Intro to metabolism II - Thermodynamics

Pages 15 – 18 and 355 – 370

The laws of thermodynamics apply to metabolism.

First law of thermodynamics – Law of conservation of energy.

- For biological systems this describes the transfer of energy from one form to another

Second law of thermodynamics – Entropy must increase if a reaction is to be spontaneous.

$$\Delta S \text{ system} + \Delta S \text{ surrounding} > 0$$

<u>A process can only occur spontaneously if the sum of the entropies of the system and its surroundings increases.</u>

- The problem with this? It is difficult to measure the changes in entropy in a biological system.
- However we can use Gibbs Free Energy of a Reaction at constant pressure and temperature:

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

$\Delta G < 0$, then…

$\Delta G = 0$, then…

$\Delta G > 0$, then…

For a reaction in equilibrium

- The direction of an enzyme catalyzed reaction and for that matter a metabolic pathway, depends of the change in free energy (ΔG).
- Reactions occur only when the free energy (Gibbs) change is negative.
- A reaction cannot occur if ΔG is positive unless coupled to a reactions whose ΔG is more negative than the first reaction is positive

For the reaction: A + B <-> C + D

$$\Delta G = \Delta G^{o\prime} + RT \ln ([C]\,[D]/[A]\,[B])$$

For a reaction at equilibrium: $\Delta G = 0$

So: $0 = \Delta G^{o\prime} + RT \ln ([C]\,[D]/[A]\,[B])$

And: $\Delta G^{o\prime} = -RT \ln ([C]\,[D]/[A]\,[B])$

The equilibrium constant, Keq is determined as: Keq = [products]/[reactants]

Thus: $\Delta G^{o\prime} = - RT \ln Keq$

This equation tells two things - that the observed ΔG for a reaction at equilibrium is zero <u>and</u> that the standard state free energy change can be determined by knowing the equilibrium constant of the reaction

The standard state in biochemistry is at pH 7 and 1 molar concentration

*** Important point***

the standard state free energy change ($\Delta G^{o\prime}$) is fixed and determined experimentally while the value of the free energy change (ΔG) depends on the concentration of reactants (substrate) and product.

At equilibrium the change in free energy is about zero, so the reaction is easily reversible.

- Enzymes near equilibrium can easily return flux of substrate or product back to equilibrium.
- Enzymes whose ΔG is highly negative are far from equilibrium and those are considered irreversable, committed steps in the metabolic pathway. These enzymes control the overall flux of metabolites through a pathway.

Energy Coupling

- A spontaneous reaction can drive a non-spontaneous reaction
- The free energy change of coupled reactions are additive

▪ Some enzyme-catalyzed reactions are interpretable as two coupled half-reactions, one spontaneous and the other non-spontaneous. At the enzyme active site, the coupled reaction is kinetically facilitated, while the individual half-reactions are prevented. The free energy changes of the half-reactions may be summed, to yield the free energy of the coupled reaction.

For example, in the reaction catalyzed by the enzyme Hexokinase, the two half-reactions are:

$ATP + H_2O \leftrightarrow ADP + P_i$ $\Delta G^{o\prime} = -31$ kJoules/mol

P_i + glucose $\leftrightarrow$ glucose-6-P + H_2O $\Delta G^{o\prime} = +14$ kJoules/mol

Coupled reaction: ATP + glucose $\leftrightarrow$ ADP + glucose-6-P .. $\Delta G^{o\prime} = -17$ kJoules/mol

The structure of the enzyme active site, from which water is excluded, prevents the individual hydrolytic reactions, while favoring the coupled reaction.

▪ Two separate enzyme-catalyzed reactions occurring in the same cellular compartment, one spontaneous and the other non-spontaneous, may be coupled by a common intermediate (reactant or product).

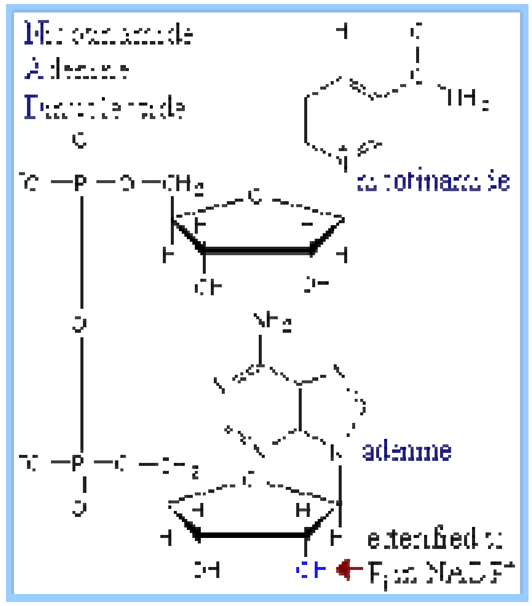
enzyme 1: A + ATP $\leftrightarrow$ B + AMP + PP_i $\Delta G^{o\prime} = +15$ kJ/mol

enzyme 2: PP_i ´ 2 P_i $\Delta G^{o\prime} = -33$ kJ/mol

Overall: A + ATP $\leftrightarrow$ B + AMP + 2P_i $\Delta G^{o\prime} = -18$ kJ/mol

Oxidation Reduction

NAD$^+$ (**N**icotinamide **A**denine **D**inucleotide) functions as an electron acceptor in catabolic pathways.



The **nicotinamide** ring of NAD$^+$, which is derived from the vitamin **niacin**, accepts 2 e$^-$ and one H$^+$ (a hydride) in going to the reduced state, as NAD$^+$ becomes **NADH**.

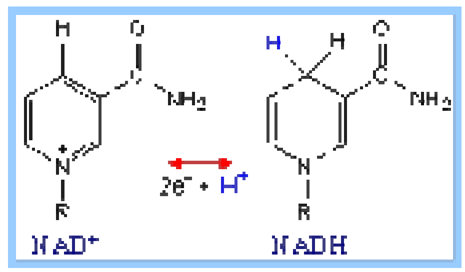
NADP$^+$**/NADPH** is similar, except for an additional phosphate esterified to a hydroxyl group on the adenosine ribose. NADPH functions as an electron donor in synthetic pathways.

The electron transfer reaction may be summarized as:

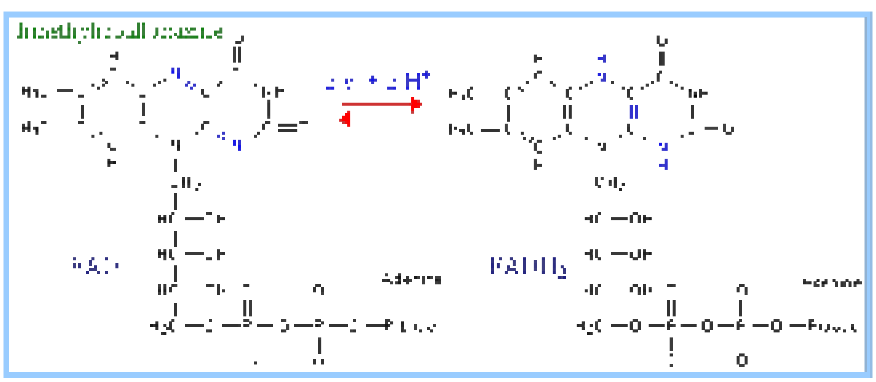
NAD$^+$ **+ 2 e**$^-$ **+ H**$^+$ **↔ NADH**

It may also be written as:

NAD$^+$ **+ 2 e**$^-$ **+ 2H**$^+$ **↔ NADH + H**$^+$



FAD (**F**lavin **A**denine **D**inucleotide) also functions as an electron acceptor. The portion of FAD that undergoes reduction/oxidation is the dimethylisoalloxazine ring, derived from the vitamin **riboflavin**.



FAD normally accepts 2 e$^-$ and 2 H$^+$ in going to its reduced state:

FAD + 2 e$^-$ **+ 2 H**$^+$ **↔ FADH**$_2$

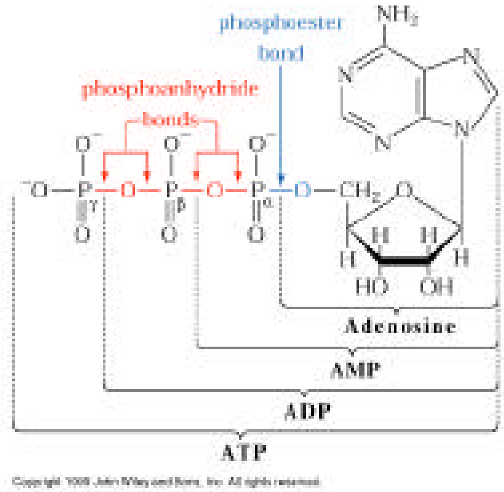
NAD$^+$ is a **coenzyme**, that reversibly binds to enzymes. FAD is a **prosthetic group**, that usually remains tightly bound at the active site of an enzyme.

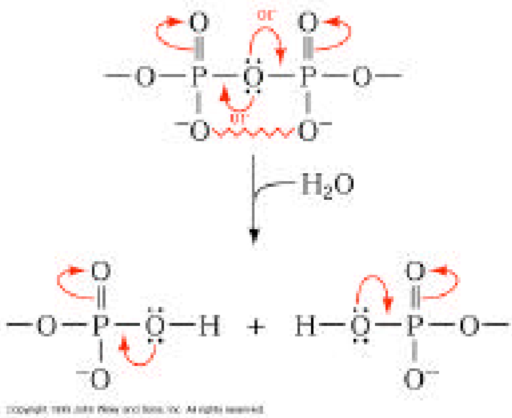
High Energy Compounds

- Energy from the metabolism of food and storage molecules such as fats and carbos are temporarily transferred to high energy compounds such as ATP, Creatine phosphate and Acetyl-CoA.
- The energy from these intermediates are used to drive otherwise endergonic ($\Delta G = +$) reactions.

How is ATP the energy current of the cell?

- Ester vs anhydrides of phosphates
- The free energy is due to the phosphoanhydryde bonds - the group transfer of the phosphate group to water.





- resonance of the products increase the product stability (ADP and AMP) over the reactant (ATP)
- the electrostatic repulsion between the charged phosphates of ATP is unstable compared to AMP and ADP
- There is an increased solvation energy of the products - entropy at work
- Compounds with "high energy" bonds are said to have **high group transfer potential**. For example, P_i may be spontaneously removed from ATP for transfer to another compound (e.g., to a hydroxyl on glycerol).

Pyrophosphate vs inorganic phosphate - PPi vs. Pi

PPi $\Delta G^{o\prime} = -33.5$ (costs an extra ATP to replace PPi)

Often the driving force of many reactions

Pyrophosphate (PP_i) is often the product of a reaction that needs a driving force. Its spontaneous hydrolysis, catalyzed by a pyrophosphatase enzyme, drives the reaction for which PP_i is a product.

Potentially **two** high energy bonds can be cleaved, as two phosphates are released by hydrolysis from **ATP** (adenosine triphosphate), yielding **ADP** (adenosine diphosphate), and ultimately **AMP** (adenosine monophosphate). This may be represented as follows:

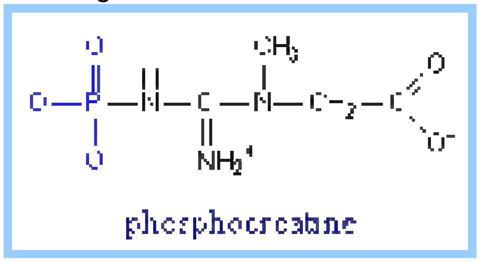
AMP~P~P → AMP~P + P_i (ATP → ADP + P_i)

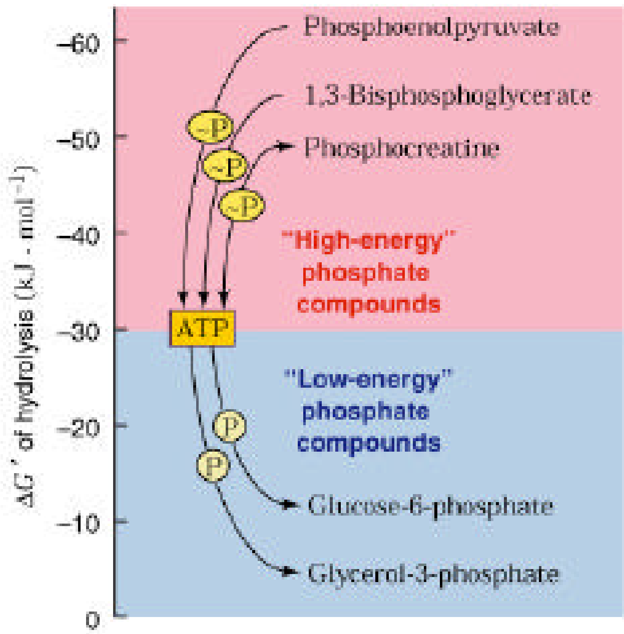
AMP~P → AMP + P_i (ADP → AMP + P_i)

AMP~P~P → AMP + P~P_i (ATP → AMP + PP_i)

P~P → 2 P_i

- The standard state free energy of ATP hydrolysis is less than some molecules and higher than others? Does this make sense?





- Phosphorylation of ATP by a kinase is called **substrate level phosphorylation**. Uses a higher energy compound than ATP. (phosphate group transfer from a high transfer potential to a lower (ATP) transfer potential compound)
- Indierect generation of ATP by H+ gradients (via the electron transfer pathway) is **oxidative phosphorylation**

Formation of triphosphate nucleotides (NTP) can occur by the actions of the enzyme NDPK.

- the change in free energy is nearly zero so the reaction is easily reversed
- reaction direction then depends on concentration of NTP and NDP nucleotides

Adenylate kinase (also called adenylyl kinase) regulates the conversion of AMP to ADP at the cost of ATP

- allows ATP to be formed by substrate level phosphorylation
- Freely reversible reaction
- AMP often an allosteric modifier signifying a low energy state of the cell

- Transfer of acyl (carbon chains) require an activation step
- These reactions involved a thioester high energy bond.
- Use the compound Coenzyme A (derived from panthanoic acid, vitamin B_5)
- The hydrolysis of thioesters is as energetically favorable as ATP hydrolysis
- Used in fatty acid synthesis, lipid production, lipid modification of proteins and others