Carolina Biological Supply/Phototake, Inc.

Leaf of a roundleaf sundew leaf *(Drosera rotundifolia)*. Enzymes secreted by the hairs on the leaf digest trapped insects, providing nutrients to the plant.

Study Plan

4 Energy, Enzymes, and Biological Reactions

Why It Matters

The rotting trunk of a fallen tree is a reminder that death comes to all organisms. Yet, the rotten hulk is crowded with living organisms. Various insects and fungi live on the organic matter of the decaying tree, and with a microscope, you could see that it is also teeming with bacteria and other microorganisms.

If the tree fell in a forest in eastern North America, one of the fungi you might find would be the "old man of the woods" mushroom, known scientifically as *Strobilomyces floccopus* **(Figure 4.1).** The cap and stalk are the most visible parts of the mushroom, but the fungus also includes slender filaments that thread into the rotting tree. Collectively, the filaments represent the mycelium, the part of the fungus devoted to securing nutrients. Similar to that of other fungi, the mycelium of the old man of the woods secretes enzymes for the extracellular digestion of complex compounds—in this case, those of the tree—and absorbs the simple molecules that are produced, converting them into simpler molecules that can be absorbed for use as raw materials and as an energy source. If you look on the underside of the mushroom's mottled brown cap, you will see hundreds of minute tubes holding the mushroom's reproductive spores. They will be re-

David Work

Figure 4.1
Old man of the woods mushroom *(Strobilomyces floccopus)* growing on a rotting tree trunk. Enzymes produced by the fungus help convert the wood to sugars that can be used as an energy source.

leased, producing other mushrooms of the same type if they fall into a favorable environment.

Thus, in death, the fallen tree becomes a basis for new life. The energy and raw materials derived from its organic molecules allow other organisms to grow, maintain their highly organized state, and reproduce.

You would have arrived at the same fundamental understanding of the connection between energy and life if you had focused your attention on a living tree in the forest, a robin, a squirrel, an earthworm, a fly, or any other living organism. **Metabolism—**the biochemical modification and use of organic molecules and energy to support the activities of life—happens only in living organisms. Metabolism comprises thousands of biochemical reactions that accomplish the special activities we associate with life, such as growth, reproduction, movement, and the ability to respond to stimuli. Metabolism depends on enzymes, that is, proteins that speed the rate of cellular chemical reactions. Without enzymes, the pace of life would be very slow indeed.

Understanding how biological reactions occur and how enzymes work requires knowledge of the basic laws of chemistry and physics. All reactions, whether they occur inside living organisms or in the outside, inanimate world, obey the same chemical and physical laws that operate everywhere in the universe. These fundamental laws are the subject of this chapter, which is our starting point for exploring the nature of energy and how cells use it to conduct their activities.

4.1 Energy, Life, and the Laws of Thermodynamics

Life, like all chemical and physical activities, is an energy-driven process. Energy cannot be measured or weighed directly. We can detect it only through its effects on matter, including its ability to move objects against opposing forces, such as friction, gravity, or pressure, or to push chemical reactions toward completion. Therefore, **energy** is most conveniently defined as *the capacity to do work*. Even when you are asleep, cells of your muscles, brain, and other parts of your body are at work and using energy.

Energy Exists in Different Forms and States

Energy takes several different forms, including heat, chemical, electrical, mechanical, and radiant energy. Visible light, infrared and ultraviolet light, gamma rays, and X-rays are all types of radiant energy. Although the forms of energy are different, energy can be converted readily from one form to another. For example, chemical energy is transformed into electrical energy in a flashlight battery, and electrical energy is transformed into light and heat energy in the flashlight bulb. In green plants, the radiant energy of sunlight is converted into chemical energy in the form of complex sugars and other organic molecules.

Kinetic and Potential Energy. All forms of energy can exist in one of two states: kinetic and potential. **Kinetic energy** (*kinetikos* = putting in motion) is the energy of motion, for example, of waves, electrons, atoms, molecules, substances, and objects. Electrical energy, radiant energy, thermal energy, sound, and motion energy are forms of kinetic energy. For instance, a moving object can transfer some of its energy to other objects, as when a baseball is hit with a bat. **Potential energy** is stored energy; it is energy present in a nonmoving location or in the specific arrangement of atoms. Chemical energy, nuclear energy, gravitational energy, and stored mechanical energy are forms of potential energy. Heavy snow located high on a mountainside represents an example of potential energy because it is readily converted into the kinetic energy of an avalanche if it begins to slide downward. A compressed spring provides another example of potential energy; it converts its potential energy to kinetic form when it is released. The reverse conversion, from kinetic to potential energy, also takes place readily. For example, a cyclist converts kinetic energy to potential energy when pushing a bicycle uphill.

Energy Conversions in Living Organisms: Catabolic and Anabolic Reactions. Conversions between potential and kinetic energy also occur in living organisms. For example, sugar has potential energy in the form of the complex arrangement of atoms and chemical bonds in the sugar molecules. All living organisms break down sugar molecules to convert their potential energy into kinetic energy; they then use the kinetic energy to do the metabolic work of life.

Cellular reactions that break down complex molecules such as sugar to make their energy available for cellular work are called **catabolic reactions** (*cata* = downward, as in the sense of a rock releasing energy as it rolls down a hill). Metabolic reactions of the opposite type, which require energy to assemble simple substances into more complex molecules, are termed **anabolic reactions** (*ana* = upward, as in the sense of using energy to push a rock up a hill). An example of

an anabolic reaction is the assembly of proteins from amino acids. Typically, living organisms use energy released in catabolic reactions to drive their anabolic reactions.

The Laws of Thermodynamics Describe the Energy Flow in Natural Systems

The study of the energy flow during chemical and physical reactions, including the catabolic and anabolic reactions of living organisms, is called **thermodynamics.** The results of this study are summarized in two fundamental laws of thermodynamics, which allow us to predict whether reactions of any kind, including biological reactions, can occur. That is, if particular groups of molecules are placed together, are they likely to react chemically and change into a different group of molecules? The laws also give us the information necessary to trace energy flows in biological reactions: they allow us to estimate the amount of energy released or required as a reaction proceeds.

The group of reacting molecules studied in thermodynamics is called a *system*. A system is whatever we define it to be; it can be as small as a single molecule or as large as the universe. Everything outside a system is its *surroundings*. In undergoing any type of change, such as a chemical reaction, a system goes from an *initial* state before the reaction begins to a *final* state when the reaction ends. There are two main types of systems: *closed* and *open*. Closed systems **(Figure 4.2a)** can exchange energy but not matter with their surroundings, whereas open systems **(Figure 4.2b)** can exchange both energy and matter with their surroundings. Living organisms are open systems because they constantly exchange matter with their surroundings. However, within a living organism, many individual biochemical reactions operate as closed systems.

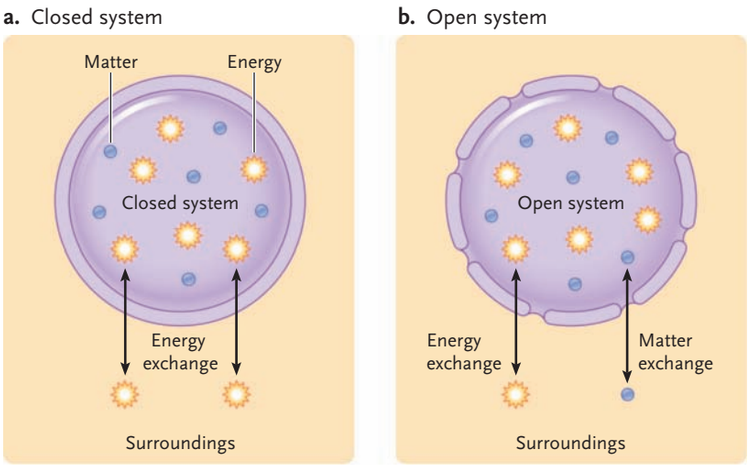


Figure 4.2
Closed and open systems in thermodynamics.

The First Law of Thermodynamics Addresses the Energy Content of Systems and Their Surroundings

The **first law of thermodynamics**, also called the principle of the conservation of energy, states that *energy can be transferred and transformed but it cannot be created or destroyed*. That is, in any process that involves an energy change, the *total amount of energy in a system and its surroundings remains constant*.

If energy can be neither created nor destroyed, what is the ultimate source of the energy we and other living organisms use? For almost all organisms, the ultimate source is the sun **(Figure 4.3).** Plants capture the kinetic energy of the light radiating from the sun by absorbing it and converting it to the potential chemical energy of complex organic molecules—primarily sugars, starches, and lipids. These substances are used as fuels by the plants themselves, by animals that feed on plants, and by organisms (such as fungi and bacteria) that break down the bodies of dead organisms. The potential energy stored in sugars and other organic molecules is used for growth, reproduction, and other work of living organisms.

Eventually, most of the solar energy absorbed by green plants is converted into heat energy as the activities of life take place. Heat energy (a form of kinetic energy) is largely unusable by living organisms; as a result, most of the heat released by the reactions of living organisms radiates to their surroundings, and then from Earth into space.

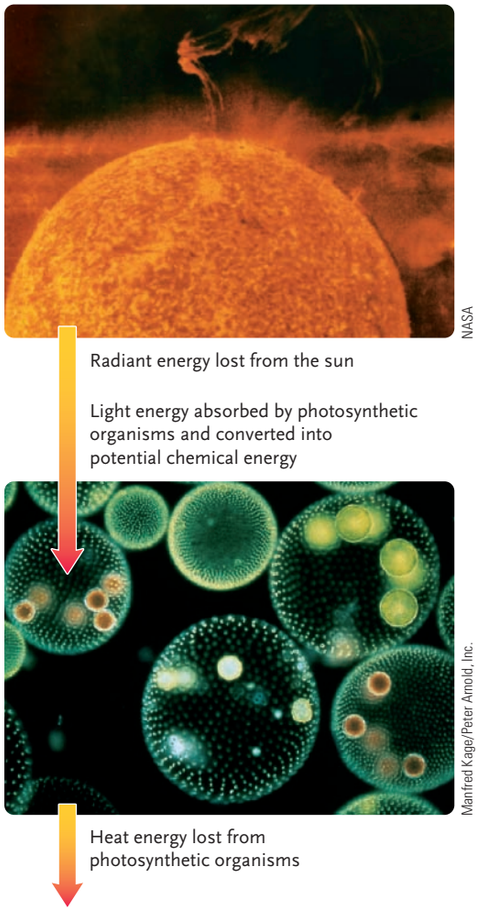


Figure 4.3
Energy flow from the sun to photosynthetic organisms (colonies of the green alga *Volvox*), which capture the kinetic radiant energy of sunlight and convert it to potential chemical energy in the form of complex organic molecules.

How does the principle of conservation of energy apply to biochemical reactions? Molecules have both kinetic and potential energy. Kinetic energy for molecules above absolute zero (−273°C) is reflected in the constant motion of the molecules, whereas potential energy for molecules is the energy contained in the arrangement of atoms and chemical bonds. The energy content of reacting systems provides part of the information required to predict the likelihood and direction of chemical reactions. Usually, the energy content of the reactants in a chemical reaction (see Section 2.3) is larger than the energy content of the products. Thus, reactions usually progress to a state in which the products have *minimum energy content*. When this is the case, the difference in energy content between reactants and products in the reacting system is released to the surroundings.

The Second Law of Thermodynamics Considers Changes in the Degree of Order in Reacting Systems

The second law of thermodynamics explains why, as any energy change occurs, the objects (matter) involved in the change typically become more disordered. (Your room and the kitchen at home are probably the best examples of this phenomenon.) You know from experience that it takes energy to straighten out (decrease) the disorder (as when you have to clean up your room).

The **second law of thermodynamics** states this tendency toward disorder formally, in terms of a system and its surroundings: in any process in which a system changes from an initial to a final state, *the total disorder of the system and its surroundings always increases*. In thermodynamics, disorder is called **entropy.** If the system and its surroundings are defined as the entire universe, the second law means that as changes occur anywhere in the universe, the total disorder or entropy of the universe constantly increases. As the first law of thermodynamics asserts, however, the total energy in the universe does not change.

At first glance, living organisms appear to violate the second law of thermodynamics. As a fertilized egg develops into an adult animal, it becomes more highly ordered (decreases its entropy) as it synthesizes organic molecules from less complex substances. However, the entropy of the whole system—the surroundings, as well as the organism—must be considered as growth proceeds. For a fertilized egg—the initial state—its surroundings include all the carbohydrates, fats, and other complex organic molecules that the developing animal uses to develop into an adult. When development is complete—the final state—the surroundings include the animal's waste products (water, carbon dioxide, and many relatively simple organic molecules), which are collectively much less complex than the organic molecules used as fuels. When the total reactants, including all the nutrients, and the total products, including all the waste materials, are included, the total change satisfies both laws of thermodynamics—the total energy content remains constant, and the entropy of the system and its surroundings increases.

Applying the first and second laws of thermodynamics together allows us to predict whether any particular chemical or physical reaction will occur without outside help. Such reactions are called **spontaneous reactions** in thermodynamics. In this usage, the word *spontaneous* means only that a reaction will occur. It does not describe the rate of a reaction; indeed, spontaneous reactions may proceed very slowly, such as the formation of rust on a nail, or very quickly, such as a match bursting into flame. This concept is important in biology, because enzymes cannot make a reaction take place if it is not already spontaneous—*enzymes can only make spontaneous reactions go faster.*

Change in Free Energy Indicates Whether a Reaction Is Spontaneous

Free energy is the energy in a system that is available to do work. In living organisms, free energy accomplishes the chemical and physical work involved in activities such as the synthesis of molecules, movement, and reproduction.

A free energy equation combines the energy and entropy changes in a system going from initial to final states (such as reactants to products):

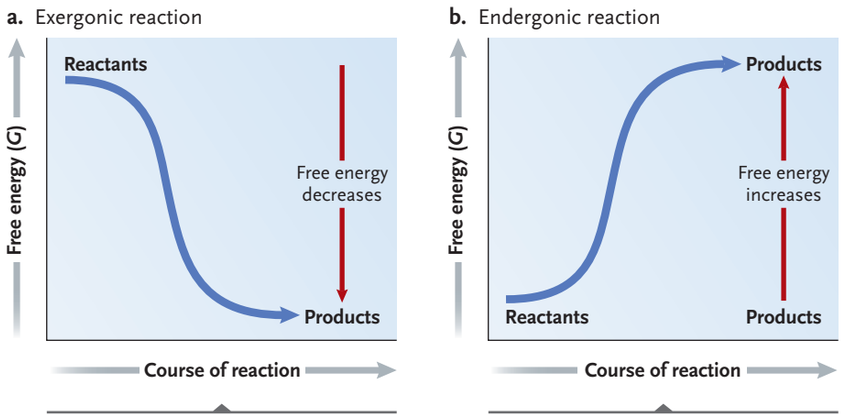
$$\Delta G = \Delta H - T\Delta S$$

in which Δ (pronounced delta) means "change in." ΔG is the change in free energy in the system (where the G recognizes physicist Josiah Willard Gibbs, the creator of the concept), ΔH is the change in energy content (considered as heat, H), T is the absolute temperature in degrees Kelvin (K, where K = °C + 273.16), and ΔS is the change in entropy. The equation states that *the free energy change as a system goes from initial to final states is the sum of the changes in energy content and entropy.* For a reaction to be spontaneous, ΔG must be negative. This negative value indicates that the free energy released by the reaction is lost from the system and is gained by the surroundings as the reaction goes from the initial to the final state. Overall, entropy has increased.

Reactions that have a negative ΔG because they release free energy are termed **exergonic reactions** (*ergon* = work) **(Figure 4.4a).** For biological systems, the free energy released by exergonic reactions accomplishes growth, movement, and all the other activities of life. If ΔG is positive, a reaction proceeds only if free energy is added to the system. Reactions that can proceed only if free energy is supplied are termed **endergonic reactions (Figure 4.4b).** Typically, the free energy for such reactions is supplied from other, exergonic, reactions.

In practical applications of the free energy equation, free energy changes (ΔG) are determined under standard conditions with the results given in kilocalories per mole (kcal/mol) of reactants converted to products. The value obtained allows the energy released or required by one reacting system to be compared directly with another.

The calculated free energy can determine the likelihood of a reaction occurring. For example, if sucrose is placed in a test tube with water, will it break down (hydrolyze) into glucose and fructose? Or, if glucose and fructose are placed together with water in a test tube, will they combine to form sucrose? The hydrolysis reaction has a ΔG of -5.5 kcal/mol, which means that for each mole of sucrose hydrolyzed, 5.5 kcal of energy is *released*. By contrast, the synthesis reaction has a ΔG of $+5.5$ kcal/mol; 5.5 kcal of energy must be *added* to convert 1 mole of reactants into 1 mole of products. Therefore, the hydrolysis of sucrose to glucose and fructose can proceed spontaneously because ΔG is negative for this reaction, but the synthesis of sucrose from glucose and fructose cannot proceed spontaneously because it has a positive ΔG. However, plants in particular perform this synthesis reaction on a regular basis. How do they do it? The next section describes how plants, and in fact all living organisms, carry out synthetic reactions without violating the laws of thermodynamics.



In an exergonic reaction, free energy is released. The products have less free energy than was present in the reactants, and the reaction proceeds spontaneously.

In an endergonic reaction, free energy is gained. The products have more free energy than was present in the reactants. An endergonic reaction is not spontaneous: it proceeds only if energy is supplied by an exergonic reaction.

Figure 4.4
Exergonic **(a)** and endergonic **(b)** reactions.

Study Break

1. Distinguish between kinetic and potential energy.
2. Distinguish between catabolic and anabolic reactions.
3. In thermodynamics, what is meant by an open system and a closed system?

4.2 How Living Organisms Couple Reactions to Make Synthesis Spontaneous

Many individual reactions of living organisms, particularly those that involve the assembly of complex molecules from less complex building blocks, have a positive ΔG and therefore are not spontaneous. For example, cells commonly carry out reactions in which ammonia (NH_3) is added to glutamic acid, an amino acid with one amino group, to produce glutamine, an amino acid with two amino groups:

$$\text{glutamic acid} + NH_3 \rightarrow \text{glutamine} + H_2O$$
$$\Delta G = +3.4 \text{ kcal/mol}$$

The glutamine is used in the assembly of proteins and is a donor of nitrogen for other reactions in the cell. The positive value for ΔG shows that the reaction cannot proceed spontaneously.

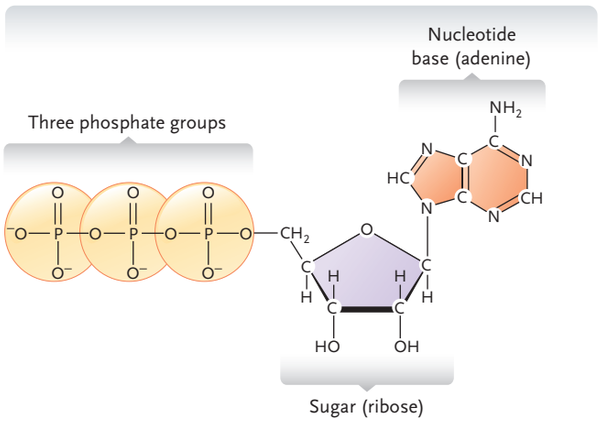
How, then, do cells carry out this reaction? They join it to another reaction with a large negative ΔG. The combined reaction, called a **coupled reaction**, has a negative ΔG, which indicates that it is spontaneous and will release free energy. In effect, the coupling system works by joining an exergonic reaction to the endergonic reaction, producing an overall reaction that is exergonic. All the endergonic reactions of living organisms, including those of growth, reproduction, movement, and response to stimuli, are made possible by coupling reactions in this way.

ATP Is the Primary Coupling Agent in All Living Organisms

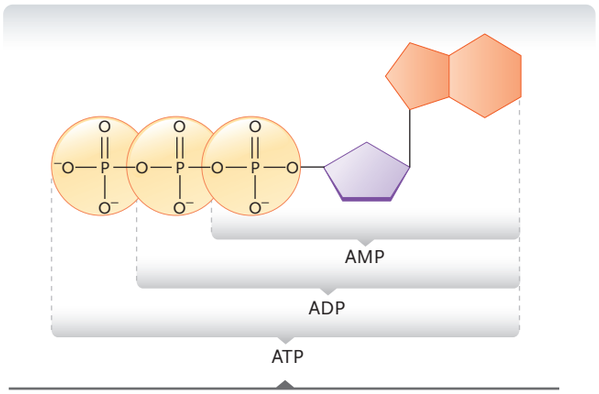
All cells, from bacteria to those of plants and animals, use the nucleotide **ATP** as the primary agent that couples exergonic and endergonic reactions. ATP provides an injection of free energy that does biological work.

ATP consists of a five-carbon sugar, ribose, linked to the nitrogenous base adenine and a chain of three phosphate groups **(Figure 4.5a).** Much of the potential energy of ATP is associated with the arrangement of the three phosphate groups, which carry a strongly negative charge in the cellular environment. Because of the close alignment of the three

a. Chemical structure of ATP



b. Adenine nucleotides



With one phosphate group, the molecule is known as AMP; with two phosphates, the molecule is called ADP. Each added phosphate packs additional potential chemical energy into the molecular structure.

c. Hydrolysis reaction removing a phosphate group from ATP

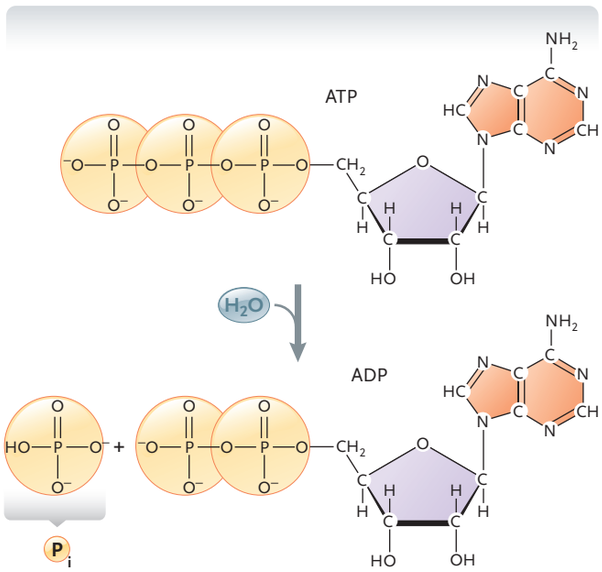


Figure 4.5
ATP, the primary molecule that couples energy-requiring reactions to energy-releasing reactions in living organisms. (P_i is the symbol used in this book for inorganic phosphate.)

phosphate groups, the negative charges repel each other strongly. Removal of one or two of the three phosphate groups is a spontaneous reaction that relieves the repulsion and releases large amounts of free energy **(Figure 4.5b).**

For example, removal of just one phosphate group (a hydrolysis reaction; see **Figure 4.5c**) releases a large increment of free energy:

$$ATP + H_2O \rightarrow ADP + P_i$$
$$\Delta G = -7.3 \text{ kcal/mol}$$

The products of this reaction are ADP and inorganic phosphate (P_i). Removal of two of the phosphate groups produces adenosine monophosphate (AMP) and almost doubles the amount of free energy released.

Cells Couple Reactions Directly by Linking Phosphate Groups from ATP to Other Molecules

Although ATP hydrolysis releases a large burst of free energy, cells do not use ATP hydrolysis directly as a mechanism to couple reactions and release energy. Instead, the ADP or phosphate group produced by ATP breakdown is temporarily linked to one of the reacting molecules or to an enzyme that accelerates a coupled reaction. In effect, the linkage transfers potential chemical energy to the molecule binding the ADP or phosphate group and, in this way, conserves much of the free energy released by ATP hydrolysis.

The reactions that couple ATP breakdown to the synthesis of glutamine from glutamic acid illustrate the process. As a first step, the phosphate group removed from ATP is transferred to glutamic acid, forming glutamyl phosphate:

$$\text{glutamic acid} + ATP \rightarrow \text{glutamyl phosphate} + ADP$$

The addition of a phosphate group to a molecule is called **phosphorylation.** The ΔG for this reaction is negative, making the reaction spontaneous, but much less free energy is released than in the hydrolysis of ATP to ADP + P_i. In the second step, glutamyl phosphate reacts with NH_3:

$$\text{glutamyl phosphate} + NH_3 \rightarrow \text{glutamine} + P_i$$

This second reaction also has a negative value for ΔG and is spontaneous.

Even though the reaction proceeds in two steps, it is usually written for convenience as one reaction, with a combined negative value for ΔG:

$$\text{glutamic acid} + NH_3 + ATP \rightarrow$$
$$\text{glutamine} + ADP + P_i$$
$$\Delta G = -3.9 \text{ kcal/mol}$$

Because ΔG is negative, the coupled reaction is spontaneous and releases energy. The difference between −3.9 kcal/mol and the −7.3 kcal/mol released by hydrolyzing ATP to ADP + P_i represents potential chem-

ical energy transferred to the glutamine molecules produced by the reaction.

Cells also Couple Reactions to Replenish Their ATP Supplies

How do cells replace the ATP used in coupling reactions? The reaction has a positive ΔG and is therefore endergonic:

$$ADP + P_i \rightarrow ATP + H_2O$$
$$\Delta G = +7.3 \text{ kcal/mol}$$

Cells accomplish this feat by coupling reactions that link ATP synthesis to catabolic reactions such as the breakdown of energy-rich sugar molecules. For example, if glucose is simply burned by igniting it in air, large quantities of free energy are released:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$
$$\Delta G = -686 \text{ kcal/mol}$$

Rather than burning glucose directly, cells couple the reaction breaking down glucose to the synthesis of ATP from ADP and P_i:

$$C_6H_{12}O_6 + 30\ ADP + 30\ P_i + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 30\ ATP$$
$$\Delta G = -476 \text{ kcal/mol}$$

The coupled reaction, shown here in greatly simplified form, is spontaneous and releases free energy, but much less than when glucose is burned in air; the difference represents energy conserved in ATP.

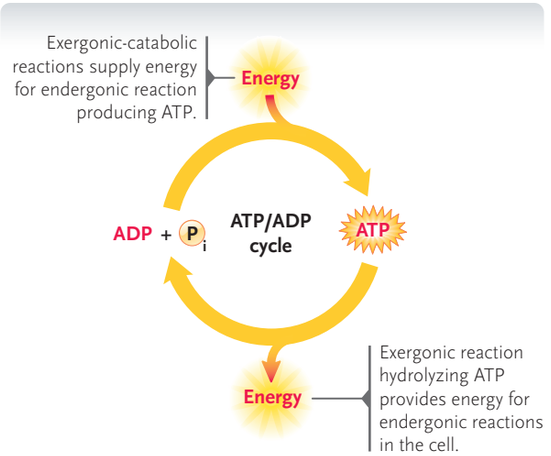
ATP thus cycles between reactions that release free energy and reactions that require free energy **(Figure 4.6a).** Adding or removing phosphate groups in the ATP/ADP/AMP system is similar to compressing or releasing a spring. Adding phosphate groups, up to a limit of three, compresses the spring and stores potential energy in the molecule. Removing one or two phosphate groups releases the spring and makes free energy available for cellular work. Examples of cellular events driven by ATP hydrolysis are shown in **Figure 4.6b;** additional examples appear in many other chapters of this book.

The discussion up to this point has assumed that spontaneous reactions go to completion—that is, that reactants are converted completely into products. However, as the next section shows, other factors can oppose completion, stopping the progress of a reaction at a point where both reactants and products are present and remain in the system.

Study Break

1. How are coupled reactions important to cell function?
2. Explain the composition of the ATP molecule. What happens to ATP when it is used in a phosphorylation reaction?

a. The ATP/ADP cycle, which couples reactions that release free energy to reactions that require free energy



b. Examples of cellular events driven by ATP hydrolysis

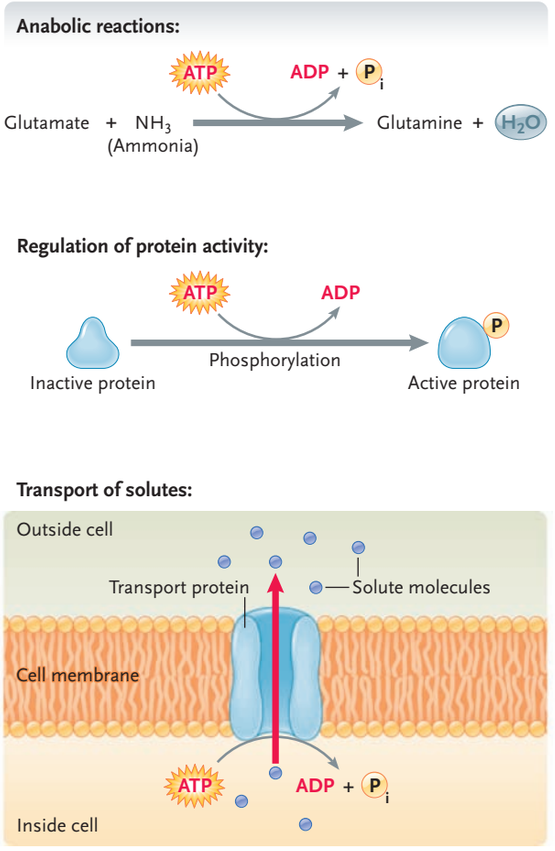


Figure 4.6 Formation and hydrolysis of ATP, which is centrally important for biological reactions. **(a)** ATP/ADP cycle. This cycle couples reactions that release free energy to reactions that require free energy. **(b)** Examples of cellular events driven by ATP hydrolysis.

4.3 Thermodynamics and Reversible Reactions

Several conditions can oppose the completion of spontaneous biological reactions, even though the reactions have a negative ΔG. Instead, the reactions run in the

direction of completion (toward reactants or toward products) until they reach the **equilibrium point**, a state of balance between the opposing factors pushing the reaction in either direction. At the equilibrium point, both reactants and products are present and the reactions typically are reversible.

The Concentration of Reactants and Products Often Establishes an Equilibrium Point

The relative concentrations (concentration = number of molecules per unit volume) of reactant and product molecules in the solution containing a reaction can oppose completion of the reaction. A solution containing reactants and products of a reaction is at a state of maximum entropy (disorder) when all the molecules are evenly distributed in equal concentrations. In terms of your room, this situation is equivalent to having all your books and clothing in a complete state of disorder on the floor. As a reaction runs past the point when the concentrations of reactants and products are equal, it begins to reduce entropy as it decreases the number of reactant molecules and adds additional product molecules, which is equivalent to beginning to hang clothes on hooks and place books on shelves.

This entropy reduction requires energy, and it begins to use some of the free energy released by the reaction. As the reaction continues, eventually the free energy released by the reaction is no longer sufficient to reduce entropy further—the equilibrium point has been reached **(Figure 4.7).** At this balance point, reactant molecules constantly change into products, and products change into reactants, at equal rates. In other words, the rates of the forward and backward reactions are equal at the equilibrium point. (For chemical reactions of all types, *rate* means the number of reactant molecules converted to products, or products to reactants, per unit time.)

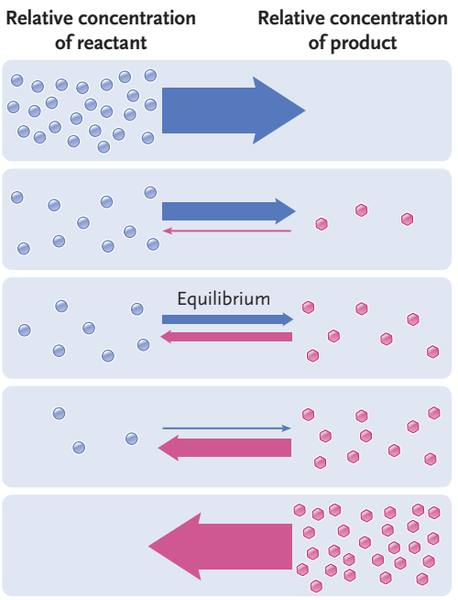


Figure 4.7
The equilibrium point of a reaction. Conditions opposing completion of spontaneous reactions, such as relative concentrations of reactants and products, stop the progress of reactions at an equilibrium point. At this point, the number of reactant molecules being converted to products equals the number of product molecules being converted back to reactants. The reaction at the equilibrium point is reversible; it may be made to run to the right (forward) by adding more reactants or to the left (backward) by adding more products.

The concentrations of reactants and products at the equilibrium point are not necessarily equal. Generally, the greater the negative value of ΔG, the further a reaction will proceed toward completion, with proportionately greater numbers of product molecules than reactant molecules at the equilibrium point.

At the equilibrium point, small changes in conditions can push a reaction in either direction, toward reactants or toward products. Thus, reactions that reach an equilibrium point are **reversible** (see Figure 4.7). Reversible reactions are written with a double arrow to indicate this feature:

$$\underset{\text{reactants}}{A + B} \rightleftharpoons \underset{\text{products}}{C + D}$$

Many Biological Reactions Keep Running Because They Never Reach Equilibrium

Most biological reactions have an equilibrium point and are reversible. However, many individual reactions in living organisms never reach an equilibrium point because they are parts of a *metabolic pathway*—a series of sequential reactions in which the products of one reaction are used immediately as the reactants for the next reaction in the series. (An example of a pathway is shown in Figure 4.18.) This immediate use of reactants keeps the individual reactions, and the entire metabolic pathway, running as long as the final products do not accumulate in excess. Metabolic pathways may be anabolic, synthesizing complex molecules from simpler substances, or catabolic, degrading complex molecules to simpler forms.

Like all biological reactions, each reversible reaction of a metabolic pathway is speeded by an enzyme. The role of enzymes in biological reactions is described in the next section.

Study Break

What is the relation between ΔG and the concentrations of reactants and products at the equilibrium point of a reaction?

4.4 Role of Enzymes in Biological Reactions

Many reactions, although spontaneous, proceed so slowly that their rate is essentially zero at the temperatures characteristic of living organisms. Enzymes increase the rate of biological reactions to levels that sustain the activities of life. For reversible reactions, enzymes speed progress toward the equilibrium point. For most enzymes, the increase ranges from a minimum of about a million to as much as a trillion times faster than the same reaction would proceed without its enzyme.

The majority of enzymes have names ending in *-ase*. The rest of the name typically relates to the substrate of the enzyme or to the type of reaction with which the enzyme is associated. For example, enzymes that break down proteins are called *proteinases* or *proteases*.

Enzymes are not the only biological molecules capable of accelerating reaction rates. Some RNA molecules (see Section 4.6) also have this capacity. To distinguish between the two types of molecules, most biologists reserve the term *enzyme* for protein molecules that can accelerate reaction rates and call the RNA molecules with this capacity *ribozymes*. We follow this usage in this book.

Many inorganic substances, particularly metallic ions, function as catalysts. One common example is platinum, which is used in the catalytic converter of automobiles to speed the breakdown of smog-forming substances in the exhaust. Substances with the ability to accelerate spontaneous reactions without being changed by the reactions, including enzymes, ribozymes, and their inorganic counterparts, are called **catalysts**—that is, they *catalyze* reactions. The acceleration of a reaction by a catalyst is called *catalysis*.

Enzymes Accelerate Reactions by Reducing Activation Energy

Enzymes and other catalysts accelerate reactions by reducing the **activation energy** of a reaction—that is, the initial input of energy required to start a reaction. Even though a reaction is spontaneous, with a negative ΔG, it may not actually start unless a relatively small boost of energy is added **(Figure 4.8a).** After it starts, the reaction becomes self-sustaining as it releases more than enough free energy to compensate for the original boost.

A rock resting in a depression at the top of a hill provides a physical example of activating energy **(Figure 4.8b).** The rock will not roll downhill spontaneously, even though its position represents considerable potential energy and the total "reaction"—the downward movement of the rock—is spontaneous and releases free energy. (Trying to stop the rock halfway down the hill would give an idea of the free energy being released.) In this physical example, the activation energy is the effort required to raise the rock over the rim of the depression and start its downhill roll. For chemical reactions, the activation energy is the energy required to disturb the existing bonds in the reactants enough to begin the conversion of reactants to products.

What provides the activation energy in chemical reactions? The molecules that participate in chemical reactions are in constant motion at temperatures above absolute zero. Although the average amount of kinetic energy may be less than the amount required for activation, collisions between the moving molecules may raise some of them to the energy level required for the reaction to proceed.

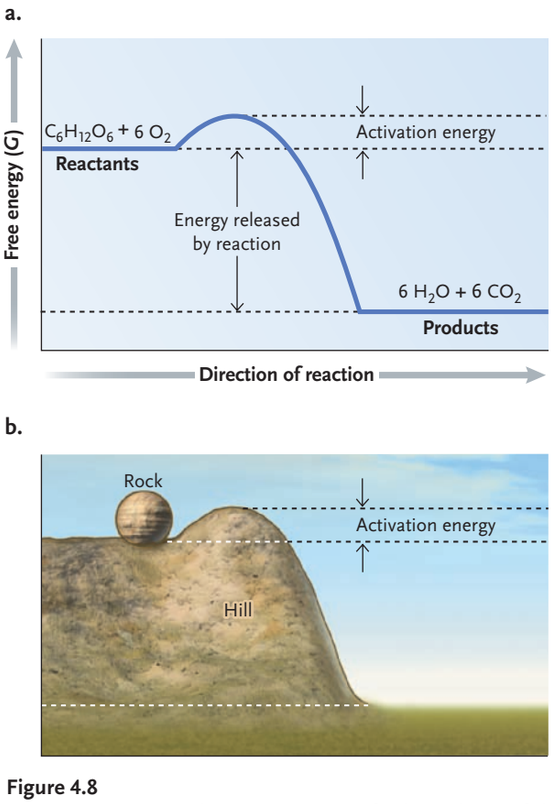


Figure 4.8
Activation energy. **(a)** The activation energy for the oxidation of glucose is an energy barrier over which glucose molecules must be raised before they can react to form H_2O and CO_2. **(b)** In an analogous physical situation, a rock poised in a depression at the top of a hill will not roll downhill unless activating energy is added to raise it over the rim of the depression.

For nonbiological reactions, heat is often added to reacting systems to supply activation energy. The addition of heat increases both the speed of individual molecules and the rate of their collisions, making it more likely that molecules will gain enough energy to react. Heating biological reactions enough to make them self-sustaining would be an unsatisfactory condition for living organisms. Instead, enzymes decrease the activation energy **(Figure 4.9),** greatly increasing the probability that molecules will gain enough energy to react at the rela-

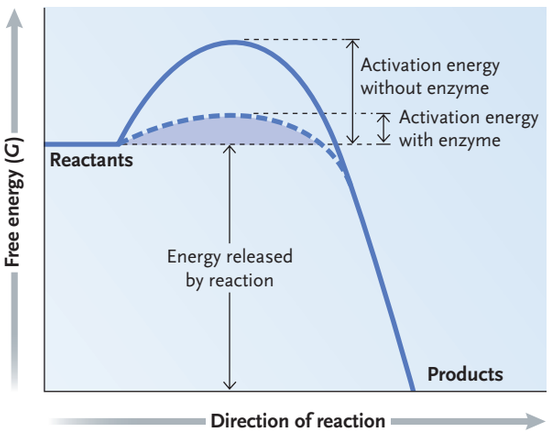


Figure 4.9
Effect of enzymes in reducing the activation energy. The reduction allows biological reactions to proceed rapidly at the relatively low temperatures that can be tolerated by living organisms.

tively low temperatures tolerated by living organisms. For the rock resting on the side of a hill, the role of an enzyme would be equivalent to reducing the depth of the depression so that the rock is finely balanced and only the slightest push is needed to start its journey downhill.

Enzymes Combine with Reactants and Are Released Unchanged

In catalysis, an enzyme combines briefly with reacting molecules and is released unchanged when the reaction is complete. For example, the enzyme in **Figure 4.10,** hexokinase, catalyzes the following reaction:

$$\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

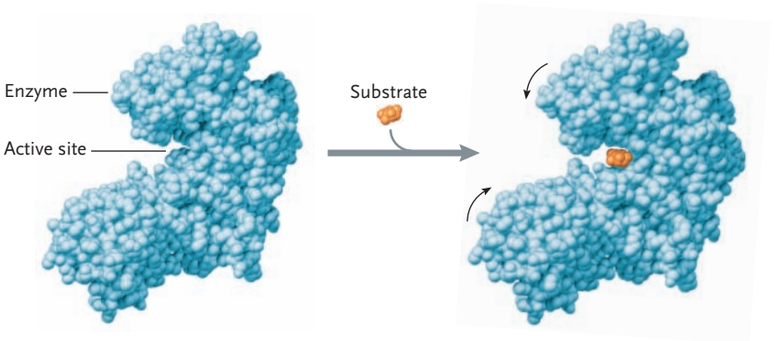


Figure 4.10
Space-filling models showing the combination of an enzyme, hexokinase (in blue), with its substrate, glucose (in yellow). Hexokinase catalyzes the phosphorylation of glucose to form glucose-6-phosphate. The phosphate group that enters the reaction is not shown. Note how the enzyme undergoes a conformational change, closing the active site more tightly as it binds the substrate.

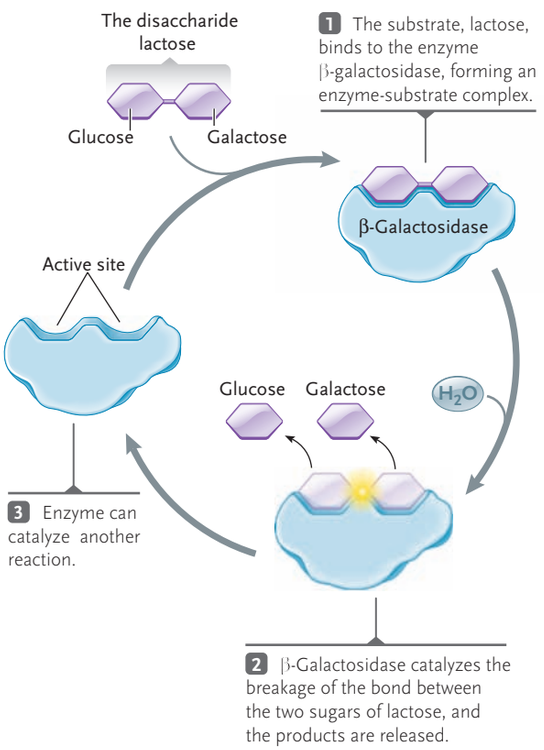


Figure 4.11
The catalytic cycle of enzymes. Shown is the enzyme β-galactosidase, which cleaves the sugar lactose to produce glucose and galactose.

Writing the enzyme name (for example, hexokinase) above the reaction arrow indicates that it is required but not involved as a reactant or a product.

Because enzymes are released unchanged after a reaction, enzyme molecules cycle repeatedly through reactions, combining with reactants and releasing products **(Figure 4.11).** Depending on the enzyme, the rate at which reactants are bound and catalyzed and at which products are released varies from 100 times to 10 million times per second. These astoundingly high rates of catalysis mean that a small number of enzyme molecules can catalyze large numbers of reactions.

Each type of enzyme catalyzes the reaction of only a single type of molecule or group of closely related molecules. This characteristic is known as **enzyme specificity.** The particular reacting molecule or molecular group that an enzyme catalyzes is known as the **substrate.** The region of an enzyme that recognizes and combines with a substrate molecule is the **active site.** In most enzymes, the active site is located in a cavity or pocket on the enzyme surface (as in the active site in Figure 4.10).

Cells have thousands of different enzymes. They vary from relatively small molecules, with single polypeptide chains containing as few as 100 amino acids, to large complexes that include many polypeptide chains totaling thousands of amino acids. Different enzymes are found in all areas of the cell, from the aqueous cell solution to the cell membranes. Other enzymes are released to catalyze reactions outside the cell. For example, enzymes that catalyze reactions breaking down food molecules are released from cells into the digestive cavity in all animals.

Many enzymes include a **cofactor,** an inorganic or organic nonprotein group that is necessary for catalysis to take place. Cofactors function in a variety of ways. Inorganic cofactors, which are all metallic ions, include iron, copper, magnesium, zinc, potassium, and manganese. Organic cofactors, also called **coenzymes,** are complex chemical groups of various kinds; in higher animals, many coenzymes are derived from vitamins.

Enzymes Reduce Activation Energy by Inducing the Transition State

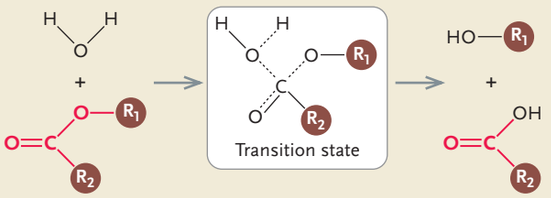
The central question of enzyme activity is: How do enzymes reduce the activation energy to speed biological reactions? Evidence from many years of experiments indicates that enzymes reduce activation energy by altering the reacting molecules to a form known as the transition state of a reaction. The **transition state** is an intermediate arrangement of atoms and bonds that both the reactants and the products of a reaction can assume. It is an activated state that is highly unstable and can move forward toward products or backward toward reactants with relatively little change in energy. The *Focus on Research* outlines an experiment showing that a transition state is involved in enzymatic catalysis.

Focus on Research

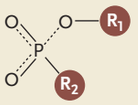
Basic Research: Testing the Transition State

One of the most inventive researchers of the twentieth century, American biochemist Linus Pauling, first proposed the idea that pushing molecules toward the transition state might be the mechanism that underlies enzymatic catalysis. Another biochemist, W. P. Jencks of Brandeis University, proposed a way of using antibodies to test Pauling's hypothesis. Animals produce proteins called antibodies when they are exposed to a foreign substance called an *antigen;* as part of their structure, antibodies contain a binding site that exactly fits the antigen. Combining an antibody with its antigen leads to the destruction or removal of the antigen from the body (as described in Chapter 43). Jencks reasoned that if antibodies could be made with a binding site that, like an enzyme, can fit the transition state of a reaction, they might act as enzymes and speed the rate of the reaction. If this occurred, the experiment would provide strong support for the idea that the transition state is part of the mechanism of enzyme action. In 1986, two groups working independently, one led by R. A. Lerner and the other by P. G. Schultz, successfully performed Jencks' proposed experimental test.

Lerner's group, at Scripps Research Institute, studied a common biological interaction called an acyl transfer reaction, in which an acyl group (shown in red in the figure) is transferred from one organic side group to another:



(R_1 and R_2 designate the organic side groups.) The transition state for this reaction is mimicked by a group of stable, unrelated molecules called phosphonate esters:



By injecting phosphonate esters into test animals, Lerner's group induced formation of antibodies tailored to fit the transition state for the acyl transfer reaction. These antibodies, as predicted by the Pauling–Jencks hypotheses, acted as enzymes speeding the rate of acyl transfer reactions.

The results directly support the proposal that achievement of the transition state is an important part of enzymatic catalysis. The technique also opened an entire new field of chemistry, the manufacture of "designer enzymes"—artificial enzymes made by developing antibodies that bind the transition state for a reaction desired in research, medicine, or industry.

Because enzymes bind the transition state, they can bind either the reactants or products of a reaction and can catalyze reversible reactions in either direction **(Figure 4.12).** However, the binding does not alter the equilibrium point of a reversible reaction; the enzyme simply increases the rate at which reversible reactions reach equilibrium.

Research has shown that at least three mechanisms contribute to the formation of the transition state. One of the key mechanisms enzymes use to induce the transition state is *bringing the reacting molecules into close proximity.* Reacting molecules can assume the transition state only when they collide; binding to an enzyme's active site brings the reactants so close together that a collision is almost certain to occur.

A second mechanism enzymes use is *orienting the reactants in positions that favor the transition state.* Binding at the active site positions the substrate molecules so that they are much more likely to collide at exactly the correct sites and angles required for achievement of the transition state.

A third mechanism is *exposing the reactant molecules to altered environments that promote their interaction.* For example, in some reactions, the active site of the enzyme may contain ionic groups with positive or negative charges that help distort reactants toward the transition state.

Many conditions and factors alter the rates at which enzymes catalyze their reactions, and enzymes rarely work at their maximum possible rates inside cells. Instead, their rates are regulated and adjusted to match the requirements of a cell for the products of the reactions they catalyze. The next section describes conditions and factors that alter enzyme activity and outlines some of the most important regulatory mechanisms that key enzymatic catalysis to cellular requirements.

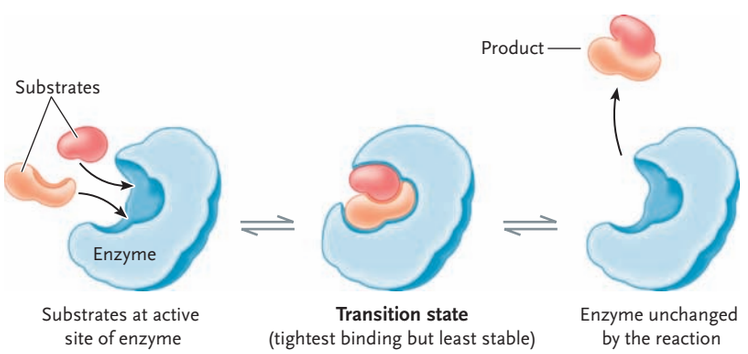


Figure 4.12
Fit of the active site to reactants, products, and the transition state. The strongest binding is to the transition state.

Study Break

Explain how enzymes accelerate reactions.

4.5 Conditions and Factors That Affect Enzyme Activity

Several conditions can alter enzyme activity, including changes in temperature and pH and changes in the concentration of substrate and other molecules that can bind to enzymes. The activity of enzymes is also regulated by control mechanisms that modify enzyme activity, thereby adjusting reaction rates to meet a cell's requirements for chemical products.

Most Enzymes Reach Maximum Activity within a Narrow Range of Temperature and pH

The activity of most enzymes is strongly altered by changes in pH and temperature. Characteristically, enzymes reach maximal activity within a narrow range of temperature or pH; at levels outside this range, enzyme activity drops off. These effects produce a typically peaked curve when enzyme activity is plotted, with the peak where temperature or pH produces maximal activity.

Effects of Temperature Changes. The effects of temperature changes on enzyme activity reflect two distinct processes. First, temperature has a general effect on chemical reactions of all kinds. As the temperature rises, the rate of chemical reactions typically increases. This effect reflects increases in the kinetic motion of all molecules, with more frequent and stronger collisions as the temperature rises. Second, temperature has an effect on all proteins, including enzymes. As the temperature rises, the kinetic motions of the amino acid chains of an enzyme increase, along with the strength and frequency of collisions between enzymes and surrounding molecules. At some point, these disturbances become strong enough to denature the enzyme: the hydrogen bonds and other forces that maintain its three-dimensional structure break, making the enzyme unfold and lose its function.

The two effects of temperature act in opposition to each other to produce characteristic changes in the rate of enzymatic catalysis **(Figure 4.13).** In the range of 0° to about 40°C, the reaction rate doubles for every 10°C increase in temperature. Above 40°C, the increasing kinetic motion begins to unfold the enzyme, reducing the rate of increase in enzyme activity. At some point, as temperature continues to rise, the unfolding causes the reaction rate to level off at a peak. Further increases cause such extensive unfolding that the reaction rate decreases rapidly to zero. For most enzymes, the peak in activity lies between 40° and 50°C; the drop-off becomes steep at 55°C and falls to zero at about 60°C. Thus, the rate of an enzyme-catalyzed reaction peaks at a temperature at which kinetic motion is greatest but no significant unfolding of the enzyme has occurred.

Although most enzymes have a temperature optimum between 40° and 50°C, some have activity peaks below or above this range. For example, the enzymes of maize (corn) pollen function best near 30°C and undergo steep reductions in activity above 32°C. As a result, environmental temperatures above 32°C can seriously inhibit the growth of corn crops. Many animals living in frigid regions have enzymes with much lower temperature optima than average. For example, the enzymes of arctic snow fleas are most active at −10°C. At the other extreme are the enzymes of archaeans that live in hot springs, which are so resistant to denaturation that they remain active at temperatures of 85°C or more.

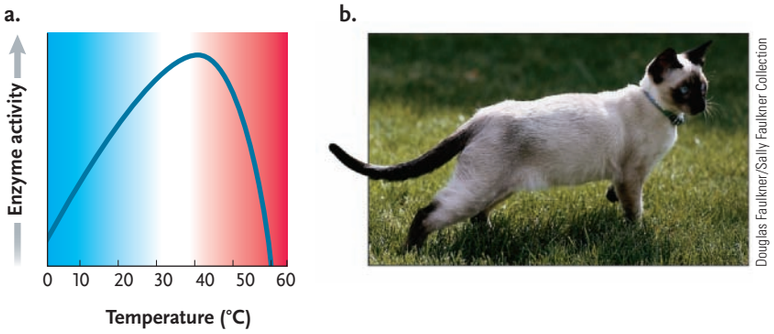


Figure 4.13

Effect of temperature on enzyme activity. **(a)** As the temperature rises, the rate of the catalyzed reaction increases proportionally until the temperature reaches the point at which the enzyme begins to denature. The rate drops off steeply as denaturation progresses and becomes complete. **(b)** Visible effects of environmental temperature on enzyme activity in Siamese cats. The fur on the extremities—ears, nose, paws, and tail—contains more dark brown pigment (melanin) than the rest of the body. A heat-sensitive enzyme controlling melanin production is denatured in warmer body regions, so dark pigment is not produced and fur color is lighter.

Effects of pH Changes. Typically, each enzyme has an optimal pH where it operates at peak efficiency in speeding the rate of its biochemical reaction **(Figure 4.14).** On either side of this pH optimum, the rate of the catalyzed reaction decreases because of the resulting alterations in charged groups. The effects on the structure and function of the active site become more extreme at pH values farther from the optimum, until the rate drops to zero.

Most enzymes have a pH optimum near the pH of the cellular contents, about pH 7. Enzymes that are secreted from cells may have pH optima farther from neutrality. An example is pepsin, a protein-digesting enzyme secreted into the stomach. This enzyme's pH optimum is 1.5, close to the acidity of stomach contents. Similarly, trypsin, also a protein-digesting enzyme, has a pH optimum at about pH 8, allowing it to function well in the somewhat alkaline contents of the intestine where it is secreted.

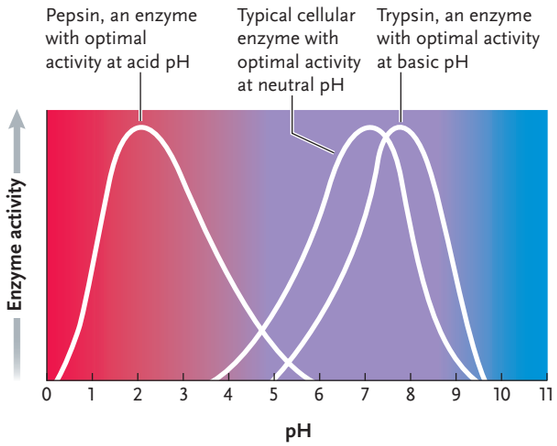


Figure 4.14
Effects of pH on enzyme activity. An enzyme typically has an optimal pH at which it is most active; at pH values above or below the optimum, the rate of enzyme activity drops off. At extreme pH values, the rate drops to zero.

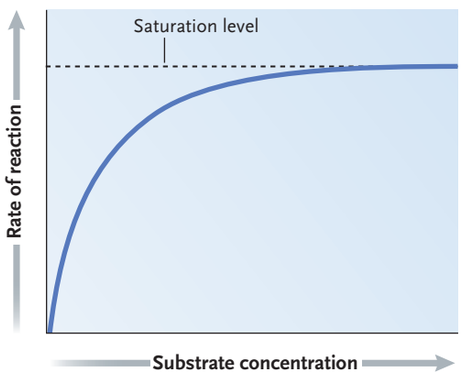


Figure 4.15
Effect of increasing substrate concentration on the rate of an enzyme-catalyzed reaction. At saturation (horizontal dashed line), further increases in substrate concentration do not increase the rate of the reaction.

Enzyme-Catalyzed Reactions Reach a Saturation Level beyond Which Increasing Substrate Concentration Does Not Increase the Reaction Rate

When substrate concentration is altered experimentally from low to high and the temperature and concentration of enzyme molecules are held constant, the rate of enzyme catalysis eventually levels off **(Figure 4.15).** At very low concentrations, substrate molecules collide so infrequently with enzyme molecules that the reaction proceeds slowly. As the substrate concentration increases, the reaction rate initially increases as enzyme and substrate molecules collide more frequently. But, as the enzyme molecules approach the maximum rate at which they can combine with reactants and release products, increasing substrate concentration has a smaller and smaller effect and the rate of reaction eventually levels off. When the enzymes are cycling as rapidly as possible, further increases in substrate concentration have no effect on the reaction rate. At this point, the enzymes are said to be **saturated**, and the reaction rate remains constant at the saturation level (see the horizontal dashed line in Figure 4.15).

By contrast, uncatalyzed reactions do not reach a saturation level. Therefore, if researchers perform the type of experiment presented in Figure 4.15 and observe that saturation occurs, they will conclude that an enzyme catalyzes the reaction.

Enzyme Inhibitors Have Characteristic Effects on Enzyme Activity

The rate at which enzymes catalyze reactions is reduced by *enzyme inhibitors,* substances that reduce enzyme activity by combining with enzyme molecules. Some inhibitors work by combining with the active site of an enzyme; others combine with critical sites located elsewhere in the structure of an enzyme **(Figure 4.16).**

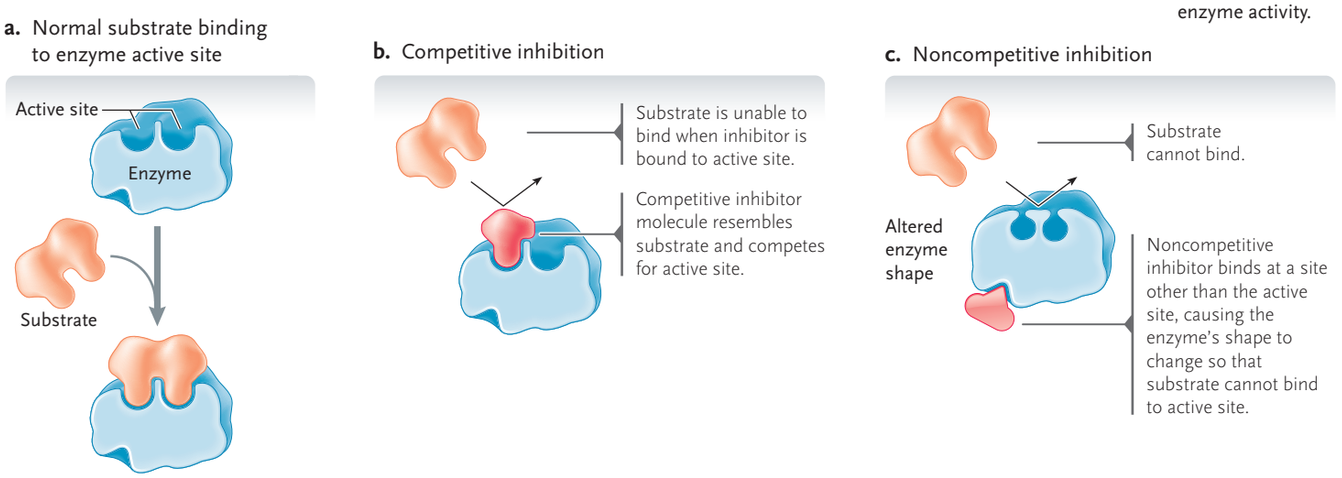


Figure 4.16
Actions of competitive and noncompetitive inhibitors of enzyme activity.

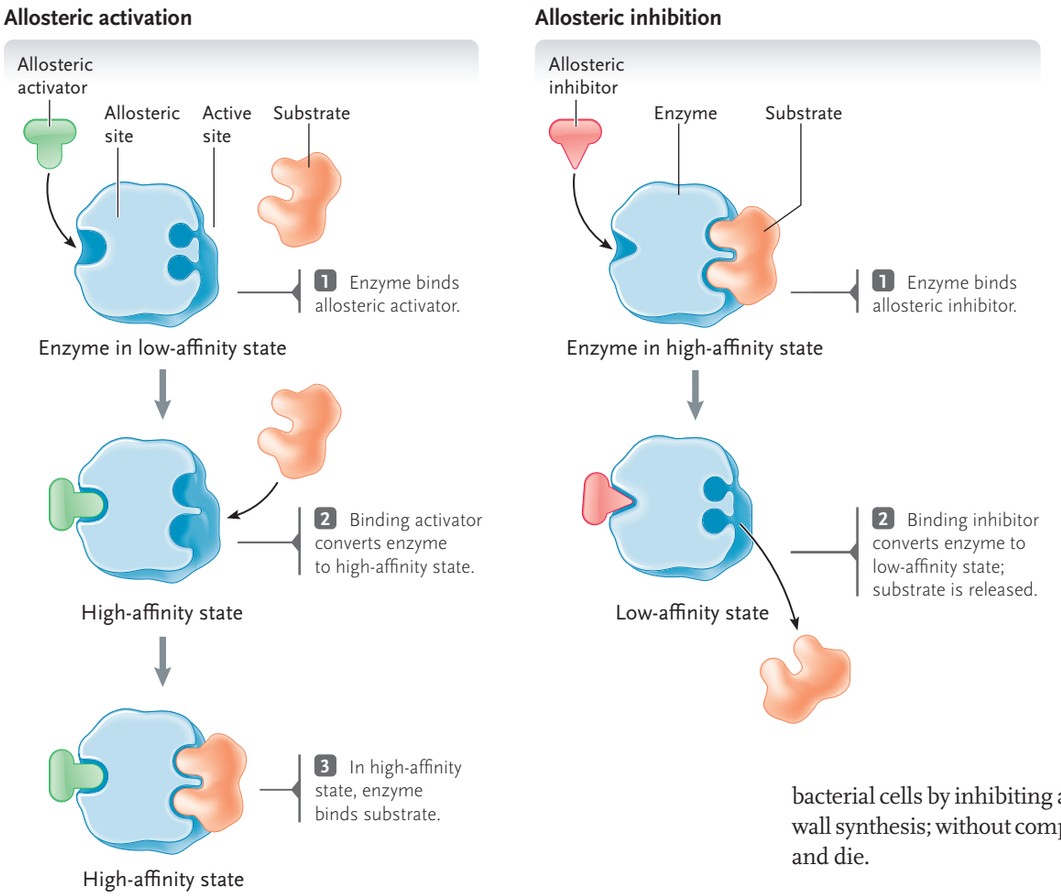


Figure 4.17
Allosteric regulation.

Inhibitors that combine with the active site have molecular structures that resemble the normal substrate closely enough to fit into and occupy the site, thereby blocking access for the normal substrate and slowing the reaction rate. If the concentration of the inhibitor is high enough, the reaction may stop completely. Inhibition of this type is called **competitive inhibition** because the inhibitor *competes* with the normal substrate for binding to the active site. Competitive inhibitors are useful in enzyme research because their structure helps identify the region of a normal substrate that binds to an enzyme.

Inhibitors that combine with enzymes at locations other than the active site often alter the conformation of the enzyme. The alterations reduce the ability of the active site to bind the normal substrate and thus induce the transition state in the substrate. Because such inhibitors do not compete directly with the substrate for binding to the active site, their pattern of inhibition is called **noncompetitive inhibition.**

Both competitive inhibitors and noncompetitive inhibitors bind to enzymes with varying strength, depending on type. Some bind so strongly that their linkage is essentially permanent and the enzyme becomes completely disabled. Others bind more loosely, so their attachment is reversible.

Some foreign molecules, such as poisons and toxins, act as inhibitors of enzyme activity. Such molecules often bind so strongly to enzymes that their inhibitory effects are essentially irreversible. For example, cyanide is a potent poison because it binds strongly to and inhibits cytochrome oxidase, the enzyme that catalyzes the use of oxygen in cellular metabolism. Humans and other animals die quickly if exposed to cyanide because of the almost instant and complete inhibition of cytochrome oxidase by the poison. Many antibiotics are toxins that inhibit enzyme activity in bacteria. Penicillin, a toxin made by a fungus, kills bacterial cells by inhibiting an enzyme necessary for cell wall synthesis; without complete walls, the bacteria burst and die.

Cellular Regulatory Pathways Use Several Mechanisms to Adjust Enzyme Activity to Meet Metabolic Requirements

Cells adjust the activity of many enzymes upward or downward to meet their needs for reaction products. Several mechanisms are used in this regulation, including competitive and noncompetitive inhibition, a form of noncompetitive control called *allosteric regulation,* and covalent modification of enzyme structure by the addition or removal of chemical groups.

Regulation by Inhibitors. Many cellular enzymes are regulated by natural inhibitors, including inhibitors that work either competitively or noncompetitively. Typically, the combination between these inhibitors and the enzyme is fully reversible. If the concentration of the inhibitor increases, it combines with the enzymes in greater numbers, thereby interfering with enzyme activity and decreasing the rate of the reaction. If the concentration of the inhibitor decreases, its combination with enzymes decreases proportionately and the rate of the reaction increases. Control by the inhibitors changes enzyme activity precisely to meet the needs of the cell for the products of the reaction catalyzed by the enzyme.

For example, the specialized control mechanism called **allosteric regulation** (*allo* = different; *stereo* =

shape) occurs by the reversible combination of a regulatory molecule with the **allosteric site**, a location on the enzyme outside the active site. The mechanism, first discovered in 1965 by French biologist Jacques Monod and his colleagues J. P. Changeux and J. Wyman at the Pasteur Institut, Paris, France, may either slow or accelerate enzyme activity.

Enzymes controlled by allosteric regulation typically have two alternate conformations controlled from the allosteric site. In one conformation, called the *high-affinity state* (the active form), the enzyme binds strongly to its substrate; in the other conformation, the *low-affinity state* (the inactive form), the enzyme binds the substrate weakly or not at all. Binding with regulatory substances may induce either state: binding an **allosteric inhibitor** converts an allosteric enzyme from the high- to low-affinity state, and binding an **allosteric activator** converts it from the low- to high-affinity state **(Figure 4.17).** Because allosteric inhibitors work by binding to sites separate from the active site, their action is noncompetitive.

Frequently, allosteric inhibitors are a product of the metabolic pathway that they regulate. If the product accumulates in excess, its effect as an inhibitor automatically slows or stops the enzymatic reaction producing it, typically by inhibiting the enzyme that catalyzes the first reaction of the pathway. If the product becomes too scarce, the inhibition is reduced and its production increases. Regulation of this type, in which the product of a reaction acts as a regulator of the reaction, is termed **feedback inhibition** (also called **end-product inhibition**). Feedback inhibition prevents cellular resources from being wasted in the synthesis of molecules made at intermediate steps of the pathway.

For instance, a biochemical pathway that makes the amino acid isoleucine from threonine proceeds in five steps, each catalyzed by an enzyme **(Figure 4.18).** The end product of the pathway, isoleucine, is an allosteric inhibitor of the first enzyme of the pathway, threonine deaminase. If the cell makes more isoleucine than it needs, isoleucine combines reversibly with threonine deaminase at the allosteric site, converting the enzyme to the low-affinity state and inhibiting its ability to combine with threonine, the substrate for the first reaction in the pathway. If isoleucine levels drop too low, the allosteric site of threonine deaminase is vacated, the enzyme is converted to the high-affinity state, and isoleucine production increases.

Regulation by Chemical Modification. Many key enzymes are regulated by chemical linkage to other substances, typically ions, functional groups such as phosphate or methyl groups, or units derived from nucleotides. The regulatory substances induce folding changes in the enzyme that adjust its activity upward or downward.

For example, chemical modification by the addition or removal of phosphate groups is a highly significant mechanism of cellular regulation that is used by

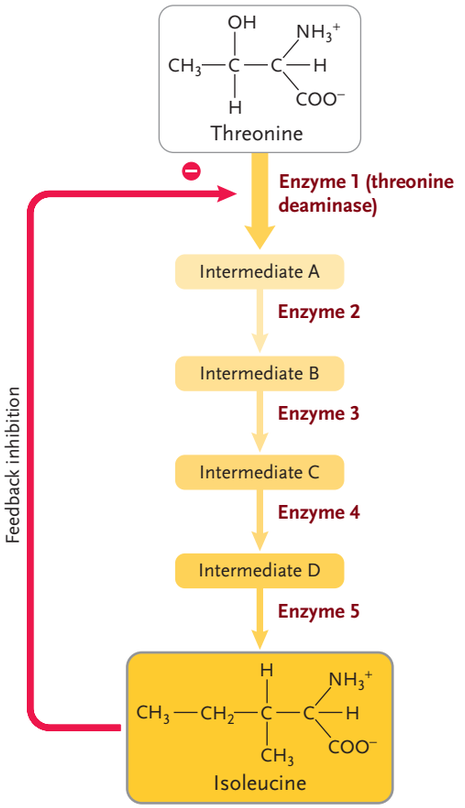


Figure 4.18
Feedback inhibition in the pathway that produces isoleucine from threonine. If the product of the pathway, isoleucine, accumulates in excess, it slows or stops the pathway by acting as an allosteric inhibitor of the enzyme that catalyzes the first step in the pathway.

all organisms from bacteria to humans. Typically, regulatory phosphate groups derived from ATP or other nucleotides are added to the regulated enzymes by other enzymes known as *protein kinases*. The addition of a phosphate group (phosphorylation) either increases or decreases enzyme activity or activates or deactivates the enzyme, depending on the particular enzyme and where the phosphate group is added to the enzyme.

Regulatory phosphate groups are removed (a process called dephosphorylation), reversing the effects of the protein kinases, by a different group of enzymes called *protein phosphatases*. The balance between phosphorylation and dephosphorylation of the enzymes modified by the kinases and protein phosphatases closely regulates cellular activity, often as a part of the response to external signal molecules (see Chapter 7).

Enzymes have been actively investigated since their discovery in the late 1800s. The word *enzyme* means "in yeast," in reference to the discovery of these protein-based catalysts in extracts of yeast cells. A hundred years of intensive research after the discovery of enzymatic proteins gave no hint that other molecules could act as biological catalysts. Thus, it came as a big surprise when RNA-based catalysts were discovered.

Insights from the Molecular Revolution

Ribozymes Take the First Step in Protein Synthesis

Harry Noller's experiment showed that if proteins were removed from ribosomes, the remaining RNA molecules could still catalyze the central reaction of protein synthesis, linkage of amino acids into chains via peptide bonds. However, his work did not eliminate the possibility that undetectable small amounts of ribosomal proteins in the preparations might be catalyzing peptide bond formation.

Billiang Zhang and Thomas R. Cech performed a definitive experiment that eliminated the possibility of protein contamination. They synthesized RNA molecules artificially, in solutions that had never been exposed to ribosomal proteins, and then tested the ability of the artificial RNA to catalyze formation of peptide bonds.

As a first step in their experiments, Zhang and Cech synthesized a large pool of artificial RNA molecules. Part of the nucleotide sequence was the same in every molecule and part differed randomly from molecule to molecule, but all were the same length. The investigators then linked an amino acid, phenylalanine, to one end of each RNA molecule by a disulfide (—S—S—) bond.

To that pool, they next added the amino acid methionine linked to the nucleotide AMP. In the cell, single amino acids linked to AMP are used in the pathway that makes proteins. The methionine–AMP combination was "tagged" by combining it with *biotin,* a small organic molecule, so that it could be identified in the reaction solution.

All the ingredients were mixed together and allowed to react. If any of the RNA molecules could act as ribozymes, catalyzing formation of a peptide bond, some of the tagged methionine should become linked to the phenylalanine at the end of the ribozyme. To find out if this had happened, the investigators poured the reaction mixture through a column packed with plastic beads that could bind to the biotin tag. Binding between the beads and the biotin tag trapped any RNA molecules that were able to catalyze linkage of the two amino acids, whereas unreactive RNA molecules flowed out of the bottom of the column. The RNA molecules with the biotin tag were then washed from the column and separated from the linked amino acids by adding a reagent that breaks disulfide bonds. Chemical tests showed that peptide bonds formed between the amino acids, the same type of linkage that joins amino acids in natural proteins.

The RNA molecules that had functioned successfully as ribozymes were then separated from their dipeptide product for further study and refinement. Eventually, the researchers obtained ribozymes that catalyzed peptide bond formation at rates 100,000 times faster than the same reaction without a catalyst.

Zhang and Cech's experiments confirmed a feature of ribozyme activity that is critical to the role proposed for these RNA-based catalysts in the primitive RNA world—their ability to catalyze formation of the fundamental linkage tying amino acids together in proteins. Thus, during the evolution of life, proteins could have been made first in quantity by RNA, with no requirement for either DNA or enzymatic proteins.

Study Break

1. Explain why the activity of an enzyme will eventually decrease to zero as the temperature rises.
2. Why do enzyme-catalyzed reactions reach a saturation level when substrate concentration is increased?
3. Distinguish between competitive and noncompetitive inhibition.

4.6 RNA-Based Biological Catalysts: Ribozymes

Ribozymes Catalyze Certain Biological Reactions

In 1981, biochemist Thomas R. Cech of the University of Colorado, Boulder, discovered a group of RNA molecules that appeared to be capable of accelerating the rate of certain biological reactions without being changed by the reactions. Further work demonstrated that these RNA-based catalysts, now called **ribozymes**, are part of the biochemical machinery of all cells. Cech and another scientist, Yale University biochemist Sidney Altman, received the Nobel Prize in 1989 for their research establishing that ribozymes are essential cellular catalysts.

Most of the known ribozymes speed the cutting and splicing reactions that remove surplus segments from RNA molecules as part of their conversion into finished form. Some have other functions, however. For example, Harry F. Noller and his coworkers at the University of California at Santa Cruz found that ribosomes, the cell structures that assemble amino acids into proteins, can still link amino acids together even if their proteins are removed. Only RNA molecules are left in the ribosomes after the proteins are extracted, suggesting that ribozymes might catalyze this central reaction of protein synthesis. After Noller's discovery, Cech and his colleague, Billiang Zhang, confirmed that ribozymes can actually catalyze this reaction (see the *Insights from the Molecular Revolution* for an outline of Cech and Zhang's experiment).

Unanswered Questions

Many biological processes rely on enzymes to catalyze key reactions. A complete understanding of those processes requires knowledge about the structure and function of the enzymes involved. Much research continues to be done to elucidate enzyme structure and function.

How does protein structure relate to enzyme function?

Many researchers are studying protein structure and its relation to protein function. For example, Janet Smith at the University of Michigan uses X-ray crystallography to determine the structures of proteins. The patterns of diffraction of X-rays shone at a protein crystal give information about how the protein's atoms are organized. The crystal structure is "solved" once a model for the protein's structure is achieved in this way.

Smith's group uses information about the structure of solved proteins to predict the functions of other proteins. Even though it is possible to solve protein structures rapidly, it is not practical to solve the structures of all proteins involved in important biological processes. Instead, Smith, as well as other researchers, draws on the current understanding of the evolution of proteins. In particular, genes for useful proteins often have been duplicated during evolution and the duplicate copy adapted to a new function. Therefore, proteins can be related in an evolutionary sense. An understanding of the molecular mechanisms of particular enzymes may then be transferable to other proteins, which is an underlying theme of Smith's research.

How does ribozyme structure relate to function, and how might ribozymes be used as therapeutic agents?

Ribozymes are catalytic RNA molecules. Various types of ribozymes exist, each type differing in its three-dimensional structure and mechanism of catalysis.

Researcher John Burke at the University of Vermont and his group are studying hairpin ribozymes and hammerhead ribozymes, which are catalytically active once they fold into those two shapes (the hammerhead shape is similar to that of the head of a hammerhead shark). Their research has four directions: determining the molecular structure of ribozymes, characterizing RNA conformational changes during catalysis, elucidating the mechanisms of catalysis, and exploring ways to use ribozymes as therapeutic agents.

For example, Burke's group has shown that the hairpin ribozyme undergoes a dramatic conformational change when the substrate binds to the active site. Furthermore, they have engineered hairpin ribozymes that can inhibit viral replication in mammalian cells. The particular viruses targeted have RNA genomes and include HIV-1 (the causative agent of AIDS) and hepatitis B virus. To achieve their goal, they had to identify appropriate target sites within the viral RNA molecules and to express the engineered ribozymes efficiently within the cell. Current research focuses on optimizing the inhibition of viral replication by the ribozymes, determining the mechanism of antiviral activity, and extending this technology to develop therapeutic approaches for significant infectious diseases such as AIDS and hepatitis B.

Peter J. Russell

Ribozymes provide a possible solution to a long-standing "chicken-or-egg" paradox about the evolution of life: Did proteins or nucleic acids come first in evolution? It is difficult to understand how DNA could exist without the enzymatic proteins required for its duplication. At the same time, it is difficult to understand how enzymes could exist without nucleic acids, which contain the information required to make them. Ribozymes offer a way around this dilemma because they could have acted as *both* enzymes and informational molecules when cellular life first appeared. The earliest forms of life therefore might have inhabited an "RNA world" in which neither DNA nor proteins played critical roles (see discussion in Chapter 24). If so, ribozymes—the most recently discovered biological catalysts—may have existed for the longest time!

This chapter concludes our survey of the chemical underpinnings of biology. In the next chapter, we survey the structure of cells, the fundamental units into which biological molecules are organized and where molecules interact to produce the characteristics of life.

Study Break

What is a ribozyme, and how does it fit the definition of an enzyme?

Review

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4.1 Energy, Life, and the Laws of Thermodynamics

- Energy, the capacity to do work, exists in kinetic and potential states. Kinetic energy is the energy of motion; potential energy is energy represented in the nonmoving location of matter or the specific arrangement of atoms. Energy may be readily converted between potential and kinetic states.
- Metabolism is the biochemical modification and use of energy in the synthesis and breakdown of organic molecules. Catabolic reactions release the potential energy of complex molecules to do cellular work. Anabolic reactions convert simple substances into more complex forms.

- Thermodynamics is the study of energy flow between a system and its surroundings during chemical and physical reactions. A system that exchanges energy but not matter with its surroundings is a closed system. A system that exchanges both energy and matter with its surroundings is an open system (Figure 4.2).
- The first law of thermodynamics states that the total amount of energy in a system and its surroundings remains constant. The second law states that in any process involving a spontaneous (possible) change from an initial to a final state, the total entropy (disorder) of the system and its surroundings always increases.
- Energy released by reactions that move spontaneously to the final state is free energy, that is, energy available to do work. The free energy equation, $\Delta G = \Delta H - T\Delta S$, states that the free energy change, ΔG, is the sum of the changes in energy content and entropy of the system as a reaction goes to completion.
- Reactions with a negative ΔG are spontaneous; they release free energy and are known as exergonic reactions. Reactions with a positive ΔG require free energy and are known as endergonic reactions (Figure 4.4).

4.2 How Living Organisms Couple Reactions to Make Synthesis Spontaneous

- Cells carry out endergonic reactions by using ATP to couple them to exergonic reactions, producing an overall reaction that proceeds spontaneously. In the coupled reactions, ATP is hydrolyzed to ADP and P_i, and one of these molecules is temporarily linked to reactants or the enzyme (Figure 4.5).
- The ATP used in coupling reactions is replenished by reactions that link ATP synthesis to catabolic reactions. ATP thus cycles between reactions that release free energy and reactions that require free energy (Figure 4.6).

Animation: Structure of ATP

Animation: Active transport

4.3 Thermodynamics and Reversible Reactions

- Factors that oppose the completion of spontaneous reactions, such as the relative concentrations of reactants and products, produce an equilibrium point at which reactants are converted to products, and products are converted back to reactants, at equal rates. Small changes in reaction conditions can easily reverse the overall progress of the reaction (Figure 4.7).

Animation: Chemical equilibrium

4.4 Role of Enzymes in Biological Reactions

- Enzymes are catalysts; they greatly speed the rate at which spontaneous reactions occur, and for reversible reactions, they increase the rate at which a reaction reaches equilibrium.
- Enzymes usually are specific: they catalyze reactions of only a single type of molecule or a group of closely related molecules.
- The active site of an enzyme combines briefly with the reactants (the substrates); the enzyme is released unchanged when the reaction is complete.
- Many enzymes include a cofactor, which is an inorganic ion or an organic nonprotein group called a coenzyme that is necessary for catalysis to occur.
- Enzymes work by decreasing the activation energy required for a chemical reaction to proceed. They reduce the activation energy by inducing the transition state of the reaction, from which the reaction can move easily in the direction of either products or reactants (Figures 4.8–4.12).
- Several mechanisms contribute to enzymatic catalysis by helping to induce the transition state. They include bringing the reactant molecules into close proximity, orienting the reactants in positions that favor the transition state, and exposing the reactants to altered environments that promote their interaction.

Animation: Activation energy

Animation: How catalase works

Animation: Enzymes and their role in lowering activation energy

4.5 Conditions and Factors That Affect Enzyme Activity

- Typically, enzymes have optimal activity at a certain temperature and a certain pH; at temperature and pH values above and below the optimum, reaction rates fall off (Figures 4.13 and 4.14).
- At high substrate concentrations, enzymes become saturated with reactants, and further increases in substrate concentration do not increase the rate of the reaction (Figure 4.15).
- Enzymes may be inhibited by nonsubstrate molecules. Competitive inhibitors interfere with reaction rates by combining with the active site of an enzyme; noncompetitive inhibitors combine with sites elsewhere on the enzyme (Figure 4.16).
- Many cellular enzymes are regulated by inhibitors. A special type of regulation, allosteric regulation, resembles noncompetitive inhibition, except that regulatory molecules may either increase or decrease enzyme activity. Allosteric regulation often carries out feedback inhibition, in which a product of an enzyme-catalyzed pathway acts as an allosteric inhibitor of the first enzyme in the pathway (Figures 4.17 and 4.18).
- Enzymes also are regulated by chemical modification, in many cases by reversible addition or removal of phosphate groups.

Animation: Allosteric activation

Animation: Allosteric inhibition

Interaction: Feedback inhibition

Interaction: Enzymes and temperature

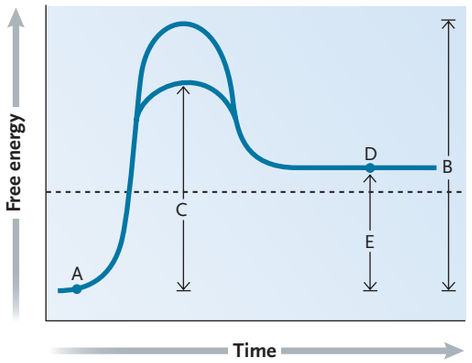
4.6 RNA-Based Biological Catalysts: Ribozymes

- RNA-based catalysts called ribozymes speed some types of biological reactions; these include cutting and splicing reactions in which surplus segments are removed from RNA molecules and linking reactions that combine amino acids into polypeptide chains.

Questions

Self-Test Questions

1. The capacity to do work best defines:
   a. a metabolic pathway.
   b. entropy.
   c. kinetic or potential energy.
   d. a catabolic reaction.
   e. thermodynamics.
2. When two glucose molecules combine:
   a. the reaction represents a negative ΔG.
   b. free energy had to be available to allow the reaction to proceed.
   c. the reaction is exothermic.
   d. it supports the second law of thermodynamics, which states there is tendency of the universe toward disorder.
   e. the resulting product has less potential energy than the reactants.
3. When glucose is converted to glucose-6-phosphate:
   a. the synthesis of glucose-6-phosphate is exergonic.
   b. ADP is at a higher energy level than ATP.
   c. glucose-6-phosphate is at a higher energy level than glucose.
   d. because ATP donates a phosphate to glucose, this is not a coupled reaction.
   e. this is a spontaneous reaction.
4. In the following graph:



   a. A represents the product.
   b. B represents the energy of activation when enzymes are present.
   c. C is the free energy difference between A and D.
   d. C is the energy of activation without enzymes.
   e. E is the difference in free energy between the reactant and the products.
5. Subtilisin, a component in many laundry detergents, removes chocolate (which contains protein) from clothes in hot water. If used regularly on your silk shirt, the silk shirt might emulsify. From this information, which of the following is *not* a reasonable deduction?
   a. Subtilisin must be heat-stable.
   b. Subtilisin is an enzyme that must have a broad range of activity.
   c. Chocolate is composed of an α helix, and silk has a β-sheet structure; thus, subtilisin probably attacks a certain amino acid group linkage rather than a specifically shaped molecule.
   d. Subtilisin is not a protein.
   e. Be careful eating chocolate if wearing a silk shirt.
6. Which of the following methods is *not* used by enzymes to increase the rate of reactions?
   a. covalent bonding with the substrate at their active site
   b. bringing reacting molecules into close proximity
   c. orienting reactants into positions to favor transition states
   d. changing charges on reactants to hasten their reactivity
   e. increasing fit of enzyme and substrate that reduces the energy of activation
7. In an enzymatic reaction:
   a. the enzyme leaves the reaction chemically unchanged.
   b. if the enzyme molecules approach maximal rate, and the substrate is continually increased, the rate of the reaction does not reach saturation.
   c. in the stomach, enzymes would have an optimal activity at a neutral pH.
   d. increasing temperature above the optimal value slows the reaction rate.
   e. the least important level of organization for an enzyme is its tertiary structure.
8. Which of the following statements about the allosteric site is true?
   a. The allosteric site is a second active site on a substrate in a metabolic pathway.
   b. The allosteric site on an enzyme can allow the product of a metabolic pathway to inhibit that enzyme and stop the pathway.
   c. When the allosteric site of an enzyme is occupied, the reaction is irreversible and the enzyme cannot react again.
   d. An allosteric activator prevents binding at the active site.
   e. An enzyme that possesses allosteric sites does not possess an active site.
9. Which of the following statements about inhibition is true?
   a. Allosteric inhibitors and allosteric activators are competitive for a given enzyme.
   b. If an inhibitor binds the active site, it is considered noncompetitive.
   c. If an inhibitor binds to a site other than the active site, this is competitive inhibition.
   d. A noncompetitive inhibitor is believed to change the shape of the enzyme, making its active site inoperable.
   e. Competitive inhibition is usually not reversible.
10. Which of the following statements is *incorrect?*
    a. Ribozymes can link amino acids to form protein.
    b. Ribozymes can act as enzymes.
    c. Ribozymes can act as informational molecules.
    d. Ribozymes are suggested as the first molecules of life.
    e. Ribozymes are proteins.

Questions for Discussion

1. Trees become more complex as they develop spontaneously from seeds to adults. Does this process violate the second law of thermodynamics? Why or why not?
2. Trace the flow of energy through your body. What products increase the entropy of you and your surroundings?
3. You have found a molecular substance that accelerates the rate of a particular reaction. What kind of information would you need to demonstrate that this molecular substance is an enzyme?

4. The addition or removal of phosphate groups from ATP is a fully reversible reaction. In what way does this reversibility facilitate the use of ATP as a coupling agent for cellular reactions?
5. Researchers once hypothesized that an enzyme and its substrate fit together like a lock and key but that the products do not fit the enzyme. Examine this idea with respect to reversible reactions.

Experimental Analysis

Succinate dehydrogenase is part of the cellular biochemical machinery for breaking down sugars, fatty acids, and amino acids into carbon dioxide and water, with the capture of their chemical energy as ATP. Suppose you are measuring the activity of this enzyme extracted from cells in test-tube reactions. You find that the rate of the reaction converting succinate to fumarate catalyzed by succinate dehydrogenase is inhibited by the addition of malonate to the reaction mixture. Design an experiment that will tell you whether malonate is acting as a competitive or a noncompetitive inhibitor.

Evolution Link

If RNA appeared first in evolution, establishing an RNA world, which do you think would evolve next: DNA or proteins? Why?