JAMES COOK UNIVERSITY
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Department of Chemistry
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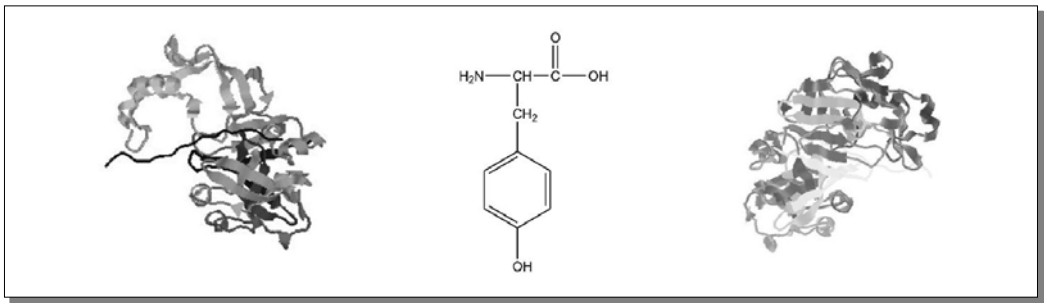
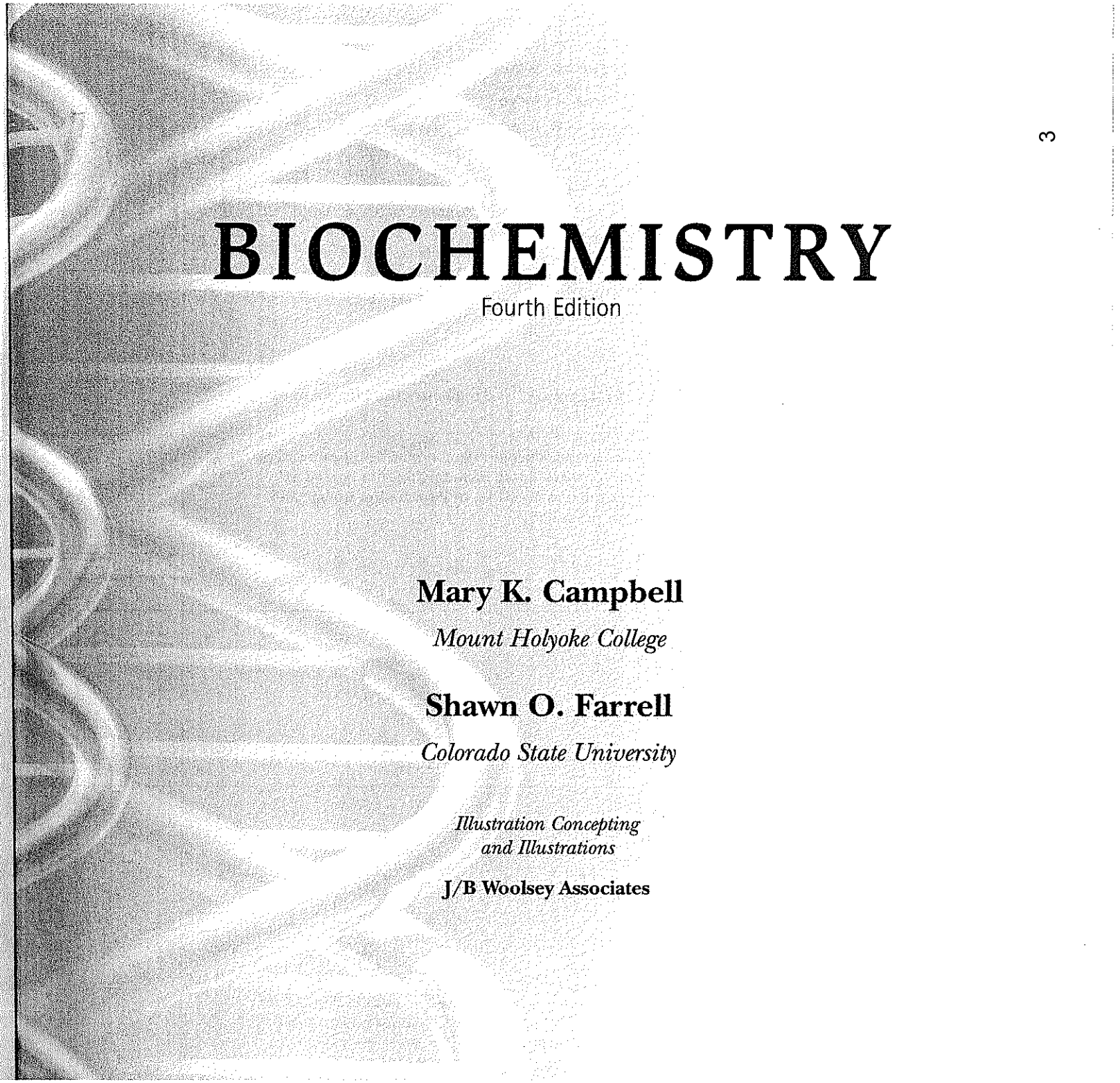


CH1010
BOOK
OF
READINGS

Table of Contents

	Page
Campbell and Farrell. *Biochemistry 4^{e}* - Purification of macromolecules, Polysomes, Lignins, Pyruvate reactions, oxidative phosphorylation, [4Fe-4S] clusters, Photosynthesis.	3
Brow, Le May, Bursten *Chemistry: The Central Science* - Formulae from analyses, spectrophotometry, coordination compounds, ligands, metal complexes.	30
Silberberg, M. *Chemistry: The molecular nature ...* - purification of organic compounds, spectrophotometry, IR and mass spectroscopy.	41
McMurray, J. *Fundamentals of Organic Chemistry 5th edition* - IR and UV spectroscopy.	48



BIOCHEMISTRY

Fourth Edition

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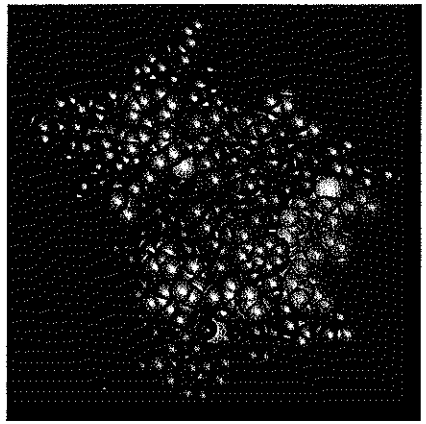
INTERCHAPTER A

Protein Purification and Characterization Techniques



A model of the protein phospholipase A_2. Proteins such as this one can be purified and studied by using the techniques of this interchapter. *(Charles Grisham.)*

OUTLINE

A.1 Purification of Proteins
A.2 Column Chromatography
A.3 Electrophoresis
A.4 Determining the Primary Structure of a Protein

Because a cell contains thousands of different protein molecules, the task of separating them and determining the structure of a single protein is exceedingly difficult. There are many techniques for purifying and characterizing a protein, ranging from strategies for determining such physical characteristics as molecular weight, isoelectric point, and number of subunits to discovering the number and type of its constituent amino acids and elucidating its complete amino acid sequence. When a protein has been degraded to its amino acids, they can be identified by chromatography according to their charge and polarity. The amino acids at the ends of a protein can be established by chemical labeling. The whole chain can be degraded by specific cleavage to give related peptide fragments. Each peptide can then be degraded one amino acid at a time to discover its sequence. In a final step of structure determination, a complete protein can be subjected to X-ray diffraction analysis to determine its three-dimensional conformation. However, the protein must first be purified by such techniques as column chromatography and electrophoresis and then crystallized.

A.1 Purification of Proteins

Many different proteins exist in a single cell. A detailed study of the properties of any one protein requires a homogeneous sample consisting of only one kind of molecule. The separation and isolation, or purification, of proteins constitutes an essential first step to further experimentation. In general, separation techniques focus on size, charge, and polarity—the sources of differences between molecules. Many techniques are performed to eliminate contaminants and arrive at a pure sample of the protein of interest. As the purification steps are followed, we make a table of the recovery and purity of the protein to gauge our success. Table A.1 shows a typical purification for an enzyme. The **percent recovery** tracks how much of the protein of interest has been retained at each step. This number usually drops steadily during the purification; however, we hope that by the time the protein is pure, sufficient product will be left for study and characterization. The **fold purification** compares the purity of the protein at each step, and this value should go up if the purification is successful.

Isolation of Proteins from Cells

Before the real purification steps can begin, the protein must be released from the cells and subcellular organelles. The first step is called **homogenization** and involves the breaking open of the cells. This can be done with a wide variety of techniques. The simplest approach is grinding the tissue in a blender with a suitable buffer. The cells are broken open, releasing soluble proteins. This process also breaks many of the subcellular organelles, such as mitochondria, peroxisomes, and endoplasmic reticulum. A gentler technique is to use a Potter–Elvejhem homogenizer, a thick-walled test tube through which a tight-fitting plunger is passed. The squeezing of the homogenate around the plunger breaks open cells, but leaves many of the organelles intact. Another

TABLE A.1 Example of a Protein Purification Scheme: Purification of the Enzyme Xanthine Dehydrogenase from a Fungus

Fraction	Volume (mL)	Total Protein (mg)	Total Activity	Specific Activity	Percent Recovery
1. Crude extract	3,800	22,800	2,460	0.108	100
2. Salt precipitate	165	2,800	1,190	0.425	48
3. Ion exchange chromatography	65	100	720	7.2	29
4. Molecular sieve chromatography	40	14.5	555	38.3	23
5. Immunoaffinity chromatography	6	1.8	275	152.108	11

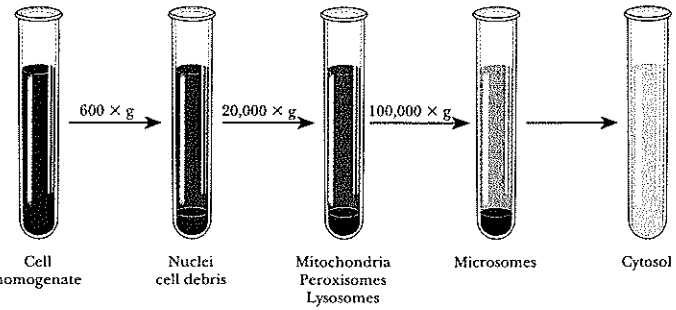


FIGURE A.1
Differential centrifugation used to separate cell components. Starting with a cell homogenate, increasing g forces will cause different cell components to be pellated.

technique, called sonication, involves using sound waves to break open the cells. Cells can also be ruptured by cycles of freezing and thawing. If the protein of interest is solidly attached to a membrane, detergents may have to be added to detach the proteins.

After the cells are homogenized, they are subjected to **differential centrifugation.** Spinning the sample at 500 times the force of gravity (500 × *g*) will result in a pallet of unbroken cells and nuclei. If the protein of interest is not found in the nuclei, this precipitate is discarded. The supernatant can then be centrifuged at higher speed; such as 10,000 × *g*, to bring down the mitochondria. If the protein of interest is soluble, the supernatant from this spin will be collected and will already be partially purified because the nuclei and mitochondria will have been removed. Figure A.1 shows a typical separation via differential centrifugation.

After the proteins are solubilized, they are often subjected to a crude purification based on solubility. Ammonium sulfate is the most common reagent to use at this step, and this procedure is referred to as **salting out.** Proteins have varying solubilities in polar and ionic compounds. Proteins remain soluble due to their interactions with water. When ammonium sulfate is added to a protein solution, some of the water is taken away from the protein to make ion–dipole bonds with the salts. With less water available to hydrate the proteins, they begin to interact with each other through hydrophobic bonds. At a defined amount of ammonium sulfate, a precipitate that contains contaminating proteins forms. These proteins are centrifuged down and discarded. Then more salt is added, and a different set of proteins, which usually contain the protein of interest, will precipitate. This precipitate is collected by centrifugation and saved. The quantity of ammonium sulfate is usually measured compared to a 100% saturated solution. A common procedure involves bringing the solution to around 40% saturation and then spinning down the precipitate that forms. Next, more ammonium sulfate is added to the supernatant, often to a level of 60% to 70% saturation. The precipitate that forms often contains the protein of interest. These preliminary techniques will not generally give a sample that is very pure, but they serve the important task of preparing the crude homogenate for the more effective procedures that follow.

A.2 Column Chromatography

The word "chromatography" comes from the Greek *chroma,* "color," and *graphein,* "to write"; the technique was first used around the beginning of the 20th century to separate plant pigments with easily visible colors. It has long since been possible to separate colorless compounds, as long as there are methods for detecting them. Chromatography is based on the fact that different compounds can distribute themselves to varying extents between different phases, or separable portions of matter. One phase is the **stationary** phase, and the other is the **mobile** phase. The mobile phase flows over the stationary material and carries the sample to be separated along with it. The components of the sample interact with the stationary phase to different extents. Some components interact relatively strongly with the stationary phase and are therefore carried along more slowly by the mobile phase than are those that interact less strongly. The differing mobilities of the components are the basis of the separation.

Many chromatographic techniques used for research on proteins are forms of **column chromatography,** where the material that makes up the stationary phase is packed in a column. The sample is a small volume of concentrated solution that is applied to the top of the column; the mobile phase, called the *eluent,* is passed through the column. The sample is diluted by the eluent, and the separation process also increases the volume occupied by the sample. In a successful experiment the entire sample eventually comes off the column. Figure A.2 diagrams an example of column chromatography.

Size exclusion chromatography, also called **gel filtration chromatography,** separates molecules on the basis of size, making it a useful way to sort proteins of varied molecular weights. It is a form of column chromatography in which the stationary phase consists of cross-linked gel particles. The gel particles are

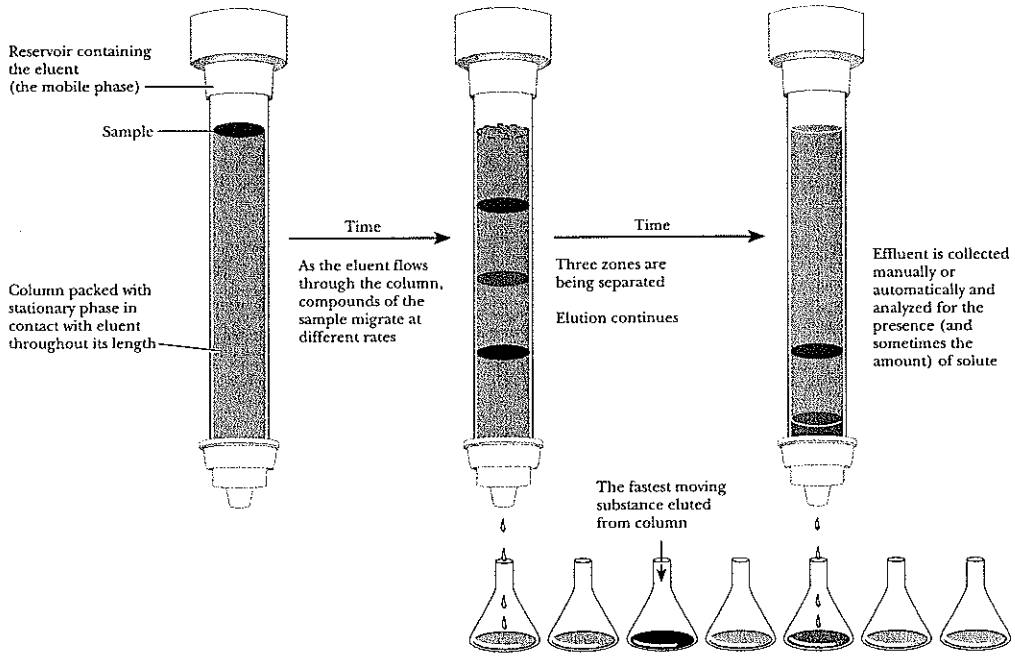


FIGURE A.2
An example of column chromatography. A sample containing several components is applied to the column. The various components travel at different rates and can be collected individually.

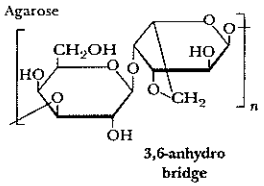


FIGURE A.3
The repeating disaccharide unit of agarose that is used for column chromatography.

usually in bead form and consist of one of two kinds of polymers. The first is a carbohydrate polymer such as **dextran** or **agarose** and often goes by the trade name Sephadex or Sepharose, respectively (Figure A.3). The second is based on **polyacrylamide** (Figure A.4), and is found under the trade name Bio-Gel. The cross-linked structure of these polymers produces pores in the material. The extent of cross-linking can be controlled to select a desired pore size. When a sample is applied to the column, smaller molecules can enter the pores and thus tend to be delayed in their progress down the column, unlike the larger molecules. As a result, the larger molecules are eluted first, followed later by the smaller ones, after the escape from the pores. Molecular sieve chromatography is represented schematically in Figure A.5. The advantages of this type of chromatography are its convenience as a way to separate molecules on the basis of size and the fact that it can be used to estimate molecular weight by comparing the sample with a set of standards. Each type of gel used has a specific range of sizes that will separate linearly with the log of the molecular weight. Each gel also has an exclusion limit, a size of protein that is too large to fit inside the pores. Any protein that size or larger will elute first and simultaneously.

Affinity chromatography uses the specific binding properties of many proteins. It is another form of column chromatography, with a polymeric material used as the stationary phase. The distinguishing feature of affinity chromatography is that the polymer is covalently linked to some compound, called a *ligand,* that binds specifically to the desired protein (Figure A.6). The other proteins in the sample do not bind to the column and can easily be eluted with buffer, while the bound protein remains on the column. The bound protein can then be eluted from the column by adding high concentrations of the ligand in soluble form thus competing for the binding of the protein with the stationary phase. The protein binds to the ligand in the mobile phase and is recovered from the column. This protein-ligand interaction can also be disrupted with a change in pH or ionic strength. Affinity chromatography is a convenient

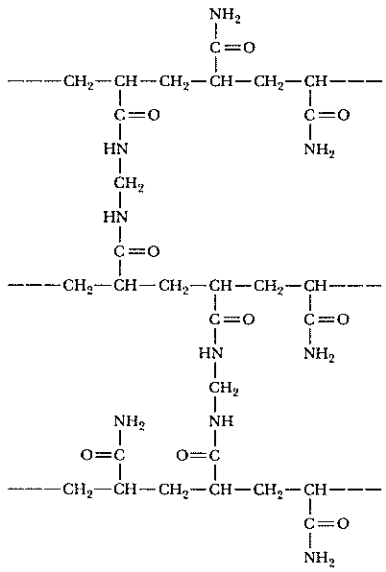


FIGURE A.4
The structure of cross-linked polyacrylamide, a polymer used in column chromatography.

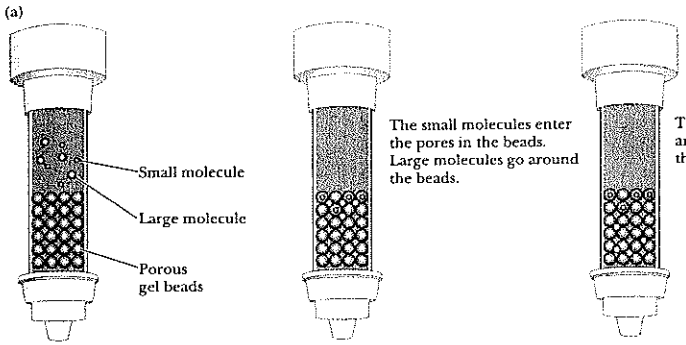


FIGURE A.5
(a) Gel filtration chromatography. Larger molecules are excluded from the gel and move more quickly through the column. Small molecules have access to the interior of the gel beads, so they take a longer time to elute. (b) V_o is the void volume, the volume of elution for a molecule excluded from the gel bead. V_e is the elution volume for a particular molecule that can enter the bead. V_t is the total volume, the elution volume for a very small molecule that enters the bead unhindered.

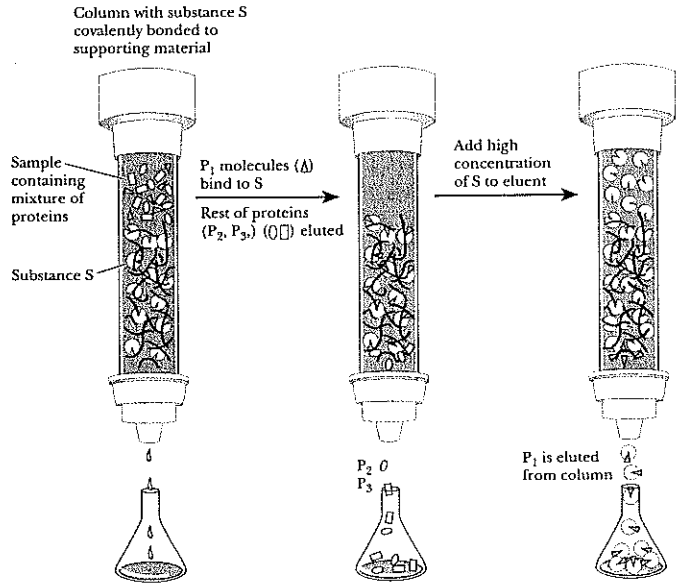
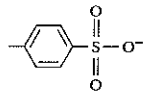
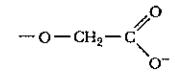
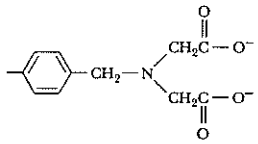


FIGURE A.6
The principle of affinity chromatography. In a mixture of proteins, only one designated P_1, will bind to a substance, S, called the substrate. The substrate is attached to the column matrix. Once the other proteins, P_2 and P_3, have been washed out, P_1 can be eluted either by adding a solution of high salt concentration, or by adding free S.

(a) Cation Exchange Media	Structure
Strongly acidic, polystyrene resin (Dowex-50)	
Weakly acidic, carboxymethyl (CM) cellulose	
Weakly acidic, chelating, polystyrene resin (Chelex-100)	

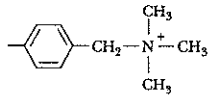
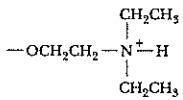
(b) Anion Exchange Media	Structure
Strongly basic, polystyrene resin (Dowex-1)	
Weakly basic, diethylaminoethyl (DEAE) cellulose	

FIGURE A.7
(a) Cation exchange resins and (b) anion exchange resins commonly used for biochemical separations.

separation method and has the advantage of producing very pure proteins. The Biochemical Connections box in Interchapter B describes an interesting way that affinity chromatography can be combined with molecular biological techniques to offer a one-step purification of a protein.

Ion exchange chromatography is logistically similar to affinity chromatography. Both use a column resin that binds the protein of interest. With ion exchange chromatography, however, the interaction is less specific and is based on net charge. An ion exchange resin will have a ligand with a positive charge or a negative charge. A negatively charged resin is a **cation exchanger,** and a positively charged one is an **anion exchanger.** Figure A.7 shows some typical ion exchange ligands. Figure A.8 shows how cation exchange chromatography would separate proteins. The column is initially equilibrated with a buffer of suitable pH and ionic strength. The exchange resin is bound to counterions. A cation exchange resin is usually bound to Na^+ or K^+ ions, and an anion exchanger is usually bound to Cl^- ions. A mixture of proteins is loaded on the column and allowed to flow through it. Those proteins that have a net charge opposite to that of the exchanger will stick to the column, *exchanging* places with the bound counterions. Those proteins that have no net charge or have the same charge as the exchanger will elute. After all the nonbinding proteins are eluted, the eluent will be changed to either a buffer that has a pH that will remove the charge on the bound proteins or to one with a higher salt concentration. The latter will outcompete the bound proteins for the limited binding space on the column. The once-bound molecules will then elute having been separated from many of the contaminating ones.

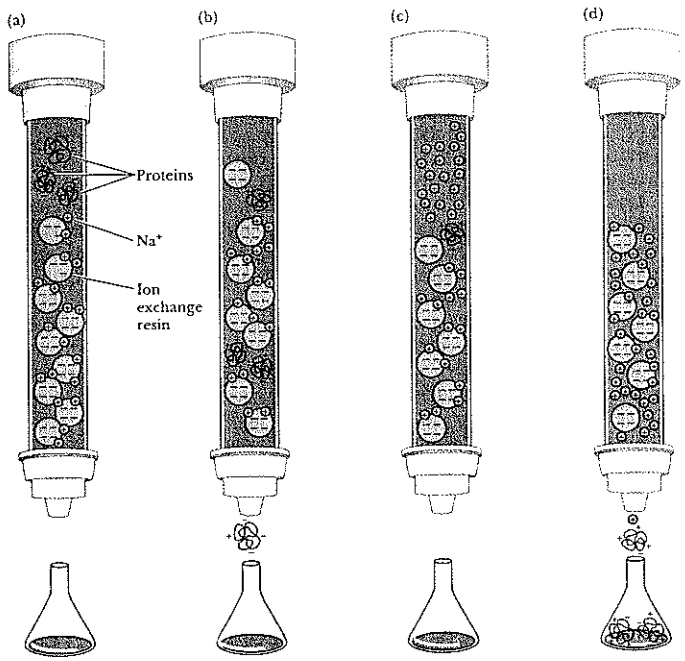


FIGURE A.8
Ion exchange chromatography using a cation exchanger. (a) At the beginning of the separation, various proteins are applied to the column. The column resin is bound to Na^+ counterions (small red spheres). (b) Proteins that have no net charge or a net negative charge pass through the column. Proteins that have a net positive charge stick to the column displacing the Na^+. (c) An excess of Na^+ ion is then added to the column. (d) The Na^+ ions outcompete the bound proteins for the binding sites on the resin and the proteins elute.

A.3 Electrophoresis

Electrophoresis is based on the motion of charged particles in an electric field toward an electrode of opposite charge. Macromolecules have differing mobilities based on their charge, shape, and size. Although many supporting media have been used for electrophoresis, including paper and liquid, the most common support is a polymer of agarose or acrylamide that is similar to those used for column chromatography. A sample is applied to wells that are formed in the supporting medium. An electric current is passed through the medium at a controlled voltage to achieve the desired separation (Figure A.9). After the proteins are separated on the gel, the gel is stained to reveal the protein locations as shown in Figure A.10.

Agarose-based gels are most often used to separate nucleic acids and will be discussed in Interchapter B. For proteins, the most common electrophoretic support is polyacrylamide (Figure A.4). The gel is prepared and cast as a continuous cross-linked matrix (rather than the bead form employed in column chromatography). In one variation of polyacrylamide gel electrophoresis, the protein sample is treated with the detergent sodium dodecyl sulfate (SDS) before it is applied to the gel. The structure of SDS is $CH_3(CH_2)_{10}CH_2OSO_3^-Na^+$. The anion binds strongly to proteins via nonspecific adsorption. The larger the protein, the more of the anion it will adsorb. SDS completely denatures proteins, breaking all the noncovalent interactions that determine tertiary and quaternary structure. This means that multisubunit proteins can be analyzed as the component polypeptide chains. All the proteins in a sample have a negative charge as a result of adsorption of the anionic SO_3^-. The proteins will also have roughly the same shape, which will be a random coil. In

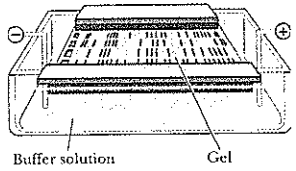


FIGURE A.9
The experimental setup for gel electrophoresis. The samples are placed on the left side of the gel. When the current is applied, the negatively charged molecules migrate toward the positive electrode.

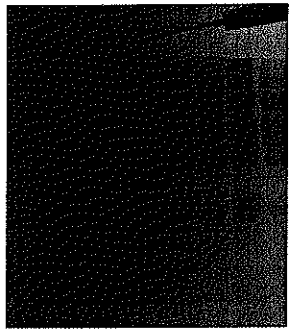


FIGURE A.10
Separation of proteins by gel electrophoresis. Each band seen in the gel represents a different protein. In the SDS–PAGE technique, the sample is treated with detergent before being applied to the gel. In isoelectric focusing, a pH gradient runs the length of the gel. *(Michael Gabridge/Visuals Unlimited.)*

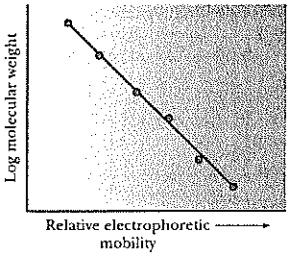


FIGURE A.11
A plot of the relative electrophoretic mobility of proteins in SDS–PAGE versus the log of the molecular weights of the individual polypeptides.

SDS–polyacrylamide gel electrophoresis (SDS–PAGE), the acrylamide offers more resistance to large molecules than to small molecules. Because the shape and charge are approximately the same for all the proteins in the sample, the size of the protein becomes the determining factor in the separation: small proteins move faster than large ones. Like molecular sieve chromatography, SDS–PAGE can be used to estimate the molecular weights of proteins by comparing the sample with standard samples. For most proteins, the log of the molecular weight is linearly related to its mobility on SDS–PAGE, as shown in Figure A.11.

Isoelectric focusing is another variation of gel electrophoresis. Since different proteins have different titratable groups, they also have different isoelectric points. Recall (Section 3.3) that the isoelectric pH (pI) is the pH at which a protein (or amino acid or peptide) has no net charge. At the pI, the number of positive charges exactly balances the number of negative charges. In an isoelectric focusing experiment, the gel is prepared with a pH gradient that parallels the electric field gradient. As proteins migrate through the gel under the influence of the electric field, they encounter regions of different pH so the charge on the protein changes. Eventually each protein reaches the point at which it has no net charge, its isoelectric point, and no longer migrates. Each protein remains at the position on the gel corresponding to its pI, allowing for an effective method of separation.

An ingenious combination, known as two-dimension gel electrophoresis (2-D gels), allows for enhanced separation by using isoelectric focusing in one dimension and SDS–PAGE run at 90° to the first (Figure A.12).

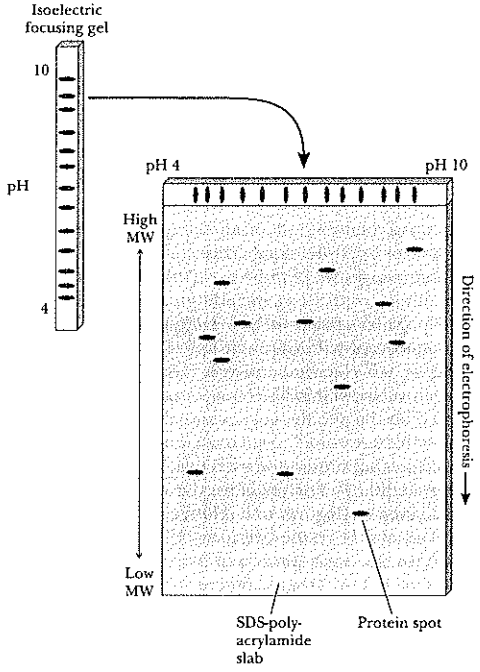


FIGURE A.12
Two-dimensional electrophoresis. A mixture of proteins is separated by isoelectric focusing in one direction. The focused proteins are then run using SDS–PAGE perpendicular to the direction of the isoelectric focusing. Thus the bands that appear on the gel have been separated first by charge and then by size.

A.4 Determining the Primary Structure of a Protein

Essential Information

The primary structure of a protein is the sequence of amino acids. The sequence is determined by cleaving the protein into smaller peptides, verifying the sequence of the individual peptides, and combining overlapping peptide sequences to obtain that of the protein.

Determining the sequence of amino acids in a protein is a routine, but not trivial, operation in classical biochemistry. Its several parts must be carried out carefully to obtain accurate results (Figure A.13).

Step 1 in determining the primary structure of a protein is to establish which amino acids are present and in what proportions. Breaking a protein down to its component amino acids is relatively easy: Heat a solution of the protein in acid, usually 6 *M* HCl, at 100 to 110°C for 12 to 36 hours to hydrolyze the peptide bonds. Separation and identification of the products are somewhat more difficult and are best done by an amino acid analyzer. This automated instrument gives both qualitative information about the identities of the amino acids present and quantitative information about the relative amounts of those amino acids. Not only does it analyze amino acids, but it also allows informed decisions to be made about which procedures to choose later in the sequencing (see Steps 3 and 4). An amino acid analyzer separates the

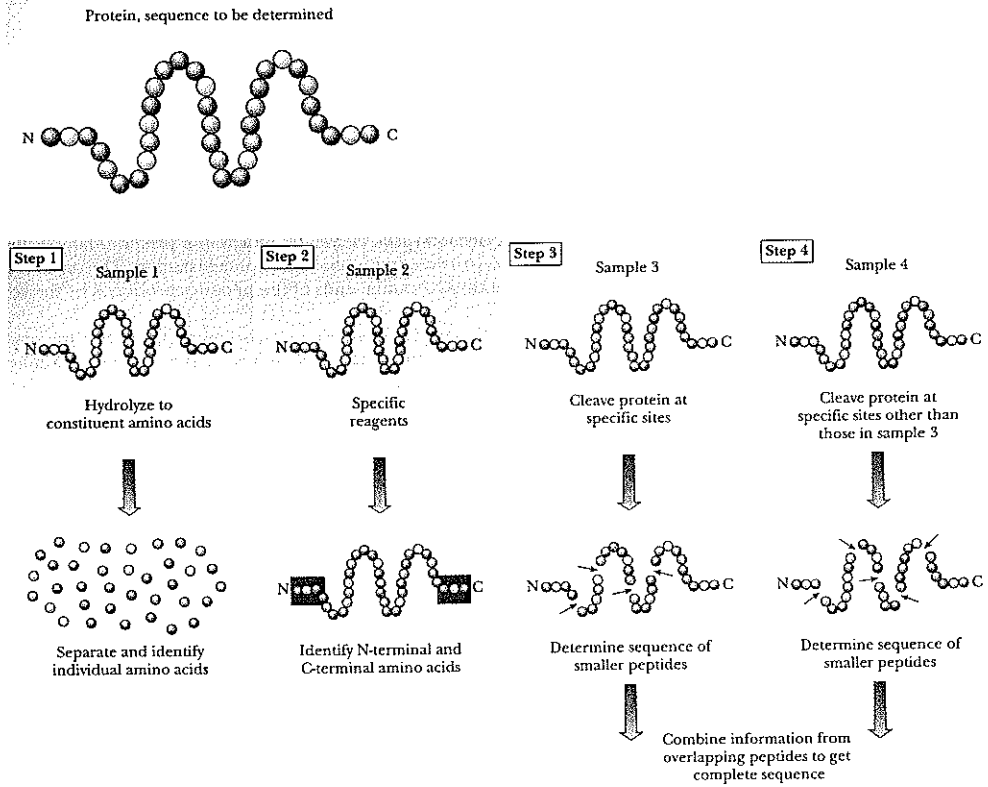


FIGURE A.13
The strategy for determining the primary structure of a given protein. The amino acid sequence can be determined by four different analyses performed on four separate samples of the same protein.

TABLE 11.3 Components Required for Each Step of Protein Synthesis in *Escherichia coli*

Step	Components
Amino acid activation	Amino acids
	tRNAs
	Aminoacyl-tRNA synthetases
	ATP, Mg^{2+}
Chain initiation	fmet-$tRNA^{fmet}$
	Initiation codon (AUG) of mRNA
	30S ribosomal subunit
	50S ribosomal subunit
	Initiation factors (IF-1, IF-2, and IF-3)
	GTP, Mg^{2+}
Chain elongation	70S ribosome
	Codons of mRNA
	Aminoacyl-tRNAs
	Elongation factors (EF-Tu, EF-Ts, and EF-G)
	GTP, Mg^{2+}
Chain termination	70S ribosome
	Termination codons (UAA, UAG, and UGA) of mRNA
	Release factors (RF-1, RF-2, and RF-3)
	GTP, Mg^{2+}

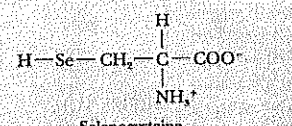
The Ribosome Is a Ribozyme

Until recently, proteins were thought to be the only molecules with catalytic ability. Then the self-splicing ability of the *Tetrahymena* snRNP showed that RNA can also catalyze reactions. In 2000, the complete structure of the large ribosomal subunit was determined by X-ray crystallography to 2.4-Å (0.24-nm) resolution (Figure 11.16). Ribosomes had been studied for 40 years, but the complete structure had been elusive. When the active sites for peptidyl transferase were looked at, it turned out that there is no protein in the vicinity of the new peptide bond, proving once again that RNA has catalytic ability. This is an exciting finding because it answers questions that have been plaguing scientists for decades. It was assumed that RNA was the first genetic material, and RNA can code for proteins that act as catalysts, but it takes proteins to do the translation, so how could the first proteins have been created? With the discovery of an RNA-based peptidyl transferase, it was suddenly possible to imagine an "RNA world" where the RNA both carried the message and processed it. This discovery is very intriguing, but it has not yet been accepted by many researchers, and some evidence questions the nature of catalytic RNA. One study showed that mutations of the putative RNA bases involved in the catalytic mechanism do not significantly reduce the efficiency of peptidyl transferase, throwing into question whether the RNA is chemically involved in the catalysis (see the article by Polacek et al. in the bibliography at the end of the chapter).

BIOCHEMICAL CONNECTIONS

The 21st Amino Acid?

Many amino acids, such as citrulline and ornithine found in the urea cycle, are not building blocks of proteins. Other nonstandard amino acids such as hydroxyproline are formed after translation by posttranslational modification. When discussing amino acids and translation, the magic number was always 20. Only 20 standard amino acids were put onto tRNA molecules for protein synthesis. In the late 1980s, another amino acid was found in proteins from eukaryotes and prokaryotes alike, including humans. It is selenocysteine, a cysteine residue that has the sulfur replaced by selenium.



Selenocysteine

It was later determined that selenocysteine is formed by placing a serine onto a special tRNA molecule called $tRNA^{sec}$. Once bound, the oxygen in the serine side chain is replaced by selenium. This tRNA molecule has an anticodon that matches the UGA stop codon. In special cases, the UGA is not read as a stop, rather the selenocysteine-$tRNA^{sec}$ is loaded into the A site and translation continues. Some are therefore calling selenocysteine the 21st amino acid. The methods by which the cell knows when to put selenocysteine into the protein instead of reading UGA as a stop codon are still being investigated.

Polysomes

In our description of protein synthesis, we have considered, up to now, the reactions that take place at one ribosome. It is, however, not only possible but quite usual for several ribosomes to be attached to the same mRNA. Each of these ribosomes will bear a polypeptide in one of various stages of completion, depending on the position of the ribosome as it moves along the mRNA (Figure 11.17). This complex of mRNA with several ribosomes is called a **polysome**, an alternative name is polyribosome.

In prokaryotes, translation begins very soon after mRNA transcription. It is possible for a molecule of mRNA that is still being transcribed to have a number of ribosomes attached to it that are in various stages of translating that mRNA. It is also possible for DNA to be in various stages of being transcribed. In this situation, several molecules of RNA polymerase are attached to a single gene, giving rise to several mRNA molecules, each of which has a number of ribosomes attached to it. The prokaryotic gene is being simultaneously transcribed and translated. This process is called *coupled translation* (Figure 11.18); it is possible in prokaryotes because of the lack of cell compartmentalization. In eukaryotes, mRNA is produced in the nucleus, and the majority of protein synthesis takes place in the cytosol.

Essential Information

The biosynthesis of proteins depends on four important steps. (1) In the activation step, amino acids are bonded to tRNAs in a reaction catalyzed by aminoacyl-tRNA synthetases. (2) The initiation step requires assembly of ribosomes, mRNA, and aminoacyl-tRNAs into a functional unit. (3) In chain elongation, the ribosome moves along the mRNA, and the protein is assembled as new amino acids are added. (4) Chain termination requires protein release factors as well as stop signals on the mRNA.

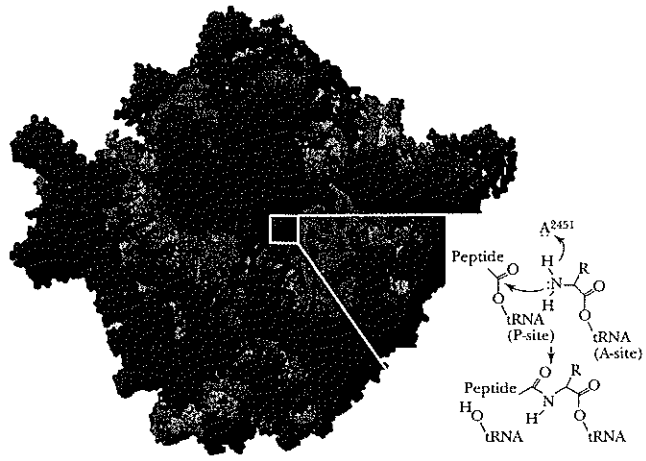


FIGURE 11.16
The large subunit of the ribosome seen from the viewpoint of the small subunit, with proteins in purple, 23S rRNA in orange and white, 5S rRNA (at the top) in pink and white, and A-site tRNA (green) and P-site tRNA (red) docked. (Box) The peptidyl transfer mechanism is catalyzed by RNA. The general base (adenine 2451 in *E. coli* 23S rRNA) is rendered unusually basic by its environment within the folded structure; it could abstract a proton at any of several steps, one of which is shown here. *(Reprinted by permission from* Science *289 [2000], p. 878.* The ribosome is a ribozyme, *by Thomas Cech.)*

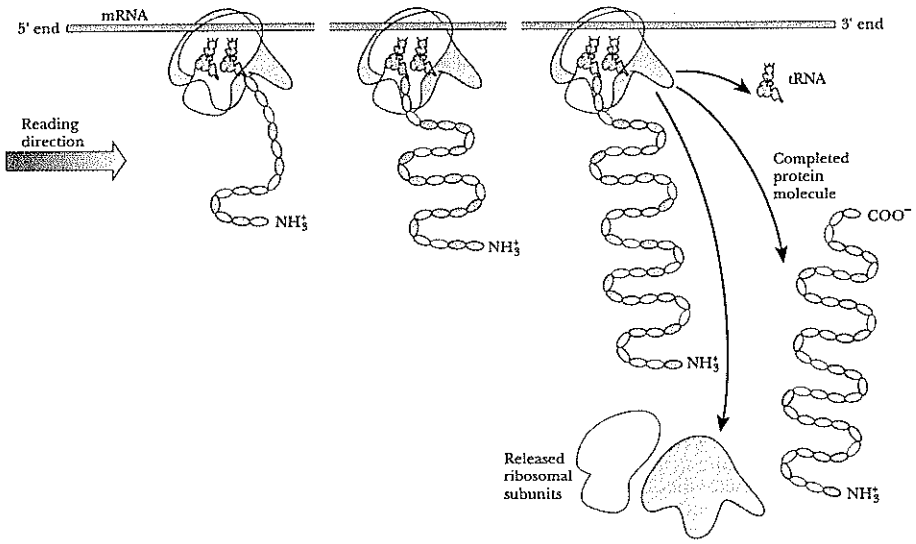


FIGURE 11.17
Simultaneous protein synthesis on polysomes. A single mRNA molecule is translated by several ribosomes simultaneously. Each ribosome produces one copy of the polypeptide chain specified by the mRNA. When the protein has been completed, the ribosome dissociates into subunits that are used in further rounds of protein synthesis.

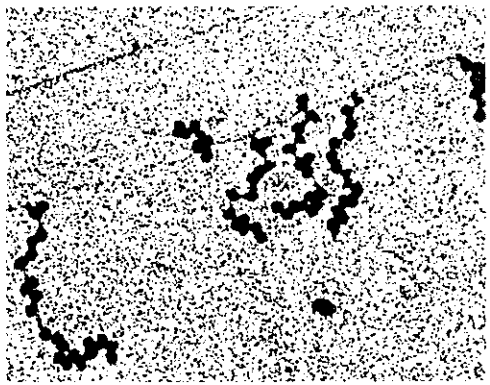


FIGURE 11.18
Electron micrograph showing coupled translation. The dark spots are ribosomes, arranged in clusters on a strand of mRNA. Several mRNAs have been transcribed from one strand of DNA, the diagonal line from center to upper right.

11.5 Translation in Eukaryotes

The main features of translation are the same in prokaryotes and eukaryotes, but the details differ. The messenger RNAs of eukaryotes are characterized by two major posttranscriptional modifications. The first is the 5′ cap, and the second is the 3′ poly-A tail (Figure 11.19). Both modifications are essential to eukaryotic translation.

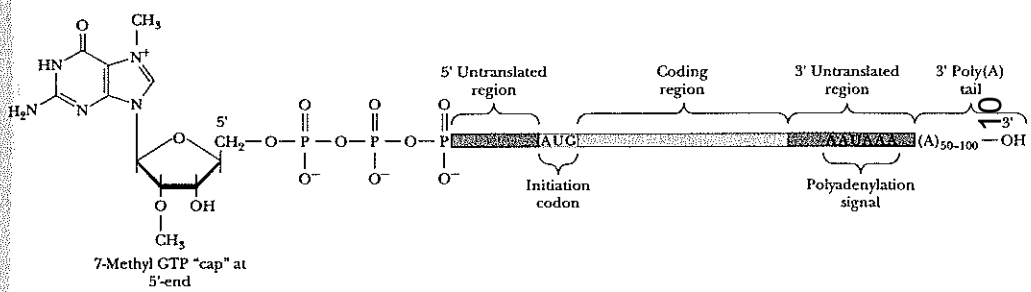


FIGURE 11.19
The characteristic structure of eukaryotic mRNAs. Untranslated regions ranging between 40 and 150 bases in length occur at both the 5′ and 3′ ends of the mature mRNA. An initiation codon at the 5′ end, invariably AUG, signals the translation start site.

Chain Initiation

This is the part of eukaryotic translation that is the most different from that in prokaryotes. Thirteen more initiation factors are given the designation **eIF,** for **eukaryotic initiation factor.** Many of them are multisubunit proteins. Table 11.4 summarizes pertinent information about these initiation factors.

Step 1 in chain initiation involves the assembly of a 43S preinitiation complex (Figure 11.20). The initial amino acid is methionine, which is attached to a special $tRNA_i$ that serves only as the initiator tRNA. There is no fmet in eukaryotes. The met-$tRNA_i$ is delivered to the 40S ribosomal subunit as a complex with GTP and eIF2. The 40S ribosome is also bound to eIF1A and eIF3. This order of events is different from that in prokaryotes in that the first tRNA binds to the ribosome without the presence of the mRNA. In Step 2, the mRNA is recruited. There is no Shine–Dalgarno sequence for location of the start codon. The 5′ cap orients the ribosome to the correct AUG via what is called a *scanning mechanism* that is driven by ATP hydrolysis. The eIF4E is also a cap-binding protein, which forms a complex with several other eIFs. A poly A binding protein **(Pab1p)** links the poly A tail to eIF4G. The eIF-40S complex is initially positioned upstream of the start codon (Figure 11.21). It moves downstream until it encounters the first AUG in the correct *context.* The context is determined by a few bases surrounding the start codon, called the **Kozak sequence.** It is characterized by the consensus sequence −3ACCAUGG+4. The ribosome may skip the first AUG it finds if the next one has the Kozak sequence. Another factor is the presence of mRNA secondary structure. If hairpin loops form downstream of an AUG, an earlier AUG may be chosen. The mRNA and the seven eIFs constitute the 48S preinitiation complex. In Step 3, the 60S ribosome is recruited, forming the 80S initiation complex. GTP is hydrolyzed, and the initiation factors are released.

Chain Elongation

Peptide chain elongation in eukaryotes is very similar to that of prokaryotes. The same mechanism of peptidyl transferase and ribosome translocation is seen. The structure of the eukaryotic ribosome is different in that there is no E site, only the A and P sites. There are two eukaryotic elongation factors, eEF1 and eEF2. The eEF1 consists of two subunits, eEF1A and eEF1B. The 1A subunit is the counterpart of EF-Tu in prokaryotes. The 1B subunit is the

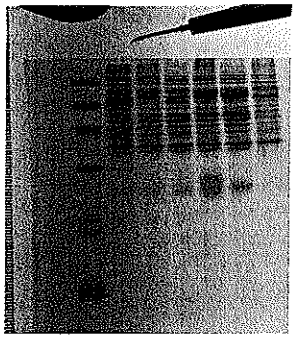


FIGURE B.1
Separation of oligonucleotides by gel electrophoresis. Each band seen in the gel represents a different oligonucleotide.
(Cryptographics/Visuals Unlimited.)

Experiments on nucleic acids frequently involve extremely small quantities of materials of widely varying molecular size. Two of the primary necessities are to separate the components of a mixture and to detect the presence of nucleic acids; fortunately, powerful methods exist for accomplishing both goals.

Separation Techniques

Any separation method uses the differences between the items to be separated. Charge and size are two properties of molecules that are frequently used for separation. One of the most widely used techniques in molecular biology, **gel electrophoresis,** uses both these properties. Electrophoresis is based on the motion of charged particles in an electric field. For our purposes, it is enough to know that the motion of a charged molecule in an electric field depends on the ratio of its charge to its mass. A sample is applied to a supporting medium. With the use of electrodes, an electric current is passed through the medium to achieve the desired separation. Polymeric gels, such as agarose and polyacrylamide, are frequently used as supporting media for electrophoresis (Figure B.1). They are prepared and cast as a continuous cross-linked matrix. The cross-linking gives rise to pores, and the choice of agarose versus polyacrylamide gels depends on the size of the molecules to be separated—agarose for larger fragments (thousands of oligonucleotides) and polyacrylamide for smaller (hundreds of oligonucleotides).

The charge on the molecules to be separated leads them to move through the gel toward an electrode of opposite charge. Nucleic acids and oligonucleotide fragments are negatively charged at neutral pH because of the presence of the phosphate groups. When these negatively charged molecules are placed in an electric field between two electrodes, they all migrate toward the positive electrode. In nucleic acids, each nucleotide residue contributes a negative charge from the phosphate to the overall charge of the fragment, but the mass of the nucleic acid or oligonucleotide increases correspondingly. Thus, the ratio of charge to mass remains approximately the same regardless of the size of the molecule in question. As a result, the separation takes place simply on the basis of size and is due to the sieving action of the gel. In a given amount of time, with a sample consisting of a mixture of oligonucleotides, a smaller oligonucleotide moves farther than a larger one in an electrophoretic separation. The oligonucleotides move in the electric field because of their charges; the distances they move in a given time depend on their sizes.

Most separations are done with an agarose gel in a horizontal position, called a submarine gel because it is actually underneath the buffer in the chamber. However, when DNA sequencing is done (see Section B.12), a polyacrylamide gel is run in a vertical position. Many different samples can be separated on a single gel. Each sample is loaded at a given place (a distinct well) at the negative electrode end of the gel, and an electric current flows until the separation is complete (Figure B.2).

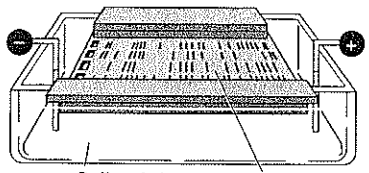


FIGURE B.2
The experimental setup for gel electrophoresis. The samples are placed on the left side of the gel. When the current is applied, the negatively charged oligonucleotides migrate toward the positive electrode.

Detection Methods

After the DNA pieces have been separated, they must be treated in some way that will allow them to be seen. Some of these techniques will allow all of the DNA to be seen, but others are more specific for certain DNA pieces.

The original method for detecting the separated products is based on radioactive labeling of the sample. A label, or tag, is an atom or molecule that allows visualization of another molecule. The isotope of phosphorus of mass number 32 (^{32}P, spoken as "P-thirty-two") was widely used in the past for this purpose. More recently, ^{35}S, or the isotope of sulfur of mass number 35 (spoken as "S-thirty-five"), has been used extensively. The DNA molecules undergo a reaction that incorporates the radioactive isotope into the DNA. When the labeled oligonucleotides have been separated, the gel is placed in contact with a piece of X-ray film. The radioactively labeled oligonucleotides expose the portions of the film with which they are in contact. When the film is developed, the positions of the labeled substances show up as dark bands. This technique is called **autoradiography,** and the resulting film image is an autoradiogram (Figure B.3).

Many examples of autoradiographs can be seen in the scientific literature, but as time goes on, autoradiography is being replaced by detection methods that do not use radioactive materials and their associated hazards. Many of these methods depend on emission of light (**luminescence**) by a chemical label attached to the fragments, and they can detect amounts of substances measured in picomoles. The way in which the label emits light depends on the application. When the base sequence of DNA is to be determined, the label is a series of four fluorescent compounds, one for each base. The gel with the separated products is irradiated with a laser; the wavelength of the laser light is one that is absorbed by each of the four labels. Each of the four labeled compounds re-emits light at a different, characteristic, longer wavelength. This is called **fluorescence.** Another detection method that uses fluorescence involves the compound ethidium bromide. Its molecular structure includes a planar portion that can slip between the bases of DNA, giving ethidium bromide different fluorescence properties when it binds to DNA from those observed when it is free in solution. An ethidium bromide solution is used as a stain for DNA in a gel. The solution soaks into the gel, and the DNA fragments in the gel can be seen as orange bands by shining ultraviolet light on the gel.

11

Essential Information

Manipulation of nucleic acids requires methods for separating polynucleotides and oligonucleotides of different sizes. This is done by gel electrophoresis. Molecules of different sizes appear as separate bands on the gels. The bands can be seen by labeling the molecules with radioactive or luminescent "tags."

FIGURE B.3
An example of an autoradiogram.
(Hank Morgan/Photo Researchers, Inc.)

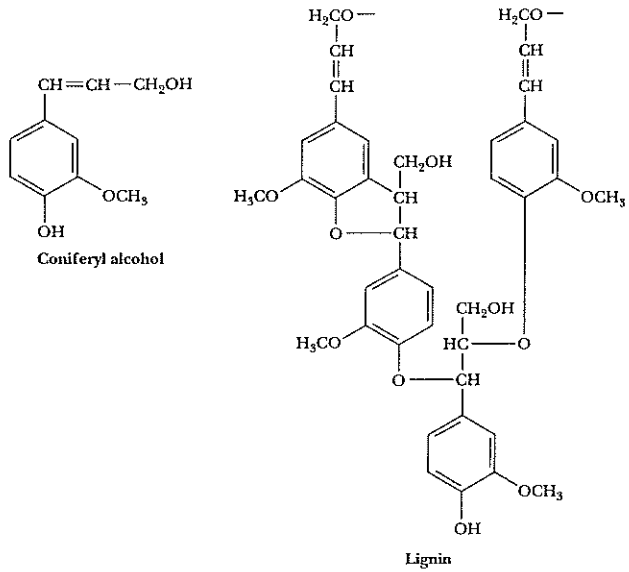
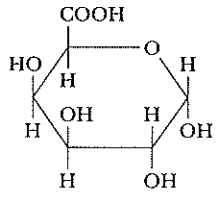


FIGURE 13.25
The structure of lignin, a polymer of coniferyl alcohol.

Plant Cell Walls

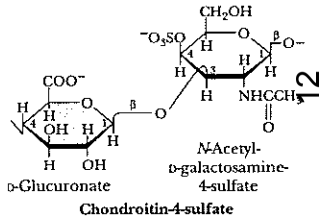
Plant cell walls consist largely of **cellulose.** The other important polysaccharide component found in plant cell walls is **pectin,** a polymer made up mostly of D-galacturonic acid, a derivative of galactose in which the hydroxyl group on carbon 6 has been oxidized to a carboxyl group.



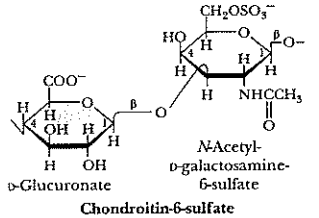
D-Galacturonic acid

Pectin is extracted from plants because it has commercial importance in the food processing industry as a gelling agent in yogurt, fruit preserves, jams, and jellies. The major nonpolysaccharide component in plant cell walls, especially in woody plants, is **lignin** (Latin *lignum,* "wood"). Lignin is a polymer of coniferyl alcohol, and it is a very tough and durable material (Figure 13.25). Unlike bacterial cell walls, plant cell walls contain comparatively little peptide or protein.

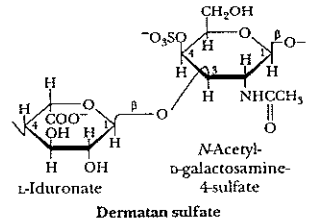
FIGURE 13.26
Glycosaminoglycans are formed from repeating disaccharide units and often occur as components of the proteoglycans.



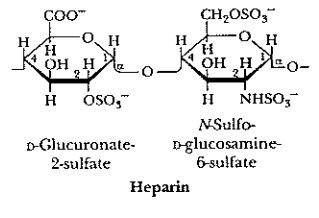
Chondroitin-4-sulfate



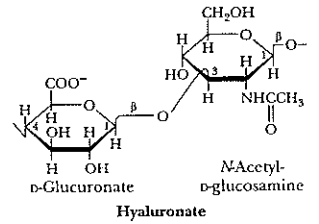
Chondroitin-6-sulfate



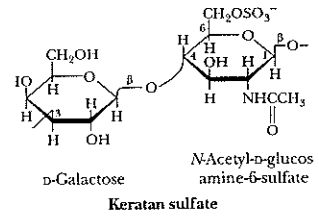
Dermatan sulfate



Heparin



Hyaluronate



Keratan sulfate

13.5 Glycoproteins

Glycoproteins contain carbohydrate residues in addition to the polypeptide chain. Some of the most important examples of glycoproteins are involved in the immune response; for example, **antibodies,** which bind to and immobilize antigens (the substances attacking the organism), are glycoproteins. Carbohydrates also play an important role as **antigenic determinants,** the portions of an antigenic molecule that antibodies recognize and to which they bind.

An example of the role of the oligosaccharide portion of glycoproteins as antigenic determinants is found in human blood groups. There are four human blood groups, A, B, AB, and O (see the Biochemical Connections box on the following page). The distinctions between the groups depend on the oligosaccharide portions of the glycoproteins on the surfaces of the blood cells called erythrocytes. In all blood types, the oligosaccharide portion of the molecule contains the sugar L-fucose, mentioned earlier in this chapter as an example of a deoxy sugar. *N*-acetylgalactosamine is found at the nonreducing end of the oligosaccharide in the type A blood-group antigen. In type B blood, α-D-galactose takes the place of *N*-acetylgalactosamine. In type O blood neither of these terminal residues is present, and in type AB blood both kinds of oligosaccharide are present (Figure 13.27).

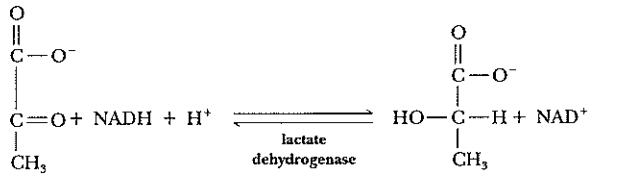
Glycosaminoglycans

Glycosaminoglycans are a type of polysaccharide based on a repeating disaccharide in which one of the sugars is an amino sugar and at least one of them has a negative charge due to a sulfate group or a carboxyl group. These polysaccharides are involved in a wide variety of cellular functions and tissues. Figure 13.26 shows the disaccharide structure of the most common ones. Heparin is a natural anticoagulant that helps prevent blood clots. Hyaluronic acid is a component of the vitreous humor of the eye and of the lubricating fluid of joints. The chondroiton sulfates and keratan sulfate are components of connective tissue. Glucosamine sulfate and chondroiton sulfate are sold in large quantities as over-the-counter drugs used to help replace frayed or otherwise damaged cartilage, especially in knees. Many people who are advised that they need knee surgery for damaged ligaments look for improvement first with a two- or three-month regimen of these glycosaminoglycans. Questions exist about the efficacy of this treatment, so it will be interesting to see what future it may have.

14.4 Anaerobic Reactions of Pyruvate

The Conversion of Pyruvate to Lactate in Muscle

The final reaction of anaerobic glycolysis is the reduction of pyruvate to lactate.



(14.11)

This reaction is also exergonic ($\Delta G^{\circ\prime} = -25.1$ kJ mol^{-1} $= -6.0$ kcal mol^{-1}); as before, we need to multiply this value by two to find the energy yield for each molecule of glucose that enters the pathway. Lactate is a dead end in muscle metabolism, but it can be recycled in the liver to form pyruvate and even glucose by a pathway called gluconeogenesis ("new synthesis of glucose"), which we will discuss in Section 15.2.

Lactate dehydrogenase (LDH) is the enzyme that catalyzes this reaction. Like glyceraldehyde-3-phosphate dehydrogenase, LDH is an NADH-linked dehydrogenase and consists of four subunits. There are two kinds of subunits, designated M and H, which vary slightly in amino acid composition. The quaternary structure of the tetramer can vary according to the relative amounts of the two kinds of subunits, yielding five possible isozymes. In human skeletal muscle, the homogeneous tetramer of the M_4 type predominates, and in heart the other homogeneous possibility, the H_4 tetramer, is the predominant form. The heterogeneous forms, M_3H, M_2H_2, and MH_3, occur in blood serum. A very sensitive clinical test for heart disease is based on the existence of the various isozymic forms of this enzyme. The relative amounts of the H_4 and MH_3 isozymes in blood serum increase drastically after myocardial infarction (heart attack) compared with normal serum. The different isozymes have slightly different kinetic properties due to their subunit compositions. The H_4 isozyme (also called LDH 1) has a higher affinity for lactate as a substrate. The M_4 isozyme (LDH 5) is allosterically inhibited by pyruvate. These differences reflect the isozymes' general roles in metabolism. The muscle is a highly anaerobic tissue, where the heart is not.

At this point one might ask why the reduction of pyruvate to lactate (a waste product in aerobic organisms) is the last step in anaerobic glycolysis, a pathway that provides energy for the organism by oxidation of nutrients. There is another point to consider about the reaction, one that involves the relative amounts of NAD^+ and NADH in a cell. The half reaction of reduction can be written

$$\text{Pyruvate} + 2\,H^+ + 2\,e^- \rightarrow \text{Lactate}$$

and the half reaction of oxidation is

$$\text{NADH} + H^+ \rightarrow NAD^+ + 2\,e^- + 2\,H^+$$

The overall reaction is, as we saw earlier,

$$\text{Pyruvate} + \text{NADH} + H^+ \rightarrow \text{Lactate} + NAD^+$$

The NADH produced from NAD^+ by the earlier oxidation of glyceraldehyde 3-phosphate is used up with no net change in the relative amounts of

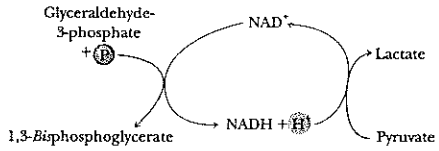


FIGURE 14.11
The recycling of NAD^+ and NADH in anaerobic glycolysis.

NADH and NAD^+ in the cell (Figure 14.11). This regeneration is needed under anaerobic conditions in the cell so that NAD^+ will be present for further glycolysis to take place. Without this regeneration, the oxidation reactions in anaerobic organisms would soon come to a halt because of the lack of NAD^+ to serve as an oxidizing agent in fermentative processes. The production of lactate buys time for the organism experiencing anaerobic metabolism and shifts some of the load away from the muscles and onto the liver where gluconeogenesis can reconvert lactate to pyruvate and glucose (Chapter 15). On the other hand, NADH is a frequently encountered reducing agent in many reactions, and it is lost to the organism in lactate production. Aerobic metabolism makes more efficient use of reducing agents ("reducing power") such as NADH because the conversion of pyruvate to lactate does not occur in aerobic metabolism. The NADH produced in the stages of glycolysis leading to the production of pyruvate is available for use in other reactions in which a reducing agent is needed.

Alcoholic Fermentation

Two other reactions related to the glycolytic pathway lead to the production of ethanol by *alcoholic fermentation*. This process is one of the alternative fates of pyruvate (Section 14.1). In the first of the two reactions that lead to the production of ethanol, pyruvate is decarboxylated (loses carbon dioxide) to produce acetaldehyde. The enzyme that catalyzes this reaction is *pyruvate decarboxylase*.

This enzyme requires Mg^{2+} and a cofactor we have not seen before, **thiamine pyrophosphate** (TPP). (Thiamine itself is vitamin B_1.) In TPP the carbon atom between the nitrogen and the sulfur in the thiazole ring (Figure 14.12) is highly reactive. It forms a carbanion (an ion with a negative charge on a

BIOCHEMICAL CONNECTIONS

Anaerobic Metabolism and Tooth Decay

Dental caries, tooth decay, is one of the most prevalent diseases in the United States and possibly in the world, although modern treatments such as fluoride and flossing have greatly reduced its incidence in young people. Contributing factors in tooth decay are a combination of a diet high in refined sugars, the development of dental plaque, and anaerobic metabolism. The high-sugar diet allows for ready growth of bacteria in the mouth, and sucrose is perhaps the most efficiently used sugar because the bacteria can make their polysaccharide "glue" more efficiently from this nonreducing sugar. The bacteria grow in expanding sticky colonies, forming plaque on the tooth surface. The bacteria growing under the surface of the plaque must utilize anaerobic metabolism because oxygen does not diffuse readily through the waxy surface of dental plaque. The two predominant by-products, lactate and pyruvate, are relatively strong organic acids, and these acid products actually cause destruction of the enamel surface. The bacteria, of course, grow rapidly in the pock holes. If the enamel is eaten all the way through, the bacteria grow even more readily in the softer dentin layer beneath the enamel.

Fluoridation results in a much harder enamel surface, and the fluoride may actually inhibit metabolism. Daily flossing disrupts the plaque and the anaerobic conditions never get started.

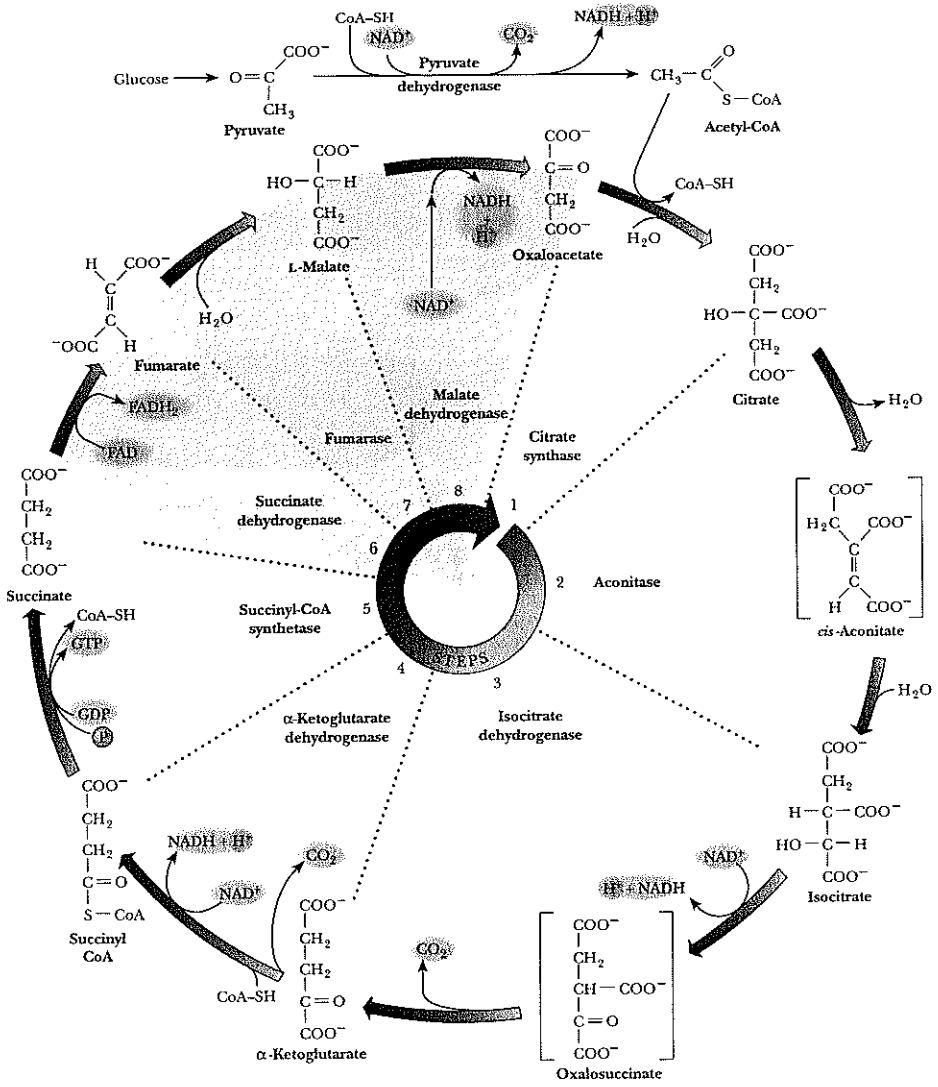


FIGURE 16.3
An overview of the citric acid cycle. Note the names of the enzymes. The loss of CO_2 is indicated, as is the phosphorylation of GDP to GTP. The production of NADH and $FADH_2$ is also indicated.

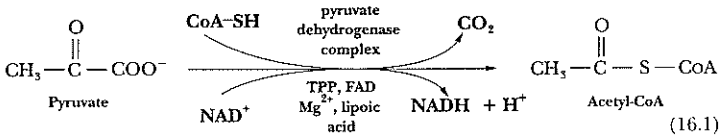
16.3 Conversion of Pyruvate to Acetyl-CoA

Pyruvate can come from several sources including glycolysis as we have seen. It moves from the cytosol into the mitochondrion via a specific transporter. There, an enzyme system called the **pyruvate dehydrogenase complex** is responsible for the conversion of pyruvate to carbon dioxide and the acetyl portion of acetyl-CoA. There is an —SH group at one end of the CoA molecule, which is the point at which the acetyl group is attached. As a result, CoA is frequently shown in equations as CoA-SH. Because CoA is a thiol (the sulfur [thio] analog of an alcohol), acetyl-CoA is a **thioester**, with a sulfur atom replacing an oxygen of the usual carboxylic ester. This difference is important, since thioesters are high-energy compounds (Chapter 12). In other words, the hydrolysis of thioesters releases enough energy to drive other reactions. An oxidation reaction precedes the transfer of the acetyl group to the CoA. The whole process involves several enzymes, all of which are part of the pyruvate dehydrogenase complex. The overall reaction

$$\text{Pyruvate} + \text{CoA-SH} + \text{NAD}^+ \rightarrow \text{Acetyl-CoA} + \text{CO}_2 + \text{H}^+ + \text{NADH}$$

is exergonic ($\Delta G^{\circ\prime} = -33.4$ kJ mol^{-1} $= -8.0$ kcal mol^{-1}), and NADH can then be used to generate ATP via the electron transport chain (Chapter 17).

The overall reaction of the pyruvate dehydrogenase complex



(16.1)

Five enzymes make up the pyruvate dehydrogenase complex in mammals. They are *pyruvate dehydrogenase (PDH), dihydrolipoyl transacetylase, dihydrolipoyl dehydrogenase, pyruvate dehydrogenase kinase,* and *pyruvate dehydrogenase phosphatase.* The first three are involved in the conversion of pyruvate to acetyl-CoA. The kinase and the phosphatase are enzymes used in the control of PDH (Section 16.5) and are present on a single polypeptide. The reaction takes place in five steps. Two enzymes catalyze reactions of *lipoic acid,* a compound that has a disulfide group in its oxidized form and two sulfhydryl groups in its reduced form. Lipoic acid differs in one respect from other coenzymes. It is a vitamin, rather than a metabolite of a vitamin, as is the case with many other coenzymes (Table 5.3). (The classification of lipoic acid as a vitamin is open to question. There is no evidence of a requirement for it in the human diet, but it is required for the growth of some bacteria and protists.)

Lipoic acid can act as an oxidizing agent; the reaction involves hydrogen transfer, which frequently accompanies biological oxidation–reduction reactions (Section 12.9). Another reaction of lipoic acid is the formation of a thioester linkage with the acetyl group before it is transferred to the acetyl-CoA. Lipoic acid can act simply as an oxidizing agent, or it can simultaneously take part in two reactions—a redox reaction and the shift of an acetyl group by transesterification.

The first step in the reaction sequence that converts pyruvate to carbon dioxide and acetyl-CoA is catalyzed by pyruvate dehydrogenase, as shown in Figure 16.4. This enzyme requires thiamine pyrophosphate (TPP; a metabolite of

CHAPTER 17

Electron Transport and Oxidative Phosphorylation

Mitochondria, shown here, are the sites of the citric acid cycle, electron transport, and oxidative phosphorylation. *(Dr. Dennis Kunkel, Phototake.)*

OUTLINE

17.1 The Role of Electron Transport in Metabolism

17.2 Reduction Potentials

17.3 Electron Transport from NADH to O_2 Requires Four Membrane-Bound Complexes

17.4 The Coupling of Oxidation to Phosphorylation

17.5 The Mechanism of Coupling in Oxidative Phosphorylation

17.6 Respiratory Inhibitors Block the Flow of Electrons in Electron Transport

17.7 Shuttle Mechanisms Mediate Transport of Metabolites Between Mitochondria and the Cytosol

17.8 The ATP Yield from Complete Oxidation of Glucose

Energy derived from the oxidation of metabolic fuels is ultimately converted to ATP, the energy currency of the cell. In eukaryotic cells, under aerobic conditions, ATP is generated by the power of electron transport along the inner membrane of the mitochondrion coupled with proton transport across the inner membrane. The electron transport chain is actually four closely related enzyme complexes embedded in the inner mitochondrial membrane. In a series of oxidation-reduction transfers, they conduct electrons along the membrane from one complex to another until the electrons reach their final destination where they combine with molecular oxygen to reduce O_2 to 2 H_2O. The energy of electron transport can then be used by three of these same enzyme complexes to pump protons across the inner membrane out into the intermembrane space. The reverse flow of protons back through the membrane into the inner matrix drives the production of ATP. An ATP synthase complex embedded in the inner membrane binds ADP and phosphate to catalyze the formation of ATP. The flow of protons through the ATP synthase from the intermembrane space to the inner matrix releases the new ATP that has been synthesized. This process is very similar to the production of ATP by photosynthesis (Chapter 19) in the thylakoid membrane of the chloroplast in green plants.

17.1 The Role of Electron Transport in Metabolism

Aerobic metabolism is a highly efficient way for an organism to extract energy from nutrients. In eukaryotic cells, the aerobic processes (including conversion of pyruvate to acetyl-CoA, the citric acid cycle, and electron transport) all occur in the mitochondria, while the anaerobic process, glycolysis, takes place outside the mitochondria in the cytosol. We have not yet seen any reactions in which oxygen plays a part, but in this chapter we shall discuss the role of oxygen in metabolism as the final acceptor of electrons in the **electron transport chain.** The reactions of the electron transport chain take place in the inner mitochondrial membrane.

The energy released by the oxidation of nutrients is used by organisms in the form of the chemical energy of ATP. Production of ATP in the mitochondria is the result of **oxidative phosphorylation,** in which ADP is phosphorylated to give ATP. The production of ATP by oxidative phosphorylation (an endergonic process) is separate from electron transport to oxygen (an exergonic process), but the reactions of the electron transport chain are strongly linked to one another and are tightly coupled to the synthesis of ATP by phosphorylation of ADP. The operation of the electron transport chain leads to pumping of protons (hydrogen ions) across the inner mitochondrial membrane, creating a

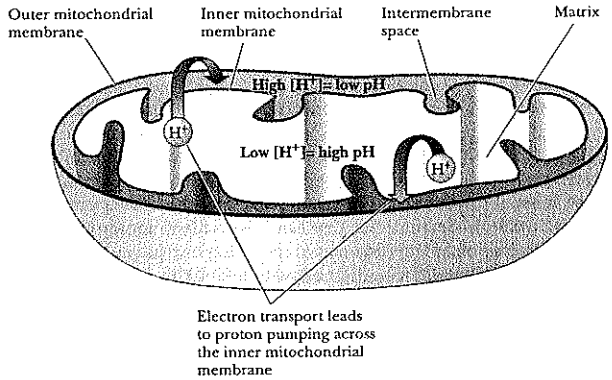
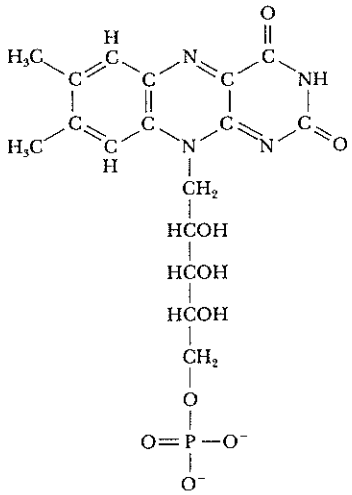


FIGURE 17.1
A proton gradient is established across the inner mitochondrial membrane as a result of electron transport. Transfer of electrons through the electron transport chain leads to the pumping of protons from the matrix to the intermembrane space. The proton gradient (also called the pH gradient), together with the membrane potential (a voltage across the membrane), provides the basis of the coupling mechanism that drives ATP synthesis.

17.3 Electron Transport from NADH to O_2 Requires Four Membrane-Bound Complexes

Intact mitochondria isolated from cells can carry out all the reactions of the electron transport chain; the electron transport apparatus can also be resolved into its component parts by a process called fractionation. Four separate **respiratory complexes** can be isolated from the inner mitochondrial membrane. These complexes are multienzyme systems. In the last chapter, we encountered other examples of such multienzyme complexes, such as the pyruvate dehydrogenase complex and the α-ketoglutarate dehydrogenase complex. Each of the respiratory complexes can carry out the reactions of a portion of the electron transport chain.

Complex I The first complex, **NADH-CoQ oxidoreductase,** catalyzes the first steps of electron transport, namely the transfer of electrons from NADH to **coenzyme Q.** This complex is an integral part of the inner mitochondrial membrane and includes, among other subunits, several proteins that contain an iron–sulfur cluster and the flavoprotein that oxidizes NADH. (The total number of subunits is over 20. This complex is a subject of active research, which has proven to be a challenging task because of its complexity. It is particularly difficult to generalize about the nature of the iron–sulfur clusters because they vary from species to species.) The flavoprotein has a flavin coenzyme, called flavin mononucleotide or FMN, which differs from FAD in not having an adenine nucleotide.

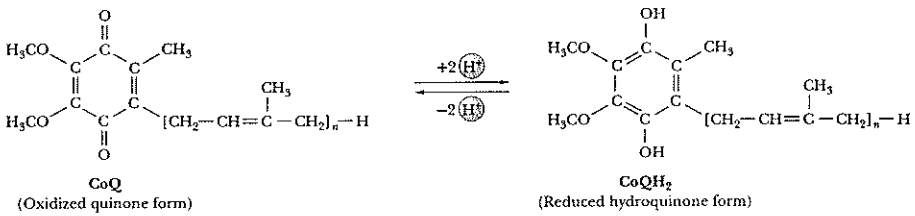


The structure of FMN
(Flavin mononucleotide)

The reaction occurs in several steps with successive oxidation and reduction of the flavoprotein and the iron–sulfur moiety. The first step is the transfer of electrons from NADH to the flavin portion of the flavoprotein:

$$NADH + H^+ + E{-}FMN \rightarrow NAD^+ + E{-}FMNH_2$$

where the notation E—FMN indicates that the flavin is covalently bonded to the enzyme. In the second step, the reduced flavoprotein is reoxidized, and the



CoQ
(Oxidized quinone form)

$CoQH_2$
(Reduced hydroquinone form)

FIGURE 17.4
The oxidized and reduced forms of coenzyme Q. Coenzyme Q is also called ubiquinone.

oxidized form of the iron–sulfur protein is reduced. The reduced iron–sulfur protein then donates its electrons to coenzyme Q (represented simply as CoQ), which becomes reduced to $CoQH_2$ (Figure 17.4). Coenzyme Q is also called ubiquinone. The equations for the second and third steps are shown here:

$$E{-}FMNH_2 + 2\ Fe\text{-}S_{oxidized} \rightarrow E{-}FMN + 2\ Fe{-}S_{reduced} + 2H^+$$

$$2\ Fe{-}S_{reduced} + CoQ + 2H^+ \rightarrow 2\ Fe{-}S_{oxidized} + CoQH_2$$

The notation Fe — S indicates the iron–sulfur clusters. The overall equation for the reaction is

$$NADH + H^+ + CoQ \rightarrow NAD^+ + CoQH_2$$

This reaction is one of the three responsible for the proton pumping (Figure 17.5) that creates the pH (proton) gradient. The standard free energy change ($\Delta G^{\circ\prime} = -81$ kJ mol^{-1} $= -19.4$ kcal mol^{-1}) indicates that the reaction is strongly exergonic, releasing enough energy to drive the phosphorylation of

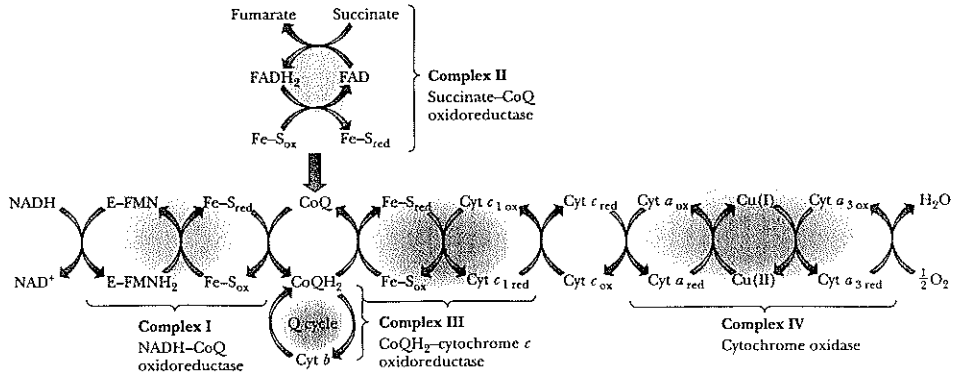
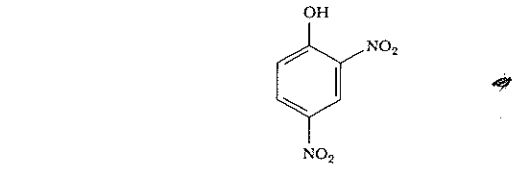
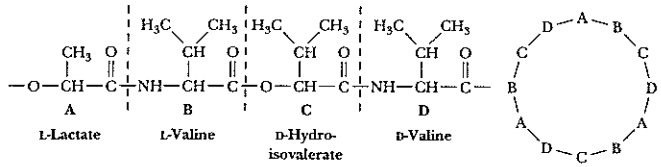


FIGURE 17.5
The electron transport chain, showing the respiratory complexes. In the reduced cytochromes, the iron is in the Fe(II) oxidation state; in the oxidized cytochromes, the oxygen is in the Fe(III) oxidation state.

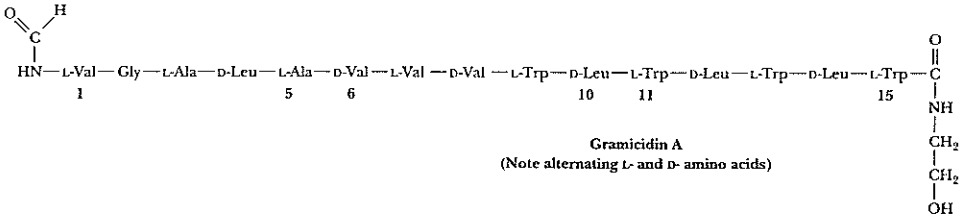


2,4-Dinitrophenol (DNP)



Repeating unit of valinomycin

(Valinomycin is a cyclic trimer of four repeating units.)



Gramicidin A

(Note alternating L- and D- amino acids)

FIGURE 17.12

Some uncouplers of oxidative phosphorylation: 2,4-dinitrophenol, valinomycin, and gramicidin A.

complex is called **ATP synthase.** It is also known as mitochondrial ATPase because the reverse reaction of ATP hydrolysis, as well as phosphorylation, can be catalyzed by the enzyme. The hydrolytic reaction was discovered before the reaction of the synthesis of ATP, hence the name. The 1997 Nobel Prize in chemistry was shared by an American scientist, Paul Boyer of UCLA, and a British scientist, John Walker of the Medical Research Council in Cambridge, England, for their elucidation of the structure and mechanism of this enzyme. (The other half of this prize went to a Danish scientist, Jens Skou, for his work on the sodium–potassium pump [Section 6.6], which also functions as an ATPase.)

Compounds known as **uncouplers** inhibit the phosphorylation of ADP without affecting electron transport. A well-known example of an uncoupler is *2,4-dinitrophenol.* Various antibiotics such as *valinomycin* and *gramicidin A* are also uncouplers (Figure 17.12). When mitochondrial oxidation processes are operating normally, electron transport from NADH or $FADH_2$ to oxygen results in the production of ATP. When an uncoupler is present, oxygen is still reduced to H_2O, but ATP is not produced. If the uncoupler is removed, ATP synthesis linked to electron transport resumes.

A term called the **P/O ratio** is used to indicate the coupling of ATP production to electron transport. The P/O ratio gives the number of moles of P_i consumed in the reaction ADP + $P_i \rightarrow$ ATP for each mole of oxygen atoms consumed in the reaction $\frac{1}{2}\,O_2 + 2H^+ + 2e^- \rightarrow H_2O$. As we have already mentioned, 2.5 moles of ATP are produced when 1 mole of NADH is oxidized to

NAD^+. Recall that oxygen is the ultimate acceptor of the electrons from NADH and that $\frac{1}{2}$ mole of O_2 molecules (one mole of oxygen atoms) is reduced for each mole of NADH oxidized. The experimentally determined P/O ratio is 2.5 when NADH is the substrate oxidized. The P/O ratio is 1.5 when $FADH_2$ is the substrate oxidized (also an experimentally determined value). Until recently, biochemists had used integral values of 3 and 2 for the P/O ratios for reoxidation of NADH and $FADH_2$, respectively. The nonintegral consensus values given here clearly underscore the complexity of electron transport, oxidative phosphorylation, and the manner in which they are coupled.

Essential Information

The coupling of electron transport to oxidative phosphorylation requires a multisubunit membrane-bound enzyme, ATP synthase. This enzyme has a channel for protons to flow from the intermembrane space into the mitochondrial matrix. The proton flow is coupled to ATP production in a process that appears to involve a conformational change of the enzyme.

17.5 The Mechanism of Coupling in Oxidative Phosphorylation

Several mechanisms have been proposed to account for the coupling of electron transport and ATP production. The mechanism that served as the point of departure in all discussions is chemiosmotic coupling, which was later modified to include a consideration of conformational coupling.

Chemiosmotic Coupling

As originally proposed, the **chemiosmotic coupling** mechanism was based entirely on the difference in proton concentration between the intermembrane space and the matrix of an actively respiring mitochondrion. In other words, the proton (hydrogen ion, H^+) gradient across the inner mitochondrial membrane is the crux of the matter. The proton gradient exists because the various proteins that serve as electron carriers in the respiratory chain are not symmetrically oriented with respect to the two sides of the inner mitochondrial membrane, nor do they react in the same way with respect to the matrix and the intermembrane space (Figure 17.13). Note that Figure 17.13 repeats the information found in Figure 17.7 with the addition of the flow of protons. The number of protons transported by respiratory complexes is uncertain and even a matter of some controversy. Figure 17.13 shows a consensus estimate for each

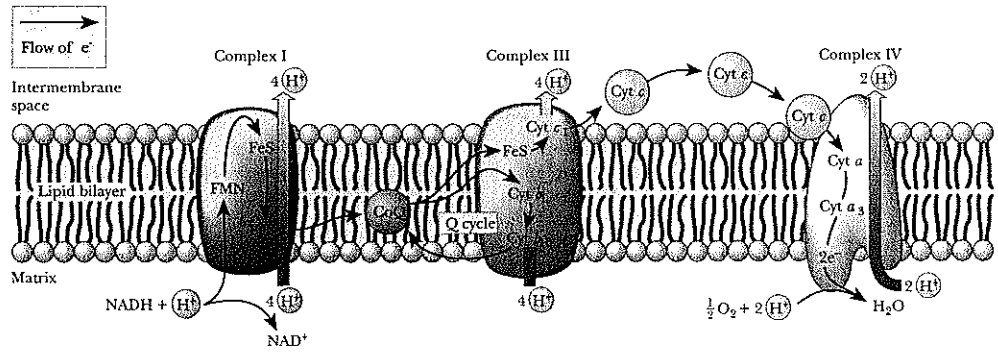


FIGURE 17.13

The creation of a proton gradient in chemiosmotic coupling. The overall effect of the electron transport reaction series is to move protons (H^+) out of the matrix into the intermembrane space, creating a difference in pH across the membrane.

30.5 kJ mol^{-} = 7.3 kcal mol^{-1}, and the reaction catalyzed by the third complex supplies enough energy to drive the production of ATP.

Complex IV The fourth complex, **cytochrome *c* oxidase,** catalyzes the final steps of electron transport, the transfer of electrons from cytochrome *c* to oxygen. The overall reaction is

$$2\ \text{Cyt}\ c[\text{Fe(II)}] + 2\ \text{H}^+ + \tfrac{1}{2}\ \text{O}_2 \longrightarrow 2\ \text{Cyt}\ c[\text{Fe(III)}] + \text{H}_2\text{O}$$

Proton pumping also takes place as a result of this reaction. Like the other respiratory complexes, cytochrome oxidase is an integral part of the inner mitochondrial membrane and contains cytochromes *a* and a_3, as well as two Cu^{2+} ions that are involved in the electron transport process. Taken as a whole, this complex contains about ten subunits. In the flow of electrons, the copper ions are intermediate electron acceptors that lie between the two *a*-type cytochromes in the sequence

$$\text{Cyt}\ c \longrightarrow \text{Cyt}\ a \longrightarrow \text{Cu}^{2+} \longrightarrow \text{Cyt}\ a_3 \longrightarrow \text{O}_2$$

To show the reactions of the cytochromes more explicitly,

$$\text{Cyt}\ c\ [\text{reduced, Fe(II)}] + \text{Cyt}\ aa_3\ [\text{oxidized, Fe(III)}] \longrightarrow \text{Cyt}\ aa_3\ [\text{reduced, Fe(II)}] + \text{Cyt}\ c\ [\text{oxidized, Fe(III)}]$$

Cytochromes *a* and a_3 taken together form the complex known as cytochrome oxidase. The reduced cytochrome oxidase is then oxidized by oxygen, which is itself reduced to water. The half reaction for the reduction of oxygen (oxygen acts as an oxidizing agent) is

$$\tfrac{1}{2}\ \text{O}_2 + 2\text{H}^+ + 2e^- \longrightarrow \text{H}_2\text{O}$$

The overall reaction is

$$2\ \text{Cyt}\ aa_3\ [\text{reduced, Fe(II)}] + \tfrac{1}{2}\ \text{O}_2 + 2\text{H}^+ \longrightarrow 2\ \text{Cyt}\ aa_3\ [\text{oxidized, Fe(III)}] + \text{H}_2\text{O}$$

Note that in this final reaction we have finally seen the link to molecular oxygen in aerobic metabolism.

Essential Information

The electron transport chain consists of four multisubunit membrane-bound complexes and two mobile electron carriers (coenzyme Q and cytochrome *c*). The reactions that take place in three of these complexes generate enough energy to drive the phosphorylation of ADP to ATP.

The standard free energy change ($\Delta G^{\circ\prime}$) is −110 kJ = −26.3 kcal for each mole of NADH that enters the electron transport chain (see Figure 17.6). We have now seen the three places in the respiratory chain where electron transport is coupled to ATP production by proton pumping. These three places are the NADH dehydrogenase reaction, the oxidation of cytochrome *b*, and the reaction of cytochrome oxidase with oxygen, although the mechanism for proton transfer in cytochrome oxidase remains a mystery. Table 17.2 summarizes the energetics of electron transport reactions.

Cytochromes and Other Iron-Containing Proteins of Electron Transport

In contrast to the electron carriers in the early stages of electron transport, such as NADH, FMN, and CoQ, the cytochromes are macromolecules. These proteins are found in all types of organisms and are typically located in membranes. In eukaryotes, the usual site is the inner mitochondrial membrane, but cytochromes can also occur in the endoplasmic reticulum.

All cytochromes contain the heme group, a part of the structure of hemoglobin and myoglobin (Section 4.4). In the cytochromes, the iron of the heme

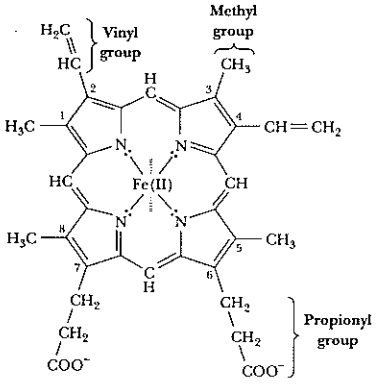
TABLE 17.2 The Energetics of Electron Transport Reactions

Reaction	$\Delta G^{\circ\prime}$	
	kJ (mol NADH)$^{-1}$	*kcal (mol/NADH)$^{-1}$*
$\text{NADH} + \text{H}^+ + \text{E—FMN} \longrightarrow \text{NAD}^+ + \text{E—FMNH}_2$	−38.6	−9.2
$\text{E—FMNH}_2 + \text{CoQ} \longrightarrow \text{E—FMN} + \text{CoQH}_2$	−42.5	−10.2
CoQH_2 + 2 Cyt *b*[Fe(III)] → CoQ + 2H^+ + 2 Cyt *b*[Fe(II)]	+11.6	+2.8
2 Cyt *b*[Fe(II)] + 2 Cyt c_1[Fe(III)] → 2 Cyt c_1[Fe(II)] + 2 Cyt *b*[Fe(III)]	−34.7	−8.3
2 Cyt c_1[Fe(II)] + 2 Cyt *c*[Fe(III)] → 2 Cyt *c*[Fe(II)] + 2 Cyt c_1[Fe(III)]	−5.8	−1.4
2 Cyt *c*[Fe(II)] + 2 Cyt (aa_3) [Fe(III)] → 2 Cyt (aa_3) [Fe(II)] + 2 Cyt *c*[Fe(III)]	−7.7	−1.8
2 Cyt (aa_3)[Fe(II)] + $\frac{1}{2}\ \text{O}_2 + 2\ \text{H}^+$ → 2 Cyt (aa_3)[Fe(III)] + H_2O	−102.3	−24.5
Overall reaction: $\text{NADH} + \text{H}^+ + \frac{1}{2}\ \text{O}_2 \longrightarrow \text{NAD}^+ + \text{H}_2\text{O}$	−220	−52.6

group does not bind to oxygen; instead, the iron is involved in the series of redox reactions, which we have already seen. There are differences in the side chains of the heme group of the cytochromes involved in the various stages of electron transport (Figure 17.9). These structural differences, combined with the variations in the polypeptide chain and in the way the polypeptide chain is attached to the heme, account for the differences in properties among the cytochromes in the electron transport chain.

Nonheme iron proteins do not contain a heme group, as their name indicates. Many of the most important proteins in this category contain sulfur, as is

(a)



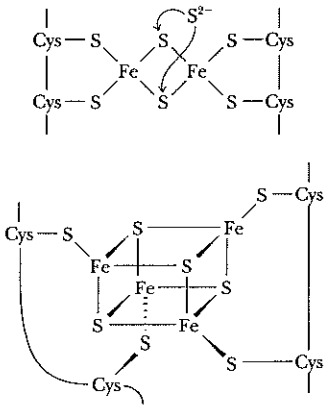
(b)

POSITION	*a* CYTOCHROMES	*c* CYTOCHROMES
1	Same	Same
2 (in *a*)	—CH—CH_2—$(CH_2$—CH=C—$CH_2)_3$H (OH; CH_3)	
2 (in *c*)		—CHCH$_3$ (S— protein; Covalent attachment)
3	Same	Same
4	Same	—CHCH$_3$ (S— protein)
5	Same	Same
6	Same	Same
7	Same	Same
8	—C=O (Formyl group) H	Same

FIGURE 17.9
The heme group of cytochromes. (a) Structures of the heme of all *b* cytochromes and of hemoglobin and myoglobin. The wedge bonds show the fifth and sixth coordination sites of the iron atom. (b) A comparison of the side chains of *a* and *c* cytochromes to those of *b* cytochromes.

the case with the iron–sulfur proteins that are components of the respiratory complexes. The iron is usually bound to cysteine or to S^{2-}. There are still many questions about the location and mode of action of iron–sulfur proteins in mitochondria.

Iron–sulfur bonding in nonheme iron proteins



17.4 The Coupling of Oxidation to Phosphorylation

Some of the energy released by the oxidation reactions in the electron transport chain is used to drive the phosphorylation of ADP. The phosphorylation of each mole of ADP requires 30.5 kJ = 7.3 kcal, and we have seen how each of the reactions catalyzed by three of the four respiratory complexes provides more than enough energy to drive this reaction, although it is by no means a direct usage of this energy. It is a common theme in metabolism that energy to be used by cells is converted to the chemical energy of ATP as needed. The energy-releasing oxidation reactions give rise to proton pumping and thus to the pH gradient across the inner mitochondrial membrane. In addition to the pH gradient, there is a voltage difference across the membrane generated by the concentration differences of ions inside and out. The energy of the electrochemical potential (voltage drop) across the membrane is converted to the chemical energy of ATP by the coupling process.

A coupling factor is needed to link oxidation and phosphorylation. A complex protein oligomer, separate from the electron transport complexes, serves this function; the complete protein spans the inner mitochondrial membrane and projects into the matrix as well. The portion of the protein that spans the membrane is called F_0. It consists of three different kinds of polypeptide chains (a, b, and c), and research is in progress to characterize it further. The portion that projects into the matrix is called F_1; it consists of five different kinds of polypeptide chains in the ratio $\alpha_3\beta_3\gamma\delta\epsilon$. Electron micrographs of mitochondria show the projections into the matrix from the inner mitochondrial membrane (Figure 17.10). The schematic organization of the protein can be seen in Figure 17.11. The F_1 sphere is the site of ATP synthesis. The whole protein

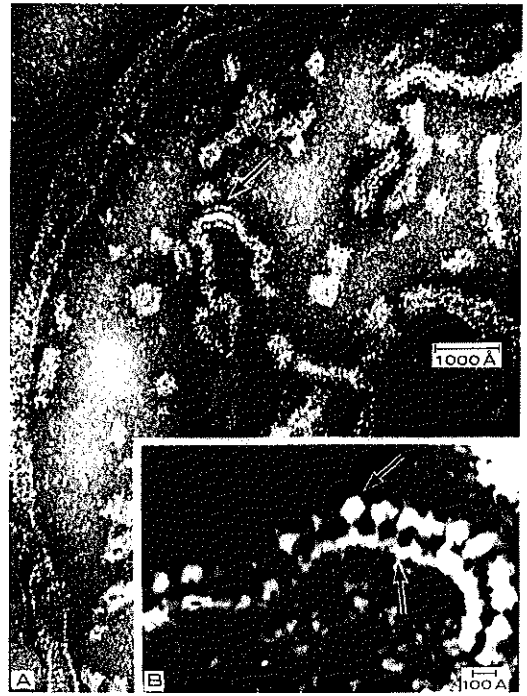


FIGURE 17.10
Electron micrograph of projections into the matrix space of a mitochondrion. Note the difference in scale between Part a and Part b. The top arrows indicate the matrix side and the F_1 subunit. The bottom arrow in part b indicates the intermembrane space. *(Photo Researchers, Inc.)*

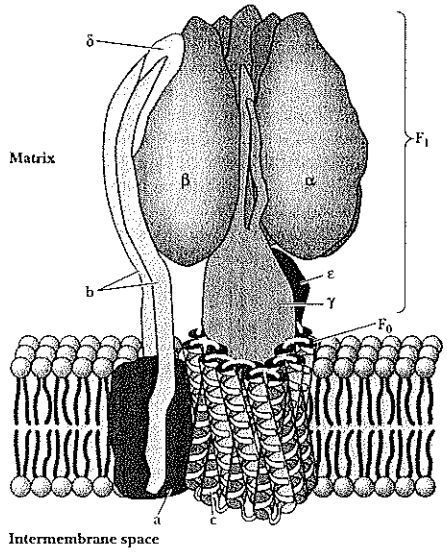


FIGURE 17.11
A model of the F_1 and F_0 components of the ATP synthase, a rotating molecular motor. The a, b, α, β, δ subunits constitute the stator of the motor, and the c, γ, and ε subunits form the rotor. Flow of protons through the structure turns the rotor.

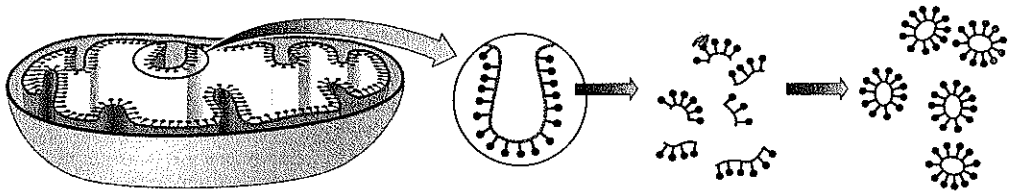


FIGURE 17.14
Closed vesicles prepared from mitochondria can pump protons and produce ATP.

complex. In the process of electron transport, the proteins of the respiratory complexes take up protons from the matrix to transfer them in redox reactions; these electron carriers subsequently release protons into the intermembrane space when they are reoxidized, creating the proton gradient. As a result, there is a higher concentration of protons in the intermembrane space than in the matrix, which is precisely what we mean by a proton gradient. It is known that the intermembrane space has a lower pH than the matrix, which is another way of saying that there is a higher concentration of protons in the intermembrane space than in the matrix. The proton gradient in turn can drive the production of ATP that occurs when the protons flow back into the matrix.

Since chemiosmotic coupling was first suggested by the British scientist Peter Mitchell in 1961, a considerable body of experimental evidence has accumulated to support it.

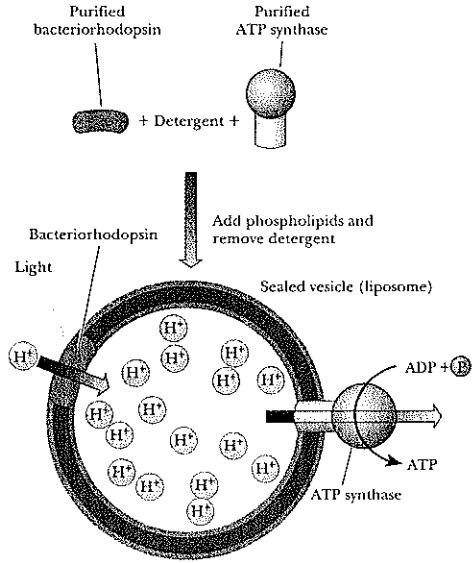


FIGURE 17.15
ATP can be produced by closed vesicles with bacteriorhodopsin as a proton pump.

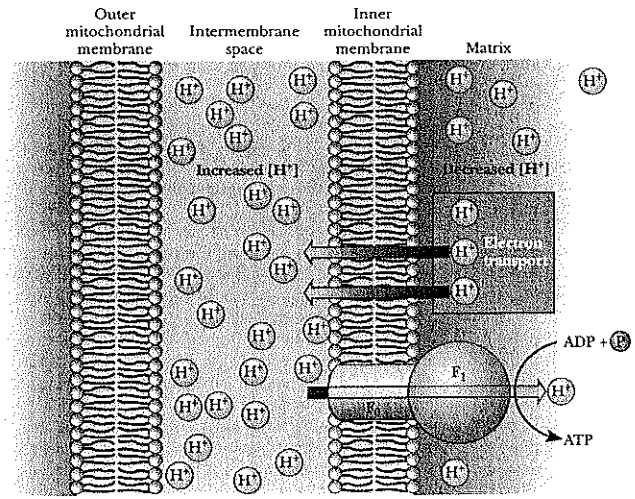


FIGURE 17.16
Formation of ATP accompanies the flow of protons back into the mitochondrial matrix.

1. A system with definite inside and outside compartments (closed vesicles) is essential for oxidative phosphorylation. The process does not occur in soluble preparations or in membrane fragments without compartmentalization.
2. Submitochondrial preparations that contain closed vesicles can be prepared; such vesicles can carry out oxidative phosphorylation, and the asymmetrical orientation of the respiratory complexes with respect to the membrane can be demonstrated (Figure 17.14).
3. A model system for oxidative phosphorylation can be constructed with proton pumping in the absence of electron transport. The model system consists of reconstituted membrane vesicles, mitochondrial ATP synthase, and a proton pump. The pump is bacteriorhodopsin, a protein found in the membrane of halobacteria. The proton pumping takes place when the protein is illuminated (Figure 17.15).
4. The existence of the pH gradient has been demonstrated and confirmed experimentally.

The way in which the proton gradient leads to the production of ATP depends on ion channels through the inner mitochondrial membrane; these channels are a feature of the structure of ATP synthase. Protons flow back into the matrix through ion channels in the ATP synthase; the F_0 part of the protein is the proton channel. The flow of protons is accompanied by formation of ATP, which takes place in the F_1 unit (Figure 17.16). The unique feature of chemiosmotic coupling is the direct linkage of the proton gradient to the phosphorylation reaction. The details of the way in which phosphorylation takes place as a result of the linkage to the proton gradient are not explicitly specified in this mechanism.

A reasonable mode of action for uncouplers can be proposed in light of the existence of a proton gradient. Dinitrophenol is an acid; its conjugate base, dinitrophenolate anion, is the actual uncoupler because it can react with protons in the intermembrane space, reducing the difference in proton concentration between the two sides of the inner mitochondrial membrane. The antibiotic uncouplers such as gramicidin A and valinomycin are **ionophores**, creating a channel through which ions such as H^+, K^+, and Na^+ can pass

CHAPTER 19

Photosynthesis

Lush rain forest vegetation. Photosynthesis linked to oxygen plays an essential role in all life, plant and animal. *(Paul Harris/Tony Stone Images.)*

OUTLINE

19.1 Chloroplasts and Chlorophylls

19.2 The Light Reactions of Photosynthesis: Photosystems I and II

19.3 A Proton Gradient Drives the Production of ATP in Photosynthesis

19.4 A Comparison of Photosynthesis with and without Oxygen: Evolutionary Implications

19.5 The Dark Reactions of Photosynthesis: Path of Carbon

19.6 An Alternative Pathway for Carbon Dioxide Fixation

The drama of photosynthesis, converting sunlight to energy-rich carbohydrates, is played out in the chloroplast "theater" of the green plant. In each chloroplast, there are stacks of thylakoid disks. The thylakoid membrane inside each disk is the lighted stage where the drama of Act I is performed. Here the energy of light is captured by electrons of chlorophyll molecules. The excited electrons are passed along a series of acceptors in an electron transport chain. In the process, a molecule of water is split, and oxygen is released into the atmosphere. At the same time, protons pumped out of the thylakoid membrane drive the production of ATP. Excited electrons reduce $NADP^+$ to NADPH, and the stored energy is used in Act II for the biosynthesis of glucose, which takes place in the dark of the stroma outside the thylakoid membrane. Carbon dioxide from the atmosphere is combined with a five-carbon sugar to produce, through an intermediate, two 3-carbon sugars and eventually the six-carbon molecule of glucose. The energy to drive this biosynthesis comes from ATP and the reducing power of NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate. Plants, at the bottom of the food chain, toil in the sun to store energy and generate oxygen for the benefit of all animals on earth.

19.1 Chloroplasts and Chlorophylls

It is well known that photosynthetic organisms, such as green plants, convert carbon dioxide (CO_2) and water to carbohydrates such as glucose (written here as $C_6H_{12}O_6$) and molecular oxygen (O_2).

$$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$$

The equation actually represents two processes. One process, the oxidation of water to produce oxygen (the light reactions), requires light energy from the sun. The light reactions of photosynthesis in prokaryotes and eukaryotes depend on solar energy, which is absorbed by chlorophyll to supply the energy needed in the light reactions. The light reactions also generate NADPH, which is the reducing agent needed in the dark reactions. The other process, the fixation of CO_2 to give sugars (the dark reactions), does not use solar energy directly but rather uses it indirectly in the form of the ATP and NADPH produced in the course of the light reactions.

In prokaryotes such as cyanobacteria, photosynthesis takes place in granules bound to the plasma membrane. The site of photosynthesis in eukaryotes such as green plants and green algae is the **chloroplast** (Figure 19.1), a membrane-bounded organelle that we discussed in Section 1.5.

Like the mitochondrion, the chloroplast has inner and outer membranes and an intermembrane space. In addition, within the chloroplast are bodies called **grana,** which consist of stacks of flattened membranes called **thylakoid disks.** The grana are connected by membranes called intergranal lamellae. The thylakoid disks are formed by the folding of a third membrane within the chloroplast. The folding of the thylakoid membrane creates two spaces in the chloroplast in addition to the intermembrane space. The **stroma** lies within the inner membrane and outside the thylakoid membrane. In addition to the stroma, there is a **thylakoid space** within the thylakoid disks themselves. The trapping of light and the production of oxygen take place in the thylakoid disks. The dark reactions, in which CO_2 is fixed to carbohydrates, take place in the stroma.

It is well established that the primary event in photosynthesis is the absorption of light by **chlorophyll.** The high energy states (excited states) of chlorophyll are useful in photosynthesis because the light energy can be passed along and converted to chemical energy in the light reaction. There are two

Essential Information

In eukaryotes, photosynthesis takes place in chloroplasts. The light reactions take place in the thylakoid membrane, a third membrane in chloroplasts in addition to the inner and outer membrane. The dark reactions take place in the stroma, the space between the thylakoid membrane and the inner membrane of the chloroplast.

21

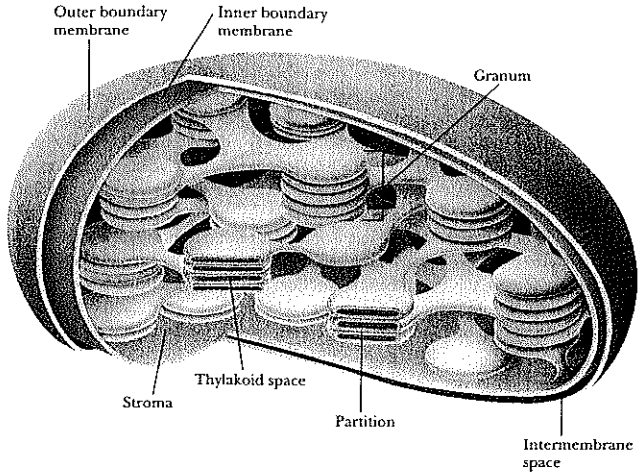


FIGURE 19.1
Membrane structures in chloroplasts.

principal types of chlorophyll, *chlorophyll a* and *chlorophyll b*. Eukaryotes such as green plants and green algae contain both chlorophyll *a* and chlorophyll *b*. Prokaryotes such as cyanobacteria (formerly called blue-green algae) contain only chlorophyll *a*. Photosynthetic bacteria other than cyanobacteria have bacteriochlorophylls, with *bacteriochlorophyll a* being the most common. Organisms such as green and purple sulfur bacteria, which contain bacteriochlorophylls, do not use water as the ultimate source of electrons for the redox reactions of photosynthesis, nor do they produce oxygen. Instead, they use other electron sources such as H_2S, which produces elemental sulfur instead of oxygen. Organisms that contain bacteriochlorophyll are anaerobic and have only one photosystem, whereas green plants have two different photosystems as we shall see.

The structure of chlorophyll is similar to that of the heme group of myoglobin, hemoglobin, and the cytochromes in that it is based on the tetrapyrrole

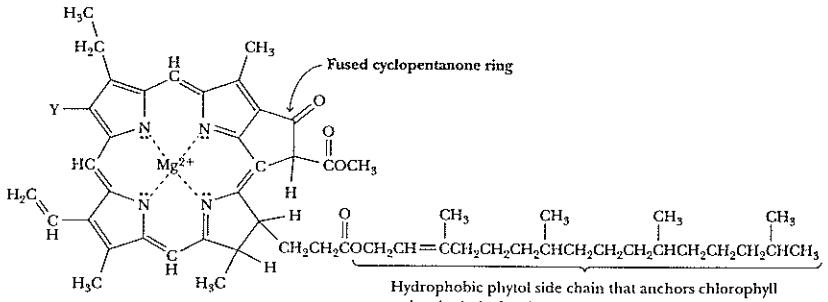


FIGURE 19.2
Molecular structures of chlorophyll *a*, chlorophyll *b*, and bacteriochlorophyll *a*.

ring of porphyrins (Figure 19.2). (See Section 4.4.) The metal ion bound to the tetrapyrrole ring is magnesium, Mg(II), rather than iron, which occurs in heme. Another difference between chlorophyll and heme is the presence of a cyclopentanone ring fused to the tetrapyrrole ring. There is a long hydrophobic side chain, the phytol group, which contains four isoprenoid units (five-carbon units that are basic building blocks in many lipids; Section 18.8) and which binds to the thylakoid membrane by hydrophobic interactions. The phytol group is covalently bound to the rest of the chlorophyll molecule by an ester linkage between the alcohol group of the phytol and a propionic acid side chain on the porphyrin ring. The difference between chlorophyll *a* and chlorophyll *b* lies in the substitution of an aldehyde group for a methyl group on the porphyrin ring. The difference between bacteriochlorophyll *a* and chlorophyll *a* is that a double bond in the porphyrin ring of chlorophyll *a* is saturated in bacteriochlorophyll *a*. The lack of a conjugated system (alternating double and single bonds) in the porphyrin ring of bacteriochlorophylls causes a significant difference in the absorption of light by bacteriochlorophyll *a* compared with chlorophyll *a* and *b*.

The absorption spectra of chlorophyll *a* and chlorophyll *b* differ slightly (Figure 19.3). Both absorb light in the red and blue portions of the visible spectrum (600 to 700 nm and 400 to 500 nm, respectively), and the presence of both types of chlorophyll guarantees that more wavelengths of the visible spectrum are absorbed than would be the case with either one individually. Recall that chlorophyll *a* is found in all photosynthetic organisms that produce oxygen. Chlorophyll *b* is found in eukaryotes such as green plants and green algae, but it occurs in smaller amounts than chlorophyll *a*. The presence of chlorophyll *b*, however, increases the portion of the visible spectrum that is absorbed and thus enhances the efficiency of photosynthesis in green plants compared with cyanobacteria. In addition to chlorophyll, various **accessory pigments**

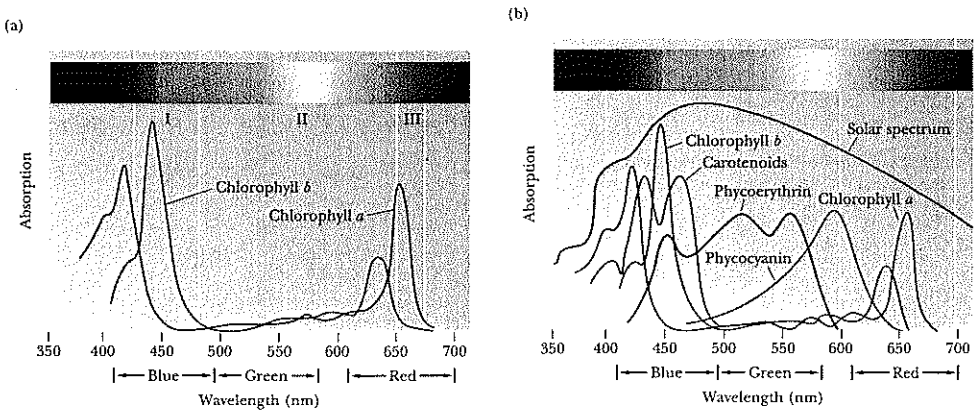


FIGURE 19.3
(a) The absorption of visible light by chlorophylls *a* and *b*. The areas marked I, II, and III are regions of the spectrum that give rise to chloroplast activity. There is greater activity in regions I and III, which are close to major absorption peaks. There are high levels of O_2 production when light from regions I and III is absorbed by chloroplasts. Lower (but measurable) activity is seen in region II, where some of the accessory pigments absorb. (b) The absorption of light by accessory pigments (superimposed on the absorption of chlorophylls *a* and *b*). The accessory pigments absorb light and transfer their energy to chlorophyll.

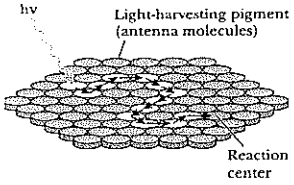


FIGURE 19.4
Schematic diagram of a photosynthetic unit. The light-harvesting pigments, or antenna molecules (green), absorb and transfer light energy to the specialized chlorophyll dimer that constitutes the reaction center (orange).

absorb light and transfer energy to chlorophylls (Figure 19.3b). Bacteriochlorophylls, the molecular form characteristic of photosynthetic organisms that do not produce oxygen, absorb light at longer wavelengths. The wavelength of maximum absorption of bacteriochlorophyll *a* is 780 nm; other bacteriochlorophylls have absorption maxima at still longer wavelengths, such as 870 or 1050 nm. Light of wavelength longer than 800 nm is part of the infrared, rather than the visible, region of the spectrum. The wavelength of light absorbed plays a critical role in the light reaction of photosynthesis because the energy of light is inversely related to wavelength (see the Biochemical Connections box below).

Most of the chlorophyll molecules in a chloroplast simply gather light (antennae chlorophylls). All chlorophylls are bound to proteins, either in antennae complexes or in one of two kinds of **photosystems** (membrane-bound protein complexes that carry out the light reactions). The light-harvesting molecules then pass their excitation energy along to a specialized pair of chlorophyll molecules at a **reaction center** characteristic of each photosystem (Figure 19.4). When the light energy reaches the reaction center, the chemical reactions of photosynthesis begin. The different environments of the antennae chlorophylls and the reaction-center chlorophylls give different properties to the two different kinds of molecules. In a typical chloroplast, there are several hundred light-harvesting antennae chlorophylls for each unique chlorophyll at a reaction center. The precise nature of reaction centers in both prokaryotes and eukaryotes is the subject of active research.

BIOCHEMICAL CONNECTIONS

The Relationship Between Wavelength and Energy of Light

A well-known equation relates the wavelength and energy of light, a point of crucial importance for our purposes. Max Planck established in the early 20th century that the energy of light is directly proportional to its frequency.

$$E = h\upsilon$$

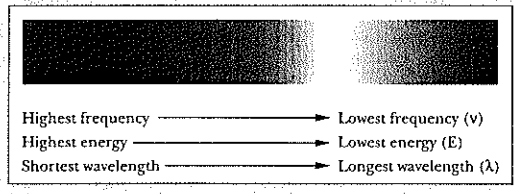
where E is energy, h is a constant (Planck's constant), and υ is the frequency of the light. The wavelength of light is related to the frequency.

$$\upsilon = \frac{c}{\lambda}$$

where λ is wavelength, υ is frequency, and c is the velocity of light. We can rewrite the expression for the energy of light in terms of wavelength rather than frequency.

$$E = h\upsilon = \frac{hc}{\lambda}$$

Light of shorter wavelength (higher frequency) is higher in energy than light of longer wavelength (lower frequency).



In the visible spectrum, blue light has a shorter wavelength (λ), higher frequency (ν), and higher energy (*E*) than red light. Intermediate values of all these quantities are observed for other colors of the visible spectrum.

19.2 The Light Reactions of Photosynthesis: Photosystems I and II

In the light reactions of photosynthesis, water is converted to oxygen by oxidation and $NADP^+$ is reduced to NADPH. The series of redox reactions is coupled to the phosphorylation of ADP to ATP in a process called **photophosphorylation.**

$$H_2O + NADP^+ \longrightarrow NADPH + H^+ + \tfrac{1}{2}O_2$$

$$ADP + P_i \longrightarrow ATP$$

The light reactions consist of two parts, accomplished by two distinct but related photosystems. One part of the reaction is the reduction of $NADP^+$ to NADPH, carried out by **Photosystem I (PSI).** The second part of the reaction is the oxidation of water to produce oxygen, carried out by **Photosystem II (PSII).** Both photosystems carry out redox (electron transfer) reactions. The two photosystems interact with each other indirectly through an electron transport chain that links the two photosystems. The production of ATP is linked to electron transport in a process similar to that seen in the production of ATP by mitochondrial electron transport.

In the dark reactions, the ATP and NADPH produced in the light reaction provide the energy and reducing power for the fixation of CO_2. The dark reactions also constitute a redox process, since the carbon in carbohydrates is in a more reduced state than the highly oxidized carbon in CO_2. The light and dark reactions do not take place separately, but they are separated for purposes of discussion only.

The net electron transport reaction of the two photosystems taken together is, except for the substitution of NADPH for NADH, the reverse of mitochondrial electron transport. The half-reaction of reduction is that of $NADP^+$ to NADPH, whereas the half-reaction of oxidation is that of water to oxygen.

$$NADP^+ + 2\,H^+ + 2\,e^- \longrightarrow NADPH + H^+$$

$$H_2O \longrightarrow \tfrac{1}{2}\,O_2 + 2\,H^+ + 2\,e^-$$

$$NADP^+ + H_2O \longrightarrow NADPH + H^+ + \tfrac{1}{2}\,O_2$$

This is an endergonic reaction with a positive $\Delta G^{\circ\prime} = +220$ kJ $mol^{-1} = +52.6$ kcal mol^{-1}. The light energy absorbed by the chlorophylls in both photosystems provides the energy that allows this endergonic reaction to take place. A series of electron carriers imbedded in the thylakoid membrane link these reactions. The electron carriers have an organization very similar to the carriers in the electron transport chain.

Photosystem I can be excited by light of wavelengths shorter than 700 nm, but Photosystem II requires light of wavelengths shorter than 680 nm for excitation. Both photosystems must operate for the chloroplast to produce NADPH, ATP, and O_2, because the two photosystems are connected by the electron transport chain. The two systems are, however, structurally distinct in the chloroplast; Photosystem I can be released preferentially from the thylakoid membrane by treatment with detergents. The reaction centers of the two photosystems provide different environments for the unique chlorophylls

Essential Information

The absorption of light by chlorophyll supplies the energy required for the reactions of photosynthesis. Several different kinds of chlorophyll are known. All have a tetrapyrrole ring structure similar to that of the porphyrins of heme, but they also have differences that affect the wavelength of light they absorb. This property allows more wavelengths of sunlight to be absorbed than would be the case with a single kind of chlorophyll.

Essential Information

Photosynthesis consists of two processes. The light reactions are electron transfer processes, in which water is oxidized to produce oxygen and $NADP^+$ is reduced to produce NADPH. The dark reactions are also electron transfer processes, but here carbon dioxide is reduced to carbohydrates.

involved. The unique chlorophyll of Photosystem I is referred to as P_{700}, where P is for pigment and 700 is for the longest wavelength of absorbed light (700 nm) that initiates the reaction. Similarly, the reaction-center chlorophyll of Photosystem II is designated P_{680} because the longest wavelength of absorbed light that initiates the reaction is 680 nm. Note particularly that the path of electrons starts with the reactions in Photosystem II rather than in Photosystem I. The reason for the nomenclature is that Photosystem I was studied extensively at an earlier date than Photosystem II because it is easier to extract Photosystem I from the thylakoid membrane than it is to do so with Photosystem II. There are two places in the reaction scheme of the two photosystems where the absorption of light supplies energy to make endergonic reactions take place (Figure 19.5)

Neither reaction-center chlorophyll is a strong-enough reducing agent to pass electrons to the next substance in the reaction sequence, but the absorption of light by the chlorophylls of both photosystems provides enough energy for such reactions to take place. The absorption of light by Chl_{II} (P_{680}) allows electrons to be passed to the electron transport chain that links Photosystem II and Photosystem I and generates a strong-enough oxidizing agent to split water, producing oxygen. When Chl_I (P_{700}) absorbs light, enough energy is provided to allow the ultimate reduction of $NADP^+$ to take place. (Note that the energy difference is shown on the vertical axis of Figure 19.5. This type of diagram is also called a Z scheme. The "Z" is rather lopsided and lies on its side, but the name is common.) In both photosystems, the result of supplying energy (light) is analogous to pumping water uphill.

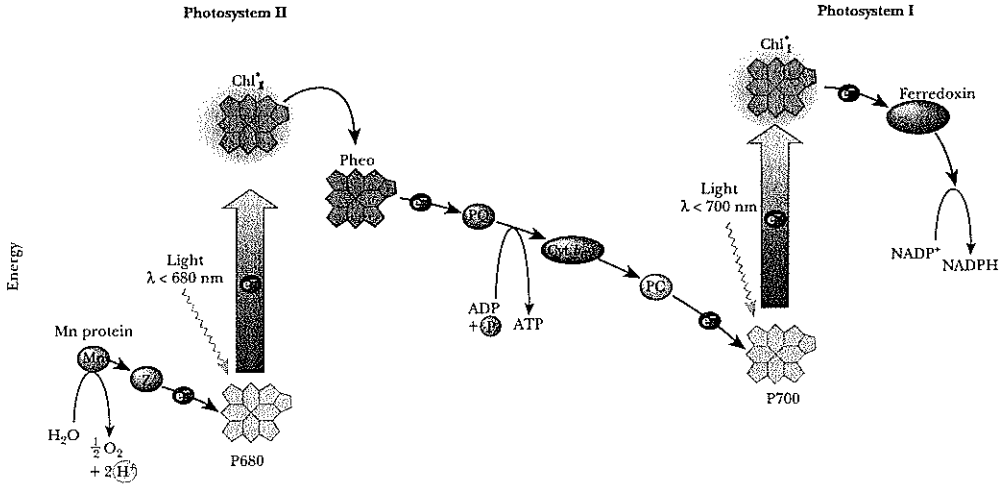


FIGURE 19.5
Electron flow in Photosystems I and II. The energy needed to transfer electrons from H_2O to $NADP^+$ is provided by the absorption of light by Photosystems I and II (vertical [up] arrows). After each absorption of light, the electrons can then flow "downhill" (diagonal [down] arrows). Photophosphorylation of ADP to yield ATP is coupled to the electron transport chain that links Photosystem II to Photosystem I. (Chl is chlorophyll; Pheo is pheophytin; PQ is plastoquinone; PC is plastocyanin.) The electron carriers that mediate the transfer of electrons from H_2O to Photosystem II include a manganese-containing protein and a protein with an essential tyrosine residue, referred to as component Z.

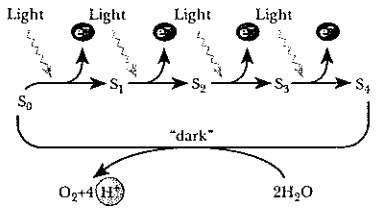


FIGURE 19.6
The PSII reaction center passes through five different oxidation states, S_0 through S_4, in the course of oxygen evolution.

Photosystem II: Water Is Split to Produce Oxygen

The oxidation of water by Photosystem II to produce oxygen is the ultimate source of electrons in photosynthesis. These electrons are subsequently passed from Photosystem II to Photosystem I by the electron transport chain. The electrons from water are needed to "fill the hole" that is left when the absorption of one photon of light leads to donation of an electron from Photosystem II to the electron transport chain.

The electrons released by the oxidation of water are first passed to Chl_{II}, which is reduced.

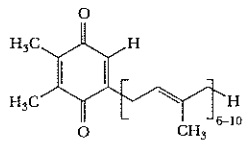
$$2\ H_2O + 4\ Chl_{II}\cdot \longrightarrow O_2 + 4\ H^+ + 4\ Chl_{II}$$

There are intermediate steps in this reaction because four electrons are required for the oxidation of water, and Chl_{II}^+ (P_{680}^+) can accept only one electron at a time. A manganese-containing protein and several other protein components are required. The **oxygen-evolving complex** of Photosystem II passes through a series of five oxidation states (designated as S_0 through S_4) in the transfer of four electrons in the process of evolving oxygen (Figure 19.6). One electron is passed from water to PSII for each quantum of light. In the process, the components of the reaction center go successively through oxidation states S_1 through S_4. The S_4 decays spontaneously to the S_0 state and in the process oxidizes two water molecules to one oxygen molecule. Note that four protons are released simultaneously. The immediate electron donor, designated Z or D depending on the source one consults, to the P_{680} chlorophyll is a tyrosine residue of one of the protein components that does not contain manganese. Several quinones serve as intermediate electron transfer agents to accommodate four electrons donated by one water molecule. Redox reactions of manganese also play a role here. (See the article by Govindjee and Coleman listed in the bibliography at the end of this chapter for a discussion of the workings of this complex.)

In Photosystem II, as in Photosystem I, the absorption of light by chlorophyll in the reaction center produces an excited state of chlorophyll. The wavelength of light is 680 nm; the reaction-center chlorophyll of Photosystem II is also referred to as P_{680}.

$$Chl_{II} + h\nu\ (680\ nm) \longrightarrow Chl_{II}\cdot$$

The excited chlorophyll passes an electron to a primary acceptor. In Photosystem II, the primary electron acceptor is a molecule of **pheophytin** (Pheo), one of the accessory pigments of the photosynthetic apparatus. The structure of pheophytin differs from that of chlorophyll only in the substitution of two hydrogens for the magnesium. The transfer of electrons is mediated by events that take place at the reaction center. The next electron acceptor is **plastoquinone** (PQ). The structure of plastoquinone (Figure 19.7) is similar to that of coenzyme Q (ubiquinone), a part of the respiratory electron transport chain (Section 17.4), and serves a very similar purpose in the transfer of electrons and hydrogen ions.



Plastoquinone

FIGURE 19.7
The structure of plastoquinone. The length of the aliphatic side chain varies in different organisms.

The electron transport chain that links the two photosystems consists of pheophytin, plastoquinone, a complex of plant cytochromes (the b_6-f complex), a copper-containing protein called **plastocyanin** (PC), and the oxidized form of P_{700} (Chl_I^+) (see Figure 19.5). The b_6-f complex of plant cytochromes consists of two *b*-type cytochromes (cytochrome b_6) and a *c*-type cytochrome (cytochrome *f*). This complex is similar in structure to the bc_1 complex in mitochondria and occupies a similar central position in an electron transport chain. This part of the photosynthetic apparatus is the subject of active research. There is a possibility that a Q-cycle (recall this from Section 17.3) may operate here as well, and the object of some of this research is to establish definitely whether this is so. In plastocyanin, the copper ion is the actual electron carrier; the copper ion exists as Cu(II) and Cu(I) in the oxidized and reduced forms, respectively. This electron transport chain has another similarity to that in mitochondria, that of coupling to ATP generation.

When the oxidized chlorophyll of P_{700} accepts electrons from the electron transport chain, it is reduced and subsequently passes an electron to Photosystem I, which absorbs a second photon of light. Absorption of light by Photosystem II does not raise the electrons to a high enough energy level to reduce $NADP^+$; the second photon absorbed by Photosystem I provides the needed energy. This difference in energy makes the "Z" of the Z scheme thoroughly lopsided, but the transfer of electrons is complete.

Essential Information

The path of electrons in the light reactions of photosynthesis can be considered to have three parts. The first is the transfer of electrons from water to the reaction-center chlorophyll of Photosystem II. The second part is the transfer of electrons from the excited-state chlorophyll of Photosystem II to an electron-transport chain consisting of accessory pigments and cytochromes, with energy provided by absorption of a photon of light. The components of this electron-transport chain resemble those of the mitochondrial electron-transport chain; they pass the electrons to the reaction-center chlorophyll of Photosystem I. The third and last part of the path of the electrons is their transfer from the excited-state chlorophyll of Photosystem I to the ultimate electron acceptor $NADP^+$, producing NADPH; again energy is provided by absorption of a photon of light.

Photosystem I: Reduction of $NADP^+$

The absorption of light by Chl_I then leads to the series of electron transfer reactions of Photosystem I. The substance to which the excited-state chlorophyll, Chl_I^*, gives an electron is apparently a molecule of chlorophyll *a*; this transfer of electrons is mediated by processes that take place in the reaction center. The next electron acceptor in the series is bound ferredoxin, an iron–sulfur protein occurring in the membrane in Photosystem I. The bound ferredoxin passes its electron to a molecule of soluble ferredoxin. Soluble ferredoxin in turn reduces an FAD-containing enzyme called ferredoxin-NADP reductase. The FAD portion of the enzyme reduces $NADP^+$ to NADPH (Figure 19.5). We can summarize the main features of the process in two equations, in which the notation ferredoxin refers to the soluble form of the protein.

$$Chl_I^* + Ferredoxin_{oxidized} \longrightarrow Chl_I^+ + Ferredoxin_{reduced}$$

$$2\ Ferredoxin_{reduced} + H^+ + NADP^+ \xrightarrow[\text{reductase}]{\text{Ferredoxin-NADP}} 2\ Ferredoxin_{oxidized} + NADPH$$

Chl_I^* donates one electron to ferredoxin, but the electron transfer reactions of FAD and $NADP^+$ involve two electrons. Thus, an electron from each of two ferredoxins is required for the production of NADPH.

The net reaction for the two photosystems together is the flow of electrons from H_2O to $NADP^+$ (see Figure 19.5).

$$2\ H_2O + 2\ NADP^+ \longrightarrow O_2 + 2\ NADPH + 2\ H^+$$

Cyclic Electron Transport in Photosystem I

In addition to the electron transfer reactions just described, it is possible for cyclic electron transport in Photosystem I to be coupled to the production of ATP (Figure 19.8). No NADPH is produced in this process. Photosystem II is not involved, and no O_2 is generated. Cyclic phosphorylation takes place when there is a high NADPH/$NADP^+$ ratio in the cell: there is not enough $NADP^+$ present in the cell to accept all the electrons generated by the excitation of Chl_{II}.

Structure of a Photosystem

The molecular structure of photosystems is a subject of intense interest to biochemists. The most extensively studied system is that from bacteria of the genus *Rhodopseudomonas*. These bacteria do not produce molecular oxygen as a result of their photosynthetic activities, but enough similarities exist between the photosynthetic reactions of *Rhodopseudomonas* and photosynthesis linked to oxygen to lead scientists to draw conclusions about the nature of reaction centers in all organisms. The detailed process that goes on at the reaction center of *Rhodopseudomonas* is important enough to warrant further discussion.

It is well established that there is a pair of bacteriochlorophyll molecules in the reaction center of *Rhodopseudomonas viridis;* the critical pair of chlorophylls is embedded in a protein complex that is in turn an integral part of the photosynthetic membrane. (We shall refer to the bacteriochlorophylls simply as chlorophylls in the interest of simplifying the discussion.) Accessory pigments, which also play a role in the light-trapping process, have specific positions close to the special pair of chlorophylls. The absorption of light by the special pair of chlorophylls raises one of their electrons to a higher energy level (Figure 19.9a). This electron is passed to a series of accessory pigments (Figure 19.9b). The first of these accessory pigments is pheophytin, which is structurally similar to chlorophyll, differing only by having two hydrogens in place of the magnesium. The electron is passed along to the pheophytin, raising it in turn to an excited energy level. (Note that the electron travels on only one of two possible paths, to one pheophytin but not the other. Research is in progress to determine why this is so.) The next electron acceptor is menaquinone (Q_A); it is structurally similar to coenzyme Q, which plays a role in the mitochondrial electron transport chain. The final electron acceptor, which is also raised to an excited state, is coenzyme Q itself (ubiquinone, called Q_B here). The electron that had been passed to Q_B is replaced by an electron donated by a cytochrome, which acquires a positive charge in the process (Figure 19.9c). The cytochrome is not bound to the membrane and diffuses away, carrying its positive charge with it. The whole process takes place in less than 10^{-9} second. The positive and negative charges have traveled in opposite directions from the chlorophyll pair and are separated from each other (Figure 19.9d). This situation is similar to the proton gradient in mitochondria, where the existence of the proton gradient is ultimately responsible for oxidative phosphorylation. The separation of charge is equivalent to a battery, a form of stored energy. The reaction center has acted as a transducer, converting light energy to a form

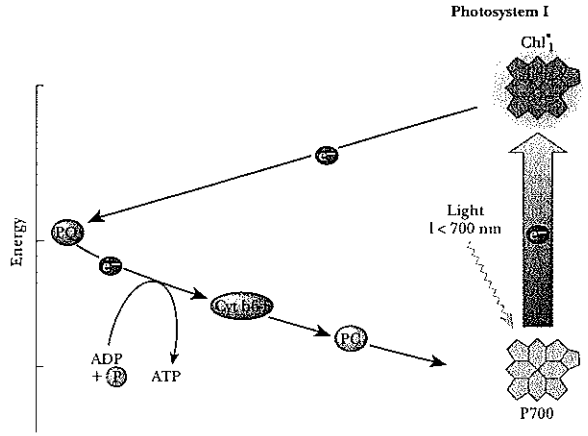


FIGURE 19.8
Cyclic electron flow coupled to photophosphorylation in Photosystem I. Note that water is not split and that no NADPH is produced. (Chl is chlorophyll; PQ is plastoquinone; PC is plastocyanin.)

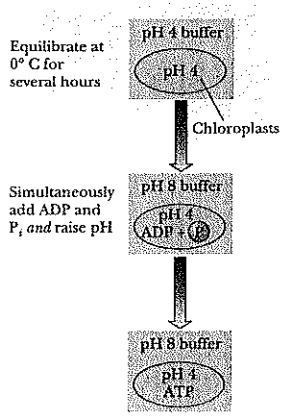


FIGURE 19.10
ATP is synthesized by chloroplasts in the dark in the presence of a proton gradient, ADP, and P_i.

19.3 A Proton Gradient Drives the Production of ATP in Photosynthesis

In Chapter 17, we saw that a proton gradient across the inner mitochondrial membrane drives the phosphorylation of ADP in respiration. The mechanism of photophosphorylation is essentially the same as that of the production of ATP in the respiratory electron transport chain. In fact, some of the strongest evidence for the chemiosmotic coupling of phosphorylation to electron transport has been obtained from experiments on chloroplasts rather than mitochondria. Chloroplasts can synthesize ATP from ADP and P_i *in the dark* if they are provided with a pH gradient.

If isolated chloroplasts are allowed to equilibrate in a pH 4 buffer for several hours, their internal pH will be equal to 4. If the pH of the buffer is raised rapidly to 8 and if ADP and P_i are added simultaneously, ATP will be produced (Figure 19.10). The production of ATP does not require the presence of light; the proton gradient produced by the pH difference supplies the driving force for phosphorylation. This experiment provides solid evidence for the chemiosmotic coupling mechanism.

Several reactions contribute to the generation of a proton gradient in chloroplasts in an actively photosynthesizing cell. The oxidation of water releases H^+ into the thylakoid space. Electron transport from Photosystem II and Photosystem I also helps create the proton gradient by involving plastoquinone and cytochromes in the process. Then Photosystem I reduces $NADP^+$ by using H^+ in the stroma to produce NADPH. As a result, the pH of the thylakoid space is lower than that of the stroma (Figure 19.11). We saw a similar situation in Chapter 17 when we discussed the pumping of protons from the mitochondrial matrix into the intermembrane space. The ATP synthase in chloroplasts is similar to the mitochondrial enzyme; in particular, it consists of two parts, CF_1 and CF_0, where the C serves to distinguish them from their mitochondrial counterparts, F_1 and F_0, respectively. Evidence exists that the components of the electron chain in chloroplasts are arranged asymmetrically in the thylakoid membrane, as is the case in mitochondria. An important consequence of this asymmetrical arrangement is the release of the ATP and NADPH produced by

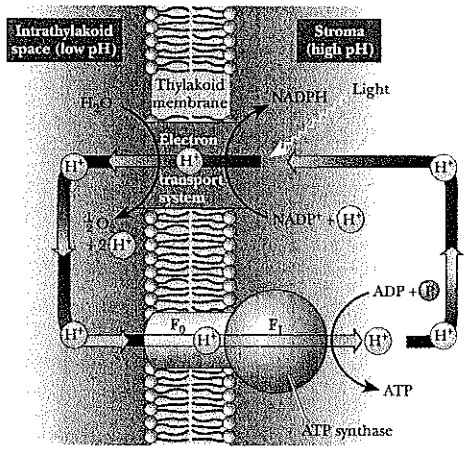


FIGURE 19.11
The relationship between photophosphorylation and the proton gradient in chloroplasts. Photosynthetic electron transport pumps H^+ out of the stroma to the intrathylakoid space to form the proton gradient (high pH in the stroma, low pH in the intrathylakoid space). The flow of H^+ back to the stroma through the ATP synthase provides the energy for synthesis of ATP from ADP and P_i.

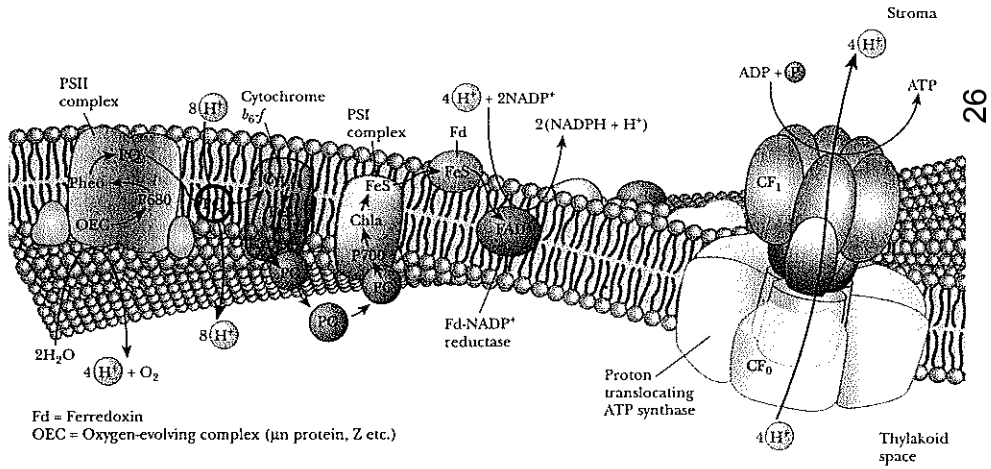


FIGURE 19.12
The components of the electron transport chain of the thylakoid membrane. This schematic representation shows Photosystem II (PSII), the cytochrome b_6-f complex , and Photosystem I (PSI), along with the soluble electron carriers plastoquinone (PQ) and plastocyanin (PC). The action of the electron transport chain sets up a proton gradient across the thylakoid membrane, coupled to synthesis of ATP by the CF_0-CF_1 ATP synthase. *(After D. R. Ort and N. E. Good, 1988.* Trends Biochem. Sci. *13, 469.)*

the light reaction into the stroma, where they provide energy and reducing power for the dark reaction of photosynthesis.

In mitochondrial electron transport, there are four respiratory complexes connected by soluble electron carriers. The electron transport apparatus of the thylakoid membrane is similar in that it consists of several large membrane-bound complexes. They are PSII (the Photosystem II complex), the cytochrome b_6-f complex, and PSI (the Photosystem I complex). As in mitochondrial electron transport, several soluble electron carriers form the connection between the protein complexes. In the thylakoid membrane, the soluble carriers are plastoquinone and plastocyanin, which have a role similar to that of coenzyme Q and cytochrome *c* in mitochondria (Figure 19.12). The proton gradient created by electron transport drives the synthesis of ATP in chloroplasts, as in mitochondria.

19.4 A Comparison of Photosynthesis with and Without Oxygen: Evolutionary Implications

Photosynthetic prokaryotes other than cyanobacteria have only one photosystem and do not produce oxygen. The chlorophyll in these organisms is different from that found in photosystems linked to oxygen (Figure 19.13). Anaerobic photosynthesis is not as efficient as photosynthesis linked to oxygen, but the anaerobic version of the process appears to be an evolutionary way station. Anaerobic photosynthesis is a means for organisms to use solar energy to satisfy their needs for food and energy. Although it is efficient in the production of ATP, its efficiency is less than that of aerobic photosynthesis for carbon fixation.

A possible scenario for the development of photosynthesis starts with heterotrophic bacteria that contain some form of chlorophyll, probably bacteriochlorophyll (*heterotrophs* are organisms that depend on their environment for organic nutrients and for energy). In such organisms, the light energy absorbed by chlorophyll can be trapped in the forms of ATP and NADPH. The important point about such a series of reactions is that photophosphorylation takes place, ensuring an independent supply of ATP for the organism. In addition, the supply of NADPH facilitates synthesis of biomolecules from simple sources such as CO_2. Under conditions of limited food supply, organisms that can synthesize their own nutrients have a selective advantage. Organisms of this sort are *autotrophs* (not dependent on an external source of biomolecules) but are also anaerobes. The ultimate electron source that they use is not water but some more easily oxidized substance, such as H_2S, as is the case with present-day green sulfur bacteria (and purple sulfur bacteria), or various organic compounds, as is the case with present-day purple nonsulfur bacteria. These organisms do not possess an oxidizing agent powerful enough to split water, which is a far more abundant electron source than H_2S or organic compounds. The ability to use water as an electron source confers a further evolutionary advantage.

As is frequently the case in biological oxidation–reduction reactions, hydrogens as well as electrons are transferred from a donor to an acceptor. In green plants, green algae, and cyanobacteria, the hydrogen donor and acceptor are H_2O and CO_2, respectively, with oxygen as a product. Other organisms such as bacteria and fungi carry out photosynthesis in which there is a hydrogen donor other than water. Some possible donors include H_2S, $H_2S_2O_3$, and succinic acid. As an example, if H_2S is the source of hydrogens and electrons, a schematic equation for photosynthesis can be written with sulfur, rather than oxygen, as a product.

$$\underset{\text{H-acceptor}}{CO_2} + \underset{\text{H-donor}}{2\,H_2S} \rightarrow \underset{\text{Carbohydrate}}{(CH_2O)} + 2\,S + H_2O$$

It is also possible for the hydrogen acceptor to be NO_2^- or NO_3^-, in which case NH_3 is a product. Photosynthesis linked to oxygen with carbon dioxide as the ultimate hydrogen acceptor is a special case of a far more general process, widely distributed among many different organisms.

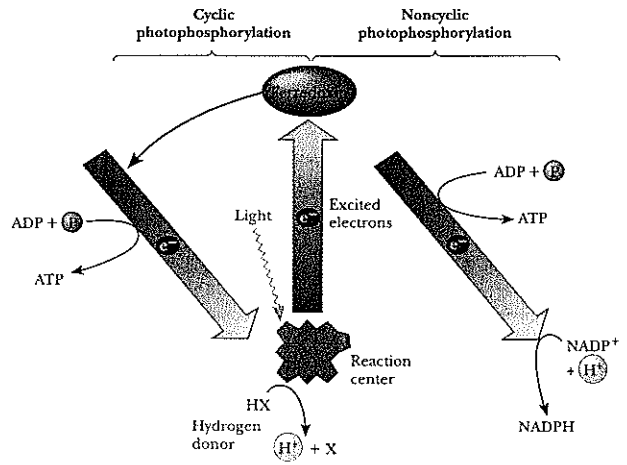


FIGURE 19.13
The two possible electron transfer pathways in a photosynthetic anaerobe. Both cyclic and noncyclic forms of photophosphorylation are shown. HX is any compound (such as H_2S) that can be a hydrogen donor. *(From L. Margulis, 1985.* Early Life, *Science Books International, Boston, p. 45.)*

Cyanobacteria were apparently the first organisms that developed the ability to use water as the ultimate reducing agent in photosynthesis. As we have seen, this feat required the development of a second photosystem as well as a new variety of chlorophyll, chlorophyll *a* rather than bacteriochlorophyll in this case. Chlorophyll *b* had not yet appeared on the scene, since it occurs only in eukaryotes. The basic system of aerobic photosynthesis was in place with cyanobacteria. As a result of aerobic photosynthesis by cyanobacteria, the earth acquired its present atmosphere with its high levels of oxygen. The existence of all other aerobic organisms depended ultimately on the activities of cyanobacteria.

19.5 The Dark Reactions of Photosynthesis: Path of Carbon

The actual storage form of the carbohydrates produced from carbon dioxide by photosynthesis is not glucose but disaccharides (e.g., sucrose in sugarcane and sugar beets) and polysaccharides (starch and cellulose). However, it is customary and convenient to write the carbohydrate product as glucose, and we shall follow this time-honored practice.

Carbon dioxide fixation takes place in the stroma. The equation for the overall reaction, like all equations for photosynthetic processes, is deceptively simple.

$$6\,CO_2 + 12\,NADPH + 18\,ATP \xrightarrow{\text{Enzymes}} C_6H_{12}O_6 + 12\,NADP^+ + 18\,ADP + 18\,P_i$$

The actual reaction pathway has some features in common with glycolysis and some in common with the pentose phosphate pathway.

The net reaction of six molecules of carbon dioxide to produce one molecule of glucose requires the carboxylation of six molecules of a five-carbon key intermediate, **ribulose-1,5-*bis*phosphate,** to form six molecules of an unstable six-carbon intermediate, which then splits to give 12 molecules of **3-phosphoglycerate.** Of these, two molecules of 3-phosphoglycerate react in turn, ultimately producing glucose. The remaining ten molecules of 3-phosphoglycerate are used to regenerate the six molecules of ribulose-1,5-*bis*phosphate. The overall reaction pathway is cyclic and is called the **Calvin cycle** (Figure 19.14) after the scientist who first investigated it, Melvin Calvin, winner of the 1961 Nobel Prize in chemistry.

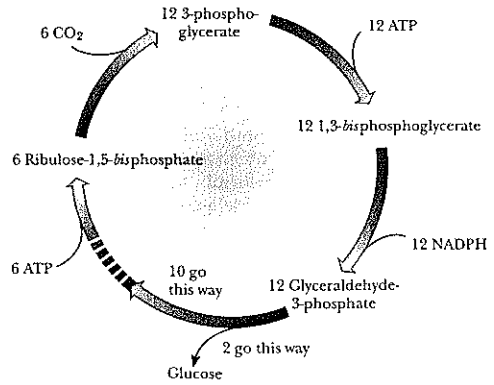


FIGURE 19.14
The main features of the Calvin cycle. Glucose is produced, and ribulose-1,5-*bis*phosphate is regenerated.

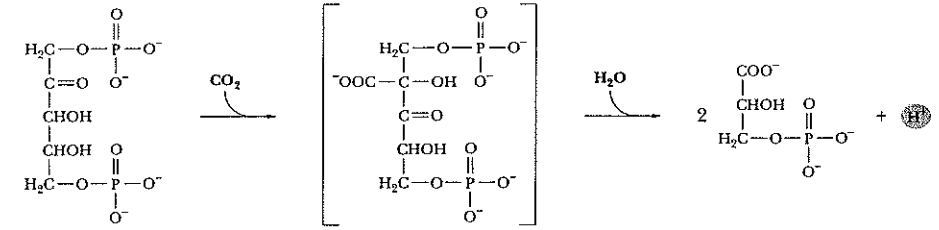


FIGURE 19.15
The reaction of ribulose-1,5-*bis*phosphate with CO_2 ultimately produces two molecules of 3-phosphoglycerate.

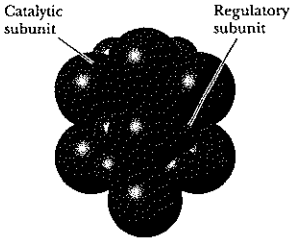


FIGURE 19.16
The subunit structure of ribulose-1,5-*bis*phosphate carboxylase.

The first reaction of the Calvin cycle is the condensation of ribulose-1,5-*bis*phosphate with carbon dioxide to form a six-carbon intermediate, 2-carboxy-3-ketoribitol-1,5-*bis*phosphate, which quickly hydrolyzes to give two molecules of 3-phosphoglycerate (Figure 19.15). The reaction is catalyzed by the enzyme *ribulose-1,5-bisphosphate carboxylase* **(Rubisco).** This enzyme is located on the stromal side of the thylakoid membrane and is probably one of the most abundant proteins in nature, since it accounts for about 15 percent of the total protein in chloroplasts. The molecular weight of ribulose-1,5-*bis*phosphate carboxylase is about 560,000, and it consists of eight large subunits (molecular weight, 55,000) and eight small subunits (molecular weight, 15,000) (Figure 19.16). The sequence of the large subunit is encoded by a chloroplast gene, and that of the small subunit is encoded by a nuclear gene. The endosymbiotic theory for the development of eukaryotes (Section 1.7) is consistent with the idea of independent genetic material in organelles. The large subunit (chloroplast gene) is catalytic, whereas the small subunit (nuclear gene) plays a regulatory role, an observation that is consistent with an endosymbiotic origin for organelles such as chloroplasts.

The incorporation of CO_2 into 3-phosphoglycerate represents the actual fixation process; the remaining reactions are those of carbohydrates. The next two reactions lead to the reduction of 3-phosphoglycerate to form glyceraldehyde-3-phosphate. The reduction takes place in the same fashion as in gluconeogenesis, except for one unique feature (Figure 19.17a): the reactions in chloroplasts require NADPH rather than NADH for the reduction of 1,3-*bis*phosphoglycerate to glyceraldehyde-3-phosphate. When glyceraldehyde-3-phosphate is formed, it can have two alternative fates: one is the production of six-carbon sugars, and the other is the regeneration of ribulose 1,5-*bis*phosphate.

Essential Information

In the dark reactions of photosynthesis, the fixation of carbon dioxide takes place when the key intermediate ribulose-1,5-*bis*phosphate reacts with carbon dioxide to produce two molecules of 3-phosphoglycerate. This reaction is catalyzed by the enzyme ribulose-1,5-*bis*phosphate carboxylase (Rubisco), one of the most abundant proteins in nature. The remainder of the dark reaction is the regeneration of ribulose-1,5-*bis*phosphate in the Calvin cycle.

Production of Six-Carbon Sugars

The formation of glucose from glyceraldehyde-3-phosphate takes place in the same manner as in gluconeogenesis (Figure 19.17b). The conversion of glyceraldehyde-3-phosphate to dihydroxyacetone phosphate takes place easily (Section 14.2). Dihydroxyacetone phosphate in turn reacts with glyceraldehyde-3-phosphate, in a series of reactions we have already seen, to give rise to fructose-6-phosphate and ultimately to glucose. Because we have already seen these reactions, we shall not discuss them again.

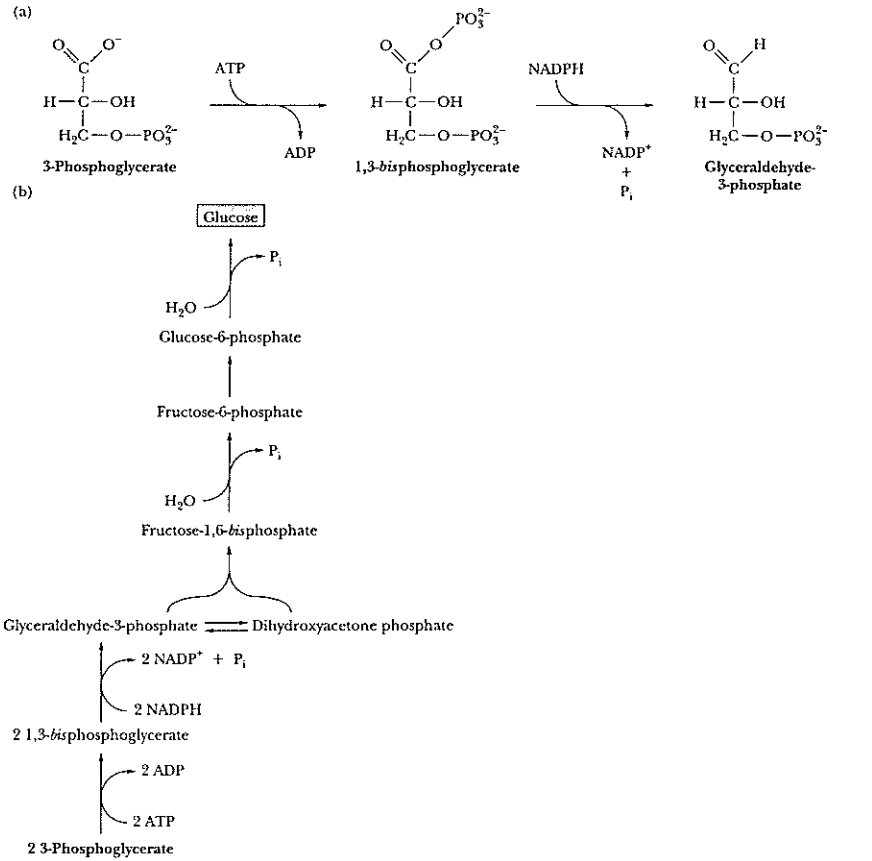


FIGURE 19.17
(a) Reduction of 3-phosphoglycerate to glyceraldehyde-3-phosphate. (b) The production of glucose from 3-phosphoglycerate in the Calvin cycle. Note the use of NADPH and ATP generated in the light reaction to provide energy for the dark reaction.

Regeneration of Ribulose-1,5-Bisphosphate

This process is readily divided into four steps: *preparation, reshuffling, isomerization,* and *phosphorylation.* The preparation begins with conversion of some of the glyceraldehyde-3-phosphate to dihydroxyacetone phosphate (catalyzed by triosephosphate isomerase). This reaction also functions in the production of six-carbon sugars. Portions of both the glyceraldehyde-3-phosphate and the dihydroxyacetone phosphate are then condensed to form fructose-1,6-*bis*phosphate (catalyzed by aldolase). Fructose-1,6-*bis*phosphate is hydrolyzed to fructose-6-phosphate (catalyzed by fructose-1,6-*bis*phosphatase). With a supply of glyceraldehyde-3-phosphate, dihydroxyacetone phosphate, and fructose-6-phosphate now available, the reshuffling can begin.

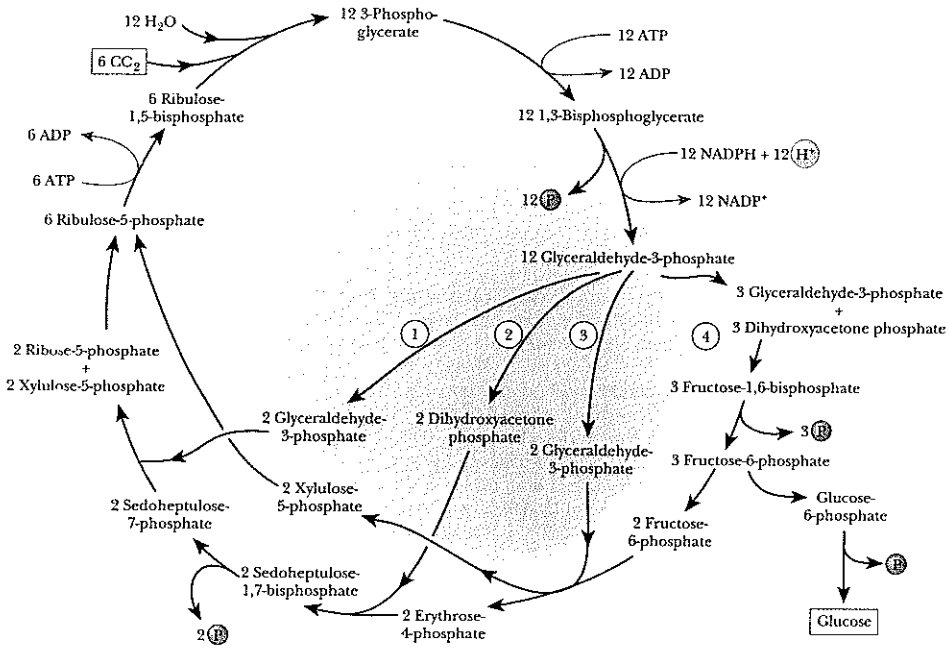


FIGURE 19.20
The complete Calvin cycle, showing the regeneration of ribulose-1,5-*bis*phosphate. Note that when glyceraldehyde-3-phosphate is formed, it (or dihydroxyacetone phosphate, to which it is easily converted) can have all four possible fates. The possible pathways are numbered. (See Figure 19.19 for the balanced equation.)

In the final step, ribulose-1,5-*bis*phosphate is regenerated by the phosphorylation of ribulose-5-phosphate (Figure 19.18b). This reaction requires ATP and is catalyzed by the enzyme *phosphoribulokinase*. The reactions leading to the regeneration of ribulose-1,5-*bis*phosphate are summarized in Figure 19.19, in which a net equation is obtained by adding all the reactions. Now we are in a position to examine the stoichiometry of the dark reaction of photosynthesis.

Stoichiometry of the Calvin Cycle

It will be convenient to refer to Figures 19.14 and 19.20 during our discussion. We shall follow what happens to six molecules of CO_2 in the course of one turn of the Calvin cycle.

For each CO_2 that reacts with one molecule of ribulose-1,5-*bis*phosphate, two molecules of 3-phosphoglycerate are produced. Conversion of each molecule of 3-phosphoglycerate to glyceraldehyde-3-phosphate requires 1 ATP and 1 NADPH. For six molecules of CO_2, we can write the equation

$$6\ CO_2 + 6\ \text{ribulose-1,5-}bis\text{phosphate} + 12\ ATP + 12\ NADPH + 12\ H^+ + 12\ H_2O$$

(6 carbons) (30 carbons)

$$\text{O} \longrightarrow 12\ \text{glyceraldehyde-3-phosphate} + 12\ ADP + 12\ P_i + 12\ NADP^+$$

(36 carbons)

The important point here is the requirement for 12 ATP and 12 NADPH for each molecule of glucose. Ten of the 12 glyceraldehyde-3-phosphate (C_3) molecules are regenerated to ribulose-1,5-*bis*phosphate (Figure 19.20), accounting for 30 of the 36 carbon atoms in 12 molecules of glyceraldehyde-3-phosphate. The remaining six carbon atoms (two glyceraldehyde-3-phosphates) are converted to glucose. The regeneration of ribulose-1,5-*bis*phosphate also requires six ATP in the process of the net conversion of six CO_2 to one molecule of glucose. See Figure 19.19 to see how this figure of six ATP is obtained; in one turn of the Calvin cycle, the overall process shown in this figure occurs twice.

Taking these points into consideration, we arrive at the *net* equation for the path of carbon in photosynthesis.

$$6\ CO_2 + 18\ ATP + 12\ NADPH + 12\ H^+ + 12\ H_2O \longrightarrow$$

$$\text{Glucose} + 12\ NADP^+ + 18\ ADP + 18\ P_i$$

The efficiency of energy use in photosynthesis can be calculated fairly easily. The $\Delta G^{\circ\prime}$ for the reduction of CO_2 to glucose is +478 kJ (+114 kcal) for each mole of CO_2 (see Exercise 25), and the energy of light of 600-nm wavelength is 1593 kJ mol^{-1} (381 kcal) mol^{-1}. We shall not explain in detail here how this figure for the energy of the light is obtained, but it comes ultimately from the equation $E = h\nu$. Light of wavelength 680 or 700 nm has lower energy than light at 600 nm. Thus, the efficiency of photosynthesis is at least (477/1593) × 100, or 30%.

19.6 An Alternative Pathway for Carbon Dioxide Fixation

In tropical plants there is a C_4 pathway (Figure 19.21), so named because it involves four-carbon compounds. The operation of this pathway (also called the **Hatch–Slack pathway**) ultimately leads to the C_3 (based on 3-phosphoglycerate) pathway of the Calvin cycle. (There are other C_4 pathways, but this one is most widely studied. Corn [maize] is an important example of a C_4 plant, and it is certainly not confined to the tropics.)

When CO_2 enters the leaf through pores in the outer cells, it reacts first with phosphoenolpyruvate to produce oxaloacetate and P_i in the mesophyll cells of the leaf. Oxaloacetate is reduced to malate, with the concomitant oxidation of NADPH. Malate is then transported to the bundle-sheath cells (the next layer) through channels that connect the two kinds of cells.

In the bundle-sheath cells, malate is decarboxylated to give pyruvate and CO_2. In the process, $NADP^+$ is reduced to NADPH (Figure 19.22). The CO_2 reacts with ribulose-1,5-*bis*phosphate to enter the Calvin cycle. Pyruvate is transported back to the mesophyll cells, where it is phosphorylated to phosphoenolpyruvate, which can react with CO_2 to start another round of the C_4

29

Chemistry

The Central Science

Ninth Edition

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SAMPLE EXERCISE 3.11

Calculate the mass, in grams, of 0.433 mol of calcium nitrate.

Solution

Analyze: We are given the number of moles of calcium nitrate and asked to calculate the mass of the sample in grams.

Plan: In order to convert moles to grams, we need the molar mass, which we can calculate using the chemical formula and atomic weights.

Solve: Because the calcium ion is Ca^{2+} and the nitrate ion is NO_3^-, calcium nitrate is $Ca(NO_3)_2$. Adding the atomic weights of the elements in the compound gives a formula weight of 164.1 amu. Using 1 mol $Ca(NO_3)_2$ = 164.1 g $Ca(NO_3)_2$ to write the appropriate conversion factor, we have

$$\text{Grams } Ca(NO_3)_2 = 0.433 \text{ mol } Ca(NO_3)_2 \left(\frac{164.1 \text{ g } Ca(NO_3)_2}{1 \text{ mol } Ca(NO_3)_2}\right) = 71.1 \text{ g } Ca(NO_3)_2$$

Check: The number of moles is less than 1, so the number of grams must be less than the molar mass, 164.1 g. Using rounded numbers to estimate, we have 0.5 × 150 = 75 g. Thus, the magnitude of our answer is reasonable. Both the units (g) and the number of significant figures (3) are correct.

PRACTICE EXERCISE

What is the mass, in grams, of **(a)** 6.33 mol of $NaHCO_3$ and **(b)** 3.0×10^{-5} mol of sulfuric acid?

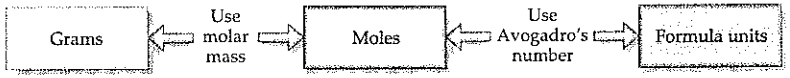
Answers: **(a)** 532 g; **(b)** 2.9×10^{-3} g

The mole concept provides the bridge between masses and numbers of particles. To illustrate how we can interconvert masses and numbers of particles, let's calculate the number of copper atoms in an old copper penny. Such a penny weighs about 3 g, and we'll assume that it is 100% copper:

$$\text{Cu atoms} = (3 \text{ g Cu})\left(\frac{1 \text{ mol Cu}}{63.5 \text{ g Cu}}\right)\left(\frac{6.02 \times 10^{23} \text{ Cu atoms}}{1 \text{ mol Cu}}\right)$$

$$= 3 \times 10^{22} \text{ Cu atoms}$$

Notice how dimensional analysis (Section 1.6) provides a straightforward route from grams to numbers of atoms. The molar mass and Avogadro's number are used as conversion factors to convert grams ⟶ moles ⟶ atoms. Notice also that our answer is a very large number. Any time you calculate the number of atoms, molecules, or ions in an ordinary sample of matter, you can expect the answer to be very large. In contrast, the number of moles in a sample will usually be much smaller, often less than 1. The general procedure for interconverting mass and number of formula units (atoms, molecules, ions, or whatever is represented by the chemical formula) of a substance is summarized in Figure 3.10 ▼.



▲ **Figure 3.10** Outline of the procedure used to interconvert the mass of a substance in grams and the number of formula units of that substance. The number of moles of the substance is central to the calculation; thus, the mole concept can be thought of as the bridge between the mass of a substance and the number of formula units.

SAMPLE EXERCISE 3.12

How many glucose molecules are in 5.23 g of $C_6H_{12}O_6$?

Solution

Analyze: We are given the number of grams of glucose and its chemical formula and asked to calculate the number of glucose molecules.

Plan: The strategy for determining the number of molecules in a given quantity of a substance is summarized in Figure 3.10. We must convert 5.23 g $C_6H_{12}O_6$ to moles $C_6H_{12}O_6$, which can then be converted to molecules $C_6H_{12}O_6$. The first conversion uses the molar mass of $C_6H_{12}O_6$: 1 mol $C_6H_{12}O_6$ = 180.0 g $C_6H_{12}O_6$. The second conversion uses Avogadro's number.

Solve:

Molecules $C_6H_{12}O_6$

$$= (5.23 \text{ g } C_6H_{12}O_6)\left(\frac{1 \text{ mol } C_6H_{12}O_6}{180.0 \text{ g } C_6H_{12}O_6}\right)\left(\frac{6.023 \times 10^{23} \text{ molecules } C_6H_{12}O_6}{1 \text{ mol } C_6H_{12}O_6}\right)$$

$$= 1.75 \times 10^{22} \text{ molecules } C_6H_{12}O_6$$

Check: The magnitude of the answer is reasonable. Because the mass we began with is less than a mole, there should be less than 6.02×10^{23} molecules. We can make a ballpark estimate of the answer: 5/200 = 2.5×10^{-2} mol; $2.5 \times 10^{-2} \times 6 \times 10^{23} = 15 \times 10^{21} = 1.5 \times 10^{22}$ molecules. The units (molecules) and significant figures (3) are appropriate.

Comment: If you were also asked for the number of atoms of a particular element, an additional factor would be needed to convert the number of molecules to the number of atoms. For example, there are six O atoms in a molecule of $C_6H_{12}O_6$. Thus, the number of O atoms in the sample is

$$\text{Atoms O} = (1.75 \times 10^{22} \text{ molecules } C_6H_{12}O_6)\left(\frac{6 \text{ atoms O}}{1 \text{ molecule } C_6H_{12}O_6}\right)$$

$$= 1.05 \times 10^{23} \text{ atoms O}$$

PRACTICE EXERCISE

(a) How many nitric acid molecules are in 4.20 g of HNO_3? **(b)** How many O atoms are in this sample?

Answers: **(a)** 4.01×10^{22} molecules HNO_3; **(b)** 1.20×10^{23} atoms O

3.5 Empirical Formulas from Analyses

The empirical formula for a substance tells us the relative number of atoms of each element it contains. Thus, the formula H_2O indicates that water contains two H atoms for each O atom. This ratio also applies on the molar level; thus, 1 mol of H_2O contains 2 mol of H atoms and 1 mol of O atoms. Conversely, the ratio of the number of moles of each element in a compound gives the subscripts in a compound's empirical formula. Thus, the mole concept provides a way of calculating the empirical formulas of chemical substances, as shown in the following examples.

Mercury forms a compound with chlorine that is 73.9% mercury and 26.1% chlorine by mass. This means that if we had a 100.0-g sample of the solid, it would contain 73.9 g of mercury (Hg) and 26.1 g of chlorine (Cl). (Any size sample can be used in problems of this type, but we will generally use 100.0 g to simplify the calculation of mass from percentage.) Using the atomic weights of the elements to give us molar masses, we then calculate the number of moles of each element in the sample:

P. K. Thamburaj, "A Known-to-Unknown Approach to Teach About Empirical and Molecular Formulas," *J. Chem. Educ.*, Vol. 78, 2001, 915–916.

Stephen DeMeo, "Making Assumptions Explicit: How the Law of Conservation of Matter Can Explain Empirical Formula Problems," *J. Chem. Educ.*, Vol. 78, 2001, 1050–1052.

An easy way to remember the strategy for converting percentage composition to an empirical formula. Joel S. Thompson, "Percent to mass, mass to mol, divide by small, multiply 'til whole". From "A Simple Rhyme for a Simple Formula," *J. Chem. Educ.*, Vol. 65, 1988, 704.

$$(73.9\ \text{g-Hg})\left(\frac{1\ \text{mol Hg}}{200.6\ \text{g-Hg}}\right) = 0.368\ \text{mol Hg}$$

$$(26.1\ \text{g-Cl})\left(\frac{1\ \text{mol Cl}}{35.5\ \text{g-Cl}}\right) = 0.735\ \text{mol Cl}$$

We then divide the larger number of moles (0.735) by the smaller (0.368) to obtain a Cl:Hg mole ratio of 1.99:1:

$$\frac{\text{moles of Cl}}{\text{moles of Hg}} = \frac{0.735\ \text{mol Cl}}{0.368\ \text{mol Hg}} = \frac{1.99\ \text{mol Cl}}{1\ \text{mol Hg}}$$

Because of experimental errors, the results may not lead to exact integers for the ratios of moles. The number 1.99 is very close to 2, so we can confidently conclude that the empirical formula for the compound is $HgCl_2$. This is the simplest, or empirical, formula because its subscripts are the smallest integers that express the *ratios* of atoms present in the compound. · · · · (Section 2.6) The general procedure for determining empirical formulas is outlined in Figure 3.11 ▶.

SAMPLE EXERCISE 3.13

Ascorbic acid (vitamin C) contains 40.92% C, 4.58% H, and 54.50% O by mass. What is the empirical formula of ascorbic acid?

Solution

Analyze: We are given the mass percentages of the elements in ascorbic acid and asked for its empirical formula.

Plan: The strategy for determining the empirical formula of a substance from its elemental composition involves the four steps given in Figure 3.11.

Solve: We first assume, for simplicity, that we have exactly 100 g of material (although any number can be used). In 100 g of ascorbic acid, we will have 40.92 g C, 4.58 g H, and 54.50 g O.

Second, we calculate the number of moles of each element in this sample:

$$\text{Moles C} = (40.92\ \text{g-C})\left(\frac{1\ \text{mol C}}{12.01\ \text{g-C}}\right) = 3.407\ \text{mol C}$$

$$\text{Moles H} = (4.58\ \text{g-H})\left(\frac{1\ \text{mol H}}{1.008\ \text{g-H}}\right) = 4.54\ \text{mol H}$$

$$\text{Moles O} = (54.50\ \text{g-O})\left(\frac{1\ \text{mol O}}{16.00\ \text{g-O}}\right) = 3.406\ \text{mol O}$$

Third, we determine the simplest whole-number ratio of moles by dividing each number of moles by the smallest number of moles, 3.406:

$$\text{C: } \frac{3.407}{3.406} = 1.000 \qquad \text{H: } \frac{4.54}{3.406} = 1.33 \qquad \text{O: } \frac{3.406}{3.406} = 1.000$$

The ratio for H is too far from 1 to attribute the difference to experimental error; in fact, it is quite close to $1\frac{1}{3}$. This suggests that if we multiply the ratio by 3, we will obtain whole numbers:

$$\text{C:H:O} = 3(1:1.33:1) = 3:4:3$$

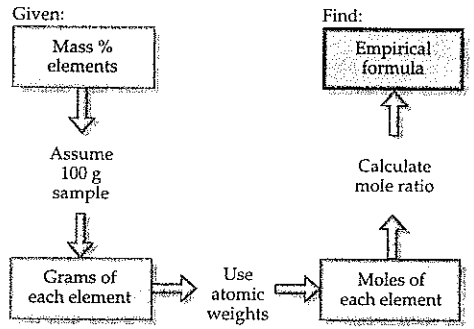
The whole-number mole ratio gives us the subscripts for the empirical formula. Thus, the empirical formula is $C_3H_4O_3$

Check: It is reassuring that the subscripts are moderately sized whole numbers. Otherwise, we have little by which to judge the reasonableness of our answer.

PRACTICE EXERCISE

A 5.325-g sample of methyl benzoate, a compound used in the manufacture of perfumes, is found to contain 3.758 g of carbon, 0.316 g of hydrogen, and 1.251 g of oxygen. What is the empirical formula of this substance?

Answer: C_4H_4O



◀ **Figure 3.11** Outline of the procedure used to calculate the empirical formula of a substance from its percentage composition. The procedure is also summarized as "percent to mass, mass to mole, divide by small, multiply 'til whole."

ACTIVITY
Molecular Formula Determination: C_8H_6O

Molecular Formula from Empirical Formula

The formula obtained from percentage compositions is always the empirical formula. We can obtain the molecular formula from the empirical formula if we know the molecular weight of the compound. *The subscripts in the molecular formula of a substance are always a whole-number multiple of the corresponding subscripts in its empirical formula.* · · · · (Section 2.6) The multiple is found by comparing the empirical formula weight with the molecular weight. In Sample Exercise 3.13, for example, the empirical formula of ascorbic acid was determined to be $C_3H_4O_3$, giving an empirical formula weight of 3(12.0 amu) + 4(1.0 amu) + 3(16.0 amu) = 88.0 amu. The experimentally determined molecular weight is 176 amu. Thus, the molecule has twice the mass (176/88.0 = 2.00) and must therefore have twice as many atoms of each kind as are given in the empirical formula. Consequently, the subscripts in the empirical formula must be multiplied by 2 to obtain the molecular formula: $C_6H_8O_6$.

An exploration of color change associated with the dehydration of copper sulfate. Lee R. Summerlin, Christie L. Borgford, and Julie B. Ealy, "Copper Sulfate: Blue to White," *Chemical Demonstrations, A Sourcebook for Teachers*, Vol. 2 (American Chemical Society, Washington, DC, 1988) pp. 69–70.

SAMPLE EXERCISE 3.14

Mesitylene, a hydrocarbon that occurs in small amounts in crude oil, has an empirical formula of C_3H_4. The experimentally determined molecular weight of this substance is 121 amu. What is the molecular formula of mesitylene?

Solution

Analyze: We are given the empirical formula and molecular weight of mesitylene and asked to determine its molecular formula.

Plan: The subscripts in a molecular formula are whole-number multiples of the subscripts in its empirical formula. To find the appropriate multiple, we must compare the molecular weight with the formula weight of the empirical formula.

Solve: First, we calculate the formula weight of the empirical formula, C_3H_4:

$$3(12.0\ \text{amu}) + 4(1.0\ \text{amu}) = 40.0\ \text{amu}$$

Next, we divide the molecular weight by the empirical formula weight to obtain the factor used to multiply the subscripts in C_3H_4:

$$\frac{\text{molecular weight}}{\text{empirical formula weight}} = \frac{121}{40.0} = 3.02$$

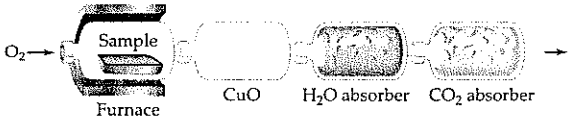
Only whole-number ratios make physical sense because we must be dealing with whole atoms. The 3.02 in this case results from a small experimental error in the molecular weight. We therefore multiply each subscript in the empirical formula by 3 to give the molecular formula: C_9H_{12}.

Check: We can have confidence in the result because dividing the molecular weight by the formula weight yields nearly a whole number.

PRACTICE EXERCISE

Ethylene glycol, the substance used in automobile antifreeze, is composed of 38.7% C, 9.7% H, and 51.6% O by mass. Its molar mass is 62.1 g/mol. **(a)** What is the empirical formula of ethylene glycol? **(b)** What is its molecular formula?

Answers: **(a)** CH_3O; **(b)** $C_2H_6O_2$



▶ **Figure 3.12** Apparatus to determine percentages of carbon and hydrogen in a compound. Copper oxide helps to oxidize traces of carbon and carbon monoxide to carbon dioxide and to oxidize hydrogen to water.

Combustion analysis works only if complete combustion occurs:
Fuel + O_2 ⟶ CO_2 + H_2O

MOVIE
Reduction of CuO

The combustion of methane, propane, and butane are compared in this simple demonstration of stoichiometry. M. Dale Alexander and Wayne C. Wolsey, "Combustion of Hydrocarbons: A Stoichiometry Demonstration," *J. Chem. Educ.*, Vol. 70, 1993, 327–328.

Combustion Analysis

The empirical formula of a compound is based on experiments that give the number of moles of each element in a sample of the compound. That is why we use the word "empirical," which means "based on observation and experiment." Chemists have devised a number of different experimental techniques to determine the empirical formulas of compounds. One of these is combustion analysis, which is commonly used for compounds containing principally carbon and hydrogen as their component elements.

When a compound containing carbon and hydrogen is completely combusted in an apparatus such as that shown in Figure 3.12 ▲, all the carbon in the compound is converted to CO_2, and all the hydrogen is converted to H_2O. (Section 3.2) The amounts of CO_2 and H_2O produced are determined by measuring the mass increase in the CO_2 and H_2O absorbers. From the masses of CO_2 and H_2O we can calculate the number of moles of C and H in the original compound and thereby the empirical formula. If a third element is present in the compound, its mass can be determined by subtracting the masses of C and H from the compound's original mass. Sample Exercise 3.15 shows how to determine the empirical formula of a compound containing C, H, and O.

SAMPLE EXERCISE 3.15

Isopropyl alcohol, a substance sold as rubbing alcohol, is composed of C, H, and O. Combustion of 0.255 g of isopropyl alcohol produces 0.561 g CO_2 and 0.306 g H_2O. Determine the empirical formula of isopropyl alcohol.

Solution

Analyze: We are given the quantities of CO_2 and H_2O produced when a given quantity of isopropyl alcohol is combusted. We must use this information to determine the empirical formula for the isopropyl alcohol, a task that requires us to calculate the number of moles of C, H, and O in the sample.

Plan: We can use the mole concept to calculate the number of grams of C present in the CO_2 and the number of grams of H present in the H_2O. These are the quantities of C and H present in the isopropyl alcohol before combustion. The number of grams of O in the compound equals the mass of the isopropyl alcohol minus the sum of the C and H masses. Once we have the number of grams of C, H, and O in the sample, we can then proceed as in Sample Exercise 3.13: Calculate the number of moles of each element, and determine the mole ratio, which gives the subscripts in the empirical formula.

Solve: To calculate the number of grams of C, we first use the molar mass of CO_2, 1 mol CO_2 = 44.0 g CO_2, to convert grams of CO_2 to moles of CO_2. Because there is only 1 C atom in each CO_2 molecule, there is 1 mol of C atoms per mole of CO_2 molecules. This fact allows us to convert the moles of CO_2 to moles of C. Finally, we use the molar mass of C, 1 mol C = 12.0 g C, to convert moles of C to grams of C. Combining the three conversion factors, we have

$$\text{Grams C} = (0.561\ \text{g}\ \cancel{\text{CO}_2})\left(\frac{1\ \text{mol}\ \cancel{\text{CO}_2}}{44.0\ \text{g}\ \cancel{\text{CO}_2}}\right)\left(\frac{1\ \text{mol}\ \cancel{\text{C}}}{1\ \text{mol}\ \cancel{\text{CO}_2}}\right)\left(\frac{12.0\ \text{g C}}{1\ \text{mol}\ \cancel{\text{C}}}\right) = 0.153\ \text{g C}$$

The calculation of the number of grams of H from the grams of H_2O is similar, although we must remember that there are 2 mol of H atoms per 1 mol of H_2O molecules:

$$\text{Grams H} = (0.306\ \text{g}\ \cancel{\text{H}_2\text{O}})\left(\frac{1\ \text{mol}\ \cancel{\text{H}_2\text{O}}}{18.0\ \text{g}\ \cancel{\text{H}_2\text{O}}}\right)\left(\frac{2\ \text{mol}\ \cancel{\text{H}}}{1\ \text{mol}\ \cancel{\text{H}_2\text{O}}}\right)\left(\frac{1.01\ \text{g H}}{1\ \text{mol}\ \cancel{\text{H}}}\right) = 0.0343\ \text{g H}$$

If the compound contains only C, H, and O, the mass of the oxygen is the difference between the total mass and the masses of C and H.

The total mass of the sample, 0.255 g, is the sum of the masses of the C, H, and O. Thus, we can calculate the mass of O as follows:

$$\text{Mass of O} = \text{mass of sample} - (\text{mass of C} + \text{mass of H})$$
$$= 0.255\ \text{g} - (0.153\ \text{g} + 0.0343\ \text{g}) = 0.068\ \text{g O}$$

We then calculate the number of moles of C, H, and O in the sample:

$$\text{Moles C} = (0.153\ \text{g}\ \cancel{\text{C}})\left(\frac{1\ \text{mol C}}{12.0\ \text{g}\ \cancel{\text{C}}}\right) = 0.0128\ \text{mol C}$$

$$\text{Moles H} = (0.0343\ \text{g}\ \cancel{\text{H}})\left(\frac{1\ \text{mol H}}{1.01\ \text{g}\ \cancel{\text{H}}}\right) = 0.0340\ \text{mol H}$$

$$\text{Moles O} = (0.068\ \text{g}\ \cancel{\text{O}})\left(\frac{1\ \text{mol O}}{16.0\ \text{g}\ \cancel{\text{O}}}\right) = 0.0043\ \text{mol O}$$

To find the empirical formula, we must compare the relative number of moles of each element in the sample. The relative number of moles of each element is found by dividing each number by the smallest number, 0.0043. The mole ratio of C : H : O so obtained is 2.98 : 7.91 : 1.00. The first two numbers are very close to the whole numbers 3 and 8, giving the empirical formula C_3H_8O.

PRACTICE EXERCISE

(a) Caproic acid, which is responsible for the foul odor of dirty socks, is composed of C, H, and O atoms. Combustion of a 0.225-g sample of this compound produces 0.512 g CO_2 and 0.209 g H_2O. What is the empirical formula of caproic acid? **(b)** Caproic acid has a molar mass of 116 g/mol. What is its molecular formula?

Answers: **(a)** C_3H_6O; **(b)** $C_6H_{12}O_2$

Due to experimental or round-off errors, the coefficients may come out close to whole numbers (see text). You should then round them off to that whole number. However, if the coefficients come out close to common fractions (e.g., $\frac{1}{4}$, $\frac{1}{3}$, $\frac{1}{2}$), the formula should be multiplied by the least common denominator (4, 3, 2, respectively) and *not* rounded.

3.6 Quantitative Information from Balanced Equations

The mole concept allows us to use the quantitative information available in a balanced equation on a practical macroscopic level. Consider the following balanced equation:

$$2H_2(g) + O_2(g) \longrightarrow 2H_2O(l) \qquad [3.11]$$

The coefficients tell us that two molecules of H_2 react with each molecule of O_2 to form two molecules of H_2O. It follows that the relative numbers of moles are identical to the relative numbers of molecules:

$2H_2(g)$	+	$O_2(g)$	⟶	$2H_2O(l)$
2 molecules		1 molecule		2 molecules
$2(6.02 \times 10^{23}$ molecules)		6.02×10^{23} molecules		$2(6.02 \times 10^{23}$ molecules)
2 mol		1 mol		2 mol

The coefficients in a balanced chemical equation can be interpreted both as the relative numbers of molecules (or formula units) involved in the reaction and as the relative numbers of moles.

The quantities 2 mol H_2, 1 mol O_2, and 2 mol H_2O, which are given by the coefficients in Equation 3.11, are called *stoichiometrically equivalent quantities*. The relationship between these quantities can be represented as

$$2\ \text{mol}\ H_2 \triangleq 1\ \text{mol}\ O_2 \triangleq 2\ \text{mol}\ H_2O$$

where the symbol $\triangleq$ means "stoichiometrically equivalent to." In other words, Equation 3.11 shows 2 mol of H_2 and 1 mol of O_2 forming 2 mol of H_2O. These stoichiometric relations can be used to convert between quantities of reactants

A Closer Look Using Spectroscopic Methods to Measure Reaction Rates

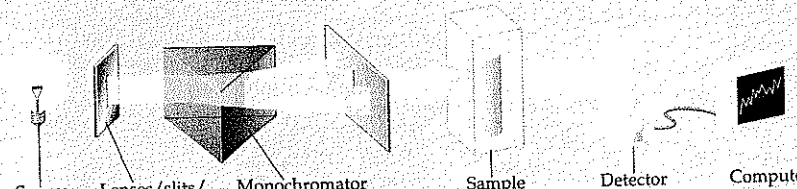
A variety of techniques can be used to monitor the concentration of a reactant or product during a reaction. Spectroscopic methods, which rely on of the ability of substances to absorb (or emit) electromagnetic radiation, are some of the most useful. Spectroscopic kinetic studies are often performed with the reaction mixture in the sample compartment of the spectrometer. The spectrometer is set to measure the light absorbed at a wavelength characteristic of one of the reactants or products. In the decomposition of HI(g) into $H_2(g)$ and $I_2(g)$, for example, both HI and H_2 are colorless, whereas I_2 is violet. During the course of the reaction, the color increases in intensity as I_2 forms. Thus, visible light of appropriate wavelength can be used to monitor the reaction.

Figure 14.5 ▼ shows the basic components of a spectrometer. The spectrometer measures the amount of light absorbed by the sample by comparing the intensity of the light emitted from the light source with the intensity of the light that emerges from the sample. As the concentration of I_2 increases and its color becomes more intense, the amount of light absorbed by the reaction mixture increases, causing less light to reach the detector.

Beer's law relates the amount of light being absorbed to the concentration of the substance absorbing the light:

$$A = abc \qquad [14.5]$$

In this equation A is the measured absorbance, a is the molar absorptivity constant (a characteristic of the substance being monitored), b is the path length through which the radiation must pass, and c is the molar concentration of the absorbing substance. Thus, the concentration is directly proportional to absorbance.



▲ **Figure 14.5** Basic components of a spectrometer.

14.3 Concentration and Rate

One way of studying the effect of concentration on reaction rate is to determine the way in which the rate at the beginning of a reaction (the initial rate) depends on the starting concentrations. To illustrate this approach, consider the following reaction:

$$NH_4^+(aq) + NO_2^-(aq) \longrightarrow N_2(g) + 2H_2O(l)$$

We might study the rate of this reaction by measuring the concentration of NH_4^+ or NO_2^- as a function of time or by measuring the volume of N_2 collected. Because the stoichiometric coefficients on NH_4^+, NO_2^-, and N_2 are all the same, all of these rates will be equal.

Once we determine the initial reaction rate for various starting concentrations of NH_4^+ and NO_2^-, we can tabulate the data as shown in Table 14.2 ▼.

Lionello Pogliani and Mario N. Berberan-Santos, "Inflation Rates, Car Devaluation, and Chemical Kinetics," *J. Chem. Educ.*, Vol. 73, 1996, 950–952.

ACTIVITY
Rates of Reactions

TABLE 14.2 Rate Data for the Reaction of Ammonium and Nitrite Ions in Water at 25 °C

Experiment Number	Initial NH_4^+ Concentration (M)	Initial NO_2^- Concentration (M)	Observed Initial Rate (M/s)
1	0.0100	0.200	5.4×10^{-7}
2	0.0200	0.200	10.8×10^{-7}
3	0.0400	0.200	21.5×10^{-7}
4	0.0600	0.200	32.3×10^{-7}
5	0.200	0.0202	10.8×10^{-7}
6	0.200	0.0404	21.6×10^{-7}
7	0.200	0.0606	32.4×10^{-7}
8	0.200	0.0808	43.3×10^{-7}

These data indicate that changing either $[NH_4^+]$ or $[NO_2^-]$ changes the reaction rate. If we double $[NH_4^+]$ while holding $[NO_2^-]$ constant, the rate doubles (compare experiments 1 and 2). If $[NH_4^+]$ is increased by a factor of 4 (compare experiments 1 and 3), the rate changes by a factor of 4, and so forth. These results indicate that the rate is proportional to $[NH_4^+]$ raised to the first power. When $[NO_2^-]$ is similarly varied while $[NH_4^+]$ is held constant, the rate is affected in the same manner. We conclude that the rate is also directly proportional to the concentration of NO_2^-. We can express the overall concentration dependence as follows:

$$\text{Rate} = k[NH_4^+][NO_2^-] \qquad [14.6]$$

An equation such as Equation 14.6, which shows how the rate depends on the concentrations of reactants, is called a **rate law**. For a general reaction,

$$aA + bB \longrightarrow cC + dD$$

the rate law generally has the form

$$\text{Rate} = k[A]^m[B]^n \qquad [14.7]$$

The constant k in the rate law is called the **rate constant**. The magnitude of k changes with temperature and therefore determines how temperature affects rate, as we will see in Section 14.5. The exponents m and n are typically small whole numbers (usually 0, 1, or 2). We will consider these exponents more closely very shortly.

If we know the rate law for a reaction and its rate for a set of reactant concentrations, we can calculate the value of the rate constant, k. For example, using the data in Table 14.2 and the results from experiment 1, we can substitute into Equation 14.6:

$$5.4 \times 10^{-7}\ M/\text{s} = k(0.0100\ M)(0.200\ M)$$

Solving for k gives

$$k = \frac{5.4 \times 10^{-7}\ M/\text{s}}{(0.0100\ M)(0.200\ M)} = 2.7 \times 10^{-4}\ M^{-1}\,\text{s}^{-1}$$

You may wish to verify that this same value of k is obtained using any of the other experimental results given in Table 14.2.

Once we have both the rate law and the value of the rate constant for a reaction, we can calculate the rate of reaction for any set of concentrations. For example, using Equation 14.6 and $k = 2.7 \times 10^{-4}\ M^{-1}\text{s}^{-1}$, we can calculate the rate for $[NH_4^+] = 0.100\ M$ and $[NO_2^-] = 0.100\ M$:

$$\text{Rate} = (2.7 \times 10^{-4}\ M^{-1}\text{s}^{-1})(0.100\ M)(0.100\ M) = 2.7 \times 10^{-6}\ M/\text{s}$$

Exponents in the Rate Law

The rate laws for most reactions have the general form

$$\text{Rate} = k[\text{reactant 1}]^m[\text{reactant 2}]^n \ldots \qquad [14.8]$$

The exponents m and n in a rate law are called **reaction orders**. For example, consider again the rate law for the reaction of NH_4^+ with NO_2^-:

$$\text{Rate} = k[NH_4^+][NO_2^-]$$

Because the exponent of $[NH_4^+]$ is one, the rate is *first order* in NH_4^+. The rate is also first order in NO_2^-. (The exponent "1" is not shown explicitly in rate laws.) The **overall reaction order** is the sum of the orders with respect to each reactant in the rate law. Thus, the rate law has an overall reaction order of $1 + 1 = 2$, and the reaction is *second order overall*.

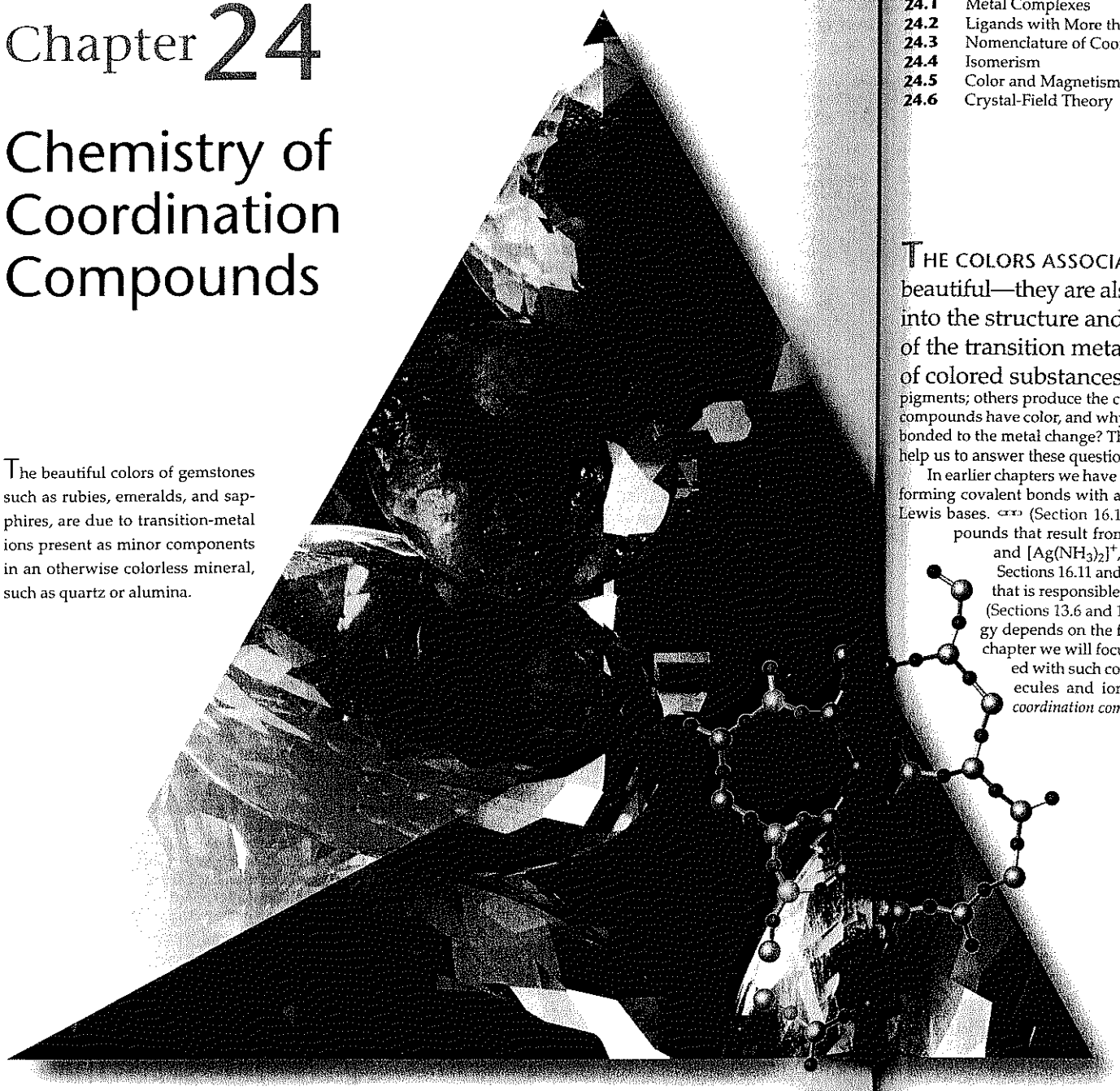
Charles J. Marzzacco, "An Analogy to Help Students Understand Reaction Orders," *J. Chem. Educ.*, Vol. 75, 1998, 482.

34

Equation 14.6 follows from the original observation that the rate $\propto [NH_4^+][NO_2]$.

Chapter 24

Chemistry of Coordination Compounds



The beautiful colors of gemstones such as rubies, emeralds, and sapphires, are due to transition-metal ions present as minor components in an otherwise colorless mineral, such as quartz or alumina.

24.1 Metal Complexes
24.2 Ligands with More than One Donor Atom
24.3 Nomenclature of Coordination Chemistry
24.4 Isomerism
24.5 Color and Magnetism
24.6 Crystal-Field Theory

THE COLORS ASSOCIATED with chemistry are not only beautiful—they are also informative, providing insights into the structure and bonding of matter. Compounds of the transition metals constitute an important group of colored substances. Some of them are used in paint pigments; others produce the colors in glass and precious gems. Why do these compounds have color, and why do these colors change as the ions or molecules bonded to the metal change? The chemistry that we explore in this chapter will help us to answer these questions.

In earlier chapters we have seen that metal ions can function as Lewis acids, forming covalent bonds with a variety of molecules and ions that function as Lewis bases. (Section 16.11) We have encountered many ions and compounds that result from such interactions. We discussed $[Fe(H_2O)_6]^{3+}$ and $[Ag(NH_3)_2]^+$, for example, in our coverage of equilibria in Sections 16.11 and 17.5. Hemoglobin, an important iron compound that is responsible for the oxygen-carrying capacity of blood. (Sections 13.6 and 18.4) In Section 23.3 we saw that hydrometallurgy depends on the formation of species such as $[Au(CN)_2]^-$. In this chapter we will focus on the rich and important chemistry associated with such complex assemblies of metals surrounded by molecules and ions. Metal compounds of this kind are called *coordination compounds*.

▶ What's Ahead ◀

- We begin by introducing the concepts of *metal complexes* and *ligands*, and providing a brief history of the development of *coordination chemistry*.
- Next we examine some of the common geometries exhibited by coordination complexes for different *coordination numbers*.
- Our discussion then turns to *polydentate ligands*, which are ligands with more than one *donor atom*, and to some of their special properties, including their important roles in biological systems.
- We introduce the *nomenclature* used to name coordination compounds.
- Coordination compounds exhibit *isomerism*, in which two compounds have the same composition but different structures. The types of isomerism exhibited by coordination compounds are described, including *structural isomers*, *geometric isomers*, and *optical isomers*, which are two isomers of a compound that are mirror images of one another.
- We will discuss the basic notions of *color* and *magnetism* in coordination compounds.
- In order to explain some of the interesting spectral and magnetic properties of coordination compounds, we present the *crystal-field theory*.

24.1 Metal Complexes

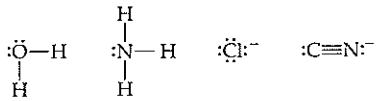
The term *coordination* comes from the fact that ligands form coordinate bonds with the metal. That is, both bonding electrons come from one of the atoms of the ligand. Chemists also use the term *dative* to describe the bonding of a ligand to a metal center.

R. Bruce Martin, "A Stability Ruler for Metal Ion Complexes," *J. Chem. Educ.*, Vol. 64, 1987, 402.

Ligands bind to metal ions through unshared pairs of electrons (lone pairs) on the donor atom.

Species such as $[Ag(NH_3)_2]^+$ that are assemblies of a central metal ion bonded to a group of surrounding molecules or ions are called **metal complexes** or merely *complexes*. If the complex carries a net charge, it is generally called a *complex ion*. (Section 17.5) Compounds that contain complexes are known as **coordination compounds**. Most of the coordination compounds that we will examine contain transition-metal ions, although ions of other metals can form complexes as well.

The molecules or ions that surround the metal ion in a complex are known as **ligands** (from the Latin word *ligare*, meaning "to bind"). There are two NH_3 ligands bonded to Ag^+ in $[Ag(NH_3)_2]^+$. Ligands are normally either anions or polar molecules. Every ligand has at least one unshared pair of valence electrons, as illustrated in the following examples:



In forming a complex, the ligands are said to *coordinate* to the metal.

The Development of Coordination Chemistry: Werner's Theory

Because compounds of the transition metals exhibit beautiful colors, the chemistry of these elements greatly fascinated chemists even before the periodic table was introduced. In the late 1700s through the 1800s many coordination compounds were isolated and studied. These compounds showed properties that seemed puzzling in light of the bonding theories at the time. Table 24.1 ▼, for example, lists a series of compounds that result from the reaction of cobalt(III) chloride with ammonia. These compounds have strikingly different colors. Even the last two listed, which were both formulated as $CoCl_3 \cdot 4NH_3$, have different colors.

Lee R. Summerlin and James L. Ealy, Jr., "Cobalt Complexes: Changing Coordination Numbers," *Chemical Demonstrations, A Sourcebook for Teachers*, Vol. 1 (American Chemical Society, Washington, DC, 1988) pp. 41–42. The coordination number of cobalt in cobalt chloride solutions is changed as the solutions are mixed with varying amounts of ethanol.

All the compounds in Table 24.1 are strong electrolytes (Section 4.1), but they yield different numbers of ions when dissolved in water. For example, dissolving $CoCl_3 \cdot 6NH_3$ in water yields four ions per formula unit, whereas $CoCl_3 \cdot 5NH_3$ yields only three ions per formula unit. Furthermore, the reaction of the compounds with excess aqueous silver nitrate leads to the precipitation of variable amounts of AgCl(*s*); the precipitation of AgCl(*s*) in this way is often used to test for the number of "free" Cl^- ions in an ionic compound. When $CoCl_3 \cdot 6NH_3$ is treated with excess $AgNO_3(aq)$, three moles of AgCl(*s*) are produced per mole of complex, so all three Cl^- ions in the formula can react to form AgCl(*s*). By contrast, when $CoCl_3 \cdot 5NH_3$ is treated with $AgNO_3(aq)$ in an analogous fashion, only two moles of AgCl(*s*) precipitate per mole of complex; one of the Cl^- ions in the compound does not react to form AgCl(*s*). These results are summarized in Table 24.1.

TABLE 24.1 Properties of Some Ammonia Complexes of Cobalt(III)

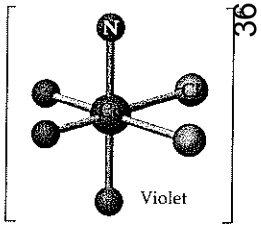
Original Formulation	Color	Ions per Formula Unit	"Free" Cl^- Ions per Formula Unit	Modern Formulation
$CoCl_3 \cdot 6NH_3$	Orange	4	3	$[Co(NH_3)_6]Cl_3$
$CoCl_3 \cdot 5NH_3$	Purple	3	2	$[Co(NH_3)_5Cl]Cl_2$
$CoCl_3 \cdot 4NH_3$	Green	2	1	*trans*-$[Co(NH_3)_4Cl_2]Cl$
$CoCl_3 \cdot 4NH_3$	Violet	2	1	*cis*-$[Co(NH_3)_4Cl_2]Cl$

In 1893 the Swiss chemist Alfred Werner (1866–1919) proposed a theory that successfully explained the observations in Table 24.1, and it became the basis for understanding coordination chemistry. Werner proposed that metal ions exhibit both "primary" and "secondary" valences. The primary valence is the oxidation state of the metal, which for the complexes in Table 24.1 is +3. (Section 4.4) The secondary valence is the number of atoms directly bonded to the metal ion, which is also called the **coordination number**. For these cobalt complexes, Werner deduced a coordination number of six with the ligands in an octahedral arrangement (Figure 9.9) around the Co ion.

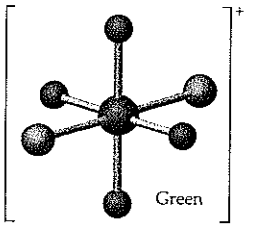
Werner's theory provided a beautiful explanation for the results in Table 24.1. The NH_3 molecules in the complexes are ligands that are bonded to the Co ion; if there are fewer than six NH_3 molecules, the remaining ligands are Cl^- ions. The central metal and the ligands bound to it constitute the **coordination sphere** of the complex. In writing the chemical formula for a coordination compound, Werner suggested using square brackets to set off the groups within the coordination sphere from other parts of the compound. He therefore proposed that $CoCl_3 \cdot 6NH_3$ and $CoCl_3 \cdot 5NH_3$ are better written as $[Co(NH_3)_6]Cl_3$ and $[Co(NH_3)_5Cl]Cl_2$, respectively. He further proposed that the chloride ions that are part of the coordination sphere are bound so tightly that they do not become freed up when the complex is dissolved in water. Thus, dissolving $[Co(NH_3)_5Cl]Cl_2$ in water produces a $[Co(NH_3)_5Cl]^{2+}$ ion and two Cl^- ions; only the two "free" Cl^- ions are able to react with $Ag^+(aq)$ to form AgCl(*s*).

Werner's ideas also explained why there are two distinctly different forms of $CoCl_3 \cdot 4NH_3$. Using Werner's postulates, we formulate the compound as $[Co(NH_3)_4Cl_2]Cl$. As shown in Figure 24.1 ▶, there are two different ways to arrange the ligands in the $[Co(NH_3)_4Cl_2]^+$ complex, called the *cis* and *trans* forms. In *cis*-$[Co(NH_3)_4Cl_2]^+$ the two chloride ligands occupy adjacent vertices of the octahedral arrangement. In *trans*-$[Co(NH_3)_4Cl_2]^+$ the chlorides are opposite one another. As seen in Table 24.1, the difference in these arrangements causes the complexes to have different colors.

The insight into the bonding in coordination compounds that Werner provided is even more remarkable when we realize that his theory predated Lewis's ideas of covalent bonding by more than 20 years! Because of his tremendous contributions to coordination chemistry, Werner was awarded the 1913 Nobel Prize in chemistry.



(a)



(b)

▲ **Figure 24.1** The two forms (isomers) of the complex $[Co(NH_3)_4Cl_2]^+$. In (a) *cis*-$[Co(NH_3)_4Cl_2]^+$ the two Cl ligands occupy adjacent vertices of the octahedron, whereas in (b) *trans*-$[Co(NH_3)_4Cl_2]^+$ they are opposite one another. (The blue spheres represent the coordinated NH_3 ligands.)

SAMPLE EXERCISE 24.1

Palladium(II) tends to form complexes with a coordination number of 4. One such compound was originally formulated as $PdCl_2 \cdot 3NH_3$. **(a)** Suggest the appropriate coordination compound formulation for this compound. (b) Suppose an aqueous solution of the compound is treated with excess $AgNO_3(aq)$. How many moles of AgCl(*s*) are formed per mole of $PdCl_2 \cdot 3NH_3$?

Solution **(a) Analyze and Plan:** We are given the coordination number of Pd(II) and the other groups in the compound. To write the formula correctly, we need to determine what ligands are attached to Pd(II) in the compound.

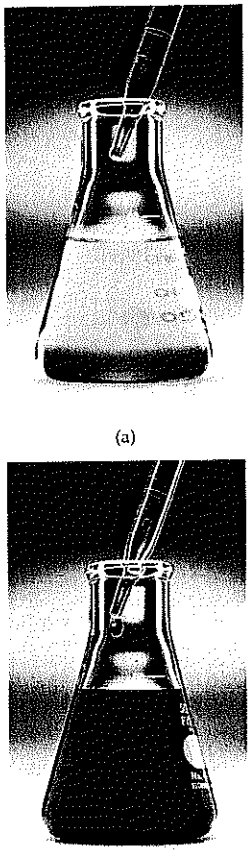
Solve: By analogy to the ammonia complexes of cobalt(III), we might expect that the three NH_3 groups of $PdCl_2 \cdot 3NH_3$ serve as ligands attached to the Pd(II) ion. The fourth ligand around Pd(II) is one of the chloride ions. The second chloride ion is not a ligand; it serves only as an anion in this ionic compound. We conclude that the correct formulation is $[Pd(NH_3)_3Cl]Cl$.

(b) We expect that the chloride ion that serves as a ligand will not be precipitated as AgCl(*s*) following the reaction with $AgNO_3(aq)$. Thus, only the single "free" Cl^- can react. We therefore expect to produce one mole of AgCl(*s*) per mole of complex. The balanced equation is the following:

$$[Pd(NH_3)_3Cl]Cl(aq) + AgNO_3(aq) \longrightarrow [Pd(NH_3)_3Cl]NO_3(aq) + AgCl(s)$$

This is a metathesis reaction (Section 4.2) in which one of the cations is the $[Pd(NH_3)_3Cl]^+$ complex ion.

Metal-ligand bonds have some covalent character in addition to ionic character.



(a)

(b)

▲ **Figure 24.2** When an aqueous solution of NH_4SCN is added to an aqueous solution of Fe^{3+}, the intensely colored $[Fe(H_2O)_5NCS]^{2+}$ ion is formed.

PRACTICE EXERCISE

Predict the number of ions produced per formula unit in an aqueous solution of $CoCl_2 \cdot 6H_2O$.
Answer: three (the complex ion and two chloride ions)

The Metal-Ligand Bond

The bond between a ligand and a metal ion is an example of an interaction between a Lewis base and a Lewis acid. (Section 16.11) Because the ligands have unshared pairs of electrons, they can function as Lewis bases (electron-pair donors). Metal ions (particularly transition-metal ions) have empty valence orbitals, so they can act as Lewis acids (electron-pair acceptors). We can picture the bond between the metal ion and ligand as the result of their sharing a pair of electrons that was initially on the ligand:

$$Ag^+(aq) + 2{:}\underset{\displaystyle H}{\overset{\displaystyle H}{\underset{|}{\overset{|}{N}}}}{-}H(aq) \longrightarrow \left[\begin{array}{c} H \quad H \\ | \quad | \\ H{-}N{:}Ag{:}N{-}H \\ | \quad | \\ H \quad H \end{array}\right]^+ (aq) \qquad [24.1]$$

The formation of metal-ligand bonds can markedly alter the properties we observe for the metal ion. A metal complex is a distinct chemical species that has physical and chemical properties different from the metal ion and the ligands from which it is formed. Complexes, for example, may have colors that differ dramatically from those of their component metal ions and ligands. Figure 24.2 ◀ shows the color change that occurs when aqueous solutions of SCN^- and Fe^{3+} are mixed, forming $[Fe(H_2O)_5NCS]^{2+}$.

Complex formation can also significantly change other properties of metal ions, such as their ease of oxidation or reduction. Ag^+, for example, is readily reduced in water.

$$Ag^+(aq) + e^- \longrightarrow Ag(s) \qquad E° = +0.799\ V \qquad [24.2]$$

In contrast, the $[Ag(CN)_2]^-$ ion is not so easily reduced because complexation by CN^- ions stabilizes silver in the +1 oxidation state.

$$[Ag(CN)_2]^-(aq) + e^- \longrightarrow Ag(s) + 2CN^-(aq) \qquad E° = -0.31\ V \qquad [24.3]$$

Hydrated metal ions are actually complex ions in which the ligand is water. Thus, $Fe^{3+}(aq)$ consists largely of $[Fe(H_2O)_6]^{3+}$. (Section 16.11) Complex ions form in aqueous solutions from reactions in which ligands such as NH_3, SCN^-, and CN^- replace H_2O molecules in the coordination sphere of the metal ion.

Charges, Coordination Numbers, and Geometries

The charge of a complex is the sum of the charges on the central metal and on its surrounding ligands. In $[Cu(NH_3)_4]SO_4$ we can deduce the charge on the complex if we first recognize that SO_4 represents the sulfate ion and therefore has a 2− charge. Because the compound is neutral, the complex ion must have a 2+ charge, $[Cu(NH_3)_4]^{2+}$. We can then use the charge of the complex ion to deduce the oxidation number of copper. Because the NH_3 ligands are neutral molecules, the oxidation number of copper must be +2.

$$\begin{array}{c} +2 + 4(0) = +2 \\ \searrow \ \downarrow \ \swarrow \\ [Cu(NH_3)_4]^{2+} \end{array}$$

SAMPLE EXERCISE 24.2

What is the oxidation number of the central metal in $[Rh(NH_3)_5Cl](NO_3)_2$?

Solution
Analyze and Plan: In order to determine the oxidation number of the Rh atom, we need to figure out what charges are contributed by the other groups in the substance. The overall charge is zero, so the oxidation number of the metal must balance the charge due to the rest of the compound.
Solve: The NO_3 group is the nitrate anion, which has a 1− charge, NO_3^-. The NH_3 ligands are neutral and the Cl is a coordinated chloride ion, which has a 1− charge, Cl^-. The sum of all the charges must be zero.

$$\begin{array}{c} x + 5(0) + (-1) + 2(-1) = 0 \\ \searrow \ \downarrow \ \downarrow \ \swarrow \\ [Rh(NH_3)_5Cl](NO_3)_2 \end{array}$$

The oxidation number of rhodium, x, must therefore be +3.

PRACTICE EXERCISE

What is the charge of the complex formed by a platinum(II) metal ion surrounded by two ammonia molecules and two bromide ions?
Answer: zero

SAMPLE EXERCISE 24.3

A complex ion contains a chromium(III) bound to four water molecules and two chloride ions. What is its formula?

Solution The oxidation state of the metal is +3, water is neutral, and chloride has a 1− charge:

$$\begin{array}{c} +3 + 4(0) + 2(-1) = +1 \\ \searrow \ \downarrow \ \swarrow \\ Cr(H_2O)_4Cl_2 \end{array}$$

The charge on the ion is 1+, $[Cr(H_2O)_4Cl_2]^+$.

PRACTICE EXERCISE

Write the formula for the complex described in the Practice Exercise accompanying Sample Exercise 24.2.
Answer: $[Pt(NH_3)_2Br_2]$

Recall that the number of atoms directly bonded to the metal atom in a complex is called the *coordination number*. The atom of the ligand bound directly to the metal is called the **donor atom**. Nitrogen, for example, is the donor atom in the $[Ag(NH_3)_2]^+$ complex shown in Equation 24.1. The silver ion in $[Ag(NH_3)_2]^+$ has a coordination number of 2, whereas each cobalt ion in the Co(III) complexes in Table 24.1 has a coordination number of 6.

The most common *donor atoms* are nitrogen, oxygen, and the halogens.

Some metal ions exhibit constant coordination numbers. The coordination number of chromium(III) and cobalt(III) is invariably 6, for example, and that of platinum(II) is always 4. The coordination numbers of most metal ions vary with the ligand, however. The most common coordination numbers are 4 and 6.

The coordination number of a metal ion is often influenced by the relative sizes of the metal ion and the surrounding ligands. As the ligand gets larger, fewer can coordinate to the metal ion. Thus, iron(III) is able to coordinate to six fluorides in $[FeF_6]^{3-}$ but coordinates to only four chlorides in $[FeCl_4]^-$. Ligands that transfer substantial negative charge to the metal also produce reduced coordination numbers. For example, six neutral ammonia molecules can coordinate to nickel(II), forming $[Ni(NH_3)_6]^{2+}$, but only four negatively charged cyanide ions can coordinate, forming $[Ni(CN)_4]^{2-}$.

D. Venkataraman, Yuhua Du, Scott R. Wilson, A. Hirsch, Peng Zhang, and Jeffrey S. Moore, "A Coordination Geometry Table of the *d*-Block Elements and Their Ions," *J. Chem. Educ.*, Vol. 74, 1997, 915–918.

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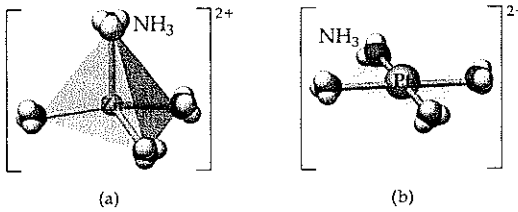
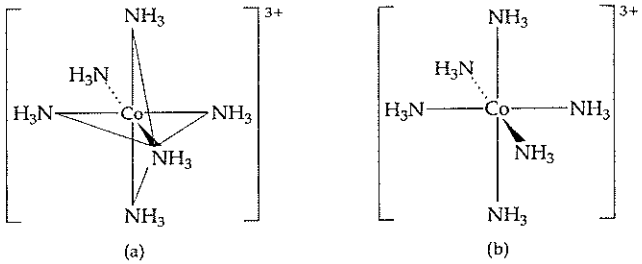


▶ **Figure 24.3** Structures of (a) $[Zn(NH_3)_4]^{2+}$ and (b) $[Pt(NH_3)_4]^{2+}$, illustrating the tetrahedral and square-planar geometries, respectively. These are the two common geometries for complexes in which the metal ion has a coordination number of 4.

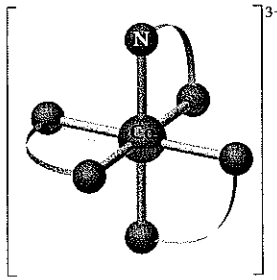


▶ **Figure 24.4** Two representations of an octahedral coordination sphere, the common geometric arrangement for complexes in which the metal ion has a coordination number of 6.

Bassam Z. Shakhashiri, "Precipitates and Complexes of Copper(II)," *Chemical Demonstrations: A Handbook for Teachers of Chemistry*, Vol. 1 (The University of Wisconsin Press, Madison, 1983) pp. 318–323. The sequential addition of various agents to beakers of $CuSO_4$ or $Cu(NO_3)_2$ yield a variety of colored copper complexes and precipitates.

Daniel T. Haworth, "Some Linguistic Detail on Chelation," *J. Chem. Educ.*, Vol. 75, 1998, 47.

3-D MODEL
Ethalyenediamine



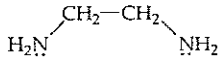
▲ **Figure 24.5** The $[Co(en)_3]^{3+}$ ion, showing how each bidentate ethylenediamine ligand is able to occupy two positions in the coordination sphere.

Four-coordinate complexes have two common geometries—tetrahedral and square planar—as shown in Figure 24.3 ▲. The tetrahedral geometry is the more common of the two and is especially common among nontransition metals. The square-planar geometry is characteristic of transition-metal ions with eight *d* electrons in the valence shell, such as platinum(II) and gold(III).

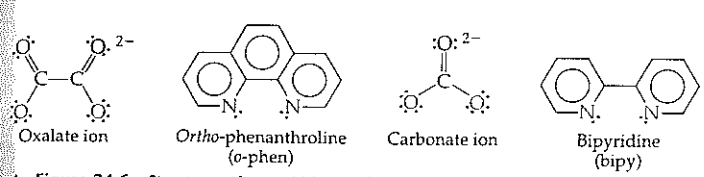
The vast majority of 6-coordinate complexes have an octahedral geometry, as shown in Figure 24.4(a) ▲. The octahedron is often represented as a planar square with ligands above and below the plane, as in Figure 24.4(b). Recall, however, that all positions on an octahedron are geometrically equivalent. (Section 9.2)

24.2 Ligands with More than One Donor Atom

The ligands that we have discussed so far, such as NH_3 and Cl^-, are called **monodentate ligands** (from the Latin, meaning "one-toothed"). These ligands possess a single donor atom and are able to occupy only one site in a coordination sphere. Some ligands have two or more donor atoms that can simultaneously coordinate to a metal ion, thereby occupying two or more coordination sites. They are called **polydentate ligands** ("many-toothed"). Because they appear to grasp the metal between two or more donor atoms, polydentate ligands are also known as **chelating agents** (from the Greek word *chele*, "claw"). One such ligand is *ethylenediamine*.



Ethylenediamine, which is abbreviated en, has two nitrogen atoms (shown in color) that have unshared pairs of electrons. These donor atoms are sufficiently far apart that the ligand can wrap around a metal ion with the two nitrogen atoms simultaneously bonding to the metal in adjacent positions. The $[Co(en)_3]^{3+}$ ion, which contains three ethylenediamine ligands in the octahedral coordination sphere of cobalt(III), is shown in Figure 24.5 ◀. Notice that the ethylenediamine has been written in a shorthand notation as two nitrogen atoms connected by an

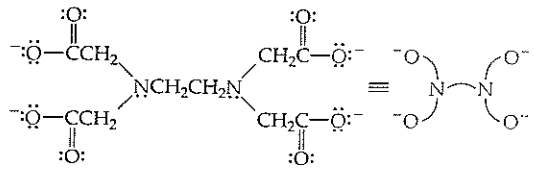


▲ **Figure 24.6** Structures of some bidentate ligands. The coordinating atoms are shown in blue.

3-D MODEL
Oxalate ion

arc. Ethylenediamine is a **bidentate ligand** ("two-toothed" ligand) because it can occupy two coordination sites. The structures of several other bidentate ligands are shown in Figure 24.6 ▲.

The ethylenediaminetetraacetate ion, abbreviated $[EDTA]^{4-}$, is an important polydentate ligand that has six donor atoms.



It can wrap around a metal ion using all six of these donor atoms, as shown in Figure 24.7 ▶, although it sometimes binds to a metal using only five of its six donor atoms.

In general, chelating ligands form more stable complexes than do related monodentate ligands. The formation constants for $[Ni(NH_3)_6]^{2+}$ and $[Ni(en)_3]^{2+}$, shown in Equations 24.4 and 24.5, illustrate this observation.

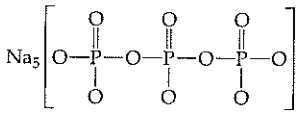
$$[Ni(H_2O)_6]^{2+}(aq) + 6NH_3(aq) \rightleftharpoons [Ni(NH_3)_6]^{2+}(aq) + 6H_2O(l) \qquad K_f = 1.2 \times 10^9 \qquad [24.4]$$

$$[Ni(H_2O)_6]^{2+}(aq) + 3en(aq) \rightleftharpoons [Ni(en)_3]^{2+}(aq) + 6H_2O(l) \qquad K_f = 6.8 \times 10^{17} \qquad [24.5]$$

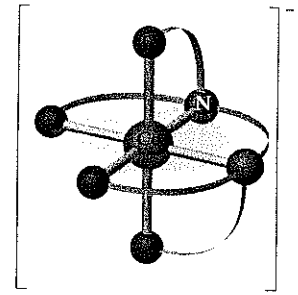
Although the donor atom is nitrogen in both instances, $[Ni(en)_3]^{2+}$ has a formation constant that is more than 10^8 times larger than that of $[Ni(NH_3)_6]^{2+}$. The generally larger formation constants for polydentate ligands as compared with the corresponding monodentate ligands is known as the **chelate effect**. We examine the origin of this effect in greater detail in "A Closer Look" in this section.

Chelating agents are often used to prevent one or more of the customary reactions of a metal ion without actually removing it from solution. For example, a metal ion that interferes with a chemical analysis can often be complexed and its interference thereby removed. In a sense, the chelating agent hides the metal ion. For this reason, scientists sometimes refer to these ligands as sequestering agents. (The word *sequester* means to remove, set apart, or separate.)

Phosphates such as sodium tripolyphosphate, shown here, are used to complex or sequester metal ions in hard water so these ions cannot interfere with the action of soap or detergents: (Section 18.6)



Mark Conway, Smallwood Holoman, Ladell Jones, Ray Leenhouts, and Gerald Williamson, "Selecting and Using Chelating Agents," *Chem. Eng.*, Vol. 106 (3), 1999, 86–90.

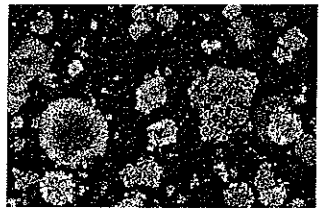


▲ **Figure 24.7** The $[CoEDTA]^-$ ion, showing how the ethylenediaminetetraacetate ion is able to wrap around a metal ion, occupying six positions in the coordination sphere.

J. Roger Hart, "EDTA-Type Chelating Agents in Everyday Consumer Products: Some Medicinal and Personal Care Products," *J. Chem. Educ.*, Vol. 61, 1984, 1060–1061.

3-D MODEL
$[EDTA]^{4-}$

38



▲ **Figure 24.8** Lichens growing on a rock surface. Lichens obtain the nutrients needed for growth from a variety of sources. Using chelating agents, they are able to extract needed metallic elements from the rocks on which they grow.

Chelating agents such as EDTA are used in consumer products, including many prepared foods such as salad dressings and frozen desserts, to complex trace metal ions that catalyze decomposition reactions. Chelating agents are used in medicine to remove metal ions such as Hg^{2+}, Pb^{2+}, and Cd^{2+}, which are detrimental to health. One method of treating lead poisoning is to administer $Na_2[Ca(EDTA)]$. The EDTA chelates the lead, allowing it to be removed from the body via urine. Chelating agents are also quite common in nature. Mosses and lichens secrete chelating agents to capture metal ions from the rocks they inhabit (Figure 24.8 ◀).

Metals and Chelates in Living Systems

Ten of the 29 elements known to be necessary for human life are transition metals. "Chemistry and Life," Section 2.7 These ten—V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, and Cd—owe their roles in living systems mainly to their ability to form complexes with a variety of donor groups present in biological systems. Metal ions are integral parts of many enzymes, which are the body's catalysts. (Section 14.7)

Although our bodies require only small quantities of metals, deficiencies can lead to serious illness. A deficiency of manganese, for example, can lead to convulsive disorders. Some epilepsy patients have been helped by the addition of manganese to their diets.

Among the most important chelating agents in nature are those derived from the *porphine* molecule, which is shown in Figure 24.10 ▶. This molecule can coordinate to a metal using the four nitrogen atoms as donors. Upon coordination to a metal, the two H atoms shown bonded to nitrogen are displaced. Complexes derived from porphine are called **porphyrins**. Different porphyrins contain different metal ions and have different substituent groups attached to the carbon atoms at the ligand's periphery. Two of the most important porphyrin or porphyrin-like compounds are *heme*, which contains Fe(II), and *chlorophyll*, which contains Mg(II).

Ei-Ichiro Ochiai, "Toxicity of Heavy Metals and Biological Defense: Principles and Applications in Bioinorganic Chemistry, Part VII," *J. Chem. Educ.*, Vol. 72, 1995, 479–484.

Colin J. Rix, "The Biochemistry of Some Iron Porphyrin Complexes," *J. Chem. Educ.*, Vol. 59, 1982, 389–392.

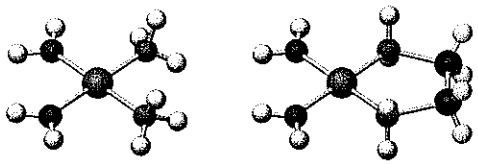
A Closer Look Entropy and the Chelate Effect

When we examined thermodynamics more closely in Chapter 19, we learned that many chemical processes are driven by positive changes in the entropy of the system. (Section 19.3) The special stability associated with the formation of chelates, called the *chelate effect*, can also be explained by looking at the entropy changes that occur when polydentate ligands bind to a metal ion. To understand this effect better, let's look at some reactions in which two H_2O ligands of the square-planar Cu(II) complex $[Cu(H_2O)_4]^{2+}$ are replaced by other ligands. First, let's consider replacing the H_2O ligands with NH_3 ligands at 27°C to form $[Cu(H_2O)_2(NH_3)_2]^{2+}$, the structure of which is shown in Figure 24.9(a) ▶.

$$[Cu(H_2O)_4]^{2+}(aq) + 2NH_3(aq) \rightleftharpoons [Cu(H_2O)_2(NH_3)_2]^{2+}(aq) + 2H_2O(l)$$

$$\Delta H° = -46\ \text{kJ};\quad \Delta S° = -8.4\ \text{J/K};\quad \Delta G° = -43\ \text{kJ}$$

The thermodynamic data provide us with information about the relative abilities of H_2O and NH_3 to serve as ligands in these systems. In general, NH_3 binds more tightly to metal ions than H_2O, so these kinds of substitution reactions are exothermic ($\Delta H < 0$). The stronger bonding of the NH_3 ligands also causes $[Cu(H_2O)_2(NH_3)_2]^{2+}$ to be more rigid, which is probably the reason that the entropy change for the reaction is slightly negative. By using Equation 19.18, we can use the value of $\Delta G°$ to calculate the equilibrium constant of the reaction at 27°C. The resulting value, $K_{eq} = 3.1 \times 10^7$, tells us that the equilibrium lies far to the right, favoring substitution of H_2O by NH_3. For this equilibrium, the change in the enthalpy is large and negative enough to overcome the negative change in the entropy.



(a) (b)

▲ **Figure 24.9** Ball-and-stick representations of the square-planar complexes (a) $[Cu(H_2O)_2(NH_3)_2]^{2+}$ and (b) $[Cu(H_2O)_2(en)]^{2+}$. The red spheres represent the H_2O ligands, and the blue spheres represent the NH_3 or en ligands.

How does this situation change if instead of two NH_3 ligands we use a single bidentate ethylenediamine (en) ligand and form $[Cu(H_2O)_2(en)]^{2+}$ [Figure 24.9(b)]? The equilibrium reaction and thermodynamic data are

$$[Cu(H_2O)_4]^{2+}(aq) + en(aq) \rightleftharpoons [Cu(H_2O)_2(en)]^{2+}(aq) + 2H_2O(l)$$

$$\Delta H° = -54\ \text{kJ};\quad \Delta S° = +23\ \text{J/K};\quad \Delta G° = -61\ \text{kJ}$$

The en ligand binds slightly more strongly to a Cu^{2+} ion than two NH_3 ligands, so the enthalpy change on forming $[Cu(H_2O)_2(en)]^{2+}$ is slightly more negative than for $[Cu(H_2O)_2(NH_3)_2]^{2+}$. There is a big difference in the entropy change, however. Whereas the entropy change for forming $[Cu(H_2O)_2(NH_3)_2]^{2+}$ is negative, the entropy change for forming $[Cu(H_2O)_2(en)]^{2+}$ is positive, indicating a greater degree of disorder. We can explain this observation by using the concepts we discussed in Section 19.4. Because a single en ligand occupies two coordination sites, two molecules of H_2O are released upon binding one en ligand. Thus, there are three molecules on the right side of the equation, whereas there are only two on the left side, all of which are part of the same aqueous solution. The greater number of molecules on the right leads to the positive entropy change for the equilibrium. The slightly more negative value of $\Delta H°$ coupled with the positive entropy change leads to a much more negative value of $\Delta G°$ and a correspondingly larger equilibrium constant: $K_{eq} = 4.2 \times 10^{10}$.

We can combine the earlier equations to show that the formation of $[Cu(H_2O)_2(en)]^{2+}$ is thermodynamically preferred over the formation of $[Cu(H_2O)_2(NH_3)_2]^{2+}$. If we add the second reaction to the reverse of the first reaction, we obtain

$$[Cu(H_2O)_2(NH_3)_2]^{2+}(aq) + en(aq) \rightleftharpoons [Cu(H_2O)_2(en)]^{2+}(aq) + 2NH_3(aq)$$

The thermochemical data for this equilibrium reaction can be obtained from those given earlier.

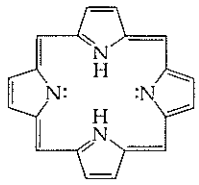
$$\Delta H° = (-54\ \text{kJ}) - (-46\ \text{kJ}) = -8\ \text{kJ}$$

$$\Delta S° = (+23\ \text{J/K}) - (-8.4\ \text{J/K}) = +31\ \text{J/K}$$

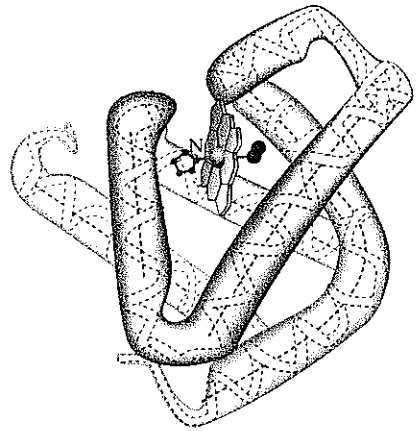
$$\Delta G° = (-61\ \text{kJ}) - (-43\ \text{kJ}) = -18\ \text{kJ}$$

Notice that at 27°C (300 K), the entropic contribution ($-T\Delta S°$) to the free-energy change is negative and greater in magnitude than the enthalpic contribution ($\Delta H°$). The resulting value of K_{eq} for this reaction, 1.4×10^3, shows that the formation of the chelate complex is much more favorable.

The chelate effect is important in biochemistry and molecular biology. The additional thermodynamic stabilization provided by entropic effects helps to stabilize biological metal-chelate complexes, such as porphyrins, and can allow changes in the oxidation state of the metal ion while retaining the structural integrity of the complex.



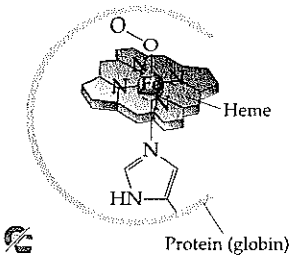
▲ **Figure 24.10** Structure of the porphine molecule. This molecule forms a tetradentate ligand with the loss of the two protons bound to nitrogen atoms. Porphine is the basic component of porphyrins, complexes that play a variety of important roles in nature.



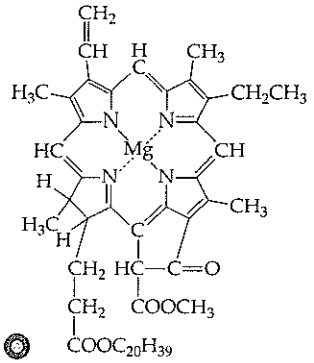
▲ **Figure 24.11** A schematic structure of myoglobin, a protein that stores oxygen in cells. Myoglobin has a molecular weight of about 18,000 amu and contains one heme unit, symbolized by the red disk. The heme unit is bound to the protein through a nitrogen-containing ligand, represented by the blue N on the left. In the oxygenated form an O_2 molecule is coordinated to the heme group, as shown. The three-dimensional structure of the protein chain is represented by the continuous purple cylinder. The helical sections are denoted by the dashed lines. The protein wraps around to make a kind of pocket for the heme group.

Figure 24.11 ▲ shows a schematic structure of myoglobin, a protein that contains one heme group. Myoglobin is a *globular protein*, one that folds into a compact, roughly spherical shape. Globular proteins are generally soluble in water and are mobile within cells. Myoglobin is found in the cells of skeletal muscle,

N. M. Senozan and R. L. Hunt, "Hemoglobin: Its Occurrence, Structure, and Adaptation," *J. Chem. Educ.*, Vol. 59, 1982, 173–178.



▲ **Figure 24.12** Schematic representation of oxymyoglobin or oxyhemoglobin. The iron is bound to four nitrogen atoms of the porphyrin, to a nitrogen from the surrounding protein, and to an O_2 molecule.



▲ **Figure 24.13** Structure of chlorophyll *a*. All chlorophyll molecules are essentially alike; they differ only in details of the side chains.

particularly in seals, whales, and porpoises. It stores oxygen in cells until it is needed for metabolic activities. Hemoglobin, the protein that transports oxygen in human blood, is made up of four heme-containing subunits, each of which is very similar to myoglobin.

The coordination environment of the iron in myoglobin and hemoglobin is illustrated schematically in Figure 24.12 ◀. The iron is coordinated to the four nitrogen atoms of the porphyrin and to a nitrogen atom from the protein chain. The sixth position around the iron is occupied either by O_2 (in oxyhemoglobin, the bright red form) or by water (in deoxyhemoglobin, the purplish red form). The oxy form is shown in Figure 24.12. Some substances, such as CO, are poisonous because they bind to iron more strongly than does O_2. (Section 18.4)

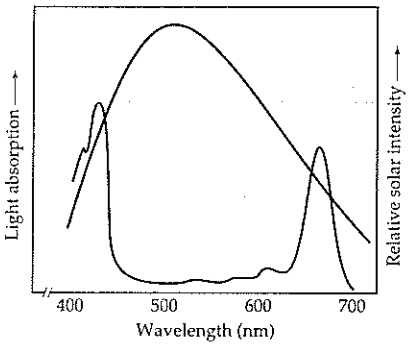
The **chlorophylls**, which are porphyrins that contain Mg(II), are the key components in the conversion of solar energy into forms that can be used by living organisms. This process, called **photosynthesis**, occurs in the leaves of green plants. In photosynthesis, carbon dioxide and water are converted to carbohydrate, with the release of oxygen.

$$6CO_2 + 6H_2O \xrightarrow{48\,h\nu} C_6H_{12}O_6 + 6O_2 \qquad [24.6]$$

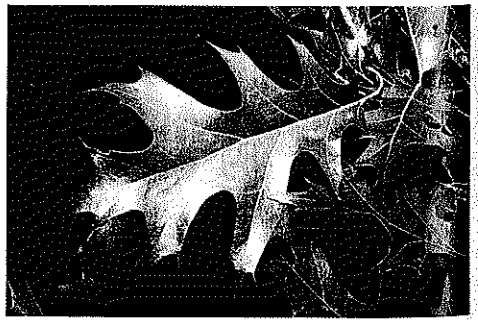
The product of this reaction is the sugar glucose, $C_6H_{12}O_6$, which serves as a fuel in biological systems. (Section 5.8) The formation of one mole of glucose requires the absorption of 48 mol of photons from sunlight or other sources of light. The photons are absorbed by chlorophyll-containing pigments in the leaves of plants. The structure of the most abundant chlorophyll, called chlorophyll *a*, is shown in Figure 24.13 ◀.

Chlorophylls contain a Mg^{2+} ion bound to four nitrogen atoms arranged around the metal in a planar array. The nitrogen atoms are part of a porphine-like ring (Figure 24.10). The series of alternating, or *conjugated*, double bonds in the ring surrounding the metal ion is similar to ones found in many organic dyes. ("Chemistry at Work," Section 9.8) This system of conjugated double bonds makes it possible for chlorophyll to absorb light strongly in the visible region of the spectrum. Figure 24.14 ▼ compares the absorption spectrum of chlorophyll to the distribution of visible solar energy at Earth's surface. Chlorophyll is green because it absorbs red light (maximum absorption at 655 nm) and blue light (maximum absorption at 430 nm) and transmits green light.

The solar energy absorbed by chlorophyll is converted by a complex series of steps into chemical energy. This stored energy is then used to drive the reaction in Equation 24.6 to the right, a direction in which it is highly endothermic. Plant photosynthesis is nature's solar-energy-conversion machine; all living systems on Earth depend on it for continued existence (Figure 24.15 ▼).



▲ **Figure 24.14** Absorption spectrum of chlorophyll (green curve), in comparison with the solar radiation at ground level (red curve).



▲ **Figure 24.15** The absorption and conversion of solar energy that occurs in leaves provides the energy necessary to drive all the living processes of the plant, including growth.

Chemistry and Life The Battle for Iron in Living Systems

Although iron is the fourth most abundant element in Earth's crust, living systems have difficulty assimilating enough iron to satisfy their needs. Consequently, iron-deficiency anemia is a common problem in humans. In plants, chlorosis, an iron deficiency that results in yellowing of leaves, is also commonplace. Living systems have difficulty assimilating iron because most iron compounds in nature have a very low solubility in water. Microorganisms have adapted to this problem by secreting an iron-binding compound, called a *siderophore*, that forms an extremely stable water-soluble complex with iron(III). One such complex is called *ferrichrome*; its structure is shown in Figure 24.16 ▼. The iron-binding strength of a siderophore is so great that it can extract iron from Pyrex™ glassware, and it readily solubilizes the iron in iron oxides.

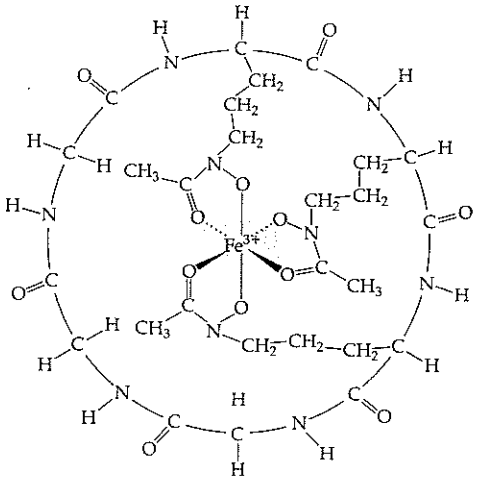
The overall charge of ferrichrome is zero, which makes it possible for the complex to pass through the rather hydrophobic walls of cells. When a dilute solution of ferrichrome is added to a cell suspension, iron is found entirely within the cells in an hour. When ferrichrome enters the cell, the iron is removed through an enzyme-catalyzed reaction that reduces the iron to iron(II). Iron in the lower oxidation state is not strongly complexed by the siderophore. Microorganisms thus acquire iron by excreting a siderophore into their immediate environment and then taking the resulting iron complex into the cell. The overall process is illustrated in Figure 24.17 ▶.

In humans, iron is assimilated from food in the intestine. A protein called *transferrin* binds iron and transports it across the intestinal wall to distribute it to other tissues in the body. The normal adult carries a total of about 4 g of iron. At any one time, about 3 g, or 75%, of this iron is in the blood, mostly in the form of hemoglobin. Most of the remainder is carried by transferrin.

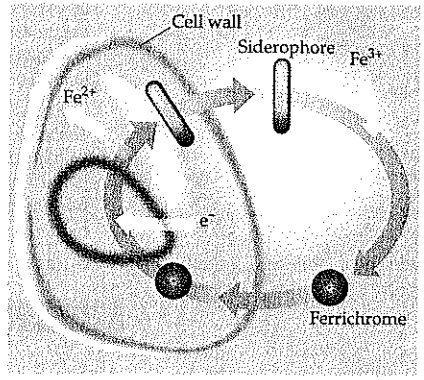
A bacterium that infects the blood requires a source of iron if it is to grow and reproduce. The bacterium excretes a siderophore into the blood to compete with transferrin for the iron it holds. The formation constants for iron binding are about the same for transferrin and siderophores. The more iron available to the bacterium, the more rapidly it can reproduce, and, thus, the more harm it can do. Several years ago, New Zealand clinics regularly gave iron supplements to infants soon after birth. However, the incidence of certain bacterial infections was eight times higher in treated than in untreated infants. Presumably, the presence of more iron in the blood than absolutely necessary makes it easier for bacteria to obtain the iron needed for growth and reproduction.

In the United States it is common medical practice to supplement infant formula with iron sometime during the first year of life because human milk is virtually devoid of iron. Given what is now known about iron metabolism by bacteria, many research workers in nutrition believe that iron supplementation is not generally justified or wise.

For bacteria to continue to multiply in the bloodstream, they must synthesize new supplies of siderophores. Synthesis of siderophores in bacteria slows, however, as the temperature is increased above the normal body temperature of 37°C, and it stops completely at 40°C. This suggests that fever in the presence of an invading microbe is a mechanism used by the body to deprive bacteria of iron.



▲ **Figure 24.16** The structure of ferrichrome. In this complex an Fe^{3+} ion is coordinated by six oxygen atoms. The complex is very stable; it has a formation constant of about 10^{30}. The overall charge of the complex is zero.



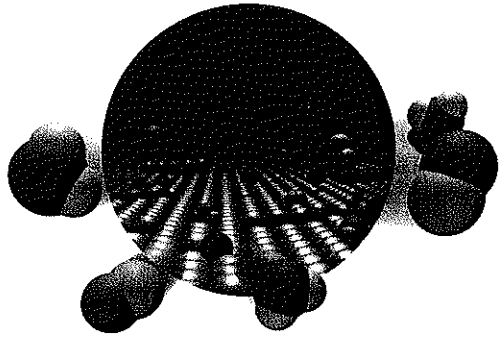
▲ **Figure 24.17** The iron-transport system of a bacterial cell. The iron-binding ligand, called a siderophore, is synthesized inside the cell and excreted into the surrounding medium. It reacts with Fe^{3+} ion to form ferrichrome, which is then absorbed by the cell. Inside the cell the ferrichrome is reduced, forming Fe^{2+}, which is not tightly bound by the siderophore. Having released the iron for use in the cell, the siderophore may be recycled back into the medium.

Martin S. Silberberg

Third Edition

CHEMISTRY

The Molecular Nature of Matter and Change



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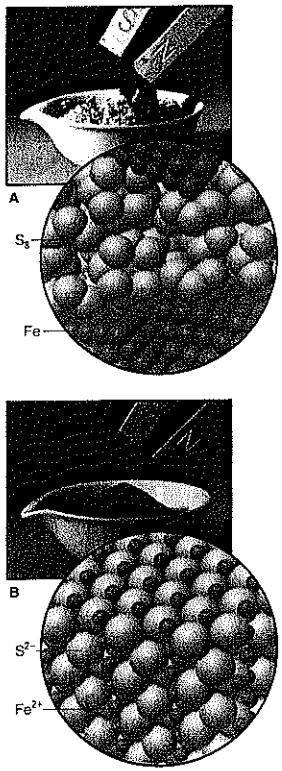


Figure 2.21 **The distinction between mixtures and compounds. A,** A *mixture* of iron and sulfur can be separated with a magnet because only the iron is magnetic. The blow-up shows separate regions of the two elements. **B,** After strong heating, the *compound* iron(II) sulfide forms, which is no longer magnetic. The blow-up shows the structure of the compound, in which there are no separate regions of the elements.

2.9 MIXTURES: CLASSIFICATION AND SEPARATION

Although we pay a great deal of attention to pure substances, they almost never occur around us. In the natural world, *matter usually occurs as mixtures*. A sample of clean air, for example, consists of many elements and compounds physically mixed together, including oxygen (O_2), nitrogen (N_2), carbon dioxide (CO_2), the six noble gases [Group 8A(18)], and water vapor (H_2O). The oceans are complex mixtures of dissolved ions and covalent substances, including Na^+, Mg^{2+}, Cl^-, SO_4^{2-}, O_2, CO_2, and of course H_2O. Rocks and soils are mixtures of numerous compounds—calcium carbonate ($CaCO_3$), silicon dioxide (SiO_2), aluminum oxide (Al_2O_3), iron(III) oxide (Fe_2O_3)—perhaps a few elements (gold, silver, and carbon in the form of diamond), and petroleum and coal, which are complex mixtures themselves. Living things contain thousands of substances: carbohydrates, lipids, proteins, nucleic acids, and many simpler ionic and covalent compounds.

There are two broad classes of mixtures. A **heterogeneous mixture** has one or more visible boundaries between the components. Thus, its composition is *not* uniform. Many rocks are heterogeneous, showing individual grains and flecks of different minerals. In some cases, as in milk and blood, the boundaries can be seen only with a microscope. A **homogeneous mixture** has no visible boundaries because the components are mixed as individual atoms, ions, and molecules. Thus, its composition *is* uniform. A mixture of sugar dissolved in water is homogeneous, for example, because the sugar molecules and water molecules are uniformly intermingled on the molecular level. We have no way to tell visually whether an object is a substance (element or compound) or a homogeneous mixture.

A homogeneous mixture is also called a **solution.** Although we usually think of solutions as liquid, they can exist in all three physical states. For example, air is a gaseous solution of mostly oxygen and nitrogen molecules, and wax is a solid solution of several fatty substances. Solutions in water, called **aqueous solutions,** are especially important in chemistry and comprise a major portion of the environment and of all organisms.

Recall that mixtures differ fundamentally from compounds in three ways: (1) the proportions of the components can vary; (2) the individual properties of the components are observable; and (3) the components can be separated by physical means. In some cases, if we apply enough energy to the components of the mixture, they react with each other chemically and form a compound, after which their individual properties are no longer observable. Figure 2.21 shows such a case with a mixture of iron and sulfur.

In order to investigate the properties of substances, chemists have devised many procedures for separating a mixture into its component elements and compounds. Indeed, the laws and models of chemistry could never have been formulated without this ability. Many of Dalton's critics, who thought they had found compounds with varying composition, were unknowingly studying mixtures! The upcoming Tools of the Laboratory essay describes some of the more common laboratory separation methods.

SECTION SUMMARY

Heterogeneous mixtures have visible boundaries between the components. Homogeneous mixtures have no visible boundaries because mixing occurs at the molecular level. A solution is a homogeneous mixture and can occur in any physical state. Mixtures (not compounds) can have variable proportions, can be separated physically, and retain their components' properties. Common physical separation processes include filtration, crystallization, extraction, chromatography, and distillation.

Tools of the Laboratory

Basic Separation Techniques

Some of the most challenging and time-consuming laboratory procedures involve separating mixtures and purifying the components. Several common separation techniques are described here. Note that all these methods depend on the *physical properties* of the substances in the mixture; no chemical changes occur.

Filtration separates the components of a mixture on the basis of *differences in particle size*. It is used most often to separate a liquid (smaller particles) from a solid (larger particles). Figure B2.3 shows simple filtration of a solid reaction product. In vacuum filtration, reduced pressure within the flask speeds the flow of the liquid through the filter. Filtration is a key step in the purification of the tap water you drink.

Figure B2.3 Filtration.

Crystallization is based on *differences in solubility*. The *solubility* of a substance is the amount that dissolves in a fixed volume of solvent at a given temperature. The procedure shown in Figure B2.4 applies the fact that many substances are more soluble in hot solvent than in cold. Purified compound is shown crystallizing out of the solution as it is cooled. Essential substances in computer chips and other modern electronic devices are purified by a type of crystallization.

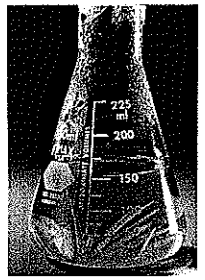


Figure B2.4 Crystallization.

Distillation separates components through *differences in volatility*, the tendency of a substance to become a gas. Ether, for example, is more volatile than water, which is much more volatile than sodium chloride. As the mixture boils, the vapor is richer in the more volatile component, which can be condensed and collected separately. The simple distillation apparatus shown in Figure B2.5 is used to separate components with *large* differences in volatility, such as water from dissolved ionic compounds. Separating components with small volatility differences requires many vaporization-condensation steps (as discussed in Chapter 13).

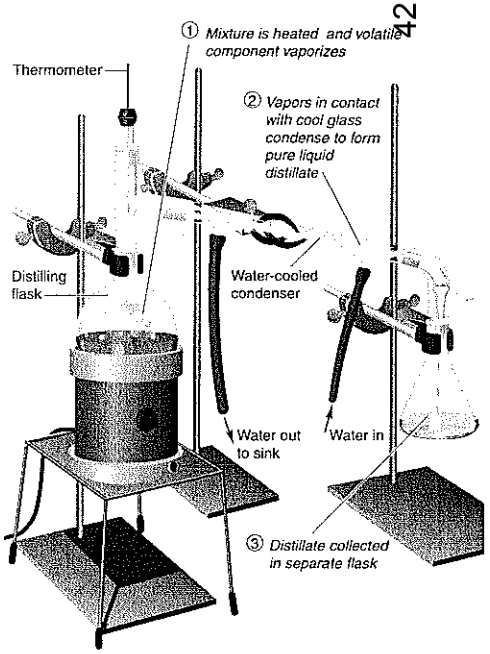


Figure B2.5 Distillation.

Extraction is also based on *differences in solubility*. In a typical procedure, a natural (often plant or animal) material is ground in a blender with a solvent that extracts (dissolves) soluble compound(s) embedded in insoluble material. This extract is separated further by the addition of a second solvent that does not dissolve in the first. After shaking in a separatory funnel, some components are extracted into the new solvent. Figure B2.6 shows the extraction of plant pigments from water into hexane, an organic solvent.

(continued)

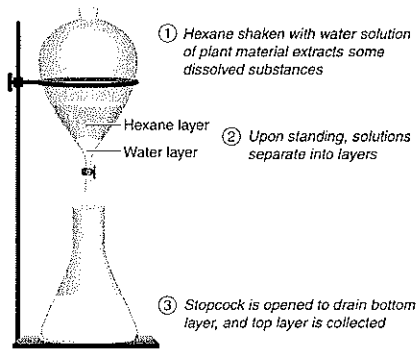


Figure B2.6 Extraction.

Tools of the Laboratory *(continued)*

Chromatography is a third technique based on *differences in solubility*. The mixture is dissolved in a gas or liquid called the *mobile phase*, and the components are separated as this phase moves over a solid (or viscous liquid) surface called the *stationary phase*. A component with low solubility in the stationary phase spends less time there, thus moving faster, than a component that is highly soluble in it. Figure B2.7 depicts the separation of a mixture of pigments in ink. Many types of chromatography are used to separate a wide variety of substances, from simple gases to biological macromolecules. In *gas-liquid chromatography (GLC)*, the mobile phase is an inert gas, such as helium, that carries the previously vaporized components into a long tube that contains the stationary phase (Figure B2.8, part A). The components emerge separately and reach a detector to create a chromatogram. A typical chromatogram has numerous peaks of specific position and height, each of which represents the amount of a given component (Figure B2.8, part B). The principle of *high-performance (high-pressure) liquid chromatography (HPLC)* is very similar, but the mixture is not vaporized, so a more diverse group of mixtures, which may include nonvolatile compounds, can be separated (Figure B2.9).

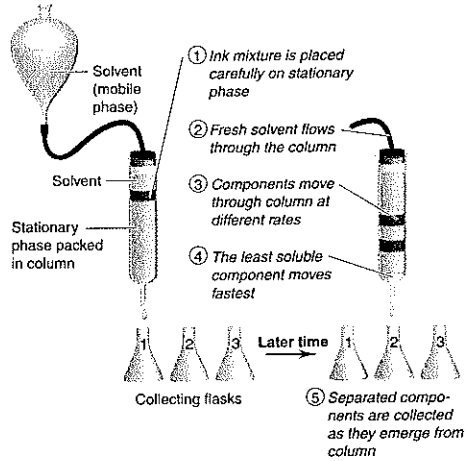
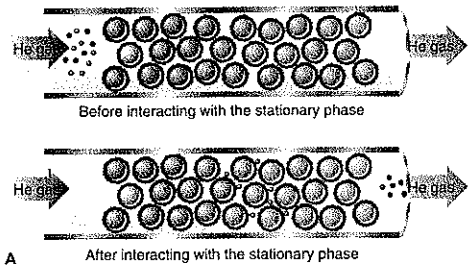


Figure B2.7 Procedure for column chromatography.



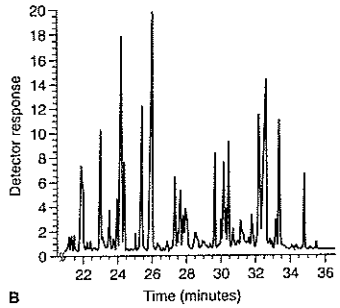


Figure B2.8 Principle of gas-liquid chromatography (GLC). **A,** The moblile phase *(purple arrow)* carries the sample mixture into a tube packed with the stationary phase *(gray outline on yellow spheres)*, and each component dissolves in the stationary phase to a different extent. A component *(red)* that dissolves less readily than another *(blue)* emerges from the tube sooner. **B,** A typical gas-liquid chromatogram of a complex mixture displays each component as a peak.

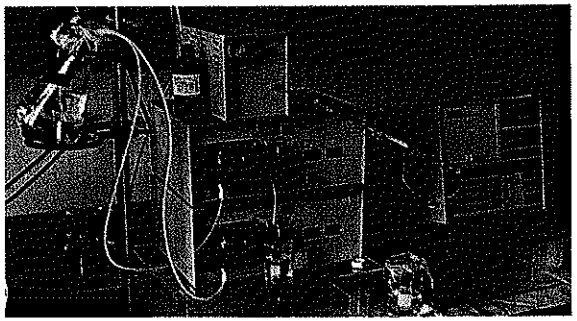


Figure B2.9 A high-performance liquid chromatograph.

Chapter Perspective

An understanding of matter at the observable and atomic levels is the essence of chemistry. In this chapter, you have learned how matter is classified in terms of its composition and how it is named in words and formulas, which are major steps toward that understanding. Figure 2.22 provides a visual review of many key terms and ideas in this chapter. In Chapter 3, we explore one of the central quantitative ideas in chemistry: how the observable amount of a substance relates to the number of atoms, molecules, or ions that make it up.

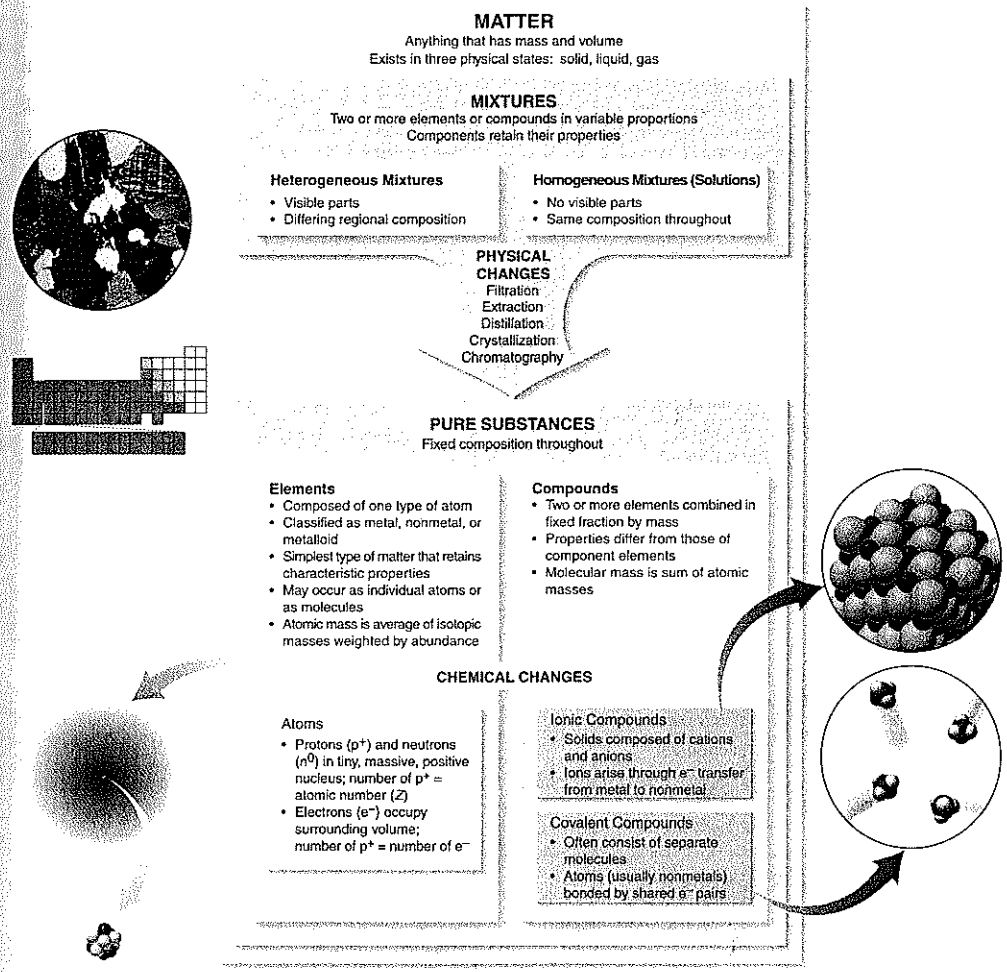


Figure 2.22 The classification of matter from a chemical point of view. Mixtures are separated by physical changes into elements and compounds. Chemical changes are required to convert elements into compounds, and vice versa.

This equation is easily adapted to find the energy difference between any two levels:

$$\Delta E = E_{final} - E_{initial} = -2.18\times10^{-18}\ \text{J}\left(\frac{1}{n^2_{final}} - \frac{1}{n^2_{initial}}\right) \quad (7.4)$$

With it, we can predict the wavelengths of the spectral lines of the H atom. (In fact, Bohr obtained a value for the Rydberg constant that differed from the spectroscopists' value by only 0.05%!) Note that if we combine Equation 7.4 with Planck's expression for the change in an atom's energy (Equation 7.2), we obtain the Rydberg equation (Equation 7.3):

$$\Delta E = h\nu = \frac{hc}{\lambda} = -2.18\times10^{-18}\ \text{J}\left(\frac{1}{n^2_{final}} - \frac{1}{n^2_{initial}}\right)$$

Therefore,
$$\frac{1}{\lambda} = -\frac{2.18\times10^{-18}\ \text{J}}{hc}\left(\frac{1}{n^2_{final}} - \frac{1}{n^2_{initial}}\right)$$
$$= -\frac{2.18\times10^{-18}\ \text{J}}{(6.626\times10^{-34}\ \text{J·s})(3.00\times10^{8}\ \text{m/s})}\left(\frac{1}{n^2_{final}} - \frac{1}{n^2_{initial}}\right)$$
$$= 1.10\times10^{7}\ \text{m}^{-1}\left(\frac{1}{n^2_{final}} - \frac{1}{n^2_{initial}}\right)$$

where $n_{final} = n_2$, $n_{initial} = n_1$, and $1.10\times10^7\ \text{m}^{-1}$ is the Rydberg constant ($1.096776\times10^7\ \text{m}^{-1}$) to three significant figures. Thus, from classical relationships of charge and of motion combined with the idea that the H atom can have only certain values of energy, we obtain an equation that leads directly to the empirical one!

We can use Equation 7.4 to find the quantity of energy needed to completely remove the electron from an H atom. In other words, what is ΔE for the following change?

$$H(g) \longrightarrow H^+(g) + e^-$$

We substitute $n_{final} = \infty$ and $n_{initial} = 1$ and obtain

$$\Delta E = E_{final} - E_{initial} = -2.18\times10^{-18}\ \text{J}\left(\frac{1}{\infty^2} - \frac{1}{1^2}\right)$$
$$= -2.18\times10^{-18}\ \text{J}(0 - 1) = 2.18\times10^{-18}\ \text{J}$$

ΔE is positive because energy is *absorbed* to remove the electron from the vicinity of the nucleus. For 1 mol of H atoms,

$$\Delta E = \left(2.18\times10^{-18}\ \frac{\text{J}}{\text{atom}}\right)\left(6.022\times10^{23}\ \frac{\text{atoms}}{\text{mol}}\right)\left(\frac{1\ \text{kJ}}{10^3\ \text{J}}\right) = 1.31\times10^{3}\ \text{kJ/mol}$$

This is the *ionization energy* of the H atom, the quantity of energy required to form 1 mol of gaseous H^+ ions from 1 mol of gaseous H atoms. We return to this idea in Chapter 8.

Spectroscopic analysis of the H atom led to the Bohr model, the first step toward our current model of the atom. From its use by 19th-century chemists as a means of identifying elements and compounds, spectrometry has developed into a major tool of modern chemistry (see the Tools of the Laboratory essay on the next two pages).

What Are Stars Made Of? In 1868, the French astronomer Pierre Janssen noted a bright yellow line in the solar emission spectrum. After he and other scientists could not reproduce the line from any known element, they considered it to be due to an element unique to the Sun and named it helium (Greek *helios*, "sun"). In 1888, the British chemist William Ramsay examined the spectrum of an inert gas obtained by heating uranium-containing minerals, and it showed the same bright yellow line. Analysis of light from stars has shown many of the elements already known on Earth, but helium is the only element that was first discovered on a star.

SECTION SUMMARY

To explain the line spectrum of atomic hydrogen, Bohr proposed that the atom's energy is quantized because the electron's motion is restricted to fixed orbits. The electron can move from one orbit to another only if the atom absorbs or emits a photon whose energy equals the difference in energy levels (orbits). Line spectra are produced because these energy changes correspond to photons of specific wavelength. Bohr's model predicted the hydrogen atomic spectrum but could not predict that of any other atom because electrons do not have fixed orbits. Despite this, Bohr's idea that atoms have quantized energy levels is a cornerstone of our current atomic model. Spectrophotometry is an instrumental technique in which emission and absorption spectra are used to identify and measure concentrations of substances.

Tools of the Laboratory

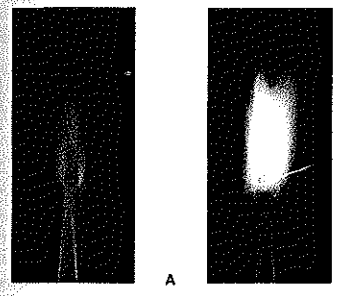
Spectrophotometry in Chemical Analysis

The use of spectral data to identify and quantify substances is essential to modern chemical analysis. The terms *spectroscopy*, *spectrometry*, and **spectrophotometry** denote a large group of instrumental techniques that obtain spectra corresponding to a substance's atomic and molecular energy levels.

The two types of spectra most often obtained are emission and absorption spectra. An **emission spectrum**, such as the H atom line spectrum, is produced when atoms in an excited state *emit* photons characteristic of the element as they return to lower energy states. Some elements produce a very intense spectral line (or several closely spaced ones) that serves as a marker of their presence. Such an intense line is the basis of **flame tests**, rapid qualitative procedures performed by placing a granule of an ionic compound or a drop of its solution in a flame (Figure B7.1, A). Some of the colors of fireworks and flares are due to emissions from the same elements shown in the flame tests: crimson from strontium salts and blue-green from copper salts (Figure B7.1, B). The characteristic colors of sodium-vapor and mercury-vapor streetlamps, seen in many towns and cities, are due to one or a few prominent lines in their emission spectra.

An **absorption spectrum** is produced when atoms *absorb* photons of certain wavelengths and become excited from lower to higher energy states. Therefore, the absorption spectrum of an element appears as dark lines against a bright background. When white light passes through sodium vapor, for example, it gives rise to a sodium absorption spectrum, and the dark lines appear at the same wavelengths as those for the yellow-orange lines in the sodium emission spectrum (Figure B7.2).

(continued)



A

B

Figure B7.1 **Flame tests and fireworks. A,** In general, the color of the flame is created by a strong emission in the line spectrum of the element and therefore is often taken as preliminary evidence of the presence of the element in a sample. Shown here are the crimson of strontium and the blue-green of copper. **B,** The same emissions from compounds that contain these elements often appear in the brilliant displays of fireworks.

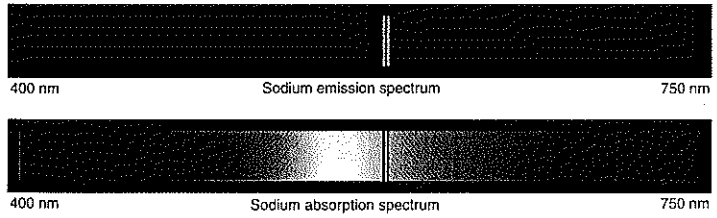


Figure B7.2 **Emission and absorption spectra of sodium atoms.** The wavelengths of the bright emission lines correspond to those of the dark absorption lines because both are created by the same energy change: $\Delta E_{emission} = -\Delta E_{absorption}$. (Only the two most intense lines in the sodium atomic spectra are shown here.)

Tools of the Laboratory (continued)

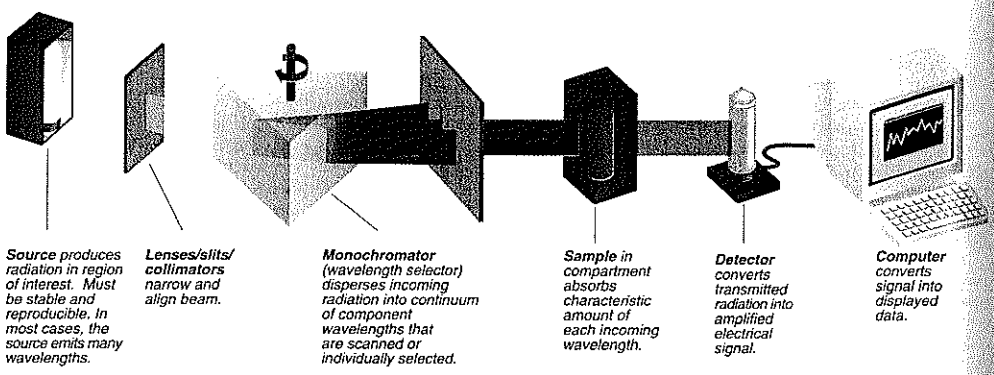


Figure B7.3 The main components of a typical spectrometer.

Instruments based on absorption spectra are much more common than those based on emission spectra, for several reasons. When a solid, liquid, or dense gas is excited, it *emits* so many lines that the spectrum is a continuum (recall the continuum of colors in sunlight). Absorption is also less destructive of fragile organic and biological molecules.

Despite differences that depend on the region of the electromagnetic spectrum used to irradiate the sample, all modern spectrometers have components that perform the same basic functions (Figure B7.3). (We discuss infrared spectroscopy and nuclear magnetic resonance spectroscopy in later chapters.)

Visible light is often used to study colored substances, which absorb only some of the wavelengths from white light. A leaf looks green, for example, because its chlorophyll absorbs red and blue wavelengths strongly and green weakly, so most of the green light is reflected. The absorption spectrum of chlorophyll *a* in ether solution appears in Figure B7.4.

The overall shape of the curve and the wavelengths of the major peaks are characteristic of chlorophyll *a*, so its spectrum serves as a means of identifying it from an unknown source. The curve varies in height because chlorophyll *a* absorbs incoming wavelengths to different extents. The absorptions appear as broad bands, rather than as the distinct lines we saw earlier for individual gaseous atoms, because dissolved substances, as well as pure solids and liquids, absorb many more wavelengths due to the greater numbers and types of energy levels within a molecule, among molecules, and between molecules and solvent.

In addition to identifying a substance, a spectrometer can be used to measure its concentration because *the absorbance, the amount of light of a given wavelength absorbed by a substance, is proportional to the number of molecules.* Suppose you want to determine the concentration of chlorophyll in an ether solution of leaf extract. You select a strongly absorbed wavelength from the chlorophyll spectrum (such as 663 nm in Figure B7.4), measure the absorbance of the leaf-extract solution, and compare it with the absorbances of a series of ether solutions with known chlorophyll concentrations.

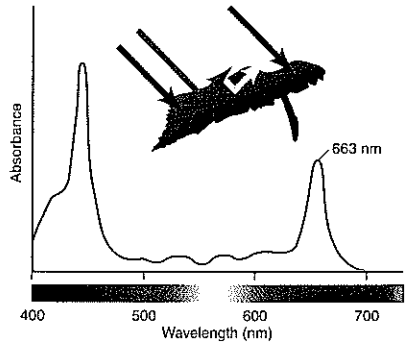


Figure B7.4 The absorption spectrum of chlorophyll *a*. Chlorophyll *a* is one of several leaf pigments. It absorbs red and blue wavelengths strongly but almost no green or yellow wavelengths. Thus, leaves containing large amounts of chlorophyll *a* appear green. The strong absorption at 663 nm can be used to quantify the amount of chlorophyll *a* present in a plant extract.

7.3 THE WAVE-PARTICLE DUALITY OF MATTER AND ENERGY

The year 1905 was a busy one for Albert Einstein. In addition to presenting the photon theory of light and explaining the photoelectric effect, he found time to explain Brownian motion (Chapter 13), which helped establish the molecular view of matter, and to introduce a new branch of physics with his theory of relativity. One of its many startling revelations was that matter and energy are alternate forms of the same entity. This idea is embodied in his famous equation $E = mc^2$, which relates the quantity of energy equivalent to a given mass, and vice versa. Relativity theory does not depend on quantum theory, but together they have completely blurred the sharp divisions we normally perceive between matter (chunky and massive) and energy (diffuse and massless).

The early proponents of quantum theory demonstrated that *energy is particle-like.* Physicists who developed the theory turned this proposition upside down and showed that *matter is wavelike.* Strange as this idea may seem, it is the key to our modern atomic model.

The Wave Nature of Electrons and the Particle Nature of Photons

Bohr's efforts were a perfect case of fitting theory to data: he *assumed* that an atom has only certain allowable energy levels in order to *explain* the observed line spectrum. However, his assumption had no basis in physical theory. Then, in the early 1920s, a young French physics student named Louis de Broglie proposed a startling reason for fixed energy levels: *if energy is particle-like, perhaps matter is wavelike.* De Broglie had been thinking of other systems that display only certain allowed motions, such as the wave of a plucked guitar string. Figure 7.13 shows that, because the ends of the string are fixed, only certain vibrational frequencies (and wavelengths) are possible. De Broglie reasoned that *if electrons have wavelike motion* and are restricted to orbits of fixed radii, that would explain why they have only certain possible frequencies and energies.

"He'll Never Make a Success of Anything" This comment, attributed to the principal of young Albert Einstein's primary school, remains a classic of misperception. Contrary to myth, the greatest physicist of the 20th century (some say of all time) was not a poor student but an independent one, preferring his own path to that prescribed by authority—a trait that gave him the intense focus characteristic of all his work. A friend recalls finding him in his small apartment, rocking his baby in its carriage with one hand while holding a pencil stub and scribbling on a pad with the other. At age 26, he was working on one of the four papers he published in 1905 that would revolutionize the way the universe is perceived and lead to his 1921 Nobel Prize.

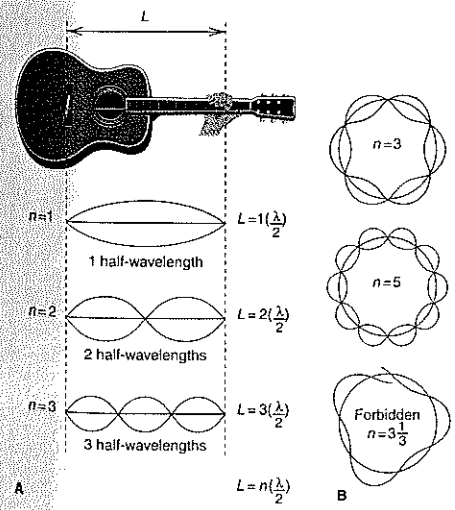


Figure 7.13 Wave motion in restricted systems. **A,** In a musical analogy to electron waves, one half-wavelength ($\lambda/2$) is the "quantum" of the guitar string's vibration. The string length L is fixed, so the only allowed vibrations occur when L is a whole-number multiple (n) of $\lambda/2$. **B,** If an electron occupies a circular orbit, only whole numbers of wavelengths are allowed ($n = 3$ and $n = 5$ are shown). A wave with a fractional number of wavelengths (such as $n = 3\frac{1}{3}$) is "forbidden" because it rapidly dies out through overlap of crests and troughs.

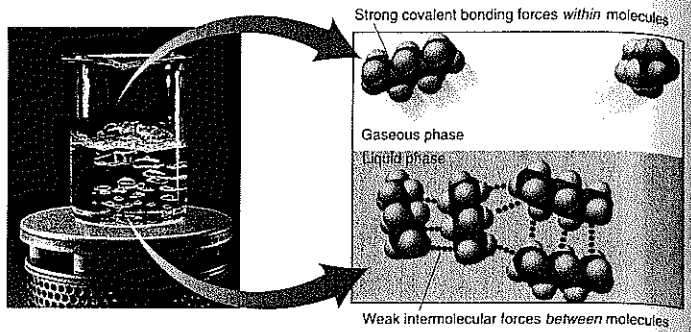
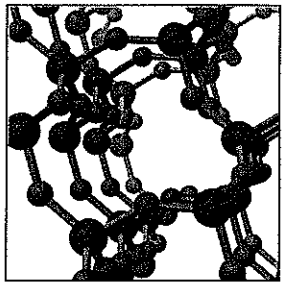


Figure 9.14 **Strong forces within molecules and weak forces between them.** When pentane boils, weak forces *between* molecules (intermolecular forces) are overcome, but the strong covalent bonds holding the atoms together within each molecule remain unaffected. Thus, the pentane molecules leave the liquid phase as intact units.



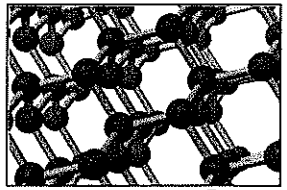


Figure 9.15 **Covalent bonds of network covalent solids. A,** In quartz (SiO_2), each Si atom is bonded covalently to four O atoms and each O atom is bonded to two Si atoms in a pattern that extends throughout the sample. Because no separate SiO_2 molecules are present, the melting point and the hardness are very high. **B,** In diamond, each C atom is covalently bonded to four other C atoms throughout the crystal. Diamond is the hardest natural substance known and has an extremely high melting point.

Consider, for example, what happens when pentane (C_5H_{12}) boils. As Figure 9.14 shows, the weak interactions *between* the pentane molecules are affected, not the strong C—C and C—H covalent bonds *within* each molecule.

Some covalent substances, called *network covalent solids,* do not consist of separate molecules. Rather, they are held together by covalent bonds that extend in three dimensions *throughout* the sample. If the model is correct, the properties of these substances *should* reflect the strength of their covalent bonds, and this is indeed the case. Two examples, quartz and diamond, are shown in Figure 9.15. Quartz (SiO_2) is very hard and melts at 1550°C. It is composed of silicon and oxygen atoms connected by covalent bonds that extend throughout the sample; no separate SiO_2 molecules exist. Diamond consists of covalent bonds connecting each carbon atom to four others throughout the sample. It is the hardest substance known and melts at around 3550°C. Clearly, covalent bonds *are* strong, but because most covalent substances consist of separate molecules with weak forces between them, their physical properties do not reflect this strength. (We discuss intermolecular forces in detail in Chapter 12.)

Unlike ionic compounds, most covalent substances are poor electrical conductors, even when melted or when dissolved in water. An electric current is carried by either mobile electrons or mobile ions. In covalent substances, the electrons are localized as either shared or unshared pairs, so they are not free to move, and no ions are present. The Tools of the Laboratory essay describes an important laboratory tool for studying covalent substances.

SECTION SUMMARY

A shared pair of valence electrons attracts the nuclei of two atoms and holds them together in a covalent bond while filling each atom's outer shell. The number of shared pairs between the two atoms is the bond order. For a given type of bond, the bond energy is the average energy required to completely separate the bonded atoms; the bond length is the average distance between their nuclei. For a given pair of bonded atoms, bond order is directly related to bond energy and inversely related to bond length. Substances that consist of separate molecules are generally soft and low melting because of the weak forces between molecules. Those held together throughout by covalent bonds are extremely hard and high melting. Most covalent substances have low electrical conductivity because electrons are localized and ions are absent. The atoms in a covalent bond vibrate, and the energy of these vibrations can be studied with IR spectroscopy.

Tools of the Laboratory

Infrared Spectroscopy

Infrared (IR) spectroscopy is an instrumental technique used primarily for measuring the absorption of IR radiation by covalently bonded molecules. It plays a major role in our understanding of molecular structure and bonding. IR spectrometers are found in most research laboratories, particularly where organic molecules are studied, and are essential aids in identifying unknown substances. Their key components are the same as those of any spectrometer (see pages 267–268). The source emits radiation of many wavelengths, and those in the IR region are selected and directed at the sample. Certain wavelengths are absorbed more than others, and the IR spectrum of the compound is generated.

What property of a molecule is displayed in its IR spectrum? All molecules, whether in a gas, liquid, or solid, undergo continual rotations and vibrations. Consider, for instance, a sample of ethane gas. The $H_3C—CH_3$ molecules zoom throughout the container, colliding with the walls and each other. If we could look closely at one molecule, however, and disregard its motion through space, we would see the molecule rotating and its two CH_3 groups rotating relative to each other about the C—C bond. The bonded atoms also vibrate, that is, move back and forth as though their bonds were flexible springs: stretching and compressing, twisting, bending, rocking, and wagging (Figure B9.1). (Thus, the length of a given bond even within a given substance is actually the *average* distance between nuclei, analogous to the average length of a spring stretching and compressing.)

Each vibrational motion has its own natural frequency, which is based on the type of motion, the masses of the atoms, and the strengths of the bonds between them. These frequencies correspond to wavelengths between 2.5 and 25 μm, which make up a part of the IR region of the electromagnetic spectrum (see Figure 7.3). The energy of each of these vibrations is quantized. Just as an atom can absorb a photon whose energy corresponds to the difference between two quantized *electron* energy levels, a molecule can absorb an IR photon whose energy corresponds to the difference between two of its quantized *vibrational* energy levels.

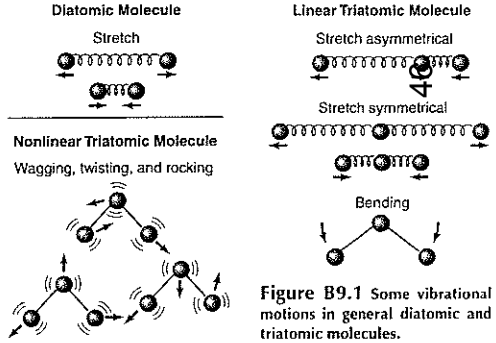


Figure B9.1 **Some vibrational motions in general diatomic and triatomic molecules.**

The IR spectrum is important in compound identification because of two related factors. First, *each kind of bond has a characteristic range of IR wavelengths it can absorb.* For example, a C—C bond absorbs IR photons in a different wavelength range from those absorbed by a C=C bond, a C—H bond, a C=O bond, and so forth. Second, the exact wavelength and quantity of IR radiation absorbed by a given bond depend on the *overall structure* of the molecule. This means that *each compound has a characteristic IR spectrum* that can be used to identify it, much as a fingerprint identifies a person. The spectrum appears as a series of downward pointing peaks, varying in depth and sharpness, that correspond to absorption frequencies across the IR region scanned. Figure B9.2 shows the IR spectrum of acrylonitrile, a compound that is used to manufacture synthetic rubber and plastics; no other compound has exactly the same IR spectrum.

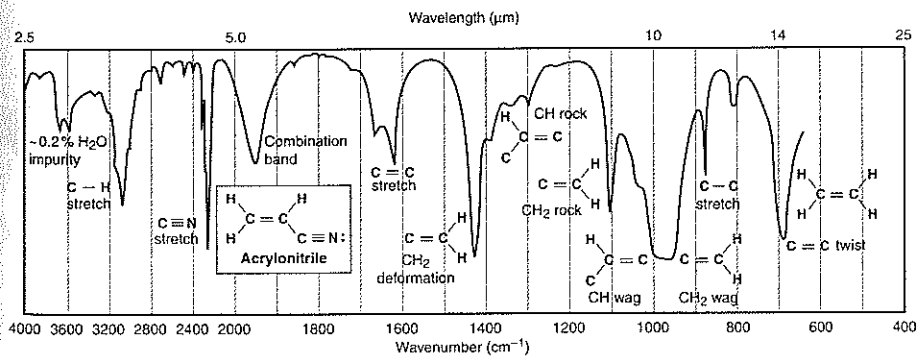


Figure B9.2 **The infrared (IR) spectrum of acrylonitrile.** The IR spectrum of acrylonitrile is typical of a molecule with several types of covalent bonds. There are many absorption bands (peaks) of differing depth and sharpness. Most peaks correspond to a particular type of vibration in a particular group of bonded atoms. Some broad peaks (for example, "combination band") represent several overlapping types of vibrations. The spectrum is reproducible and unique for acrylonitrile. (The bottom axis shows wavenumbers, the inverse of wavelength, so its units are those of length^{-1}. The scale expands to the right of 2000 cm^{-1}.)

Tools of the Laboratory

Mass Spectrometry

Mass spectrometry, the most powerful technique for measuring the mass and abundance of charged particles, emerged from electric and magnetic deflection studies on particles formed in cathode ray experiments. When a high-energy electron collides with an atom of neon-20, for example, one of the atom's electrons is knocked away and the resulting particle has one positive charge, Ne^+ (Figure B2.1). Thus, its mass/charge ratio (*m/e*) equals the mass divided by 1+. The *m/e* values are measured to identify the masses of different isotopes of an element.

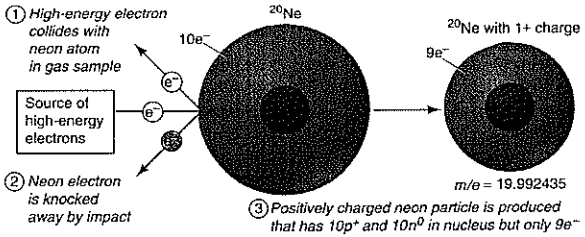
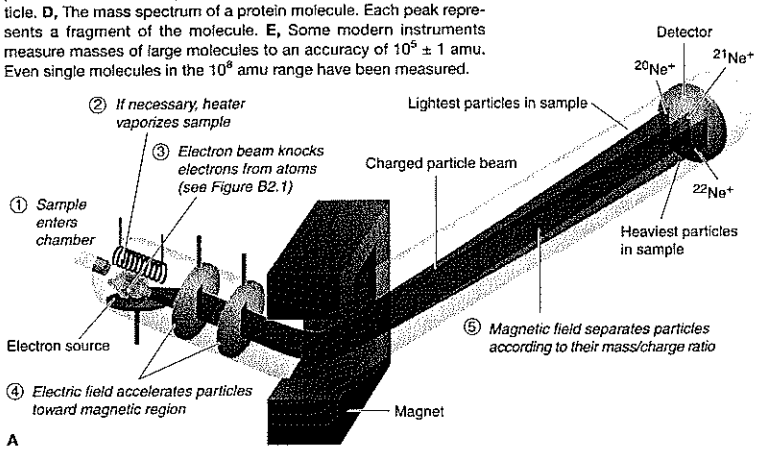


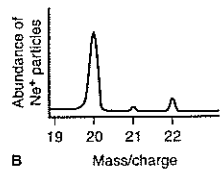
Figure B2.1 Formation of a positively charged neon (Ne) particle.

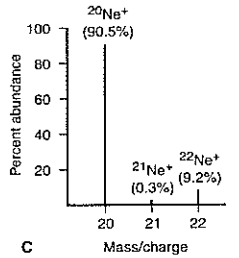
Figure B2.2, parts A–C, depicts the core of one type of mass spectrometer and the data it provides. The sample is introduced and vaporized (if liquid or solid), then bombarded by high-energy electrons to form positively charged particles. These are attracted toward a series of negatively charged plates with slits in them, and some particles pass through into an evacuated tube exposed to a magnetic field. As the particles zoom through this region, they are deflected (their paths are bent) according to their *m/e*: the lightest particles are deflected most and the heaviest particles least. At the end of the magnetic region, the particles strike a detector, which records their relative positions and abundances. For very precise work, such as determining isotopic masses and abundances, the instrument is calibrated with a substance of known amount and mass.

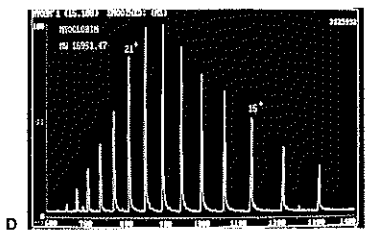
Mass spectrometry is also used in structural chemistry and separations science to measure the mass of virtually any atom, molecule, or fragment of a molecule. Among its many applications, mass spectrometry is employed by biochemists determining protein structures (Figure B2.2, parts D and E), materials scientists examining catalyst surfaces, forensic chemists analyzing criminal evidence, organic chemists designing new drugs, and industrial chemists investigating petroleum components.

Figure B2.2 The mass spectrometer and its data. **A,** Charged particles are separated on the basis of their *m/e* values. Ne is the sample here. **B,** The data show the abundance of each particle. The three peaks are three Ne isotopes. **C,** The percent abundance of each particle. **D,** The mass spectrum of a protein molecule. Each peak represents a fragment of the molecule. **E,** Some modern instruments measure masses of large molecules to an accuracy of $10^5 \pm 1$ amu. Even single molecules in the 10^8 amu range have been measured.





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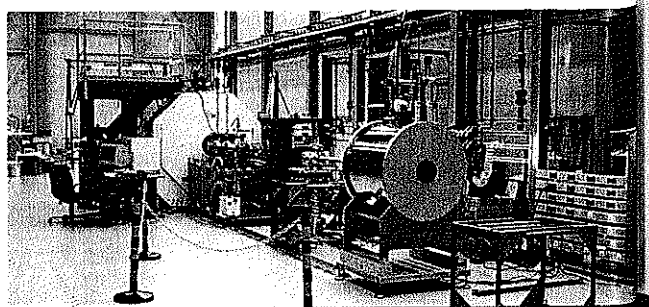
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this percent abundance as a fraction and multiplying by its isotopic mass gives the portion contributed by ^{28}Si:

Portion of Si atomic mass from ^{28}Si = 27.97693 amu × 0.9223 = 25.8031 amu
(retaining two additional significant figures)

Similar calculations give the portions contributed by ^{29}Si (28.976495 amu × 0.0467 = 1.3532 amu) and by ^{30}Si (29.973770 amu × 0.0310 = 0.9292 amu), and adding the three portions together (rounding to two decimal places at the end) gives the atomic mass of silicon:

Atomic mass of Si = 25.8031 amu + 1.3532 amu + 0.9292 amu
= 28.0855 amu = 28.09 amu

SAMPLE PROBLEM 2.3 Calculating the Atomic Mass of an Element

Problem Silver (Ag; Z = 47) has 46 known isotopes, but only two occur naturally, ^{107}Ag and ^{109}Ag. Given the following mass spectrometric data, calculate the atomic mass of Ag:

Isotope	Mass (amu)	Abundance (%)
^{107}Ag	106.90509	51.84
^{109}Ag	108.90476	48.16

Plan From the mass and abundance of the two Ag isotopes, we have to find the atomic mass of Ag (weighted average of the isotopic masses). We multiply each isotopic mass by its fractional abundance to find the portion of the atomic mass contributed by each isotope. The sum of the isotopic portions is the atomic mass.

Solution Finding the portion of the atomic mass from each isotope:

Portion of atomic mass from ^{107}Ag: = isotopic mass × fractional abundance
= 106.90509 amu × 0.5184 = 55.42 amu

Portion of atomic mass from ^{109}Ag: = 108.90476 amu × 0.4816 = 52.45 amu

Finding the atomic mass of silver:

Atomic mass of Ag = 55.42 amu + 52.45 amu = 107.87 amu

Mass (g) of each isotope
multiply by fractional abundance of each isotope
Portion of atomic mass from each isotope
add isotopic portions
Atomic mass

Check The individual portions seem right: ~100 amu × 0.50 = 50 amu. The portions are almost the same because the two isotopic abundances are almost the same. We rounded each portion to four significant figures because that is the number of significant figures in the abundance values. This is the correct atomic mass (to two decimal places), as shown in the list of elements (*inside front cover*).

Comment Averages must be interpreted carefully. The average number of children in an American family in 1985 was 2.4. You know that no family actually has 2.4 children; you should also know that no individual silver atom has a mass of 107.87 amu. But for most laboratory purposes, we consider a sample of silver to consist of atoms with this average mass.

FOLLOW-UP PROBLEM 2.3 Boron (B; Z = 5) has two naturally occurring isotopes. Calculate the percent abundances of ^{10}B and ^{11}B from the following: atomic mass of B = 10.81 amu; isotopic mass of ^{10}B = 10.0129 amu; isotopic mass of ^{11}B = 11.0093 amu. (*Hint:* The sum of the fractional abundances is 1. If x = abundance of ^{10}B, then $1 - x$ = abundance of ^{11}B.)

A Modern Reassessment of the Atomic Theory

We began discussing the atomic basis of matter with Dalton's model, which proved inaccurate in several respects. What happens to a model whose postulates are found by later experiment to be incorrect? No model can predict every possible future observation, but a powerful model evolves to retain its usefulness. Let's reexamine the atomic theory in light of what we know now:

1. *All matter is composed of atoms.* We now know that atoms *are* divisible and composed of smaller, subatomic particles (electrons, protons, and neutrons), but the atom is still the smallest body that *retains the unique identity* of an element.

Fundamentals of Organic Chemistry

Fifth Edition

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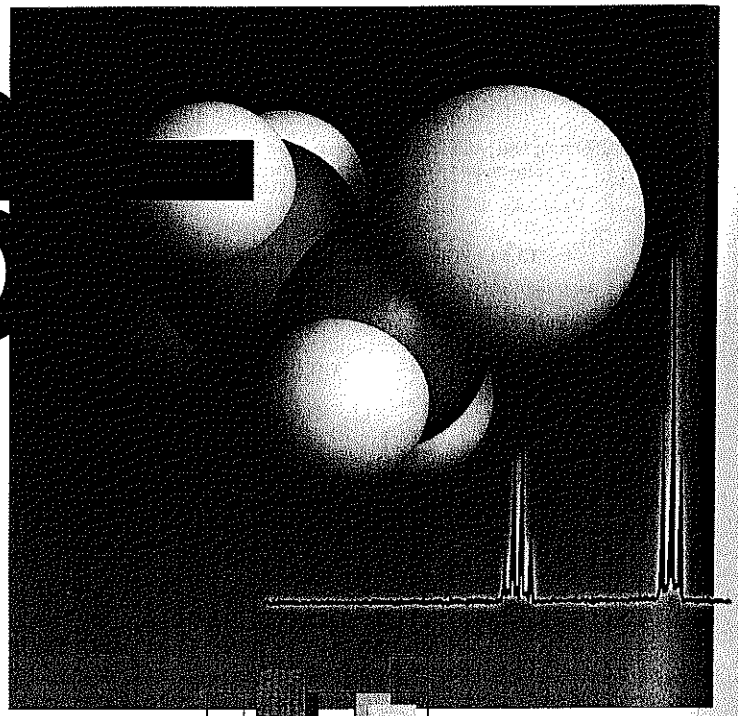


Structure Determination

Every time a reaction is run, the products must be identified. Determining the structure of an organic molecule was a difficult and time-consuming process in the nineteenth and early twentieth centuries, but extraordinary advances have been made in the past few decades. Powerful techniques and specialized instruments that greatly simplify structure determination are now available. We'll look at three of the most useful such techniques—infrared spectroscopy (IR), ultraviolet spectroscopy (UV), and nuclear magnetic resonance spectroscopy (NMR)—each of which yields a different kind of structural information.

Infrared spectroscopy	What functional groups are present?
Ultraviolet spectroscopy	Is a conjugated π electron system present?
Nuclear magnetic resonance spectroscopy	What carbon–hydrogen framework is present?

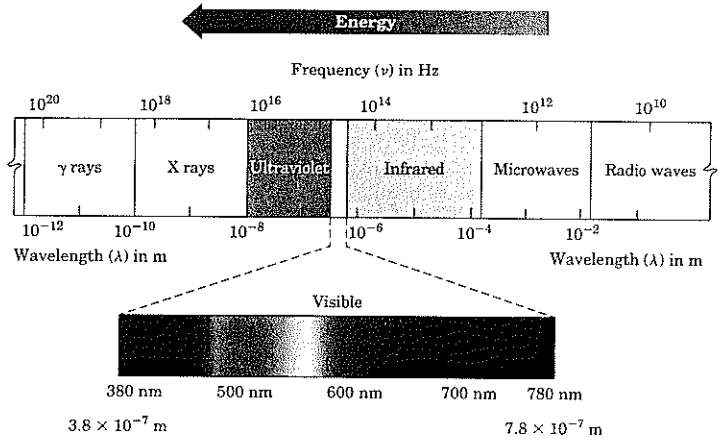
49

13.1 Infrared Spectroscopy and the Electromagnetic Spectrum

Infrared (IR) spectroscopy is a method of structure determination that depends on the interaction of molecules with infrared radiant energy. Before beginning a study of infrared spectroscopy, however, we need to look into the nature of radiant energy and the electromagnetic spectrum.

Visible light, X rays, microwaves, radio waves, and so forth, are all different kinds of *electromagnetic radiation*. Collectively, they make up the **electromagnetic spectrum**, shown in Figure 13.1. The electromagnetic spectrum is loosely divided into regions, with the familiar visible region accounting for only a small portion of the overall spectrum, from 3.8×10^{-7} to 7.8×10^{-7} m in wavelength. The visible region is flanked by the infrared and ultraviolet regions.

FIGURE 13.1 ▽
The electromagnetic spectrum.

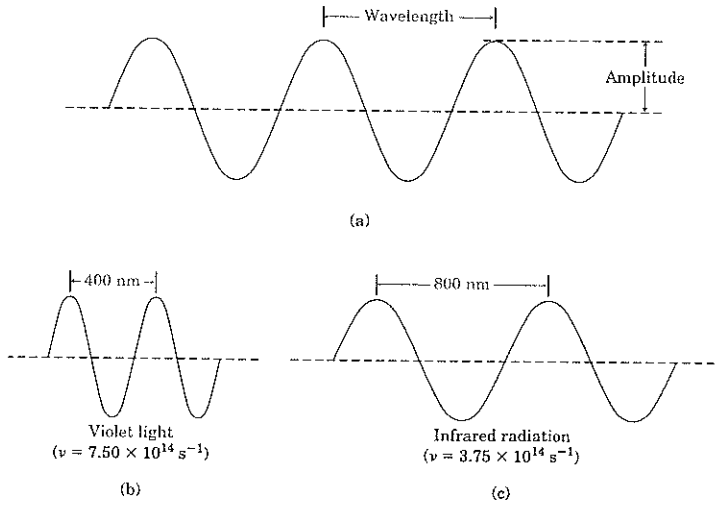


Electromagnetic radiation has dual behavior. In some respects it has the properties of a particle (called a *photon*), yet in other respects it behaves as an energy wave traveling at the speed of light. Like all waves, electromagnetic radiation is characterized by a *frequency*, a *wavelength*, and an *amplitude* (Figure 13.2). The **frequency**, ν (Greek nu), is the number of

wave peaks that pass by a fixed point per unit time, usually given in reciprocal seconds (s^{-1}), or **hertz, Hz** (1 Hz = 1 s^{-1}). The **wavelength**, λ (Greek lambda), is the distance from one wave maximum to the next. The **amplitude** is the height of the wave, measured from the midpoint between peak and trough to the maximum. The intensity of radiant energy, whether a feeble beam or a blinding glare, is proportional to the square of the wave's amplitude.

FIGURE 13.2 ▽

(a) Wavelength (λ) is the distance between two successive wave maxima. Amplitude is the height of the wave measured from the center. (b)–(c) What we perceive as different kinds of electromagnetic radiation are simply waves with different wavelengths and frequencies.



Multiplying the length of a wave in meters (m) by its frequency in reciprocal seconds (s^{-1}) gives the speed of the wave in meters per second (m/s). The rate of travel of all electromagnetic radiation in a vacuum is a constant value, commonly called the "speed of light" and abbreviated *c*. It's numerical value is defined as exactly 2.997 924 58 × 10^8 m/s, usually rounded off to 3.00 × 10^8 m/s.

$$\text{Wavelength} \times \text{Frequency} = \text{Speed}$$

$$\lambda\ (\text{m}) \times \nu\ (\text{s}^{-1}) = c\ (\text{m/s})$$

which can be rewritten as

$$\lambda = \frac{c}{\nu} \quad \text{or} \quad \nu = \frac{c}{\lambda}$$

Electromagnetic energy is transmitted only in discrete amounts, called *quanta*. The amount of energy ϵ corresponding to 1 quantum of energy (or 1 photon) with a given frequency ν is expressed by the equation

$$\epsilon = h\nu = \frac{hc}{\lambda}$$

where

ϵ = Energy of 1 photon (1 quantum)

h = Planck's constant (6.62 × 10^{-34} J · s = 1.58 × 10^{-34} cal · s)

ν = Frequency (s^{-1})

λ = Wavelength (m)

c = Speed of light (3.00 × 10^8 m/s)

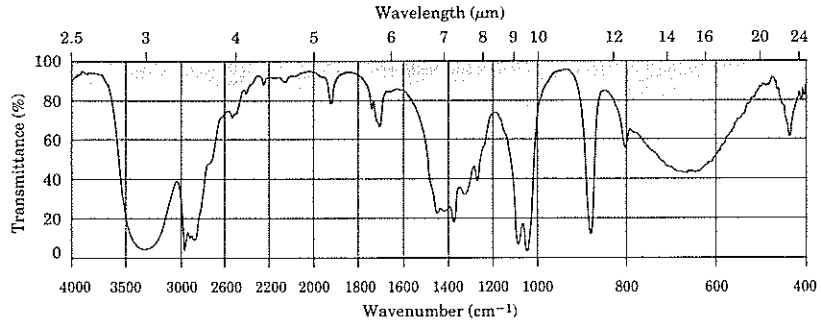
This equation says that the energy of a given photon varies *directly* with its frequency ν but *inversely* with its wavelength λ. High frequencies and short wavelengths correspond to high-energy radiation such as gamma rays; low frequencies and long wavelengths correspond to low-energy radiation such as radio waves.

When an organic compound is exposed to electromagnetic radiation, it absorbs energy of certain wavelengths but passes, or transmits, energy of other wavelengths. If we irradiate an organic compound with energy of many wavelengths and determine which are absorbed and which are transmitted, we can determine the **absorption spectrum** of the compound. The results are displayed on a plot of wavelength versus the amount of radiation transmitted.

The spectrum of ethanol irradiated with infrared radiation is shown in Figure 13.3. The horizontal axis shows the wavelength in micrometers, and the vertical axis shows the intensity of the various energy absorptions in percent transmittance. The baseline corresponding to 0% absorption (or 100% transmittance) runs along the top of the chart, and a downward spike means that energy absorption has occurred at that wavelength.

FIGURE 13.3 ▽

The infrared absorption spectrum of ethanol, CH_3CH_2OH. A transmittance of 100% means that all the energy is passing through the sample. A lower transmittance means that some energy is being absorbed. Thus, each downward spike corresponds to an energy absorption.



Practice Problem 13.1 Which is higher in energy, FM radio waves with a frequency of 1.015 × 10^8 Hz (101.5 MHz) or visible light with a frequency of 5 × 10^{14} Hz?

Strategy Remember the equations $\epsilon = h\nu$ and $\epsilon = hc/\lambda$, which say that energy increases as frequency increases and as wavelength decreases.

Solution Since visible light has a higher frequency than radio waves, it is higher in energy.

Practice Problem 13.2 What is the wavelength in meters of visible light with a frequency of 4.5×10^{14} Hz?

Strategy Frequency and wavelength are related by the equation $\lambda = c/\nu$, where c is the speed of light (3.0×10^8 m/s).

Solution $$\lambda = \frac{3.0 \times 10^8 \text{ m/s}}{4.5 \times 10^{14} \text{ s}^{-1}} = 6.7 \times 10^{-7} \text{ m}$$

Problem 13.1 How does the energy of infrared radiation with $\lambda = 1.0 \times 10^{-6}$ m compare with that of an X ray having $\lambda = 3.0 \times 10^{-9}$ m?

Problem 13.2 Which is higher in energy, radiation with $\nu = 4.0 \times 10^9$ Hz or radiation with $\lambda = 9.0 \times 10^{-6}$ m?

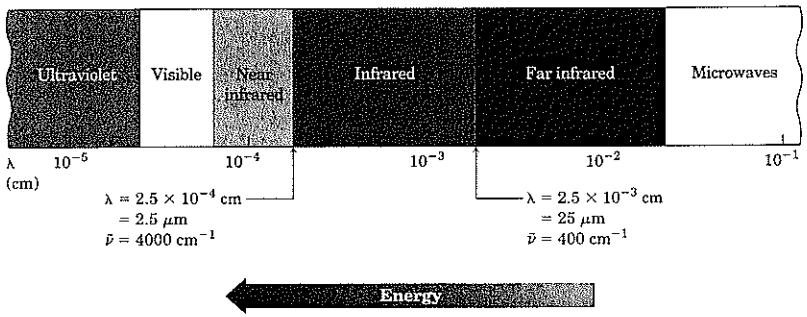
13.2 Infrared Spectroscopy of Organic Molecules

The infrared region of the electromagnetic spectrum covers the range from just above the visible (7.8×10^{-7} m) to approximately 10^{-4} m, but only the middle of the region is used by organic chemists (Figure 13.4). This midportion extends from 2.5×10^{-5} to 2.5×10^{-6} cm, and wavelengths are usually given in *micrometers* (μm; 1 μm = 10^{-6} m). Frequencies are usually given in **wavenumbers** ($\tilde{\nu}$), rather than in hertz. The wavenumber is equal to the reciprocal of the wavelength in centimeters and is thus expressed in units of reciprocal centimeters (cm^{-1}):

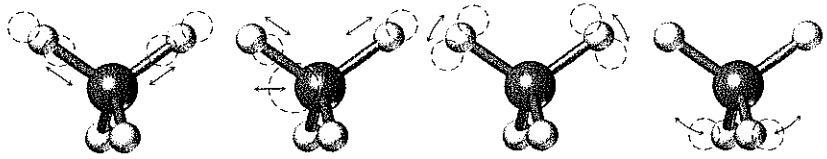
$$\text{Wavenumber: } \tilde{\nu}\ (\text{cm}^{-1}) = \frac{1}{\lambda\ (\text{cm})}$$

FIGURE 13.4 ▽

The infrared (IR) region of the electromagnetic spectrum.



Why does a molecule absorb some wavelengths of infrared energy but not others? All molecules have a certain amount of energy, which causes bonds to stretch and contract, atoms to wag back and forth, and other molecular motions to occur. Some of the kinds of allowed vibrations are shown below:



Symmetric stretching **Antisymmetric stretching** **In-plane bending** **Out-of-plane bending**

The amount of energy a molecule contains is not continuously variable but is *quantized*. That is, a molecule can vibrate only at specific frequencies corresponding to specific energy levels. Take bond stretching, for example. Although we usually speak of bond lengths as if they were fixed, the numbers given are really averages because bonds are constantly stretching and bending, lengthening and contracting. Thus, a typical C—H bond with an average bond length of 110 pm is actually vibrating at a specific frequency, alternately stretching and compressing as if there were a spring connecting the two atoms.

When the molecule is irradiated with electromagnetic radiation, *energy is absorbed if the frequency of the radiation matches the frequency of the vibration*. The result of energy absorption is an increased amplitude for the vibration; in other words, the "spring" connecting the two atoms stretches and compresses a bit further. Since each frequency absorbed by a molecule corresponds to a specific molecular motion, we can find what kinds of motions a molecule has by measuring its IR spectrum. By then interpreting those motions, we can find out what kinds of bonds (functional groups) are present in the molecule.

IR spectrum ⟶ What molecular motions? ⟶ **What functional groups?**

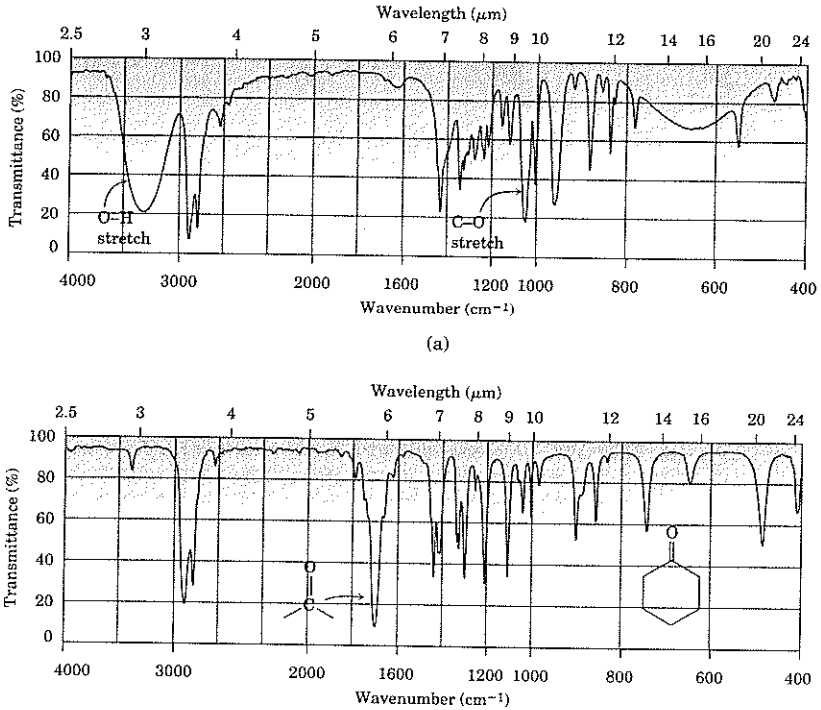
The full interpretation of an IR spectrum is difficult because most organic molecules are so large that they have dozens of different bond stretching and bending motions. Thus, an IR spectrum usually contains dozens of absorptions. Fortunately, we don't need to interpret an IR spectrum fully to get useful information because *functional groups have characteristic IR absorptions that don't change from one compound to another.* The C=O absorption of a ketone is almost always in the range 1680 to 1750 cm^{-1}, the O—H absorption of an alcohol is almost always in the range 3400 to 3650 cm^{-1}, the C=C absorption of an alkene is almost always in the range 1640 to 1680 cm^{-1}, and so forth. By learning to recognize where characteristic functional-group absorptions occur, it's possible to get structural information from IR spectra.

Look at the IR spectra of cyclohexanol and cyclohexanone in Figure 13.5 to see how they can be used. Although both spectra contain many peaks, the characteristic absorptions of the different functional groups allow the compounds to be distinguished. Cyclohexanol shows a characteristic alcohol

O—H absorption at 3300 cm^{-1} and a C—O absorption at 1060 cm^{-1}; cyclohexanone shows a characteristic ketone C=O peak at 1715 cm^{-1}.

FIGURE 13.5 ▼

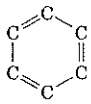
Infrared spectra of (a) cyclohexanol and (b) cyclohexanone. Such spectra are easily obtained in minutes with milligram amounts of material.



One further point about infrared spectroscopy: It's also possible to obtain structural information from an IR spectrum by noticing which absorptions are *not* present. If the spectrum of an unknown does *not* have an absorption near 3400 cm^{-1}, the unknown is not an alcohol; if the spectrum does not have an absorption near 1715 cm^{-1}, the unknown is not a ketone; and so on. Table 13.1 lists characteristic IR absorption frequencies of some common functional groups.

It helps in remembering the positions of various IR absorptions to divide the infrared range from 4000 to 200 cm^{-1} into four parts, as shown in Figure 13.6.

TABLE 13.1 Characteristic Infrared Absorptions of Some Functional Groups

Functional group class	Band position (cm^{-1})	Intensity of absorption
Alkanes; alkyl groups		
C—H	2850–2960	Medium to strong
Alkenes		
=C—H	3020–3100	Medium
C=C	1640–1680	Medium
Alkynes		
≡C—H	3300	Strong
—C≡C—	2100–2260	Medium
Alkyl halides		
C—Cl	600–800	Strong
C—Br	500–600	Strong
C—I	500	Strong
Alcohols		
O—H	3400–3650	Strong, broad
C—O	1050–1150	Strong
Aromatics		
	3030	Weak
	1660–2000	Weak
	1450–1600	Medium
Amines		
N—H	3300–3500	Medium
C—N	1030–1230	Medium
Carbonyl compounds[a]		
C=O	1670–1780	Strong
Carboxylic acids		
O—H	2500–3100	Strong, very broad
Nitriles		
C≡N	2210–2260	Medium
Nitro compounds		
NO_2	1540	Strong

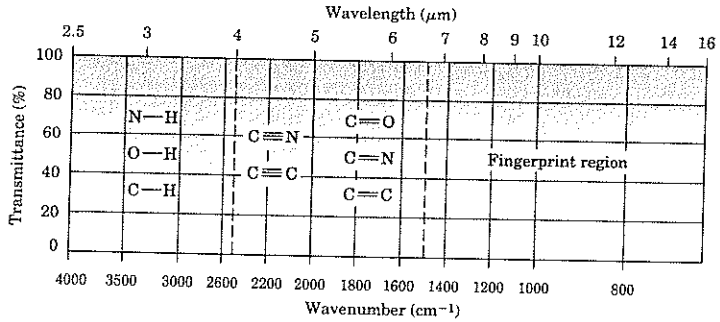
[a]Carboxylic acids, esters, aldehydes, and ketones.

52

• The region from 4000 to 2500 cm^{-1} corresponds to N—H, C—H, and O—H bond stretching motions. Both N—H and O—H bonds absorb in the 3300 to 3600 cm^{-1} range, whereas C—H bond stretching occurs near 3000 cm^{-1}. Since almost all organic compounds have C—H bonds, almost all IR spectra have an intense absorption in this region.

FIGURE 13.6 ▽

Single-bond, double-bond, triple-bond, and fingerprint regions in the infrared spectrum.



- The region from 2500 to 2000 cm^{-1} is where triple-bond stretching occurs. Both nitriles (RC≡N) and alkynes (RC≡CR′) absorb here.
- The region from 2000 to 1500 cm^{-1} is where C=O, C=N, and C=C bonds absorb. Carbonyl groups generally absorb from 1670 to 1780 cm^{-1}, and alkene stretching normally occurs in the narrow range from 1640 to 1680 cm^{-1}. The exact position of a C=O absorption is often diagnostic of the exact kind of carbonyl group in the molecule. Esters usually absorb at 1735 cm^{-1}, aldehydes at 1725 cm^{-1}, and open-chain ketones at 1715 cm^{-1}.
- The region below 1500 cm^{-1} is the so-called *fingerprint region*. A large number of absorptions due to various C—O, C—C, and C—N single-bond vibrations occur here, forming a unique pattern that acts as an identifying "fingerprint" of each organic molecule.

Practice Problem 13.3 Refer to Table 13.1 and make educated guesses about the functional groups that cause the following IR absorptions:
(a) 1735 cm^{-1} (b) 3500 cm^{-1}

Solution (a) An absorption at 1735 cm^{-1} is in the carbonyl-group region of the IR spectrum, probably an ester.
(b) An absorption at 3500 cm^{-1} is in the —OH (alcohol) region.

Practice Problem 13.4 Acetone and 2-propen-1-ol ($H_2C{=}CHCH_2OH$) are isomers. How could you distinguish them by IR spectroscopy?

Strategy Identify the functional groups in each molecule, and refer to Table 13.1.

Solution Table 13.1 shows that acetone has a strong C=O absorption at 1715 cm^{-1}, while 2-propen-1-ol has an —OH absorption at 3500 cm^{-1} and a C=C absorption at 1660 cm^{-1}.

Problem 13.3 What functional groups might molecules contain if they show IR absorptions at the following frequencies?
(a) 1715 cm^{-1} (b) 1540 cm^{-1}
(c) 2210 cm^{-1} (d) 1720 and 2500–3100 cm^{-1}
(e) 3500 and 1735 cm^{-1}

53

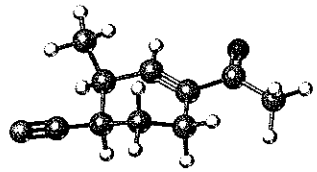
Problem 13.4 How might you use IR spectroscopy to help distinguish between the following pairs of isomers?

(a) CH_3CH_2OH and CH_3OCH_3

(b)  and $CH_3CH_2CH_2CH_2CH{=}CH_2$

(c) $CH_3CH_2\overset{O}{\overset{\|}{C}}OH$ and $HOCH_2CH_2\overset{O}{\overset{\|}{C}}H$

Problem 13.5 Where might the following molecule have IR absorptions?



13.3 Ultraviolet Spectroscopy

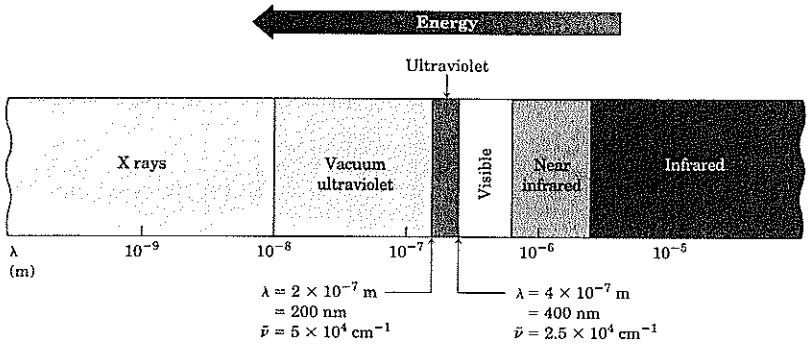
The **ultraviolet (UV)** region of the electromagnetic spectrum extends from the low-wavelength end of the visible region (4×10^{-7} m) to 10^{-8} m. The portion of greatest interest to organic chemists, though, is the narrow range from 2×10^{-7} m to 4×10^{-7} m. Absorptions in this region are measured in *nanometers* (nm), where 1 nm = 10^{-9} m = 10^{-7} cm. Thus, the ultraviolet range of interest is from 200 to 400 nm (Figure 13.7).

We saw in Section 13.1 that an organic molecule either absorbs or transmits electromagnetic energy when irradiated, depending on the radiation's energy level. With IR radiation, the energy absorbed corresponds to the amount necessary to raise the amplitude of molecular bending or stretching vibrations. With UV radiation, the energy absorbed corresponds to the amount necessary to raise the energy level of a π electron in an unsaturated molecule.

Ultraviolet spectra are recorded by irradiating a sample with UV light of continuously changing wavelength. When the wavelength of light corresponds to the amount of energy required to promote a π electron in an unsaturated molecule to a higher level, energy is absorbed. The absorption is detected and displayed on a chart that plots wavelength versus percent radiation absorbed.

FIGURE 13.7 ▽

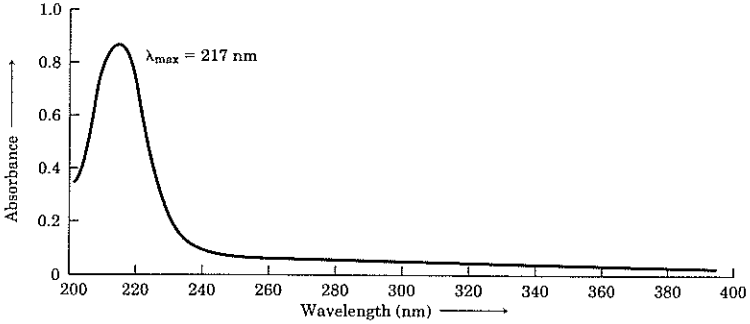
The ultraviolet (UV) region of the electromagnetic spectrum.



A typical UV spectrum—that of 1,3-butadiene—is shown in Figure 13.8. Unlike IR spectra, which generally have many peaks, UV spectra are usually quite simple. Often, there is only a single broad peak, which is identified by noting the wavelength at the very top (λ_{max}). For 1,3-butadiene, λ_{max} = 217 nm.

FIGURE 13.8 ▽

Ultraviolet spectrum of 1,3-butadiene.



13.4 Interpreting Ultraviolet Spectra: The Effect of Conjugation

The wavelength of radiation necessary to raise the energy of a π electron in an unsaturated molecule depends on the nature of the π electron system in the molecule. One of the most important factors is the extent of conjugation

(Section 4.10). It turns out that the energy required for an electronic transition *decreases* as the extent of conjugation *increases*. Thus, 1,3-buta*di*ene shows an absorption at λ_{max} = 217 nm, 1,3,5-hexa*tri*ene absorbs at λ_{max} = 258 nm, and 1,3,5,7-octa*tetra*ene has λ_{max} = 290 nm. (Remember: Longer wavelength means lower energy.)

Other kinds of conjugated π electron systems besides dienes and polyenes also show ultraviolet absorptions. Conjugated enones, such as 3-buten-2-one, and aromatic molecules, such as benzene, also have characteristic UV absorptions that aid in structure determination. The UV absorption maxima of some representative conjugated molecules are given in Table 13.2.

TABLE 13.2 Ultraviolet Absorption Maxima of Some Conjugated Molecules

Name	Structure	λ_{max} (nm)
Ethylene	$H_2C{=}CH_2$	171
2-Methyl-1,3-butadiene	$H_2C{=}C(CH_3){-}CH{=}CH_2$	220
1,3-Cyclohexadiene		256
1,3,5-Hexatriene	$H_2C{=}CH{-}CH{=}CH{-}CH{=}CH_2$	258
3-Buten-2-one	$H_2C{=}CH{-}C(CH_3){=}O$	219
Benzene		254

Practice Problem 13.5 1,5-Hexadiene and 1,3-hexadiene are isomers. How can you distinguish them by UV spectroscopy?

$H_2C{=}CHCH_2CH_2CH{=}CH_2$ **1,5-Hexadiene** $\quad$ $CH_3CH_2CH{=}CHCH{=}CH_2$ **1,3-Hexadiene**

Solution 1,3-Hexadiene is a conjugated diene, but 1,5-hexadiene is nonconjugated. Only the conjugated isomer shows a UV absorption above 200 nm.