

Bioenergetics

Catabolism involves more than the simple breakdown of compounds. Combustion of glucose yields large amounts of energy ($\Delta G^\circ = -2870 \text{ kJ/mol}$ for glucose conversion to carbon dioxide and water); however, most of this energy is released as heat. Metabolic processes occur in **many** steps to allow both the diversion of energy in usable form and the diversion of catabolic pathway intermediates for biosynthetic processes.

One major method for diverting energy in usable form involves the use of “high-energy phosphate” compounds.

High-energy phosphate compounds

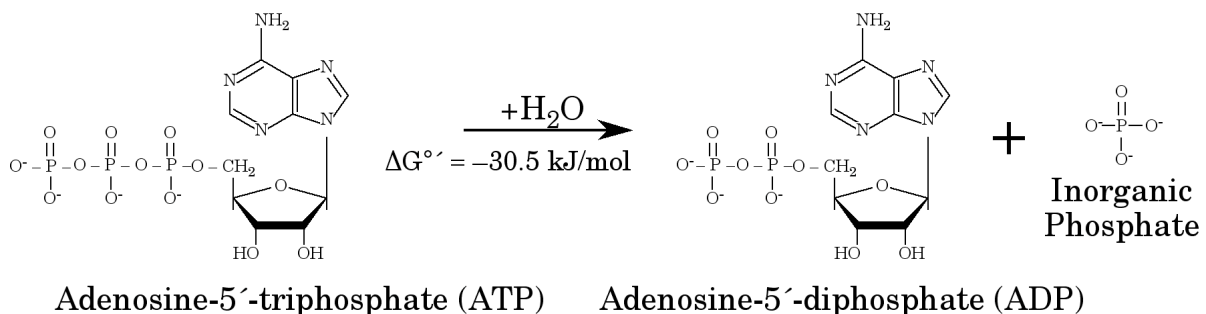
Phosphate-containing compounds are considered “high-energy” if they have large $-\Delta G^\circ$ for hydrolysis (“large” meaning “more negative than -20 to -25 kJ/mol ”). High-energy phosphate compounds are **not** used for long-term energy storage. They are temporary forms of stored energy, and are used to carry energy from one reaction to another.

High-energy phosphate compounds are **not** necessarily unstable. Remember that ΔG° does **not** indicate anything about the rate of a reaction; most “high-energy compounds” are quite stable, because the hydrolysis reaction activation energy is quite large. In most cases, the conversion of a high-energy compound to a low-energy compound at detectable rates requires the intervention of an enzyme.

Examples of high-energy compounds

Organisms use a number of high-energy compounds. The following includes a few examples. The ΔG° for the hydrolysis reaction are included for each reaction. Note that, in most cases, the hydrolysis reaction as drawn rarely occurs in cells.

One of these compounds deserves your special attention: **ATP**. **Anyone studying biochemistry will become very familiar with ATP**. ATP is an extremely useful molecule for exchanging energy between enzymes.

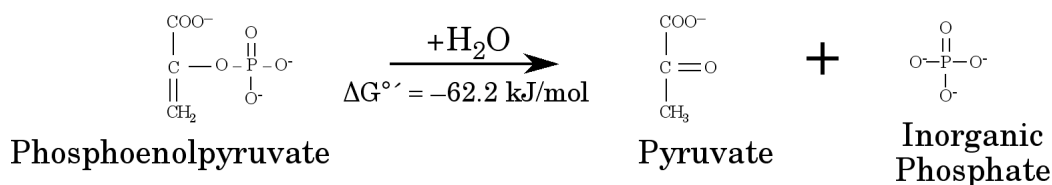


The hydrolysis of ATP has a ΔG° of -30.5 kJ/mol (note that the precise value is somewhat variable, depending on the presence of magnesium and other non-reactant species that alter the energetics of the reaction). In addition, because in cells the concentration of ATP is typically considerably higher than that of ADP, the ΔG for the reaction is more negative than the ΔG° value. Recall that ΔG is a measure of the amount of energy available to do work; ATP hydrolysis can therefore

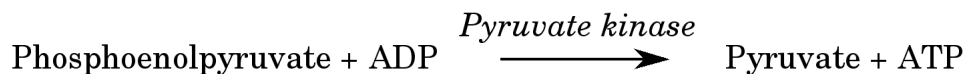
donate energy to other systems to allow those systems to perform reactions that would otherwise be thermodynamically unfavorable.

Among phosphate-containing molecules, ATP has a **central location**. Although it contains high-energy phosphate bonds, the energy in each of the ATP phosphoanhydride bonds is somewhat lower than the energy of a few other biological molecules.

For example, the hydrolysis of phosphoenolpyruvate has a large negative ΔG° , due to the fact that the covalent bond to the phosphate traps pyruvate in the energetic enol configuration.

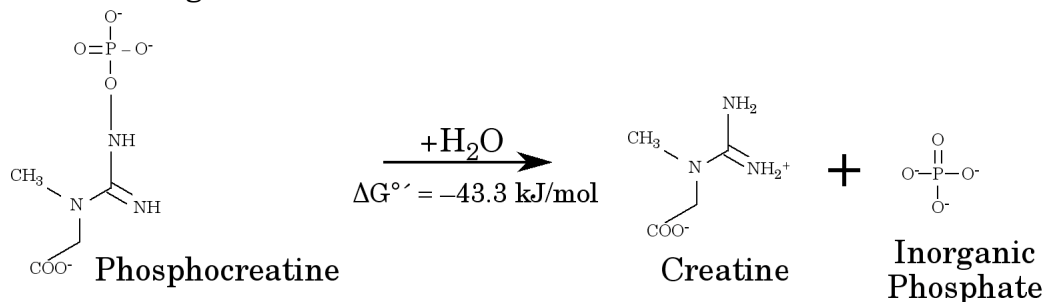


Phosphoenolpyruvate has a much higher ΔG° than ATP. In cells, the direct hydrolysis of phosphoenolpyruvate does not occur; instead, the energy stored in this molecule is transferred to ATP in a reaction catalyzed by the enzyme **pyruvate kinase**.

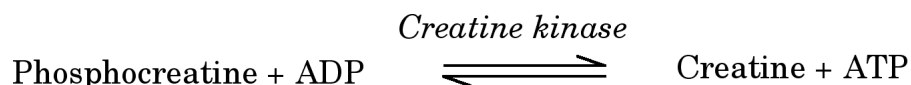


In this case, the two reactions (*i.e.* the hydrolysis of phosphoenolpyruvate and the reverse of the hydrolysis of ATP) are **coupled**. The normally unfavorable reaction (the conversion of ADP + phosphate to ATP) becomes a spontaneous process.

A second interesting reaction is:



Note that the ΔG° for phosphocreatine hydrolysis is also strongly negative. In cells, the reaction catalyzed by creatine kinase (below) is reversible (this reaction is important in muscle as a method for rapidly generating ATP under conditions of rapid utilization; resting muscles replenish their supply of phosphocreatine using the right-to-left reaction).



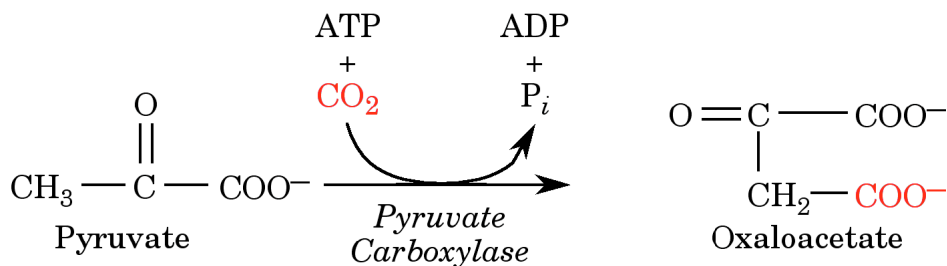
The ΔG° for the reaction is -12.8 kJ/mol. Note that this is the sum of the hydrolysis ΔG° values: -43.3 kJ/mol + 30.5 kJ/mol. For this calculation, the ADP conversion to ATP is the reverse of the hydrolysis reaction, and therefore has a $+\Delta G^\circ$.

If you consider the moderately large ΔG° for the creatine kinase reaction, it is somewhat surprising that this reaction is reversible. Once again, however, it is necessary to remember that **ΔG° does not determine whether a reaction is spontaneous**. Instead, it is **the ΔG for the reaction that determines whether a reaction is spontaneous**. The normal concentration of ATP in the cell is **much** higher than the concentration of ADP. In resting cells, the relative concentrations of ADP and ATP are such that the ΔG for hydrolysis of ATP is about -50 kJ/mol.

The creatine kinase reaction thus illustrates two important points. The first point is that the actual physiological value of ΔG varies depending on the cellular conditions. Reactions may therefore be reversible or irreversible under cellular conditions, in spite of sizable ΔG° values. The second point is that, for multisubstrate reactions, it is possible to **couple** a favorable reaction to an unfavorable one. When this is done, the overall ΔG° is the sum of the ΔG° for the individual reactions (as shown above).

The coupled reactions shown thus far involve the transfer of phosphate from one molecule to another (or the hydrolysis of phosphate compounds). ATP is heavily involved in these phosphate transfer reactions, and most kinase² reactions use ATP as the phosphate donor.

However, in some cases, cleavage of the high-energy bond is used entirely as a driving force, and the reaction does result in hydrolysis of the bond. Once again, ATP is the most commonly used energy source for these reactions. An example of this is the reaction catalyzed by the biotin-dependent enzyme *pyruvate carboxylase*. The product oxaloacetate does not contain a phosphate. Instead, the thermodynamically unfavorable addition of carbonate to the pyruvate is coupled to the thermodynamically favorable ATP hydrolysis by the enzyme.



ATP hydrolysis is also widely used by **active transport pump proteins** that move

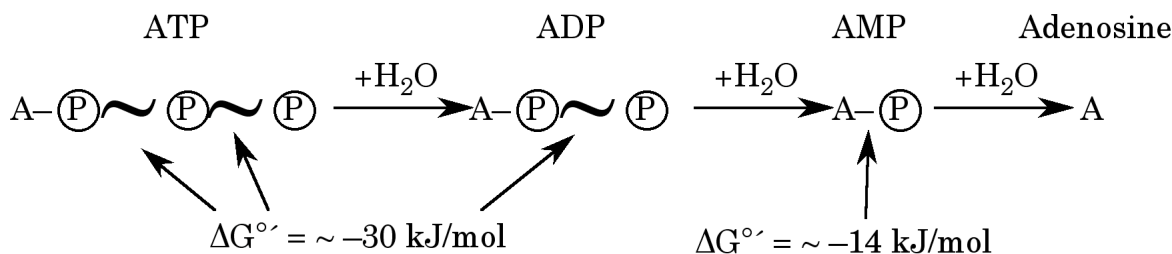
² A “kinase” is an enzyme that phosphorylates its substrate. The name of the enzyme usually comes from the phosphate acceptor. In some cases, confusion as to the physiological substrate results in confusing nomenclature. As an example, the name pyruvate kinase implies that the enzyme phosphorylates pyruvate, while in fact this physiologically irreversible enzyme produces pyruvate and ATP from phosphoenolpyruvate and ADP.

molecules across membranes against electrochemical gradients. Once again, this process uses the ΔG released by ATP hydrolysis to perform a process that would otherwise be thermodynamically unfavorable.

Representations of high-energy phosphate bonds

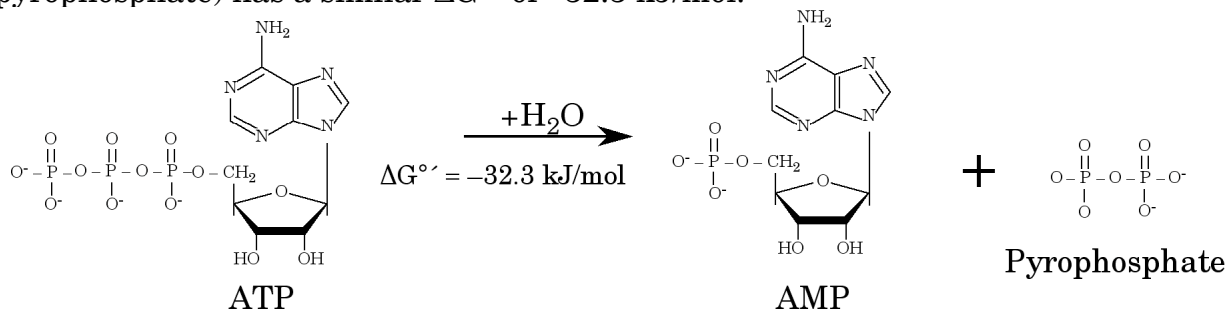
In some cases, the actual structure of the compound is of secondary importance; instead it may be more useful to emphasize the fact that the phosphate bond contains usable energy.

The reaction diagram below shows the hydrolysis events possible for ATP. Note the “~” between the \square and \square phosphates (*i.e.* the last two phosphates in the molecule). This “~” indicates the presence of a high-energy phosphate bond. Note also the hydrolysis of the \square phosphate (which releases free adenosine from AMP) has a ΔG° of only about -14 kJ/mol; this is not considered to be a high-energy phosphate bond; this fact is emphasized by drawing the bond as a straight line.



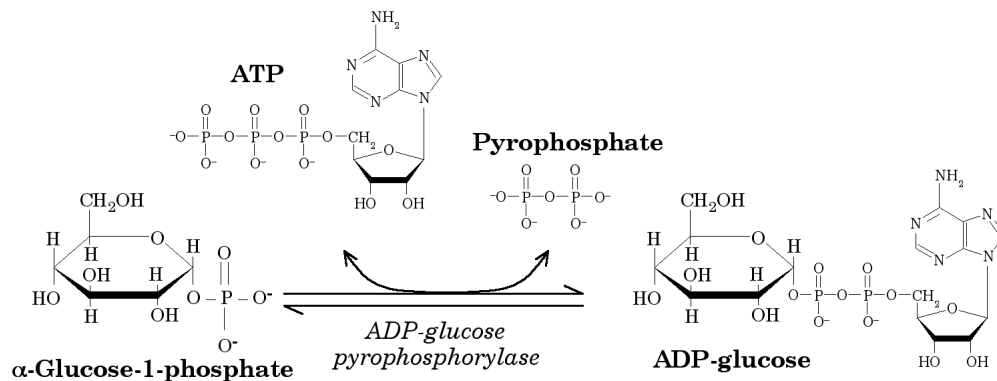
ATP conversion to ADP or AMP

As noted above, ATP contains two high-energy phosphate bonds. Depending on the process, the hydrolysis of ATP can result in release or transfer of either one or two phosphates. Releasing one phosphate (conversion of ATP to ADP) has a ΔG° of -30.5 kJ/mol. Releasing two phosphates (conversion of ATP to AMP, with release of pyrophosphate) has a similar ΔG° of -32.3 kJ/mol.



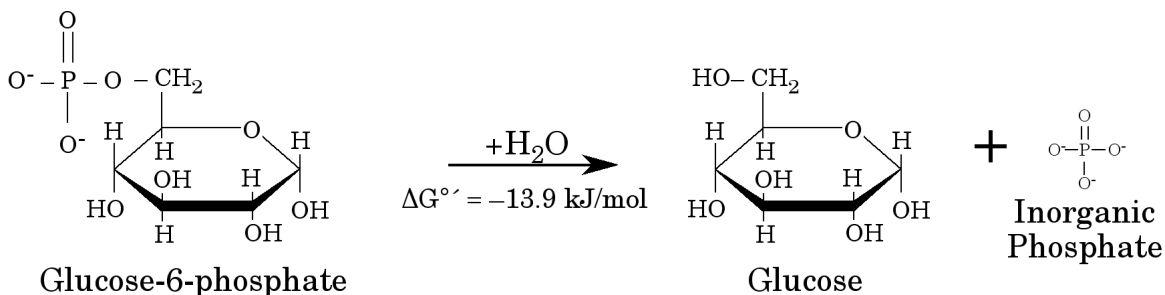
In living organisms, however, the release of pyrophosphate can increase the *effective* ΔG of the reaction. As an example, the reaction catalyzed by ADP-glucose pyrophosphorylase is reversible. In the bacterial and plant cells that perform the process, however, the pyrophosphate is rapidly hydrolyzed by *pyrophosphatase* to inorganic phosphate (a reaction with a ΔG° of -33.6 kJ/mol). The result is an irreversible reaction, in which the ADP-glucose product cannot be converted back to ATP + glucose-1-phosphate because the levels of pyrophosphate are too low to allow it.

Thus, the release of pyrophosphate and AMP can drive the reaction more strongly than can release of ADP and phosphate, in spite of the fact that the directly coupled reaction (the release of pyrophosphate from ATP) has a very similar ΔG° to that of ATP hydrolysis to ADP.



Low-energy phosphates

Not all phosphate-containing molecules contain enough energy to drive other reactions. One example of this, mentioned above, is the hydrolysis of AMP to adenosine. Another example is the hydrolysis of glucose-6-phosphate to glucose:

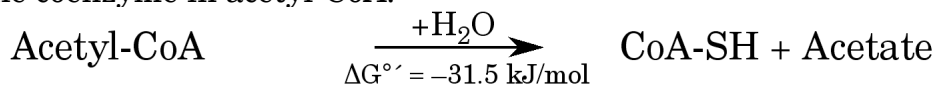


The ΔG° for this reaction is also about -14 kJ/mol . This ΔG° is sufficient to result in a negative ΔG for the hydrolysis reaction under most physiological conditions; however, the negative ΔG is not usually enough to drive other reactions.

For cells, it is fortunate that this reaction has a relatively low ΔG° ; the phosphate present in glucose-6-phosphate is donated by ATP. The phosphorylation of glucose using ATP is therefore usually spontaneous in cells (the ΔG° for ATP-dependent glucose phosphorylation is -16.6 kJ/mol).

Other high-energy bonds

Although high-energy phosphates are very commonly used, they are not the only physiologically relevant high-energy compounds. Another common high-energy bond is present in thioester compounds. An example is the bond that links the acetyl group to the coenzyme in acetyl-CoA.



In addition, some other molecules can act as energy storage molecules, although they do not obviously contain high-energy bonds. A good example of this is NADH, which stores a significant amount of energy. To see how, we need to look at the energy involved in oxidation and reduction reactions.

Energy in redox reactions:

Oxidation and reduction of molecules involves transfer of energy. The amount of energy depends on the reduction potential of the molecules involved. The energy can be calculated from the equation:

$$\Delta G^{\circ} = -nF\Delta E^{\circ}$$

In this equation, ΔE° is the standard reduction potential difference, n is the number of electrons transferred, and F is the Faraday constant 96485 J/(V•mol). Note that this equation applies to standard conditions; concentrations of reactants and products alter the actual ΔE for the process (and therefore the ΔG for the process).

Side Note: Reduction Potentials and the Nernst equation

Note that most tables give **reduction potentials**. The reduction potential for NAD is -0.32 V. For the process of converting NADH to NAD, the reaction involves oxidizing NADH; the oxidation potential is $(-1)(\text{reduction potential})$.

The equation given above for calculating ΔG° is for standard conditions (as you can tell from the “ ΔG° ”). The Nernst equation allows the determination of the free energy change under the actual conditions of reagent concentration and temperature that exist in the reaction.

The Nernst equation is shown at right.

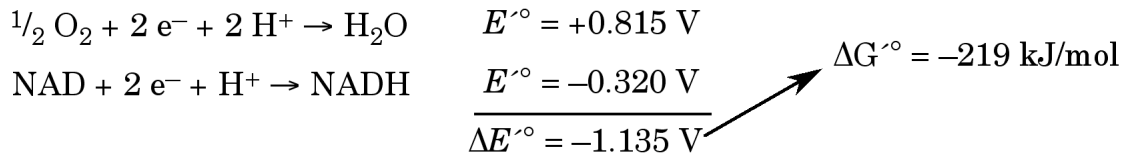
$$\Delta E = \Delta E^{\circ} - \frac{RT}{nF} \ln \left(\frac{[\text{Products}]}{[\text{Reactants}]} \right)$$

How much energy does NADH contain?

Under standard conditions, the transfer of electrons from NADH to oxygen (resulting in production of NAD and water) results in a large negative ΔG° . To see how large, it is necessary to look at the parts of the reaction (the technical term is the “half-reaction”). The first “half-reaction” is the oxidation of NADH, which releases two electrons; the ΔG° for NADH oxidation = $-(2 e^{-})(96485 \text{ J/(V}\cdot\text{mol)})(0.32 \text{ V}) = -61.75 \text{ kJ/mol}$. Note that, in physiological systems it is highly unlikely that any compound will release electrons freely into the environment; instead the electrons released by one compound will be used to reduce another compound. The compound being reduced in this case is an oxygen molecule. This is the second “half-reaction” the addition of two electrons to oxygen. The ΔG° for oxygen reduction = $-2(96485 \text{ J/(V}\cdot\text{mol)})(0.816 \text{ V}) = -157.5 \text{ kJ/mol}$.

The overall reaction has a ΔG° which is the sum of the two “half-reactions” = -219.2 kJ/mol . This is considerably larger than the ΔG° for ATP hydrolysis (-30.5 kJ/mol). Clearly NADH has a large amount of energy stored in the molecule. (Note: the

reduction potential for these compounds varies slightly depending on the source you consult. Note also that NADH is not always oxidized by oxygen; the energy in any redox molecule depends on the specific redox reaction involved.)



Membrane Biophysics

One final method for temporarily storing energy is frequently used by cells. This method is an electrochemical gradient. Because membranes act as barriers to the free movement of molecules, it is possible for differences in concentration across membranes to exist; for entropic reasons, this concentration difference has potential energy. If the molecules involved are charged, they will also interact with any charge gradient across the membrane. The tendency for a molecule to **enter** a cell (or cellular compartment) as the result of an electrochemical gradient is given by the equation:

$$\Delta G = \underbrace{RT \ln\left(\frac{C_{in}}{C_{out}}\right)}_{\text{Concentration gradient}} + \underbrace{ZF\Delta\Psi}_{\text{Potential gradient}}$$

This equation has multiple terms. The first term is the concentration gradient, which is due to the difference in concentration of the molecule between the two sides of the membrane. The second term describes the interaction between a charged species and the membrane potential gradient. In this equation, Z is the charge on the ion (including both magnitude and sign, so Na^+ has $Z = +1$, while HPO_4^{2-} has $Z = -2$), F is the Faraday constant (96485 Coulombs/mol = 96485 joules/volts/mol), and $\Delta\Psi$ is the potential difference across the membrane (in volts). Note that, in this equation, both Z and $\Delta\Psi$ can vary in sign. The direction of the ion flow therefore depends on the concentration gradient for that specific ion, and on the electrical effects of the total ion gradient for the membrane. Note also that the potential gradient only applies to ions; uncharged molecules have $Z = 0$, and therefore do not experience a potential gradient term.

These concentration gradient and potential gradient terms together are the electrochemical gradient. Note that **it is possible for the signs of the concentration and potential gradients to oppose one another**; thus the magnitude of the electrochemical gradient may be smaller than the individual component gradients.

Unless the overall electrochemical gradient is zero, the molecule **will** attempt to move in some direction **spontaneously**. The sign of the electrochemical gradient

indicates the direction: for the formulism used in the above equation **negative means that the molecule will tend to move into the cell.**³ The magnitude of the electrochemical gradient is the energy stored in the gradient; this is the energy that must be overcome in order to pump a molecule against the gradient; it is also the energy that is stored in the gradient and is available to drive some other process.

Summary

One common method for the temporary storage of energy is in the form of high-energy phosphate compounds. The most important of these compounds is ATP, which acts as an energy shuttle. ATP is a kinetically stable, thermodynamically energetic molecule. ATP stores energy obtained from catabolic reactions, and supplies it to anabolic reactions and other cellular processes.

Although ATP is considered to be a “high-energy” compound, it is actually intermediate in energy. ADP can therefore act as a phosphate acceptor to form ATP in a few physiological reactions; in these cases, molecules with higher ΔG° of hydrolysis act as phosphate donors. More frequently, however, ATP acts as the phosphate donor during the synthesis of low-energy phosphate bonds.

ATP hydrolysis can also supply as a thermodynamic driving force to allow otherwise thermodynamically unfavorable reactions that do not involve phosphate transfer to proceed.

Although ATP is the most widely used high-energy compound, other high-energy compounds are also physiologically relevant. One of these is the high-energy thioester bond of acetyl-CoA.

Reduced coenzymes and electrochemical gradients also contain large amounts of potential energy.

The energy stored in high-energy compounds, reduced coenzymes, and electrochemical gradients is used to drive biosynthetic processes and otherwise support life.

³ Note: some textbooks use different formulism. In the 3rd edition of *Lehninger Principles of Biochemistry*, the equation given is incorrect, because it has one of the signs reversed. As always, when performing calculations using this equation, a reality check on your final answer is highly beneficial.