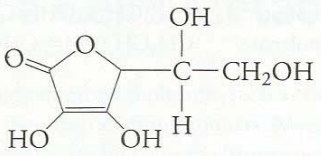
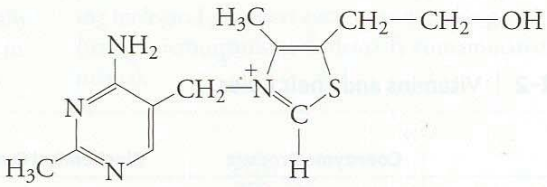
whose diet lacked fresh fruit and vegetables. Eventually, the active ingredient was identified as ascorbic acid (vitamin C):



Ascorbic acid (vitamin C)

Ascorbic acid is a cofactor for the enzyme that hydroxylates Pro residues in collagen (see Section 5-2). A deficiency of vitamin C prevents normal formation of collagen fibrils, producing the symptoms of scurvy.

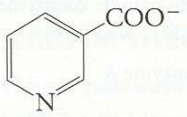
A study of the disease beriberi led to the discovery of the first B vitamin. Beriberi, characterized by leg weakness and swelling, is caused by a deficiency of thiamine (vitamin B_1).



Thiamine (vitamin B_1)

Thiamine acts as a prosthetic group in some essential enzymes, including the one that converts pyruvate to acetyl-CoA. Rice husks are rich in thiamine, and individuals whose diet consists largely of polished (huskless) rice can develop beriberi. The disease was originally thought to be infectious, until the same symptoms were observed in chickens and prisoners fed a diet of polished rice. Thiamine deficiency can occur in chronic alcoholics and others with a limited diet and impaired nutrient absorption.

Niacin, a component of NAD^+ and $NADP^+$, was first identified as the factor missing in the vitamin-deficiency disease pellagra.



Niacin

The symptoms of pellagra, including diarrhea and dermatitis, can be alleviated by boosting the intake of the essential amino acid tryptophan, which humans can convert to niacin. Niacin deficiency was once common in certain populations whose diet consisted largely of maize (corn). This grain is low in tryptophan and its niacin is covalently bound to other molecules; hence, it is not easily absorbed during digestion. In South America, where maize originated, the kernels are traditionally prepared by soaking or boiling them in an alkaline solution, a treatment that releases niacin and prevents pellagra. Unfortunately, this food-preparation custom did not spread to other parts of the world that adopted maize farming.

Most vitamins are readily obtained from a balanced diet, although poor nutrition, particularly in impoverished parts of the world, still causes vitamin-deficiency diseases. Intestinal bacteria, as well as plant- and animal-derived foods, are the natural sources of vitamins. However, plants do not contain cobalamin, so individuals who follow a strict vegetarian diet are at higher risk for developing a cobalamin deficiency.

CONCEPT REVIEW

- Why are compounds such as glyceraldehyde-3-phosphate, pyruvate, and acetyl-CoA so important in metabolism?
- What role do cofactors such as NAD^+ and ubiquinone play in metabolic reactions?
- What is the importance of reoxidizing NADH and QH_2 by molecular oxygen?
- Summarize the main features of metabolic pathways.
- Explain the relationship between vitamins and coenzymes.

12-3 Free Energy Changes in Metabolic Reactions

KEY CONCEPTS

- The free energy change for a reaction depends on the equilibrium constant for the reaction and on the actual concentrations of the reacting species.
- A reaction with a large negative change in free energy can be coupled to another unfavorable reaction.
- A reaction that breaks a phosphoanhydride bond in ATP occurs with a large change in free energy.
- Cells also use the free energy of other phosphorylated compounds, thioesters, reduced cofactors, and electrochemical gradients.
- Nonequilibrium reactions often serve as metabolic control points.

We have introduced the idea that catabolic reactions tend to release free energy and anabolic reactions tend to consume it (Fig. 12-1), but in fact, *all reactions in vivo occur with a net decrease in free energy; that is, ΔG is always less than zero* (free energy is discussed in Section 1-3). In a cell, metabolic reactions are not isolated but are linked, so that the free energy of a thermodynamically favorable reaction can be transferred to a second, unfavorable reaction to allow it to proceed. What is the nature of this free energy and how is it transferred? Free energy is not a substance or the property of a single molecule, so it is misleading to refer to a molecule or a bond within that molecule as having a large amount of free energy. Rather, *free energy is a property of a system, and it changes when the system undergoes a chemical reaction.*

The free energy change depends on reactant concentrations

The change in free energy of a system is related to the concentrations of the reacting substances. When a reaction such as $A + B \rightleftharpoons C + D$ is at equilibrium, the concentrations of the four reactants define the **equilibrium constant, K_{eq},** for the reaction:

$$K_{eq} = \frac{[C]_{eq}[D]_{eq}}{[A]_{eq}[B]_{eq}} \qquad \textbf{[12-1]}$$

(the brackets indicate the molar concentration of each substance). Recall that at equilibrium, the rates of the forward and reverse reactions are balanced, so there is no net change in the concentration of any reactant (equilibrium does *not* mean that the concentrations of the reactants and products are equal).

*When the system is not at equilibrium, the reactants experience a driving force to reach their equilibrium values. This force is the **standard free energy change for the reaction,** $\Delta G^{\circ\prime}$, which is defined as*

$$\Delta G^{\circ\prime} = -RT \ln K_{eq} \qquad \textbf{[12-2]}$$

Use Equation 12-2 to calculate $\Delta G^{\circ\prime}$ from K_{eq} and vice versa.

R is the gas constant (8.3145 $J \cdot K^{-1} \cdot mol^{-1}$) and T is the temperature in Kelvin. Recall from Section 1-3 that free energy has units of joules per mole.

By convention, measurements of standard free energy are valid under **standard conditions,** where the temperature is 25°C (298 K) and the pressure is 1 atm (these conditions are indicated by the degree symbol after ΔG). For a chemist, standard conditions specify an initial activity of 1 for each reactant (activity is the reactant's concentration corrected for its nonideal behavior). However, these conditions are impractical for biochemists since most biochemical reactions occur near neutral pH (where $[H^+] = 10^{-7}$ M rather than 1 M) and in aqueous solution (where $[H_2O] =$ 55.5 M). The biochemical standard conditions are summarized in Table 12-3. Biochemists use a prime symbol to indicate the standard free energy change for a reaction under biochemical standard conditions. In most equilibrium expressions, $[H^+]$ and

TABLE 12-3 | Biochemical Standard State

Temperature	25°C (298 K)
Pressure	1 atm
Reactant concentration	1 M
pH	7.0 ($[H^+] = 10^{-7}$ M)
Water concentration	55.5 M

$[H_2O]$ are set to 1 so that these terms can be ignored. And because biochemical reactions typically involve dilute solutions of reactants, molar concentrations can be used instead of activities.

Like K_{eq}, $\Delta G^{\circ\prime}$ is a constant for a particular reaction. It may be a positive or negative value, and it indicates whether the reaction can proceed spontaneously ($\Delta G^{\circ\prime} < 0$) or not ($\Delta G^{\circ\prime} > 0$) under standard conditions. In a living cell, reactants and products are almost never present at standard-state concentrations and the temperature may not be 25°C, yet reactions do occur with some change in free energy. Thus, it is important to distinguish the standard free energy change of a reaction from its actual free energy change, ΔG. ΔG is a function of the actual concentrations of the reactants and the temperature (37°C or 310 K in humans). ΔG is related to the standard free energy change for the reaction:

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[C][D]}{[A][B]} \qquad [12\text{-}3]$$

Use Equation 12-3 to calculate the actual free energy change for a reaction under cellular conditions of temperature and reactant concentration.

Here, the bracketed quantities represent the actual, nonequilibrium concentrations of the reactants. The concentration term in Equation 12-3 is sometimes called the **mass action ratio.**

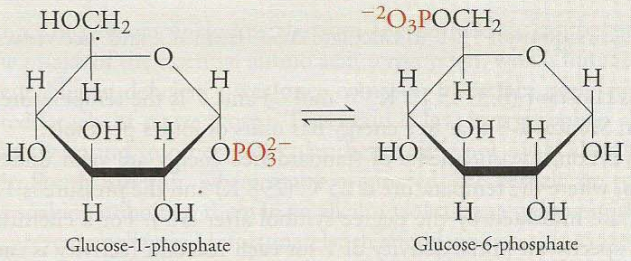
When the reaction is at equilibrium, $\Delta G = 0$ and

$$\Delta G^{\circ\prime} = -RT \ln \frac{[C]_{eq}[D]_{eq}}{[A]_{eq}[B]_{eq}} \qquad [12\text{-}4]$$

which is equivalent to Equation 12-2. Note that Equation 12-3 shows that *the criterion for spontaneity for a reaction is ΔG, a property of the actual concentrations of the reactants, not the constant* $\Delta G^{\circ\prime}$. Thus, a reaction with a positive standard free energy change (a reaction that cannot occur when the reactants are present at standard concentrations) may proceed *in vivo*, depending on the concentrations of reactants in the cell (see Sample Calculation 12-1). Keep in mind that thermodynamic spontaneity does not imply a rapid reaction. Even a substance with a strong tendency to undergo reaction ($\Delta G \ll 0$) will usually not react until acted upon by an enzyme that catalyzes the reaction.

SAMPLE CALCULATION 12-1

PROBLEM The standard free energy change for the reaction catalyzed by phosphoglucomutase



is $-7.1\ kJ \cdot mol^{-1}$. Calculate the equilibrium constant for the reaction. Calculate ΔG at 37°C when the concentration of glucose-1-phosphate is 1 mM and the concentration of glucose-6-phosphate is 25 mM. Is the reaction spontaneous under these conditions?

The equilibrium constant K_{eq} can be derived by rearranging Equation 12-2.

SOLUTION

$$K_{eq} = e^{-\Delta G^{\circ\prime}/RT}$$
$$= e^{-(-7100\ \text{J}\cdot\text{mol}^{-1})/(8.3145\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1})(298\ \text{K})}$$
$$= e^{2.87} = 17.6$$

At 37°C, $T = 310$ K.

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[\text{glucose-6-phosphate}]}{[\text{glucose-1-phosphate}]}$$
$$= -7.1\ \text{kJ}\cdot\text{mol}^{-1} + (8.3145\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1})(310\ \text{K}) \ln (0.025/0.001)$$
$$= -7.1\ \text{kJ}\cdot\text{mol}^{-1} + 8.3\ \text{kJ}\cdot\text{mol}^{-1}$$
$$= +1.2\ \text{kJ}\cdot\text{mol}^{-1}$$

The reaction is not spontaneous, because ΔG is greater than zero.

Unfavorable reactions are coupled to favorable reactions

A biochemical reaction may at first seem to be thermodynamically forbidden because its free energy change is greater than zero. Yet the reaction can proceed *in vivo* when it is coupled to a second reaction whose value of ΔG is very large and negative, so that the *net* change in free energy for the combined reactions is less than zero. *ATP is often involved in such coupled processes because its reactions occur with a relatively large negative change in free energy.*

Adenosine triphosphate (ATP) contains two phosphoanhydride bonds (Fig. 12-11). Cleavage of either of these bonds—that is, transfer of one or two of its phosphoryl groups to another molecule—is a reaction with a large negative standard free energy change (under physiological conditions, ΔG is even more negative). As a reference point, biochemists use the reaction in which a phosphoryl group is transferred to water—in other words, hydrolysis of the phosphoanhydride bond, such as

$$\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i$$

This is a spontaneous reaction with a $\Delta G^{\circ\prime}$ value of $-30\ \text{kJ}\cdot\text{mol}^{-1}$.

The following example illustrates the role of ATP in a coupled reaction. Consider the phosphorylation of glucose by inorganic phosphate (HPO_4^{2-} or P_i), a thermodynamically unfavorable reaction ($\Delta G^{\circ\prime} = +13.8\ \text{kJ}\cdot\text{mol}^{-1}$):

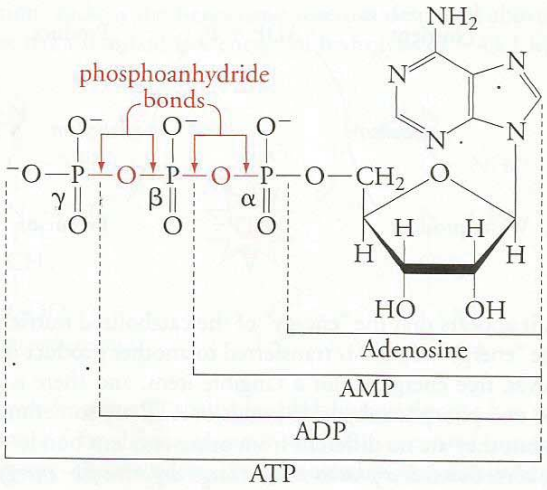
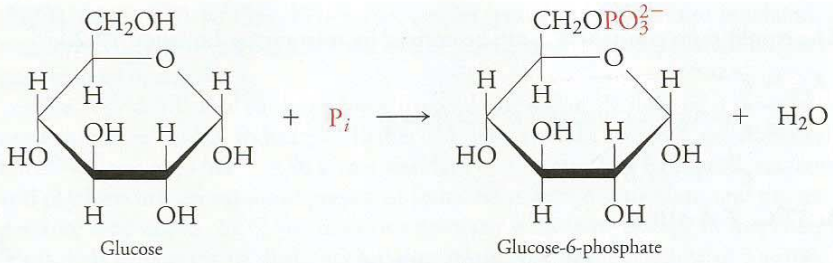


Figure 12-11 Adenosine triphosphate. The three phosphate groups are sometimes described by the Greek letters α, β, and γ. The linkage between the first (α) and second (β) phosphoryl groups, and between the second (β) and third (γ), is a phosphoanhydride bond. A reaction in which one or two phosphoryl groups are transferred to another compound (a reaction in which a phosphoanhydride bond is cleaved) has a large negative value of $\Delta G^{\circ\prime}$.



Glucose Glucose-6-phosphate

When this reaction is combined with the ATP hydrolysis reaction, the values of $\Delta G^{\circ\prime}$ for each reaction are added:

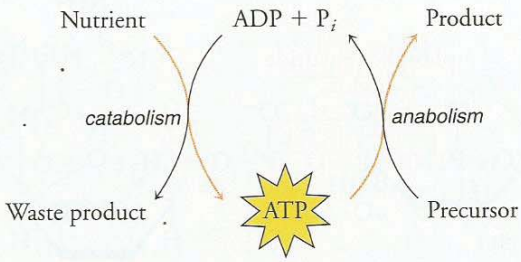
	$\Delta G^{\circ\prime}$
Glucose + $P_i \rightarrow$ Glucose-6-phosphate + H_2O	$+13.8\ kJ \cdot mol^{-1}$
ATP + $H_2O \rightarrow$ ADP + P_i	$-30.5\ kJ \cdot mol^{-1}$
Glucose + ATP $\rightarrow$ Glucose-6-phosphate + ADP	$-16.7\ kJ \cdot mol^{-1}$

The net chemical reaction, the phosphorylation of glucose, is thermodynamically favorable ($\Delta G < 0$). *In vivo,* this reaction is catalyzed by hexokinase (introduced in Section 6-3), and a phosphoryl group is transferred from ATP directly to glucose. The ATP is not actually hydrolyzed, and there is no free phosphoryl group floating around the enzyme. However, writing out the two coupled reactions, as shown above, makes it easier to see what's going on thermodynamically.

Some biochemical processes appear to occur with the concomitant hydrolysis of ATP to ADP + P_i, for example, the operation of myosin and kinesin (Section 5-3) or the Na,K-ATPase ion pump (Section 9-3). But a closer look reveals that in all these processes, ATP actually transfers a phosphoryl group to a protein. Later, the phosphoryl group is transferred to water, so the net reaction takes the form of ATP hydrolysis. The same ATP "hydrolysis" effect applies to some reactions in which the AMP moiety of ATP (rather than a phosphoryl group) is transferred to a substance, leaving inorganic pyrophosphate (PP_i). Cleavage of the phosphoanhydride bond of PP_i also has a large negative value of $\Delta G^{\circ\prime}$.

What's so special about ATP?

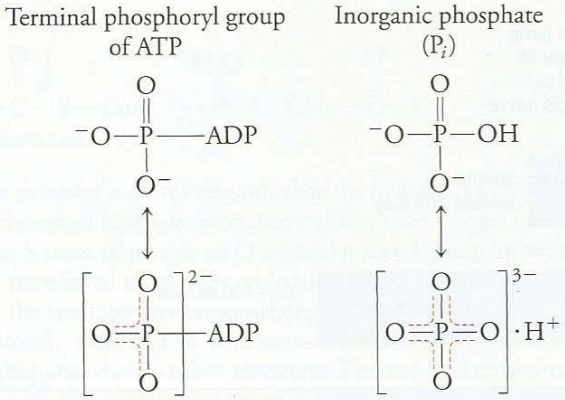
Because ATP appears to drive many thermodynamically unfavorable reactions, it is tempting to think of ATP as an agent that transfers packets of free energy around the cell. This is one reason why ATP is commonly called the energy currency of the cell. The general role of ATP in linking exergonic ATP-producing processes to endergonic ATP-consuming processes can be diagrammed as



In this scheme, it appears that the "energy" of the catabolized nutrient is transferred to ATP, then the "energy" of ATP is transferred to another product in a biosynthetic reaction. However, free energy is not a tangible item, and there is nothing magic about ATP. The two phosphoanhydride bonds of ATP are sometimes called "high-energy" bonds, but they are no different from other covalent bonds. All that matters is that *breaking these bonds is a process with a large negative free energy change.* Using

the simple example of ATP hydrolysis, we can state that a large amount of free energy is released when ATP is hydrolyzed because the products of the reaction have less free energy than the reactants. It is worth examining two reasons why this is so.

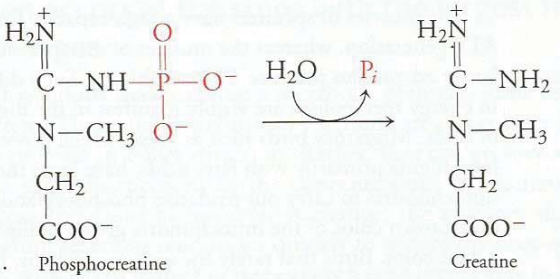
1. *The ATP hydrolysis products are more stable than the reactants.* At physiological pH, ATP has three to four negative charges (the pK is close to 7), and the anionic groups repel each other. In the products ADP and P_i, separation of the charges relieves some of this unfavorable electrostatic repulsion.
2. *A compound with a phosphoanhydride bond experiences less resonance stabilization than its hydrolysis products.* **Resonance stabilization** reflects the degree of electron delocalization in a molecule and can be roughly assessed by the number of different ways of depicting the molecule's structure. There are fewer equivalent ways of arranging the bonds of the terminal phosphoryl group of ATP than there are in free P_i.



Free energy can take different forms

ATP is not the only substance that functions as energy currency in the cell. Other compounds that participate in reactions with large negative changes in free energy can serve the same purpose. For example, a number of phosphorylated compounds other than ATP can give up their phosphoryl group to another molecule. Table 12-4 lists the standard free energy changes for some of these reactions in which the phosphoryl group is transferred to water.

Although hydrolysis of the bond linking the phosphate group to the rest of the molecule could be a wasteful process (the product would be free phosphate, P_i), the values listed in the table are a guide to how such compounds would behave in a coupled reaction, such as the hexokinase reaction described above. For example, phosphocreatine has a standard free energy of hydrolysis of -43.1 kJ $\cdot$ mol^{-1}:



Creatine has lower free energy than phosphocreatine since it has two, rather than one, resonance forms; this resonance stabilization contributes to the large negative free energy change when phosphocreatine transfers its phosphoryl

TABLE 12-4 | Standard Free Energy Change for Phosphate Hydrolysis

Compound	$\Delta G^{\circ\prime}$ (kJ $\cdot$ mol^{-1})
Phosphoenolpyruvate	−61.9
1,3-Bisphosphoglycerate	−49.4
ATP → AMP + PP_i	−45.6
Phosphocreatine	−43.1
ATP → ADP + P_i	−30.5
Glucose-1-phosphate	−20.9
Glucose-6-phosphate	−13.8
Glycerol-3-phosphate	−9.2

group to another compound. In muscles, phosphocreatine transfers a phosphoryl group to ADP to produce ATP (Box 12-B).

Like ATP, other nucleoside triphosphates have large negative standard free energies of hydrolysis. GTP rather than ATP serves as the energy currency for reactions that occur during cellular signaling (Section 10-2) and protein synthesis (Section

BOX 12-B **A CLOSER LOOK**

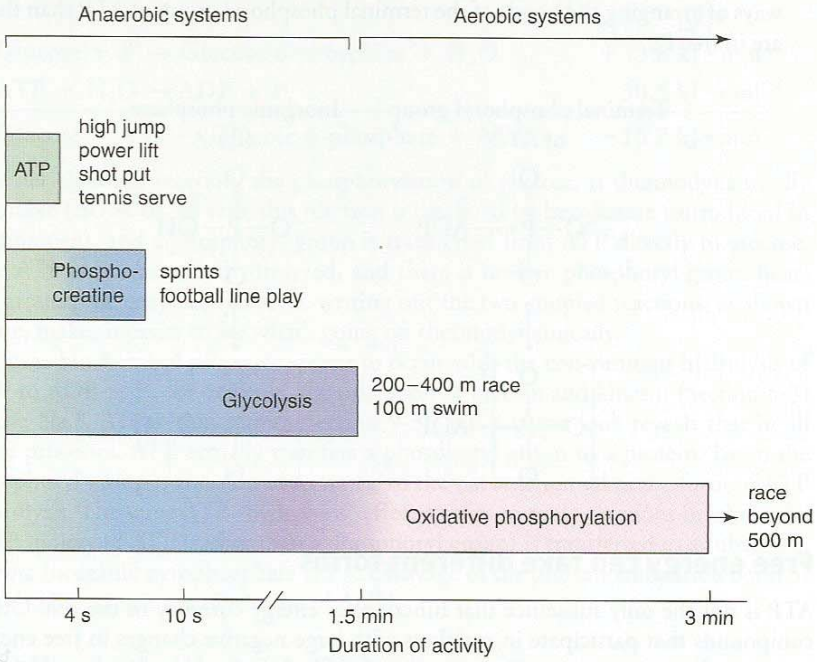
Powering Human Muscles

In resting muscles, when the demand for ATP is low, creatine kinase catalyzes the transfer of a phosphoryl group from ATP to creatine to produce phosphocreatine:

$$ATP + creatine \rightleftharpoons phosphocreatine + ADP$$

This reaction runs in reverse when ADP concentrations rise, as they do when muscle contraction converts ATP to ADP + P_i. Phosphocreatine therefore acts as a sort of phosphoryl-group reservoir to maintain the supply of ATP. Cells cannot stockpile ATP; its concentration remains remarkably stable (between 2 and 5 mM in most cells) under widely varying levels of demand. Without phosphocreatine, a muscle would exhaust its ATP supply before it could be replenished by other, slower processes.

The potential power-boosting function of phosphocreatine has attracted some commercial interest. Administering creatine intravenously appears to improve heart muscle function in individuals with congestive heart failure, but there is no strong evidence that oral creatine supplements enhance muscle performance in athletes.



[Figure adapted from McArdle, W.D., Katch, F.I., and Katch, V.L., *Exercise Physiology* (2nd ed.), p. 348, Lea & Febiger (1986).]

Different types of physical activity make different demands on a muscle's ATP-generating mechanisms. A single burst of activity is powered by the available ATP. Activities lasting up to a few seconds require phosphocreatine to maintain the ATP supply. Phosphocreatine itself is limited, so continued muscle contraction must rely on ATP produced by catabolizing glucose (obtained from the muscle's store of glycogen) via glycolysis. The end product of this pathway is lactate, the conjugate base of a weak acid, and muscle pain sets in as the acid accumulates and the pH begins to drop. Up to this point, the muscle functions anaerobically. To continue its activity, it must switch to aerobic metabolism and further oxidize glucose via the citric acid cycle. The muscle also catabolizes fatty acids, whose products also enter the citric acid cycle. Recall that the citric acid cycle generates reduced cofactors that must be reoxidized by molecular oxygen. Aerobic metabolism of glucose and fatty acids is slower than anaerobic glycolysis; but it generates considerably more ATP. Some forms of physical activity and the systems that power them are diagrammed above.

A casual athlete can detect the shift from anaerobic to aerobic metabolism after about a minute and a half. In world-class athletes, the breakpoint occurs at about 150 to 170 seconds, which corresponds roughly to the finish line in a 1000-meter race.

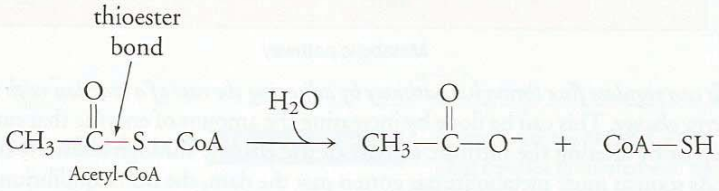
The muscles of sprinters have a high capacity for anaerobic ATP generation, whereas the muscles of distance runners are better adapted to produce ATP aerobically. Such differences in energy metabolism are visibly manifest in the flight muscles of birds. Migratory birds such as geese, which power their long flights primarily with fatty acids, have large numbers of mitochondria to carry out oxidative phosphorylation. The reddish-brown color of the mitochondria gives the flight muscles a dark color. Birds that rarely fly, such as chickens, have fewer mitochondria and lighter-colored muscles. When these birds do fly, it is usually only a short burst of activity that is powered by anaerobic mechanisms.

22-3). In the cell, nucleoside triphosphates are freely interconverted by reactions such as the one catalyzed by nucleoside diphosphate kinase, which transfers a phosphoryl group from ATP to a nucleoside diphosphate (NDP):

$$\text{ATP} + \text{NDP} \rightleftharpoons \text{ADP} + \text{NTP}$$

Because the reactants and products are energetically equivalent, $\Delta G^{\circ\prime}$ values for these reactions are near zero.

Another class of compounds that can release a large amount of free energy upon hydrolysis are **thioesters,** such as acetyl-CoA. Coenzyme A is a nucleotide derivative with a side chain ending in a sulfhydryl (SH) group (Fig. 3-4a). An acyl or acetyl group (the "A" for which coenzyme A was named) is linked to the sulfhydryl group by a thioester bond. Hydrolysis of this bond has a $\Delta G^{\circ\prime}$ value of -31.5 kJ · mol^{-1}, comparable to that of ATP hydrolysis:



Hydrolysis of a thioester is more exergonic than the hydrolysis of an ordinary (oxygen) ester because thioesters have less resonance stability than oxygen esters, owing to the larger size of an S atom relative to an O atom. An acetyl group linked to coenzyme A can be readily transferred to another molecule because formation of the new linkage is powered by the favorable free energy change of breaking the thioester bond.

We have already seen that in oxidation–reduction reactions, cofactors such as NAD^+ and ubiquinone can collect electrons. The reduced cofactors are a form of energy currency because their subsequent reoxidation by another compound occurs with a negative change in free energy. Ultimately, the transfer of electrons from one reduced cofactor to another and finally to oxygen, the final electron acceptor in many cells, releases enough free energy to drive the synthesis of ATP.

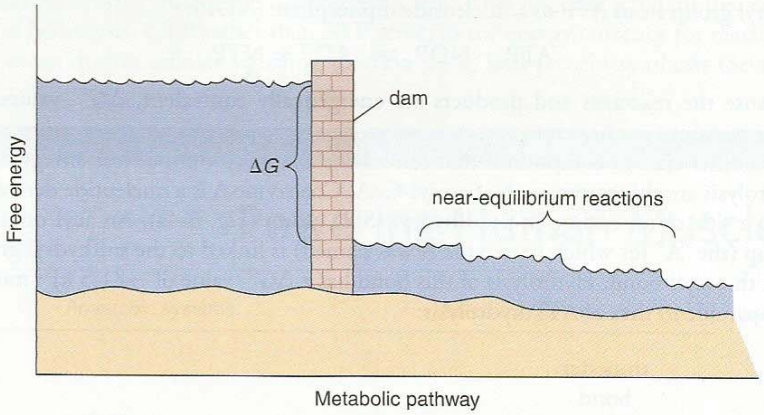
Keep in mind that free energy changes occur not just as the result of chemical changes such as phosphoryl-group transfer or electron transfer. As decreed by the first law of thermodynamics (Section 1-3), *energy can take many forms.* We will see that ATP production depends on the energy of an electrochemical gradient, that is, an imbalance in the concentration of a substance (in this case, protons) on the two sides of a membrane. The free energy change of dissipating this gradient (allowing the system to move toward equilibrium) is converted to the mechanical energy of an enzyme that synthesizes ATP. In photosynthetic cells, the chemical reactions required to generate carbohydrates are ultimately driven by the free energy changes of reactions in which light-excited molecules relax to a lower-energy state.

Regulation occurs at the steps with the largest free energy changes

In a series of reactions that make up a metabolic pathway, some reactions have ΔG values near zero. These near-equilibrium reactions are not subject to a strong driving force to proceed in either direction. Rather, flux can go forward or backward, according to slight fluctuations in the concentrations of reactants and products. When the concentrations of metabolites change, the enzymes that catalyze these near-equilibrium reactions tend to act quickly to restore the near-equilibrium state.

Reactions with large changes in free energy have a longer way to go to reach equilibrium; these are the reactions that experience the greatest "urge" to proceed forward. However, the enzymes that catalyze these reactions do not allow the reaction to reach equilibrium because they work too slowly. Often the enzymes are already saturated with substrate, so the reactions cannot go any faster (when $[S] \gg K_M$, $v \approx V_{max}$;

Section 7-2). The rates of these far-from-equilibrium reactions limit flux through the entire pathway because the reactions function like dams.



Cells can regulate flux through a pathway by adjusting the rate of a reaction with a large free energy change. This can be done by increasing the amount of enzyme that catalyzes that step or by altering the intrinsic activity of the enzyme through allosteric mechanisms. As soon as more metabolite has gotten past the dam, the near-equilibrium reactions go with the flow, allowing the pathway intermediates to move toward the final product. Most metabolic pathways do not have a single flow-control point, as the dam analogy might suggest. Instead, flux is typically controlled at several points to ensure that the pathway can work efficiently as part of the cell's entire metabolic network.

CONCEPT REVIEW

- Why must free energy changes be negative for reactions *in vivo*?
- What is the standard free energy change for a reaction and how is it related to the reaction's equilibrium constant?
- Distinguish ΔG and $\Delta G^{\circ\prime}$. How are they related?
- Why is it misleading to refer to ATP as a high-energy molecule?
- Explain why cleavage of one of ATP's phosphoanhydride bonds releases large amounts of free energy.
- How do phosphorylated compounds, thioesters, and reduced cofactors appear to transfer free energy? What other forms of energy do cells use?
- Why do cells control metabolic reactions with large free energy changes?

SUMMARY

12-1 Food and Fuel

- Polymeric food molecules such as starch, proteins, and triacylglycerols are broken down to their monomeric components (glucose, amino acids, and fatty acids), which are absorbed. These materials are stored as polymers in a tissue-specific manner.
- Metabolic fuels are mobilized from glycogen, fat, and proteins as needed.

12-2 Metabolic Pathways

- Series of reactions known as metabolic pathways break down and synthesize biological molecules. Several pathways make use of the same small molecule intermediates.
- During the oxidation of amino acids, monosaccharides, and fatty acids, electrons are transferred to carriers such as NAD^+ and ubiquinone. Reoxidation of the reduced cofactors drives the synthesis of ATP by oxidative phosphorylation.
- Metabolic pathways form a complex network, but not all cells or organisms carry out all possible metabolic processes. Humans rely on other organisms to supply vitamins and other essential materials.

12-3 Free Energy Changes in Metabolic Reactions

- The standard free energy change for a reaction is related to the equilibrium constant, but the actual free energy change is related to the actual cellular concentrations of reactants and products.
- A thermodynamically unfavorable reaction may proceed when it is coupled to a favorable process involving ATP, whose phosphoanhydride bonds release a large amount of free energy when cleaved.
- Other forms of cellular energy currency include phosphorylated compounds, thioesters, and reduced cofactors.
- Cells regulate metabolic pathways at the steps that are farthest from equilibrium.

GLOSSARY TERMS

chemoautotroph
photoautotroph
heterotroph
catabolism
anabolism
metabolism
lipoprotein
metabolic fuel
mobilization
phosphorolysis
diabetes mellitus
lysosome
proteasome
metabolic pathway
metabolite
glycolysis
citric acid cycle
oxidation
reduction
redox reaction
cofactor
coenzyme
oxidative phosphorylation
flux
metabolomics
metabolome
essential compound
vitamin
equilibrium constant (K_{eq})
standard free energy change ($\Delta G^{\circ\prime}$)
standard conditions
mass action ratio
resonance stabilization
thioester

BIOINFORMATICS PROJECT 4

Learn to use the BRENDA, KEGG, and OMIM databases to explore the enzymes, intermediates, and diseases associated with specific metabolic pathways.

Metabolic Pathways

PROBLEMS

12-1 Food and Fuel

1. Classify the following organisms as chemoautotrophs, photoautotrophs, or heterotrophs:
(a) *Hydrogenobacter*, which converts molecular hydrogen and oxygen to water
(b) *Arabidopsis thaliana*, a green plant
(c) The nitrosifying bacteria, which oxidize NH_3 to nitrite
(d) *Saccharomyces cerevisiae*, yeast
(e) *Caenorhabditis elegans*, a nematode worm
(f) The *Thiothrix* bacteria, which oxidize hydrogen sulfide
(g) Cyanobacteria (erroneously termed "blue-green algae" in the past)

2. The purple nonsulfur bacteria obtain their cellular energy from a photosynthetic process that does not produce oxygen. These bacteria also require an organic carbon source. Using the terms in this chapter, coin a new term that describes the trophic strategy of this organism.

3. Pancreatic amylase, which is similar to salivary amylase, is secreted by the pancreas into the small intestine. The active site of pancreatic amylase accommodates five glucosyl residues and cleaves the glycosidic bond between the second and third residues. What are the main products of amylase digestion?

4. Monosaccharides, the products of polysaccharide and disaccharide digestion, enter the cells lining the intestine via a specialized transport system. What is the source of free energy for this transport process?

5. Hydrolysis of proteins begins in the stomach, catalyzed by the hydrochloric acid secreted into the stomach by parietal cells. Draw the reaction that shows the hydrolysis of a peptide bond.

6. The cleavage of peptide bonds in the stomach is catalyzed both by hydrochloric acid (see Problem 5) and by the stomach enzyme pepsin. Peptide bond cleavage continues in the small intestine, catalyzed by the pancreatic enzymes trypsin and chymotrypsin. At what pH does pepsin function optimally; that is, at what pH is the V_{max} for pepsin greatest? Is the pH optimum for pepsin different from that for trypsin and chymotrypsin? Explain.

7. Free amino acid transport from the intestinal lumen into intestinal cells requires Na^+ ions. Draw a diagram that illustrates amino acid transport into these cells.

8. In oral rehydration therapy (ORT), patients suffering from diarrhea are given a solution consisting of a mixture of glucose and electrolytes. Some formulations also contain amino acids. Why are electrolytes added to the mixture?

9. Triacylglycerol digestion begins in the stomach. Gastric lipase catalyzes hydrolysis of the fatty acid from the third glycerol carbon.
(a) Draw the reactants and products of this reaction.
(b) Conversion of the triacylglycerol to a diacylglycerol and a fatty acid promotes emulsification of fats in the stomach; that is, the products are more easily incorporated into micelles. Explain why.

10. The cells lining the small intestine absorb cholesterol but not cholesteryl esters (see page 298). Draw the reaction catalyzed by cholesteryl esterase that produces cholesterol from cholesteryl stearate.

11: **(a)** Consider the physical properties of a polar glycogen molecule and an aggregation of hydrophobic triacylglycerols. On a per-weight basis, why is fat a more efficient form of energy storage than glycogen?
(b) Explain why there is an upper limit to the size of a glycogen molecule but there is no upper limit to the amount of triacylglycerols that an adipocyte can store.

12. Glycogen can be expanded quickly, by adding glucose residues to its many branches, and degraded quickly, by simultaneously

removing glucose from the ends of these branches. Are the enzymes that catalyze these processes specific for the reducing or nonreducing ends of the glycogen polymer? Explain.

13. The phosphorolysis reaction that removes glucose residues from glycogen yields as its product glucose-1-phosphate (see page 300). Why is it necessary to remove the phosphate group before the glucose exits the cell to enter the circulation?

14. Hydrolytic enzymes encased within the membrane-bound lysosomes all work optimally at pH ~5. This feature serves as a cellular "insurance policy" in the event of lysosomal enzyme leakage into the cytosol. Explain.

12-2 Metabolic Pathways

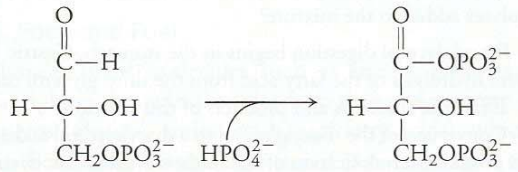
15. The common intermediates listed in the table below appear as reactants or products in several pathways. Place a checkmark in the box that indicates the appropriate pathway for each reactant.

	Glycolysis	Citric acid cycle	Fatty acid metabolism
Acetyl-CoA			
Glyceraldehyde-3-phosphate			
Pyruvate			

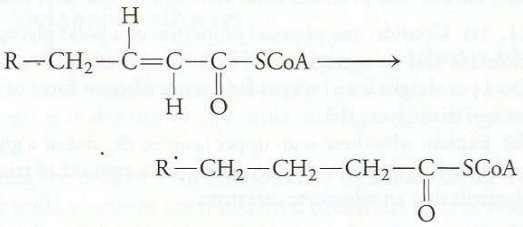
	Triacylglycerol synthesis	Photosynthesis	Transamination
Acetyl-CoA			
Glyceraldehyde-3-phosphate			
Pyruvate			

16. For each of the (unbalanced) reactions shown below, tell whether the reactant is being oxidized or reduced.

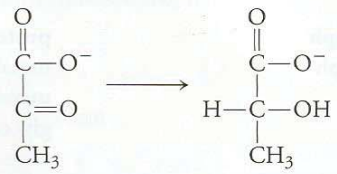
(a) A reaction from the catabolic glycolytic pathway



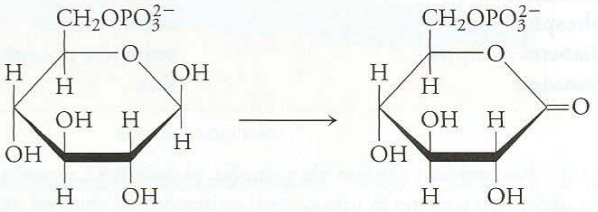
(b) A reaction from the fatty acid synthesis pathway



(c) A reaction associated with the catabolic glycolytic pathway



(d) A reaction associated with the anabolic pentose phosphate pathway



17. For each of the reactions shown in Problem 16, identify the cofactor as NAD^+, $NADP^+$, NADH, or NADPH.

18. Food scientists at Ohio State University gave volunteers salsa or salad with or without lipid-rich avocados, then drew blood samples periodically and measured levels of β-carotene (see Box 8-A). They found that serum β-carotene levels were 2 to 15 times greater when the volunteers consumed the food with avocado. Explain these results.

19. A vitamin K–dependent carboxylase enzyme catalyzes the γ-carboxylation of specific glutamate residues in blood coagulation proteins. **(a)** Draw the structure of a γ-carboxyglutamate residue. **(b)** Why does this post-translational modification assist coagulation proteins in binding the Ca^{2+} ions required for blood clotting?

20. Vitamin B_{12} is synthesized by certain gastrointestinal bacteria and is also found in foods of animal origin such as meat, milk, eggs, and fish. When vitamin B_{12}–containing foods are consumed, the vitamin is released from the food and binds to a salivary vitamin B_{12}–binding protein called haptocorrin. The haptocorrin–vitamin B_{12} complex passes from the stomach to the small intestine, where the vitamin is released from the haptocorrin and then binds to intrinsic factor (IF). The IF–vitamin B_{12} complex then enters the cells lining the intestine by receptor-mediated endocytosis. Using this information, make a list of individuals most at risk for vitamin B_{12} deficiency.

12-3 Free Energy Changes in Metabolic Reactions

21. **(a)** The $\Delta G^{\circ\prime}$ value for a hypothetical reaction is 10 kJ · mol^{-1}. Compare the K_{eq} for this reaction with the K_{eq} for a reaction whose $\Delta G^{\circ\prime}$ value is twice as large.

(b) Carry out the same exercise for a hypothetical reaction whose $\Delta G^{\circ\prime}$ value is -10 kJ · mol^{-1}.

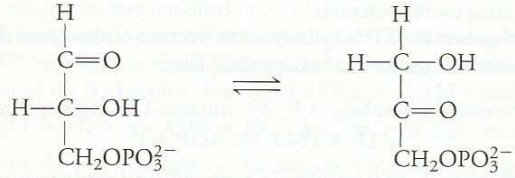
22. The equilibrium constant and the $\Delta G^{\circ\prime}$ values were recently determined for the arginine kinase reaction.

$$\text{Phosphoarginine} + \text{ADP} \underset{}{\overset{\text{arginine kinase}}{\rightleftharpoons}} \text{Arginine} + \text{ATP}$$

(a) What is the value of the K_{eq} for the reaction at 25°C when equilibrium concentrations of the reactants and products are as follows: [phosphoarginine] = 0.737 mM, [ADP] = 0.750 mM, [arginine] = 4.78 mM, and [ATP] = 3.87 mM?
(b) What is the value of the $\Delta G^{\circ\prime}$ for this reaction? Is it spontaneous under standard conditions?

23. Calculate ΔG for the hydrolysis of ATP under cellular conditions, where [ATP] = 3 mM, [ADP] = 1 mM, and $[P_i]$ = 5 mM.

24. The standard free energy change for the reaction catalyzed by triose phosphate isomerase is 7.9 kJ · mol^{-1}.



Glyceraldehyde-3-phosphate Dihydroxyacetone phosphate

(a) Calculate the equilibrium constant for the reaction.
(b) Calculate ΔG at 37°C when the concentration of glyceraldehyde-3-phosphate is 0.1 mM and the concentration of dihydroxyacetone phosphate is 0.5 mM.
(c) Is the reaction spontaneous under these conditions? Would the reverse reaction be spontaneous?

25. The $\Delta G^{\circ\prime}$ for the hydrolysis of ATP under standard conditions at pH 7 and in the presence of magnesium ions is −30.5 kJ · mol^{-1}.
(a) How would this value change if ATP hydrolysis were carried out at a pH of less than 7? Explain.
(b) How would this value change if magnesium ions were not present?

26. The $\Delta G^{\circ\prime}$ for the formation of UDP–glucose from glucose-1-phosphate and UTP is about zero. Yet the production of UDP–glucose is highly favorable. What is the driving force for this reaction?

$$\text{glucose-1-phosphate} + \text{UTP} \rightleftharpoons \text{UDP–glucose} + PP_i$$

27. **(a)** The complete oxidation of glucose releases a considerable amount of energy. The $\Delta G^{\circ\prime}$ for the reaction shown below is $-2850\ kJ \cdot mol^{-1}$.

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

How many moles of ATP could be produced under standard conditions from the oxidation of one mole of glucose, assuming about 33% efficiency?
(b) The oxidation of palmitate, a 16-carbon saturated fatty acid, releases 9781 kJ · mol^{-1}.

$$C_{16}H_{32}O_2 + 23\ O_2 \rightarrow 16\ CO_2 + 16\ H_2O$$

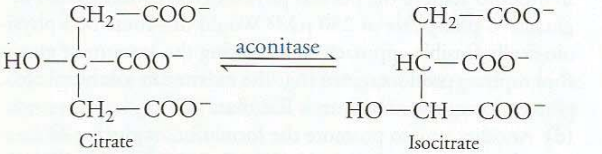
How many moles of ATP could be produced under standard conditions from the oxidation of one mole of palmitate, assuming 33% efficiency?
(c) Calculate the number of ATP molecules produced per carbon for glucose and palmitate. Explain the reason for the difference.

28. An adult female weighing 125 lb must consume 2000 Calories of food daily. (Note: A nutritional "large calorie," or "Calorie," is equal to a kilocalorie. 1 calorie = 4.184 J)
(a) If this energy is used to synthesize ATP, calculate the number of moles of ATP that would be synthesized each day under standard conditions (assuming 33% efficiency).
(b) Calculate the number of grams of ATP that would be synthesized each day. The molar mass of ATP is 505 g · mol^{-1}. What is the mass of ATP in pounds? (2.2 kg = 1 lb)
(c) There is approximately 40 g of ATP in the adult 125-lb female. Considering this fact and your answer to part (b), suggest an explanation that is consistent with these findings.

29. Which of the compounds listed in Table 12-4 could be involved in a reaction coupled to the synthesis of ATP from ADP + P_i?

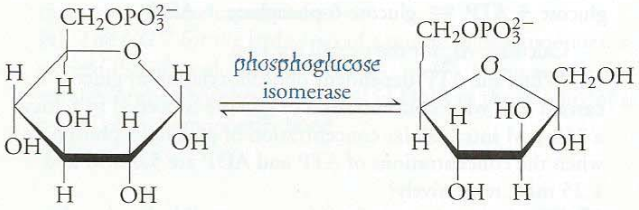
30. Which of the compounds listed in Table 12-4 could be involved in a reaction coupled to the hydrolysis of ATP to ADP + P_i?

31. Citrate is isomerized to isocitrate in the citric acid cycle (Chapter 14). The reaction is catalyzed by the enzyme aconitase. The $\Delta G^{\circ\prime}$ of the reaction is 5 kJ · mol^{-1}. The kinetics of the reaction are studied *in vitro*, where 1 M citrate and 1 M isocitrate are added to an aqueous solution of the enzyme at 25°C.
(a) What is the K_{eq} for the reaction?
(b) What are the equilibrium concentrations of the reactant and product?
(c) What is the preferred direction of the reaction under standard conditions?
(d) The aconitase reaction is the second step of an eight-step pathway and occurs in the direction shown in the figure. How can you reconcile these facts with your answer to part c?



Citrate Isocitrate

32. The equilibrium constant for the conversion of glucose-6-phosphate to fructose-6-phosphate is 0.41. The reaction is reversible and is catalyzed by the enzyme phosphoglucose isomerase.



Glucose-6-phosphate Fructose-6-phosphate

(a) What is the $\Delta G^{\circ\prime}$ for this reaction? Would this reaction proceed in the direction written under standard conditions?
(b) What is the ΔG for this reaction at 37°C when the concentration of glucose-6-phosphate is 2.0 mM and the concentration of the fructose-6-phosphate is 0.5 mM? Would the reaction proceed in the direction written under these cellular conditions?

33. The conversion of glutamate to glutamine is unfavorable. In order for this transformation to occur in the cell, it must be coupled to the hydrolysis of ATP. Consider two possible mechanisms:

Mechanism 1: glutamate + $NH_3 \rightleftharpoons$ glutamine

$$ATP + H_2O \rightleftharpoons ADP + P_i$$

Mechanism 2: glutamate + ATP $\rightleftharpoons$ γ-glutamylphosphate + ADP

γ-glutamylphosphate + H_2O + NH_3 $\rightleftharpoons$ glutamine + P_i

Write the overall equation for the reaction for each mechanism. Is one mechanism more likely than the other? Or are both mechanisms equally feasible for the conversion of glutamate to glutamine? Explain.

34. The phosphorylation of glucose to glucose-6-phosphate is the first step of glycolysis (Chapter 13). The phosphorylation of glucose by phosphate is described by the following equation.

glucose + P_i $\rightleftharpoons$ glucose-6-phosphate + H_2O

$\Delta G^{\circ\prime} = +13.8\ \text{kJ} \cdot \text{mol}^{-1}$.

(a) Calculate the equilibrium constant for the above reaction.
(b) What would the equilibrium concentration of glucose-6-phosphate be under cellular conditions of [glucose] = [P_i] = 5 mM if glucose were phosphorylated according to the reaction above? Does this reaction provide a feasible route for the production of glucose-6-phosphate for the glycolytic pathway?
(c) One way to increase the amount of a product of a particular reaction is to increase the concentrations of the reactants. This would shift the equilibrium to the right, according to LeChâtelier's principle. If the cellular concentration of phosphate were 5 mM, what concentration of glucose would be required in order to achieve the normal physiological concentration of glucose-6-phosphate of 250 μM? Would this route be a physiologically feasible approach to increasing the amount of glucose-6-phosphate product, given that the maximum solubility of glucose in aqueous medium is less than 1 M?
(d) Another way to promote the formation of glucose-6-phosphate is to couple the phosphorylation of glucose to the hydrolysis of ATP:

glucose + P_i $\rightleftharpoons$ glucose-6-phosphate + H_2O $\quad \Delta G^{\circ\prime} = 13.8\ \text{kJ} \cdot \text{mol}^{-1}$

ATP + H_2O $\rightleftharpoons$ ADP + P_i $\quad \Delta G^{\circ\prime} = -30.5\ \text{kJ} \cdot \text{mol}^{-1}$

glucose + ATP $\rightleftharpoons$ glucose-6-phosphate + ADP

Calculate K_{eq} for the overall reaction.
(e) When the ATP-dependent phosphorylation of glucose is carried out, what concentration of glucose is needed to achieve a 250-μM intracellular concentration of glucose-6-phosphate when the concentrations of ATP and ADP are 5.0 mM and 1.25 mM, respectively?
(f) Which route is more feasible to accomplish the phosphorylation of glucose to glucose-6-phosphate: the direct phosphorylation by P_i or the coupling of this phosphorylation to ATP hydrolysis? Explain.

35. Fructose-6-phosphate is phosphorylated to fructose-1,6-bisphosphate as part of the glycolytic pathway. The phosphorylation of fructose-6-phosphate by phosphate is described by the following equation:

fructose-6-phosphate + P_i $\rightleftharpoons$ fructose-1,6-bisphosphate

$\Delta G^{\circ\prime} = 47.7\ \text{kJ} \cdot \text{mol}^{-1}$

(a) What is the ratio of fructose-1,6-bisphosphate to fructose-6-phosphate at equilibrium if the concentration of phosphate in the cell is 5 mM?
(b) Suppose that the phosphorylation of fructose-6-phosphate is coupled to the hydrolysis of ATP.

ATP + H_2O $\rightleftharpoons$ ADP + P_i $\quad \Delta G^{\circ\prime} = -30.5\ \text{kJ} \cdot \text{mol}^{-1}$

Write the new equation that describes the phosphorylation of fructose-6-phosphate coupled with ATP hydrolysis. Calculate the $\Delta G^{\circ\prime}$ for the reaction.
(c) What is the ratio of fructose-1,6-bisphosphate to fructose-6-phosphate at equilibrium for the reaction you wrote in part (b) if the equilibrium concentration of ATP = 3 mM and [ADP] = 1 mM?
(d) Write a concise paragraph that summarizes your findings above.
(e) One can envision two mechanisms for coupling ATP hydrolysis to the phosphorylation of fructose-6-phosphate, yielding the same overall reaction:
Mechanism 1: ATP is hydrolyzed as fructose-6-phosphate is transformed to fructose-1,6-bisphosphate:

fructose-6-phosphate + P_i $\rightleftharpoons$ fructose-1,6-bisphosphate

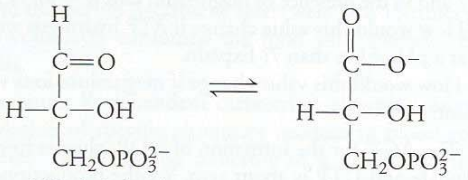
ATP + H_2O $\rightleftharpoons$ ADP + P_i

Mechanism 2: ATP transfers its γ phosphate directly to fructose-6-phosphate in one step, producing fructose-1,6-bisphosphate.

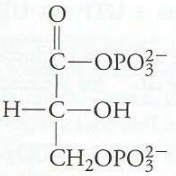
fructose-6-phosphate + ATP + H_2O $\rightleftharpoons$ fructose-1,6-bisphosphate + ADP

Choose one of the above mechanisms as the more biochemically feasible and provide a rationale for your choice.

36. Glyceraldehyde-3-phosphate (GAP) is eventually converted to 3-phosphoglycerate (3PG) in the glycolytic pathway.



Glyceraldehyde-3-phosphate $\quad$ 3-Phosphoglycerate



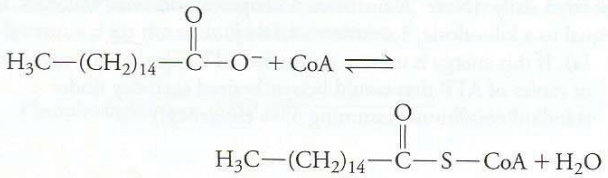
1,3-Bisphosphoglycerate (1,3-BPG)

Consider these two scenarios:

I. GAP is oxidized to 1,3-BPG ($\Delta G^{\circ\prime} = 6.7\ \text{kJ} \cdot \text{mol}^{-1}$), which is subsequently hydrolyzed to yield 3PG ($\Delta G^{\circ\prime} = -49.3\ \text{kJ} \cdot \text{mol}^{-1}$)
II. GAP is oxidized to 1,3BPG, which then transfers its phosphate to ADP, yielding ATP ($\Delta G^{\circ\prime} = -18.8\ \text{kJ} \cdot \text{mol}^{-1}$).

Write the overall equations for the two scenarios. Which is more likely to occur in the cell, and why?

37. Palmitate is activated in the cell by forming a thioester bond to coenzyme A. The $\Delta G^{\circ\prime}$ for the synthesis of palmitoyl-CoA from palmitate and coenzyme A is $31.5\ \text{kJ} \cdot \text{mol}^{-1}$.



(a) What is the ratio of products to reactants at equilibrium for the reaction? Is the reaction favorable? Explain.
(b) Suppose the synthesis of palmitoyl-CoA were coupled with ATP hydrolysis to ADP. The standard free energy for the hydrolysis of the γ phosphate linkage of ATP is $-30.5\ kJ \cdot mol^{-1}$.

$$ATP + H_2O \rightleftharpoons ADP + P_i \quad \Delta G^{\circ\prime} = -30.5\ kJ \cdot mol^{-1}$$

Write the new equation for the activation of palmitate when coupled with ATP hydrolysis to ADP. Calculate $\Delta G^{\circ\prime}$ for the reaction. What is the ratio of products to reactants at equilibrium for the reaction? Is the reaction favorable? Compare your answer to the answer you obtained in part (a).
(c) Suppose the reaction described in part (a) were coupled with ATP hydrolysis to AMP. The standard free energy for the hydrolysis of the β phosphate linkage of ATP is $-32.2\ kJ \cdot mol^{-1}$.

$$ATP + H_2O \rightleftharpoons AMP + PP_i \quad \Delta G^{\circ\prime} = -32.2\ kJ \cdot mol^{-1}$$

Write the new equation for the activation of palmitate when coupled with ATP hydrolysis to AMP. Calculate $\Delta G^{\circ\prime}$ for the reaction. What is the ratio of products to reactants at equilibrium for the reaction? Is the reaction favorable? Compare your answer to the answer you obtained in part (b).
(d) The enzyme pyrophosphatase hydrolyzes pyrophosphate, PP_i:

$$PP_i + H_2O \rightleftharpoons 2\,P_i \quad \Delta G^{\circ\prime} = -33.5\ kJ \cdot mol^{-1}$$

The activation of palmitate, as described in part (c), is coupled to the hydrolysis of pyrophosphate. Write the equation for this coupled reaction. Calculate $\Delta G^{\circ\prime}$ for the reaction. What is the ratio of products to reactants at equilibrium for the reaction? Is the reaction favorable? Compare your answer to the answers you obtained in parts (b) and (c).

38. DNA containing broken phosphodiester bonds ("nicks") can be repaired by the action of a ligase enzyme. Formation of a new phosphodiester bond in DNA requires the free energy of ATP phosphoanhydride bond cleavage. In the ligase-catalyzed reaction, ATP is hydrolyzed to AMP:

$$ATP + \text{nicked bond} \xrightleftharpoons{\text{ligase}} AMP + PP_i + \text{phosphodiester bond}$$

The equilibrium constant expression for this reaction can be rearranged to define a constant, C, as follows:

$$K_{eq} = \frac{[\text{phosphodiester bond}][AMP][PP_i]}{[\text{nick}][ATP]}$$

$$\frac{[\text{nick}]}{[\text{phosphodiester bond}]} = \frac{[AMP][PP_i]}{K_{eq}[ATP]}$$

$$C = \frac{[PP_i]}{K_{eq}[ATP]}$$

$$\frac{[\text{nick}]}{[\text{phosphodiester bond}]} = C[AMP]$$

Researchers have determined the ratio of nicked bonds to phosphodiester bonds at various concentrations of AMP.
(a) Using the data provided, construct a plot of [nick]/[phosphodiester bond] versus [AMP] and determine the value of C from the plot.

[AMP] (mM)	[nick]/[phosphodiester bond]
10	4.0×10^{-5}
15	4.3×10^{-5}
20	5.47×10^{-5}
25	6.67×10^{-5}
30	8.67×10^{-5}
35	9.47×10^{-5}
40	9.30×10^{-5}
45	1.0×10^{-4}
50	1.13×10^{-4}

(b) Determine the value of K_{eq} for the reaction, given that the concentrations of PP_i and ATP were held constant at 1.0 mM and 14 μM, respectively.
(c) What is the value of $\Delta G^{\circ\prime}$ for the reaction?
(d) What is the value of $\Delta G^{\circ\prime}$ for the following reaction?

$$\text{nicked bond} \rightleftharpoons \text{phosphodiester bond}$$

Note that the $\Delta G^{\circ\prime}$ for the hydrolysis of ATP to AMP and PP_i is $-48.5\ kJ \cdot mol^{-1}$ in the presence of 10 mM Mg^{2+}, the conditions used in these experiments.
(e) The $\Delta G^{\circ\prime}$ for the hydrolysis of a typical phosphomonoester to yield P_i and an alcohol is $-13.8\ kJ \cdot mol^{-1}$. Compare the stability of the phosphodiester bond in DNA to the stability of a typical phosphomonoester bond.

SELECTED READINGS

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