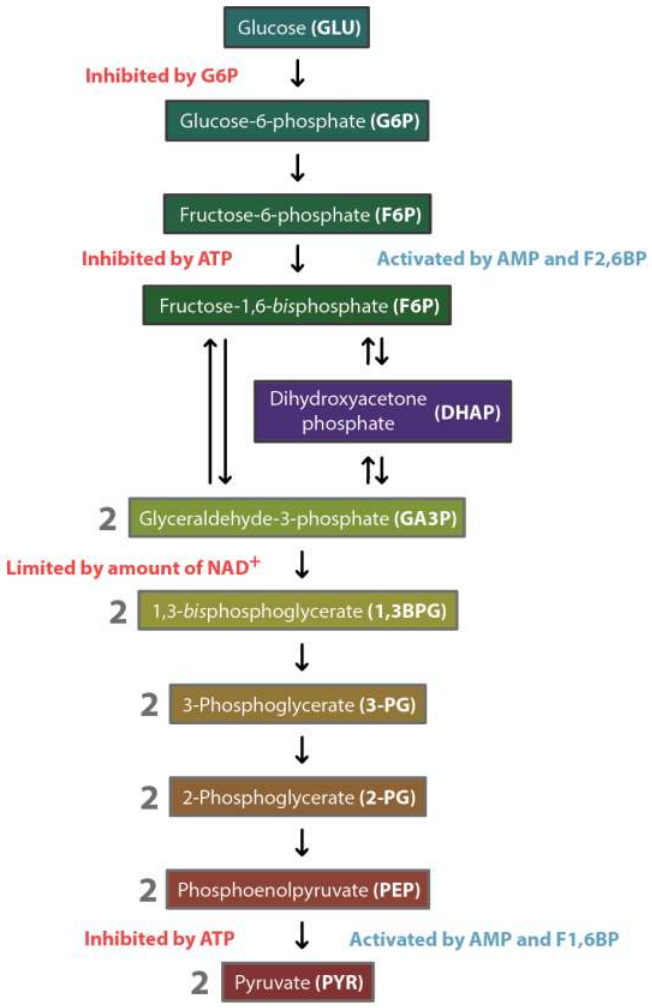


Figure 9.1.3**:** Glycolysis Regulation

Glycolysis is regulated in a reciprocal fashion compared to its corresponding anabolic pathway, **gluconeogenesis.** Reciprocal regulation occurs when the same molecule or treatment (phosphorylation, for example) has opposite effects on catabolic and anabolic pathways. Reciprocal regulation is important when anabolic and corresponding catabolic pathways are occurring in the same cellular location.

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8.4: Fermentation

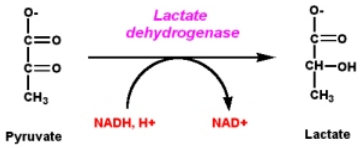
Fermentation is the process by which living organisms recycle $NADH \rightarrow NAD^+$. NAD^+ is a required molecule necessary for the oxidation of Glyceraldehyde-3-phosphate to produce the high energy molecule 1,3-bisphosphoglycerate (Step 6 of Glycolysis). Fermentation occurs in the cytosol of cells.

Introduction

Because NAD^+ is used in Glycolysis it is important that living cells have a way of recycling NAD^+ from $NADH$. One way that a cell recycles NAD^+ is through the process of respiration, a set of sequential electron transfers involving an electron transport chain to a terminal electron acceptor. In aerobic organisms, the terminal electron acceptor is oxygen. In anaerobic organisms, the terminal electron acceptor can vary from species to species and include but are not limited to various metals like Fe(III), Mn(IV) and Co(III), CO_2, nitrate, sulfur This process reduces NADH back to NAD^+ which can then be used again in step 6 of Glycolysis or other red/ox reactions in the cell. Another way that NAD^+ is recycled from $NADH$ is by a process called fermentation.

Example: Lactic acid fermentation in contracting muscle

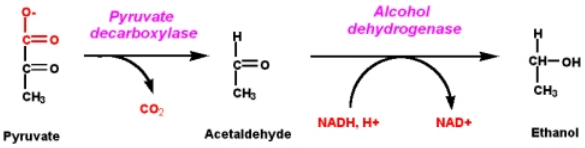
Lactic acid fermentation occurs by converting pyruvate into lactate using the enzyme Lactate dehydrogenase and producing NAD^+ in the process. This process takes place in oxygen depleted muscle and some bacteria. It is responsible for the sour taste of sauerkraut and yogurt. NAD^+ is required for the oxidation of glyceraldehyde-3-P to produce 1,3-Bisphosphoglycerate (Step 6 of Gycolysis). If the supply of NAD^+ is not replenished by the ETC or fermentation, glycolysis is unable to proceed. Fermentation is a necessary process for anaerobic organisms to produce energy. The yield of energy is much less than if the organism were to continue on through the TCA cycle and ETC, but energy is produce nonetheless.



Example: Alcoholic fermentation in yeast

The purpose of fermentation in yeast is the same as that in muscle and bacteria, to replenish the supply of NAD^+ for glycolysis, but this process occurs in two steps:

1. Alcoholic fermentation consists of pyruvate being first converted into acetaldehyde by the enzyme pyruvate decarboxylase and releasing CO_2.
2. In the second step acetaldehyde is converted into ethanol using alcohol dehydrogenase and producing NAD^+ in the process. It is this recycled NAD^+ that can be used to continue on with glycolysis.



References

- Garrett, H., Reginald and Charles Grisham. Biochemistry. Boston: Twayne Publishers, 2008.
- Raven, Peter. Biology. Boston: Twayne Publishers, 2005.

Problems

1. Draw the chemical structures of pyruvate, ethanol and lactate (the reactant and products of fermentation)
2. Why is fermentation necessary? (Hint: see step 6 of Glycolysis)
3. What type of environment is necessary for fermentation to occur?
4. Where does fermentation occur? What part of the cell?
5. Explain the alternative to fermentation and why it is able to proceed. (Hint: Final electron acceptor)

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8.5: 8.5-Stage III of carbohydrate catabolism. The Krebs Cycle (Citric Acid Cycle)

The primary catabolic pathway in the body is the **citric acid cycle (CAC)** because it is here that oxidation to CO_2 occurs for breakdown products of the cell's major building blocks - sugars, fatty acids, amino acids. The pathway is cyclic (***Figure 10.1***) and thus, doesn't really have a starting or ending point. All of the reactions occur in the mitochondrion, though one enzyme is embedded in the organelle's membrane. As needs change, cells may use a subset of the reactions of the cycle to produce a desired molecule rather than to run the entire cycle.

The primary catabolic pathway in the body is the citric acid cycle, also known as the tricarboxylic acid cycle and the Krebs cycle, completes the oxidation of glucose by taking the pyruvates from glycolysis (and other pathways), and completely breaking them down into CO_2 molecules, H_2O molecules, and generating additional ATP by oxidative phosphorylation. In prokaryotic cells, the citric acid cycle occurs in the cytoplasm; in eukaryotic cells the citric acid cycle takes place in the matrix of the mitochondria.

The overall reaction for the citric acid cycle is:

$$\textit{acetylCoA} + 3\ NAD^+ + FAD + GDP + Pi + H_2O \rightarrow 2\ CO_2 + CoA + 3\ NADH + 2H^+ + FADH_2 + GTP$$

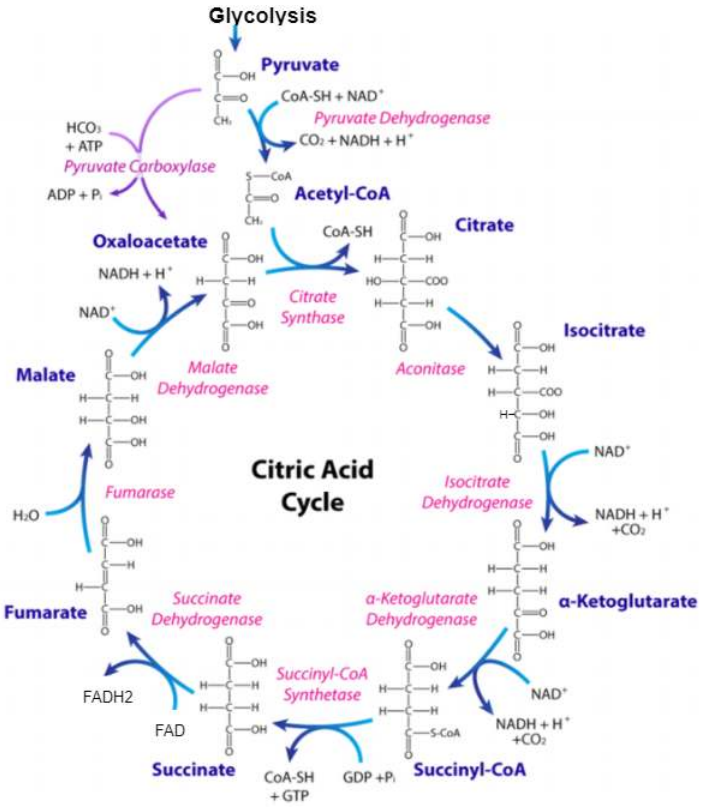


Figure 10.1: *The citric acid cycle*

Steps in the Citric Acid Cycle and Regulation

Step 1. The first step is a condensation step, combining the two-carbon acetyl group (from acetyl CoA) with a four-carbon oxaloacetate molecule to form a six-carbon molecule of citrate. CoA is bound to a sulfhydryl group (-SH) and diffuses away to eventually combine with another acetyl group. This step is irreversible because it is highly exergonic. **The rate of this reaction is controlled by negative feedback and the amount of ATP and NADH available.** If ATP or NADH levels increase, the rate of this reaction decreases. If ATP is in short supply and concentration of ADP increases, the rate increases.

Step 2. Citrate loses one water molecule and gains another as citrate is converted into its isomer, isocitrate.

Steps 3 and 4. In step three, isocitrate is oxidized, producing a five-carbon molecule, α-ketoglutarate, together with a molecule of CO_2 and two electrons, which reduce NAD^+ to NADH. **This step is also regulated by negative feedback from ATP and NADH and by a positive effect of ADP.** Steps three and four are both oxidation and decarboxylation steps, which release electrons that reduce NAD^+ to NADH and release carboxyl groups that form CO_2 molecules. α-Ketoglutarate is the product of step three, and a succinyl group is the product of step four. CoA binds the succinyl group to form succinyl CoA. **The enzyme that catalyzes step four is regulated by feedback inhibition of ATP, succinyl CoA, and NADH.**

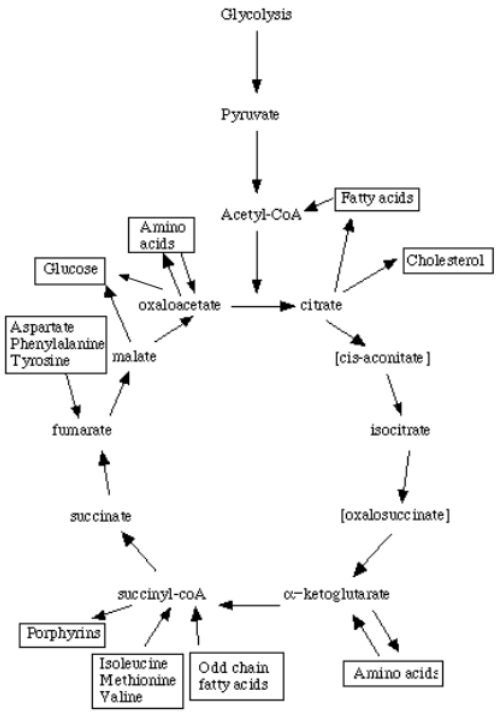
Step 5. A phosphate group is substituted for coenzyme A, and a high- energy bond is formed. This energy is used in substrate-level phosphorylation (during the conversion of the succinyl group to succinate) to form either guanine triphosphate (GTP) or ATP. There are two forms of the enzyme, called isoenzymes, for this step, depending upon the type of animal tissue in which they are found. One form is found in tissues that use large amounts of ATP, such as heart and skeletal muscle. This form produces ATP. The second form of the enzyme is found in tissues that have a high number of anabolic pathways, such as liver. This form produces GTP. GTP is energetically equivalent to ATP; however, its use is more restricted. In particular, protein synthesis primarily uses GTP.

Step 6. Step six is a dehydration process that converts succinate into fumarate with the help of an enzyme called **succinate dehydrogenate**. Two hydrogen atoms are transferred to FAD, producing $FADH_2$. The energy contained in the electrons of these atoms is insufficient to reduce NAD^+ but adequate to reduce FAD. Unlike NADH, this carrier remains attached to the enzyme and transfers the electrons to the electron transport chain directly. This process is made possible by the localization of the enzyme catalyzing this step inside the inner membrane of the mitochondrion.

Step 7. Water is added to fumarate during step seven, and malate is produced. The last step in the citric acid cycle regenerates oxaloacetate by oxidizing malate. Another molecule of NADH is produced.

Products of the Citric Acid Cycle

Two carbon atoms come into the citric acid cycle from each acetyl group, representing four out of the six carbons of one glucose molecule. Two carbon dioxide molecules are released on each turn of the cycle; however, these do not necessarily contain the most recently-added carbon atoms. The two acetyl carbon atoms will eventually be released on later turns of the cycle; thus, all six carbon atoms from the original glucose molecule are eventually incorporated into carbon dioxide. Each turn of the cycle forms three NADH molecules and one $FADH_2$ molecule. These carriers will connect with the last portion of aerobic respiration to produce ATP molecules. One GTP or ATP is also made in each cycle. Several of the intermediate compounds in the citric acid cycle can be used in synthesizing non-essential amino acids; therefore, the cycle is **amphibolic**, meaning that the Citric Acid Cycle serves a role in both catabolic and anabolic processes.



Amphibolic Properties of the Citric Acid Cycle. Image adapted from original by Sazhnyev, CC BY-SA 4.0, via Wikimedia Commons

Summary

In the citric acid cycle, the acetyl group from acetyl CoA is attached to a four-carbon oxaloacetate molecule to form a six-carbon citrate molecule. Through a series of steps, citrate is oxidized, releasing two carbon dioxide molecules for each acetyl group fed into the cycle. In the process, three NAD^+ molecules are reduced to NADH, one FAD molecule is reduced to $FADH_2$, and one ATP or GTP (depending on the cell type) is produced (by substrate-level phosphorylation). Because the final product of the citric acid cycle is also the first reactant, the cycle runs continuously in the presence of sufficient reactants.

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8.6: Oxidative Phosphorylation

Skills to Develop

- Describe how electrons move through the electron transport chain and what happens to their energy levels
- Explain how a proton (H^+) gradient is established and maintained by the electron transport chain

You have just read about two pathways in glucose catabolism—glycolysis and the citric acid cycle—that generate ATP and coenzymes NADH and FADH2. Most of the ATP generated during the aerobic catabolism of glucose, however, is not generated directly from these pathways. Rather, it is derived from a process that begins with moving electrons (e-) from NADH and FADH2 through a series of electron transporters (Electron Trasport Chain, located in the inner mitochondrial membrane) that undergo redox reactions. The Electron Transport Chain catalyzes the reduction of O_2 into H_2O:

$4H^+ + 4e- + O_2$ --> $2H_2O$

or, equivalently,

$2H^+ + 2e- + 1/2\ O_2$ --> H_2O

Simultaneously with transporting electrons and reducing oxygen, some of the electrons transporters (complexes I, III, and IV, see figure Figure **8.6.1**) are able to pump protons (hydrogen ions, H+) from the matrix into the intermembrane space, and it is in this way that the hydrogen ion gradient is established and maintained between the two compartments separated by the inner mitochondrial membrane. This causes hydrogen ions to accumulate in the intermembrane space. Therefore, a concentration gradient forms. The energy accumulated by this gradient is used by the enzyme ATP synthase to synthesize ATP . While hydrogen ions diffuse back into the matrix by passing through ATP synthase, the flux of hydrogen ions powers the catalytic action of ATP synthase, which phosphorylates ADP, producing ATP. This mechanism is called **chemiosmosis**.

Electron Transport Chain

The electron transport chain (Figure **8.6.1**) is the last component of aerobic respiration and is the only part of glucose metabolism that uses atmospheric oxygen. Oxygen continuously diffuses into plants; in animals, it enters the body through the respiratory system. Electron transport is a series of redox reactions that resemble a relay race or bucket brigade in that electrons are passed rapidly from one component to the next, to the endpoint of the chain where the electrons reduce molecular oxygen, producing water. There are four complexes composed of proteins, labeled I through IV in Figure **8.6.1**, and the aggregation of these four complexes, together with associated mobile, accessory electron carriers, is called the **electron transport chain**. The electron transport chain is present in multiple copies in the inner mitochondrial membrane of eukaryotes and the plasma membrane of prokaryotes.

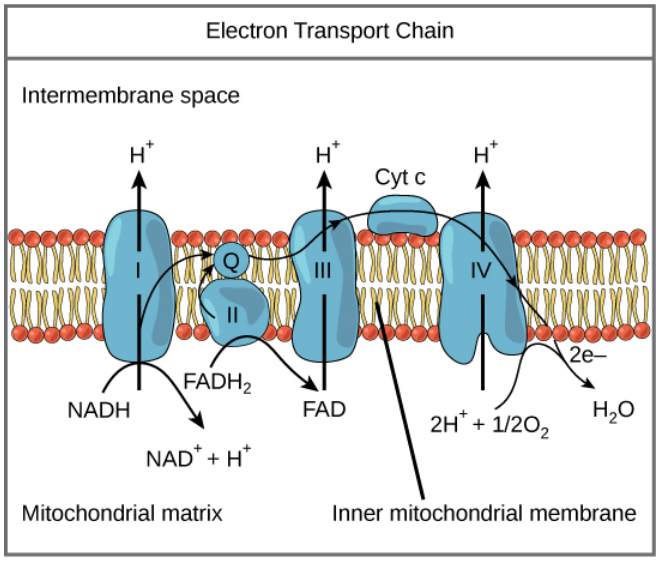


Figure **8.6.1**: The electron transport chain is a series of electron transporters embedded in the inner mitochondrial membrane that shuttles electrons from NADH and $FADH_2$ to molecular oxygen. In the process, protons are pumped from the mitochondrial matrix to the intermembrane space, and oxygen is reduced to form water.

Complex I

To start, two electrons are carried to the first complex aboard NADH. This complex, labeled I, is composed of flavin mononucleotide (FMN) and an iron-sulfur (Fe-S)-containing protein. FMN, which is derived from vitamin B_2, also called riboflavin, is one of several prosthetic groups or co-factors in the electron transport chain. A prosthetic group is a non-protein molecule required for the activity of a protein. Prosthetic groups are organic or inorganic, non-peptide molecules bound to a protein that facilitate its function; prosthetic groups include co-enzymes, which are the prosthetic groups of enzymes. The enzyme in complex I is NADH dehydrogenase and is a very large protein, containing 45 amino acid chains. Complex I can pump four hydrogen ions across the membrane from the matrix into the intermembrane space, and it is in this way that the hydrogen ion gradient is established and maintained between the two compartments separated by the inner mitochondrial membrane.

Q and Complex II

Complex II directly receives $FADH_2$, which does not pass through complex I. The compound connecting the first and second complexes to the third is ubiquinone (Q). The Q molecule is lipid soluble and freely moves through the hydrophobic core of the membrane. Once it is reduced, (QH_2), ubiquinone delivers its electrons to the next complex in the electron transport chain. Q receives the electrons derived from NADH from complex I and the electrons derived from $FADH_2$ from complex II, including succinate dehydrogenase. This enzyme and $FADH_2$ form a small complex that delivers electrons directly to the electron transport chain, bypassing the first complex. Since these electrons bypass and thus do not energize the proton pump in the first complex, fewer ATP molecules are made from the $FADH_2$ electrons. The number of ATP molecules ultimately obtained is directly proportional to the number of protons pumped across the inner mitochondrial membrane.

Complex III

The third complex is composed of cytochrome b, another Fe-S protein, Rieske center (2Fe-2S center), and cytochrome c proteins; this complex is also called cytochrome oxidoreductase. Cytochrome proteins have a prosthetic group of heme. The heme molecule is similar to the heme in hemoglobin, but it carries electrons, not oxygen. As a result, the iron ion at its core is reduced and oxidized as it passes the electrons, fluctuating between different oxidation states: Fe^{++} (reduced) and Fe^{+++} (oxidized). The heme molecules in the cytochromes have slightly different characteristics due to the effects of the different proteins binding them, giving slightly different characteristics to each complex. Complex III pumps protons through the membrane and passes its electrons to cytochrome c for transport to the fourth complex of proteins and enzymes (cytochrome c is the acceptor of electrons from Q; however, whereas Q carries pairs of electrons, cytochrome c can accept only one at a time).

Complex IV

The fourth complex is composed of cytochrome proteins c, a, and a_3. This complex contains two heme groups (one in each of the two cytochromes, a, and a_3) and three copper ions (a pair of Cu_A and one Cu_B in cytochrome a_3). The cytochromes hold an oxygen molecule very tightly between the iron and copper ions until the oxygen is completely reduced. The reduced oxygen then picks up two hydrogen ions from the surrounding medium to make water (H_2O). The removal of the hydrogen ions from the system contributes to the ion gradient used in the process of chemiosmosis.

Chemiosmosis

In chemiosmosis, the free energy from the series of redox reactions just described is used to pump hydrogen ions (protons) across the membrane. The uneven distribution of H^+ ions across the membrane establishes both concentration and electrical gradients (thus, an electrochemical gradient), owing to the hydrogen ions' positive charge and their aggregation on one side of the membrane.

If the membrane were open to diffusion by the hydrogen ions, the ions would tend to diffuse back across into the matrix, driven by their electrochemical gradient. Recall that many ions cannot diffuse through the nonpolar regions of phospholipid membranes without the aid of ion channels. Similarly, hydrogen ions in the matrix space can only pass through the inner mitochondrial membrane through an integral membrane protein called ATP synthase (Figure 8.6.2). This complex protein acts as a tiny generator, turned by the force of the hydrogen ions diffusing through it, down their electrochemical gradient. The turning of parts of this molecular machine facilitates the addition of a phosphate to ADP, forming ATP, using the potential energy of the hydrogen ion gradient.

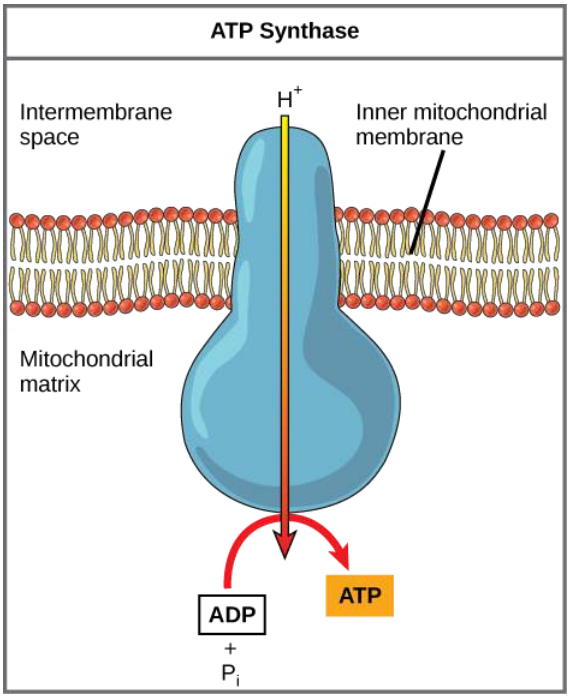


Figure 8.6.2: ATP synthase is a complex, molecular machine that uses a proton (H+) gradient to form ATP from ADP and inorganic phosphate (Pi). (Credit: modification of work by Klaus Hoffmeier)

Chemiosmosis (Figure 8.6.3) is used to generate 90 percent of the ATP made during aerobic glucose catabolism; it is also the method used in the light reactions of photosynthesis to harness the energy of sunlight in the process of photophosphorylation. Recall that the production of ATP using the process of chemiosmosis in mitochondria is called oxidative phosphorylation. The overall result of these reactions is the production of ATP from the energy of the electrons removed from hydrogen atoms. These

atoms were originally part of a glucose molecule. At the end of the pathway, the electrons are used to reduce an oxygen molecule to oxygen ions. The extra electrons on the oxygen attract hydrogen ions (protons) from the surrounding medium, and water is formed.

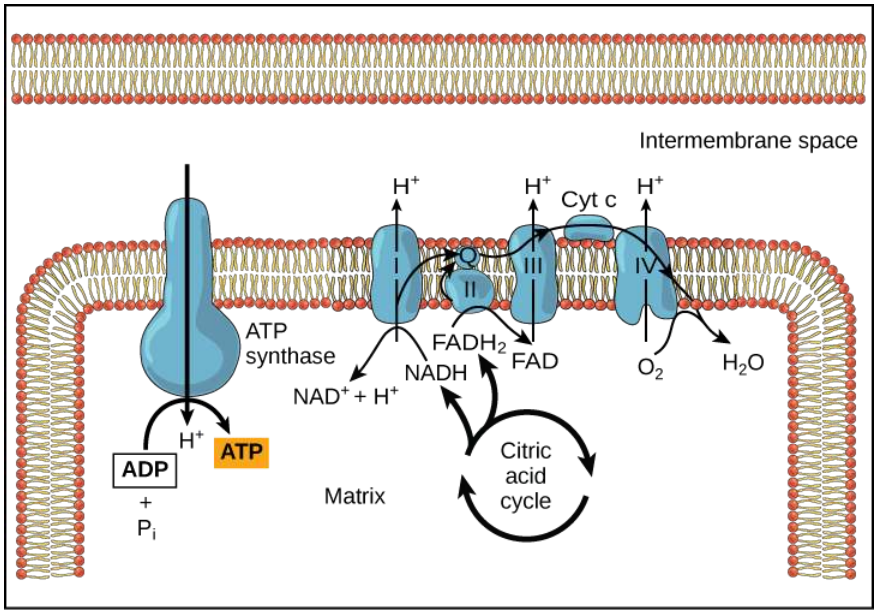


Figure **8.6.3**: In oxidative phosphorylation, the pH gradient formed by the electron transport chain is used by ATP synthase to form ATP.

Summary

The electron transport chain is the portion of aerobic respiration that uses free oxygen as the final electron acceptor of the electrons removed from the intermediate compounds in glucose catabolism. The electron transport chain is composed of four large, multiprotein complexes embedded in the inner mitochondrial membrane and two small diffusible electron carriers shuttling electrons between them. The electrons are passed through a series of redox reactions, with a small amount of free energy used at three points to transport hydrogen ions across a membrane. This process contributes to the gradient used in chemiosmosis. The electrons passing through the electron transport chain gradually lose energy, High-energy electrons donated to the chain by either NADH or $FADH_2$ complete the chain, as low-energy electrons reduce oxygen molecules and form water. The level of free energy of the electrons drops from about 60 kcal/mol in NADH or 45 kcal/mol in $FADH_2$ to about 0 kcal/mol in water. The end products of the electron transport chain are water and ATP. A number of intermediate compounds of the citric acid cycle can be diverted into the anabolism of other biochemical molecules, such as nonessential amino acids, sugars, and lipids. These same molecules can serve as energy sources for the glucose pathways.

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8.7: Energy yield by complete oxidation of glucose

Learning Objectives

- Determine the amount of ATP produced by the oxidation of glucose in the presence and absence of oxygen.

Determining the exact yield of ATP for aerobic respiration is difficult for a number of reasons. First of all, the number of ATP generated per reduced NADH or $FADH_2$ is not always a whole number. For every pair of electrons transported to the electron transport chain by a molecule of NADH, between 2 and 3 ATP are generated. For each pair of electrons transferred by $FADH_2$, between 1 and 2 ATP are generated. In eukaryotic cells, unlike prokaryotes, NADH generated in the cytoplasm during glycolysis must be transported across the mitochondrial membrane before it can transfer electrons to the electron transport chain. Muscle and brain cells use a transport mechanism that converts the NADH in the cytoplasm into FADH2. In the liver, kidneys, and heart cells, a different transport mechanism is used, and NADH in the cytoplasm is converted into NADH in the mitochondria. As a result, different numbers of ATP molecules are generated from cytoplasmatic NADH in each tissue.

For simplicity, however, we will look at the theoretical maximum yield of ATP per glucose molecule oxidized by aerobic respiration. We will assume that for each pair of electrons transferred to the electron transport chain by NADH, 2.5 ATP will be generated; for each electron pair transferred by $FADH_2$, 1.5 ATP will be generated. Keep in mind, however, that less ATP may actually be generated.

In eukaryotic cells, the theoretical maximum yield of ATP generated per glucose is 30 to 32, depending on how the 2 NADH generated in the cytoplasm during glycolysis enter the mitochondria and whether the resulting yield is 2 or 3 ATP per NADH.

Table **8.7.1**: Maximum Yield of ATP from the Complete Oxidation of 1 Mol of Glucose

Reaction	Comments	Yield of ATP (moles)
glucose → glucose 6-phosphate	consumes 1 mol ATP	−1
fructose 6-phosphate → fructose 1,6-bisphosphate	consumes 1 mol ATP	−1
glyceraldehyde 3-phosphate → BPG	produces 2 mol of cytoplasmic NADH	
BPG → 3-phosphoglycerate	produces 2 mol ATP	+2
phosphoenolpyruvate → pyruvate	produces 2 mol ATP	+2
pyruvate → acetyl-CoA + CO_2	produces 2 mol NADH	
isocitrate → α-ketoglutarate + CO_2	produces 2 mol NADH	
α-ketoglutarate → succinyl-CoA + CO_2	produces 2 mol NADH	
succinyl-CoA → succinate	produces 2 mol GTP	+2
succinate → fumarate	produces 2 mol $FADH_2$	
malate → oxaloacetate	produces 2 mol NADH	
2 cytoplasmic NADH from glycolysis	yields 2.5 or 1.5 mol ATP per NADH (depending on tissue)	+3 to +5
2 NADH from the oxidation of pyruvate	yields 2.5 mol ATP per NADH	+5
2 $FADH_2$ from the citric acid cycle	yields 1.5 ATP per $FADH_2$	+3
6 NADH from the citric acid cycle	yields 2.5 ATP per NADH	+15
Net yield of ATP:		+30 to +32

ATP Yield from Glycolysis and Oxidative Phosphorylation

When glucose is chemically "burned" as a fuel to produce carbon dioxide (CO_2) and water (H_2O), the energy released from this oxidation process is 670 kcal/mol of glucose:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6CO_2 + 6\ H_2O \quad \Delta H = -670\ \text{kcal/mol}$$

The net energy yield from anaerobic glucose metabolism can readily be calculated in moles of ATP. In the initial phosphorylation of glucose (step 1), 1 mol of ATP is expended, along with another in the phosphorylation of fructose 6-phosphate (step 3). In step 7, 2 mol of BPG (recall that 2 mol of 1,3-BPG are formed for each mole of glucose) are converted to 2 mol of 3-phosphoglycerate, and 2 mol of ATP are produced. In step 10, 2 mol of pyruvate and 2 mol of ATP are formed per mole of glucose. For every mole of glucose degraded, 2 mol of ATP are initially consumed and 4 mol of ATP are ultimately produced. The net production of ATP is thus 2 mol for each mole of glucose converted to lactate or ethanol. If 7.4 kcal of energy is conserved per mole of ATP produced, the energy conserved in the anaerobic catabolism of glucose to two molecules of lactate (or ethanol) is as follows:

$$2\times\ [7.4kcal\ /\ 670kcal]\ \times 100 = 2.2\ \%$$

Thus anaerobic cells extract only a very small fraction of the total energy of the glucose molecule by glycolysis. Contrast this result with the amount of energy obtained when glucose is completely oxidized to carbon dioxide and water through glycolysis, the citric acid cycle, the electron transport chain, and oxidative phosphorylation. Note that a variable amount of ATP is synthesized, depending on the tissue, from the NADH formed in the cytoplasm during glycolysis. This is because NADH is not transported into the inner mitochondrial membrane where the enzymes for the electron transport chain are located. Instead, brain and muscle cells use a transport mechanism that passes electrons from the cytoplasmic NADH through the membrane to flavin adenine dinucleotide (FAD) molecules inside the mitochondria, forming reduced flavin adenine dinucleotide (FADH2), which then feeds the electrons into the electron transport chain. This route lowers the yield of ATP to 1.5 molecules of ATP, rather than the usual 2.5 molecules. A more efficient transport system is found in liver, heart, and kidney cells where the formation of one cytoplasmic NADH molecule results in the formation of one mitochondrial NADH molecule, which leads to the formation of 2.5 molecules of ATP. The total amount of energy conserved in the aerobic catabolism of glucose in the liver is as follows:

$$32\times\ [7.4kcal\ /\ 670kcal]\ \times\ 100 = 35\ \%$$

Conservation of 35% of the total energy released compares favorably with the efficiency of any machine. In comparison, automobiles are only about 20%–25% efficient in using the energy released by the combustion of gasoline. As indicated earlier, the 58% of released energy that is not conserved enters the surroundings (that is, the cell) as heat that helps to maintain body temperature. If we are exercising strenuously and our metabolism speeds up to provide the energy needed for muscle contraction, more heat is produced. We begin to perspire to dissipate some of that heat. As the perspiration evaporates, the excess heat is carried away from the body by the departing water vapor.

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8.8: Carbohydrate Storage and Breakdown

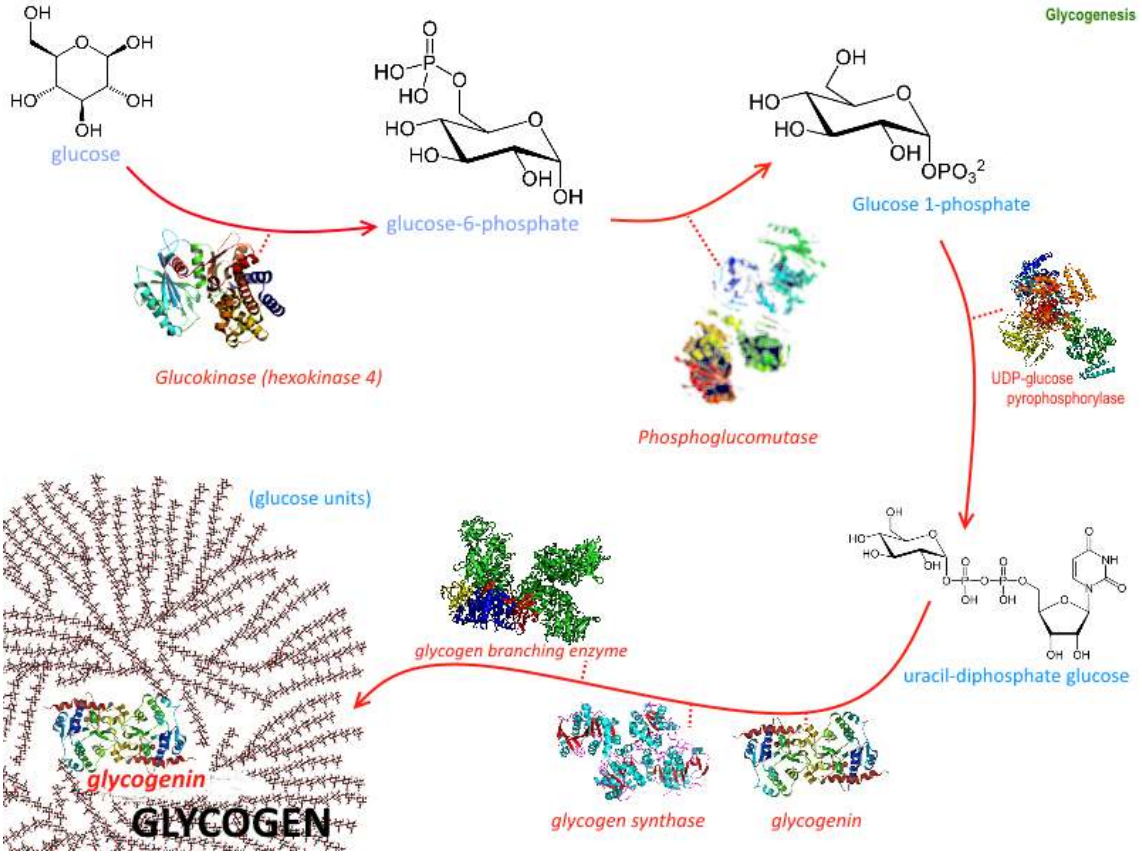
Carbohydrates are important cellular energy sources. They provide energy quickly through glycolysis and passing of intermediates to pathways, such as the citric acid cycle, and amino acid metabolism (indirectly). It is important, therefore, to understand how these important molecules are used and stored.

Plants are notable in storing glucose for energy in the form of amylose and amylopectin (see and for structural integrity in the form of cellulose. These structures differ in that cellulose contains glucoses solely joined by beta-1,4 bonds, whereas amylose has only alpha1,4 bonds and amylopectin has alpha 1,4 and alpha 1,6 bonds. Animals store glucose primary in liver and muscle in the form of a compound related to amylopectin known as glycogen. The structural differences between glycogen and amylopectin are solely due to the frequency of the alpha 1,6 branches of glucoses. In glycogen they occur about every 10 residues instead of every 30-50, as in amylopectin.

Glycogen Synthesis or **Glycogenesis**

When the glucose intake is higher than the energy demand, the body stores the glucose excess as glycogen. This process is called **glycogenesis**. Let us first consider the steps in glycogen synthesis. 1) Glycogen synthesis from glucose involves phosphorylation to form Glucose-6-Phospahte (G6P), and isomerization to form Glucose-1-Phosphate (G1P) (using phosphoglucomutase common to glycogen breakdown). G1P is reacted with UTP to form UDP-glucose in a reaction catalyzed by UDP-glucose pyrophosphorylase. Glycogen synthase catalyzes synthesis of glycogen by joining carbon #1 of the UDPG-derived glucose onto the carbon #4 of the non-reducing end of a glycogen chain. to form the familiar alpha(1,4) glycogen links. Another product of the reaction is UDP.

It is also worth noting in passing that glycogen synthase will only add glucose units from UDPG onto a preexisting glycogen chain that has at least four glucose residues. Linkage of the first few glucose units to form the minimal "primer" needed for glycogen synthase recognition is catalyzed by a protein called glycogenin, which attaches to the first glucose and catalyzes linkage of the first eight glucoses by alpha(1,4) bonds. 3) The characteristic alpha(1,6) branches of glycogen are the products of an enzyme known as Branching Enzyme. Branching Enzyme breaks alpha(1,4) chains and carries the broken chain to the carbon #6 and forms an alpha(1,6) linkage.



The steps in Glycogenesys. Image by Mark Cidade, CC BY-SA 4.0, via Wikimedia Commons

Glycogen Breakdown or **Glycogenolysis**

When the cell requires energy and there is no glucose available, the body will use its glycogen repository. This process is called **Glycogenolysis**. Glycogenolysis occurs mostly in the liver and muscle cells. Glycogen phosphorylase (sometimes simply called phosphorylase) catalyzes breakdown of glycogen into Glucose-1-Phosphate (G1P). The reaction, (see below right) that produces G1P from glycogen is a phosphorolysis, not a hydrolysis reaction. The distinction is that hydrolysis reactions use water to cleave bigger molecules into smaller ones, but phosphorolysis reactions use phosphate instead for the same purpose. Note that the phosphate is just that - it does NOT come from ATP. Since ATP is not used to put phosphate on G1P, the reaction saves the cell energy.

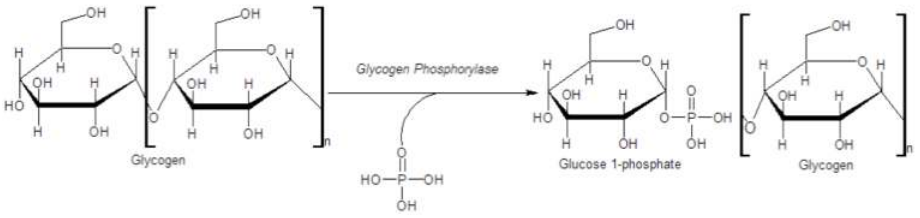


Figure 7.1.3: *Phosphorolysis of Glycogen*

Glycogen phosphorylase will only act on non-reducing ends of a glycogen chain that are at least 5 glucoses away from a branch point. A second enzyme, Glycogen Debranching Enzyme (GDE), is therefore needed to convert alpha(1-6) branches to alpha(1-4)

branches. GDE acts on glycogen branches that have reached their limit of hydrolysis with glycogen phosphorylase.

If glycogenolysis is taking place in the liver, glucose 6-phosphate can be converted to glucose by the enzyme **glucose 6-phosphatase**; the glucose produced in the liver is then released to the bloodstream for use in other organs.

$$\text{Glucose-6-phosphate} \xrightarrow{\text{glucose 6-phosphatase}} \text{Glucose} + \text{Pi}$$

Muscle cells in contrast do not have the enzyme glucose 6-phosphatase, so they cannot share their glycogen stores with the rest of the body.

Glucose can, of course, be converted to Glucose-6-Phosphate (G6P) as the first step in glycolysis by either hexokinase or glucokinase. G1P can be converted to G6P by action of an enzyme called phosphoglucomutase. This reaction is readily reversible, allowing G6P and G1P to be interconverted as the concentration of one or the other increases. This is important, because phosphoglucomutase is needed to form G1P for glycogen biosynthesis.

Regulation of Glycogen Metabolism

Regulation of glycogen metabolism is complex, occurring both allosterically and via hormone-receptor controlled events that result in protein phosphorylation or dephosphorylation. In order to avoid a futile cycle of glycogen synthesis and breakdown simultaneously, cells have evolved an elaborate set of controls that ensure only one pathway is primarily active at a time.

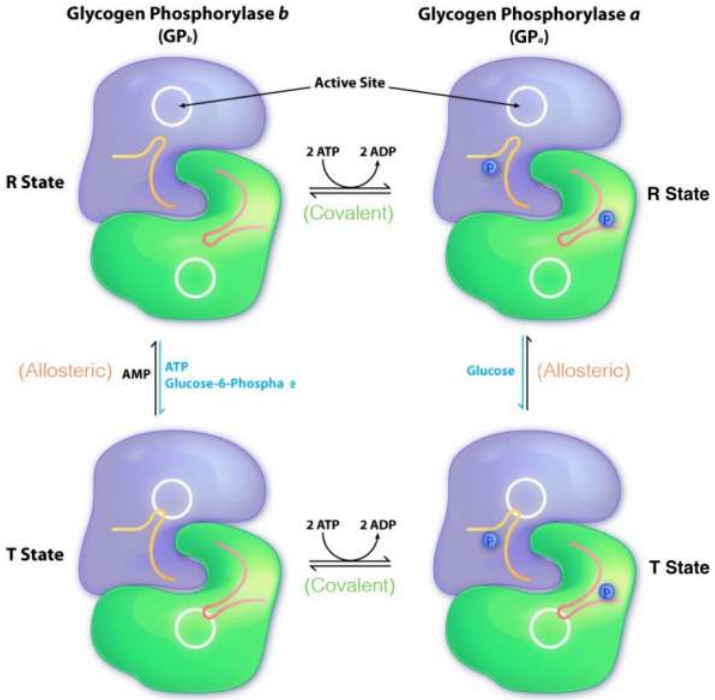


Figure 7.1.4: *Regulation of Glycogen Phosphorylase*

Regulation of glycogen metabolism is managed by the enzymes glycogen phosphorylase and glycogen synthase. Glycogen phosphorylase (GP) is regulated by both allosteric factors (ATP, G6P, AMP, and glucose) and by covalent modification (phosphorylation/dephosphorylation). Its regulation is consistent with the energy needs of the cell. High energy substrates (ATP, G6P, glucose) allosterically inhibit GP, while low energy substrates (AMP, others) allosterically activate it. Glycogen phosphorylase can be found in two different states, glycogen phosphorylase a (GP*a*) and glycogen phosphorylase b (GP*b*). The difference in the structures is due to phosphorylation of the Ser-14 residue which results in the active form (GP*a*). Protein phosphatases dephosphorylate the GP*a* to the inactive form, also known as GP*b*. Both forms of glycogen phosphorylase can also be found in T and R states where T is the inactive state because it appears to have a low affinity for substrate and R is the active state where it appears to have a greater affinity for substrate.

After a meal, blood glucose levels rise and insulin is released. It simultaneously stimulates uptake of glucose by cells and incorporation of it into glycogen by activation of glycogen synthase and inactivation of glycogen phosphorylase. When blood glucose levels fall, glycogen phosphorylase gets activated, stimulating glycogen breakdown to raise blood glucose.

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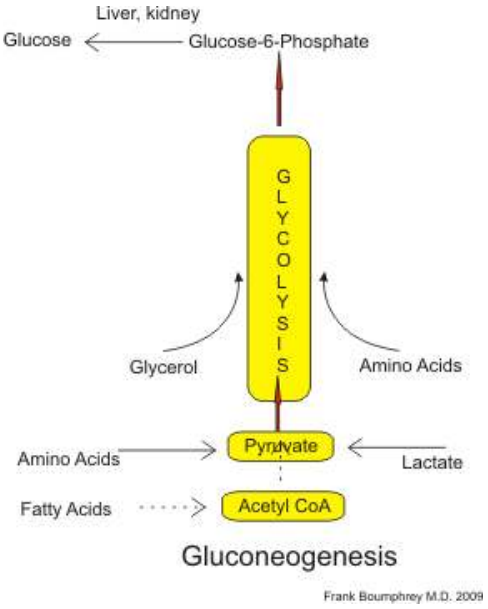
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8.9: Gluconeogenesis- Reaction and regulation

The main source of energy for eukaryotes is glucose. When glucose is unavailable, organisms are capable of metabolizing glucose from other non-carbohydrate precursors. The process that coverts pyruvate into glucose is called **gluconeogenesis**. Pyruvate can be generated from the degradation of lactate, fatty acids, certain amino acids and glycerol.

This metabolic pathway is important because the brain depends on glucose as its primary fuel and red blood cells use only glucose as a fuel. The daily glucose requirement of the brain in a typical adult human being is about 120 g, which accounts for most of the 160 g of glucose needed daily by the whole body. The amount of glucose present in body fluids is about 20 g, and that readily available from glycogen, a storage form of glucose, is approximately 190 g. Thus, the direct glucose reserves are sufficient to meet glucose needs for about a day. During a longer period of starvation, glucose must be formed from noncarbohydrate sources.

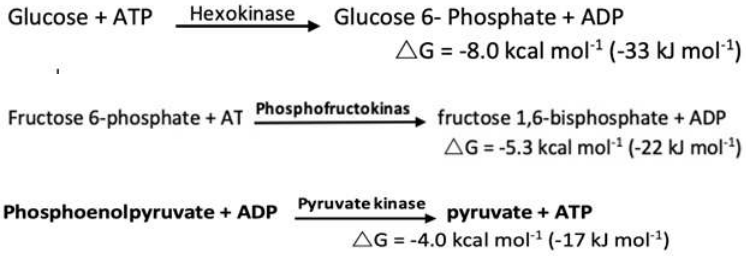
The major site of **gluconeogenesis** is the *liver*, with a small amount also taking place in the *kidney, brain, skeletal muscle, or heart muscle*.



Overview of Glucogenesis. Image by Boumphreyfr, CC BY-SA 3.0, via Wikimedia Commons

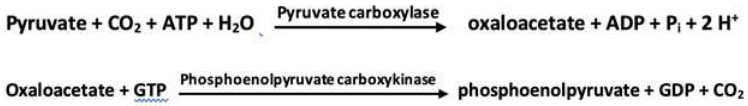
Gluconeogenesis Is Not a Reversal of Glycolysis

In glycolysis, glucose is converted into pyruvate; in **gluconeogenesis**, pyruvate is converted into glucose. However, ***gluconeogenesis*** *is not a reversal of glycolysis (see figure 8.9.1)*. Several reactions must differ because the equilibrium of glycolysis lies far on the side of pyruvate formation. The actual ΔG for the formation of pyruvate from glucose is about -20 kcal mol^{-1} (-84 kJ mol^{-1}) under typical cellular conditions. Most of the decrease in free energy in glycolysis takes place in the three essentially irreversible steps catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase.

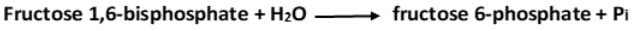


In **gluconeogenesis**, the following new steps bypass these virtually irreversible reactions of glycolysis:

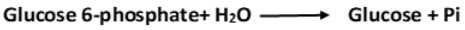
1. Phosphoenolpyruvate is formed from pyruvate by way of oxaloacetate through the action of pyruvate carboxylase and phosphoenolpyruvate carboxykinase.



2. *Fructose 6-phosphate is formed from fructose 1,6-bisphosphate by hydrolysis of the phosphate ester at carbon 1*. Fructose 1,6-bisphosphatase catalyzes this exergonic hydrolysis.



3. *Glucose is formed by hydrolysis of glucose 6-phosphate* in a reaction catalyzed by glucose 6-phosphatase.



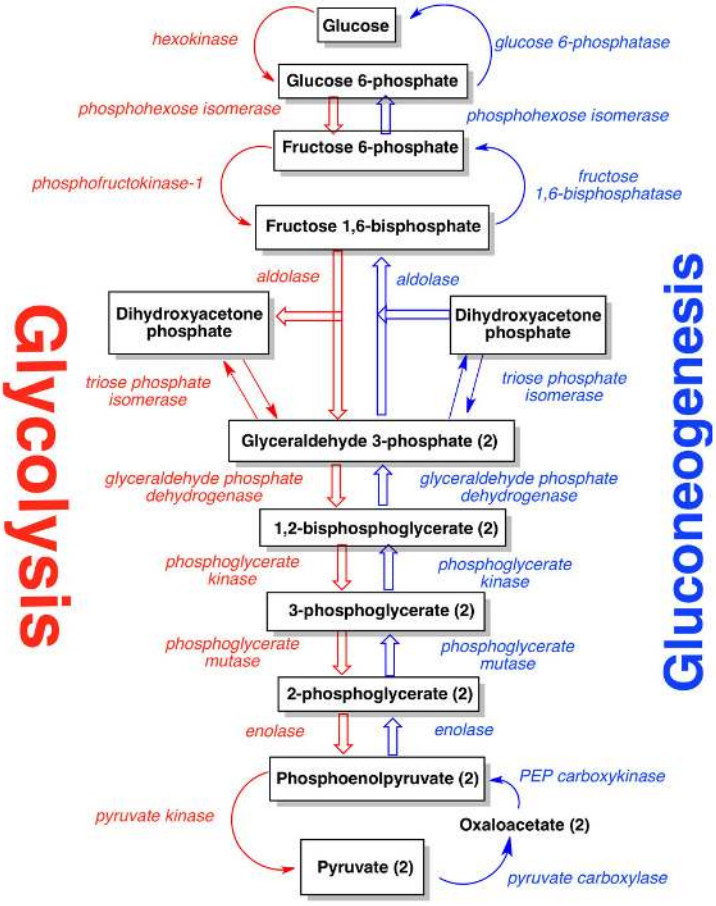


Figure 8.9.1. Glycolysis vs Glucogenogenesis. Image by Dwong527 / CC BY-SA (https://creativecommons.org/licenses/by-sa/3.0). Wikimedia Commons.

Regulation

It is important for organisms to conserve energy, they have derived ways to regulate those metabolic pathways that require and release the most energy. When there is an excess of energy available, gluconeogenesis is inhibited. When energy is required, gluconeogenesis is activated.

1. The conversion of pyruvate to PEP is regulated by acetyl-CoA. Because acetyl-CoA is an important metabolite in the TCA cycle which produces a lot of energy, when concentrations of acetyl-CoA are high organisms use pyruvate carboxylase to channel pyruvate away from the TCA cycle. If the organism does not need more energy, then it is best to divert those metabolites towards storage or other necessary processes.
2. The conversion of fructose-1,6-bisphosphate to fructose-6-phosphate with the use of fructose-1,6-phosphatase is negatively regulated and inhibited by the molecules AMP and fructose-2,6-bP. These are reciprocal regulators to glycolysis' phosphofructokinase. Phosphofructosekinase is positively regulated by AMP and fructose-2,6-bP. Once again, when the energy levels produced are higher than needed, i.e. a large ATP to AMP ratio, the organism increases gluconeogenesis and decreases glycolysis. The opposite also applies when energy levels are lower than needed, i.e. a low ATP to AMP ratio, the organism increases glycolysis and decreases gluconeogenesis.
3. The conversion of glucose-6-P to glucose with use of glucose-6-phosphatase is controlled by substrate level regulation. The metabolite responsible for this type of regulation is glucose-6-P. As levels of glucose-6-P increase, glucose-6-phosphatase increases activity and more glucose is produced. Thus glycolysis is unable to proceed.

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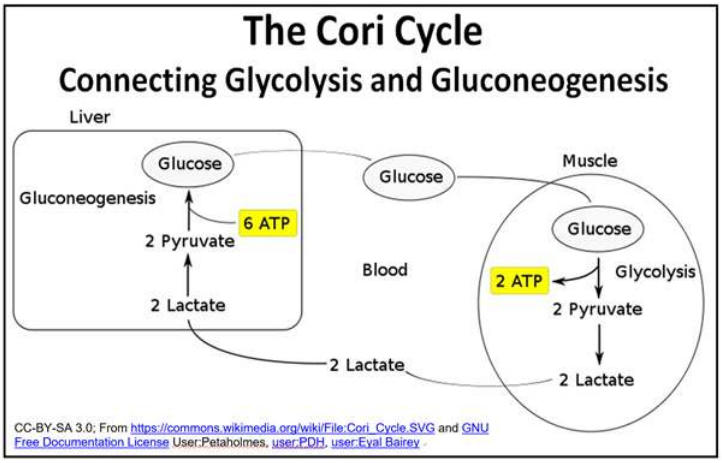
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8.10: Cori Cycle

Gluconeogenesis from lactate is particularly important during periods of intense physical activity. As discussed before, when oxygen supply is insufficient, typically during intense muscular activity, pyruvate generated during glycolysis is converted into lactic acid by lactate dehydrogenase. Instead of accumulating inside the muscle cells, lactate produced by anaerobic fermentation is taken up by the liver. This initiates the other half of the Cori cycle. In the liver, gluconeogenesis occurs.

So glycolysis in the muscle and gluconeogenesis in the liver would seem to be cyclic (see image below). In fact, this apparent cycle was recognized by Carl and Gerti Cori, who shared the 1947 Nobel Prize for Medicine or Physiology with Bernardo Houssay for discovering how glycogen is broken down to pyruvate in muscle (in fact most) cells, which can then be used to resynthesize glucose in liver cells. Named after the Coris, The Cori Cycle, shown below, recognizes the interdependence of liver and muscle in glucose breakdown and resynthesis. Glucose generated in the liver can enter the bloodstream and be used in the muscle to support the physical activity.



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8.11: Hormonal Regulation of Metabolism

The levels of glucose in the blood are regulated by the hormones insulin and glucagon from the pancreas, and T3 and T4 from the thyroid.

Learning Objectives

- Explain how the hormones glucagon and insulin regulate blood glucose

Hormonal Regulation of Metabolism

Blood glucose levels vary widely over the course of a day as periods of food consumption alternate with periods of fasting. Insulin and glucagon are the two hormones primarily responsible for maintaining homeostasis of blood glucose levels. Additional regulation is mediated by the thyroid hormones.

Regulation of Blood Glucose Levels: Insulin and Glucagon

Cells of the body require nutrients in order to function. These nutrients are obtained through feeding. In order to manage nutrient intake, storing excess intake, and utilizing reserves when necessary, the body uses hormones to moderate energy stores. Insulin is produced by the beta cells of the pancreas, which are stimulated to release insulin as blood glucose levels rise (for example, after a meal is consumed). Insulin lowers blood glucose levels by enhancing the rate of glucose uptake and utilization by target cells, which use glucose for ATP production. It also stimulates the liver to convert glucose to glycogen, which is then stored by cells for later use. As insulin binds to its target cell via insulin receptors and signal transduction, it triggers the cell to incorporate glucose transport proteins into its membrane. This allows glucose to enter the cell, where it can be used as an energy source. These actions mediated by insulin cause blood glucose concentrations to fall, called a hypoglycemic, or “low sugar” effect, which inhibits further insulin release from beta cells through a negative feedback loop.

Impaired insulin function can lead to a condition called diabetes mellitus, which has many effects on the body. It can be caused by low levels of insulin production by the beta cells of the pancreas, or by reduced sensitivity of tissue cells to insulin. This prevents glucose from being absorbed by cells, causing high levels of blood glucose, or hyperglycemia (high sugar). High blood glucose levels make it difficult for the kidneys to recover all the glucose from nascent urine, resulting in glucose being lost in urine. High glucose levels also result in less water being reabsorbed by the kidneys, causing high amounts of urine to be produced; this may result in dehydration. Over time, high blood glucose levels can cause nerve damage to the eyes and peripheral body tissues, as well as damage to the kidneys and cardiovascular system. Oversecretion of insulin can cause hypoglycemia, low blood glucose levels. This causes insufficient glucose availability to cells, often leading to muscle weakness. It can sometimes cause unconsciousness or death if left untreated.

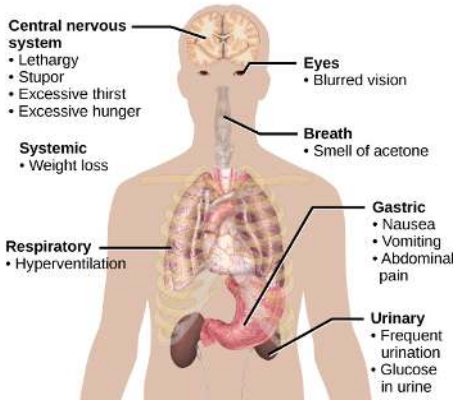


Figure **8.11.1**: **Diabetes mellitus**: Diabetes mellitus can cause a wide range of symptoms, including nausea, vomiting, blurred vision, lethargy, a frequency in urination, and high levels of glucose in the urine.

When blood glucose levels decline below normal levels, for example between meals or when glucose is utilized during exercise, the hormone glucagon is released from the pancreas. Glucagon raises blood glucose levels, eliciting what is called a hyperglycemic effect, by stimulating the breakdown of glycogen to glucose in skeletal muscle cells and liver cells in a process called

glycogenolysis. Glucose can then be utilized as energy by muscle cells and released into circulation by the liver cells. Glucagon also stimulates absorption of amino acids from the blood by the liver, which then converts them to glucose. This process of glucose synthesis is called gluconeogenesis. Rising blood glucose levels inhibit further glucagon release by the pancreas via a negative feedback mechanism. In this way, insulin and glucagon work together to maintain homeostatic glucose levels.

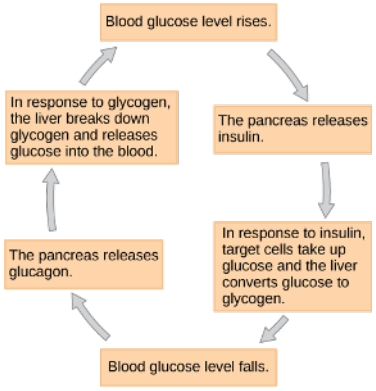


Figure **8.11.1**: **The regulation of blood glucose levels by insulin and glucagon**: As the levels of glucose in the blood rise, insulin stimulates the cells to take up more glucose and signals the liver to convert the excess glucose to glycogen, a form in which it can be stored for later use. When the levels of glucose in the blood fall, glucagon responds by stimulating the breakdown of glycogen into glucose and signals the production of additional glucose from amino acids.

Key Points

- When blood glucose levels rise, insulin is secreted by the pancreas, lowering blood glucose by increasing its uptake in cells and stimulating the liver to convert glucose to glycogen, in which form it can be stored.
- If insulin secretion is impaired, it can result in diabetes mellitus: a disease in which blood glucose levels remain high, leading to excess glucose in the urine, increased urine output, and dehydration, among other symptoms.
- When blood glucose levels fall, glucagon is secreted by the pancreas, which increases blood glucose levels by stimulating the breakdown of glycogen into glucose and the creation of glucose from amino acids.

Sources:

Hormonal Regulation of Metabolism. (2020, August 15). Retrieved April 7, 2021, from https://bio.libretexts.org/@go/page/13977

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CHAPTER OVERVIEW

9: Metabolism of Lipids

9.1: Stage I of Lipid catabolism
9.2: 9.2-Mobilization of Triacylglycerols
9.3: Glycerol Metabolism
9.4: Oxidation of Fatty Acids
9.5: Fatty Acid Synthesis
9.6: Actions of insulin and glucagon in fat metabolism

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9.1: Stage I of Lipid catabolism

Learning Objectives

- Describe the digestion of lipids.
- Know the properties and functions of the different types of lipoproteins.
- Know the sources and function of common dietary lipids.

Digestion of Lipids

Lipid digestion begins in the upper portion of the small intestine (Figure **9.1.1**). A hormone secreted in this region stimulates the gallbladder to discharge bile into the duodenum. The principal constituents of bile are the bile salts, which emulsify large, water-insoluble lipid droplets, disrupting some of the hydrophobic interactions holding the lipid molecules together and suspending the resulting smaller globules (micelles) in the aqueous digestive medium. These changes greatly increase the surface area of the lipid particles, allowing for more intimate contact with the lipases and thus rapid digestion of the fats. Another hormone promotes the secretion of pancreatic juice, which contains these enzymes.

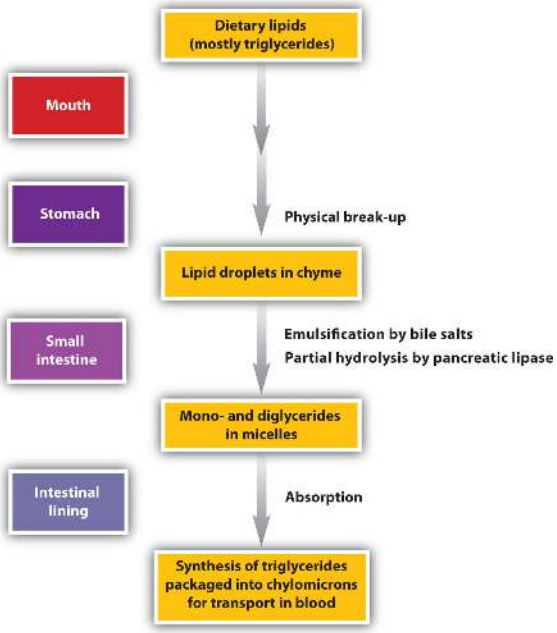
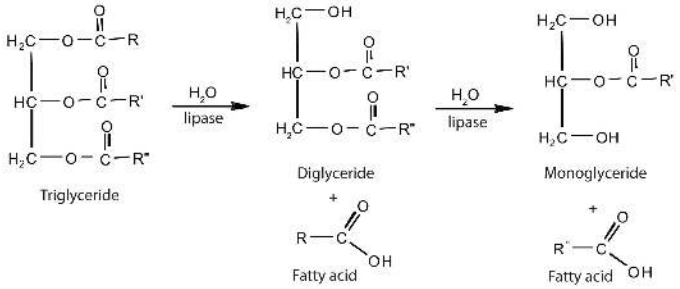


***Figure* 9.1.1** *The principal events and sites of lipid (primarily triglyceride) digestion.*

The lipases in pancreatic juice catalyze the digestion of triglycerides first to diglycerides and then to 2-monoglycerides and fatty acids:



Phospholipids and cholesterol esters undergo similar hydrolysis in the small intestine, and their component molecules are absorbed through the intestinal lining. The monoglycerides and fatty acids are absorbed by the intestinal lining cells and then they are

resynthesized into triglycerides. In order to be transported in the aqueous media of the bloodstream, triglycerides are combined with proteins and cholesterol forming lipoprotein complexes known as **chylomicrons**.

Fats, Cholesterol, and lipoproteins in the blood

Chylomicrons are are complex combination of fats (phospholipids, triacylglycerol, cholesterol, etc) and proteins (sometimes called apolipoprotein), so another way to refer to chylomicros is lipoproteins. These complexes are necessary to transport the lipids through the blood. Different types of lipoproteins have a different composition and therefore, different density.



Model of a Chylomicron. Image by OpenStax College, CC BY 3.0, via Wikimedia Commons

According tor their density, chylomicrons or liporproteins are classify as high-density lipoproteins (HDL), low-density lipoporteins (LDL), and very low-density lipoproteins (VLDL). The table shows the properties of each.

The table below also shows the difference in density, diameter, and composition of different lipoproteins. Notice that as diameter decreases, density increases.

Table Extra close brace or missing open brace *Properties and of different lipoproteins.*

Lipoprotein	Density (g/dL)	Diameter (nm)	% protein	% cholesterol	% phospholipid	% triacylglycerol	Purpose
HDL (high-density lipoproteins)	1.063-1.21	5-12	33	30	29	4-8	It is sometimes called "good" cholesterol because it carries cholesterol from other parts of your body back to your liver. Your liver then removes the cholesterol from your body.
LDL (low-density lipoproteins)	1.019-1.063	18-25	25	56-50	21-22	8-10	It is sometimes called "bad" cholesterol because a high LDL level leads to the buildup of plaque in your arteries.
VLDL (very low-density lipoproteins)	0.95-1.006	30-80	10	22	18	50	Some people also call VLDL a "bad" cholesterol because it too contributes to the buildup of plaque in your arteries. But VLDL and LDL are different; VLDL mainly carries triglycerides and LDL mainly carries cholesterol.

You are probably familiar with HDL and LDL being referred to as "good cholesterol" and "bad cholesterol," respectively. This is an oversimplification to help the public interpret their blood lipid values, because cholesterol is cholesterol; it's not good or bad. HDL and LDL both contain cholesterol but there difference is in their composition. The percenge of cholesterol in LDL is much higher What's so bad about LDL? Too much cholesterol in the blood can combine with other substances to form plaque. Plaque

sticks to the walls of the arteries. This buildup of plaque is known as atherosclerosis. It can lead to coronary artery disease, wherein the coronary arteries become narrow or even blocked.

HDL is good in that it scavenges cholesterol from other lipoproteins or cells and returns it to the liver.

Cholesterol is only found in foods of **animal origin,** frequently as a cholesterol esters, meaning that there is a fatty acid attached to the OH group. If consumers were more knowledgeable, intentionally misleading practices, such as labeling a banana “cholesterol free”, would not be as widespread as they currently are today. Although cholesterol has acquired the status of a nutrition "villain", it is a vital component of cell membranes and is used to produce vitamin D, hormones, and bile acids. You can see the similarity between the structures of vitamin D and estradiol, one of the forms of estrogen shown below.

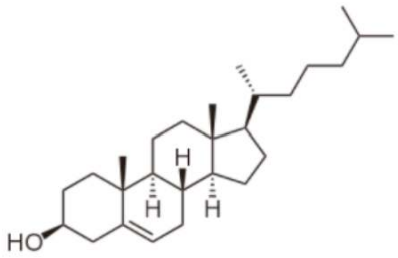


***Figure* 9.1.2** *The carbon ring structure of cholesterol*[1]

We do not need to consume any cholesterol from our diets (not essential) because our bodies have the ability to synthesize the required amounts. The figure below gives you an idea of the cholesterol content of a variety of foods.

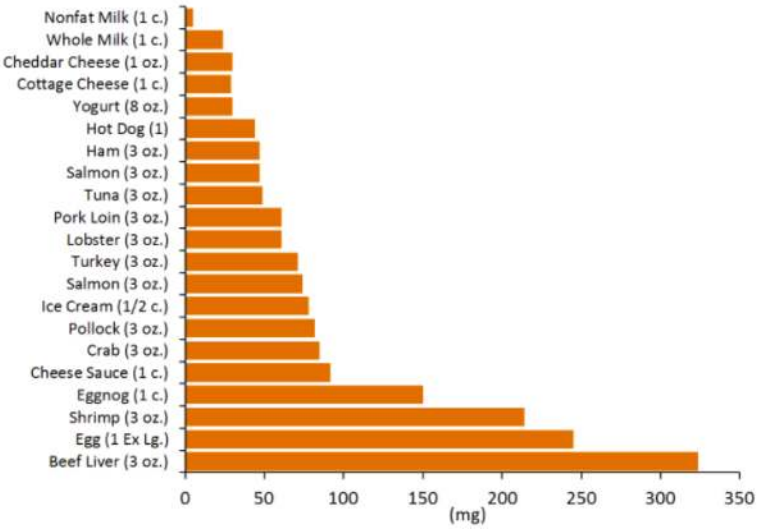


***Figure* 9.1.4** *The cholesterol content (mg) of foods*[4]

The most common cause of high cholesterol is an unhealthy lifestyle. This can include:

- **Unhealthy eating habits,** such as eating lots of bad fats. One type, saturated fat, is found in some meats, dairy products, chocolate, baked goods, and deep-fried and processed foods. Another type, trans fat, is in some fried and processed foods. Eating these fats can raise your LDL (bad) cholesterol.
- **Lack of physical activity,** with lots of sitting and little exercise. This lowers your HDL (good) cholesterol.
- **Smoking,** which lowers HDL cholesterol, especially in women. It also raises your LDL cholesterol.

Genetics may also cause people to have high cholesterol. For example, familial hypercholesterolemia (FH) is an inherited form of high cholesterol. Other medical conditions and certain medicines may also cause high cholesterol.

Summary

- Digestion of lipids by lipases in pancreatic juice occurs primarily in the small intestines. The monoglycerides and fatty acids cross the intestinal lining into the bloodstream, where they are resynthesized into triglycerides and transported as chylomicrons. Phospholipids and cholesteryl esters undergo similar hydrolysis in the small intestine, and their component molecules are also absorbed through the intestinal lining.
- Two lipoproteins (composed of a lipid and a protein) of great interest are HDL "good cholesterol transporter" and LDL "bad cholesterol transporter."

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9.2: 9.2-Mobilization of Triacylglycerols

Lipolysis and Mobilization of Triacylglycerols

The preferred source of energy for the body is carbohydrates. However, the storage of carbohydrates in the body (glycogen) is depleted within a few hours. Afterward, lipids become the next source of energy. When the body storage of glycogen is depleted, the production of certain hormones stimulates the hydrolysis of lipids. **Lipolysis** is the metabolic pathway catalyzed by lipases through which lipid triglycerides are hydrolyzed into glycerol and three fatty acids. It usually occurs in fat adipocytes, as a response to high epinephrine and low insulin levels in the blood. The free fatty acids and glycerol are then released into the blood. This process is called **mobilization of triacylglycerol or fats.**

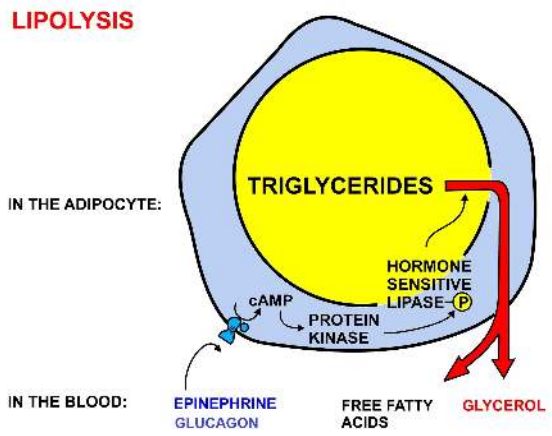


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Mobilized fatty acids in the blood are transported to the required tissue forming a lipoprotein complex with **serum albumin** protein**.** Serum albumin is produced by the liver and is the most abundant blood protein in mammals.

Sources

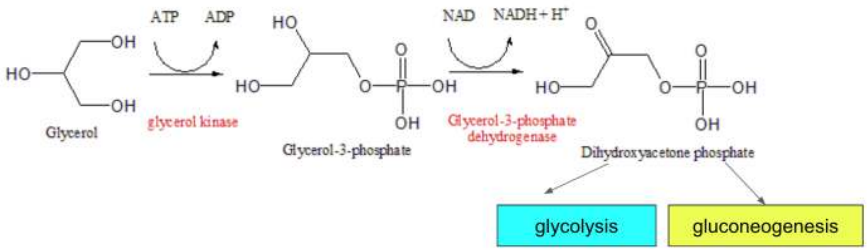
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Serum albumin on Wikipedia. Retrieved Sept 27, 2020. Content adapted under Creative Commons Attribution-ShareAlike License.

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9.3: Glycerol Metabolism

The glycerol also enters the bloodstream and is absorbed by the liver or kidney where it is converted to glycerol 3-phosphate by the enzyme **glycerol kinase,** and the resulting glycerol 3-phosphate is oxidized to dihydroxyacetone phosphate by the enzyme glycerol-3-phosphate dehydrogenase:



The glycolytic enzyme triose phosphate isomerase converts dihydroxyacetone phosphate to **glyceraldehyde 3-phosphate**, which is **oxidized via glycolysis**, or **converted to glucose via gluconeogenesis.**

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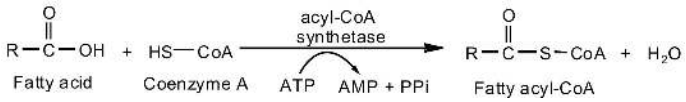
9.4: Oxidation of Fatty Acids

Learning Objectives

- To describe the reactions needed to completely oxidize a fatty acid to carbon dioxide and water.

Like glucose, the fatty acids released in the digestion of triglycerides and other lipids are broken down in a series of sequential reactions accompanied by the gradual release of usable energy. Some of these reactions are oxidative and require nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD). The enzymes that participate in fatty acid catabolism are located in the **mitochondria**, along with the enzymes of the citric acid cycle, the electron transport chain, and oxidative phosphorylation. This localization of enzymes in the mitochondria is of the utmost importance because it facilitates efficient utilization of energy stored in fatty acids and other molecules.

Fatty acid oxidation is initiated on the outer mitochondrial membrane. There the fatty acids, which like carbohydrates are relatively inert, must first be activated by conversion to an energy-rich fatty acid derivative of coenzyme A called *fatty acyl-coenzyme A* (CoA). The activation is catalyzed by *acyl-CoA synthetase*. For each molecule of fatty acid activated, one molecule of coenzyme A and one molecule of adenosine triphosphate (ATP) are used, equaling a net utilization of the two high-energy bonds in one ATP molecule (which is therefore converted to adenosine monophosphate [AMP] rather than adenosine diphosphate [ADP]):



The fatty acyl-CoA diffuses to the inner mitochondrial membrane, where it combines with a carrier molecule known as **carnitine** in a reaction catalyzed by *carnitine acyltransferase*. The acyl-carnitine derivative is transported into the mitochondrial matrix and converted back to the fatty acyl-CoA.

Carnitine is an amino acid derivative synthesized from methionine and lysine.

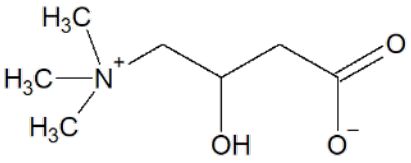


Figure **9.4.1**: Chemical structure of the non-proteinogenic amino acid carnitine



Figure **9.4.2**: Role of carnitine in the transport of Acyl-CoA from cytosol to the mitochondrial matrix. Image adapted from Original image: Slagtvectorization: Own work, CC0, via Wikimedia Commons

Steps in the β-Oxidation of Fatty Acids

Further oxidation of the fatty acyl-CoA occurs in the mitochondrial matrix via a sequence of four reactions known collectively as β-oxidation because the β-carbon undergoes successive oxidations in the progressive removal of two carbon atoms from the carboxyl end of the fatty acyl-CoA (Figure **9.4.1**).

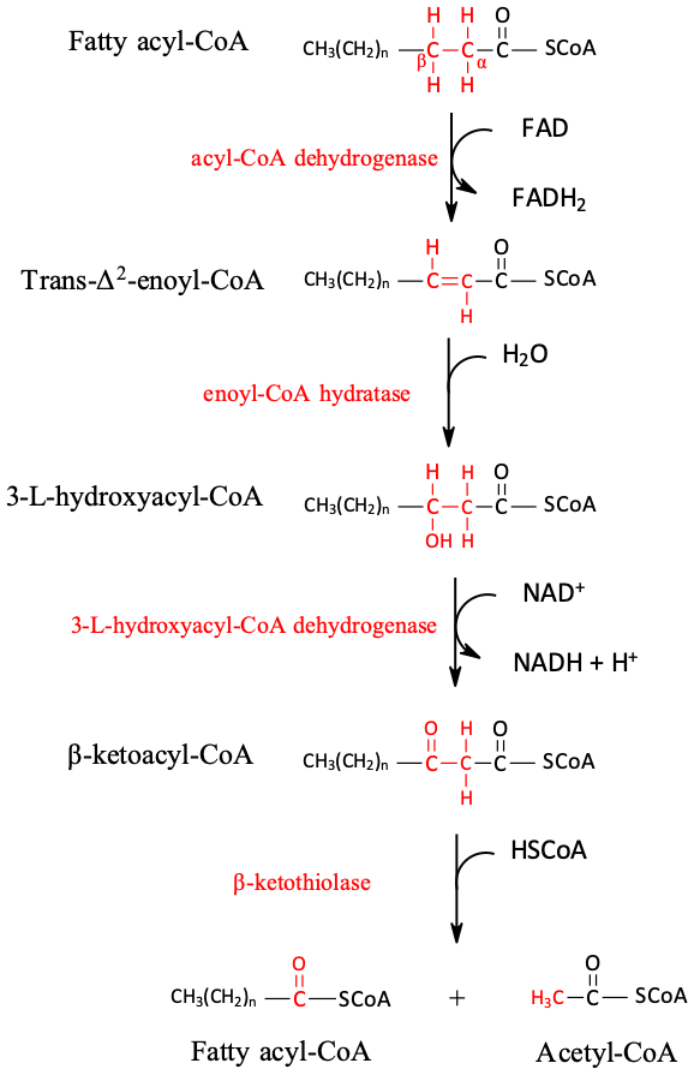


Figure 9.4.3: Fatty Acid Oxidation. The fatty acyl-CoA formed in the final step becomes the substrate for the first step in the next round of β-oxidation. β-oxidation continues until two acetyl-CoA molecules are produced in the final step.

The first step in the catabolism of fatty acids is the formation of an alkene in an oxidation reaction catalyzed by *acyl-CoA dehydrogenase*. In this reaction, the coenzyme FAD accepts two hydrogen atoms from the acyl-CoA, one from the α-carbon and one from the β-carbon, forming reduced flavin adenine dinucleotide ($FADH_2$).

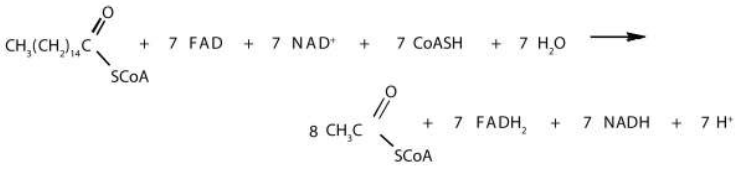
> The $FADH_2$ is reoxidized back to FAD via the electron transport chain that supplies energy to form 1.5–2 molecules of ATP.

Next, the *trans*-alkene is hydrated to form a secondary alcohol in a reaction catalyzed by *enoyl-CoA hydratase*. The enzyme forms only the L-isomer.

The secondary alcohol is then oxidized to a ketone by *β-hydroxyacyl-CoA dehydrogenase*, with NAD^+ acting as the oxidizing agent. The reoxidation of each molecule of NADH to NAD^+ by the electron transport chain furnishes 2.5–3 molecules of ATP.

The final reaction is cleavage of the β-ketoacyl-CoA by a molecule of coenzyme A. The products are acetyl-CoA and a fatty acyl-CoA that has been shortened by two carbon atoms. The reaction is catalyzed by *thiolase*.

The shortened fatty acyl-CoA is then degraded by repetitions of these four steps, each time releasing a molecule of acetyl-CoA. The overall equation for the β-oxidation of palmitoyl-CoA (16 carbon atoms) is as follows:



> *Because each shortened fatty acyl-CoA cycles back to the beginning of the pathway, β-oxidation is sometimes referred to as the fatty acid spiral.*

The fate of the acetyl-CoA obtained from fatty acid oxidation depends on the needs of an organism. It may enter the citric acid cycle and be oxidized to produce energy, it may be used for the formation of water-soluble derivatives known as ketone bodies, or it may serve as the starting material for the synthesis of fatty acids.

ATP Yield from Fatty Acid Oxidation. Comparison with Glucose Oxidation.

The amount of ATP obtained from fatty acid oxidation depends on the size of the fatty acid being oxidized. For our purposes here. we'll study palmitic acid, a saturated fatty acid with 16 carbon atoms, as a typical fatty acid in the human diet. Calculating its energy yield provides a model for determining the ATP yield of all other fatty acids.

The breakdown by an organism of 1 mol of palmitic acid requires 1 mol of ATP (for activation) and forms 8 mol of acetyl-CoA. Recall from the metabolism of carbohydrates that each mole of acetyl-CoA metabolized by the citric acid cycle yields 12 mol of ATP. The complete degradation of 1 mol of palmitic acid requires the β-oxidation reactions to be repeated seven times. Thus, 7 mol of NADH and 7 mol of $FADH_2$ are produced. Reoxidation of these compounds through respiration yields 3 and 2 mol of ATP, respectively. The energy calculations can be summarized as follows:

1 mol of ATP is split to AMP and $2P_i$	−2 ATP
8 mol of acetyl-CoA formed (8 × 12 ATP)	96 ATP
7 mol of $FADH_2$ formed (7 × 1.5)	10.5 ATP
7 mol of NADH formed (7 × 2.5)	17.5 ATP
Total	122 ATP

> *The number of times β-oxidation is repeated for a fatty acid containing n carbon atoms is n/2 – 1 because the final turn yields two acetyl-CoA molecules.*

The combustion of 1 mol of palmitic acid releases a considerable amount of energy:

$$C_{16}H_{32}O_2 + 23O_2 \rightarrow 16CO_2 + 16H_2O + 2,340\ kcal \tag{9.4.1}$$

The percentage of this energy that is conserved by the cell in the form of ATP is as follows:

$$\frac{\text{energy conserved}}{\text{total energy available}} \times 100 = \frac{(122\ \text{ATP})(7.4\ \text{kcal/ATP})}{2,340\ \text{kcal}} \times 100 = 39\% \tag{9.4.2}$$

In terms of moles of reactant, the efficiency of fatty acid metabolism is comparable to that of carbohydrate metabolism, which we calculated to be 39%. However, in terms of grams, there is a big difference between the energy that can be generated per gram of glucose and per gram of fatty acid. In the carbohydrate metabolism module, we determine that the oxidation of 1 mol of glucose produces 32 ATP moles, that is, 38 x 7.4 kcal /mol ATP = 236.8 kcal. That is the amount of energy produced by 1 mol, or 180 g of glucose. In other words, 1 gram of glucose produces 1.32 kcal of energy (1.32/g glucose). For a fatty acid, such as palmitic acid, we are able to produce 122 ATP molees per mol of palmitic acid, that is, 122 x 7.4 kcal/mol ATP = 902.8 kal. One mole of palmitic acid equals 256 grams. Therefore, the complete oxidation of palmitic acid produces 3.52 kcal/g of palmitic acid, which is **more**

than twice the amount of energy obtained per mole of glucose. The fact that carbons atoms in fatty acids are more reduced than the carbon atoms in glucose explains the difference in the amount of energy produced by their oxidation.

> *Interesting fact: the oxidation of fatty acids also produces large quantities of water. This water, which sustains migratory birds and animals (such as the camel) for long periods of time.*

Ketone Bodies

In the liver, most of the acetyl-CoA obtained from fatty acid oxidation is oxidized by the citric acid cycle. However, under certain metabolic conditions such as starvation or diabetes mellitus, the rate of fatty acid oxidation increases to provide energy. This leads to an increase in the concentration of acetyl-CoA. The increased acetyl-CoA cannot be oxidized by the citric acid cycle because of a decrease in the concentration of oxaloacetate, which is diverted to glucose synthesis. Therefore, some of the acetyl-CoA is used to synthesize a group of compounds known as ***ketone bodies*****:** acetoacetate, β-hydroxybutyrate, and acetone. Two acetyl-CoA molecules combine, in a reversal of the final step of β-oxidation, to produce acetoacetyl-CoA. The acetoacetyl-CoA reacts with another molecule of acetyl-CoA and water to form β-hydroxy-β-methylglutaryl-CoA, which is then cleaved to acetoacetate and acetyl-CoA. Most of the acetoacetate is reduced to β-hydroxybutyrate, while a small amount is decarboxylated to carbon dioxide and acetone.

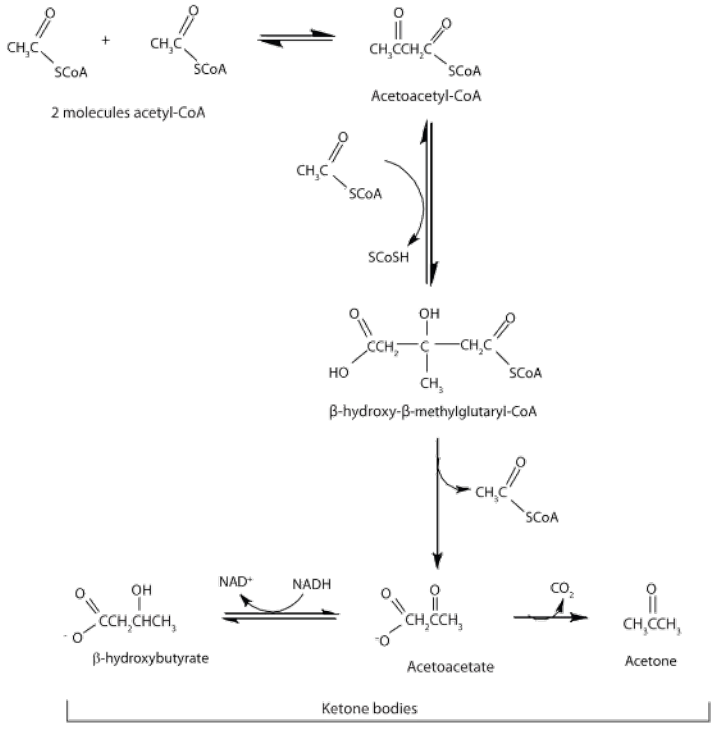


Figure **9.4.4**: Formation og ketone bodies

The acetoacetate and β-hydroxybutyrate synthesized by the liver are released into the blood for use as a metabolic fuel (to be converted back to acetyl-CoA) by other tissues, particularly the kidney and the heart. Thus, during prolonged starvation, ketone bodies provide about 70% of the energy requirements of the brain. Under normal conditions, the kidneys excrete about 20 mg of ketone bodies each day, and the blood levels are maintained at about 1 mg of ketone bodies per 100 mL of blood.

In starvation, diabetes mellitus, and certain other physiological conditions in which cells do not receive sufficient amounts of carbohydrate, the rate of ketone body formation in the liver increases further, to a level much higher than can be used by other tissues. The excess ketone bodies accumulate in the blood and the urine, a condition referred to as *ketosis*. When the acetone in the blood reaches the lungs, its volatility causes it to be expelled in the breath. The sweet smell of acetone, a characteristic of ketosis, is frequently noticed on the breath of severely diabetic patients.

Because two of the three kinds of ketone bodies are weak acids, their presence in the blood in excessive amounts overwhelms the blood buffers and causes a marked decrease in blood pH (to 6.9 from a normal value of 7.4). This decrease in pH leads to a serious condition known as ***acidosis*.** One of the effects of acidosis is a decrease in the ability of hemoglobin to transport oxygen in the blood. In moderate to severe acidosis, breathing becomes labored and very painful. The body also loses fluids and becomes dehydrated as the kidneys attempt to get rid of the acids by eliminating large quantities of water. The lowered oxygen supply and dehydration lead to depression; even mild acidosis leads to lethargy, loss of appetite, and a generally run-down feeling. Untreated patients may go into a coma. At that point, prompt treatment is necessary if the person's life is to be saved.

Summary

- Fatty acids, obtained from the breakdown of triglycerides and other lipids, are oxidized through a series of reactions known as β-oxidation.
- In each round of β-oxidation, 1 molecule of acetyl-CoA, 1 molecule of NADH, and 1 molecule of $FADH_2$ are produced.
- The acetyl-CoA, NADH, and $FADH_2$ are used in the citric acid cycle, the electron transport chain, and oxidative phosphorylation to produce ATP

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9.5: Fatty Acid Synthesis

When the intake of nutrients exceeds the energy requirements of the body, the acetyl-CoA produced by the degradation of food can be converted into fatty acids and stored as fats. This anabolic process is accomplished using a different set of enzymes than the catabolism of fatty acids discussed earlier. Another difference between the catabolic and anabolic reactions for fatty acids is the location: whereas we saw that catabolism occurs largely in the mitochondria, fatty acid synthesis is run from a single large enzymatic complex in the **cytoplasm**.

We will illustrate here the formation of palmitic acid. Fatty acid synthesis (Figure **9.5.11**) starts with the formation of malonyl-CoA. Malonyl-CoA is a 3-carbon molecule also formed from acetyl-CoA and bicarbonate by an enzyme called Acetyl-CoA carboxylase:

$$\text{acetyl-CoA} + HCO_3^- + \text{ATP} \text{ ---> } \text{malonyl-CoA} + \text{ADP} + \text{Pi}$$

The acetyl-CoA in the cytoplasm is primarily derived from the mitochondrial acetyl-CoA. Because the inner mitochondrial membrane is impermeable to acetyl-CoA, this compound is exported to the cytoplasm via a citrate-malate shuttle that couples deacetylation in the mitochondrion with acetylation in the cytosol.

Fatty acid synthesis is carried out by the **fatty acid synthase system**, comprised of seven enzymes linked together with an acyl carrier protein (ACP). As mentioned, this complex is found in the cytoplasm.

The acetyl-CoA and malonyl-CoA are linked to the synthase and ACP, then there is a sequence of acetyl group transfers that runs a total of seven times to form palmitoyl-ACP, from which the palmitic acid is finally released. Palmitic acid is the precursor for variety of long-chain fatty acids such as stearic acid, palmitoleic acid, and oleic acid. Generally, there is either an elongation or sometimes a desaturation step.

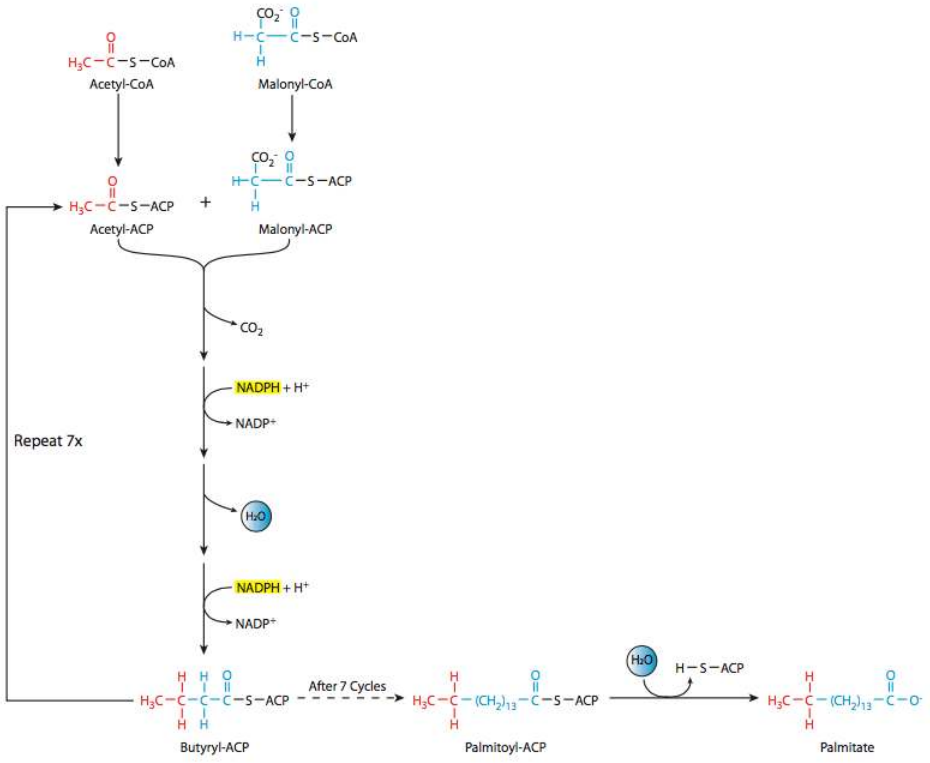


Figure **9.5.11**. Fatty acid synthesis

The overall reaction for the synthesis of palmitic acid from acetyl CoA is as follows:

$$8 \text{ Acetyl CoA (2C)} + 14 \text{ NADPH} + 13H^+ + 7 \text{ ATP} \rightarrow \text{ Palmitate (16C)} + 8 \text{ CoA-SH} + 6 H_2O + 14 NADP^+ + 7 \text{ ADP} + 7 \text{ Pi}$$

Each of the fatty acyl chain additions generates an ester bond, which requires a significant energy input: that energy comes from a linked ATP hydrolysis reaction for each chain addition. These fatty acids are then used to form the triacylglycerol and stored in adipose tissue, which contains the bulk of the energy storage molecules in most animals. Triacylglycerols are synthesized by the

reaction of fatty acyl-CoA chains with glycerol-3-phosphate. Two rounds of this reaction yields diacylglygerol-3-phosphate (phosphatidic acid). After the action of phosphatidate phosphatase, the phosphatidic acid is converted to 1,2-diacylglycerol. This reacts with fatty acyl-CoA to form the final triacyglycerol.

Humans can convert glucose into fatty acids, but we cannot convert fatty acids into glucose because we lack the enzyme necessary to transform acetyl CoA into pyruvate, the compound used in gluconeogenesis. Some plants and bacteria do have the enzyme necessary for this metabolic transformation, so they are capable of producing glucose from fats.

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9.6: Actions of insulin and glucagon in fat metabolism

Learning Objectives

- Summarize the relationship between insulin secretion and glucagon regulation in metabolism homeostasis between lipids and carbohydrates

Glucagon and insulin are peptide hormones secreted by the pancreas that play a key role in maintaining a stable blood glucose level and body homeostasis. Glucagon is produced by alpha cells in the pancreas and elevates the concentration of glucose in the blood by promoting gluconeogenesis and glycogenolysis. Glucose is stored in the liver in the form of the polysaccharide glycogen. Liver cells have glucagon receptors and when glucagon binds to the liver cells they convert glycogen into individual glucose molecules and release them into the bloodstream. As these stores become depleted, glucagon then encourages the liver and kidney to synthesize additional glucose by gluconeogenesis. Glucagon also turns off glycolysis in the liver, causing glycolytic intermediates to be shuttled to gluconeogenesis **to produce glucose from fat**.

Insulin is produced by beta cells in the pancreas and acts to oppose the functions of glucagon. Its main role is to promote the conversion of circulating glucose into glycogen via glycogenesis in the liver and muscle cells. Insulin also inhibits gluconeogenesis and promotes **the storage of glucose in fat through lipid synthesis and also by inhibiting lipolysis and beta-oxidation of fatty acids.**

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CHAPTER OVERVIEW

10: Metabolism of Amino Acids

10.1: Proteins metabolism

10.2: Amino Acids Degradation

10.3: Urea Cycle

10.4: Amino Acid Synthesis

10.5: Connections of Carbohydrate, Protein, and Lipid Metabolic Pathways

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10.1: Proteins metabolism

Learning Objectives

- Describe the metabolism of proteins.
- Know the importance of essential amino acids.
- Know the sources and function of common proteins in the diet.

Protein Metabolism

The main sources of amino acids for the human body are the proteins in our diet, the non-essential amino acids synthesized by the liver plus the amino acids that come from the own's body protein, which are being constantly degraded and resynthesized.

Protein digestion begins in the stomach (Figure 10.1.3), where the action of gastric juice hydrolyzes about 10% of the peptide bonds. Gastric juice is a mixture of water (more than 99%), inorganic ions, hydrochloric acid, and various enzymes and other proteins. The pain of a gastric ulcer is at least partially due to irritation of the ulcerated tissue by acidic gastric juice.

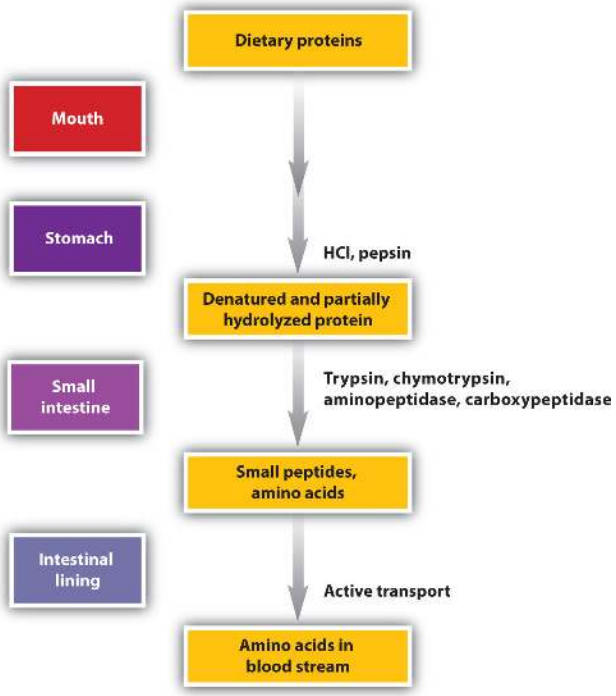


***Figure* 10.1.1** *The principal events and Sites of Protein Digestion*

The hydrochloric acid (HCl) in gastric juice is secreted by glands in the stomach lining. The pH of freshly secreted gastric juice is about 1.0, but the contents of the stomach may raise the pH to between 1.5 and 2.5. HCl helps to denature food proteins; that is, it unfolds the protein molecules to expose their chains to more efficient enzyme action. The principal digestive component of gastric juice is pepsinogen, an inactive enzyme produced in cells located in the stomach wall. When food enters the stomach after a period of fasting, pepsinogen is converted to its active form—pepsin—in a series of steps initiated by the drop in pH. Pepsin catalyzes the hydrolysis of peptide linkages within protein molecules. It has a fairly broad specificity but acts preferentially on linkages involving the aromatic amino acids tryptophan, tyrosine, and phenylalanine, as well as methionine and leucine. Protein digestion is completed in the small intestine.

Amino Acids pool

Once the proteins in the diet have been hydrolyzed, the free amino acids join the non-essential amino acid synthesized in the liver and the amino acids recycled from the body's own proteins, constituting the **amino acid pool** now available for metabolic processes. Most of the amino acid pool is used for the synthesis of protein and other nitrogen-containing compounds such as DNA

bases, neurotransmitters, hormones, etc. Under certain metabolic situations, amino acids can also be used as a source of energy by the body. It is worth mentioning that the **human body cannot store amino acids**. If the amino acids in the amino acid pool are not used for biological processes, they are degraded and the nitrogen excreted in the urine as urea.

Protein turnover

A balance between protein synthesis and protein degradation is required for good health and normal protein metabolism. Not all the amino acids needed for the biological function of the body need to be incorporated through the diet. When the proteins already present in the metabolism have complete their lifespan, they are also recycled. **Protein turnover** refers to the replacement of older proteins as they are broken down within the cell. Different types of proteins have very different turnover rates, depending on their particular function. Structural proteins such as college tend to have long half-life periods (in the range of years), while enzymatic protein have a shorter life span to adapt to the metabolic requirements of the body.

Example protein half-lives

Name	Half-Life
Collagen	117 years
Eye lens crystallin	>70 years
Replication factor C subunit 1	9 hours
40S ribosomal protein S8	3 hours
Ornithine decarboxylase	11 minutes

Once the protein have been hydrolyzed and amino acids recycled, these amino acids are added to the amino acid pool for further utilization.

Complete and Incomplete Proteins

Amino acids are classified into three groups namely: essential amino acids and nonessential amino acids

ESSENTIAL AMINO ACIDS

- Essential amino acids cannot be made by the body. As a result, they must come from food.
- The 9 essential amino acids are: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

NONESSENTIAL AMINO ACIDS

Nonessential means that our bodies produce an amino acid, even if we do not get it from the food we eat. Nonessential amino acids include: alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine.

Based on this classification of amino acids, proteins can also be classified as either complete or incomplete. **Complete proteins** provide adequate amounts of all nine essential amino acids. Animal proteins such as meat, fish, milk, and eggs are good examples of complete proteins. **Incomplete proteins** do not contain adequate amounts of one or more of the essential amino acids. For example, if a protein doesn't provide enough of the essential amino acid leucine it would be considered incomplete. Leucine would be referred to as the limiting amino acid, because there is not enough of it for the protein to be complete. Most plant foods are incomplete proteins, with a few exceptions such as soy. Table **10.1.1** shows the limiting amino acids in some plant foods.

Food	Amino Acid(s)
Beans and Most Legumes	Methionine, Tryptophan
Tree Nuts and Seeds	Methionine, Lysine
Grains	Lysine
Vegetables	Methionine, Lysine

Table 10.1.1 *Limiting Amino Acids in Some Common Plant Foods.*

Complementary Proteins

Even though most plant foods do not contain complete proteins, it does not mean that they should be sworn off as protein sources. It is possible to pair foods containing incomplete proteins with different limiting amino acids to provide adequate amounts of the essential amino acids. These two proteins are called **complementary proteins**, because they supply the amino acid(s) missing in the other protein. A simple analogy would be that of a 4 piece puzzle. If one person has 2 pieces of a puzzle, and another person has 2 remaining pieces, neither of them have a complete puzzle. But when they are combined, the two individuals create a complete puzzle.



Figure 10.1.2 Complementary proteins are kind of like puzzle pieces.

Two examples of complementary proteins are shown below.



Figure 10.1.3 .*Two complementary protein examples*

It should be noted that complementary proteins do not need to be consumed at the same time or meal. It is currently recommended that essential amino acids be met on a daily basis, meaning that if a grain is consumed at one meal, a legume could be consumed at a later meal, and the proteins would still complement one another.

Summary

- Protein digestion begins in the stomach where hydrolysis of the protein linkages occurs with the action of gastric juices (mainly HCl) and the active enzyme pepsin. Protein digestion is completed in the small intestine wherein other protein digesting enzymes are involved.
- Essential amino acids cannot be made by the body and must come from food.

- Complete proteins provide adequate amounts of all nine essential amino acids.
- Protein turnover refers to the replacement of amino acids in older proteins
- The amino acid pool is the total amount of amino acids from the diet, protein recycling, and non-essential amino acids produced by the body that is available for metabolic processing.

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10.2: Amino Acids Degradation

Learning Objectives

- To describe how excess amino acids are degraded.

The liver is the principal site of amino acid metabolism, but other tissues, such as the kidney, the small intestine, muscles, and adipose tissue, take part. Generally, the first step in the breakdown of amino acids is the separation of the amino group from the carbon skeleton, usually by a **transamination reaction**. The carbon skeletons resulting from the deaminated amino acids are used to form either glucose or fats, or they are converted to a metabolic intermediate that can be oxidized by the citric acid cycle. The latter alternative, amino acid catabolism, is more likely to occur when glucose levels are low—for example, when a person is fasting or starving.

Transamination

Transamination is an exchange of functional groups between any amino acid (except lysine, proline, and threonine) and an α-keto acid. The amino group is usually transferred to the keto carbon atom of α-ketoglutarate, converting the α-keto acid to glutamate. Transamination reactions are catalyzed by specific transaminases (also called aminotransferases), which require pyridoxal phosphate as a coenzyme.

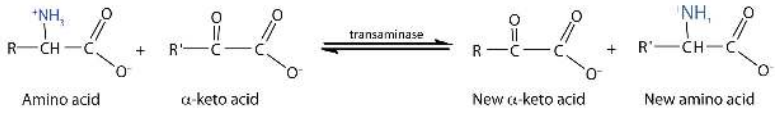


Figure **10.2.1**).

> *In an α-keto acid, the carbonyl or keto group is located on the carbon atom adjacent to the carboxyl group of the acid.*

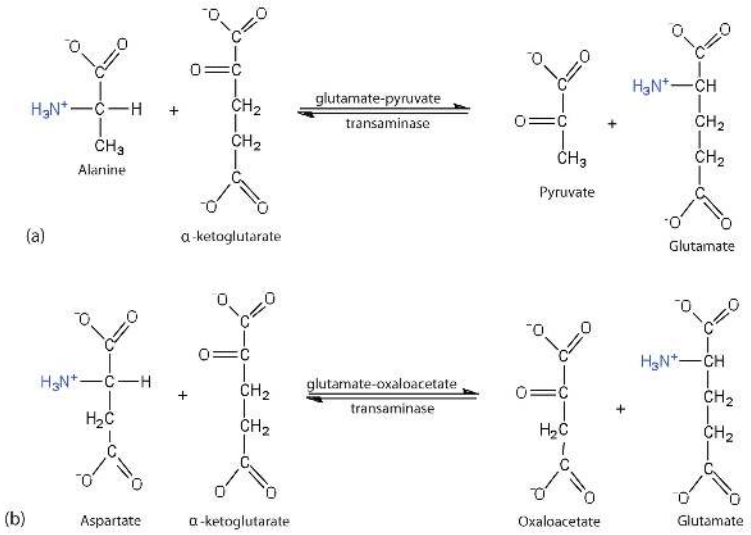


Figure **10.2.1**: Two Transamination Reactions. In both reactions, the final acceptor of the amino group is α-ketoglutarate, and the final product is glutamate.

Another important example of a transamination reaction is the formation of aspartate, which is used during urea formation. In this case, the acceptor of the amino group is oxaloacetate. For example, aspartate can be obtained from another amino acid such as alanine:

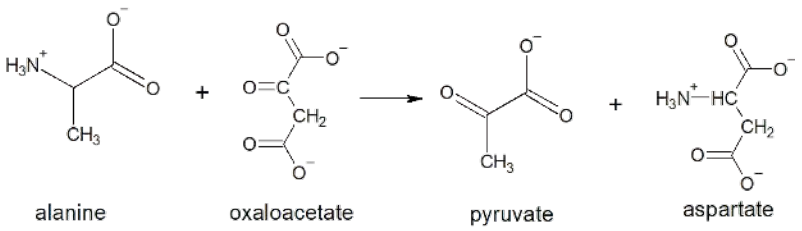
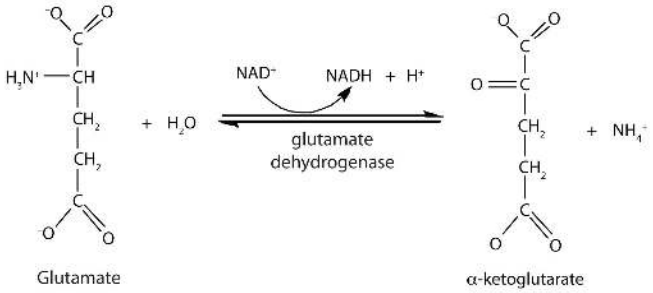


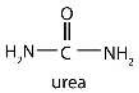
Figure **10.2.2**: A transamination reactions in which aspartate is formed. In this case, oxaloacetate is the amino group acceptor

Oxidative Deamination

In the breakdown of amino acids for energy, the final acceptor of the α-amino group is α-ketoglutarate, forming glutamate. Glutamate can then undergo oxidative deamination, in which it loses its amino group as an ammonium (NH_4^+) ion and is oxidized back to α-ketoglutarate (ready to accept another amino group):



This reaction occurs primarily in liver mitochondria. Most of the NH_4^+ ion formed by oxidative deamination of glutamate is converted to urea and excreted in the urine in a series of reactions known as the **urea cycle**.



The synthesis of glutamate occurs in animal cells by reversing the reaction catalyzed by glutamate dehydrogenase. For this reaction nicotinamide adenine dinucleotide phosphate (NADPH) acts as the reducing agent. The synthesis of glutamate is significant because it is one of the few reactions in animals that can incorporate inorganic nitrogen (NH_4^+) into an α-keto acid to form an amino acid. The amino group can then be passed on through transamination reactions, to produce other amino acids from the appropriate α-keto acids.

The Fate of the Carbon Skeleton

Any amino acid can be converted into an intermediate of the citric acid cycle. Once the amino group is removed, usually by transamination, the α-keto acid that remains is catabolized by a pathway unique to that acid and consisting of one or more reactions. For example, phenylalanine undergoes a series of six reactions before it splits into fumarate and acetoacetate. Fumarate is an intermediate in the citric acid cycle, while acetoacetate must be converted to acetoacetyl-coenzyme A (CoA) and then to acetyl-CoA before it enters the citric acid cycle.

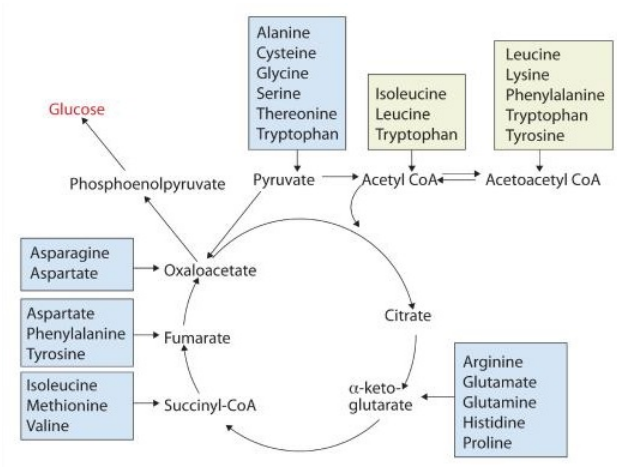


Figure **10.2.3**: Fates of the Carbon Skeletons of Amino Acids

Those amino acids that can form any of the intermediates of carbohydrate metabolism can subsequently be converted to glucose via a metabolic pathway known as gluconeogenesis. These amino acids are called **glucogenic amino acids**. Amino acids that are converted to acetoacetyl-CoA or acetyl-CoA, which can be used for the synthesis of ketone bodies but not glucose, are called **ketogenic amino acids**. Some amino acids fall into both categories. Leucine and lysine are the only amino acids that are exclusively ketogenic. Figure **10.2.2** summarizes the ultimate fates of the carbon skeletons of the 20 amino acids. The table below summarizes the Glucogenic and Ketogenic Amino Acids.

Glucogenic and Ketogenic Amino Acids

Glucogenic	Both Glucogenic and Ketogenic	Ketogenic
Aspartate	Isoleucine	Leucine
Asparagine	Phenylalanine	Lysine
Alanine	Tryptophan	
Glycine	Tyrosine	
Serine		
Threonine		
Cysteine		
Glutamate		
Glutamine		
Arginine		
Proline		
Histidine		
Valine		
Methionine		

Summary

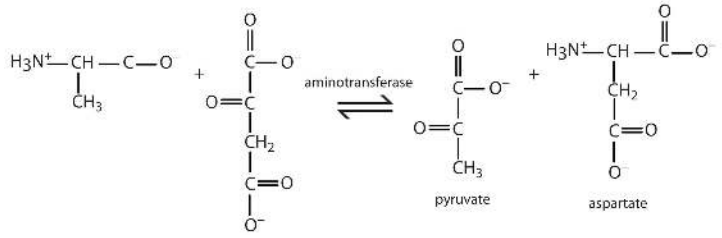
Generally the first step in the breakdown of amino acids is the removal of the amino group, usually through a reaction known as transamination. The carbon skeletons of the amino acids undergo further reactions to form compounds that can either be used for the synthesis of glucose or the synthesis of ketone bodies.

Concept Review Exercises

1. a. Write the equation for the transamination reaction between alanine and oxaloacetate.
   b. Name the two products that are formed.
2. What is the purpose of oxidative deamination?

Answers

1. a.



   b. pyruvate and aspartate

2. Oxidative deamination provides a reaction in which the amino group [as the ammonium (NH_4^+) ion] is removed from a molecule, not simply transferred from one molecule to another. Most of the NH_4^+ ion is converted to urea and excreted from the body.

Exercises

1. Write the equation for the transamination reaction between valine and pyruvate.
2. Write the equation for the transamination reaction between phenylalanine and oxaloacetate.
3. What products are formed in the oxidative deamination of glutamate?
4. Determine if each amino acid is glucogenic, ketogenic, or both.
   a. phenylalanine
   b. leucine
   c. serine
5. Determine if each amino acid is glucogenic, ketogenic, or both.
   a. asparagine
   b. tyrosine
   c. valine

Answers

1.



3. α-ketoglutarate, NADH, and NH_4^+

5. a. glucogenic
   b. both
   c. glucogenic

Sources

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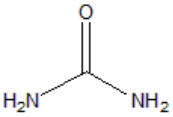
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10.3: Urea Cycle

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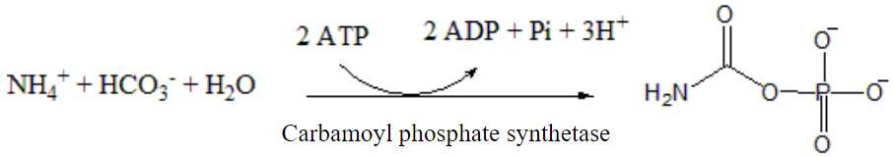
The fate of the amino group: the urea cycle

Deamination of amino acids results in the production of **ammonium** (**NH4+**). Ammonium is an extremely toxic base and its accumulation in the body would quickly be fatal. However, the **liver** contains a system of carrier molecules and enzymes which quickly converts the ammonia (and carbon dioxide) into urea. This is called the **urea cycle**.



urea

The cycle scavenges free ammonia (as ammonium ion) which is toxic if it accumulates. The capture reaction also requires ATP, and bicarbonate, and the product is **carbamoyl phosphate**. The reaction is catalyzed by the enzyme **carbamoyl phosphate synthetase**:



Carbamoyl phosphate

This molecule is combined with the non-protein amino acid known as **ornithine** to make another non-protein amino acid known as **citrulline**. Addition of aspartate to citrulline creates argninosuccinate, which splits off a fumarate, creating arginine (a source of arginine for the body). If arginine is not needed, it can be hydrolyzed to yield urea (excreted) and ornithine, thus completing the cycle. The first two reactions described here occur in the mitochondrion and the remaining ones occur in the cytoplasm. Molecules of the urea cycle intersecting other pathways include fumarate (citric acid cycle), aspartate (amino acid metabolism), arginine (amino acid metabolism), and ammonia (amino acid metabolism).

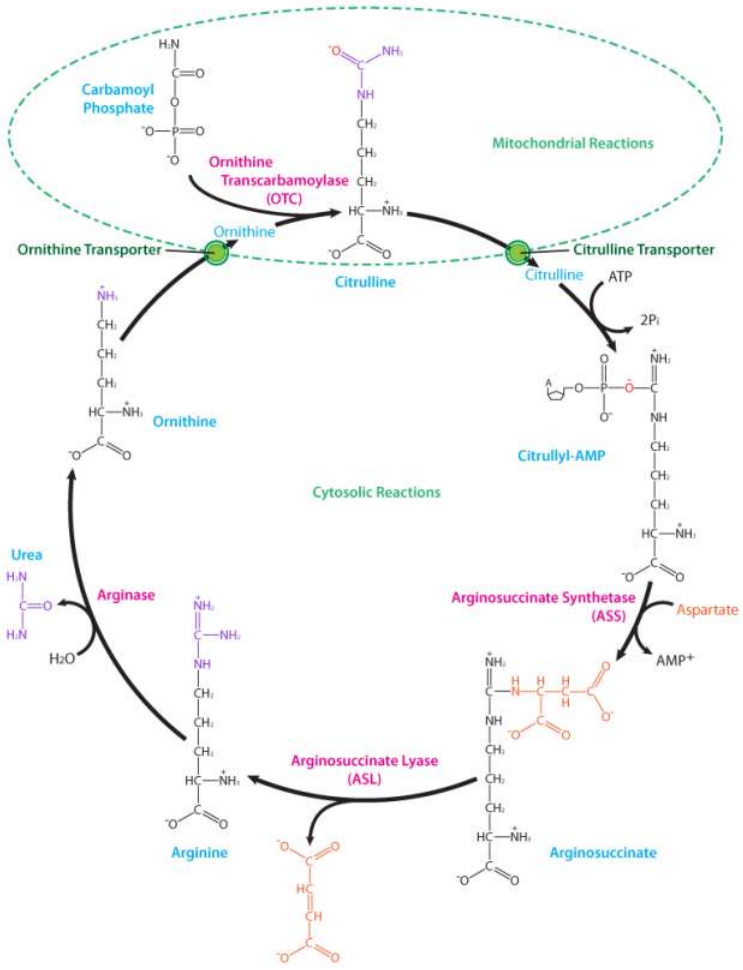
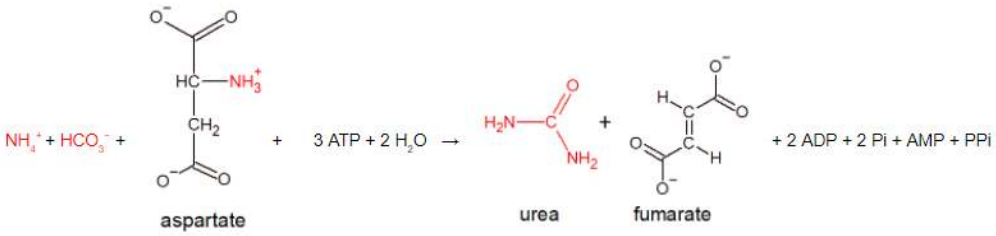


Figure 7.5.1: *The Urea Cycle*

After urea is formed, it is excreted in the urine. An adult excretes 20–30 g of urea in the urine daily.

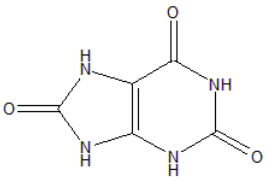
The overall equation of the urea cycle is:



Uric acid

Humans also excrete a second nitrogenous waste, **uric acid**. It is the product of **nucleic acid**, not protein, metabolism. It is produced within peroxisomes, and it is excreted in the urine, or reabsorbed by kidneys to regulate blood levels. Uric acid is a potent antioxidant and thus can protect cells from damage by reactive oxygen species (**ROS**). In human blood plasma, the reference range of uric acid is typically 3.4–7.2 mg per 100 mL for men, and 2.4–6.1 mg per 100 mL for women. Uric acid concentrations in blood plasma above and below the normal range are known as, respectively, hyperuricemia and hypouricemia.

Uric acid is only slightly soluble in water and easily precipitates out of solution forming needle-like crystals of sodium urate. These contribute to the formation of kidney stones and produce the excruciating pain of **gout** when deposited in the joints.



Uric acid

Uric acid is only slightly soluble in water and easily precipitates out of solution forming needle-like crystals of sodium urate. These contribute to the formation of kidney stones and produce the excruciating pain of **gout** when deposited in the joints. The concentration of uric acid is 100-times greater in the cytosol than in the extracellular fluid. So when lethally-damaged cells release their contents, crystals of uric acid form in the vicinity.

So the risk of kidney stones and gout may be the price we pay for the antioxidant protection provided by uric acid.

Most mammals have an enzyme - **uricas** - for breaking uric acid down into a soluble product. However, during the evolution of great apes and humans, the gene encoding uricase became inactive. A predisposition to gout is our legacy.

Contributors

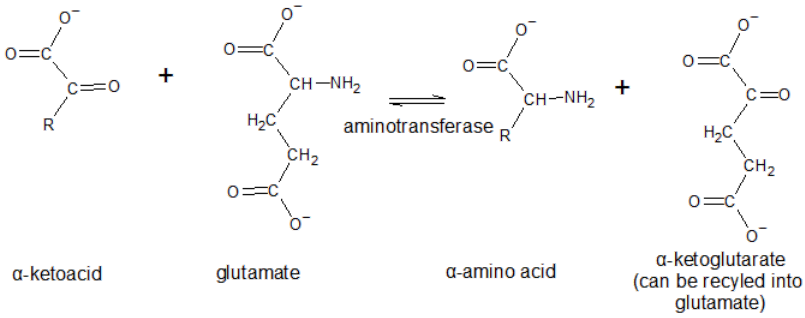
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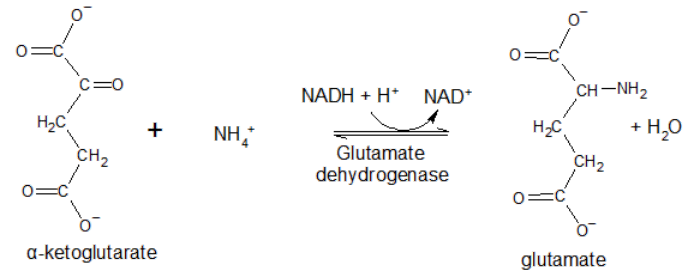
10.4: Amino Acid Synthesis

In humans, only half of the standard amino acids (Glu, Gln, Pro, Arg, Asp, Asn, Ala, Gly, Ser, Tyr, Cys) can be synthesized, and are thus classified the **nonessential amino acids.**

Most amino acids are synthesized from α-ketoacids or α-hydroxy acids (3-phosphoglycerate), and later transaminated from another amino acid (usually glutamate). The enzyme involved in this reaction is an aminotransferase. **Glutamate is usually the amino group donor** for this reaction: α-ketoacid + glutamate ⇄ amino acid + α-ketoglutarate



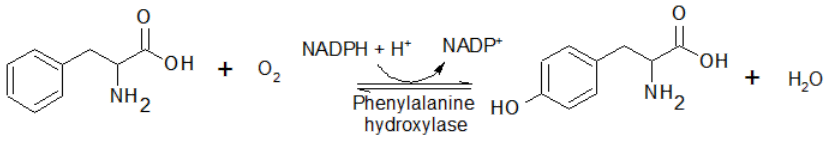
Glutamate itself is regenerated by the amination of α-ketoglutarate, catalyzed by Glutamate dehydrogenase:



The carbon skeletons used for the synthesis of amino acids are intermediates of the glycolysis pathway and the citric acid cycle (see table below)

Source of carbon skeleton used for the synthesis of nonessential amino acids	
Intermediates of glycolysis	
pyruvate is used for the synthesis of	glycine, serine, cysteine
3-phosphoglycerate is used for the synthesis of	alanine
Intermediates of citric acid cycle	
α-ketoglutarate is used for the synthesis of	glutamate, glutamine, proline, arginine
oxalacetate is used for the synthesis of	aspartate, asparagine

Tyrosine is another amino acid that depends on an essential amino acid as a precursor. In this case, phenylalanine hydroxylase oxidizes phenylalanine to produce tyrosine:



Phenylketonuria is a genetic disorder that results in low levels of the enzyme phenylalanine hydroxylase. This results in the buildup of dietary phenylalanine to potentially toxic levels. Untreated, PKU can lead to intellectual disability, seizures, behavioral problems, and mental disorders. It may also result in a musty smell and lighter skin.

In general, the synthesis of essential amino acids, usually in microorganisms, is much more complex than for the nonessential amino acids and is best left to a full-fledged biochemistry course.

Sources

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10.5: Connections of Carbohydrate, Protein, and Lipid Metabolic Pathways

Skills to Develop

- Discuss the ways in which carbohydrate metabolic pathways, glycolysis, and the citric acid cycle interrelate with protein and lipid metabolic pathways
- Explain why metabolic pathways are not considered closed systems

You have learned about the catabolism of glucose, which provides energy to living cells. But living things consume more than glucose for food. How does a turkey sandwich end up as ATP in your cells? This happens because all of the catabolic pathways for carbohydrates, proteins, and lipids eventually connect into glycolysis and the citric acid cycle pathways. Metabolic pathways should be thought of as porous—that is, substances enter from other pathways, and intermediates leave for other pathways. These pathways are not closed systems. Many of the substrates, intermediates, and products in a particular pathway are reactants in other pathways.

Connections of Other Sugars to Glucose Metabolism

Glycogen, a polymer of glucose, is an energy storage molecule in animals. When there is adequate ATP present, excess glucose is shunted into glycogen for storage. Glycogen is made and stored in both liver and muscle. The glycogen will be hydrolyzed into glucose monomers (G-1-P) if blood sugar levels drop. The presence of glycogen as a source of glucose allows ATP to be produced for a longer period of time during exercise. Glycogen is broken down into G-1-P and converted into G-6-P in both muscle and liver cells, and this product enters the glycolytic pathway.

Sucrose is a disaccharide with a molecule of glucose and a molecule of fructose bonded together with a glycosidic linkage. Fructose is one of the three dietary monosaccharides, along with glucose and galactose (which is part of the milk sugar, the disaccharide lactose), which are absorbed directly into the bloodstream during digestion. The catabolism of both fructose and galactose produces the same number of ATP molecules as glucose.

Connections of Proteins to Glucose Metabolism

Proteins are hydrolyzed by a variety of enzymes in cells. Most of the time, the amino acids are recycled into the synthesis of new proteins. If there are excess amino acids, however, or if the body is in a state of starvation, some amino acids will be shunted into the pathways of glucose catabolism (Figure 10.5.1). Each amino acid must have its amino group removed prior to entry into these pathways. The amino group is converted into ammonia. In mammals, the liver synthesizes urea from two ammonia molecules and a carbon dioxide molecule. Thus, urea is the principal waste product in mammals produced from the nitrogen originating in amino acids, and it leaves the body in urine.

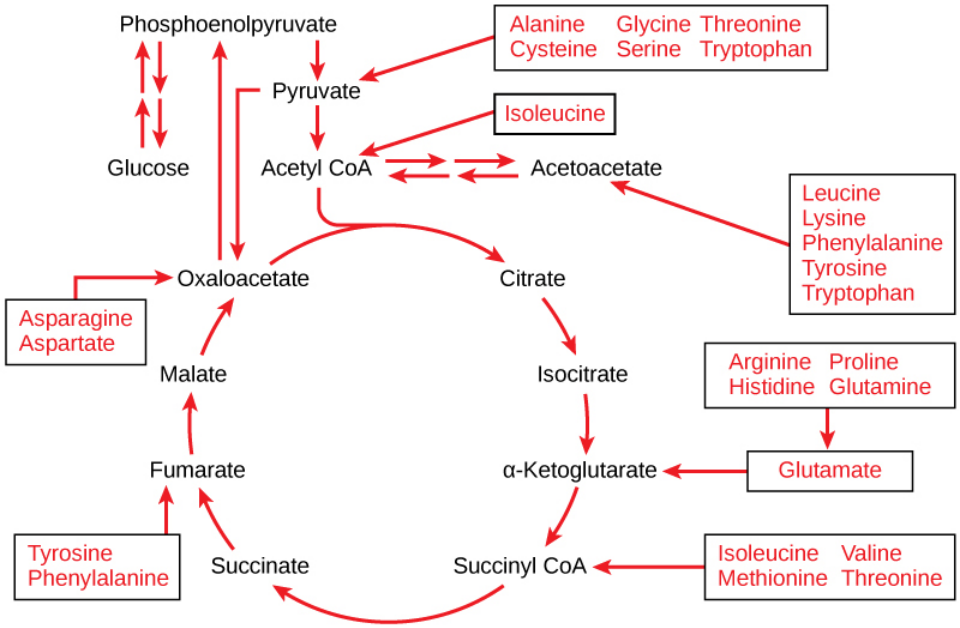


Figure **10.5.1**: The carbon skeletons of certain amino acids (indicated in boxes) derived from proteins can feed into the citric acid cycle. (credit: modification of work by Mikael Häggström)

Connections of Lipid and Glucose Metabolisms

The lipids that are connected to the glucose pathways are triglycerides. Triglycerides are a form of long-term energy storage in animals. Triglycerides are made of glycerol and three fatty acids. Animals can make most of the fatty acids they need. Triglycerides can be both made and broken down through parts of the glucose catabolism pathways. Glycerol can be phosphorylated to glycerol-3-phosphate, which continues through glycolysis. Fatty acids are catabolized in a process called beta-oxidation that takes place in the matrix of the mitochondria and converts their fatty acid chains into two carbon units of acetyl groups. The acetyl groups are picked up by CoA to form acetyl CoA that proceeds into the citric acid cycle.

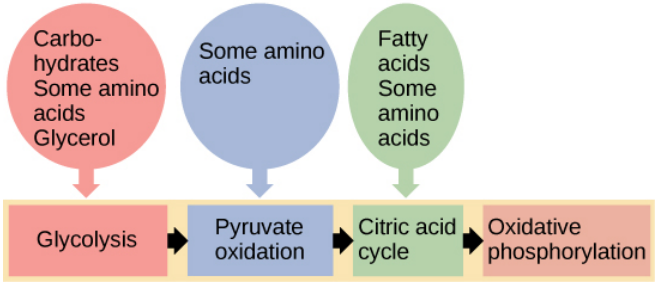


Figure **10.5.2**: Glycogen from the liver and muscles, hydrolyzed into glucose-1-phosphate, together with fats and proteins, can feed into the catabolic pathways for carbohydrates.

Summary

The breakdown and synthesis of carbohydrates, proteins, and lipids connect with the pathways of glucose catabolism. The simple sugars are catabolized during glycolysis. The fatty acids from fats connect with glucose catabolism through acetyl CoA. The amino acids from proteins connect with glucose catabolism through pyruvate, acetyl CoA, and components of the citric acid cycle.

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7.1: Nutrients

W

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7.1: Nutrients
Waxes
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Glossary

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