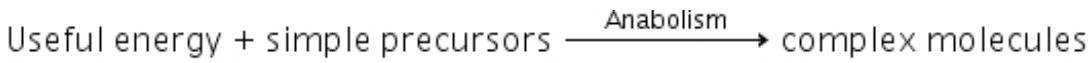
Reactions that construct a more complex molecule from simpler components are called *anabolic reactions* or **anabolism**. These reactions — such as the synthesis of glucose, fats, or DNA — usually require energy. Useful forms of energy that are produced in catabolism are employed in anabolism to generate complex structures from simple ones, or energy-rich states from energy-poor ones.



cnding on the energy conditions in the cell. These pathways are referred to as **amphibolic pathways**.

An important general principle of metabolism is that biosynthetic and degradative pathways are almost always distinct. This separation is necessary for energetic reasons, as will be evident in subsequent chapters. It also facilitates the control of metabolism.

A thermodynamically unfavorable reaction can be driven by a favorable reaction

How are specific pathways constructed from individual reactions? A pathway must satisfy minimally two criteria: (1) the individual reactions must be specific, and (2) each of the reactions that constitutes the pathway must be thermodynamically favored under real, rather than standard, conditions. A reaction that is specific will yield only one particular product or set of products from its reactants. As discussed in Section 5.1, enzymes provide this specificity.

The thermodynamics of metabolism are most readily approached in relation to free energy, which was discussed in Sections 1.3 and 5.2. A reaction can occur spontaneously only if ΔG, the change in free energy,

is negative. Recall that ΔG for the formation of products C and D from substrates A and B is given by

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

Thus, the ΔG of a reaction depends on the nature of the reactants and products (expressed by the $\Delta G^{\circ\prime}$ term, the standard free-energy change) and on their concentrations (expressed by the second term). In a metabolic pathway, reactions with a positive $\Delta G^{\circ\prime}$ can proceed under physiological conditions because the concentrations of reactants and products are far from standard conditions, such that the ΔG of the reaction is negative. Another important thermodynamic fact is that the overall free-energy change for a chemically coupled series of reactions is equal to the sum of the free-energy changes of the individual steps. Consider the following reactions:

$A \rightleftharpoons B + C$	$\Delta G^{\circ\prime} = +21$ kJ mol^{-1} (+5 kcal mol^{-1})
$B \rightleftharpoons D$	$\Delta G^{\circ\prime} = -34$ kJ mol^{-1} (–8 kcal mol^{-1})
$A \rightleftharpoons C + D$	$\Delta G^{\circ\prime} = -13$ kJ mol^{-1} (–3 kcal mol^{-1})

Under standard conditions, A cannot be spontaneously converted into B and C, because $\Delta G^{\circ\prime}$ is positive. However, the conversion of B into D under standard conditions is thermodynamically feasible; if the two reactions are coupled such that one cannot occur without the other, then the free-energy changes are additive. Thus, the conversion of A

into C and D has a $\Delta G^{\circ\prime}$ of $-13\ \text{kJ mol}^{-1}$ $(-3\ \text{kcal mol}^{-1})$, which means that it can occur spontaneously under standard conditions.

Thus, a thermodynamically unfavorable reaction can be driven by a thermodynamically favorable reaction to which it is coupled. This kind of coupling occurs in the active sites of enzymes; in this example, the reactions are coupled by the shared chemical intermediate B, which is both formed and destroyed in the course of the reaction. Metabolic pathways often employ the coupling of thermodynamically favorable reactions to unfavorable reactions to create new enzymatically catalyzed reactions that have an overall negative change in free energy.

Self-Check Question

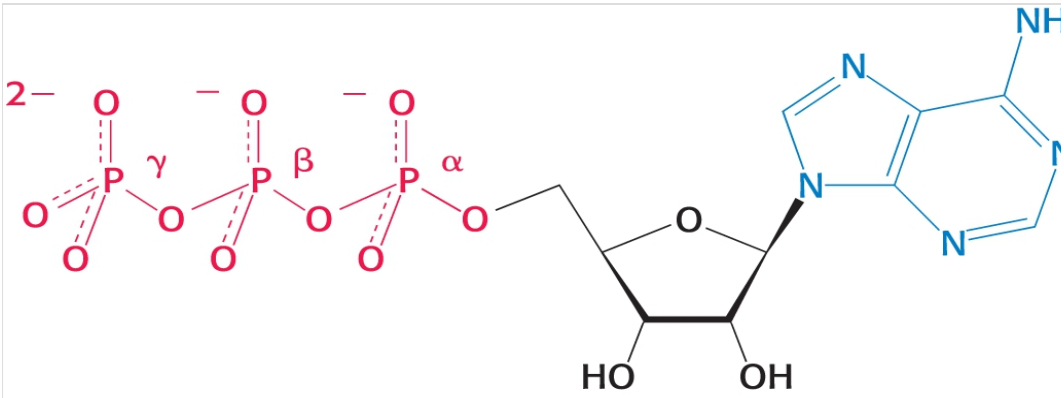
Imagine that a cell needs to produce a compound B, but the reaction converting A into B has a positive $\Delta G^{\circ\prime}$ value. There are two ways that A can still be converted to B; one involves changing the reaction itself, and the other does not. What are they?

15.2 ATP Is the Universal Currency of Free Energy in Biological Systems

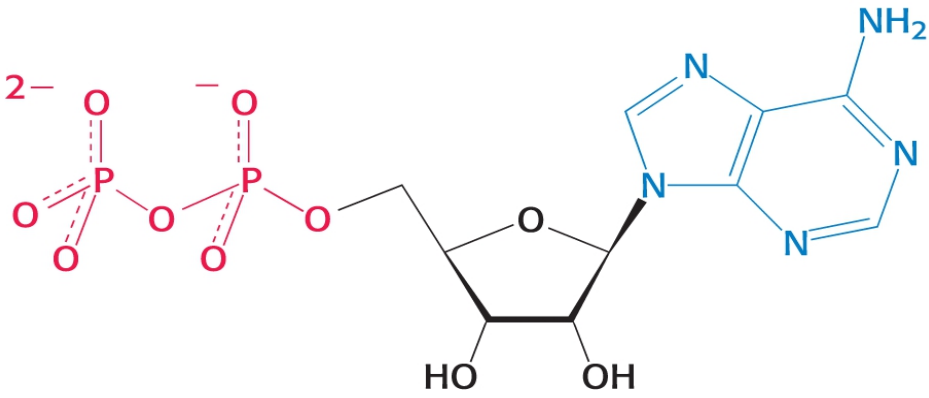
Just as commerce is facilitated by the use of a common currency, the commerce of the cell — metabolism — is facilitated by the use of a common energy currency, adenosine triphosphate (ATP). Part of the free energy derived from light or the oxidation of food is transformed into this highly accessible molecule, which acts as the free-energy donor in most energy-requiring processes such as motion, active transport, and biosynthesis. Indeed, most of catabolism consists of reactions that extract energy from fuels such as carbohydrates and fats and capture it through the production of ATP.

ATP hydrolysis is exergonic

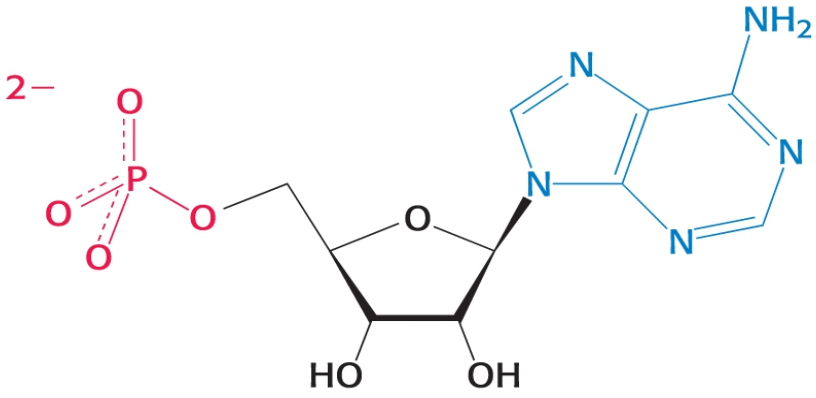
ATP is a nucleotide consisting of adenine, a ribose, and a triphosphate unit (Figure 15.3). The active form of ATP is usually a complex of ATP with Mg^{2+} or Mn^{2+}. In considering the role of ATP as an energy carrier, we can focus on its triphosphate moiety.



Adenosine triphosphate (ATP)



Adenosine diphosphate (ADP)



Adenosine monophosphate (AMP)

FIGURE 15.3 Structures of ATP, ADP, and AMP differ only by the number of phosphates. These adenylates consist of adenine (blue), a ribose (black), and a tri-, di-, or monophosphate unit (red). The innermost phosphorus atom of ATP is designated P_α, the middle one P_β, and the outermost one P_γ.

- *ATP is an energy-rich molecule because its triphosphate unit contains two phosphoanhydride linkages.* A large amount of free energy is released when ATP is hydrolyzed to adenosine diphosphate (ADP) and orthophosphate (P_i), or when ATP is hydrolyzed to adenosine monophosphate (AMP) and pyrophosphate (PP_i).

$$ATP + H_2O \rightleftharpoons ADP + P_i$$
$$\Delta G^{\circ\prime} = -30.5 \text{ kJ mol}^{-1} (-7.3 \text{ kcal mol}^{-1})$$

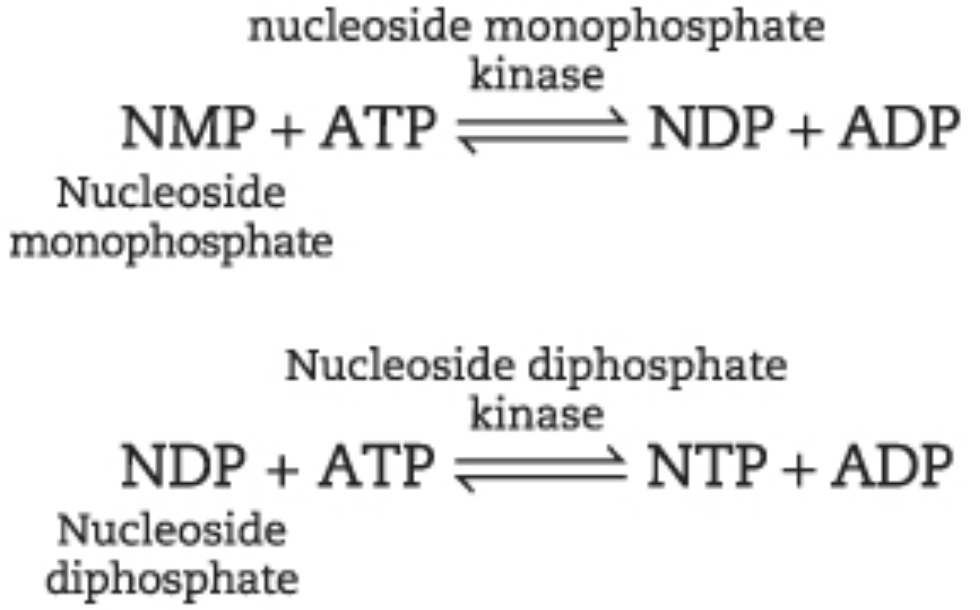
$$ATP + H_2O \rightleftharpoons AMP + PP_i$$
$$\Delta G^{\circ\prime} = -45.6 \text{ kJ mol}^{-1} (-10.9 \text{ kcal mol}^{-1})$$

However, this hydrolysis reaction is frequently misunderstood. It is imperative to note that this release of free energy does *not* derive from the cleavage of the any of the covalent bonds in ATP, as it requires energy to break a covalent bond. Instead, the energy is released by the formation of new covalent bonds and noncovalent interactions with water, and from the increase in entropy of the products relative to the reactants. Furthermore, the precise ΔG for these reactions depends on the ionic strength of the medium and on the concentrations of Mg^{2+} and other metal ions. Under typical

cellular concentrations, the ΔG for these hydrolyses is approximately $-50\ \text{kJ mol}^{-1} (-12\ \text{kcal mol}^{-1})$.

- *The ATP–ADP cycle is the fundamental mode of energy exchange in biological systems.* The free energy released in the hydrolysis of ATP is harnessed to drive reactions that require an input of free energy, such as muscle contraction. In turn, ATP is formed from ADP and P_i when fuel molecules are oxidized in chemotrophs or when light is trapped by phototrophs.

- *Enzymes catalyze the exchange of phosphoryl groups from one nucleotide to another.* Some biosynthetic reactions are driven not by ATP, but by guanosine triphosphate (GTP), uridine triphosphate (UTP), and cytidine triphosphate (CTP). The diphosphate forms of these nucleotides are denoted by GDP, UDP, and CDP, and the monophosphate forms by GMP, UMP, and CMP. The phosphorylation of nucleoside monophosphates is catalyzed by a family of nucleoside monophosphate kinases. The phosphorylation of nucleoside diphosphates is catalyzed by nucleoside diphosphate kinase, an enzyme with broad specificity.



- *Although all of the nucleoside triphosphates are energetically equivalent, ATP is nonetheless the primary cellular energy carrier and therefore the energy currency.* In addition, two important electron carriers — NAD^+ and FAD — as well the acyl group carrier, coenzyme A, are derivatives of ATP. It is intriguing to consider why evolution selected adenosine derivatives, but it may be due to differences in the stability of adenine compared to the other bases in prebiotic conditions. Regardless of the reason, the role of adenosine phosphates — particularly ATP — in energy metabolism is paramount.

ATP hydrolysis drives metabolism by shifting the equilibrium of coupled reactions

An otherwise unfavorable reaction can be made possible by coupling to ATP hydrolysis. Consider a reaction that is thermodynamically unfavorable without an input of free energy, a situation common to most biosynthetic reactions. Suppose that the standard free energy of the B is $+16.7\ \text{kJ mol}^{-1}$ or $+4.0\ \text{kcal mol}^{-1}$

$$\text{A} \rightleftharpoons \text{B} \quad \Delta G^{\circ\prime} = +16.7\ \text{kJ mol}^{-1}\ (+4.0\ \text{kcal mol}^{-1})$$

The equilibrium constant K'_{eq} of this reaction at 25°C is related to $\Delta G^{\circ\prime}$ (in units of kilojoules per mole) by

$$K'_{eq} = [\text{B}]_{eq}/[\text{A}]_{eq} = e^{-\Delta G^{\circ\prime}/2.47} = 1.15 \times 10^{-3}$$

Thus, net conversion of A into B cannot take place when the molar ratio of B to A is equal to or greater than 1.15×10^{-3}.

However, A can be converted into B under these conditions if the reaction is coupled to the hydrolysis of ATP. Under standard conditions, the $\Delta G^{\circ\prime}$ of hydrolysis is approximately $-30.5\ \text{kJ mol}^{-1}$ or $-7.3\ \text{kcal mol}^{-1}$. The new overall reaction is

$$\text{A} + \text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{B} + \text{ADP} + \text{P}_\text{i}$$

$$\Delta G^{\circ\prime} = -13.8\ \text{kJ mol}^{-1}\ (-3.3\ \text{kcal mol}^{-1})$$

Its free-energy change of $-13.8\ \text{kJ mol}^{-1}$ or $-3.3\ \text{kcal mol}^{-1}$ is the sum of the value of $\Delta G^{\circ\prime}$ for the conversion of A into B ($+16.7\ \text{kJ mol}^{-1}$ or $+4.0\ \text{kcal mol}^{-1}$) and the value of $\Delta G^{\circ\prime}$ for the hydrolysis of ATP ($-30.5\ \text{kJ mol}^{-1}$ or $-7.3\ \text{kcal mol}^{-1}$). At pH 7, the equilibrium constant of this coupled reaction is

$$K'_{eq} = \frac{[B]_{eq}}{[A]_{eq}} \times \frac{[ADP]_{eq}[P_i]_{eq}}{[ATP]_{eq}} = e^{13.8/2.47} = 2.67 \times 10^2$$

At equilibrium, the ratio of [B] to [A] is given by

$$\frac{[B]_{eq}}{[A]_{eq}} = K'_{eq} \frac{[ATP]_{eq}}{[ADP]_{eq}[P_i]_{eq}}$$

which means that the hydrolysis of ATP enables A to be converted into B until the [B]/[A] ratio reaches a value of 2.67×10^2.

This equilibrium ratio is strikingly different from the value of 1.15×10^{-3} for the reaction $A \rightarrow B$ in the absence of ATP hydrolysis. In other words, coupling the hydrolysis of ATP with the conversion of A into B under standard conditions has changed the equilibrium ratio of B to A by a factor of about 10^5. If we were to use the ΔG of hydrolysis of

ATP under cellular conditions ($-50.2\ \text{kJ mol}^{-1}$ or $-12\ \text{kcal mol}^{-1}$) in our calculations instead of $\Delta G^{\circ\prime}$, the change in the equilibrium ratio would be even more dramatic, on the order of 10^8.

We see here the thermodynamic essence of ATP's action as an energy-coupling agent. Cells maintain ATP levels by using oxidizable substrates or light as sources of free energy for synthesizing the molecule. In the cell, the hydrolysis of an ATP molecule in a coupled reaction then changes the equilibrium ratio of products to reactants by a very large factor, of the order of 10^8. More generally, the hydrolysis of *n* ATP molecules changes the equilibrium ratio of a coupled reaction (or sequence of reactions) by a factor of 10^{8n}. For example, the theoretical hydrolysis of three ATP molecules in a coupled reaction changes the equilibrium ratio by a factor of 10^{24}. Thus, a thermodynamically unfavorable process can be converted into a favorable one by coupling it to the hydrolysis of a sufficient number of ATP molecules in a new reaction.

It should also be emphasized that A and B in the preceding coupled reaction may be interpreted very generally, not only as different chemical species. For example, A and B may represent activated and unactivated conformations of a protein that is activated by phosphorylation with ATP. Through such changes in protein conformation, molecular motors such as myosin, kinesin, and dynein convert the chemical energy of ATP into mechanical energy (Section 6.5). Indeed, this conversion is the basis of muscle contraction.

Alternatively, A and B may refer to the concentrations of an ion or molecule on the outside and inside of a cell, as in the active transport of a nutrient. The active transport of Na^+ and K^+ across membranes is

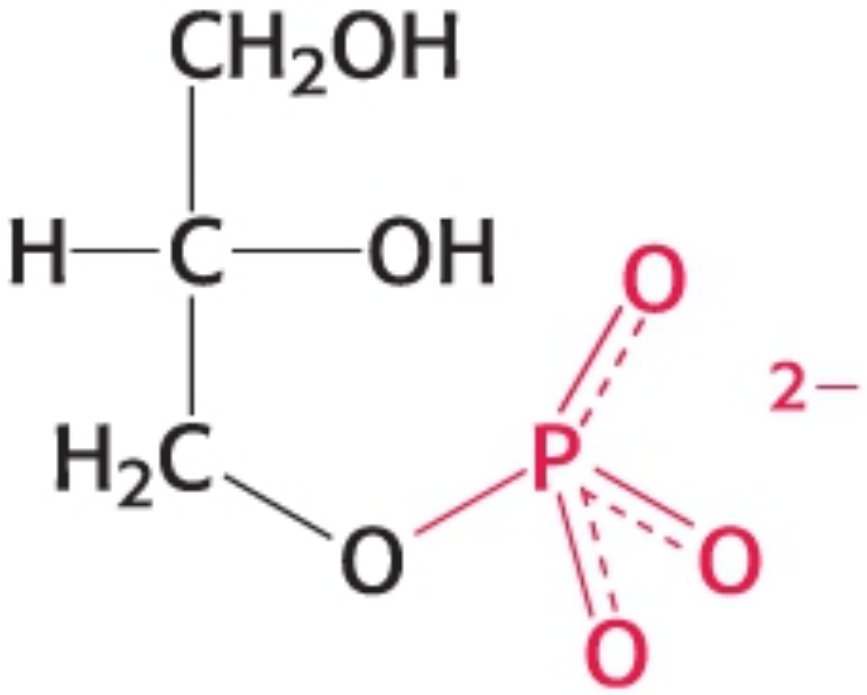
driven by the phosphorylation of the sodium–potassium pump by ATP and its subsequent dephosphorylation (Section 13.2).

The high phosphoryl potential of ATP results from structural differences between ATP and its hydrolysis products

What makes ATP an efficient phosphoryl-group donor? Let us compare the standard free energy of hydrolysis of ATP with that of a phosphate ester, such as glycerol 3-phosphate:

$$\text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{ADP} + \text{P}_\text{i}$$
$$\Delta G^{\circ\prime} = -30.5 \text{ kJ mol}^{-1} (-7.3 \text{ kcal mol}^{-1})$$

$$\text{Glycerol 3-phosphate} + \text{H}_2\text{O} \rightleftharpoons \text{glycerol} + \text{P}_\text{i}$$
$$\Delta G^{\circ\prime} = -9.2 \text{ kJ mol}^{-1} (-2.2 \text{ kcal mol}^{-1})$$



Glycerol 3-phosphate

The magnitude of $\Delta G^{\circ\prime}$ for the hydrolysis of glycerol 3-phosphate is much smaller than that of ATP, which means that ATP has a stronger tendency to transfer its terminal phosphoryl group to water than does glycerol 3-phosphate. In other words, ATP has a higher **phosphoryl-transfer potential** than glycerol 3-phosphate.

The high phosphoryl-transfer potential of ATP can be explained by features of the ATP structure. Because $\Delta G^{\circ\prime}$ depends on the difference in free energies of the products and reactants, we need to examine the

structures of both ATP and its hydrolysis products, ADP and P_i, to answer this question. Four factors are important:

1. *Resonance stabilization*. Orthophosphate (P_i), one of the products of ATP hydrolysis, has greater resonance stabilization than do any of the ATP phosphoryl groups. Orthophosphate has several resonance forms of similar energy (Figure 15.4), whereas the γ phosphoryl group of ATP has a smaller number.

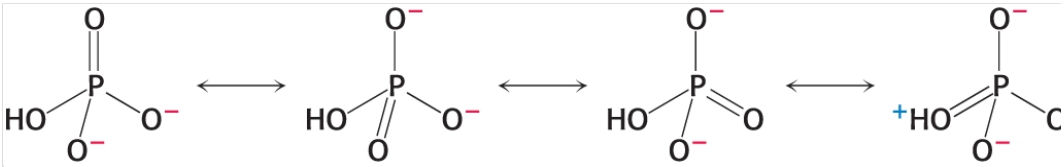


FIGURE 15.4 Orthophosphate has four favorable resonance structures.

2. *Electrostatic repulsion.* At pH 7, the triphosphate unit of ATP carries about four negative charges. These charges repel one another because they are in close proximity. The repulsion between them is reduced when ATP is hydrolyzed.

3. *Increase in entropy*. The entropy of the products of ATP hydrolysis is greater, in that there are now two molecules instead of a single ATP molecule. We disregard the molecule of water used to hydrolyze the ATP; given the high concentration (55.5 M), there is effectively no change in the concentration of water during the reaction.

4. *Stabilization due to hydration.* Water binds to ADP and P_i, stabilizing these molecules, and rendering the reverse reaction — the synthesis of ATP — more unfavorable.

ATP is often called a high-energy phosphate compound, and its phosphoanhydride linkages are sometimes referred to as high-energy

bonds. Indeed, a “squiggle” $(\sim P)$ is often used to indicate the bonds that can be broken in ATP hydrolysis. This is a misnomer, however, as there is nothing special about the bonds themselves. The phosphoanhydride linkages are high-energy in the sense that much free energy is released when they are hydrolyzed — a reaction with water — for the reasons listed above, but the energy release comes from entropy and new bond formation, rather than bond cleavage.

Phosphoryl-transfer potential is an important form of cellular energy transformation

The standard free energies of hydrolysis provide a convenient means of comparing the phosphoryl-transfer potential of phosphorylated compounds. Such comparisons reveal that ATP is not the only compound with a high phosphoryl-transfer potential. In fact, some compounds in biological systems have a higher phosphoryl-transfer potential than that of ATP. These compounds include phosphoenolpyruvate (PEP), 1,3-bisphosphoglycerate (1,3-BPG), and creatine phosphate (**Figure 15.5**).

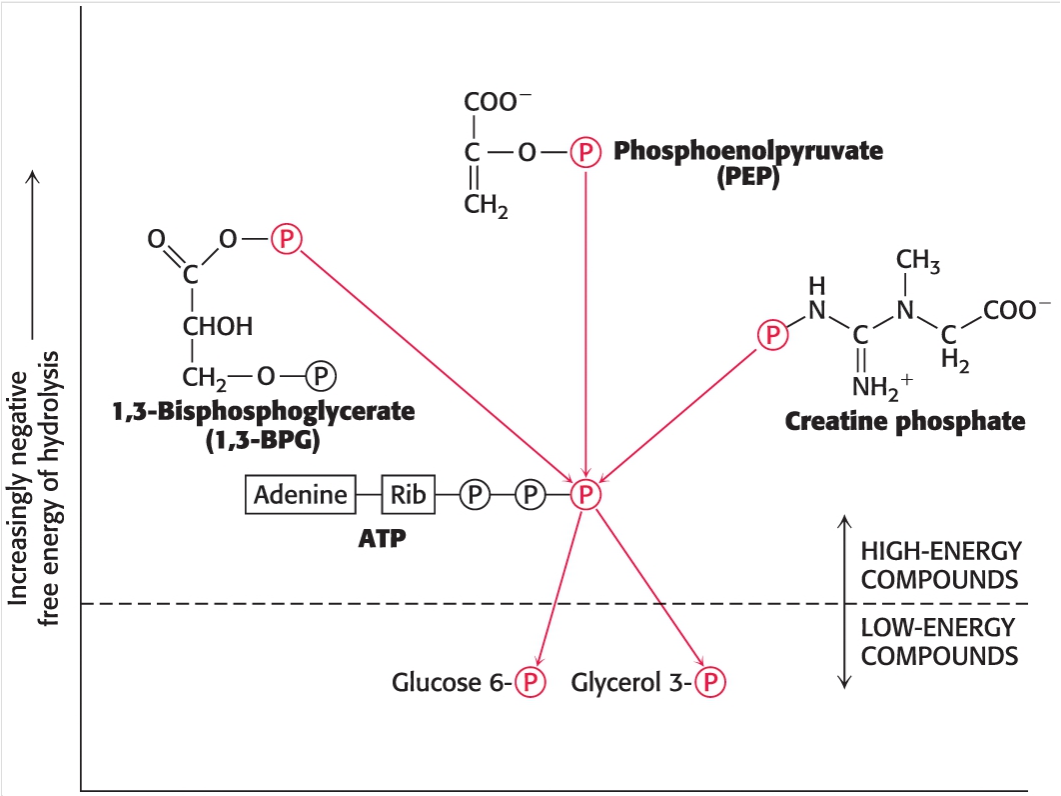


FIGURE 15.5 Compounds with high phosphoryl-transfer potential can be used to make ATP from ADP. The role of ATP as the cellular energy currency is illustrated by its relation to other phosphorylated compounds. ATP has a phosphoryl-transfer potential that is intermediate among the biologically important phosphorylated molecules. High-phosphoryl-transfer-potential compounds (1,3-BPG, PEP, and creatine phosphate) derived from the metabolism of fuel molecules are used to power ATP synthesis. In turn, ATP donates a phosphoryl group to other biomolecules to facilitate their metabolism.

[Data from D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, 5th ed. (W. H. Freeman and Company, 2009), Fig. 13-19.]

PEP can transfer its phosphoryl group to ADP to form ATP. Indeed, this transfer is one of the ways in which ATP is generated in the breakdown of sugars (Section 16.1). It is significant that ATP has a phosphoryl-transfer potential that is intermediate among the biologically important phosphorylated molecules (**Table 15.1**). This intermediate position enables ATP to function efficiently as a carrier of phosphoryl groups.

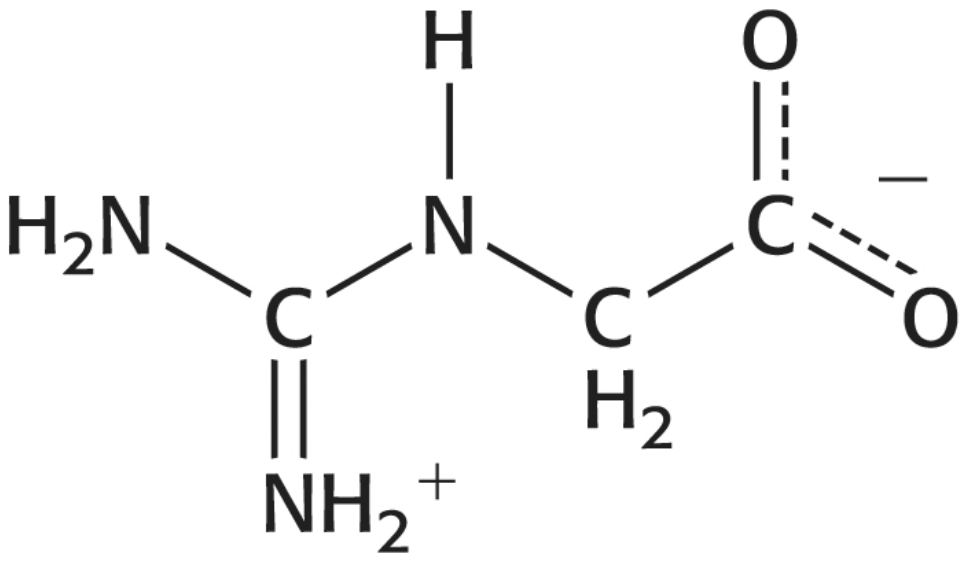
TABLE 15.1 Standard free energies of hydrolysis of some phosphorylated compounds

Compound	$kJ\ mol^{-1}$	$kJ\ mol^{-1}$
Phosphoenolpyruvate	−61.9	−14.8
1,3-Bisphosphoglycerate	−49.4	−11.8
Creatine phosphate	−43.1	−10.3
ATP (to ADP)	−30.5	−7.3
Glucose 1-phosphate	−20.9	−5.0
Pyrophosphate	−19.3	−4.6
Glucose 6-phosphate	−13.8	−3.3
Glycerol 3-phosphate	−9.2	−2.2

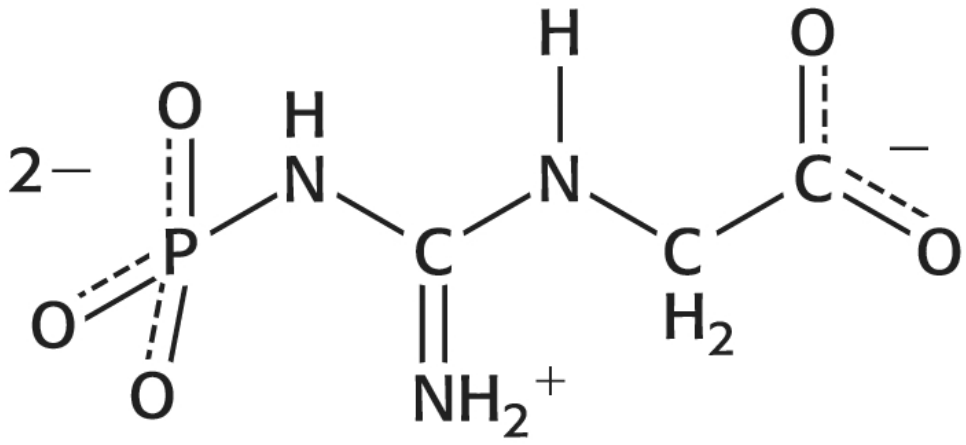
The amount of ATP in muscle suffices to sustain contractile activity for less than a second. Creatine phosphate in vertebrate muscle serves as a reservoir of high-potential phosphoryl groups that can be readily transferred to ADP. Indeed, we use creatine phosphate to regenerate ATP from ADP every time we exercise strenuously. This reaction is catalyzed by creatine kinase.

$$\text{Creatine phosphate} + \text{ADP} \xrightleftharpoons{\text{creatine kinase}} \text{ATP} + \text{creatine}$$

At pH 7, the standard free energy of hydrolysis of creatine phosphate is $-43.1\ \text{kJ mol}^{-1}\ (-10.3\ \text{kcal mol}^{-1})$ compared with $-30.5\ \text{kJ mol}^{-1}\ (-7.3\ \text{kcal mol}^{-1})$ for ATP.



Creatine



Creatine phosphate

As illustrated in the next boxed example, there is a large amount of creatine phosphate present in resting muscle. Because of its abundance and high phosphoryl-transfer potential relative to that of ATP, creatine phosphate is a highly effective phosphoryl buffer. Indeed, creatine phosphate is the major source of phosphoryl groups for ATP regeneration for a runner during the first 4 seconds of a 100-meter sprint. The fact that creatine phosphate can replenish ATP pools is the basis of the use of creatine as a dietary supplement by athletes in sports requiring short bursts of intense activity. After the creatine phosphate pool is depleted, ATP must be generated through anaerobic or aerobic metabolism (Figure 15.6).

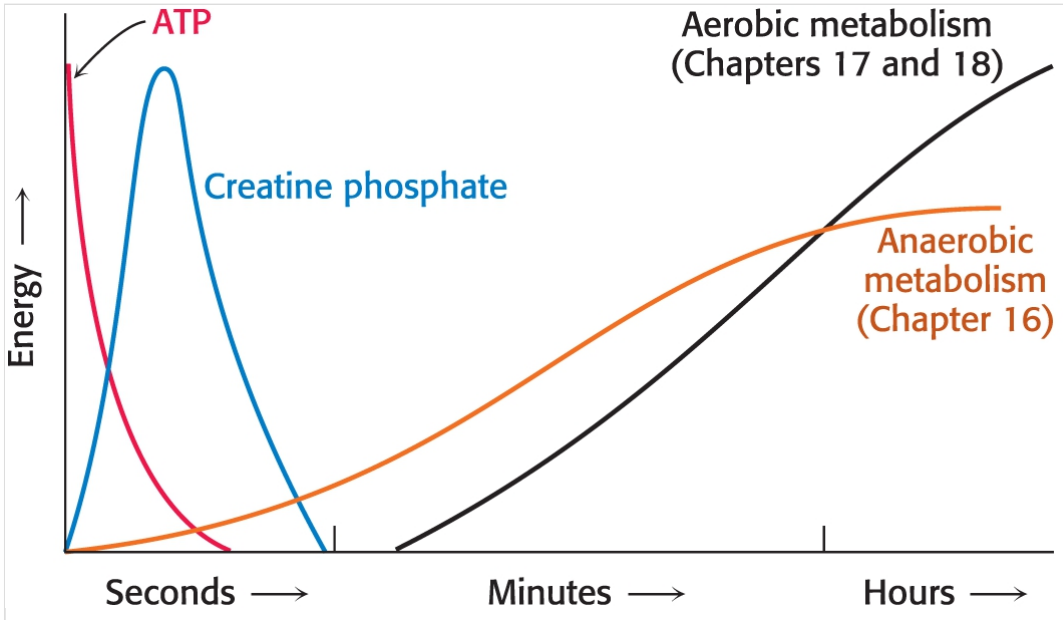


FIGURE 15.6 The sources of ATP change as exercise duration increases, even within the first few seconds. In the initial seconds of exertion, power is generated by existing high-phosphoryl-transfer compounds (ATP and creatine phosphate). Subsequently, the ATP must be regenerated by metabolic pathways.

EXAMPLE

Calculating ΔG for a Coupled Reaction Under Real Conditions

PROBLEM:

Creatine phosphate is used as a phosphoryl donor for ATP synthesis in muscle in the following reaction:

$$\text{Creatine phosphate} + \text{ADP} \overset{\text{creatine kinase}}{\rightleftharpoons} \text{ATP} + \text{creatine}$$

When muscles are at rest, the reaction proceeds in the reverse direction, synthesizing creatine phosphate. As noted above, for a skeletal muscle at rest, the following metabolites are present at the indicated concentrations.

$$[\text{ATP}] = 4\ \text{mM}; \text{ADP} = 0.013\ \text{mM}$$
$$[\text{creatine phosphate}] = 25\ \text{mM}; [\text{creatine}] = 13\ \text{mM}$$

Calculate the ΔG for the creatine kinase reaction as written above for a muscle at rest assuming body temperature is 37°C.

Getting Started:

What formula do we need to calculate ΔG, and what information are we missing to complete this calculation?

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

Analyze:

The values necessary for the right-hand term are provided, but we need to know $\Delta G^{\circ\prime}$ for the kinase reaction. How do we determine $\Delta G^{\circ\prime}$? Recall from this chapter that two component parts of a reaction have

additive $\Delta G^{\circ\prime}$ values. We need to dissect the creatine kinase reaction into its two component reactions and look up the $\Delta G^{\circ\prime}$ values for each of the component reactions. These are:

$$ADP + P_i \rightarrow ATP + H_2O$$
$$\text{Creatine phosphate} + H_2O \rightarrow \text{creatine} + P_i$$

Using Table 15.1, we can determine the $\Delta G^{\circ\prime}$ values for both reactions.

For ATP synthesis, $\Delta G^{\circ\prime} = +30.5\ \text{kJ mol}^{-1}$. For creatine phosphate hydrolysis, $\Delta G^{\circ\prime} = -43.1\ \text{kJ mol}^{-1}$.

Calculate:

What is the $\Delta G^{\circ\prime}$ of the overall creatine kinase reaction in the direction written?

$\Delta G^{\circ\prime}$ values are additive, so the $\Delta G^{\circ\prime}$ for the kinase reaction is $-43.1\ \text{kJ mol}^{-1} + 30.5\ \text{kJ mol}^{-1} = -12.6\ \text{kJ mol}^{-1}$.

Now, having determined $\Delta G^{\circ\prime}$, we can use the equation above and the values provided to determine ΔG.

$$\Delta G = -12.6\ \text{kJ mol}^{-1} + RT\ \ln\left(\frac{[\text{ATP}][\text{creatine}]}{[\text{ADP}][\text{creatine phosphate}]}\right)$$

$$= -12.6\ \text{kJ mol}^{-1} + (0.0083\ \text{kJ K}^{-1}\ \text{mol}^{-1})(310\ \text{K})\ \ln\left(\frac{[4\ \text{mM}][13\ \text{mM}]}{[0.013\ \text{mM}][25\ \text{mM}]}\right)$$

$$= -12.6\ \text{kJ mol}^{-1} + (2.573\ \text{kJ mol}^{-1})\ \ln(160)$$

$$= 0.5\ \text{kJ mol}^{-1}$$

Reflect:

Note that this value is close to zero, indicating that the reaction is very near equilibrium. Does this make sense to you, for resting muscle? Consider that upon sudden muscle contraction during exercise, the depletion of ATP shifts the reaction toward creatine and ATP. Conversely, during the recovery from exercise, the replenishment of ATP from oxidative phosphorylation shifts the reaction back toward the formation of creatine phosphate. Once replenished, and when the muscle is at rest, we should expect that the reaction will eventually settle at equilibrium concentrations and, therefore, ΔG should be near zero.