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Molecular Biochemistry

METABOLISM I: Bioenergetics

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Reading Assignment: **Textbook of Biochemistry, 6th ed.** , edited by Devlin, Chapt. 14, pp. 529-537. Some of the same material is also covered in **Molecular Biology of the Cell, 4th ed.**, by Alberts *et al.*, Chapt. 2, pp. 82-93.

KEY CONCEPTS AND *LEARNING OBJECTIVES*

1. In an oxygen-containing environment, organic molecules are energy rich because they are very unstable with respect to their oxidized products, CO_2 and H_2O. In the parlence of Alberts *et al.*, these molecules contain high-energy electrons. Consequently, the oxidation of organic molecules (transfer of their high-energy electrons to oxygen) releases energy that can be made to perform work.
   a. *Define or explain the terms **oxidation** and **reduction**.*
   b. *Recognize simple oxidation-reduction reactions and identify the participant that is oxidized and the participant that is reduced.*

2. Energy from food is captured by a multistage process in which the metabolic fuels are oxidized in small steps (give up electrons two at a time to an oxidant). Electrons are transferred spontaneously (with the help of catalysts) from reductants (glucose, fatty acids, amino acids) that have a low affinity for electrons to oxidants (NAD^+, FAD, and O_2) that have higher affinities for electrons.
   a. *Identify and recognize the proximal or immediate oxidants (oxidized cofactors) that accept electron pairs from food molecules during catabolism and become reduced.*
   b. *Explain the effect of each small stepwise oxidation of food molecules on the chemical potential energy of the partially oxidized fuel and the cofactors that carryout the oxidations.*

3. ATP is the medium through which the energy released from the oxidation of food is transferred to those physical and chemical processes that require energy in order to occur.
   a. *Recognize the structure of ATP*
   b. *Explain the relationship between the reduced cofactors generated when food is oxidized and the synthesis of ATP.*
   c. *Describe the role of mitochondria in ATP synthesis.*
   d. *Describe the relationship between ATP and the work carried out by the cell.*

4 A physical or chemical change (reaction) that occurs spontaneously can be made to perform work. The amount of work that can be performed is equal to the free energy change for the reaction (ΔG).

a. *Define the terms exergonic and endergonic*

b. *Understand the relationship between the sign (positive or negative) and magnitude of free energy changes and the favorability of chemical reactions.*

c. *Explain the relationship between free energy change (ΔG) for a chemical reaction, the equilibrium constant for that reaction, and the initial or prevailing concentration of reactants and products for that reaction.*

d. *Identify the major physical or chemical change that provides the energy (work) to form ATP within the cell*

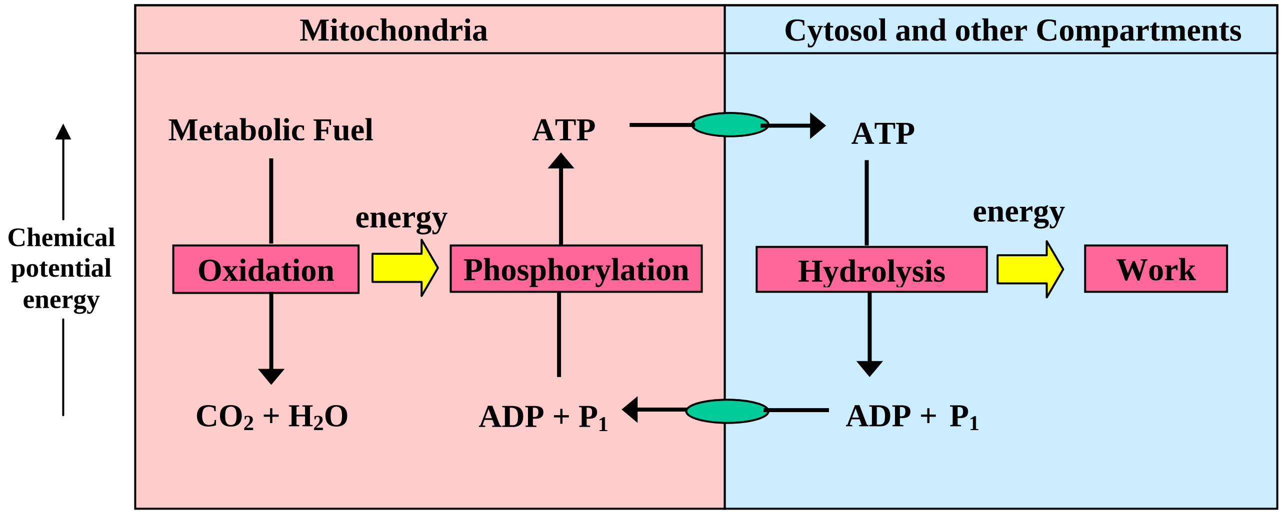
5. The actual free energy change for ATP hyrolysis under physiological conditions, $\boldsymbol{\Delta G_p}$, is often called the phosphorylation potential and is very negative. When an unfavorable physical change or chemical reaction is coupled to ATP hydrolysis (ATP + $H_2O \rightarrow$ ADP + P_i), the equilibrium constant for the unfavorable process can be made more favorable by a factor of about 120 million under the conditions that usually prevail in a mammalian cell.

a. *Explain the term **coupled reactions** and distinguish reactions that are coupled from reactions that are not coupled.*

b. *Describe the relationship between the overall equilibrium constant for a coupled process and the equilibrium constants for the individual steps in that process.*

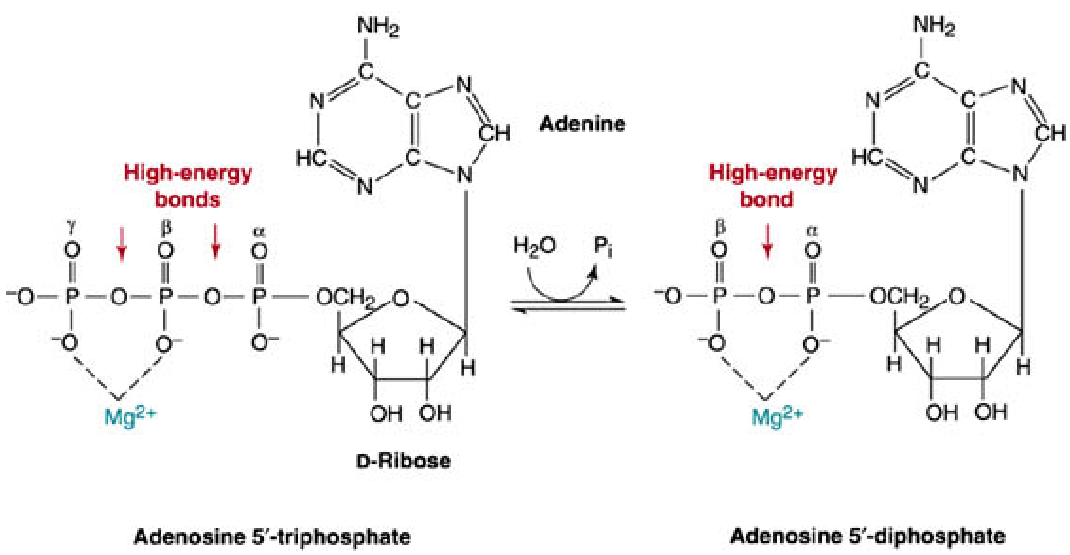
c. *Describe the relationship between the overall free energy change for a coupled process and the free energy changes for the individual steps in that process.*

1. ATP as an energy currency or medium of energy exchange that links energy-producing and energy-consuming processes (Prepared by A.Frankfater)



2. Structure of ATP (**Figs. 13-2** from **Textbook of Biochemistry, 5th ed.,** Devlin, editor, Wiley-Liss, publisher).

A. The hydrolysis of the high energy phosphoanhydride bonds in ATP is thermodynamically favorable.



$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + HPO_4^{2-} + H^+ \qquad \Delta G^{o\prime} = -7.3 \text{ kcal/mol}$$

B. Other ribonucleoside triphosphates can function as high-energy intermediates in various biosynthetic reactions. These can be regenerated from their mono- and diphosphates in reactions with ATP. In these reactions, chemical energy is transferred from ATP to the resulting ribonucleoside triphosphate product.

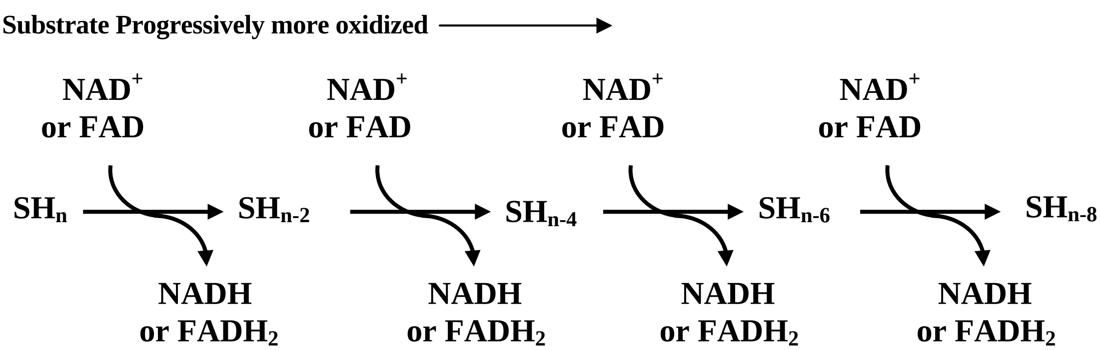
$$NMP + ATP \xrightleftharpoons[]{\text{nucleoside monophosphate kinase}} NDP + ADP \quad \text{where N can be C, G, or U}$$

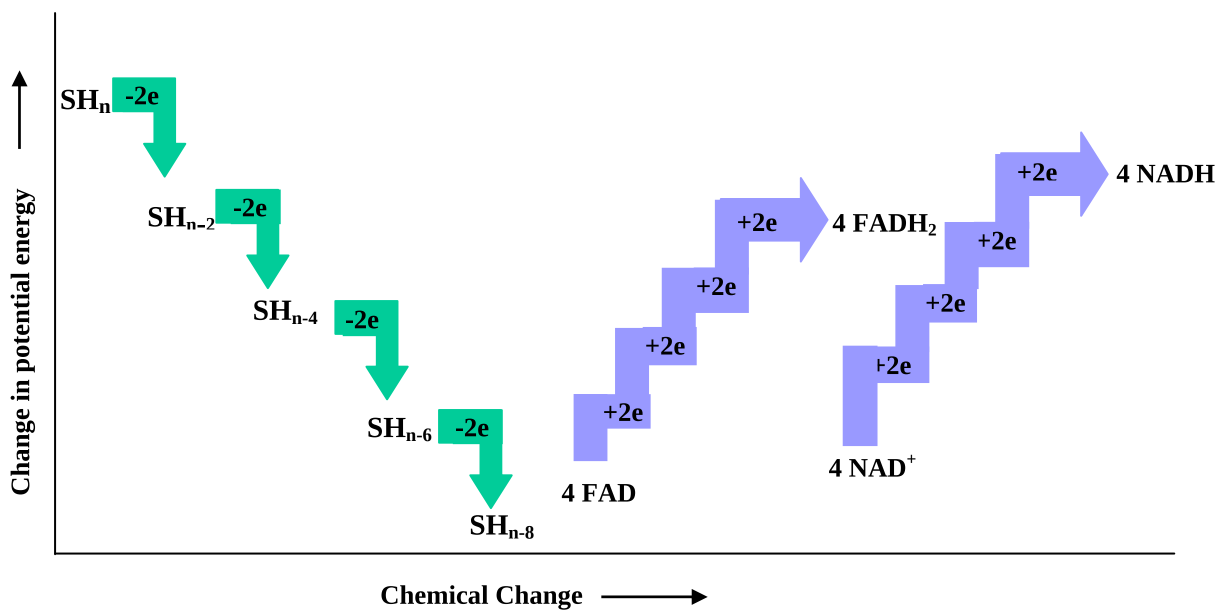
$$NDP + ATP \xrightleftharpoons[]{\text{nucleoside diphosphate kinase}} NTP + ADP$$

In muscle, adenylate kinase (myokinase) catalyzes the conversion of 2 molecules of ADP to ATP and AMP.

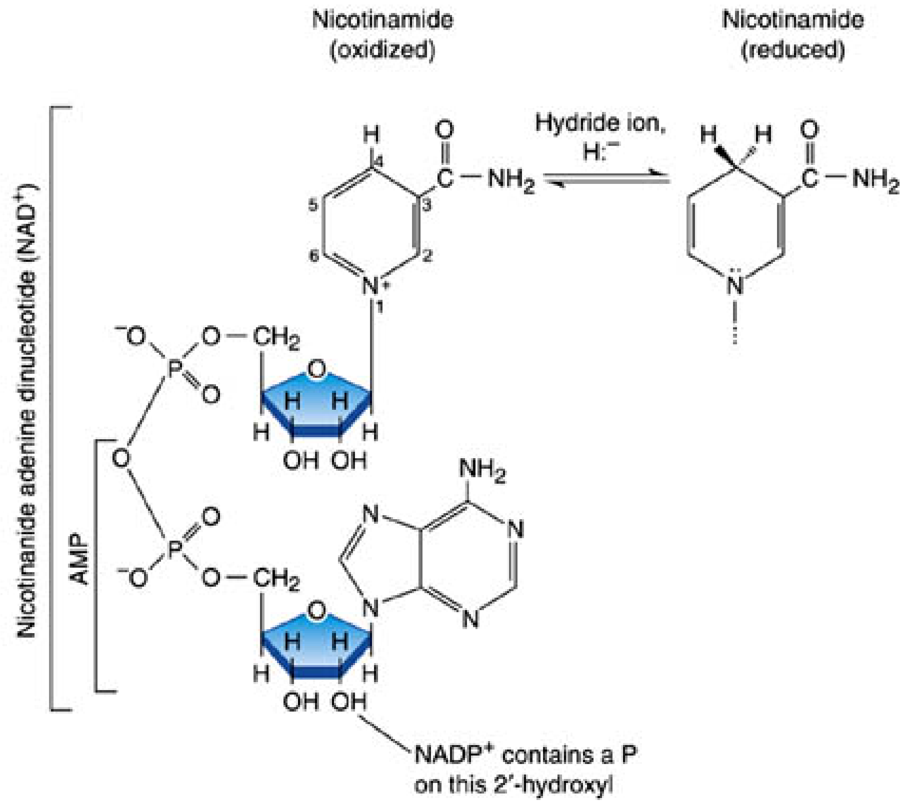
$$\text{ADP} + \text{ADP} \underset{}{\overset{\text{myokinase}}{\rightleftharpoons}} \text{ATP} + \text{AMP}$$

3. Foods are oxidized in small steps using NAD^+ or flavin coenzymes (FAD or FMN) as the primary oxidants. Each reaction involves the transfer of a pair of electrons (2 electrons) from a substrate to the oxidized coenzyme. The transfer of "high energy electrons" from substrates to nicotinamide and flavin coenzymes results in the transfer of chemical potential energy from the substrates to these coenzymes (Prepared by A. Frankfater).





4. Structures of NAD^+ and FAD/FMN (**Figs. 13-4** and **13-16** from **Textbook of Biochemistry, 5th ed.** , Devlin, editor, Wiley-Liss, publisher).



5. Gibbs Free Energy and Work.
At constant temperature and pressure (conditions that apply to biological systems), **Gibbs free energy (G) describes the total amount of energy in the system that is capable of doing work**. However, work can only be performed when a change occurs that is accompanied by a change in Gibbs free energy (symbolized by ΔG). *ΔG is the maximum amount of energy available for the performance of work for a given change*.

The sign (positive or negative) and magnetude of ΔG determines whether a particular chemical reaction can occur spontaneously and whether it can be used to perform work. *Processes that are accompanied by a decrease in Gibbs free energy (negative ΔG) are thermodynamically favorable (spontaneous) and can be made to perform work. Processes that release free energy are said to be* ***exergonic****. Processes characterized by a positive ΔG require work to be done on them in order to occur and are said to be* ***endergonic****.*

A. Relationship between standard free-energy change and equilibrium constant.

All chemical reactions are characterized by a **standard Gibbs free energy change**, $\Delta G^{\circ\prime}$, *which is related by the following equation to the equilibrium constant for the reaction at pH 7.0.*

$$\Delta G^{\circ\prime} = - RT \ln K'_{eq} = - 2.303\, RT \log K'_{eq}$$

$\Delta G^{\circ\prime}$ is negative for reactions in which the equilibrium constant is greater than 1 (products are favored over reactants).

B. The actual Gibbs free energy change for a chemical reaction depends on the initial or actual concentrations of reactants and products.

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

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

For example, ΔG for ATP hydrolysis in a cell is given by the expression

$$\Delta G_p = \Delta G^{\circ\prime} + RT \ln \frac{[ADP][P_i]}{[ATP]}$$

$\Delta G^{\circ\prime}$ for ATP hydrolysis is about – 7.7 kcal/mole at 37°C. Under conditions that prevail in most cells, the ratio of $[ADP][P_i]/[ATP]$ is maintained at about 0.0012. Consequently, ΔG_p for ATP hydrolysis is about – 12 kcal/mole under physiological condition. If the $[ADP][P_i]/[ATP]$ ratio were about 140,000, the equilibrium ratio, then ΔG_p would be equal to 0. **The further the concentrations of reactants and products are from their equilibrium concentrations, the larger the magnitude of ΔG for the reaction**.

C. Metabolic pathways within a cell are made up of series of sequential reactions. For a series of reactions, the overall Gibbs free energy change is equal to the sum of the free energy changes for the individual reactions.

$$\mathbf{A \rightarrow B \rightarrow C \rightarrow D}$$

$$\Delta G^{\circ\prime}_{a \rightarrow d} = \Delta G^{\circ\prime}_{a \rightarrow b} + \Delta G^{\circ\prime}_{b \rightarrow c} + \Delta G^{\circ\prime}_{c \rightarrow d}$$

$$K'_{eq(a \rightarrow d)} = K'_{eq(a \rightarrow b)} \times K'_{(b \rightarrow c)} \times K'_{eq(c \rightarrow d)}$$

6. The ability of ATP to function as a medium of energy exchange is facilitated by its capacity to transfer phosphate or AMP to another molecule.

Glucose + ATP $\longrightarrow$ Glucose-6-phosphate + ADP; $\Delta G^{\circ\prime}_{(\text{glucose 6-P hydrolysis})}$ = - 3.3 kcal/mol

$$\underset{\text{Palmitic acid}}{R{-}\overset{\overset{O}{\|}}{C}{-}O^{-}} + \text{ATP} \longrightarrow \underset{\text{Palmitoyl adenylate}}{R{-}\overset{\overset{O}{\|}}{C}{-}O{-}\underset{\underset{O^{-}}{|}}{\overset{\overset{O}{\|}}{P}}{-}O{-}\text{Ribose-Adenine}} + PP_i \text{ (pyrophosphate)}$$

$\Delta G^{\circ\prime}_{(\text{palmitoyl-AMP hydrolysis})} \approx$ - 10 kcal/mol

7. Example of a **coupled reaction** in which the energy of ATP hydrolysis is used to perform biosynthetic work

<u>Formation of Glutamine</u>:
glutamate + $NH_3 \rightarrow$ glutamine + H_2O — $\Delta G^{\circ\prime}_{gln}$ = + 3.4 kcal/mol; $K'_{eq(gln)}$ = 0.004 at 37°C

<u>Hydrolysis of ATP</u>:
ATP + $H_2O \rightarrow$ ADP + P_i — $\Delta G^{\circ\prime}_{ATP}$ = -7.3 kcal/mol; $K'_{eq(ATP)}$ = 140,000

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sum: glutamate + NH_3 + ATP $\rightarrow$ glutamine + ADP + P_i

These two reactions are **uncoupled** (independent) and are unable to influence each other. Were they coupled, the overall Gibbs free energy change and equilibrium constant for the combined reactions at 37°C would favor glutamine biosynthesis.

$$\Delta G^{\circ\prime}_{(\text{combined})} = \Delta G^{\circ\prime}_{gln} + \Delta G^{\circ\prime}_{ATP} = -3.9$$

$$K'_{eq(\text{combined})} = K'_{eq(gln)} \times K'_{eq(ATP)} = 560$$

These two reactions can be coupled by causing them to share a common intermediate or participant. In that way they become sequential reactions, and their free energies can be added and the equilibrium constants can be multiplied.

glutamate + ATP $\longrightarrow$ glutamyl-phosphate + ADP	$\Delta G^{o\prime}$ = + 2.8 kcal/mol
glutamyl-phosphate + NH_3 $\longrightarrow$ glutamine	$\Delta G^{o\prime}$ = - 6.7 kcal/mol
sum: glutamate + NH_3 + ATP $\longrightarrow$ glutamine + ADP + P_i	$\Delta G^{o\prime}$ = -3.9 kcal/mol K'_{eq} = 560

8. In the cell, the concentrations of ATP, ADP and P_i are kept far from their equilibrium concentrations (equilibrium ratio of $[ADP][P_i]/[ATP] \approx 140{,}000$). The actual ratio is less than 0.002 (see below).

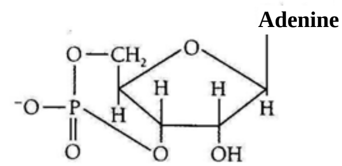
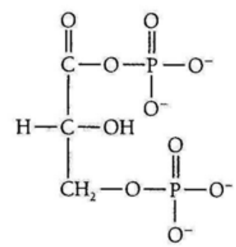
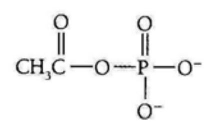
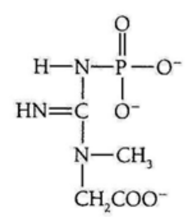
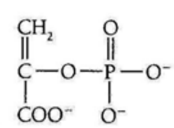
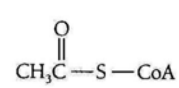
	Concentration (mM)			$\frac{[ADP][P_i]}{[ATP]}$	ΔG_p
	ATP	ADP	P_i		kcal/mol
Rat hepatocytes	3.38	1.32	4.80	0.00187	-11.2
Rat myocytes	8.05	0.93	8.05	0.00093	-11.6
Rat neurons	2.59	0.73	2.72	0.00077	-11.7
E. coli	7.90	1.04	7.90	0.00104	-11.5

Under condtions that actually prevail in the cell, the effect of coupling glutamine synthesis, or any other process, to ATP hydrolysis, is to shift its equilibrium in favor of products by a factor of about 117,000,000 (antilog of $-\Delta G_p/2.303\ RT$).

$$\Delta G_{gln} = \Delta G^{o\prime}_{gln} + \Delta G_p$$

$$K_{obs\ (gln)} = K'_{eq\ (gln)} \times 117{,}000{,}000$$

9. Examples of other high energy chemical intermediates.

	$\Delta G^{o\prime}$ of hydrolysis		
Type of bond	**Kcal/mol**	**kJ/mol**	**Example**
Phosphoric acid anhydrides	−11.9	−50.4	 3′,5′ cyclic AMP
Phosphoric–carboxylic acid anhydrides	−10.1	−49.6	 1,3-Bisphosphoglycerate
	−10.3	−43.3	 Acetyl phosphate
Phosphoguanidines	−10.3	−43.3	 Creatine phosphate
Enol phosphates	−14.8	−62.2	 Phosphoenolpyruvate
Thiol esters	−7.7	−31.5	 Acetyl CoA

10. NADH and $FADH_2$ produced during the oxidation of substrates in the mitochondrial matrix is reoxidized by molecular oxygen. The oxidations of NADH and $FADH_2$ are thermodynamically favorable, and the energy released is captured to make ATP (oxidative phosphorylation). (Prepared by A. Frankfater).