

Bioenergetics

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Basis of Thermodynamics

Every living cell and organism must perform work to stay alive, to grow and to reproduce. The ability to harvest energy from nutrients or photons of light and to channel it into biological work is the miracle of life. Living organisms carry out a remarkable variety of energy transductions. The biological energy transductions obey the physical laws that govern all natural processes, including the laws of thermodynamics.

1st Law of Thermodynamics

The energy of the universe remains constant.

2nd Law of Thermodynamics

All spontaneous processes increase the entropy of the universe.

State functions depend only on the initial and final conditions not on path taken between the initial and final conditions. **They are independent of path.** The important state functions for the study of biological systems are:

G, the **Gibbs free energy** which is equal to the total amount of energy capable of doing work during a process at constant temperature and pressure.

- If ΔG is negative, then the process is spontaneous and termed exergonic.
- If ΔG is positive, then the process is nonspontaneous and termed endergonic.
- If ΔG is equal to zero, then the process has reached equilibrium.

H, the **Enthalpy** which is the heat content of the system.

- When ΔH is negative the process produces heat and is termed exothermic.
- When ΔH is positive the process absorbs heat and is termed endothermic.

S, the **Entropy** is a quantitative expression of the degree of randomness or disorder of the system.

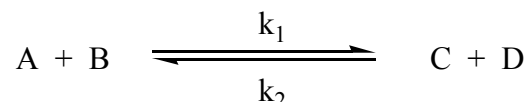
- When ΔS is positive then the disorder of the system has increased.
- When ΔS is negative then the disorder of the system has decreased.

The conditions of biological systems are constant temperature and pressure. Under such conditions the relationships between the change in free energy, enthalpy and entropy can be described by the expression where T is the temperature of the system in Kelvin.

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

Equilibrium Constants

All spontaneous processes proceed until equilibrium is reached. Consider the following chemical reaction.



The forward rate of product formation is $= k_1[A][B]$

The reverse rate of reactant formation is $= k_2[C][D]$

At equilibrium the concentrations of products and reactants are such that forward and reverse rates are equal

$$k_1[A_{eq}][B_{eq}] = k_2[C_{eq}][D_{eq}]$$

A little algebra and presto

$$K_{eq} = \frac{k_1}{k_2} = \frac{[C_{eq}][D_{eq}]}{[A_{eq}][B_{eq}]}$$

At equilibrium $\Delta G = 0$.

The biochemist standard state the concentration of reactants and products are initially set at 1 M, the temperature is 298 °K, the pressure is 1 atm, the pH is 7.0 and the concentration of water is 55 M. The biochemists constants are written as $\Delta G^{\circ'}$ and K'_{eq} . This is the only standard state we will work with in this class so forgive if I occasionally drop the prime. $\Delta G^{\circ'}$ is a constant characteristic for each reaction just as K'_{eq} is a constant characteristic for each reaction. These two constants have a simple relationship.

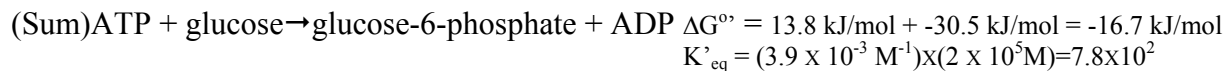
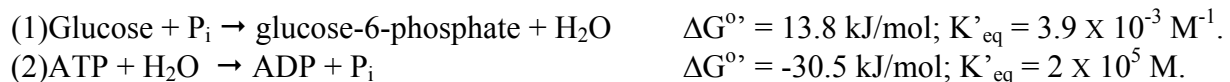
$$\Delta G^{\circ'} = -RT \ln K'_{eq} \quad \text{or} \quad K'_{eq} = e^{\left(\frac{-\Delta G^{\circ'}}{RT}\right)}$$

The actual free energy change depends on the reactant and product concentrations.

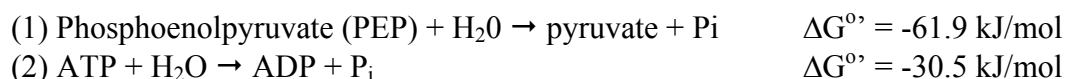
$$\Delta G = \Delta G^{\circ'} + RT \ln \left[\frac{[C][D]}{[A][B]} \right]$$

Reactions can be coupled together. The standard free energy changes are additive. Cool feature of state functions. Multiply the equilibrium constants

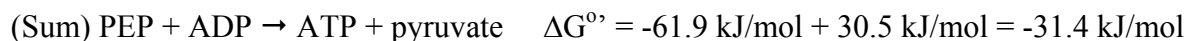
Ie.



Given



What is the free energy change for: PEP + ADP → ATP + pyruvate ?



*Note: change the direction of the reaction change the sign of the $\Delta G^{\circ'}$, invert equilibrium constant. $1/K'_{eq}$.

Thermodynamics of ATP Hydrolysis

ATP is the principle energy currency of the cell that links catabolism to anabolism. ATP has a large negative standard free energy change of hydrolysis.

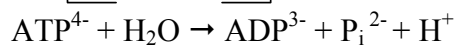
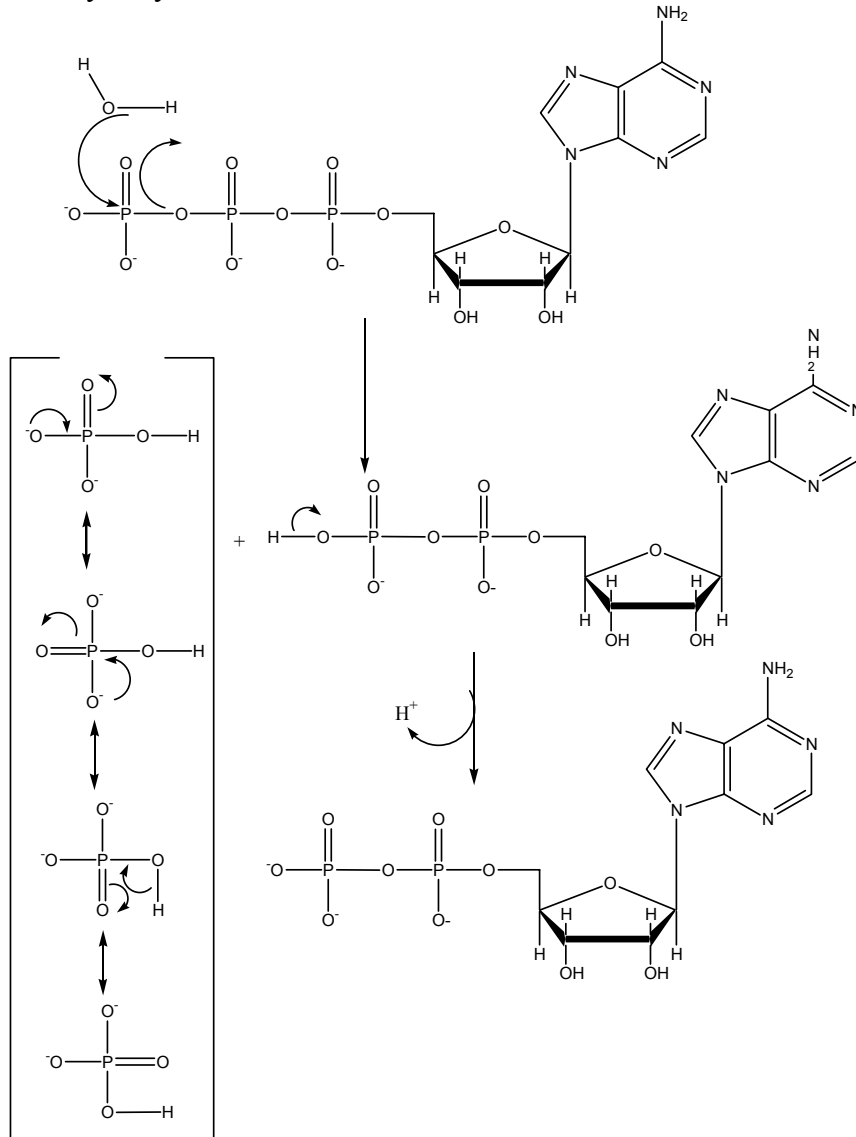


What is the chemical basis of the large, negative free energy change?

1. The hydrolytic cleavage of the γ -phosphate anhydride bond relieves electrostatic repulsion in ATP.

- The phosphate formed is stabilized by several resonance forms that are not possible in ATP.
- The ADP product, immediately ionizes, releasing H^+ in a medium with low hydrogen ion concentration, pH 7.
- ATP has a small solvation energy compared to the solvation energies of ADP, P_i and H^+ . Thus the products of hydrolysis are stabilized more by solvation than then reactant ATP.

ATP hydrolysis



$$\Delta G^{o'} = -30.5 \text{ kJ/mol}$$

In cells, the concentration of ATP, ADP and P_i are not 1M. For example in human erythrocytes the concentration of ATP is 2.25 mM, $[ADP] = 0.25 \text{ mM}$ and $[P_i] = 1.65 \text{ mM}$

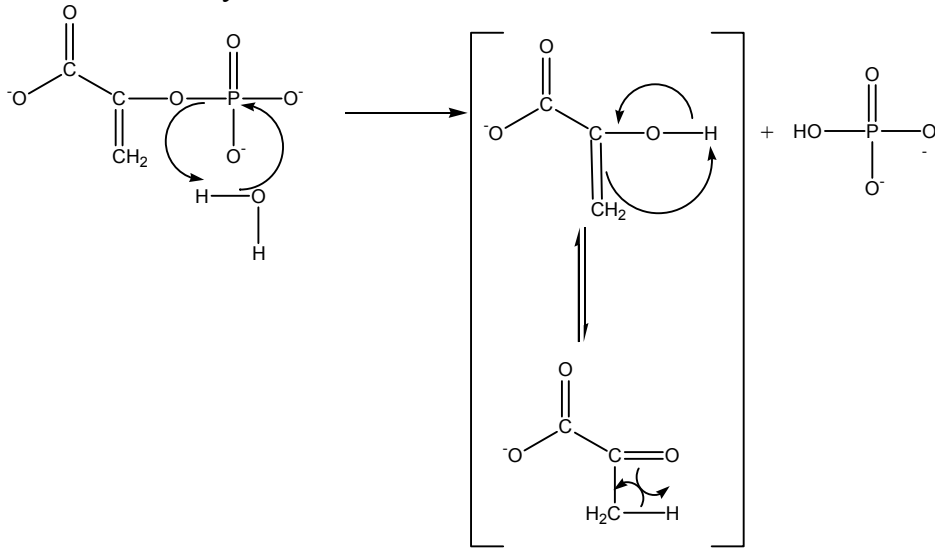
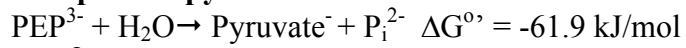
$$\Delta G = \Delta G^{o'} + RT \ln \left[\frac{[ADP][P_i]}{[ATP]} \right] = -30.5 \text{ kJ/mol} + 8.315 \text{ J/mol}^\circ K \times 298^\circ K \times \ln \left[\frac{[2.5 \times 10^{-4} M][1.65 \times 10^{-3} M]}{[2.25 \times 10^{-3} M]} \right]$$

$$\Delta G = -30.5 \text{ kJ/mol} - 21,300 \text{ J/mol} = -51.8 \text{ kJ/mol}$$

This ΔG for ATP hydrolysis in the cell is designated ΔG_p . For intact cells, ΔG_p ranges from -50 to -65 kJ/mol. This is known as the phosphorylation potential.

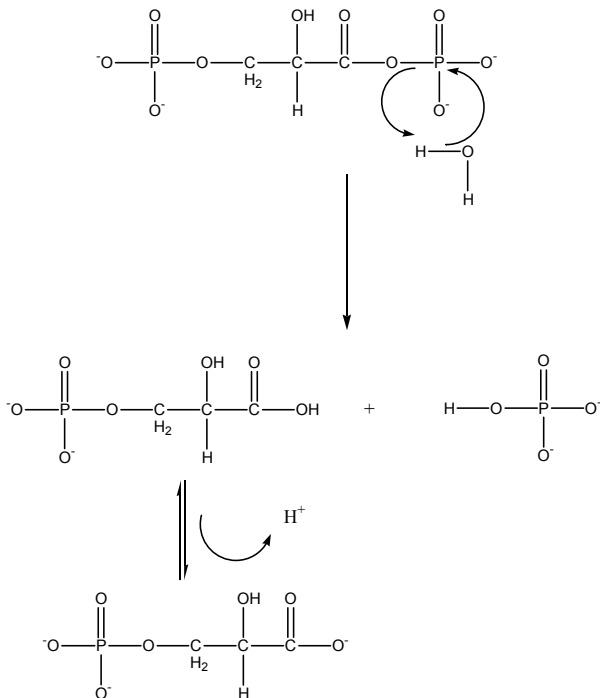
Other High Energy Phosphorylated compounds.

Phosphoenolpyruvate – PEP



Phosphoenolpyruvate contains one phosphate ester bond that can undergo hydrolysis to yield the enol form of pyruvate which immediately tautomerizes to the more stable keto form of pyruvate. The reactant PEP has only one stable form while the product pyruvate has two possible forms. This extra stabilization of the product is the greatest contributor to the high standard free energy of hydrolysis.

1,3-Bisphosphoglycerate

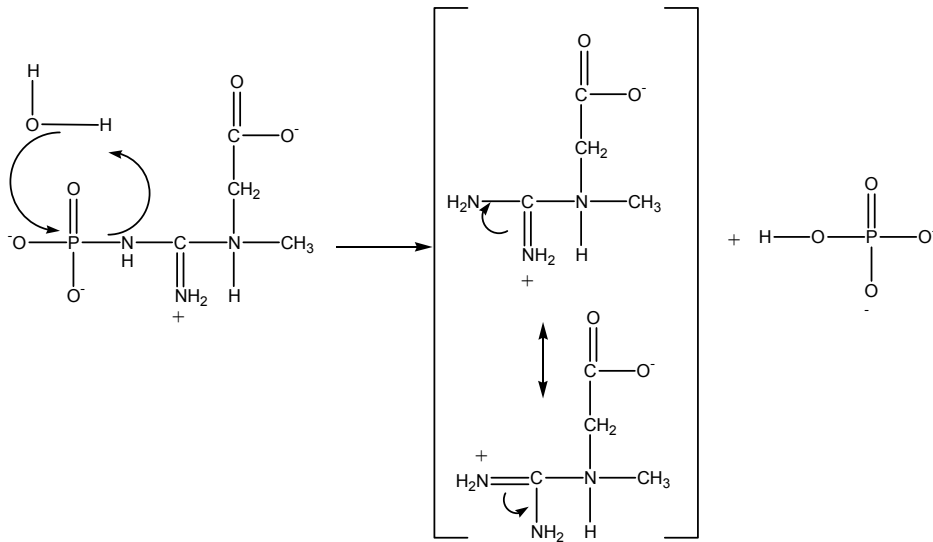


This high energy compound contains one phosphoanhydride bond.

The product 3-phosphoglyceric acid immediately ionizes to produce a carboxylate anion.

The removal of the 3-phosphoglyceric acid and the resonance stabilized phosphate favor the forward reaction.

Phosphocreatine



The release of Pi and the resonance stabilized creatine favor the forward reaction.

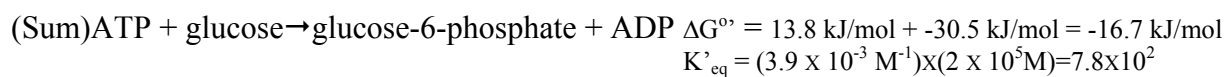
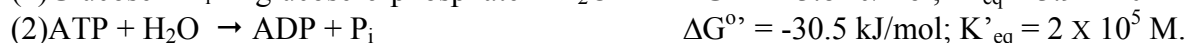
Note for all of these phosphate releasing reactions, the phosphate formed is stabilized by resonance favoring product formation.

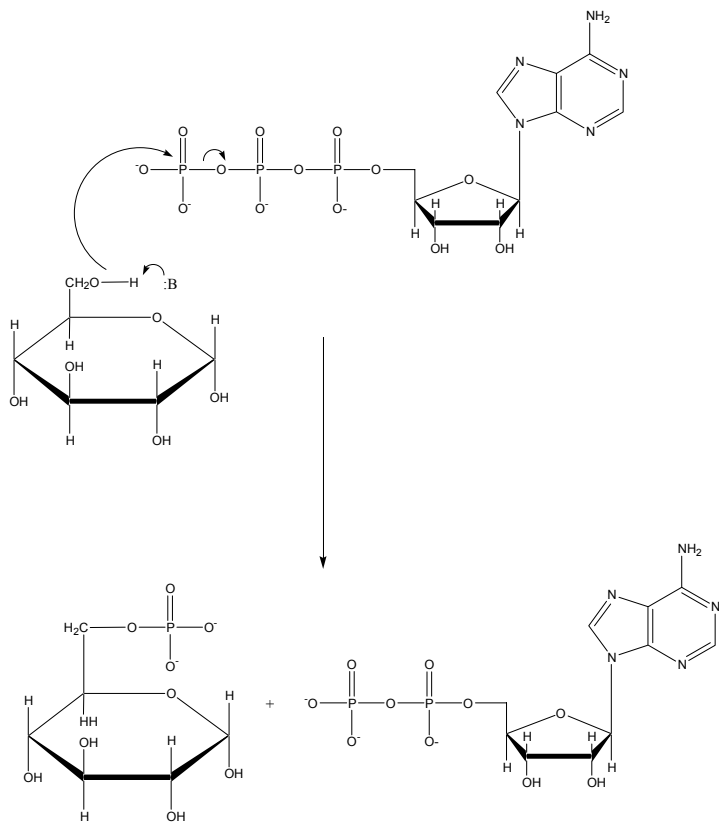
Standard Free Energies of Phosphate Ester Hydrolysis of Some Biological Compounds	
Compound	$\Delta G^{\circ'}$ (kJ/mol)
Phosphoenolpyruvate (PEP)	-61.9
1,3-Bisphosphoglycerate	-49.4
Acetyl phosphate	-43.1
Phosphocreatine	-43.1
ADP \rightarrow AMP + Pi	-35.7
PPi	-33.5
ATP \rightarrow AMP + PPi	-32.2
ATP \rightarrow ADP + Pi	-30.5
Glucose-1-phosphate	-20.9
Fructose-6-phosphate	-15.9
Glucose-6-phosphate	-13.8
Glycerol-3-phosphate	-9.2
AMP \rightarrow adenosine + Pi	-9.2

The compounds with more negative free energy of phosphate ester hydrolysis than ATP can phosphorylate ADP to form ATP. Ie. $\text{PEP} + \text{ADP} \rightarrow \text{ATP} + \text{pyruvate}$ $\Delta G^{\circ'} = -31.4 \text{ kJ/mol}$

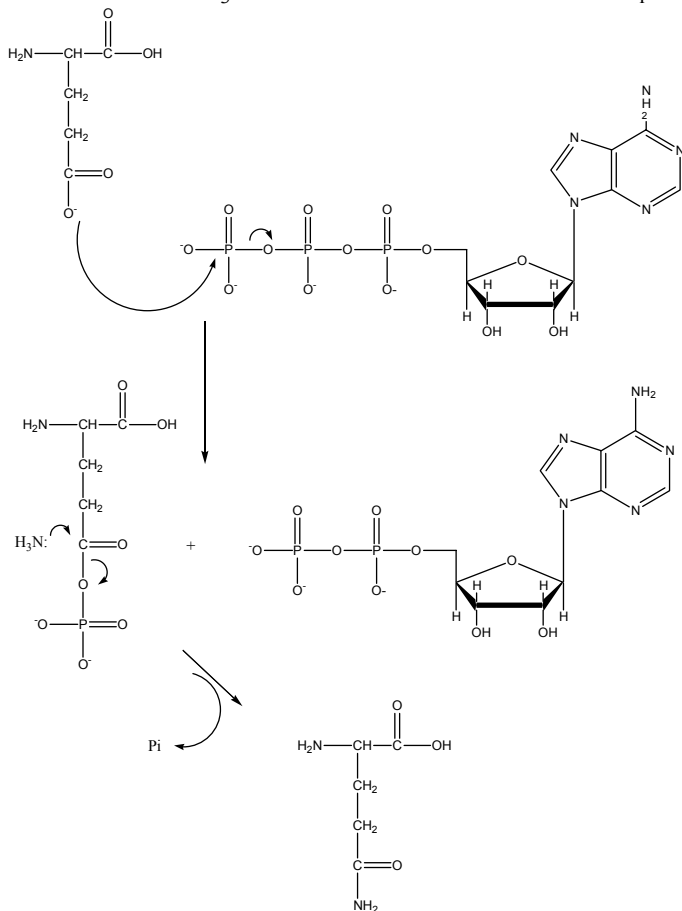
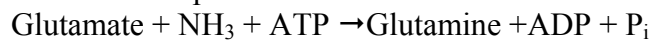
ATP provides energy by group transfers, not simple hydrolysis.

Ie.

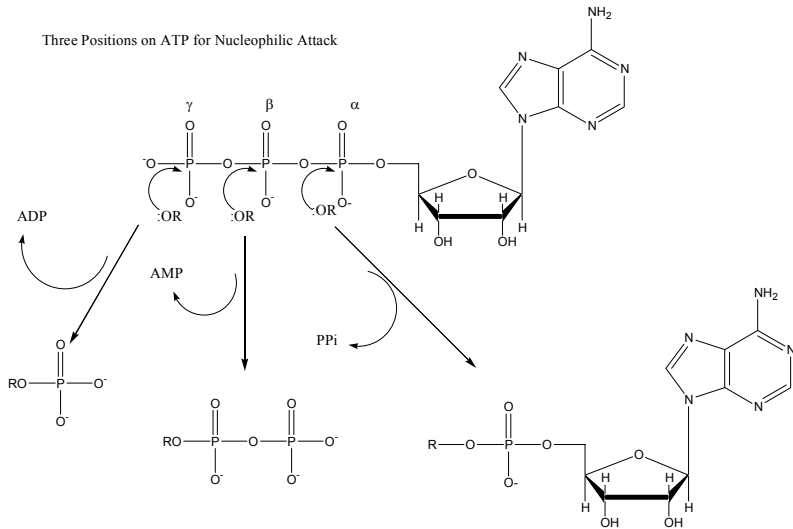




Another example



Three Positions on ATP for Nucleophilic Attack



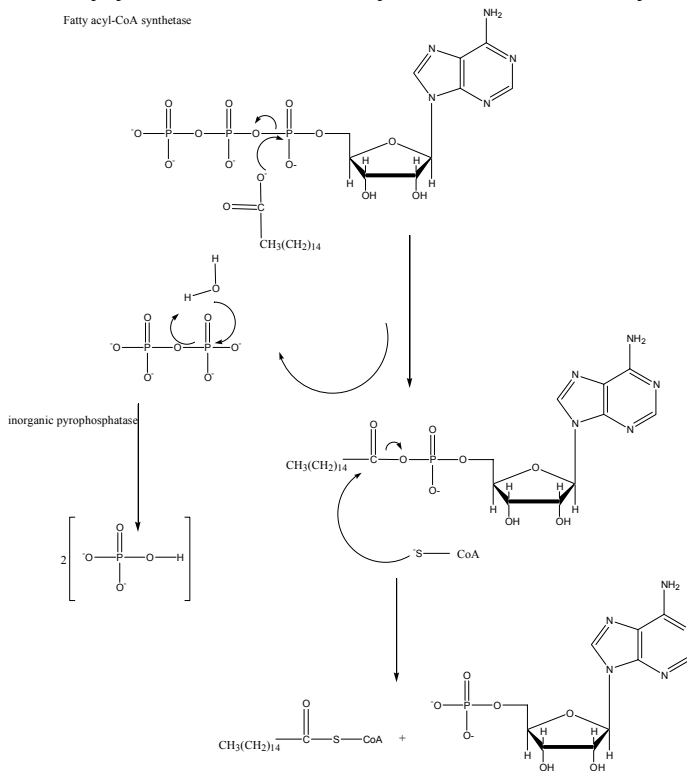
Most of the group transfer reactions of ATP are S_N2 nucleophilic substitutions. In the examples above the nucleophile is an oxygen of an alcohol. Each of the three phosphates of ATP are susceptible to nucleophilic attack. Nucleophilic attack at the γ -phosphate results in ADP and the transfer of phosphate to the nucleophile. Nucleophilic attack at the β -phosphate results in AMP and the transfer of a pyrophosphate group to the

nucleophile. Nucleophilic attack at the α -phosphate results in pyrophosphate and the adenylation of the nucleophile.

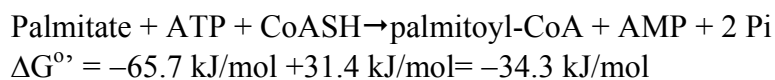
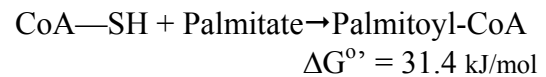
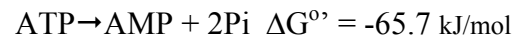
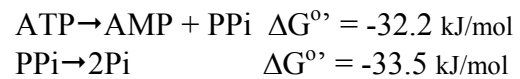
Inorganic Pyrophosphatase

The nucleophilic attack at the α -phosphate of ATP results in an adenylylated nucleophile and pyrophosphate. The ubiquitous enzyme inorganic pyrophosphatase provides an additional thermodynamic push for the adenylation reaction by catalyzing the hydrolysis of pyrophosphate into two molecules of phosphate ($\Delta G^{0'} = -33.5$ kJ/mol). This enzyme makes adenylation reactions very favorable thermodynamically.

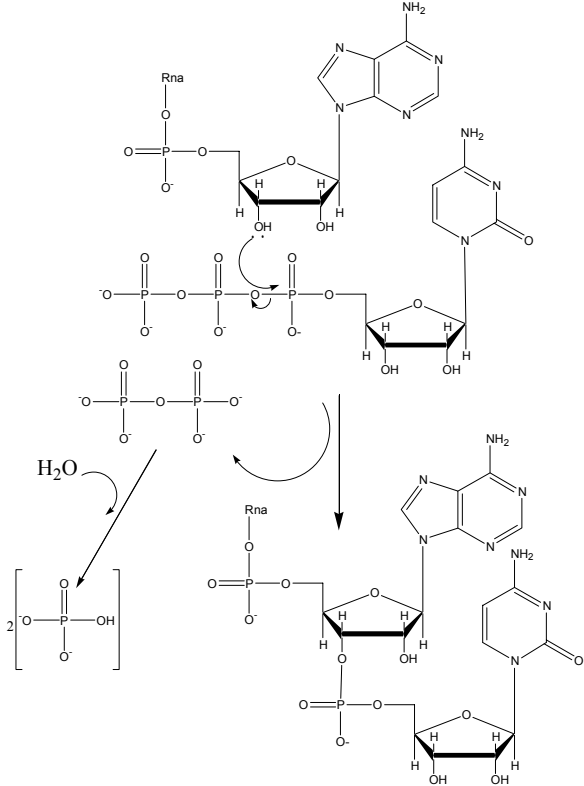
Fatty acyl-CoA synthetase



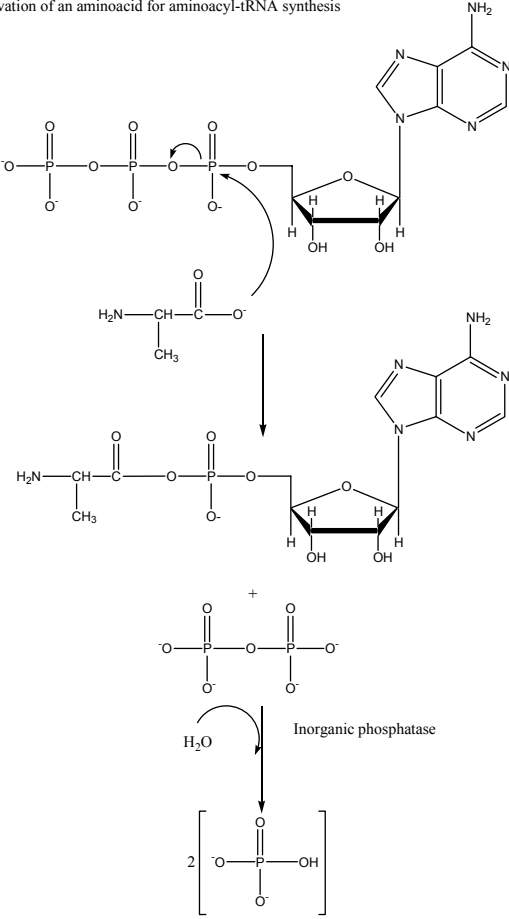
Example: Fatty acyl-CoA synthesis.



RNA Synthesis



Activation of an amino acid for aminoacyl-tRNA synthesis



Transphosphorylation

There are other nucleoside triphosphates (GTP, CTP, UTP, dATP, dGTP, dCTP and dTTP). These are all energetically equivalent to ATP. These nucleotides are generated and maintained by phosphoryl group transfer to the corresponding nucleoside diphosphates and monophosphates. ATP is the primary high energy nucleoside produced by catabolism. Several enzymes catalyze the transfer of the phosphoryl group from ATP to the other nucleotides. There are called nucleoside diphosphate kinases.



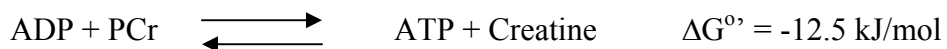
Note that this reaction is fully reversible.

When ADP accumulates as a result of phosphoryl group transfers from ATP, such as when the muscles are vigorously exercising. **Adenylate kinase** removes the ADP by the reaction:

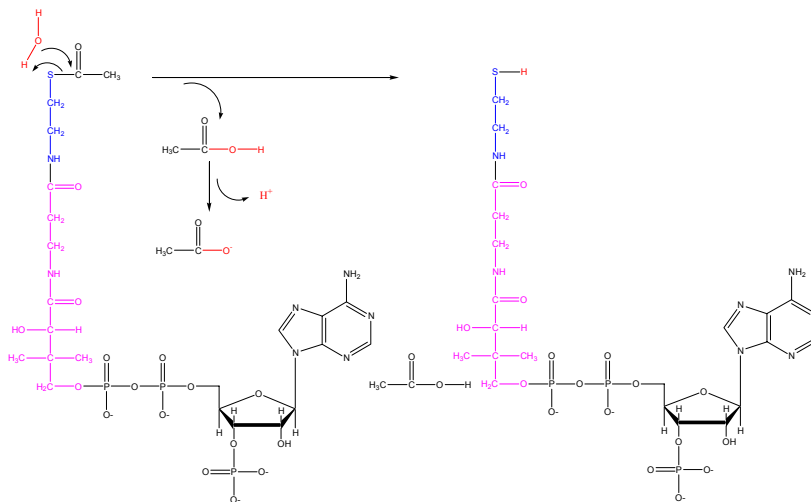


Note this reaction is also fully reversible, so that the enzyme can also convert AMP to ADP when AMP and ATP concentrations are high. There is a similar enzyme for guanosine nucleotides, **Guanylate kinase**.

Phosphocreatine (PCr) serves as a ready source of phosphoryl groups for quick synthesis of ATP from ADP. It is found in the skeletal muscle at a concentration 10 times greater than the cellular concentration of ATP. It is also found in smooth muscle, the brain and the kidney at lower concentration. **Creatine Kinase** catalyzes the following reaction.



When a sudden demand for energy depletes the concentration of ATP, the PCr reservoir is used to replenish ATP at a much faster rate than ATP can be synthesized by catabolic pathways. When the demand for ATP diminishes, ATP synthesized by catabolism replenishes the PCr reservoir.



High Energy Thioesters

Thioesters have a large negative free energy of hydrolysis. As an example, acetyl-CoA is one of many thioesters important in metabolism.

