CHAPTER **THREE** 3

Energy, Catalysis, and Biosynthesis

One property above all makes living things seem almost miraculously different from nonliving matter: they create and maintain order in a universe that is tending always toward greater disorder. To accomplish this remarkable feat, the cells in a living organism must carry out a never-ending stream of chemical reactions that produce the molecules the organism requires to meet its metabolic needs. In some of these reactions, small organic molecules—amino acids, sugars, nucleotides, and lipids—are taken apart or modified to supply the many other small molecules that the cell requires. In other reactions, these small molecules are used to construct an enormously diverse range of larger molecules, including the proteins, nucleic acids, and other macromolecules that endow living systems with all of their most distinctive properties. Each cell can be viewed as a tiny chemical factory, performing many millions of these reactions every second.

To carry out the tremendous number of chemical reactions needed to sustain it, a living organism requires both a source of atoms in the form of food molecules and a source of energy. The atoms and the energy must both come, ultimately, from the nonliving environment. In this chapter, we discuss why cells require energy, and how they use energy and atoms from their environment to create the molecular order that makes life possible.

Most of the chemical reactions that cells perform would normally occur only at temperatures that are much higher than those inside a cell. Each reaction therefore requires a major boost in chemical reactivity to enable it to proceed rapidly within the cell. This boost is provided by specialized proteins called *enzymes*, each of which accelerates, or *catalyzes*, just one

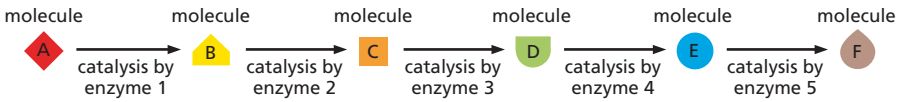


Figure 3–1 A series of enzyme-catalyzed reactions forms a metabolic pathway. Each enzyme catalyzes a chemical reaction involving a particular molecule. In this example, a set of enzymes acting in series converts molecule A to molecule F, forming a metabolic pathway.

of the many possible kinds of reactions that a particular molecule might undergo. These enzyme-catalyzed reactions are usually connected in series, so that the product of one reaction becomes the starting material for the next (**Figure 3–1**). The long linear reaction pathways, or *metabolic pathways*, that result are in turn linked to one another, forming a complex web of interconnected reactions.

Rather than being an inconvenience, the necessity for *catalysis* is a benefit, as it allows the cell to precisely control its **metabolism**—the sum total of all the chemical reactions it needs to carry out to survive, grow, and reproduce. This control is central to the chemistry of life.

Two opposing streams of chemical reactions occur in cells, the *catabolic* pathways and the *anabolic* pathways. The catabolic pathways (**catabolism**) break down foodstuffs into smaller molecules, thereby generating both a useful form of energy for the cell and some of the small molecules that the cell needs as building blocks. The anabolic, or *biosynthetic*, pathways (**anabolism**) use the energy harnessed by catabolism to drive the synthesis of the many molecules that form the cell. Together, these two sets of reactions constitute the metabolism of the cell (**Figure 3–2**).

The details regarding the individual reactions that comprise cell metabolism are part of the subject matter of *biochemistry*, and they need not concern us here. But the general principles by which cells obtain energy from their environment and use it to create order are central to cell biology. We begin this chapter with a discussion of why a constant input of energy is needed to sustain living organisms. We then discuss how enzymes catalyze the reactions that produce biological order. Finally, we describe the molecules that carry the energy that makes life possible.

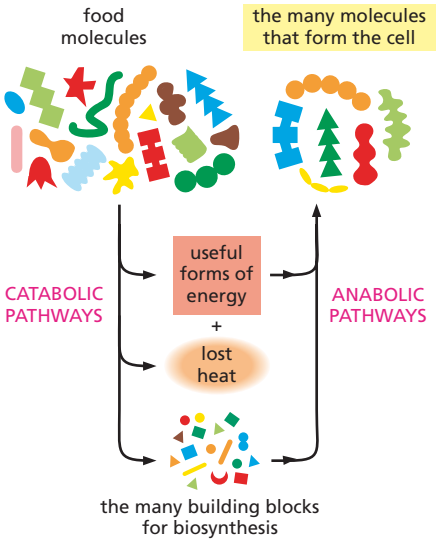


Figure 3–2 Catabolic and anabolic pathways together constitute the cell's metabolism. Note that a major portion of the energy stored in the chemical bonds of food molecules is dissipated as heat. Thus, only some of this energy can be converted to the useful forms of energy needed to drive the synthesis of new molecules.

THE USE OF ENERGY BY CELLS

Nonliving things left to themselves eventually become disordered: buildings crumble and dead organisms decay. Living cells, by contrast, not only maintain, but actually generate order at every level, from the large-scale structure of a butterfly or a flower down to the organization of the molecules that make up these organisms (**Figure 3–3**). This property of life is made possible by elaborate molecular mechanisms that extract energy from the environment and convert it into the energy stored in chemical bonds. Biological structures are therefore able to maintain their form, even though the materials of which they are made are continually being broken down, replaced, and recycled. Your body has the same basic structure it had 10 years ago, even though you now contain atoms that, for the most part, were not in your body then.

Biological Order Is Made Possible by the Release of Heat Energy from Cells

The universal tendency of things to become disordered is expressed in a fundamental law of physics, the *second law of thermodynamics.* This law states that, in the universe or in any isolated system (a collection of matter that is completely isolated from the rest of the universe), the degree of disorder can only increase. The second law of thermodynamics has such profound implications for living things that it is worth restating in several ways.

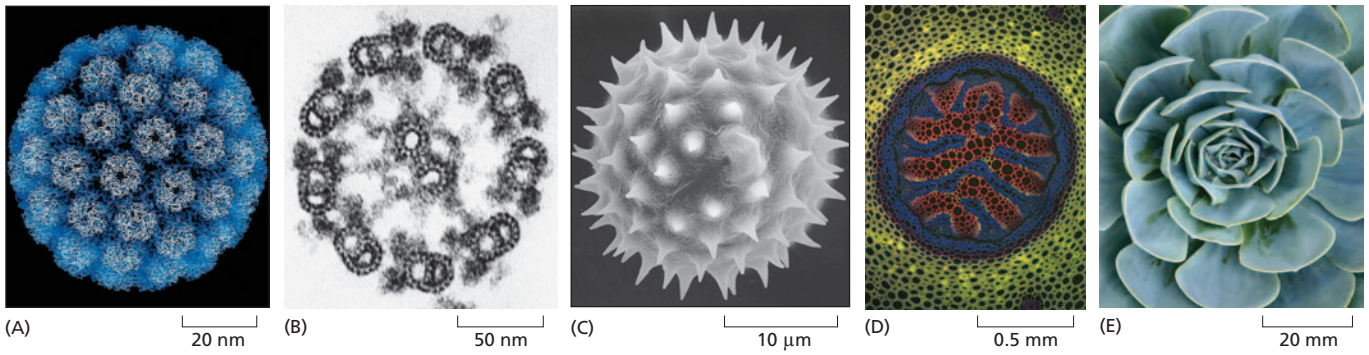


Figure 3–3 Biological structures are highly ordered. Well-defined, ornate, and beautiful spatial patterns can be found at every level of organization in living organisms. In order of increasing size: (A) protein molecules in the coat of a virus (a parasite that, although not technically alive, contains the same types of molecules as those found in living cells); (B) the regular array of microtubules seen in a cross section of a sperm tail; (C) surface contours of a pollen grain (a single cell); (D) cross section of a fern stem, showing the patterned arrangement of cells; and (E) flower with a spiral array of petals, each made of millions of cells. (A, courtesy of Robert Grant, Stéphane Crainic, and James M. Hogle; B, courtesy of Lewis Tilney; C, courtesy of Colin MacFarlane and Chris Jeffree; D, courtesy of Jim Haseloff.)

We can express the second law in terms of probability by stating that *systems will change spontaneously toward those arrangements that have the greatest probability.* Consider a box of 100 coins all lying heads up. A series of events that disturbs the box will tend to move the arrangement toward a mixture of 50 heads and 50 tails. The reason is simple: there are a huge number of possible arrangements of the individual coins that can achieve the 50–50 result, but only one possible arrangement that keeps them all oriented heads up. Because the 50–50 mixture accommodates a greater number of possibilities and places fewer constraints on the orientation of each individual coin, we say that it is more "disordered." For the same reason, one's living space will become increasingly disordered without an intentional effort to keep it organized. Movement toward disorder is a spontaneous process, requiring a periodic input of energy to reverse it (**Figure 3–4**).

The measure of a system's disorder is called the **entropy** of the system, and the greater the disorder, the greater the entropy. Thus another way to express the second law of thermodynamics is to say that systems will change spontaneously toward arrangements with greater entropy. Living cells—by surviving, growing, and forming complex communities and even whole organisms—generate order and thus might appear to defy the second law of thermodynamics. This is not the case, however,

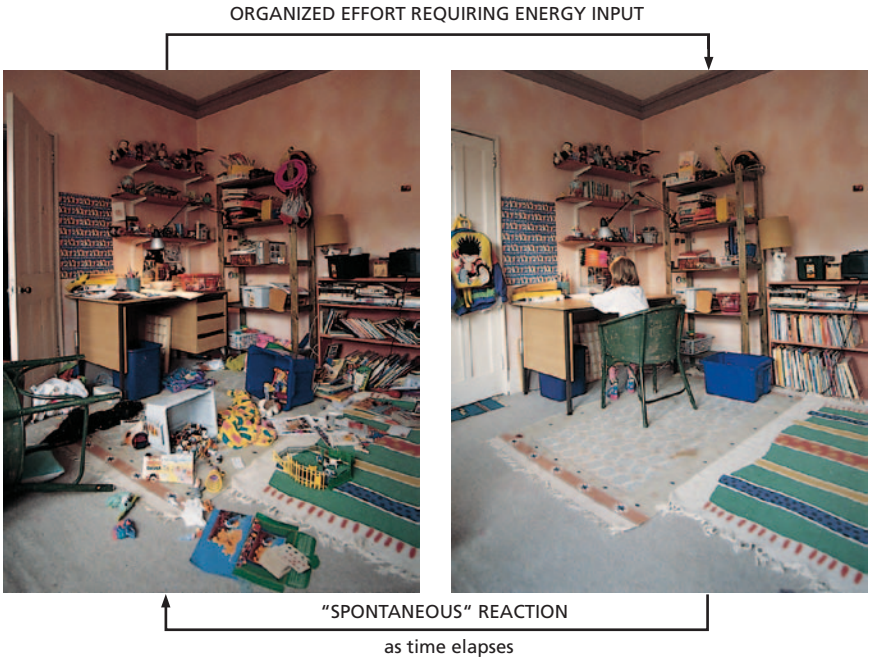


Figure 3–4 The spontaneous tendency toward disorder is an everyday experience. Reversing this natural tendency toward disorder requires an intentional effort and an input of energy. In fact, from the second law of thermodynamics, we can be certain that the human intervention required will release enough heat to the environment to more than compensate for the reestablishment of order in this room.

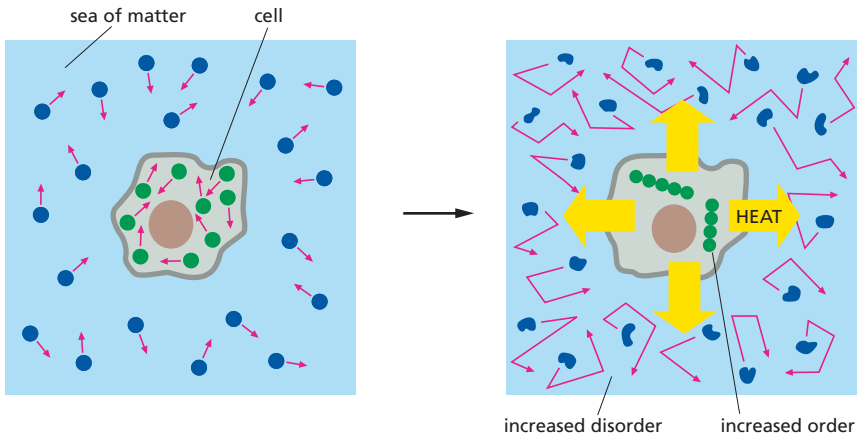


Figure 3–5 Living cells do not defy the second law of thermodynamics. In the diagram on the *left*, the molecules of both the cell and the rest of the universe (the environment) are depicted in a relatively disordered state. In the diagram on the *right*, the cell has taken in energy from food molecules and released heat by carrying out a reaction that orders the molecules that the cell contains. Because the heat increases the disorder in the environment around the cell—as depicted by the *longer, jagged red arrows*, which represent increased thermal motion, and the distorted molecules, which indicate enhanced molecular vibration and rotation—the second law of thermodynamics is satisfied, even as the cell grows and constructs larger molecules.

because a cell is not an isolated system. Rather, it takes in energy from its environment—in the form of food, inorganic molecules, or photons of light from the sun—and it then uses this energy to generate order within itself, forging new chemical bonds and building large macromolecules. In the course of performing the chemical reactions that generate order, some energy is lost in the form of heat. Heat is energy in its most disordered form—the random jostling of molecules (analogous to the random jostling of the coins in the box). Because the cell is not an isolated system, the heat energy that its reactions generate is quickly dispersed into the cell's surroundings. There, the heat increases the intensity of the thermal motions of nearby molecules, thereby increasing the entropy of the environment (**Figure 3–5**).

The amount of heat released by a cell must be great enough that the increased order generated inside the cell is more than compensated for by the increased disorder generated in the environment. Only in this case is the second law of thermodynamics satisfied, because the total entropy of the system—that of the cell plus its environment—increases as a result of the chemical reactions inside the cell.

Cells Can Convert Energy from One Form to Another

According to the *first law of thermodynamics*, energy cannot be created or destroyed—but it can be converted from one form to another (**Figure 3–6**). Cells take advantage of this law of thermodynamics, for example, when they convert the energy from sunlight into the energy in the chemical bonds of sugars and other small organic molecules during photosynthesis. Although chemical reactions that power such energy conversions can change how much energy is present in one form or another, the first law tells us that the total amount of energy in the universe must always be the same.

When an animal cell breaks down foodstuffs, some of the energy in the chemical bonds in the food molecules (chemical-bond energy) is converted into the thermal motion of molecules (heat energy). This conversion of chemical energy into heat energy causes the universe as a whole to become more disordered—as required by the second law of thermodynamics. But the cell cannot derive any benefit from the heat energy it produces unless the heat-generating reactions are directly linked to processes that maintain molecular order inside the cell. It is the tight coupling of heat production to an increase in order that distinguishes the metabolism of a cell from the wasteful burning of fuel in a fire. Later in this chapter, we illustrate how this coupling occurs. For the moment, it is

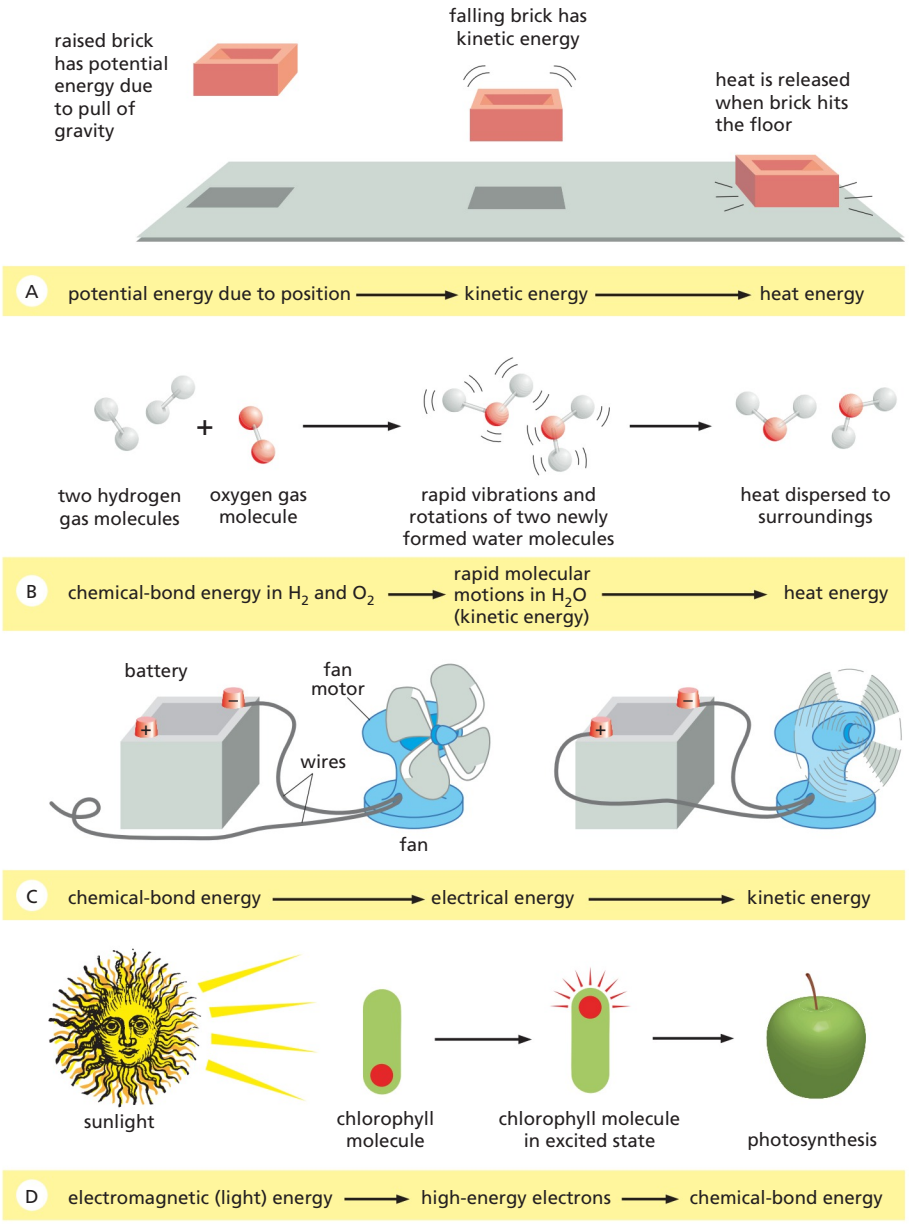


Figure 3–6 Different forms of energy are interconvertible, but the total amount of energy must be conserved. In (A), we can use the height and weight of the brick to predict exactly how much heat will be released when it hits the floor. In (B), the large amount of chemical-bond energy released when water (H_2O) is formed from H_2 and O_2 is initially converted to very rapid thermal motions in the two new H_2O molecules; however, collisions with other H_2O molecules almost instantaneously spread this kinetic energy evenly throughout the surroundings (heat transfer), making the new H_2O molecules indistinguishable from all the rest. (C) Cells can convert chemical-bond energy into kinetic energy to drive, for example, molecular motor proteins; however, this occurs without the intermediate conversion to electrical energy that a man-made appliance such as this fan requires. (D) Some cells can also harvest the energy from sunlight to form chemical bonds via photosynthesis.

sufficient to recognize that—by directly linking the "burning" of food molecules to the generation of biological order—cells are able to create and maintain an island of order in a universe tending toward chaos.

Photosynthetic Organisms Use Sunlight to Synthesize Organic Molecules

All animals live on energy stored in the chemical bonds of organic molecules, which they take in as food. These food molecules also provide the atoms that animals need to construct new living matter. Some animals obtain their food by eating other animals, others by eating plants. Plants, by contrast, obtain their energy directly from sunlight. Thus, the energy animals obtain by eating plants—or by eating animals that have eaten plants—ultimately comes from the sun (Figure 3–7).

Solar energy enters the living world through **photosynthesis**, a process that converts the electromagnetic energy in sunlight into chemical-bond energy in cells. Photosynthetic organisms—including plants, algae, and

Figure 3–7 With few exceptions, the radiant energy of sunlight sustains all life. Trapped by plants and some microorganisms through photosynthesis, light from the sun is the ultimate source of all energy for humans and other animals. (*Wheat Field Behind Saint-Paul Hospital with a Reaper* by Vincent van Gogh. Courtesy of Museum Folkwang, Essen.)

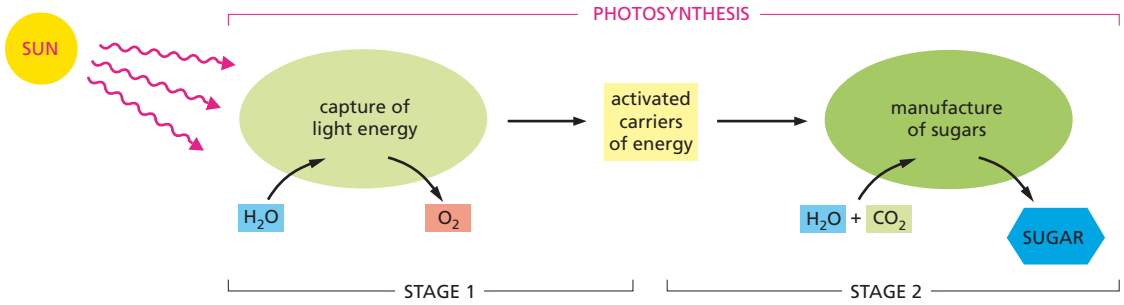


Figure 3–8 Photosynthesis takes place in two stages. The activated carriers generated in the first stage are two molecules that we will discuss shortly: ATP and NADPH.

some bacteria—use the energy they derive from sunlight to synthesize small chemical building blocks such as sugars, amino acids, nucleotides, and fatty acids. These small molecules in turn are converted into the macromolecules—the proteins, nucleic acids, polysaccharides, and lipids—that form the plant.

We describe the elegant mechanisms that underlie photosynthesis in detail in Chapter 14. Generally speaking, the reactions of photosynthesis take place in two stages. In the first stage, energy from sunlight is captured and transiently stored as chemical-bond energy in specialized molecules called *activated carriers*, which we discuss in more detail later in the chapter. All of the oxygen (O_2) in the air we breathe is generated by the splitting of water molecules during this first stage of photosynthesis.

In the second stage, the activated carriers are used to help drive a *carbon-fixation* process, in which sugars are manufactured from carbon dioxide gas (CO_2). In this way, photosynthesis generates an essential source of stored chemical-bond energy and other organic materials—for the plant itself and for any animals that eat it. The two stages of photosynthesis are summarized in **Figure 3–8**.

QUESTION 3–1

Consider the equation

$$\text{light energy} + CO_2 + H_2O \rightarrow \text{sugars} + O_2 + \text{heat energy}$$

Would you expect this reaction to occur in a single step? Why must heat be generated in the reaction? Explain your answers.

Cells Obtain Energy by the Oxidation of Organic Molecules

All animal and plant cells require the chemical energy stored in the chemical bonds of organic molecules—either the sugars that a plant has produced by photosynthesis as food for itself or the mixture of large and small molecules that an animal has eaten. To use this energy to live, grow, and reproduce, organisms must extract it in a usable form. In both plants and animals, energy is extracted from food molecules by a process of gradual *oxidation*, or controlled burning.

Earth's atmosphere is about 21% oxygen. In the presence of oxygen, the most energetically stable form of carbon is CO_2 and that of hydrogen is H_2O. A cell is therefore able to obtain energy from sugars or other organic molecules by allowing the carbon and hydrogen atoms in these molecules to combine with oxygen—that is, become *oxidized*—to produce CO_2 and H_2O, respectively—a process known as cellular **respiration**.

Photosynthesis and cellular respiration are complementary processes (**Figure 3–9**). This means that the transactions between plants and animals are not all one way. Plants, animals, and microorganisms have existed together on this planet for so long that they have become an essential part of each other's environments. The oxygen released by photosynthesis is consumed by nearly all organisms for the oxidative breakdown of organic molecules. And some of the CO_2 molecules that today are incorporated into organic molecules by photosynthesis in a green leaf were released yesterday into the atmosphere by the respiration of an animal, a fungus, or the plant itself, or by the burning of

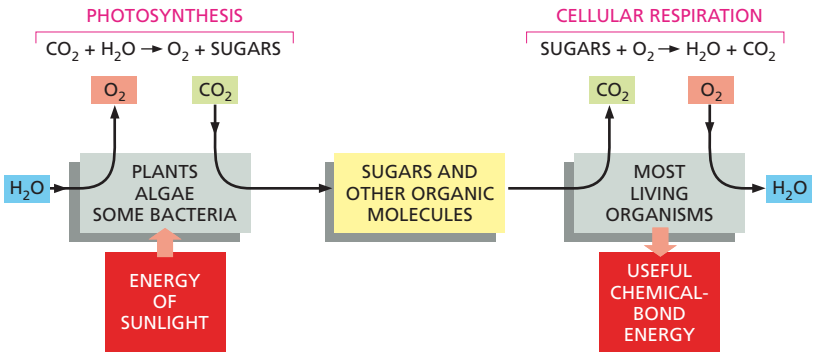


Figure 3–9 Photosynthesis and cellular respiration are complementary processes in the living world. The *left* side of the diagram shows how photosynthesis—carried out by plants and photosynthetic microorganisms—uses the energy of sunlight to produce sugars and other organic molecules from the carbon atoms in CO_2 in the atmosphere. In turn, these molecules serve as food for other organisms. The *right* side of the diagram shows how cellular respiration in most organisms—including plants and photosynthetic microorganisms—uses O_2 to oxidize food molecules, releasing the same carbon atoms in the form of CO_2 back to the atmosphere. In the process, the organisms obtain the useful chemical-bond energy that they need to survive. The first cells on Earth are thought to have been capable of neither photosynthesis nor cellular respiration (discussed in Chapter 14). However, photosynthesis must have preceded respiration on the Earth, because there is strong evidence that billions of years of photosynthesis were required to release enough O_2 to create an atmosphere that could support respiration.

fossil fuels. Carbon utilization therefore forms a huge cycle that involves the *biosphere* (all of the living organisms on Earth) as a whole, crossing boundaries between individual organisms (**Figure 3–10**).

Oxidation and Reduction Involve Electron Transfers

The cell does not oxidize organic molecules in one step, as occurs when organic material is burned in a fire. Through the use of enzyme catalysts, metabolism directs the molecules through a large number of reactions, few of which actually involve the direct addition of oxygen. Thus, before we consider some of these reactions, we should explain what is meant by oxidation.

The term **oxidation** literally means the addition of oxygen atoms to a molecule. More generally, though, oxidation is said to occur in any reaction in which electrons are transferred from one atom to another. Oxidation, in this sense, refers to the removal of electrons from an atom. The converse reaction, called **reduction**, involves the addition of electrons to an atom. Thus, Fe^{2+} is oxidized when it loses an electron to become Fe^{3+}, whereas a chlorine atom is reduced when it gains an electron to become Cl^-. Because the number of electrons is conserved in a chemical reaction (there is no net loss or gain), oxidation and reduction always occur simultaneously: that is, if one molecule gains an electron in a reaction (reduction), a second molecule must lose the electron (oxidation). When a sugar molecule is oxidized to CO_2 and H_2O, for example, the O_2 molecules involved in forming H_2O gain electrons and thus are said to have been reduced.

The terms oxidation and reduction apply even when there is only a partial shift of electrons between atoms linked by a covalent bond. When a carbon atom becomes covalently bonded to an atom with a strong affinity for electrons—oxygen, chlorine, or sulfur, for example—it gives up more

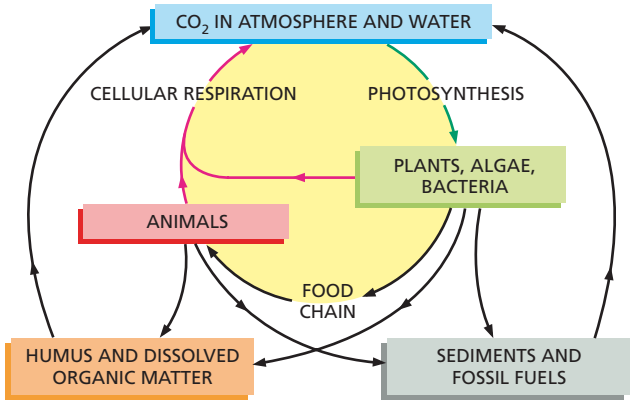


Figure 3–10 Carbon atoms cycle continuously through the biosphere. Individual carbon atoms are incorporated into organic molecules of the living world by the photosynthetic activity of plants, algae, and bacteria. They then pass to animals and microorganisms—as well as into organic material in soil and oceans—and are ultimately restored to the atmosphere in the form of CO_2 when organic molecules are oxidized by cells during respiration or burned by humans as fossil fuels.

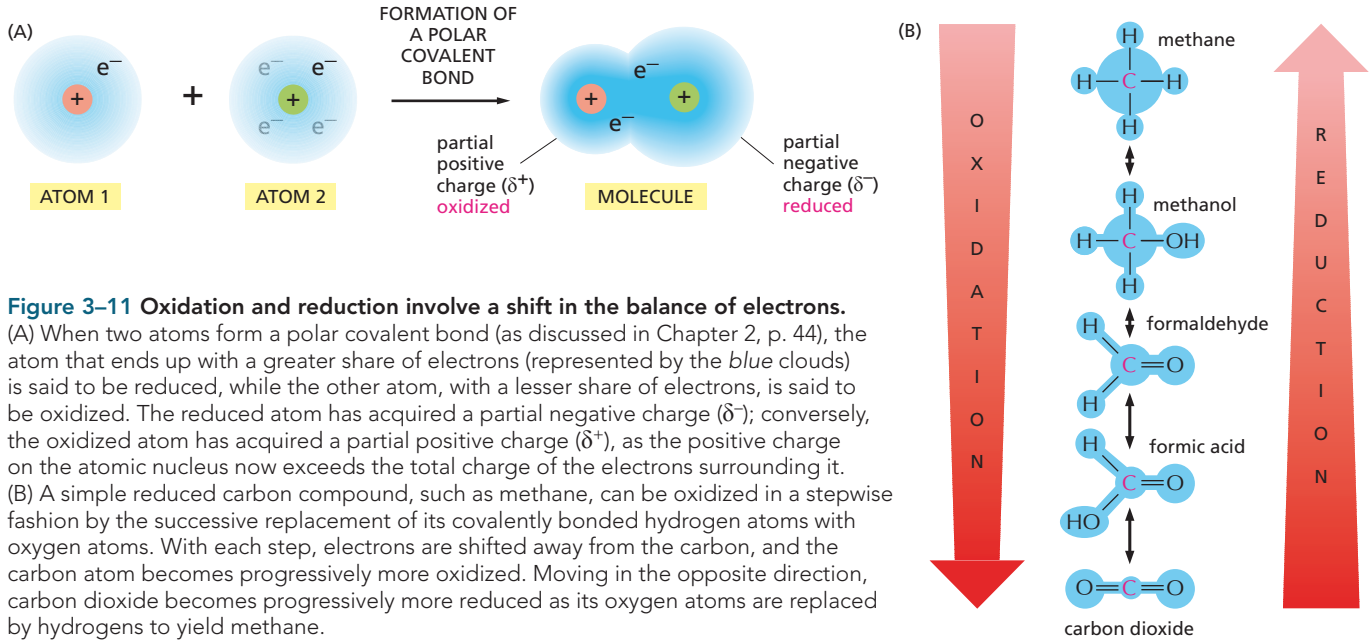


Figure 3–11 Oxidation and reduction involve a shift in the balance of electrons. (A) When two atoms form a polar covalent bond (as discussed in Chapter 2, p. 44), the atom that ends up with a greater share of electrons (represented by the *blue* clouds) is said to be reduced, while the other atom, with a lesser share of electrons, is said to be oxidized. The reduced atom has acquired a partial negative charge (δ^-); conversely, the oxidized atom has acquired a partial positive charge (δ^+), as the positive charge on the atomic nucleus now exceeds the total charge of the electrons surrounding it. (B) A simple reduced carbon compound, such as methane, can be oxidized in a stepwise fashion by the successive replacement of its covalently bonded hydrogen atoms with oxygen atoms. With each step, electrons are shifted away from the carbon, and the carbon atom becomes progressively more oxidized. Moving in the opposite direction, carbon dioxide becomes progressively more reduced as its oxygen atoms are replaced by hydrogens to yield methane.

than its equal share of electrons and forms a *polar covalent bond*. The positive charge of the carbon nucleus now slightly exceeds the negative charge of its electrons, so that the carbon atom acquires a partial positive charge (δ^+) and is said to be oxidized. Conversely, the carbon atom in a C–H bond has somewhat more than its share of electrons; it acquires a partial negative charge (δ^-), and so is said to be reduced (**Figure 3–11A**).

When a molecule in a cell picks up an electron (e^-), it often picks up a proton (H^+) at the same time (protons being freely available in water). The net effect in this case is to add a hydrogen atom to the molecule:

$$A + e^- + H^+ \rightarrow AH$$

Even though a proton plus an electron is involved (instead of just an electron), such *hydrogenation* reactions are reductions, and the reverse, *dehydrogenation*, reactions are oxidations. An easy way to tell whether an organic molecule is being oxidized or reduced is to count its C–H bonds: reduction occurs when the number of C–H bonds increases, whereas oxidation occurs when the number of C–H bonds decreases (**Figure 3–11B**).

As we will see later in this chapter—and again in Chapter 13—cells use enzymes to catalyze the oxidation of organic molecules in small steps, through a sequence of reactions that allows energy to be harvested in useful forms.

FREE ENERGY AND CATALYSIS

Enzymes, like cells, obey the second law of thermodynamics. Although they can speed up energetically favorable reactions—those that produce disorder in the universe—enzymes cannot by themselves force energetically unfavorable reactions to occur. Cells, however, must do just that in order to grow and divide—or just to survive. They must build highly ordered and energy-rich molecules from small and simple ones—a process that requires an input of energy.

To understand how enzymes promote **catalysis**—the acceleration of the specific chemical reactions needed to sustain life—we first need to examine the energetics involved. In this section, we consider how the free energy of molecules contributes to their chemistry, and we see how

free-energy changes—which reflect how much total disorder is generated in the universe by a reaction—influence whether and how the reaction will proceed. We then discuss how enzymes lower the activation energy needed to initiate reactions in the cell. And we describe how enzymes can exploit differences in the free-energy changes of different reactions to drive the energetically unfavorable reactions that produce biological order. Such enzyme-assisted catalysis is crucial for cells: without it, life could not exist.

Chemical Reactions Proceed in the Direction that Causes a Loss of Free Energy

Paper burns readily, releasing into the atmosphere water and carbon dioxide as gases, while simultaneously releasing energy as heat:

$$\text{paper} + O_2 \rightarrow \text{smoke} + \text{ashes} + \text{heat} + CO_2 + H_2O$$

This reaction occurs in only one direction: smoke and ashes never spontaneously gather carbon dioxide and water from the heated atmosphere and reconstitute themselves into paper. When paper burns, much of its chemical energy is dissipated as heat: it is not lost from the universe, since energy can never be created or destroyed; instead, it is irretrievably dispersed in the chaotic random thermal motions of molecules. At the same time, the atoms and molecules of the paper become dispersed and disordered. In the language of thermodynamics, there has been a release of *free energy*—that is, energy that can be harnessed to do work or drive chemical reactions. This release reflects a loss of orderliness in the way the energy and molecules had been stored in the paper. We will discuss free energy in more detail shortly, but the general principle can be summarized as follows: chemical reactions proceed only in the direction that leads to a loss of free energy. In other words, the spontaneous direction for any reaction is the direction that goes "downhill." A "downhill" reaction in this sense is said to be energetically favorable.

Enzymes Reduce the Energy Needed to Initiate Spontaneous Reactions

Although the most energetically favorable form of carbon under ordinary conditions is CO_2, and that of hydrogen is H_2O, a living organism will not disappear in a puff of smoke, and the book in your hands will not burst spontaneously into flames. This is because the molecules in both the living organism and the book are in a relatively stable state, and they cannot be changed to lower-energy states without an initial input of energy. In other words, a molecule requires a boost over an energy barrier before it can undergo a chemical reaction that moves it to a lower-energy (more stable) state (**Figure 3–12A**). This boost is known as the

QUESTION 3–2

In which of the following reactions does the *red* atom undergo an oxidation?

A. $Na \rightarrow Na^+$ (Na atom → Na^+ ion)
B. $Cl \rightarrow Cl^-$ (Cl atom → Cl^- ion)
C. $CH_3CH_2OH \rightarrow CH_3CHO$ (ethanol → acetaldehyde)
D. $CH_3CHO \rightarrow CH_3COO^-$ (acetaldehyde → acetic acid)
E. $CH_2{=}CH_2 \rightarrow CH_3CH_3$ (ethene → ethane)

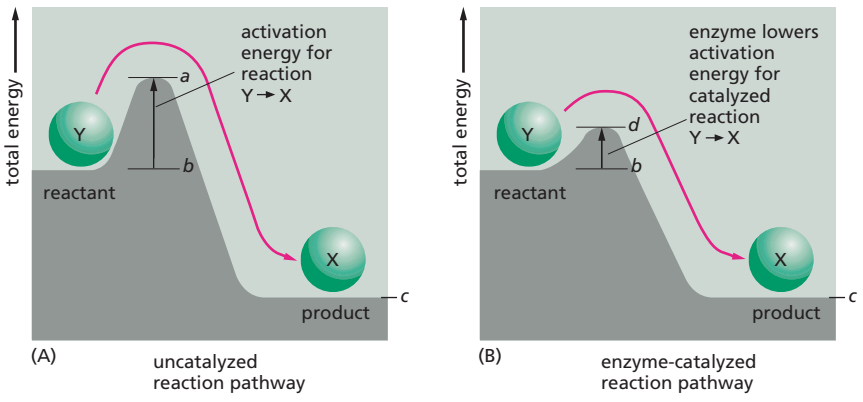


Figure 3–12 Even energetically favorable reactions require activation energy to get them started. (A) Compound Y (a reactant) is in a relatively stable state; thus energy is required to convert it to compound X (a product), even though X is at a lower overall energy level than Y. This conversion will not take place, therefore, unless compound Y can acquire enough *activation energy* (*energy a* minus *energy b*) from its surroundings to undergo the reaction that converts it into compound X. This energy may be provided by means of an unusually energetic collision with other molecules. For the reverse reaction, X → Y, the activation energy required will be much larger (*energy a* minus *energy c*); this reaction will therefore occur much more rarely. Activation energies are always positive. The total energy change for the energetically favorable reaction Y → X, is *energy c* minus *energy b*, a negative number, which corresponds to a loss of free energy. (B) Energy barriers for specific reactions can be lowered by catalysts, as indicated by the line marked *d*. Enzymes are particularly effective catalysts because they greatly reduce the activation energy for the reactions they catalyze.

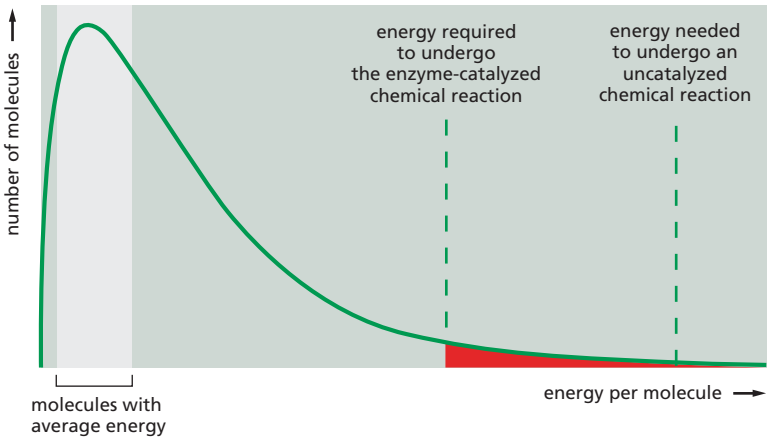


Figure 3–13 Lowering the activation energy greatly increases the probability that a reaction will occur. At any given instant, a population of identical substrate molecules will have a range of energies, distributed as shown on the graph. The varying energies come from collisions with surrounding molecules, which make the substrate molecules jiggle, vibrate, and spin. For a molecule to undergo a chemical reaction, the energy of the molecule must exceed the activation energy barrier for that reaction (*dashed lines*); for most biological reactions, this almost never happens without enzyme catalysis. Even with enzyme catalysis, only a small fraction of substrate molecules reach an energy state that is high enough for them to undergo a reaction (*red shaded* area).

activation energy. In the case of a burning book, the activation energy is provided by the heat of a lighted match. But cells can't raise their temperature to drive biological reactions. Inside cells, the push over the energy barrier is aided by specialized proteins called **enzymes**.

Each enzyme binds tightly to one or two molecules, called **substrates**, and holds them in a way that greatly reduces the activation energy needed to facilitate a specific chemical interaction between them (**Figure 3–12B**). A substance that can lower the activation energy of a reaction is termed a **catalyst**; catalysts increase the rate of chemical reactions because they allow a much larger proportion of the random collisions with surrounding molecules to kick the substrates over the energy barrier, as illustrated in **Figure 3–13** and **Figure 3–14A**. Enzymes are among the most effective catalysts known. They can speed up reactions by a factor of as much as 10^{14} (that is, trillions of times faster than the same reactions would proceed without an enzyme catalyst). Enzymes therefore allow reactions that would not otherwise occur to proceed rapidly at the normal temperature inside cells.

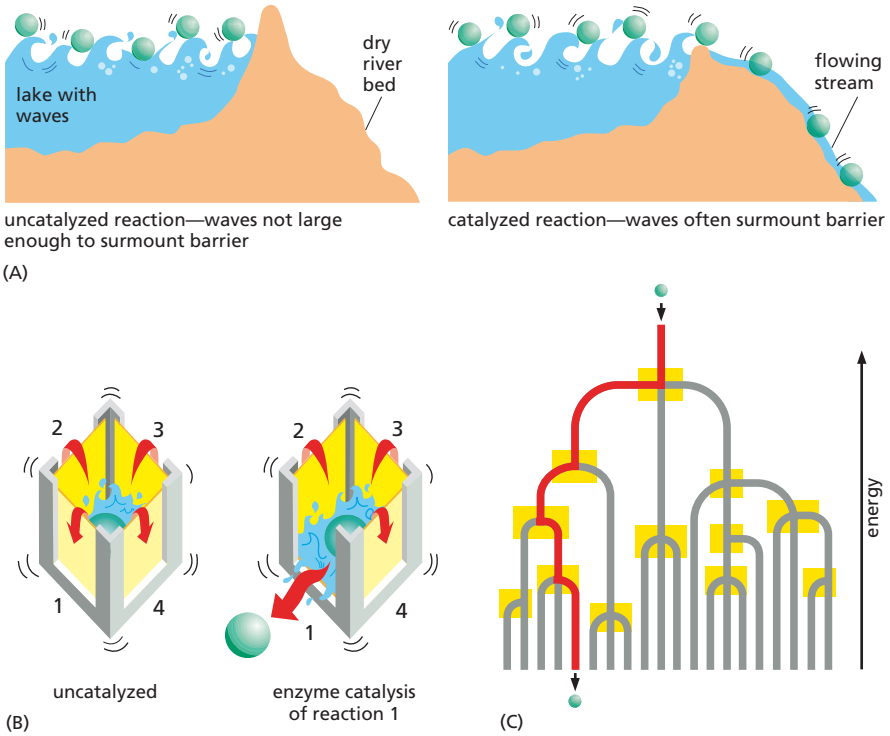


Figure 3–14 Enzymes catalyze reactions by lowering the activation energy barrier. (A) The dam represents the activation energy, which is lowered by enzyme catalysis. Each *green ball* represents a potential substrate molecule that is bouncing up and down in energy level owing to constant encounters with waves, an analogy for the thermal bombardment of substrate molecules by surrounding water molecules. When the barrier—the activation energy—is lowered significantly, the balls (substrate molecules) with sufficient energy can roll downhill, an energetically favorable movement. (B) The four walls of the box represent the activation energy barriers for four different chemical reactions that are all energetically favorable because the products are at lower energy levels than the substrates. In the left-hand box, none of these reactions occurs because even the largest waves are not large enough to surmount any of the energy barriers. In the right-hand box, enzyme catalysis lowers the activation energy for reaction number 1 only; now the jostling of the waves allows the substrate molecule to pass over this energy barrier, allowing reaction 1 to proceed (**Movie 3.1**). (C) A branching river with a set of barrier dams (*yellow* boxes) serves to illustrate how a series of enzyme-catalyzed reactions determines the exact reaction pathway followed by each molecule inside the cell by controlling specifically which reaction will be allowed at each junction.

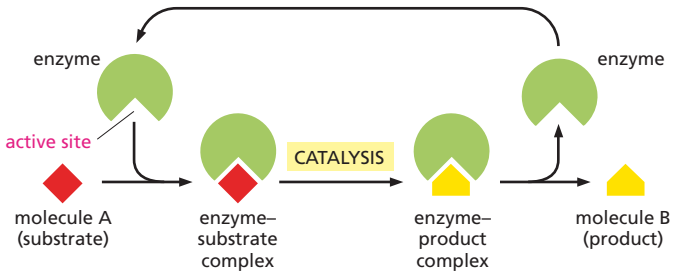


Figure 3–15 Enzymes convert substrates to products while remaining unchanged themselves. Each enzyme has an active site to which one or two substrate molecules bind, forming an enzyme–substrate complex. A reaction occurs at the active site, generating an enzyme–product complex. The product is then released, allowing the enzyme to bind additional substrate molecules and repeat the reaction. An enzyme thus serves as a catalyst, and it usually forms or breaks a single covalent bond in a substrate molecule.

Unlike the effects of temperature, enzymes are highly selective. Each enzyme usually speeds up only one particular reaction out of the several possible reactions that its substrate molecules could undergo. In this way, enzymes direct each of the many different molecules in a cell along specific reaction pathways (**Figure 3–14B and C**), thereby producing the compounds that the cell actually needs.

Like all catalysts, enzyme molecules themselves remain unchanged after participating in a reaction and therefore can function over and over again (**Figure 3–15**). In Chapter 4, we will discuss further how enzymes work, after we have looked in detail at the molecular structure of proteins.

The Free-Energy Change for a Reaction Determines Whether It Can Occur

According to the second law of thermodynamics, a chemical reaction can proceed only if it results in a net (overall) increase in the disorder of the universe (see Figure 3–5). Disorder increases when useful energy that could be harnessed to do work is dissipated as heat. The useful energy in a system is known as its **free energy**, or ***G***. And because chemical reactions involve a transition from one molecular state to another, the term that is of most interest to chemists and cell biologists is the **free-energy change**, denoted **ΔG** ("Delta *G*").

Let's consider a collection of molecules. ΔG measures the amount of disorder created in the universe when a reaction involving these molecules takes place. *Energetically favorable* reactions, by definition, are those that create disorder by decreasing the free energy of the system to which they belong; in other words, they have a *negative* ΔG (**Figure 3–16**).

A reaction can occur spontaneously only if ΔG is negative. On a macroscopic scale, an energetically favorable reaction with a negative ΔG is the relaxation of a compressed spring into an expanded state, releasing its stored elastic energy as heat to its surroundings. On a microscopic scale, an energetically favorable reaction with a negative ΔG occurs when salt (NaCl) dissolves in water. Note that, just because a reaction can occur spontaneously, does not mean it will occur quickly. The decay of diamonds into graphite is a spontaneous process—but it takes millions of years.

Energetically unfavorable reactions, by contrast, create order in the universe; they have a *positive* ΔG. Such reactions—for example, the formation of a peptide bond between two amino acids—cannot occur spontaneously; they take place only when they are coupled to a second reaction with a negative ΔG large enough that the net ΔG of the entire process is negative (**Figure 3–17**). Life is possible because enzymes can create biological order by coupling energetically unfavorable reactions with energetically favorable ones. These critical concepts are summarized, with examples, in **Panel 3–1** (pp. 96–97).

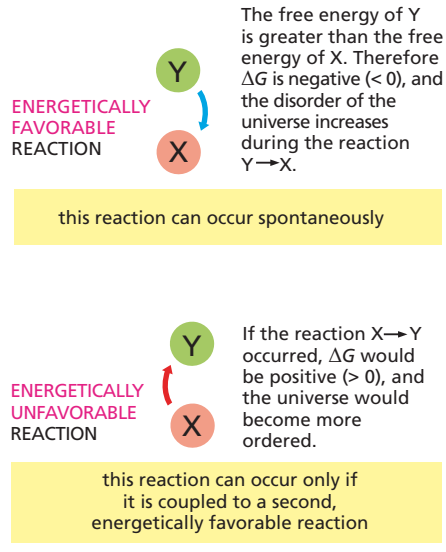


Figure 3–16 Energetically favorable reactions have a negative ΔG, whereas energetically unfavorable reactions have a positive ΔG.

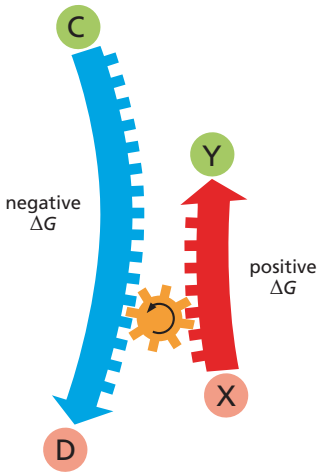


Figure 3–17 Reaction coupling can drive an energetically unfavorable reaction. The energetically unfavorable ($\Delta G > 0$) reaction X → Y cannot occur unless it is coupled to an energetically favorable ($\Delta G < 0$) reaction C → D, such that the net free-energy change for the coupled reactions is negative (less than 0).

ΔG Changes As a Reaction Proceeds Toward Equilibrium

It's easy to see how a tensed spring, when left to itself, will relax and release its stored energy to the environment as heat. But chemical reactions are a bit more complex—and harder to intuit. That's because whether a reaction will proceed depends not only on the energy stored in each individual molecule, but also on the concentrations of the molecules in the reaction mixture. Recalling our coin analogy, more coins in a jiggling box will flip from a head to a tail orientation when the box contains 90 heads and 10 tails, than when the box contains 10 heads and 90 tails.

The same is true for a chemical reaction. As the energetically favorable reaction Y → X proceeds, the concentration of the product X will increase and the concentration of the substrate Y will decrease. This change in relative concentrations of substrate and product will cause the ratio of Y to X to shrink, making the initially favorable ΔG less and less negative. Unless more Y is added, the reaction will slow and eventually stop.

Because ΔG changes as products accumulate and substrates are depleted, chemical reactions will generally proceed until they reach a state of **equilibrium**. At that point, the rates of the forward and reverse reactions are equal, and there is no further net change in the concentrations of substrate or product (**Figure 3–18**). For reactions at chemical equilibrium, $\Delta G = 0$, so the reaction will not proceed forward or backward, and no work can be done.

Such a state of chemical inactivity would be incompatible with life. Living cells avoid reaching a state of complete chemical equilibrium because they are constantly exchanging materials with their environment: replenishing nutrients and eliminating waste products. Many of the individual reactions in the cell's complex metabolic network also exist in disequilibrium because the products of one reaction are continually being siphoned off to become the substrates in a subsequent reaction. Rarely do products and substrates reach concentrations at which the forward and reverse reaction rates are equal.

The Standard Free-Energy Change, ΔG°, Makes it Possible to Compare the Energetics of Different Reactions

Because ΔG depends on the concentrations of the molecules in the reaction mixture at any given time, it is not a particularly useful value for comparing the relative energies of different types of reactions. But such energetic assessments are necessary, for example, to predict whether an energetically favorable reaction is likely to have a ΔG negative enough to drive an energetically unfavorable reaction. To compare reactions in this way, we need to turn to the *standard free-energy change* of a reaction, $\boldsymbol{\Delta G^\circ}$. The ΔG° is independent of concentration; it depends only on the intrinsic characters of the reacting molecules, based on their behavior under ideal conditions where the concentrations of all the reactants are set to the same fixed value of 1 mole/liter.

A large body of thermodynamic data has been collected from which ΔG° can be calculated for most metabolic reactions. Some common reactions are compared in terms of their ΔG° in Panel 3–1 (pp. 96–97).

The ΔG of a reaction can be calculated from ΔG° if the concentrations of the reactants and products are known. For the simple reaction Y → X, their relationship follows this equation:

$$\Delta G = \Delta G^\circ + RT \ln \frac{[X]}{[Y]}$$

where ΔG is in kilocalories per mole, [Y] and [X] denote the concentrations

QUESTION 3–3

Consider the analogy of the jiggling box containing coins that was described on page 85. The reaction, the flipping of coins that either face heads up (H) or tails up (T), is described by the equation H ↔ T, where the rate of the forward reaction equals the rate of the reverse reaction.

A. What are ΔG and ΔG° in this analogy?

B. What corresponds to the temperature at which the reaction proceeds? What corresponds to the activation energy of the reaction? Assume you have an "enzyme," called jigglase, which catalyzes this reaction. What would the effect of jigglase be and what, mechanically, might jigglase do in this analogy?

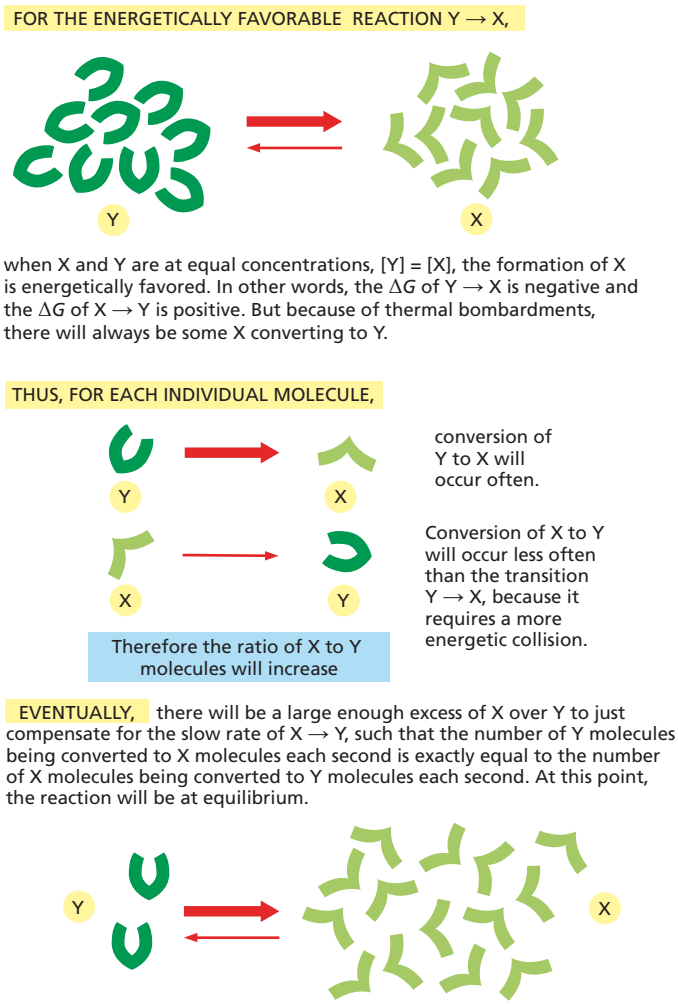


Figure 3–18 Reactions will eventually reach a chemical equilibrium. At that point, the forward and the backward fluxes of reacting molecules are equal and opposite. The widths of the arrows indicate the relative rates at which an *individual molecule* converts.

of Y and X in moles/liter, ln is the natural logarithm, and RT is the product of the gas constant, R, and the absolute temperature, T. At 37°C, $RT = 0.616$. (A mole is 6×10^{23} molecules of a substance.)

From this equation, we can see that when the concentrations of reactants and products are equal, in other words, [X]/[Y] = 1, the value of ΔG equals the value of $\Delta G°$ (because ln 1 = 0). Thus when the reactants and products are present in equal concentrations, the direction of the reaction depends entirely on the intrinsic properties of the molecules.

The Equilibrium Constant Is Directly Proportional to $\Delta G°$

As mentioned earlier, all chemical reactions tend to proceed toward equilibrium. Knowing where that equilibrium lies for any given reaction will tell you which way the reaction will proceed—and how far it will go. For example, if a reaction is at equilibrium when the concentration of the product is ten times the concentration of the substrate, and we begin with a surplus of substrate and little or no product, the reaction will proceed forward for some time. For the simple reaction Y → X, that value—the ratio of substrate to product at equilibrium—is called the reaction's **equilibrium constant**, ***K***. Expressed as an equation:

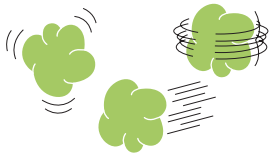
$$K = \frac{[X]}{[Y]}$$

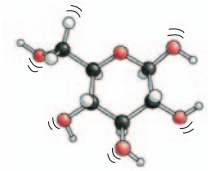
where [X] is the concentration of the product and [Y] is the concentration of the substrate at equilibrium.

FREE ENERGY

This panel reviews the concept of free energy and offers examples showing how changes in free energy determine whether—and how—biological reactions occur.

The molecules of a living cell possess energy because of their vibrations, rotations, and movement through space, and because of the energy that is stored in the bonds between individual atoms.



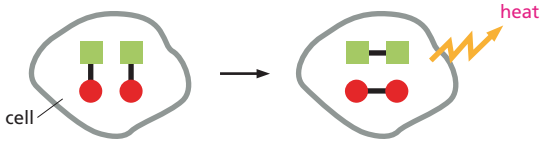


The free energy, *G* (in kcal/mole), measures the energy of a molecule which could in principle be used to do useful work at constant temperature, as in a living cell. Energy can also be expressed in joules (1 cal = 4.184 joules).

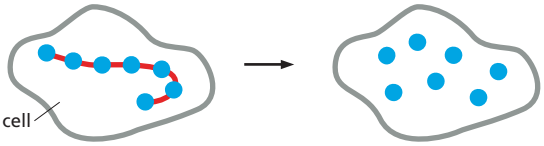
REACTIONS CAUSE DISORDER

Think of a chemical reaction occurring in a cell that has a constant temperature and volume. This reaction can produce disorder in two ways.

1 Changes of bond energy of the reacting molecules can cause heat to be released, which disorders the environment around the cell.



2 The reaction can decrease the amount of order in the cell—for example, by breaking apart a long chain of molecules, or by disrupting an interaction that prevents bond rotations.



PREDICTING REACTIONS

To predict the outcome of a reaction (Will it proceed to the right or to the left? At what point will it stop?), we must measure its standard free-energy change ($\Delta G°$). This quantity represents the gain or loss of free energy as one mole of reactant is converted to one mole of product under "standard conditions" (all molecules present at a concentration of 1 M and pH 7.0).

driving force

$\Delta G°$ for some reactions

glucose-1-P → glucose-6-P	–1.7 kcal/mole
sucrose → glucose + fructose	–5.5 kcal/mole
ATP → ADP + P_i	–7.3 kcal/mole
glucose + $6O_2$ → $6CO_2$ + $6H_2O$	–686 kcal/mole

ΔG ("DELTA G")

Changes in free energy occurring in a reaction are denoted by ΔG, where "Δ" indicates a difference. Thus, for the reaction

$$A + B \longrightarrow C + D$$

ΔG = free energy (C + D) minus free energy (A + B)

ΔG measures the amount of disorder caused by a reaction: the change in order inside the cell, plus the change in order of the surroundings caused by the heat released.

ΔG is useful because it measures how far away from equilibrium a reaction is. Thus the reaction

$$ATP \longrightarrow ADP + P_i$$

has a large negative ΔG because cells keep the reaction a long way from equilibrium by continually making fresh ATP. However, if the cell dies, then most of its ATP will be hydrolyzed, until equilibrium is reached; at equilibrium, the forward and backward reactions occur at equal rates and $\Delta G = 0$.

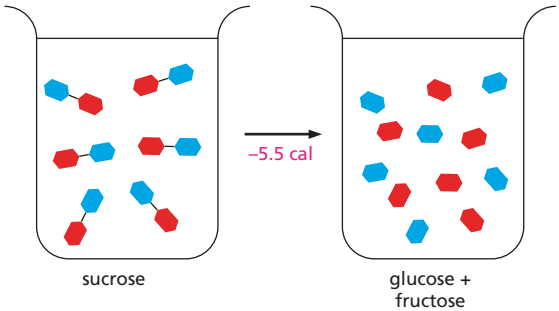
SPONTANEOUS REACTIONS

From the second law of thermodynamics, we know that the disorder of the universe can only increase. ΔG is *negative* if the disorder of the universe (reaction plus surroundings) *increases*.

In other words, a chemical reaction that occurs spontaneously must have a negative ΔG:

$$G_{products} - G_{reactants} = \Delta G < 0$$

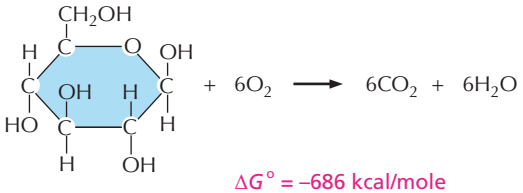
EXAMPLE: The difference in free energy of 100 ml of 10 mM sucrose (common sugar) and 100 ml of 10 mM glucose plus 10 mM fructose is about –5.5 calories. Therefore, the hydrolysis reaction that produces two monosaccharides from a disaccharide (sucrose → glucose + fructose) can proceed spontaneously.



In contrast, the reverse reaction (glucose + fructose → sucrose), which has a ΔG of +5.5 calories, could not occur without an input of energy from a coupled reaction.

REACTION RATES

A spontaneous reaction is not necessarily an instantaneous reaction: a reaction with a negative free-energy change (ΔG) will not necessarily occur rapidly by itself. Consider, for example, the combustion of glucose in oxygen:



$\Delta G^\circ = -686$ kcal/mole

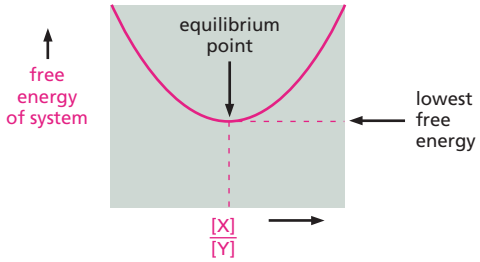
Even this highly favorable reaction may not occur for centuries unless there are enzymes to speed up the process.
Enzymes are able to catalyze reactions and speed up their rate, but they cannot change the ΔG° of a reaction.

CHEMICAL EQUILIBRIA

A fixed relationship exists between the standard free-energy change of a reaction, ΔG°, and its equilibrium constant K. For example, the reversible reaction

$$Y \rightleftarrows X$$

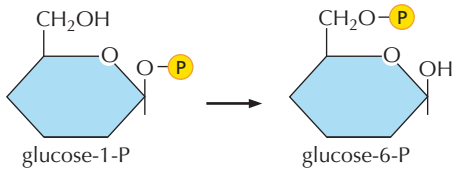
will proceed until the ratio of concentrations [X]/[Y] is equal to K (note: square brackets [ ] indicate concentration). At this point, the free energy of the system will have its lowest value.



At 37°C, $\Delta G^\circ = -1.42 \log_{10} K$ (see text, p. 98)

$K = 10^{-\Delta G^\circ/1.42}$

For example, the reaction



has $\Delta G^\circ = -1.74$ kcal/mole. Therefore, its equilibrium constant

$$K = 10^{(1.74/1.42)} = 10^{(1.23)} = 17$$

So the reaction will reach steady state when

[glucose-6-P]/[glucose-1-P] = 17

COUPLED REACTIONS

Reactions can be "coupled" together if they share one or more intermediates. In this case, the overall free-energy change is simply the sum of the individual ΔG° values. A reaction that is unfavorable (has a positive ΔG°) can for this reason be driven by a second, highly favorable reaction.

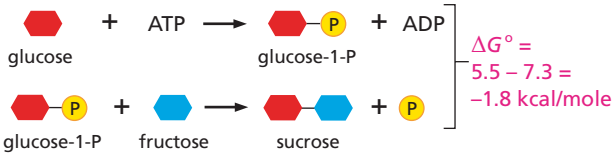
SINGLE REACTION

NET RESULT: reaction will not occur

ATP → ADP + P $\Delta G^\circ = -7.3$ kcal/mole

NET RESULT: reaction is highly favorable

COUPLED REACTIONS



NET RESULT: sucrose is made in a reaction driven by the hydrolysis of ATP

HIGH-ENERGY BONDS

One of the most common reactions in the cell is hydrolysis, in which a covalent bond is split by adding water.

The ΔG° for this reaction is sometimes loosely termed the "bond energy." Compounds such as acetyl phosphate and ATP, which have a large negative ΔG° of hydrolysis in an aqueous solution, are said to have "high-energy" bonds.

	ΔG° (kcal/mole)
acetyl P → acetate + P_i	–10.3
ATP → ADP + P_i	–7.3
glucose-6-P → glucose + P_i	–3.3

(Note that, for simplicity, H_2O is omitted from the above equations.)

But how do we know at what concentrations of substrate and product a reaction will reach equilibrium? It goes back to the intrinsic properties of the molecules involved, as expressed by $\Delta G°$. Let's see why.

At equilibrium, the rate of the forward reaction is exactly balanced by the rate of the reverse reaction. At that point, $\Delta G = 0$, and there is no net change of free energy to drive the reaction in either direction (see Panel 3–1, pp. 96–97).

Now, if we return to the equation presented on p. 94,

$$\Delta G = \Delta G° + RT \ln \frac{[X]}{[Y]}$$

we can see that, at equilibrium at 37°C, where $\Delta G = 0$ and the constant $RT = 0.616$, this equation becomes:

$$\Delta G° = -0.616 \ln \frac{[X]}{[Y]}$$

In other words, $\Delta G°$ is directly proportional to the equilibrium constant, K:

$$\Delta G° = -0.616 \ln K$$

If we convert this equation from natural log (ln) to the more commonly used base–10 logarithm (log), we get

$$\Delta G° = -1.42 \log K$$

This equation reveals how the equilibrium ratio of Y to X, expressed as the equilibrium constant K, depends on the intrinsic character of the molecules, as expressed in the value of $\Delta G°$ (**Table 3–1**). It tells us that for every 1.42 kcal/mole difference in free energy at 37°C, the equilibrium constant changes by a factor of 10. Thus, the more energetically favorable the reaction, the more product will accumulate if the reaction proceeds to equilibrium.

TABLE 3–1 RELATIONSHIP BETWEEN THE STANDARD FREE-ENERGY CHANGE, $\Delta G°$, AND THE EQUILIBRIUM CONSTANT

Equilibrium Constant $\frac{[X]}{[Y]}$	Standard Free Energy ($\Delta G°$) of X minus Free Energy of Y in kcal/mole
10^5	–7.1
10^4	–5.7
10^3	–4.3
10^2	–2.8
10	–1.4
1	0
10^{-1}	1.4
10^{-2}	2.8
10^{-3}	4.3
10^{-4}	5.7
10^{-5}	7.1

Values of the equilibrium constant were calculated for the simple chemical reaction Y ↔ X, using the equation given in the text.

The $\Delta G°$ values given here are in kilocalories per mole at 37°C. As explained in the text, $\Delta G°$ represents the free-energy difference under standard conditions (where all components are present at a concentration of 1 mole/liter).

From this table, we see that, if there is a favorable free-energy change of –4.3 kcal/mole for the transition Y → X, there will be 1000 times more molecules of X than of Y at equilibrium.

In Complex Reactions, the Equilibrium Constant Includes the Concentrations of All Reactants and Products

We have so far discussed the simplest of reactions, Y → X, in which a single substrate is converted into a single product. But inside cells, it is more common for two reactants to combine to form a single product: A + B ⇌ AB. How can we predict how this reaction will proceed?

The same principles apply, except that in this case the equilibrium constant K includes the concentrations of both of the reactants, in addition to the concentration of the product:

$$K = [AB]/[A][B]$$

As illustrated in **Figure 3–19**, the concentrations of both reactants are multiplied because the formation of product AB depends on the collision of A and B, and these encounters occur at a rate that is proportional to [A] × [B]. As with single-substrate reactions, $\Delta G° = -1.42 \log K$ at 37°C.

The Equilibrium Constant Indicates the Strength of Molecular Interactions

The concept of free-energy change does not only apply to chemical reactions where covalent bonds are being broken and formed, but also to interactions where one molecule binds to another by means of noncovalent interactions (see Chapter 2, p. 63). Noncovalent interactions are immensely important to cells. They include the binding of substrates to enzymes, the binding of gene regulatory proteins to DNA, and the binding of one protein to another to make the many different structural and functional protein complexes that operate in a living cell.

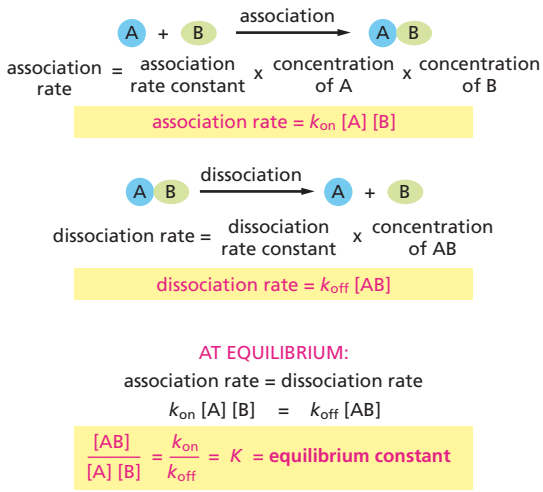


Figure 3–19 The equilibrium constant (*K*) for the reaction A + B → AB depends on both the association and dissociation rate constants. Molecules A and B must collide in order to interact, and the association rate is therefore proportional to the product of their individual concentrations [A] × [B]. As shown, the ratio of the rate constants k_{on} and k_{off} for the association and the dissociation reactions, respectively, is equal to the equilibrium constant, *K*, for the interaction. For two interacting components, *K* involves the concentrations of both substrates, in addition to that of the product. However, the relationship between *K* and $\Delta G°$ is the same as that shown in Table 3–1. The larger the value of *K*, the stronger is the binding between A and B.

Two molecules will bind to each other if the free-energy change for the interaction is negative; that is, the free energy of the resulting complex is lower than the sum of the free energies of the two partners when unbound. Because the equilibrium constant of a reaction is related directly to $\Delta G°$, *K* is commonly employed as a measure of the binding strength of a noncovalent interaction between two molecules. The binding strength is a very useful quantity to know because it also indicates how specific the interaction is between the two molecules.

Consider the reaction that was shown in Figure 3–19, where molecule A interacts with molecule B to form the complex AB. The reaction proceeds until it reaches equilibrium, at which point the number of association events precisely equals the number of dissociation events; at this point, the concentrations of reactants A and B, and of the complex AB, can be used to determine the equilibrium constant *K*.

K becomes larger as the *binding energy*—that is, the energy released in the binding interaction—increases. In other words, the larger *K* is, the greater is the drop in free energy between the dissociated and associated states, and the more tightly the two molecules will bind. Even a change of a few noncovalent bonds can have a striking effect on a binding interaction, as illustrated in **Figure 3–20**. In this example, eliminating a few hydrogen bonds from a binding interaction can be seen to cause a dramatic decrease in the amount of complex that exists at equilibrium.

For Sequential Reactions, the Changes in Free Energy Are Additive

Now we return to our original concern: how can enzymes catalyze reactions that are energetically unfavorable? One way they do so is by directly coupling energetically unfavorable reactions with energetically favorable ones. Consider, for example, two sequential reactions,

$$X \rightarrow Y \text{ and } Y \rightarrow Z$$

where the $\Delta G°$ values are +5 and –13 kcal/mole, respectively. (Recall that a mole is 6×10^{23} molecules of a substance.) The unfavorable reaction, X → Y, will not occur spontaneously. However, it can be driven by the favorable reaction Y → Z, provided that the second reaction follows the first. That's because the overall free-energy change for the coupled reaction is equal to the sum of the free-energy changes for each individual reaction. In this case, the $\Delta G°$ for the coupled reaction will be –8 kcal/mole, making the overall pathway energetically favorable.

Consider 1000 molecules of A and 1000 molecules of B in the cytosol of a eukaryotic cell. The concentration of both will be about 10^{-9} M.

If the equilibrium constant (*K*) for A + B ↔ AB is 10^{10} liters/mole, then at equilibrium there will be

270	270	730
A molecules	B molecules	AB complexes

If the equilibrium constant is a little weaker, say 10^{8} liters/mole—a value that represents a loss of 2.8 kcal/mole of binding energy from the example above, or 2–3 fewer hydrogen bonds—then there will be

915	915	85
A molecules	B molecules	AB complexes

Figure 3–20 Small changes in the number of weak bonds can have drastic effects on a binding interaction. This example illustrates the dramatic effect of the presence or absence of a few weak noncovalent bonds in the interaction between two cytosolic proteins.

Cells can therefore cause the energetically unfavorable transition, X → Y, to occur if an enzyme catalyzing the X → Y reaction is supplemented by a second enzyme that catalyzes the energetically favorable reaction, Y → Z. In effect, the reaction Y → Z acts as a "siphon," pulling the conversion of all of molecule X to molecule Y, and then to molecule Z (**Figure 3–21**). For example, several of the reactions in the long pathway that converts sugars into CO_2 and H_2O are energetically unfavorable. The pathway nevertheless proceeds rapidly to completion, however, because the total $\Delta G°$ for the series of sequential reactions has a large negative value.

Forming a sequential pathway, however, is not the answer for all metabolic needs. Often the desired reaction is simply X → Y, without further conversion of Y to some other product. Fortunately, there are other, more general ways of using enzymes to couple reactions together, involving the production of activated carriers that can shuttle energy from one reaction site to another. We discuss these systems shortly. Before we do, let's pause to look at how enzymes find and recognize their substrates and how enzyme-catalyzed reactions proceed. After all, thermodynamic considerations merely establish whether chemical reactions can occur; enzymes actually make them happen.

QUESTION 3–4

For the reactions shown in Figure 3–21, sketch an energy diagram similar to that in Figure 3–12 for the two reactions alone and for the combined reactions. Indicate the standard free-energy changes for the reactions X → Y, Y → Z, and X → Z in the graph. Indicate how enzymes that catalyze these reactions would change the energy diagram.

Thermal Motion Allows Enzymes to Find Their Substrates

Enzymes and their substrates are both present in relatively small amounts in the cytosol of a cell, yet a typical enzyme can capture and process about a thousand substrate molecules every second. This means that an enzyme can release its product and bind a new substrate in a fraction of a millisecond. How do these molecules find each other so quickly in the crowded cytosol of the cell?

Rapid binding is possible because molecular motions are enormously fast. Because of heat energy, molecules are in constant motion and consequently will explore the cytosolic space very efficiently by wandering

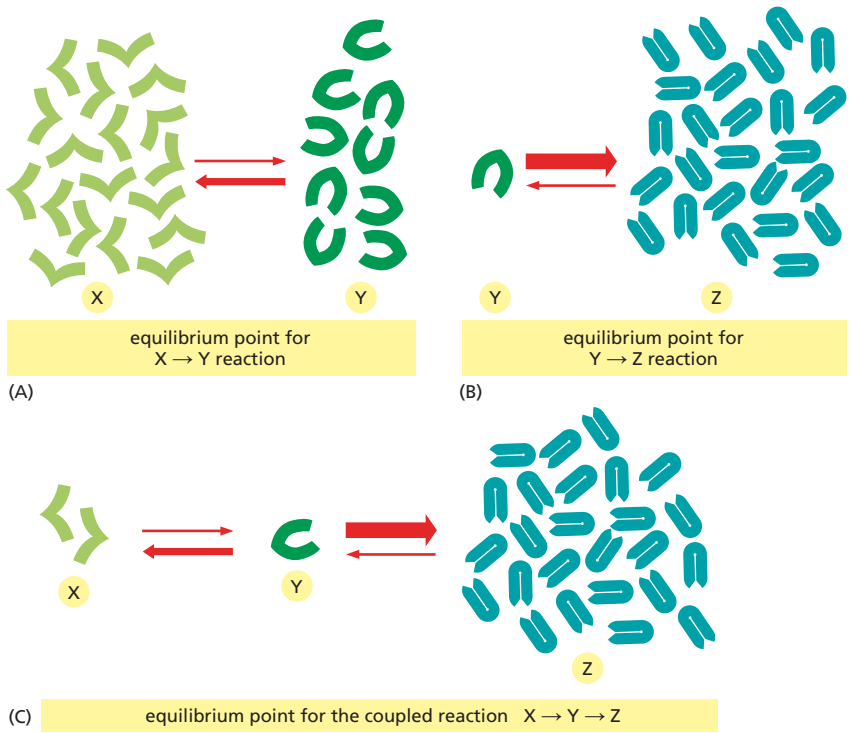


Figure 3–21 An energetically unfavorable reaction can be driven by an energetically favorable follow-on reaction that acts as a chemical siphon. (A) At equilibrium, there are twice as many X molecules as Y molecules. (B) At equilibrium, there are 25 times more Z molecules than Y molecules. (C) If the reactions in (A) and (B) are coupled, nearly all of the X molecules will be converted to Z molecules, as shown. In terms of energetics, the $\Delta G°$ of the Y → Z reaction is so negative that, when coupled to the X → Y reaction, it lowers the ΔG of X → Y, because the ΔG of X → Y decreases as the ratio of Y to X declines. As shown in Figure 3–18, arrow widths reflect the relative rates at which an individual molecule converts; the arrow lengths are the same in both directions here, indicating that there is no net flux.

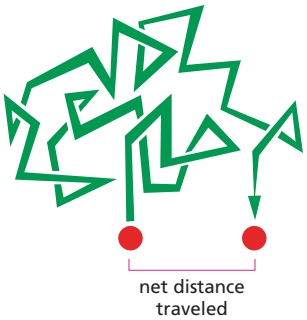


Figure 3–22 A molecule traverses the cytosol by taking a random walk. Molecules in solution move in a random fashion due to the continual buffeting they receive in collisions with other molecules. This movement allows small molecules to diffuse rapidly throughout the cell cytosol (**Movie 3.2**).

randomly through it—a process called **diffusion**. In this way, every molecule in the cytosol collides with a huge number of other molecules each second. As the molecules in a liquid collide and bounce off one another, an individual molecule moves first one way and then another, its path constituting a *random walk* (**Figure 3–22**).

Although the cytosol of a cell is densely packed with molecules of various shapes and sizes (**Figure 3–23**), experiments in which fluorescent dyes and other labeled molecules are injected into the cell cytosol show that small organic molecules diffuse through this aqueous gel nearly as rapidly as they do through water. A small organic molecule, such as a substrate, takes only about one-fifth of a second on average to diffuse a distance of 10 μm. Diffusion is therefore an efficient way for small molecules to move limited distances in the cell.

Because proteins diffuse through the cytosol much more slowly than do small molecules, the rate at which an enzyme will encounter its substrate depends on the concentration of the substrate. The most abundant substrates are present in the cell at a concentration of about 0.5 mM. Because pure water is 55 M, there is only about one such substrate molecule in the cell for every 10^5 water molecules. Nevertheless, the site on an enzyme that binds this substrate will be bombarded by about 500,000 random collisions with the substrate every second. For a substrate concentration tenfold lower (0.05 mM), the number of collisions drops to 50,000 per second, and so on.

The random encounters between an enzyme and its substrate often lead to the formation of an enzyme–substrate complex. This association is stabilized by the formation of multiple, weak bonds between the enzyme and substrate. These weak interactions—which can include hydrogen bonds, van der Waals attractions, and electrostatic attractions (discussed in Chapter 2)—persist until random thermal motion causes the molecules to dissociate again. When two colliding molecules have poorly matching surfaces, few noncovalent bonds are formed, and their total energy is negligible compared with that of thermal motion. In this case, the two molecules dissociate as rapidly as they come together (see Figure 2–33). This is what prevents incorrect and unwanted associations from forming between mismatched molecules, such as those between an enzyme and the wrong substrate. But when the enzyme and substrate are well matched, they form many weak interactions, which keep them held together long enough for a covalent bond in the substrate molecule to be formed or broken. Knowing the speed at which molecules collide and come apart, as well as how fast bonds can be formed and broken, makes the observed rate of enzymatic catalysis seem a little less amazing.

QUESTION 3–5

The enzyme carbonic anhydrase is one of the speediest enzymes known. It catalyzes the rapid conversion of CO_2 gas into the much more soluble bicarbonate ion (HCO_3^-). The reaction:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

is very important for the efficient transport of CO_2 from tissue, where CO_2 is produced by respiration, to the lungs, where it is exhaled. Carbonic anhydrase accelerates the reaction 10^7-fold, hydrating 10^5 CO_2 molecules per second at its maximal speed. What do you suppose limits the speed of the enzyme? Sketch a diagram analogous to the one shown in Figure 3–13 and indicate which portion of your diagram has been designed to display the 10^7-fold acceleration.

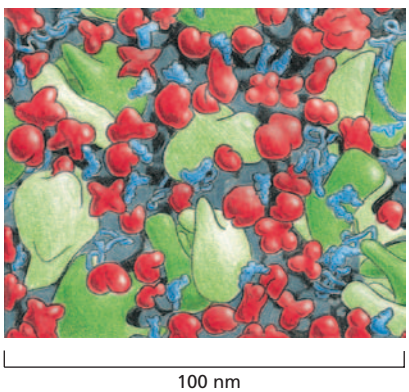


Figure 3–23 The cytosol is crowded with various molecules. Only the macromolecules, which are drawn to scale, are shown. RNAs are *blue*, ribosomes are *green*, and proteins are *red*. Enzymes and other macromolecules diffuse relatively slowly in the cytosol, in part because they interact with so many other macromolecules. Small molecules, by contrast, can diffuse nearly as rapidly as they do in water. (Adapted from D.S. Goodsell, *Trends Biochem. Sci.* 16:203–206, 1991. With permission from Elsevier.)

V_{max} and K_M Measure Enzyme Performance

To catalyze a reaction, an enzyme must first bind its substrate. The substrate then undergoes a reaction to form the product, which initially remains bound to the enzyme. Finally, the product is released and diffuses away, leaving the enzyme free to bind another substrate molecule and catalyze another reaction (see Figure 3–15). The rates of the different steps vary widely from one enzyme to another, and they can be measured by mixing purified enzymes and substrates together under carefully defined conditions in a test tube (see **How We Know**, pp. 104–106).

In such experiments, the substrate is introduced in increasing concentrations to a solution containing a fixed concentration of enzyme. At first, the concentration of the enzyme–substrate complex—and therefore the rate at which product is formed—rises in a linear fashion in direct proportion to substrate concentration. However, as more and more enzyme molecules become occupied by substrate, this rate increase tapers off, until at a very high concentration of substrate it reaches a maximum value, termed V_{max}. At this point, the active sites of all enzyme molecules in the sample are fully occupied by substrate, and the rate of product formation depends only on how rapidly the substrate molecule can undergo a reaction to form the product. For many enzymes, this **turnover number** is of the order of 1000 substrate molecules per second, although turnover numbers between 1 and 100,000 have been measured.

Because there is no clearly defined substrate concentration at which the enzyme can be deemed fully occupied, biochemists instead use a different parameter to gauge the concentration of substrate needed to make the enzyme work efficiently. This value is called the **Michaelis constant**, K_M, named after one of the biochemists who worked out the relationship. The K_M of an enzyme is defined as the concentration of substrate at which the enzyme works at half its maximum speed (**Figure 3–24**). In general, a small K_M indicates that a substrate binds very tightly to the enzyme, and a large K_M indicates weak binding.

Although an enzyme (or any catalyst) functions to lower the activation energy for a reaction such as Y → X, it is important to note that the enzyme will also lower the activation energy for the reverse reaction X → Y to exactly the same degree. The forward and backward reactions will therefore be accelerated by the same factor by an enzyme, and the equilibrium point for the reaction—and thus its $\Delta G°$—remains unchanged (**Figure 3–25**).

QUESTION 3–6

In cells, an enzyme catalyzes the reaction AB → A + B. It was isolated, however, as an enzyme that carries out the opposite reaction A + B → AB. Explain the paradox.

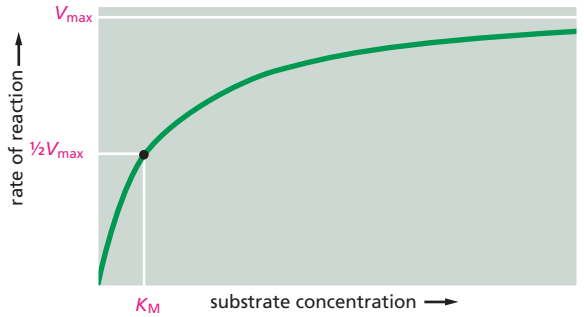


Figure 3–24 An enzyme's performance depends on how rapidly it can process its substrate. The rate of an enzyme reaction (V) increases as the substrate concentration increases, until a maximum value (V_{max}) is reached. At this point, all substrate-binding sites on the enzyme molecules are fully occupied, and the rate of the reaction is limited by the rate of the catalytic process on the enzyme surface. For most enzymes, the concentration of substrate at which the reaction rate is half-maximal (K_M) is a direct measure of how tightly the substrate is bound, with a large value of K_M (a large amount of substrate needed) corresponding to weak binding.

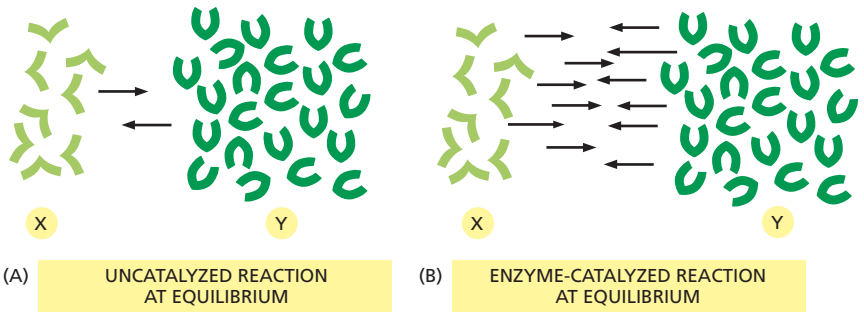


Figure 3–25 Enzymes cannot change the equilibrium point for reactions. Enzymes, like all catalysts, speed up the forward and reverse rates of a reaction by the same amount. Therefore, for both the (A) uncatalyzed and (B) catalyzed reactions shown here, the number of molecules undergoing the transition X ⟶ Y is equal to the number of molecules undergoing the transition Y ⟶ X when the ratio of Y molecules to X molecules is 3.5 to 1, as illustrated. In other words, both the catalyzed and uncatalyzed reactions will eventually reach the same equilibrium point, although the catalyzed reaction will reach equilibrium faster.

ACTIVATED CARRIERS AND BIOSYNTHESIS

The energy released by energetically favorable reactions such as the oxidation of food molecules must be stored temporarily before it can be used by cells to fuel energetically unfavorable reactions, such as the synthesis of all the other molecules needed by the cell. In most cases, the energy is stored as chemical-bond energy in a set of *activated carriers*, small organic molecules that contain one or more energy-rich covalent bonds. These molecules diffuse rapidly and carry their bond energy from the sites of energy generation to the sites where energy is used for **biosynthesis** or for other energy-requiring cell activities (**Figure 3–26**).

Activated carriers store energy in an easily exchangeable form, either as a readily transferable chemical group or as readily transferable ("high energy") electrons. They can serve a dual role as a source of both energy and chemical groups for biosynthetic reactions. The most important activated carriers are *ATP* and two molecules that are closely related to each other, *NADH* and *NADPH*. Cells use activated carriers like money to pay for the energetically unfavorable reactions that otherwise would not take place.

The Formation of an Activated Carrier Is Coupled to an Energetically Favorable Reaction

When a fuel molecule such as glucose is oxidized in a cell, enzyme-catalyzed reactions ensure that a large part of the free energy released is captured in a chemically useful form, rather than being released wastefully as heat. (Oxidizing sugar in a cell allows you to power metabolic reactions, whereas burning a chocolate bar in the street will get you nowhere, producing no metabolically useful energy.) In cells, energy capture is achieved by means of a **coupled reaction**, in which an energetically favorable reaction is used to drive an energetically unfavorable one that produces an activated carrier or some other useful molecule.

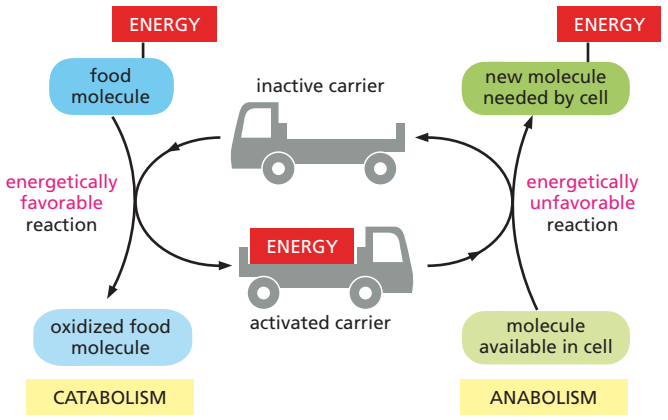


Figure 3–26 Activated carriers can store and transfer energy in a form that cells can use. By serving as intracellular energy shuttles, activated carriers perform their function as go-betweens that link the release of energy from the breakdown of food molecules (catabolism) to the energy-requiring biosynthesis of small and large organic molecules (anabolism).

MEASURING ENZYME PERFORMANCE

At first glance, it seems that a cell's metabolic pathways have been pretty well mapped out, with each reaction proceeding predictably to the next—substrate X is converted to product Y, which is passed along to enzyme Z. So why would anyone need to know exactly how tightly a particular enzyme clutches its substrate or whether it can process 100 or 1000 substrate molecules every second?

In reality, such elaborate metabolic maps merely suggest which pathways a cell might follow as it converts nutrients into small molecules, chemical energy, and the larger building blocks of life. Like a road map, they do not predict the density of traffic under a particular set of conditions: which pathways the cell will use when it is starving, when it is well fed, when oxygen is scarce, when it is stressed, or when it decides to divide. The study of an enzyme's *kinetics*—how fast it operates, how it handles its substrate, how its activity is controlled—makes it possible to predict how an individual catalyst will perform, and how it will interact with other enzymes in a network. Such knowledge leads to a deeper understanding of cell biology, and it opens the door to learning how to harness enzymes to perform desired reactions.

Speed

The first step to understanding how an enzyme performs involves determining the maximal velocity, V_{max}, for the reaction it catalyzes. This is accomplished by measuring, in a test tube, how rapidly the reaction proceeds in the presence of different concentrations of substrate (**Figure 3–27A**): the rate should increase as the amount of substrate rises until the reaction reaches its V_{max}. The velocity of the reaction is measured by monitoring either how quickly the substrate is consumed or how rapidly the product accumulates. In many cases, the appearance of product or the disappearance of substrate can be observed directly with a spectrophotometer. This instrument detects the presence of molecules that absorb light at a particular wavelength; NADH, for example, absorbs light at 340 nm, while its oxidized counterpart, NAD^+, does not. So, a reaction that generates NADH (by reducing NAD^+) can be monitored by following the formation of NADH at 340 nm in a spectrophotometer.

To determine the V_{max} of a reaction, you would set up a series of test tubes, where each tube contains a different concentration of substrate. For each tube, add the same amount of enzyme and then measure the velocity of the reaction—the number of micromoles of substrate consumed or product generated per minute. Because these numbers will tend to decrease over time, the rate used is the velocity measured early in the reaction. These initial velocity values (v) are then plotted against the substrate concentration, yielding a curve like the one shown in **Figure 3–27B**.

Looking at this plot, however, it is difficult to determine the exact value of V_{max}, as it is not clear where the reaction rate will reach its plateau. To get around this problem, the data are converted to their reciprocals

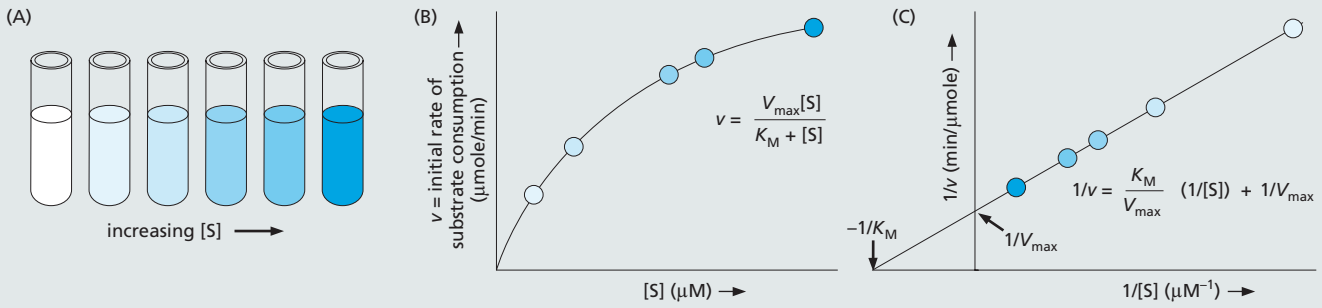


Figure 3–27 Measured reaction rates are plotted to determine V_{max} and K_M of an enzyme-catalyzed reaction. (A) A series of increasing substrate concentrations is prepared, a fixed amount of enzyme is added, and initial reaction rates (velocities) are determined. (B) The initial velocities (v) plotted against the substrate concentrations [S] give a curve described by the general equation $y = ax/(b + x)$. Substituting our kinetic terms, the equation becomes $v = V_{max}[S]/(K_M + [S])$, where V_{max} is the asymptote of the curve (the value of y at an infinite value of x), and K_M is equal to the substrate concentration where v is one-half V_{max}. This is called the *Michaelis–Menten equation*, named for the biochemists who provided evidence for this enzymatic relationship. (C) In a double-reciprocal plot, $1/v$ is plotted against 1/[S]. The equation describing this straight line is $1/v = (K_M/V_{max})(1/[S]) + 1/V_{max}$. When 1/[S] = 0, the y intercept ($1/v$) is $1/V_{max}$. When $1/v = 0$, the x intercept (1/[S]) is $-1/K_M$. Plotting the data this way allows V_{max} and K_M to be calculated more precisely. By convention, lowercase letters are used for variables (hence v for velocity) and uppercase letters are used for constants (hence V_{max}).

and graphed in a "double-reciprocal plot," where the inverse of the velocity ($1/v$) appears on the y axis and the inverse of the substrate concentration ($1/[S]$) on the x axis (**Figure 3–27C**). This graph yields a straight line whose y intercept (the point where the line crosses the y axis) represents $1/V_{max}$ and whose x intercept corresponds to $-1/K_M$. These values are then converted to values for V_{max} and K_M.

Enzymologists use this technique to determine the kinetic parameters of many enzyme-catalyzed reactions (although these days computer programs automatically plot the data and spit out the sought-after values). Some reactions, however, happen too fast to be monitored in this way; the reaction is essentially complete—the substrate entirely consumed—within thousandths of a second. For these reactions, a special piece of equipment must be used to follow what happens during the first few milliseconds after enzyme and substrate meet (**Figure 3–28**).

Control

Substrates are not the only molecules that can influence how well or how quickly an enzyme works. In many cases, products, substrate lookalikes, inhibitors, and other small molecules can also increase or decrease enzyme activity. Such regulation allows cells to control when and how rapidly various reactions occur, a process we will consider in more detail in Chapter 4.

Determining how an inhibitor decreases an enzyme's activity can reveal how a metabolic pathway is regulated—and can suggest how those control points can be circumvented by carefully designed mutations in specific genes.

The effect of an inhibitor on an enzyme's activity is monitored in the same way that we measured the enzyme's kinetics. A curve is first generated showing the velocity of the uninhibited reaction between enzyme and substrate, as described previously. Additional curves are then produced for reactions in which the inhibitor molecule has been included in the mix.

Comparing these curves, with and without inhibitor, can also reveal how a particular inhibitor impedes enzyme activity. For example, some inhibitors bind to the same site on an enzyme as its substrate. These *competitive inhibitors* block enzyme activity by competing directly with the substrate for the enzyme's attention. They resemble the substrate enough to tie up the enzyme, but they differ enough in structure to avoid getting converted to product. This blockage can be overcome by adding enough substrate so that enzymes are more likely to encounter a substrate molecule than an inhibitor molecule. From the kinetic data, we can see that competitive inhibitors do not change the V_{max} of a reaction; in other words, add enough substrate and the enzyme will encounter mostly substrate molecules and will reach its maximum velocity (**Figure 3–29**).

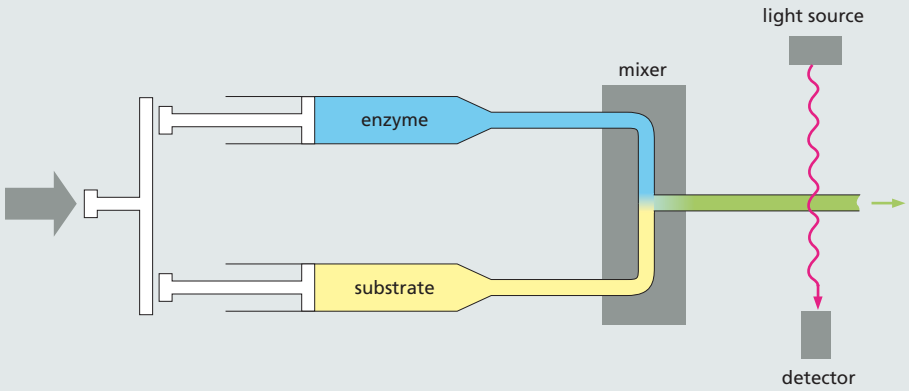


Figure 3–28 A stopped-flow apparatus is used to observe reactions during the first few milliseconds. In this piece of equipment, the enzyme and substrate are rapidly injected into a mixing chamber through two syringes. The enzyme and its substrate meet as they shoot through the mixing tube at flow rates that can easily reach 1000 cm/sec. They then enter another tube and zoom past a detector that monitors, say, the appearance of product. If the detector is located within a centimeter of where the enzyme and substrate meet, it is possible to observe reactions when they are only a few milliseconds old.

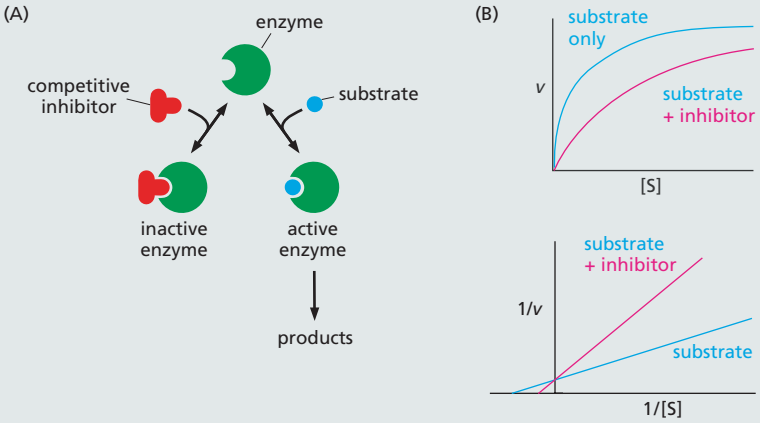


Figure 3–29 A competitive inhibitor directly blocks substrate binding to an enzyme. (A) The active site of the enzyme can bind either the competitive inhibitor or the substrate, but not both together. (B) The upper plot shows that inhibition by a competitive inhibitor can be overcome by increasing the substrate concentration. The double-reciprocal plot below shows that the V_{max} of the reaction is not changed in the presence of the competitive inhibitor: the *y* intercept is identical for both the curves.

Competitive inhibitors can be used to treat patients who have been poisoned by ethylene glycol, an ingredient in commercially available antifreeze. Although ethylene glycol is itself not fatally toxic, a by-product of its metabolism—oxalic acid—can be lethal. To prevent oxalic acid from forming, the patient is given a large (though not quite intoxicating) dose of ethanol. Ethanol competes with the ethylene glycol for binding to alcohol dehydrogenase, the first enzyme in the pathway to oxalic acid formation. As a result, the ethylene glycol goes mostly unmetabolized and is safely eliminated from the body.

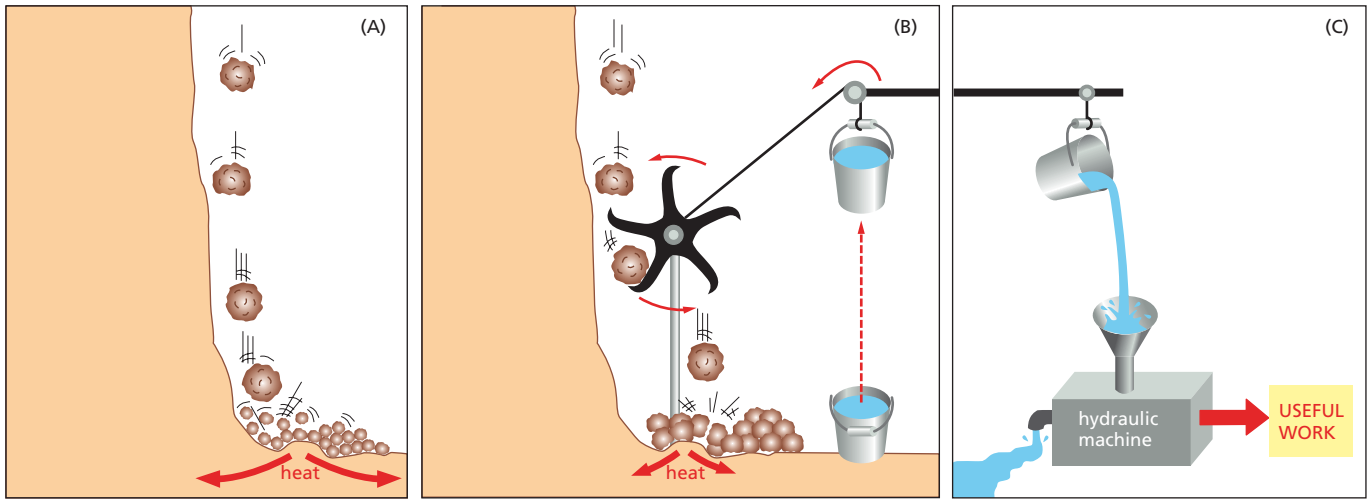
Other types of inhibitors may interact with sites on the enzyme distant from where the substrate binds. As we discuss in Chapter 4, many biosynthetic enzymes are regulated by feedback inhibition, whereby an enzyme early in a pathway will be shut down by a product generated later in the pathway. Because this type of inhibitor binds to a separate regulatory site on the enzyme, the substrate can still bind, but it might do so more slowly than it would in the absence of inhibitor. Such *noncompetitive inhibition* is not overcome by the addition of more substrate.

Design

With the kinetic data in hand, we can use computer modeling programs to predict how an enzyme will perform, and even how a cell will respond when exposed to different conditions—such as the addition of a particular sugar or amino acid to the culture medium, or the addition of a poison or a pollutant. Seeing how a cell manages its resources—which pathways it favors for dealing with particular biochemical challenges—can also suggest strategies for designing better catalysts for reactions of medical or commercial importance (e.g., for producing drugs or detoxifying industrial waste). Using such tactics, bacteria have even been genetically engineered to produce large amounts of indigo—the dye, originally extracted from plants, that makes your blue jeans blue.

Computer programs have been developed to facilitate the dissection of complex reaction pathways. They require information about the components in the pathway, including the K_M and V_{max} of the participating enzymes and the concentrations of enzymes, substrates, products, inhibitors, and other regulatory molecules. The program then predicts how molecules will flow through the pathway, which products will be generated, and where any bottlenecks might be. The process is not unlike balancing an algebraic equation, in which every atom of carbon, nitrogen, oxygen, and so on must be tallied. Such careful accounting makes it possible to rationally design ways to manipulate the pathway, such as re-routing it around a bottleneck, eliminating an important inhibitor, redirecting the reactions to favor the generation of predominantly one product, or extending the pathway to produce a novel molecule. Of course, such computer models must be validated in cells, which may not always behave as predicted.

Producing designer cells that spew out commercial products generally requires using genetic engineering techniques to introduce the gene or genes of choice into a cell, usually a bacterium, that can be manipulated and maintained in the laboratory. We discuss these methods at greater length in Chapter 10. Harnessing the power of cell biology for commercial purposes—even to produce something as simple as the amino acid tryptophan—is currently a multibillion-dollar industry. And, as more genome data come in, presenting us with more enzymes to exploit, it may not be long before vats of custom-made bacteria are churning out drugs and chemicals that represent the biological equivalent of pure gold.



kinetic energy of falling rocks is transformed into heat energy only

part of the kinetic energy is used to lift a bucket of water, and a correspondingly smaller amount is transformed into heat

the potential energy stored in the raised bucket of water can be used to drive hydraulic machines that carry out a variety of useful tasks

Figure 3–30 A mechanical model illustrates the principle of coupled chemical reactions. The spontaneous reaction shown in (A) could serve as an analogy for the direct oxidation of glucose to CO_2 and H_2O, which produces only heat. In (B), the same reaction is coupled to a second reaction, which could serve as an analogy for the synthesis of activated carriers. The energy produced in (B) is in a more useful form than in (A) and can be used to drive a variety of otherwise energetically unfavorable reactions (C).

Such coupling requires enzymes, which are fundamental to all of the energy transactions in the cell.

The nature of a coupled reaction is illustrated by a mechanical analogy in **Figure 3–30**, in which an energetically favorable chemical reaction is represented by rocks falling from a cliff. The kinetic energy of falling rocks would normally be entirely wasted in the form of heat generated by friction when the rocks hit the ground (Figure 3–30A). By careful design, however, part of this energy could be used to drive a paddle wheel that lifts a bucket of water (Figure 3–30B). Because the rocks can now reach the ground only after moving the paddle wheel, we say that the energetically favorable reaction of rocks falling has been directly coupled to the energetically unfavorable reaction of lifting the bucket of water. Because part of the energy is used to do work in (B), the rocks hit the ground with less velocity than in (A), and correspondingly less energy is wasted as heat. The energy saved in the elevated bucket of water can then be used to do useful work (Figure 3–30C).

Analogous processes occur in cells, where enzymes play the role of the paddle wheel in Figure 3–30B. By mechanisms that we discuss in Chapter 13, enzymes couple an energetically favorable reaction, such as the oxidation of foodstuffs, to an energetically unfavorable reaction, such as the generation of activated carriers. As a result, the amount of heat released by the oxidation reaction is reduced by exactly the amount of energy that is stored in the energy-rich covalent bonds of the activated carrier. That saved energy can then be used to power a chemical reaction elsewhere in the cell.

ATP Is the Most Widely Used Activated Carrier

The most important and versatile of the activated carriers in cells is **ATP** (adenosine 5′-triphosphate). Just as the energy stored in the raised bucket of water in Figure 3–30B can be used to drive a wide variety of hydraulic machines, ATP serves as a convenient and versatile store, or currency, of energy that can be used to drive a variety of chemical reactions in cells.

QUESTION 3–7

Use Figure 3–30B to illustrate the following reaction driven by the hydrolysis of ATP:
$X + ATP \rightarrow Y + ADP + P_i$
A. In this case, which molecule or molecules would be analogous to (i) rocks at top of cliff, (ii) broken debris at bottom of cliff, (iii) bucket at its highest point, and (iv) bucket on the ground?
B. What would be analogous to (i) the rocks hitting the ground in the absence of the paddle wheel in Figure 3–30A and (ii) the hydraulic machine in Figure 3–30C?

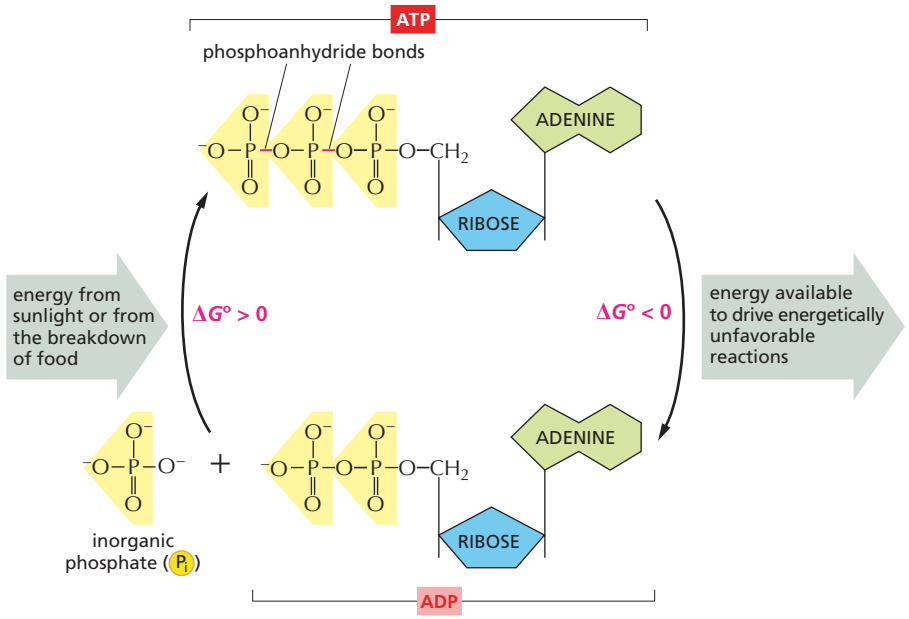


Figure 3–31 The interconversion of ATP and ADP occurs in a cycle. The two outermost phosphate groups in ATP are held to the rest of the molecule by high-energy phosphoanhydride bonds and are readily transferred to other organic molecules. Water can be added to ATP to form ADP and inorganic phosphate (P_i). Inside a cell, this hydrolysis of the terminal phosphate of ATP yields between 11 and 13 kcal/mole of usable energy. Although the $\Delta G°$ of this reaction is –7.3 kcal/mole, the ΔG is much more negative, because the ratio of ATP to the products ADP and P_i is so high inside the cell.

The large negative $\Delta G°$ of the reaction arises from a number of factors. Release of the terminal phosphate group removes an unfavorable repulsion between adjacent negative charges; in addition, the inorganic phosphate ion (P_i) released is stabilized by favorable hydrogen-bond formation with water. The formation of ATP from ADP and P_i reverses the hydrolysis reaction; because this condensation reaction is energetically unfavorable, it must be coupled to an energetically more favorable reaction to occur.

As shown in **Figure 3–31**, ATP is synthesized in an energetically unfavorable *phosphorylation* reaction, in which a phosphate group is added to **ADP** (adenosine 5′-diphosphate). When required, ATP gives up this energy packet in an energetically favorable hydrolysis to ADP and inorganic phosphate (P_i). The regenerated ADP is then available to be used for another round of the phosphorylation reaction that forms ATP, creating an ATP cycle in the cell.

The energetically favorable reaction of ATP hydrolysis is coupled to many otherwise unfavorable reactions through which other molecules are synthesized. We will encounter several of these reactions in this chapter, where we will see exactly how this is done. ATP hydrolysis is often coupled to the transfer of the terminal phosphate in ATP to another molecule, as illustrated in **Figure 3–32**. Any reaction that involves the transfer of a phosphate group to a molecule is termed a phosphorylation reaction. Phosphorylation reactions are examples of condensation reactions (see Figure 2–25), and they occur in many important cell processes: they activate substrates, mediate the exchange of chemical energy, and serve as key constituents of intracellular signaling pathways (discussed in Chapter 16).

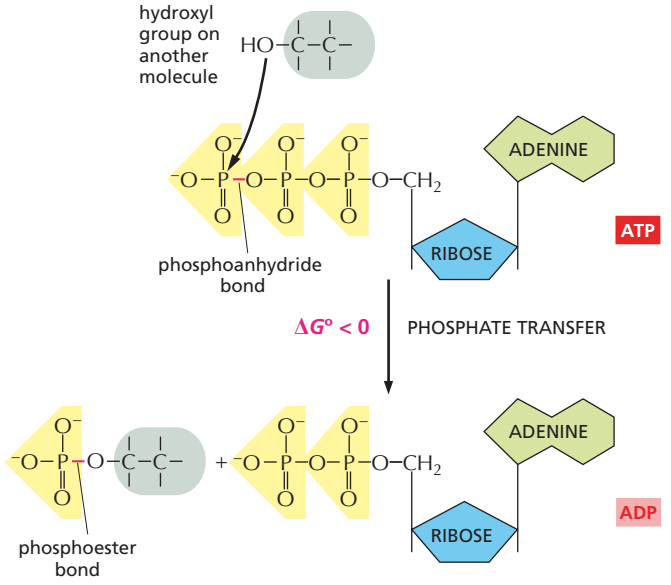


Figure 3–32 The terminal phosphate of ATP can be readily transferred to other molecules. Because an energy-rich phosphoanhydride bond in ATP is converted to a less energy-rich phosphoester bond in the phosphate-accepting molecule, this reaction is energetically favorable, having a large negative $\Delta G°$ (see Panel 3–1, pp. 96–97). Phosphorylation reactions of this type are involved in the synthesis of phospholipids and in the initial steps of the breakdown of sugars, as well as in many other metabolic and intracellular signaling pathways.

ATP is the most abundant activated carrier in cells. It is used, for example, to supply energy for many of the pumps that actively transport substances into or out of the cell (discussed in Chapter 12); it also powers the molecular motors that enable muscle cells to contract and nerve cells to transport materials along their lengthy axons (discussed in Chapter 17). Why evolution selected this particular nucleotide over the others as the major carrier of energy, however, remains a mystery. The nucleotide GTP, although similar, has very different functions in the cell, as we discuss in later chapters.

Energy Stored in ATP Is Often Harnessed to Join Two Molecules Together

A common type of reaction that is needed for biosynthesis is one in which two molecules, A and B, are joined together by a covalent bond to produce A–B in the energetically unfavorable condensation reaction:

$$A\text{–}H + B\text{–}OH \rightarrow A\text{–}B + H_2O$$

ATP hydrolysis can be coupled indirectly to this reaction to make it go forward. In this case, energy from ATP hydrolysis is first used to convert B–OH to a higher-energy intermediate compound, which then reacts directly with A–H to give A–B. The simplest mechanism involves the transfer of a phosphate from ATP to B–OH to make $B\text{–}O\text{–}PO_3$, in which case the reaction pathway contains only two steps:

1. $B\text{–}OH + ATP \rightarrow B\text{–}O\text{–}PO_3 + ADP$
2. $A\text{–}H + B\text{–}O\text{–}PO_3 \rightarrow A\text{–}B + P_i$

Net result: $B\text{–}OH + ATP + A\text{–}H \rightarrow A\text{–}B + ADP + P_i$

The condensation reaction, which by itself is energetically unfavorable, has been forced to occur by being coupled to ATP hydrolysis in an enzyme-catalyzed reaction pathway (**Figure 3–33A**).

A biosynthetic reaction of exactly this type is employed to synthesize the amino acid glutamine, as illustrated in **Figure 3–33B**. We will see later in the chapter that very similar (but more complex) mechanisms are also used to produce nearly all of the large molecules of the cell.

NADH and NADPH Are Both Activated Carriers of Electrons

Other important activated carriers participate in oxidation–reduction reactions and are commonly part of coupled reactions in cells. These activated carriers are specialized to carry both high-energy electrons and hydrogen atoms. The most important of these *electron carriers* are **NADH** (nicotinamide adenine dinucleotide) and the closely related molecule **NADPH** (nicotinamide adenine dinucleotide phosphate). Both NADH and NADPH carry energy in the form of two high-energy electrons plus a proton (H^+), which together form a hydride ion (H^-). When these activated carriers pass their energy (in the form of a hydride ion) to a donor molecule, they become oxidized to form **NAD^+** and **$NADP^+$**, respectively.

Like ATP, NADPH is an activated carrier that participates in many important biosynthetic reactions that would otherwise be energetically unfavorable. NADPH is produced according to the general scheme shown in **Figure 3–34A**. During a special set of energy-yielding catabolic reactions, a hydride ion is removed from the substrate molecule and added to the nicotinamide ring of $NADP^+$ to form NADPH. This is a typical oxidation–reduction reaction; the substrate is oxidized and $NADP^+$ is reduced.

QUESTION 3–8

The phosphoanhydride bond that links two phosphate groups in ATP in a high-energy linkage has a $\Delta G°$ of –7.3 kcal/mole. Hydrolysis of this bond in a cell liberates from 11 to 13 kcal/mole of usable energy. How can this be? Why do you think a range of energies is given, rather than a precise number as for $\Delta G°$?

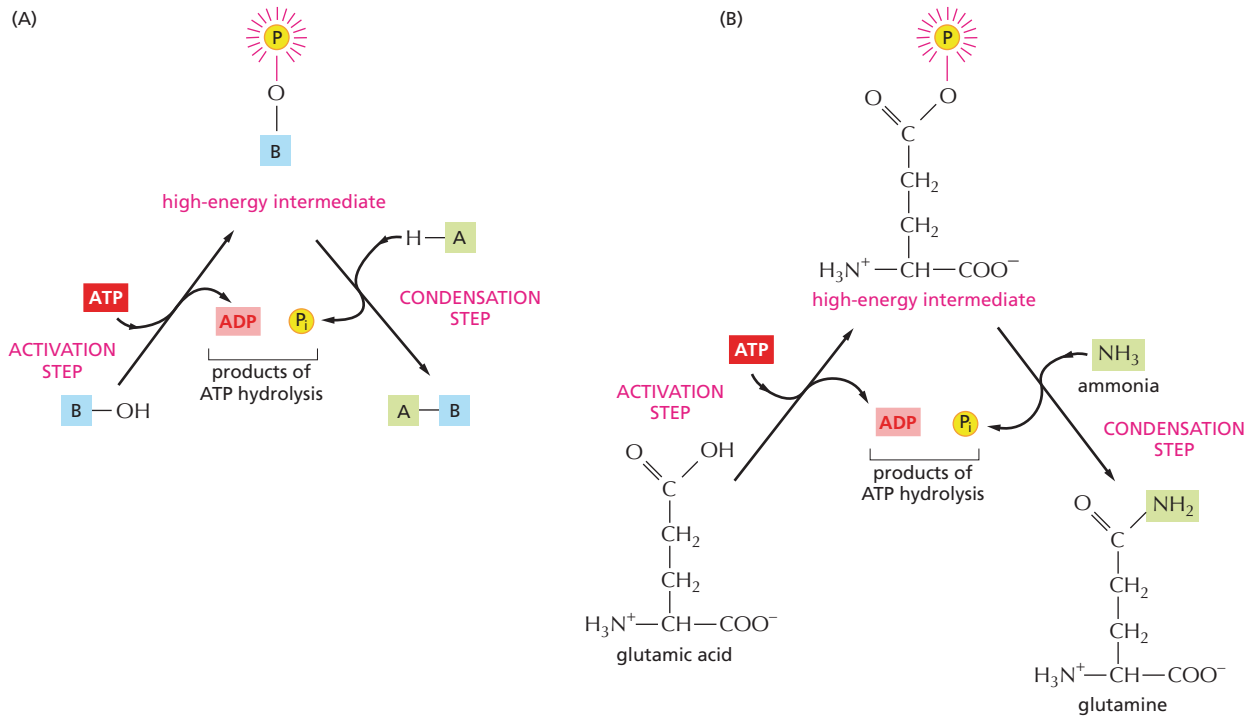


Figure 3–33 An energetically unfavorable biosynthetic reaction can be driven by ATP hydrolysis. (A) Schematic illustration of the formation of A–B in the condensation reaction described in the text. (B) The biosynthesis of the amino acid glutamine from glutamic acid. Glutamic acid is first converted to a high-energy phosphorylated intermediate (corresponding to the compound B–O–PO_3 described in the text), which then reacts with ammonia (corresponding to A–H) to form glutamine. In this example, both steps occur on the surface of the same enzyme, glutamine synthetase (not shown). For clarity, the glutamic acid side chain is shown in its uncharged form. ATP hydrolysis can drive this energetically unfavorable reaction because it yields more energy ($\Delta G°$ of –7.3 kcal/mole) than the energy required for the synthesis of glutamine from glutamic acid plus NH_3 ($\Delta G°$ of +3.4 kcal/mole).

The hydride ion carried by NADPH is given up readily in a subsequent oxidation–reduction reaction, because the ring can achieve a more stable arrangement of electrons without it. In this subsequent reaction, which regenerates $NADP^+$, the NADPH becomes oxidized and the substrate becomes reduced—thus completing the NADPH cycle. NADPH is efficient at donating its hydride ion to other molecules for the same reason that ATP readily transfers a phosphate: in both cases, the transfer is accompanied by a large negative free-energy change. One example of the use of NADPH in biosynthesis is shown in **Figure 3–35**.

NADPH and NADH Have Different Roles in Cells

NADPH and NADH differ in a single phosphate group, which is located far from the region involved in electron transfer in NADPH (Figure 3–34B). Although this phosphate group has no effect on the electron-transfer properties of NADPH compared with NADH, it is nonetheless crucial for their distinctive roles, as it gives NADPH a slightly different shape from NADH. This subtle difference in conformation makes it possible for the two carriers to bind as substrates to different sets of enzymes and thereby deliver electrons (in the form of hydride ions) to different target molecules.

Why should there be this division of labor? The answer lies in the need to regulate two sets of electron-transfer reactions independently. NADPH operates chiefly with enzymes that catalyze anabolic reactions, supplying the high-energy electrons needed to synthesize energy-rich biological molecules. NADH, by contrast, has a special role as an intermediate in

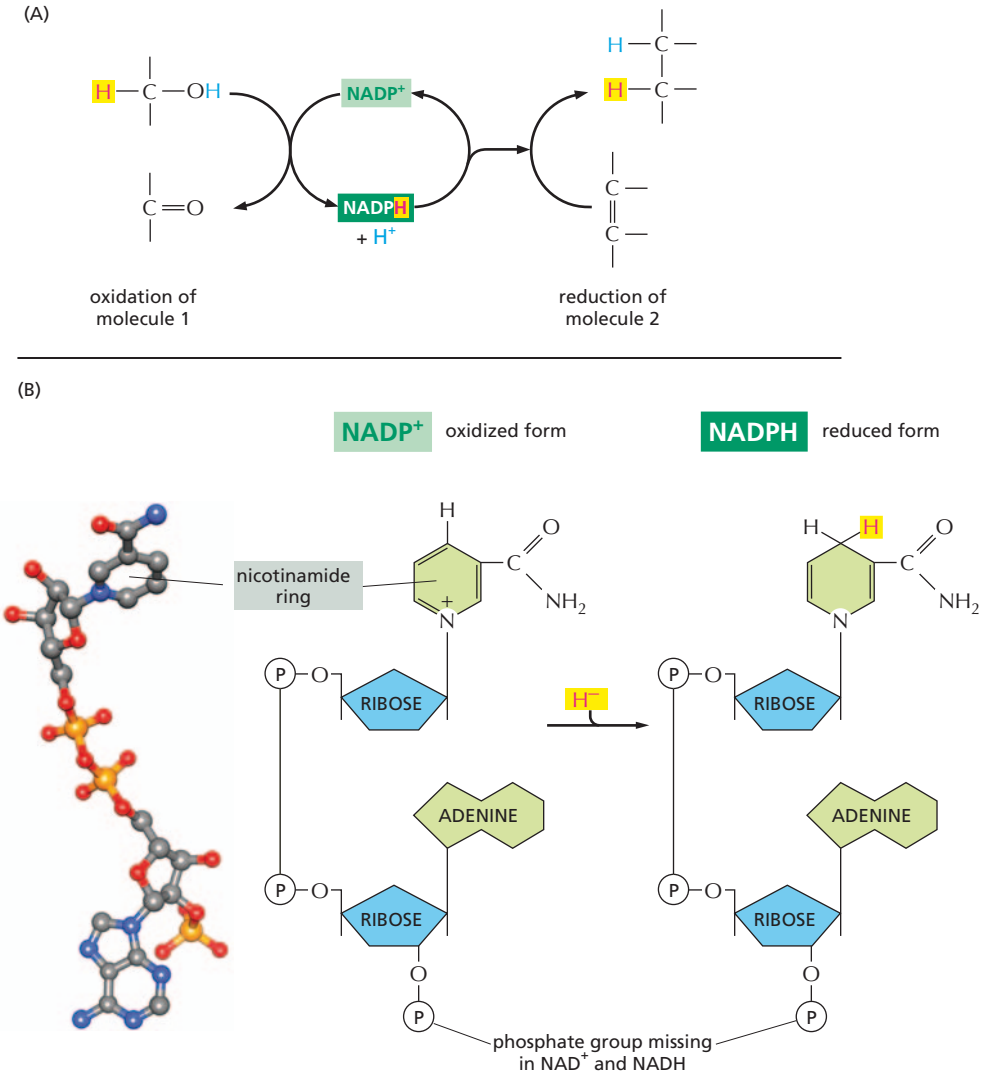


Figure 3–34 NADPH is an activated carrier of electrons. (A) NADPH is produced in reactions of the general type shown on the left, in which two hydrogen atoms are removed from a substrate. The oxidized form of the carrier molecule, $NADP^+$, receives one hydrogen atom plus an electron (a hydride ion), while the proton (H^+) from the other H atom is released into solution. Because NADPH holds its hydride ion in a high-energy linkage, the ion can easily be transferred to other molecules, as shown on the right. (B) The structure of $NADP^+$ and NADPH. On the left is a ball-and-stick model of NADP. The part of the $NADP^+$ molecule known as the nicotinamide ring accepts two electrons, together with a proton (the equivalent of a hydride ion, H^-), forming NADPH. NAD^+ and NADH are identical in structure to $NADP^+$ and NADPH, respectively, except that they lack the phosphate group, as indicated.

the catabolic system of reactions that generate ATP through the oxidation of food molecules, as we discuss in Chapter 13. The genesis of NADH from NAD^+ and that of NADPH from $NADP^+$ occurs by different pathways that are independently regulated, so that the cell can adjust the supply of electrons for these two contrasting purposes. Inside the cell, the ratio of NAD^+ to NADH is kept high, whereas the ratio of $NADP^+$ to NADPH is kept low. This arrangement provides plenty of NAD^+ to act as an oxidizing agent and plenty of NADPH to act as a reducing agent—as required for their special roles in catabolism and anabolism, respectively.

Cells Make Use of Many Other Activated Carriers

In addition to ATP (which transfers a phosphate) and NADPH and NADH (which transfer electrons and hydrogen), cells make use of other activated carriers that pick up and carry a chemical group in an easily transferred, high-energy linkage. *$FADH_2$*, like NADH and NADPH, carries hydrogen and high-energy electrons (see Figure 13–13B). But other important reactions involve the transfers of acetyl, methyl, carboxyl, and glucose groups from activated carriers for the purpose of biosynthesis (**Table 3–2**). Coenzyme A, for example, can carry an acetyl group in a readily transferable linkage. This activated carrier, called **acetyl CoA** (acetyl coenzyme A), is shown in **Figure 3–36**. It is used, for example, to add sequentially two-carbon units in the biosynthesis of the hydrocarbon tails of fatty acids.

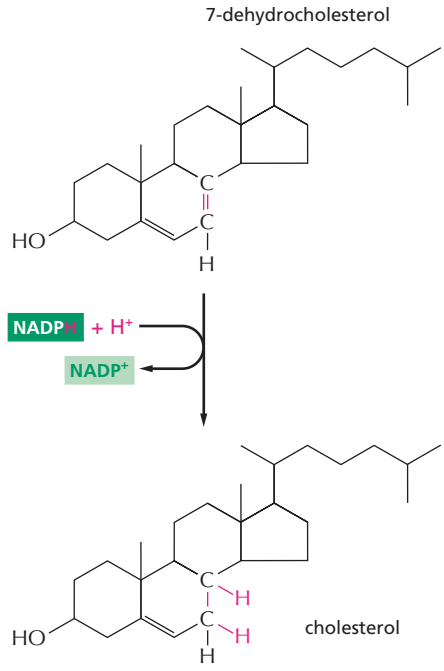


Figure 3–35 NADPH participates in the final stage of one of the biosynthetic routes leading to cholesterol. As in many other biosynthetic reactions, the reduction of the C=C bond is achieved by the transfer of a hydride ion from the activated carrier NADPH, plus a proton (H^+) from solution.

TABLE 3–2 SOME ACTIVATED CARRIERS WIDELY USED IN METABOLISM

Activated Carrier	Group Carried in High-Energy Linkage
ATP	phosphate
NADH, NADPH, $FADH_2$	electrons and hydrogens
Acetyl CoA	acetyl group
Carboxylated biotin	carboxyl group
S-adenosylmethionine	methyl group
Uridine diphosphate glucose	glucose

In acetyl CoA and the other activated carriers in Table 3–2, the transferable group makes up only a small part of the molecule. The rest consists of a large organic portion that serves as a convenient "handle," facilitating the recognition of the carrier molecule by specific enzymes. As with acetyl CoA, this handle portion very often contains a nucleotide. This curious fact may be a relic from an early stage of cell evolution. It is thought that the main catalysts for early life forms on Earth were RNA molecules (or their close relatives) and that proteins were a later evolutionary addition. It is therefore tempting to speculate that many of the activated carriers that we find today originated in an earlier RNA world, where their nucleotide portions would have been useful for binding these carriers to RNA-based catalysts, or *ribozymes* (discussed in Chapter 7).

Activated carriers are usually generated in reactions coupled to ATP hydrolysis, as shown for biotin in **Figure 3–37**. Therefore, the energy that enables their groups to be used for biosynthesis ultimately comes from the catabolic reactions that generate ATP. Similar processes occur in the synthesis of the very large macromolecules—the nucleic acids, proteins, and polysaccharides—which we discuss next.

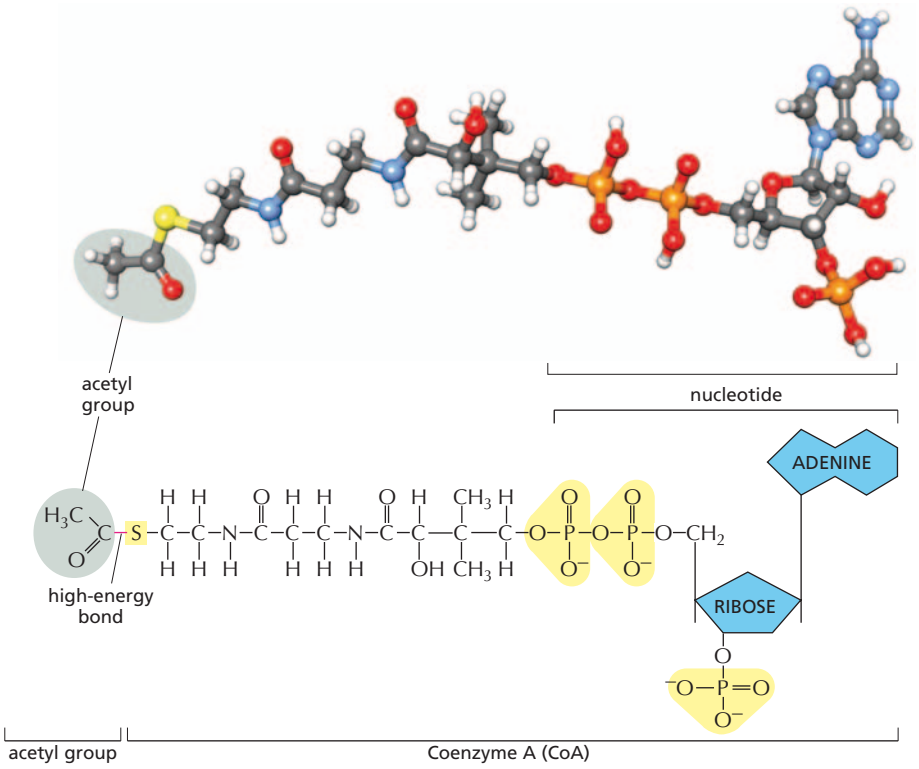


Figure 3–36 Acetyl coenzyme A (CoA) is another important activated carrier. A ball-and-stick model is shown above the structure of acetyl CoA. The sulfur atom (*yellow*) forms a thioester bond to acetate. Because the thioester bond is a high-energy linkage, it releases a large amount of free energy when it is hydrolyzed; thus the acetyl group carried by CoA can be readily transferred to other molecules.

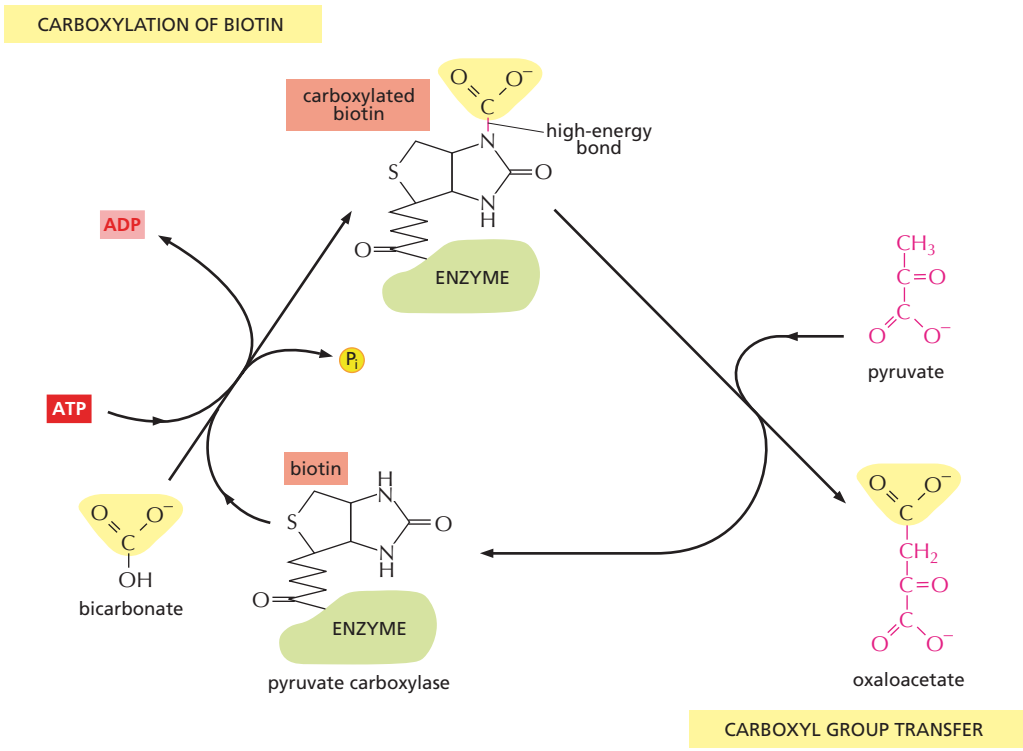


Figure 3–37 An activated carrier transfers a carboxyl group to a substrate. Biotin is a vitamin that is used by a number of enzymes, including *pyruvate carboxylase* shown here. Once it is carboxylated, biotin can transfer a carboxyl group to another molecule. Here, it transfers a carboxyl group to pyruvate, producing oxaloacetate, a molecule needed in the citric acid cycle (discussed in Chapter 13). Other enzymes use biotin to transfer carboxyl groups to other acceptor molecules. Note that the synthesis of carboxylated biotin requires energy derived from ATP hydrolysis—a general feature of many activated carriers.

The Synthesis of Biological Polymers Requires an Energy Input

The macromolecules of the cell constitute the vast majority of its dry mass—that is, the mass not due to water. These molecules are made from *subunits* (or monomers) that are linked together by bonds formed during an enzyme-catalyzed condensation reaction. The reverse reaction—the breakdown of polymers—occurs through enzyme-catalyzed hydrolysis reactions. These hydrolysis reactions are energetically favorable, whereas the corresponding biosynthetic reactions require an energy input and are more complex (**Figure 3–38**).

The nucleic acids (DNA and RNA), proteins, and polysaccharides are all polymers that are produced by the repeated addition of a subunit onto one end of a growing chain. The mode of synthesis of each of these macromolecules is outlined in **Figure 3–39**. As indicated, the condensation step in each case depends on energy provided by the hydrolysis of a nucleoside triphosphate. And yet, except for the nucleic acids, there are no phosphate groups left in the final product molecules. How, then, is the energy of ATP hydrolysis coupled to polymer synthesis?

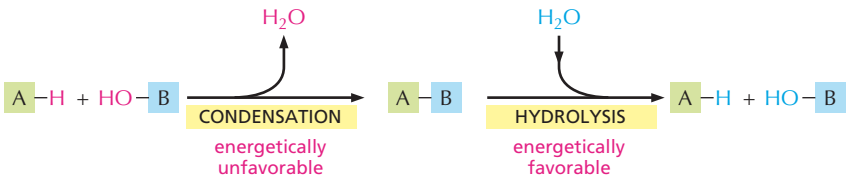


Figure 3–38 In cells, macromolecules are synthesized by condensation reactions and broken down by hydrolysis reactions. Condensation reactions are all energetically unfavorable, whereas hydrolysis reactions are all energetically favorable.

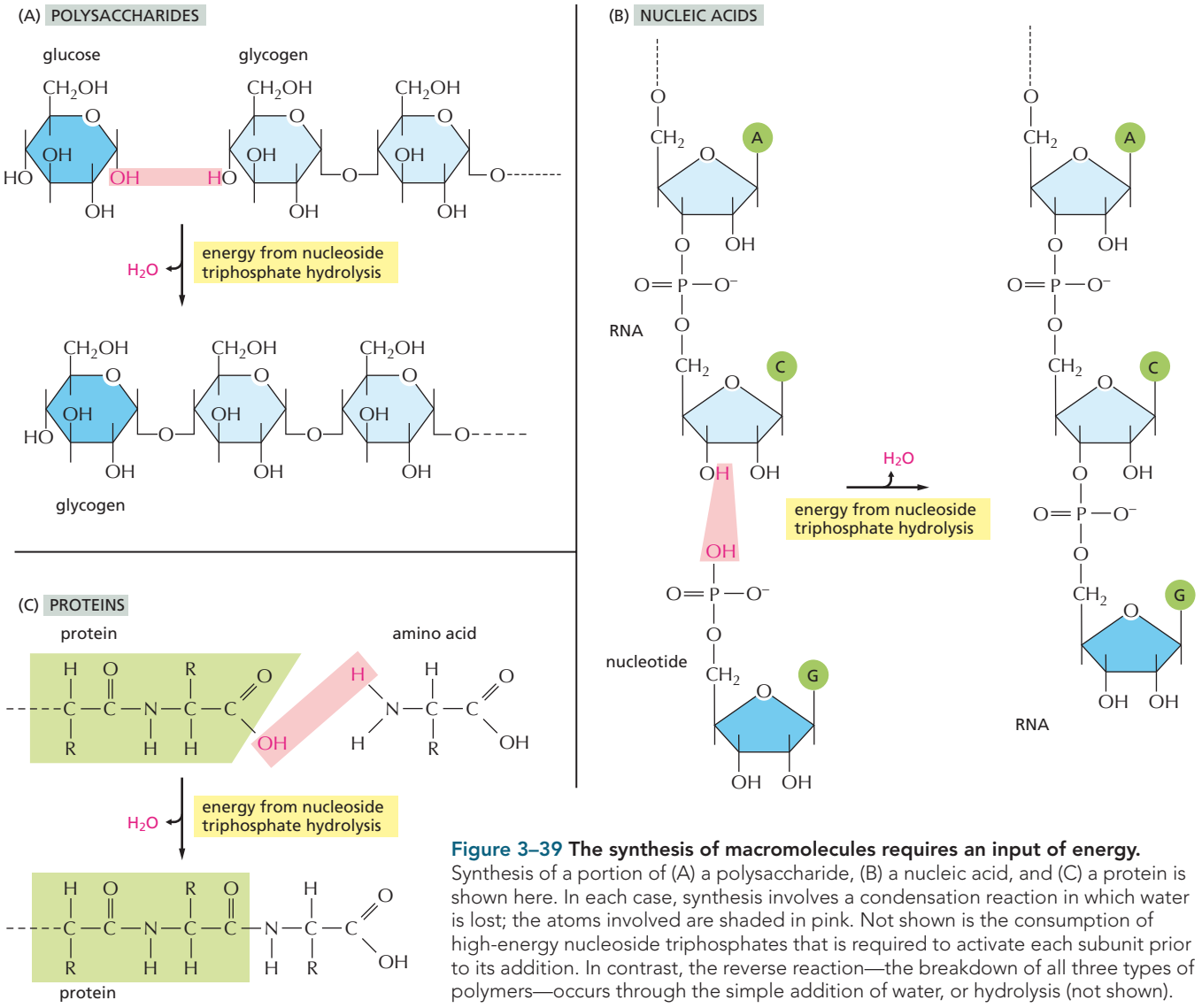


Figure 3–39 The synthesis of macromolecules requires an input of energy. Synthesis of a portion of (A) a polysaccharide, (B) a nucleic acid, and (C) a protein is shown here. In each case, synthesis involves a condensation reaction in which water is lost; the atoms involved are shaded in pink. Not shown is the consumption of high-energy nucleoside triphosphates that is required to activate each subunit prior to its addition. In contrast, the reverse reaction—the breakdown of all three types of polymers—occurs through the simple addition of water, or hydrolysis (not shown).

QUESTION 3–9

Which of the following reactions will occur only if coupled to a second, energetically favorable reaction?

A. glucose + O_2 → CO_2 + H_2O
B. CO_2 + H_2O → glucose + O_2
C. nucleoside triphosphates → DNA
D. nucleotide bases → nucleoside triphosphates
E. ADP + P_i → ATP

For each type of macromolecule, an enzyme-catalyzed pathway exists, which resembles that discussed previously for the synthesis of the amino acid glutamine (see Figure 3–33). The principle is exactly the same, in that the –OH group that will be removed in the condensation reaction is first activated by forming a high-energy linkage to a second molecule. The mechanisms used to link ATP hydrolysis to the synthesis of proteins and polysaccharides, however, are more complex than that used for glutamine synthesis. In the biosynthetic pathways leading to these macromolecules, a series of high-energy intermediates generates the final high-energy bond that is broken during the condensation step (as discussed in Chapter 7 for protein synthesis).

There are limits to what each activated carrier can do in driving biosynthesis. For example, the ΔG for the hydrolysis of ATP to ADP and inorganic phosphate (P_i) depends on the concentrations of all of the reactants, and under the usual conditions in a cell, is between –11 and –13 kcal/mole. In principle, this hydrolysis reaction can be used to drive an unfavorable reaction with a ΔG of, perhaps, +10 kcal/mole, provided that a suitable reaction path is available. For some biosynthetic reactions, however, even –13 kcal/mole may be insufficient. In these cases, the path of ATP

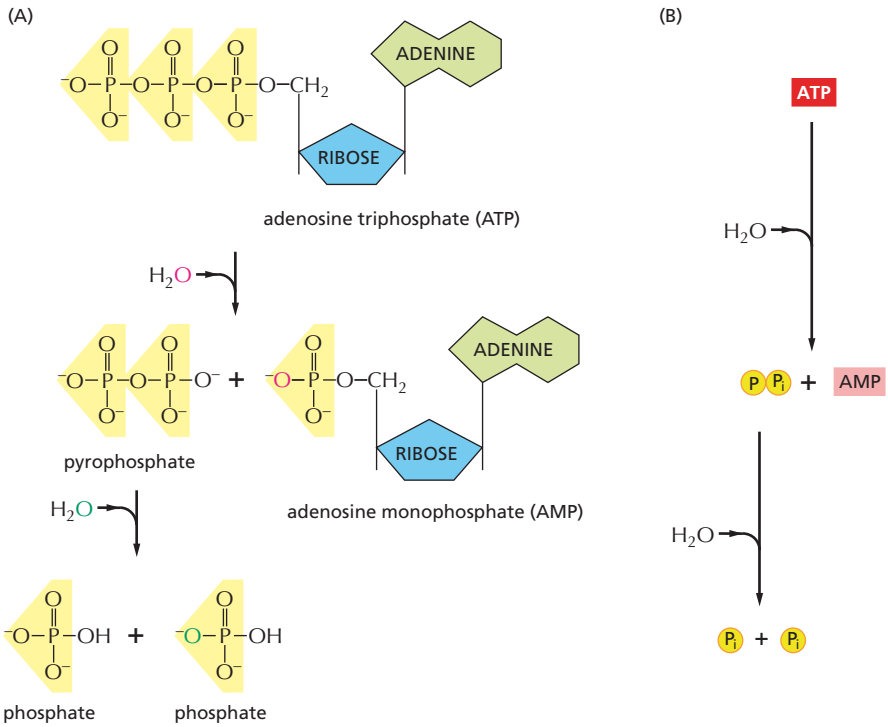


Figure 3–40 In an alternative route for the hydrolysis of ATP, pyrophosphate is first formed and then hydrolyzed in solution. This route releases about twice as much free energy as the reaction shown earlier in Figure 3–31. (A) In each of the two successive hydrolysis reactions, an oxygen atom from the participating water molecule is retained in the products, whereas the hydrogen atoms from water form free hydrogen ions, H^+. (B) The overall reaction shown in summary form.

hydrolysis can be altered so that it initially produces AMP and pyrophosphate (PP_i), which is itself then hydrolyzed in solution in a subsequent step (**Figure 3–40**). The whole process makes available a total ΔG of about –26 kcal/mole. The biosynthetic reaction involved in the synthesis of nucleic acids (polynucleotides) is driven in this way (**Figure 3–41**).

ATP will make many appearances throughout the book as a molecule that powers reactions in the cell. And in Chapters 13 and 14, we discuss how the cell uses the energy from food to generate ATP. In the next chapter, we learn more about the proteins that make such reactions possible.

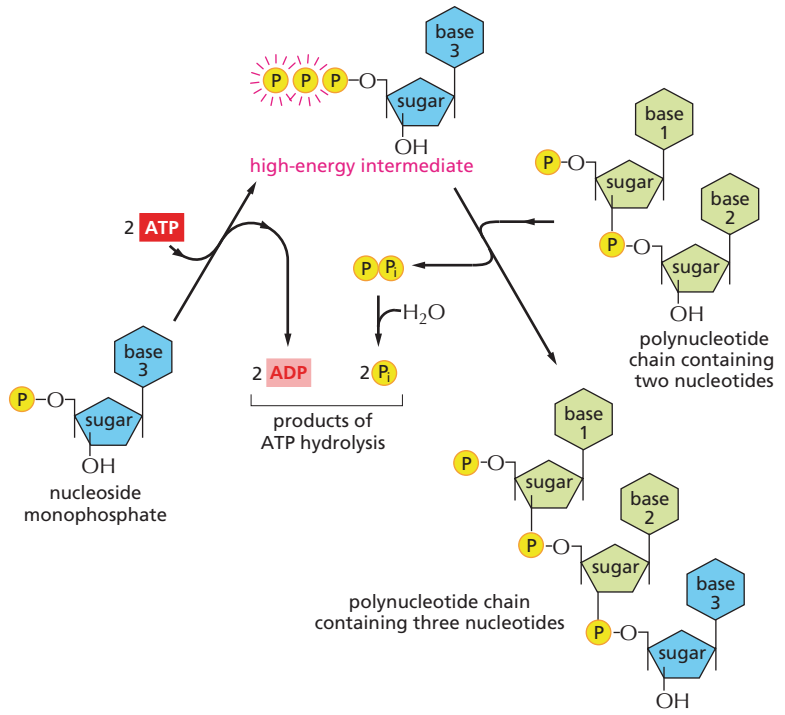


Figure 3–41 Synthesis of a polynucleotide, RNA or DNA, is a multistep process driven by ATP hydrolysis. In the first step, a nucleoside monophosphate is activated by the sequential transfer of the terminal phosphate groups from two ATP molecules. The high-energy intermediate formed—a nucleoside triphosphate—exists free in solution until it reacts with the growing end of an RNA or a DNA chain with release of pyrophosphate. Hydrolysis of the pyrophosphate to inorganic phosphate is highly favorable and helps to drive the overall reaction in the direction of polynucleotide synthesis.

ESSENTIAL CONCEPTS

- Living organisms are able to exist because of a continual input of energy. Part of this energy is used to carry out essential reactions that support cell metabolism, growth, movement, and reproduction; the remainder is lost in the form of heat.
- The ultimate source of energy for most living organisms is the sun. Plants, algae, and photosynthetic bacteria use solar energy to produce organic molecules from carbon dioxide. Animals obtain food by eating plants or by eating animals that feed on plants.
- Each of the many hundreds of chemical reactions that occur in a cell is specifically catalyzed by an enzyme. Large numbers of different enzymes work in sequence to form chains of reactions, called metabolic pathways, each performing a different function in the cell.
- Catabolic reactions release energy by breaking down organic molecules, including foods, through oxidative pathways. Anabolic reactions generate the many complex organic molecules needed by the cell, and they require an energy input. In animal cells, both the building blocks and the energy required for the anabolic reactions are obtained through catabolic reactions.
- Enzymes catalyze reactions by binding to particular substrate molecules in a way that lowers the activation energy required for making and breaking specific covalent bonds.
- The rate at which an enzyme catalyzes a reaction depends on how rapidly it finds its substrates and how quickly the product forms and then diffuses away. These rates vary widely from one enzyme to another.
- The only chemical reactions possible are those that increase the total amount of disorder in the universe. The free-energy change for a reaction, ΔG, measures this disorder, and it must be less than zero for a reaction to proceed spontaneously.
- The ΔG for a chemical reaction depends on the concentrations of the reacting molecules, and it may be calculated from these concentrations if the equilibrium constant (K) of the reaction (or the standard free-energy change, $\Delta G°$, for the reactants) is known.
- Equilibrium constants govern all of the associations (and dissociations) that occur between macromolecules and small molecules in the cell. The larger the binding energy between two molecules, the larger the equilibrium constant and the more likely that these molecules will be found bound to each other.
- By creating a reaction pathway that couples an energetically favorable reaction to an energetically unfavorable one, enzymes can make otherwise impossible chemical transformations occur.
- A small set of activated carriers, particularly ATP, NADH, and NADPH, plays a central part in these coupled reactions in cells. ATP carries high-energy phosphate groups, whereas NADH and NADPH carry high-energy electrons.
- Food molecules provide the carbon skeletons for the formation of macromolecules. The covalent bonds of these larger molecules are produced by condensation reactions that are coupled to energetically favorable bond changes in activated carriers such as ATP and NADPH.

KEY TERMS

acetyl CoA
activated carrier
activation energy
ADP, ATP
anabolism
biosynthesis
catabolism
catalysis
catalyst
condensation reaction
coupled reaction
diffusion
entropy
enzyme
equilibrium
equilibrium constant, K
free energy, G
free-energy change, ΔG
hydrolysis
K_M
metabolism
Michaelis constant (K_M)
NAD^+, NADH
$NADP^+$, NADPH
oxidation
photosynthesis
reduction
respiration
standard free-energy change, $\Delta G°$
substrate
turnover number
V_{max}

QUESTIONS

QUESTION 3–10

Which of the following statements are correct? Explain your answers.

A. Some enzyme-catalyzed reactions cease completely if their enzyme is absent.

B. High-energy electrons (such as those found in the activated carriers NADH and NADPH) move faster around the atomic nucleus.

C. Hydrolysis of ATP to AMP can provide about twice as much energy as hydrolysis of ATP to ADP.

D. A partially oxidized carbon atom has a somewhat smaller diameter than a more reduced one.

E. Some activated carrier molecules can transfer both energy and a chemical group to a second molecule.

F. The rule that oxidations release energy, whereas reductions require energy input, applies to all chemical reactions, not just those that occur in living cells.

G. Cold-blooded animals have an energetic disadvantage because they release less heat to the environment than warm-blooded animals do. This slows their ability to make ordered macromolecules.

H. Linking the reaction X → Y to a second, energetically favorable reaction Y → Z will shift the equilibrium constant of the first reaction.

QUESTION 3–11

Consider a transition of X → Y. Assume that the only difference between X and Y is the presence of three hydrogen bonds in Y that are absent in X. What is the ratio of X to Y when the reaction is in equilibrium? Approximate your answer by using Table 3–1 (p. 98), with 1 kcal/mole as the energy of each hydrogen bond. If Y instead has six hydrogen bonds that distinguish it from X, how would that change the ratio?

QUESTION 3–12

Protein A binds to protein B to form a complex, AB. At equilibrium in a cell the concentrations of A, B, and AB are all at 1 μM.

A. Referring to Figure 3–19, calculate the equilibrium constant for the reaction A + B ⇌ AB.

B. What would the equilibrium constant be if A, B, and AB were each present in equilibrium at the much lower concentrations of 1 nM each?

C. How many extra hydrogen bonds would be needed to hold A and B together at this lower concentration so that a similar proportion of the molecules are found in the AB complex? (Remember that each hydrogen bond contributes about 1 kcal/mole.)

QUESTION 3–13

Discuss the following statement: "Whether the ΔG for a reaction is larger, smaller, or the same as $\Delta G°$ depends on the concentration of the compounds that participate in the reaction."

QUESTION 3–14

A. How many ATP molecules could maximally be generated from one molecule of glucose, if the complete oxidation of 1 mole of glucose to CO_2 and H_2O yields 686 kcal of free energy and the useful chemical energy available in the high-energy phosphate bond of 1 mole of ATP is 12 kcal?

B. As we will see in Chapter 14 (Table 14–1), respiration produces 30 moles of ATP from 1 mole of glucose. Compare this number with your answer in part (A). What is the overall efficiency of ATP production from glucose?

C. If the cells of your body oxidize 1 mole of glucose, by how much would the temperature of your body (assume that your body consists of 75 kg of water) increase if the heat were not dissipated into the environment? [Recall that a kilocalorie (kcal) is defined as that amount of energy that heats 1 kg of water by 1°C.]

D. What would the consequences be if the cells of your body could convert the energy in food substances with only 20% efficiency? Would your body—as it is presently constructed—work just fine, overheat, or freeze?

E. A resting human hydrolyzes about 40 kg of ATP every 24 hours. The oxidation of how much glucose would produce this amount of energy? (Hint: Look up the structure of ATP in Figure 2–24 to calculate its molecular weight; the atomic weights of H, C, N, O, and P are 1, 12, 14, 16, and 31, respectively.)

QUESTION 3–15

A prominent scientist claims to have isolated mutant cells that can convert 1 molecule of glucose into 57 molecules of ATP. Should this discovery be celebrated, or do you suppose that something might be wrong with it? Explain your answer.

QUESTION 3–16

In a simple reaction A $\rightleftharpoons$ A*, a molecule is interconvertible between two forms that differ in standard free energy $G°$ by 4.3 kcal/mole, with A* having the higher $G°$.

A. Use Table 3–1 (p. 98) to find how many more molecules will be in state A* compared with state A at equilibrium.

B. If an enzyme lowered the activation energy of the reaction by 2.8 kcal/mole, how would the ratio of A to A* change?

QUESTION 3–17

A reaction in a single-step biosynthetic pathway that converts a metabolite into a particularly vicious poison (metabolite $\rightleftharpoons$ poison) in a mushroom is energetically highly unfavorable. The reaction is normally driven by ATP hydrolysis. Assume that a mutation in the enzyme that catalyzes the reaction prevents it from utilizing ATP, but still allows it to catalyze the reaction.

A. Do you suppose it might be safe for you to eat a mushroom that bears this mutation? Base your answer on an estimation of how much less poison the mutant mushroom would produce, assuming the reaction is in equilibrium and most of the energy stored in ATP is used to drive the unfavorable reaction in nonmutant mushrooms.

B. Would your answer be different for another mutant mushroom whose enzyme couples the reaction to ATP hydrolysis but works 100 times more slowly?

QUESTION 3–18

Consider the effects of two enzymes, A and B. Enzyme A catalyzes the reaction

$$ATP + GDP \rightleftharpoons ADP + GTP$$

and enzyme B catalyzes the reaction

$$NADH + NADP^+ \rightleftharpoons NAD^+ + NADPH$$

Discuss whether the enzymes would be beneficial or detrimental to cells.

QUESTION 3–19

Discuss the following statement: "Enzymes and heat are alike in that both can speed up reactions that—although thermodynamically feasible—do not occur at an appreciable rate because they require a high activation energy. Diseases that seem to benefit from the careful application of heat—in the form of hot chicken soup, for example—are therefore likely to be due to the insufficient function of an enzyme."

QUESTION 3–20

The curve shown in Figure 3–24 is described by the Michaelis–Menten equation:

$$\text{rate } (v) = V_{max}\,[S]/([S] + K_M)$$

Can you convince yourself that the features qualitatively described in the text are accurately represented by this equation? In particular, how can the equation be simplified when the substrate concentration [S] is in one of the following ranges: (A) [S] is much smaller than the K_M, (B) [S] equals the K_M, and (C) [S] is much larger than the K_M?

QUESTION 3–21

The rate of a simple enzyme reaction is given by the standard Michaelis–Menten equation:

$$\text{rate} = V_{max}\,[S]/([S] + K_M)$$

If the V_{max} of an enzyme is 100 μmole/sec and the K_M is 1 mM, at what substrate concentration is the rate 50 μmole/sec? Plot a graph of rate versus substrate (S) concentration for [S] = 0 to 10 mM. Convert this to a plot of 1/rate versus 1/[S]. Why is the latter plot a straight line?

QUESTION 3–22

Select the correct options in the following and explain your choices. If [S] is much smaller than K_M, the active site of the enzyme is mostly occupied/unoccupied. If [S] is very much greater than K_M, the reaction rate is limited by the enzyme/substrate concentration.

QUESTION 3–23

A. The reaction rates of the reaction S → P catalyzed by enzyme E were determined under conditions such that only very little product was formed. The following data were measured:

Substrate concentration (μM)	Reaction rate (μmole/min)
0.08	0.15
0.12	0.21
0.54	0.7
1.23	1.1
1.82	1.3
2.72	1.5
4.94	1.7
10.00	1.8

Plot the above data as a graph. Use this graph to estimate the K_M and the V_{max} for this enzyme.

B. Recall from the How We Know essay (pp. 104–106) that to determine these values more precisely, a trick is generally used in which the Michaelis–Menten equation is transformed so that it is possible to plot the data as a straight line. A simple rearrangement yields

$$1/\text{rate} = (K_M/V_{max})\,(1/[S]) + 1/V_{max}$$

which is an equation of the form $y = ax + b$. Calculate 1/rate and 1/[S] for the data given in part (A) and then plot 1/rate versus 1/[S] as a new graph. Determine K_M and V_{max} from the intercept of the line with the axis, where 1/[S] = 0, combined with the slope of the line. Do your results agree with the estimates made from the first graph of the raw data?

C. It is stated in part (A) that only very little product was formed under the reaction conditions. Why is this important?

D. Assume the enzyme is regulated such that upon phosphorylation its K_M increases by a factor of 3 without changing its V_{max}. Is this an activation or inhibition? Plot the data you would expect for the phosphorylated enzyme in both the graph for (A) and the graph for (B).

Chapter 3

ANSWER 3–1 The equation represents the "bottom line" of photosynthesis, which occurs as a large set of individual reactions that are catalyzed by many individual enzymes. Because sugars are more complicated molecules than CO_2 and H_2O, the reaction generates a more ordered state inside the cell. As demanded by the second law of thermodynamics, this increase in order must be accompanied by a greater increase in disorder, which occurs because heat is generated at many steps on the long pathway leading to the products summarized in this equation.

ANSWER 3–2 Oxidation is defined as removal of electrons, and reduction represents a gain of electrons. Therefore, (A) is an oxidation, and (B) is a reduction. The *red* carbon atom in (C) remains largely unchanged; the neighboring carbon atom, however, loses a hydrogen atom (i.e., an electron and a proton) and hence becomes oxidized. The *red* carbon atom in (D) becomes oxidized because it loses a hydrogen atom, whereas the *red* carbon atom in (E) becomes reduced because it gains a hydrogen atom.

ANSWER 3–3

A. Both states of the coin, H and T, have an equal probability. There is therefore no driving force, i.e., no energy difference, that would favor H turning to T or vice versa. Therefore, $\Delta G° = 0$ for this reaction. However, a reaction proceeds if H and T coins are not present in the box in equal numbers. In this case, the concentration difference between H and T creates a driving force and $\Delta G \neq 0$; when the reaction reaches equilibrium—i.e., when there are equal numbers of H and T—$\Delta G = 0$.

B. The amount of shaking corresponds to the temperature, as it results in the "thermal" motion of the coins. The activation energy of the reaction is the energy that needs to be expended to flip the coin, i.e., to stand it on its rim, from where it can fall back facing either side up. Jigglase would speed up the flipping by lowering the energy required for this; it could, for example, be a magnet that is suspended above the box and helps lift the coins. Jigglase would not affect where the equilibrium lies (at an equal number of H and T), but it would speed up the process of reaching the equilibrium, because in the presence of jigglase more coins would flip back and forth.

ANSWER 3–4 See **Figure A3–4**. Note that $\Delta G°_{X \rightarrow Y}$ is positive, whereas $\Delta G°_{Y \rightarrow Z}$ and $\Delta G°_{X \rightarrow Z}$ are negative. The graph also shows that $\Delta G°_{X \rightarrow Z} = \Delta G°_{X \rightarrow Y} + \Delta G°_{Y \rightarrow Z}$. We do not know from the information given in Figure 3–12 how high the activation energy barriers are; they are therefore drawn to an arbitrary height (solid lines). The activation energies would be lowered by enzymes that catalyze these reactions, thereby speeding up the reaction rates (dotted lines), but the enzymes would not change the $\Delta G°$ values.

ANSWER 3–5 The reaction rates might be limited by (1) the concentration of the substrate, i.e., how often a molecule of CO_2 collides with the active site on the enzyme; (2) how many of these collisions are energetic enough to lead to a reaction; and (3) how fast the enzyme can release

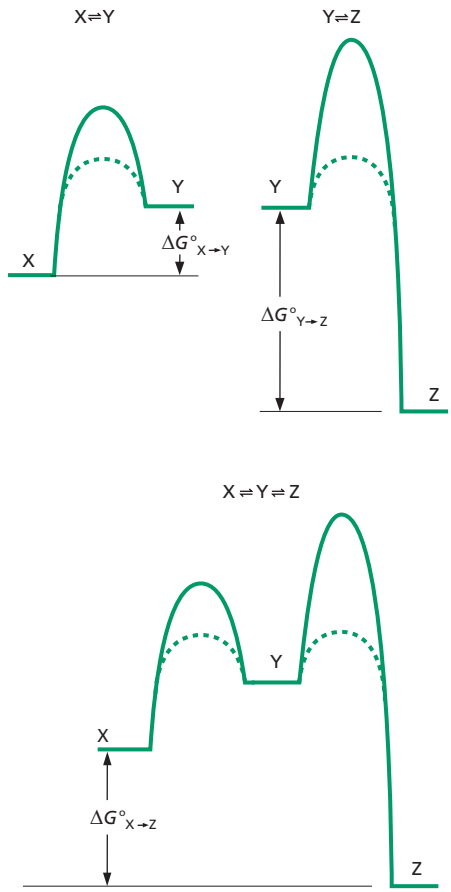


Figure A3–4

the products of the reaction and therefore be free to bind more CO_2. The diagram in **Figure A3–5** shows that the enzyme lowers the activation energy barrier, so that more CO_2 molecules have sufficient energy to undergo the reaction. The area under the curve from point A to infinite energy or from point B to infinite energy indicates the total number of molecules that will react without or with the enzyme, respectively. Although not drawn to scale, the ratio of these two areas should be 10^7.

ANSWER 3–6 All reactions are reversible. If the compound AB can dissociate to produce A and B, then it must also be possible for A and B to associate to form AB. Which of the two reactions predominates depends on the equilibrium constant of the reaction and the concentration of A, B, and AB (as discussed in Figure 3–19). Presumably, when this enzyme was isolated its activity was detected by

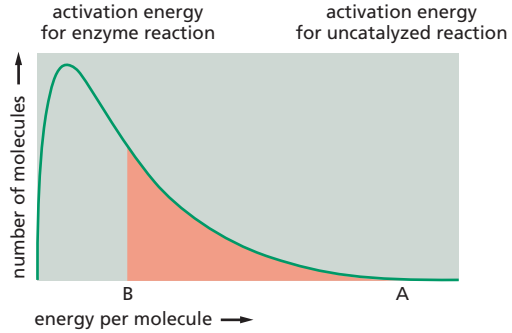


Figure A3–5

supplying A and B in relatively large amounts and measuring the amount of AB generated. But suppose, however, that in the cell there is a large concentration of AB, in which case the enzyme would actually catalyze AB → A + B. (This question is based on an actual example in which an enzyme was isolated and named according to the reaction in one direction, but was later shown to catalyze the reverse reaction in living cells.)

ANSWER 3–7

A. The rocks in Figure 3–30B provide the energy to lift the bucket of water. In the reaction X + ATP → Y + ADP + P_i, ATP hydrolysis is driving the reaction; thus ATP corresponds to the rocks on top of the cliff. The broken debris in Figure 3–30B corresponds to ADP and P_i, the products of ATP hydrolysis. In the reaction, ATP hydrolysis is coupled to the conversion of X to Y. X, therefore, is the starting material, the bucket on the ground, which is converted to Y, the bucket at its highest point.

B. (i) The rock hitting the ground would be the futile hydrolysis of ATP—for example, in the absence of an enzyme that uses the energy released by the ATP hydrolysis to drive an otherwise unfavorable reaction; in this case, the energy stored in the phosphoanhydride bond of ATP would be lost as heat. (ii) The energy stored in Y could be used to drive another reaction. If Y represented the activated form of amino acid X, for example, it could undergo a condensation reaction to form a peptide bond during protein synthesis.

ANSWER 3–8 The free energy ΔG derived from ATP hydrolysis depends on both the $\Delta G°$ and the concentrations of the substrate and products. For example, for a particular set of concentrations, one might have

$$\Delta G = -12 \text{ kcal/mole} = -7.3 \text{ kcal/mole} + 0.616 \ln \frac{[\text{ADP}] \times [P_i]}{[\text{ATP}]}$$

ΔG is smaller than $\Delta G°$, largely because the ATP concentration in cells is high (in the millimolar range) and the ADP concentration is low (in the 10 μM range). The concentration term of this equation is therefore smaller than 1 and its logarithm is a negative number.

$\Delta G°$ is a constant for the reaction and will not vary with reaction conditions. ΔG, in contrast, depends on the concentrations of ATP, ADP, and phosphate, which can be somewhat different between cells.

ANSWER 3–9 Reactions B, D, and E all require coupling to other, energetically favorable reactions. In each case, higher-order structures are formed that are more complicated and have higher-energy bonds than the starting materials. In contrast, reaction A is a catabolic reaction that leads to compounds in a lower energy state and will occur spontaneously. The nucleoside triphosphates in reaction C contain enough energy to drive DNA synthesis (see Figure 3–41).

ANSWER 3–10

A. Nearly true, but strictly speaking, false. Because enzymes enhance the rate but do not change the equilibrium point of a reaction, a reaction will always occur in the absence of the enzyme, though often at a minuscule rate. Moreover, competing reactions may use up the substrate more quickly, thus further impeding the desired reaction. Thus, in practical terms, without an enzyme, some reactions may never occur to an appreciable extent.

B. False. High-energy electrons are more easily transferred, i.e., more loosely bound to the donor molecule. This does not mean that they move any faster.

C. True. Hydrolysis of an ATP molecule to form AMP also produces a pyrophosphate (PP_i) molecule, which in turn is hydrolyzed into two phosphate molecules. This second reaction releases almost the same amount of energy as the initial hydrolysis of ATP, thereby approximately doubling the energy total yield.

D. True. Oxidation is the removal of electrons, which reduces the diameter of the carbon atom.

E. True. ATP, for example, can donate both chemical-bond energy and a phosphate group.

F. False. Living cells have a particular kind of chemistry in which most oxidations are energy-releasing events; under different conditions, however, such as in a hydrogen-containing atmosphere, reductions would be energy-releasing events.

G. False. All cells, including those of cold- and warm-blooded animals, radiate comparable amounts of heat as a consequence of their metabolic reactions. For bacterial cells, for example, this becomes apparent when a compost pile heats up.

H. False. The equilibrium constant of the reaction X ↔ Y remains unchanged. If Y is removed by a second reaction, more X is converted to Y so that the ratio of X to Y remains constant.

ANSWER 3–11 The free-energy difference ($\Delta G°$) between Y and X due to three hydrogen bonds is –3 kcal/mole. (Note that the free energy of Y is lower than that of X, because energy would need to be expended to break the bonds to convert Y to X. The value for $\Delta G°$ for the transition X → Y is therefore negative.) The equilibrium constant for the reaction is therefore about 100 (from Table 3–1, p. 98); i.e., there are about 100 times more molecules of Y than of X at equilibrium. An additional three hydrogen bonds would increase $\Delta G°$ to –6 kcal/mole and increase the equilibrium constant about another 100-fold to 10^4. Thus, relatively small differences in energy can have a major effect on equilibria.

ANSWER 3–12

A. The equilibrium constant is defined as $K = [\text{AB}]/([\text{A}] \times [\text{B}])$. The square brackets indicate the concentration. Thus, if A, B, and AB are each 1 μM (10^{-6} M), K will be 10^6 liters/mole [= $10^{-6}/(10^{-6} \times 10^{-6})$].

B. Similarly, if A, B, and AB are each 1 nM (10^{-9} M), then K will be 10^9 liters/mole.

C. This example illustrates that interacting proteins that are present in cells in lower concentrations need to bind to each other with higher affinities so that a significant fraction of the molecules are bound at equilibrium. In this particular case, lowering the concentration by 1000-fold (from μM to nM) requires an increase in the equilibrium constant by 1000-fold to maintain the AB protein complex in the same proportion (corresponding to –4.3 kcal of free energy; see Table 3–1). This corresponds to about four or five extra hydrogen bonds.

ANSWER 3–13 The statement is correct. The criterion for whether a reaction proceeds spontaneously is ΔG, not $\Delta G°$,

and takes the concentrations of the reacting components into account. A reaction with a negative $\Delta G°$, for example, would not proceed spontaneously under conditions where there is a large enough excess of products, i.e., more than at equilibrium. Conversely, a reaction with a positive $\Delta G°$ might spontaneously go forward under conditions where there is a huge excess of substrate.

ANSWER 3–14

A. A maximum of 57 ATP molecules (= 686/12) corresponds to the total energy released by the complete oxidation of glucose to CO_2 and H_2O.
B. The overall efficiency of ATP production would be about 53%, calculated as the ratio of actually produced ATP molecules (30) divided by the number of ATP molecules that could be obtained if all the energy stored in a glucose molecule could be harvested as chemical energy in ATP (57).
C. During the oxidation of 1 mole of glucose, 322 kcal (the remaining 47% of the available 686 kcal in one mole of glucose that is not stored as chemical energy in ATP) would be released as heat. This amount of energy would heat your body by 4.3°C (= 322 kcal/75 kg). This is a significant amount of heat, considering that 4°C of elevated temperature would be a quite incapacitating fever and that 1 mole (180 g) of glucose is no more than two cups of sugar.
D. If the energy yield were only 20%, then instead of 47% in the example above, 80% of the available energy would be released as heat and would need to be dissipated by your body. The heat production would be more than 1.7-fold higher than normal, and your body would certainly overheat.
E. The chemical formula of ATP is $C_{10}H_{12}O_{13}N_5P_3$, and its molecular weight is therefore 503 g/mole. Your resting body therefore hydrolyzes about 80 moles (= 40 kg/0.503 kg/mole) of ATP in 24 hours (this corresponds to about 1000 kcal of liberated chemical energy). Because every mole of glucose yields 30 moles of ATP, this amount of energy could be produced by oxidation of 480 g glucose (= 180 g/mole × 80 moles/30).

ANSWER 3–15 This scientist is definitely a fake. The 57 ATP molecules would store 684 kcal (= 57 × 12 kcal) of chemical energy, which implies that the efficiency of ATP production from glucose would have been greater than 99%. This impossible degree of efficiency would leave virtually no energy to be released as heat, and this release is required according to the laws of thermodynamics.

ANSWER 3–16

A. From Table 3–1 (p. 98) we know that a free-energy difference of 4.3 kcal/mole corresponds to an equilibrium constant of 10^{-3}, i.e., $[A^*]/[A] = 10^{-3}$. The concentration of A* is therefore 1000-fold lower than that of A at equilibrium.
B. The ratio of A to A* would be unchanged. Lowering the activation energy barrier with an enzyme would accelerate the rate of the reaction, i.e., it would allow more molecules in a given time period to convert from A → A* and from A* → A, but it would not affect the ratio of A → A* at equilibrium.

ANSWER 3–17

A. The mutant mushroom would probably be safe to eat. ATP hydrolysis can provide approximately –12 kcal/mole of energy. This amount of energy shifts the equilibrium point of a reaction by an enormous factor: about 10^8-fold (from Table 3–1, p. 98, we see that –5.7 kcal/mole corresponds to an equilibrium constant of 10^4; thus, –12 kcal/mole corresponds to about 10^8. Note that, for coupled reactions, energies are additive, whereas equilibrium constants are multiplied). Therefore, if the energy of ATP hydrolysis cannot be utilized by the enzyme, 10^8-fold less poison is made. This example illustrates that coupling a reaction to the hydrolysis of an activated carrier molecule can shift the equilibrium point drastically.
B. It would be risky to consume this mutant mushroom. Slowing down the reaction rate would not affect its equilibrium point, and if the reaction were allowed to proceed for a long enough time, the mushroom would likely be loaded with poison. It is possible that the reaction would not reach equilibrium, but it would not be advisable to take a chance.

ANSWER 3–18 Enzyme A is beneficial. It allows the interconversion of two energy-carrier molecules, both of which are required as the triphosphate form for many metabolic reactions. Any ADP that is formed is quickly converted to ATP, and thus the cell maintains a high ATP/ADP ratio. Because of enzyme A, called nucleotide phosphokinase, some of the ATP is used to keep the GTP/GDP ratio similarly high.

Enzyme B would be highly detrimental to the cell. Cells use NAD^+ as an electron acceptor in catabolic reactions and must maintain high concentrations of this form of the carrier as it is used in reactions that break down glucose to make ATP. In contrast, NADPH is used as an electron donor in biosynthetic reactions and is kept at high concentration in the cells so as to allow the synthesis of nucleotides, fatty acids, and other essential molecules. Since enzyme B would deplete the cell's reserves of both NAD^+ and NADPH, it would decrease the rates of both catabolic and biosynthetic reactions.

ANSWER 3–19 Because enzymes are catalysts, enzyme reactions have to be thermodynamically feasible; the enzyme only lowers the activation energy barrier that otherwise slows the rate with which the reaction occurs. Heat confers more kinetic energy to substrates so that a higher fraction of them can surmount the normal activation energy barrier. Many substrates, however, have many different ways in which they could react, and all of these potential pathways will be enhanced by heat. An enzyme, by contrast, acts selectively to facilitate only one particular pathway that, in evolution, was selected to be useful for the cell. Heat, therefore, cannot substitute for enzyme function, and chicken soup must exert its claimed beneficial effects by other mechanisms, which remain to be discovered.

ANSWER 3–20

A. When $[S] << K_M$, the term $([S] + K_M)$ approaches K_M. Therefore, the equation is simplified to rate = $V_{max}[S]/K_M$. Therefore, the rate is proportional to [S].
B. When $[S] = K_M$, the term $[S]/([S] + K_M)$ equals ½. Therefore, the reaction rate is half of the maximal rate V_{max}.

C. If [S] >> K_M, the term ([S] + K_M) approaches [S]. Therefore, [S]/([S] + K_M) equals 1 and the reaction occurs at its maximal rate V_{max}.

ANSWER 3–21 The substrate concentration is 1 mM. This value can be obtained by substituting values in the equation, but it is simpler to note that the desired rate (50 μmole/sec) is exactly half of the maximum rate, V_{max}, where the substrate concentration is typically equal to the K_M. The two plots requested are shown in **Figure A3–21**. A plot of 1/rate versus 1/[S] is a straight line because rearranging the standard equation yields the equation listed in Question 3–23B.

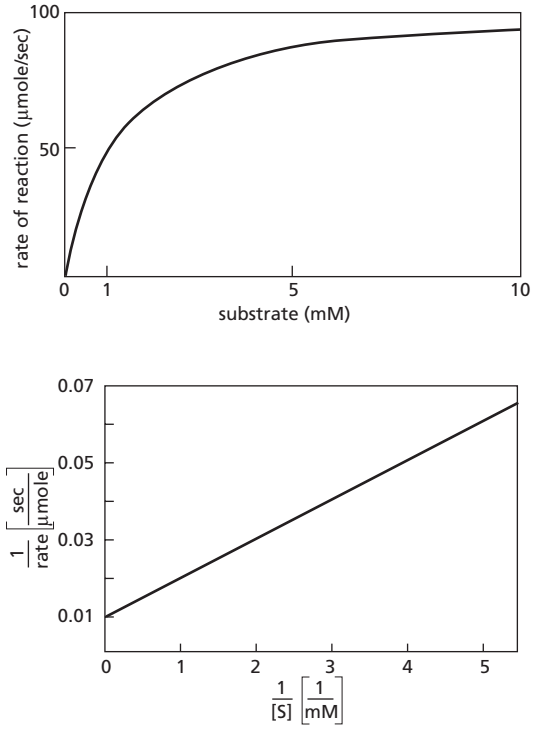


Figure A3–21

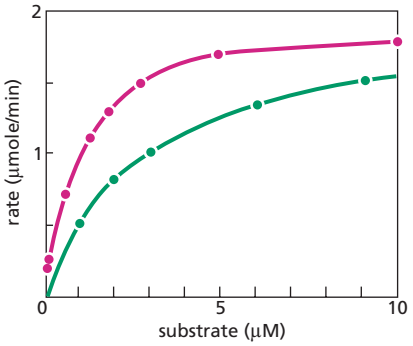
ANSWER 3–22 If [S] is very much smaller than K_M, the active site of the enzyme is mostly unoccupied. If [S] is very much greater than K_M, the reaction rate is limited by the enzyme concentration (because most of the catalytic sites are fully occupied).

ANSWER 3–23

A,B. The data in the boxes have been used to plot the red curve and red line in **Figure A3–23**. From the plotted data, the K_M is 1 μM and the V_{max} is 2 μmole/min. Note that the data are much easier to interpret in the linear plot, because the curve in (A) approaches, but never reaches, V_{max}.

C. It is important that only a small quantity of product is made, because otherwise the rate of reaction would decrease as the substrate was depleted and product accumulated. Thus the measured rates would be lower than they should be.

D. If the K_M increases, then the concentration of substrate needed to give a half-maximal rate is increased. As more substrate is needed to produce the same rate, the enzyme-catalyzed reaction has been inhibited by the phosphorylation. The expected data plots for the phosphorylated enzyme are the green curve and the green line in Figure A3–23.



DATA FOR A AND B

[S] (μM)	1/[S] [1/μM]	rate (μmole/min)	1/rate [min/μmole]
0.08	12.50	0.15	6.7
0.12	8.30	0.21	4.8
0.54	1.85	0.70	1.4
1.23	0.81	1.1	0.91
1.82	0.56	1.3	0.77
2.72	0.37	1.5	0.67
4.94	0.20	1.7	0.59
10.00	0.10	1.8	0.56

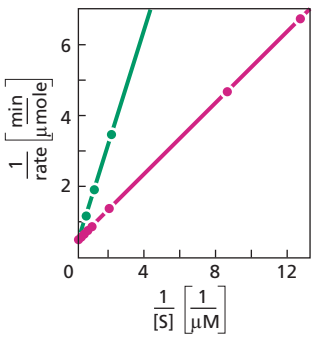


Figure A3–23

Chapter 4

ANSWER 4–1 Urea is a very small organic molecule that functions both as an efficient hydrogen-bond donor (through its $-NH_2$ groups) and as an efficient hydrogen-bond acceptor (through its –C=O group). As such, it can squeeze between hydrogen bonds that stabilize protein molecules and thus destabilize protein structures. In addition, the nonpolar side chains of a protein are held together in the interior of the folded structure because they would disrupt the structure of water if they were exposed. At high concentrations of urea, the hydrogen-bonded network of water molecules becomes disrupted so that these hydrophobic forces are significantly diminished. Proteins unfold in urea as a consequence of its effect on these two forces.

ANSWER 4–2 The amino acid sequence consists of alternating nonpolar and charged or polar amino acids. The resulting strand in a β sheet would therefore be polar on one side and hydrophobic on the other. Such a strand would probably be surrounded on either side by similar