Chapter 3

Thermodynamics of Biological Systems

Chapter Outline

- Thermodynamic concepts
  - Systems
    - Isolated systems cannot exchange matter or energy with surroundings
    - Closed systems exchange energy but not matter
    - Open systems exchange both energy and matter
  - First Law: • E = q + w
    - • E = change in internal energy (state function), q = heat absorbed, and w = work done on the system
    - H = E + PV: H = enthalpy (energy transferred at P=constant): • H = q when work limited to P• V
    - • H^o = -Rd(lnK_{eq})/d(1/T): van't Hoff plot RlnK$_{eq}$ vs 1/T (R = gas constant = 8.314J/mol K
  - Second Law: Disorder or randomness
    - S=klnW where k = Boltzmann's constant = 1.38x10^{-23}
    -
    - J/K, W = number of ways of arranging the components of a system at • E = 0.
    - dS = dq/T for reversible process
  - Third law: Entropy of a perfectly ordered, crystalline array at 0 K is exactly zero
    - $S = \int_0^T C_p \, d\ln T$, C_p = heat capacity = dH/dT for constant P process
  - Gibbs free energy: • G = • H + T• S
    - • G = • G° + RTln([P]/[R]) and • G° = -RTln([P]$_{eq}$/[R]$_{eq}$)
    - When protons involved in process: • G°'=• G° ± RTln[H$^+$]
    - ("+" if reaction produces protons; "-" if protons consumed)
- Coupled processes: Enzymatic coupling of a thermodynamically unfavorable reaction with a thermodynamically favorable reaction to drive the unfavorable reaction. Thermodynamically favorable reaction is often hydrolysis of high-energy molecule
- Energy transduction: high-energy phosphate ATP and reduced cofactor NADPH
  - Phototrophs use light energy to produce ATP and NADPH
  - Chemotrophs use chemical energy to produce ATP and NADPH
- High-energy molecules
  - Phosphoric anhydrides (ATP, ADP, GTP, UTP, etc.)
  - Enol phosphates (phosphoenolpyruvate a.k.a. PEP)
  - Phosphoric-carboxylic anhydrides (1,3-bisphosphoglycerate)
  - Guanidino phosphates (creatine phosphate)
- Why is hydrolysis of high-energy bonds favorable
  - Destabilization of reactant due to electrostatic repulsion
  - Product isomerization and resonance stabilization
  - Entropy factors
- Thermodynamics of ATP hydrolysis influenced by: pH, cation concentration, reactant and product concentrations

Chapter Objectives

Laws of Thermodynamics

The first law of thermodynamics is simply a conservation of energy statement. The internal energy changes if work and/or heat are exchanged. In biological systems, we are usually dealing with constant-pressure processes and in this case the term enthalpy, H, is used. Enthalpy is the heat exchanged at constant pressure. Since enthalpy is heat, it is readily measured using a calorimeter or from a plot of $R(\ln K_{eq})$ versus 1/T, a van't Hoff plot. (To get ahead of the story, the point is that if •G and •H are known, •S can be calculated.)

The second law of thermodynamics introduces the term entropy, S, which is a measure of disorder or randomness in a system. A spontaneous reaction is accompanied by an increase in disorder. For a reversible reaction, $dS_{reversible} = dq/T$. Also, S = k ln W where k = Boltzmann's constant, and W = the number of ways to arrange the components of a system without changing the internal energy.

Gibbs Free Energy

The change is Gibbs free energy for a reaction is the amount of energy available to do work at constant pressure and constant volume. This is an important concept and should be understood. For a general reaction of the type

$$A + B \text{ D } C + D$$

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

When a reaction is at equilibrium, clearly it can do no work (nor have work done on it) without change so it must be true that •G = O at equilibrium. Thus, $\bullet G^\circ = -RT \ln K_{eq}$, and by measuring the equilibrium concentrations of reactants and products, •G° can be evaluated. Knowing the initial concentrations of reactants and products and •G° allows a calculation of •G. Why is this important? The sign on •G tell us in which direction the reaction will proceed. A negative •G indicates that the reaction has energy to do work and will be spontaneous in the direction written. A positive •G indicates work must be done on the reaction for it to proceed as written, otherwise it will run in reverse. The magnitude of •G is the amount of energy available to do work when the reaction goes to equilibrium. Finally, the relationship •G = •H - T•S can be used to evaluate •S, the change in disorder, if •G and •H are known.

High Energy Compounds

ATP is the energy currency of cells and its hydrolysis is used to drive a large number of reactions. For ATP and other high-energy biomolecules, you should understand the properties that make them energy-rich compounds. These include: destabilization due to electrostatic repulsion, stabilization of hydrolysis products by ionization and resonance, and entropy factors. Examples of high-energy compounds (from Table 3.3 in Garrett and Grisham) include: phosphoric acid anhydrides (e.g., ATP, ADP, GTP, UTP, CTP, and PP_i), phosphoric-carboxylic anhydrides (acetyl phosphate and 1,3-bisphosphoglycerate), enol phosphates (PEP), and guanidinium phosphates (creatine and arginine phosphate. ATP is the cardinal high-energy compound. Hydrolysis of GTP is important in signal transduction and protein synthesis. UTP and CTP are used in polysaccharide and phospholipid synthesis, respectively. We will encounter 1,3-bisphosphoglycerate and PEP in glycolysis. These high-energy compounds, along with creatine and arginine phosphates, are used to replenish ATP from ADP. Other important high energy compounds include coenzyme A derivatives such as acetyl-CoA, and succinyl-CoA important in the citric acid cycle. Aminoacylated-tRNAs, the substrates used by the ribosome for protein synthesis, are high-energy compounds.

Problems and Solutions

1. An enzymatic hydrolysis of fructose-1-P

$$\text{Fructose}-1-P + H_2O \text{ D fructose} + P_i$$

was allowed to proceed to equilibrium at 25°C. The original concentration of fructose-1-P was 0.2 M, but when the system had reached equilibrium the concentration of fructose 1-P was only 6.52×10^{-5} M. Calculate the equilibrium constant for this reaction and the standard free energy of hydrolysis of fructose 1-P.

Answer: For $\text{fructose}-1-P + H_2O \text{ D } \text{fructose} + P_i$

The equilibrium constant, K_{eq}, is given by

$$K_{eq} = \frac{[\text{fructose}]_{eq}[P_i]_{eq}}{[\text{fructose}-1-P]_{eq}}$$

At 25°C or 298 K (273 + 25), $[\text{fructose-1-P}]_{eq} = 6.52 \times 10^{-5}$ M. Initially [fructose-1-P] = 0.2 M. The amount of the fructose produced is given by: 0.2 M - 6.52×10^{-5} M.

And, since an equal amount of $[P_i]$ is produced, K_{eq} may be written as follows:

$$K_{eq} = \frac{(0.2\text{M} - 6.52\times10^{-5})(0.2\text{M} - 6.52\times10^{-5})}{6.52\times10^{-5}}$$

$$K_{eq} = 613 \text{ M}$$

$$\Delta G^\circ = -RT\ln K_{eq}$$

$$\Delta G^\circ = -(8.314 \text{ J/mol K}) \times 298 \text{ K} \times \ln 613$$

$$\Delta G^\circ = -15.9 \text{ kJ/mol}$$

2. The equilibrium constant for some process ADB is 0.5 at 20°C and 10 at 30°C. Assuming that ΔH° is independent of temperature, calculate ΔH° for this reaction. Determine ΔG° and ΔS° at 20° and at 30°C. Why is it important in this problem to assume that ΔH° is independent of temperature?

Answer:

At 20°C

$$\Delta G^\circ = -RT\ln K_{eq}$$

$$\Delta G^\circ = -(8.314 \text{ J/mol K}) \times (273+20) \text{ K} \times \ln 0.5$$

$$= 1.69 \text{ kJ/mol}$$

At 30°C

$$\Delta G^\circ = -RT\ln K_{eq}$$

$$\Delta G^\circ = -(8.314 \text{ J/mol K}) \times (273+30) \text{K} \times \ln 10$$

$$= -5.80 \text{ kJ/mol}$$

From the equation $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$, we see that ΔG° is linearly related to T when ΔH° is independent of temperature. If this is the case, then $d\Delta H^\circ/dT = 0$ (i.e., the heat capacity is zero). A plot of ΔG° versus T will be linear with a slope =- ΔS° and a y intercept = ΔH°.

$$-\Delta S^\circ = \text{slope} = \frac{\Delta G^\circ_{30°C} - \Delta G^\circ_{20°C}}{T_{30°C} - T_{20°C}} = \frac{-5.8 - 1.69}{30 - 20}$$

$$\Delta S^\circ = 0.75 \text{ kJ/mol K}$$

ΔH° can be calculated using $\Delta H^\circ = \Delta G^\circ + T\Delta S^\circ$.

For 20°C, $\Delta H^\circ = 1.69(\text{kJ/mol}) + 293 \text{ K} \times 0.75(\text{kJ/mol K}) = 221.5 \text{kJ/mol}$

For 30°C, $\Delta H^\circ = -5.80(\text{kJ/mol}) + 303 \text{ K} \times 0.75(\text{kJ/mol K}) = 221.5 \text{kJ/mol}$

Therefore, ΔH° = 221.5 kJ/mol and ΔS° = 0.75 kJ/mol K at both temperatures, and, ΔG° = 1.69 kJ/mol at 20° C and ΔG° = -5.80 kJ/mol at 30° C.

3. The standard state free energy of hydrolysis of acetyl phosphate is ΔG° = -42.3 kJ/mol.

$$\text{Acetyl} - P + H_2O \rightarrow \text{acetate} + P_i$$

Calculate the free energy change for the acetyl phosphate hydrolysis in a solution of 2 mM acetate, 2 mM phosphate and 3 nM acetyl phosphate.

Answer:

$$\Delta G = \Delta G^\circ + RT\ln\frac{[\text{Product}]_{initial}}{[\text{Reactant}]_{initial}}$$

$$\Delta G = \Delta G^\circ + 8.314(\text{J/mol K})\times(273+20)\ \text{K}\times\ln\frac{[\text{acetate}][P_i]}{[\text{acetyl-P}]}$$

$$\Delta G = -42{,}300(\text{J/mol}) + 8.314(\text{J/mol K})\times(273+20)\ \text{K}\times\ln\frac{(2\times10^{-3})(2\times10^{-3})}{(3\times10^{-9})}$$

$$\Delta G = -24.8\ \text{kJ/mol}$$

4. Define a state function. Name three thermodynamic quantities that are state functions and three that are not.

Answer: State functions are quantities that depend on the state of a system not on the process or path taken to reach this state. For example, volume is a state function as is the change in volume ΔV. The quantity ΔV depends only on the final and initial value of V; ΔV is independent of the path taken from V_i to V_f. Pressure and temperature are also state functions. The total internal energy of a system is a state function. Recall from the first law of thermodynamics, the total internal energy changes by heat being absorbed or released and by work being done on or by the system. In going from one state to another, heat may or may not be exchanged and work may or may not be done. Thus, heat and work are not state functions. For example, using temperature as a measure of the total internal energy of a system, a change in temperature can occur upon absorption of heat with no work or by work with no heat. The amount of work done by a system does not indicate the final state of the system.

5. ATP hydrolysis at pH 7.0 is accompanied by release of a hydrogen ion to the medium

$$ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + HPO_4^{2-} + H^+$$

If the $\Delta G^{\circ\prime}$ for this reaction is -30.5 kJ/mol, what is ΔG° (that is, the free energy change for the same reaction with all components, including H^+, at a standard state of 1 M)?

Answer: The reaction produces H^+ and we can use the following equation to calculate ΔG°: $\Delta G^\circ = \Delta G^{\circ\prime} - RT\ln[H^+]$ where $\Delta G^{\circ\prime} = -30.5$ kJ/mol, T = 298 K, R = 8.314 J/mol K and $[H^+] = 10^{-7}$. Thus,

$$\Delta G^\circ = -30.5(\text{kJ/mol}) - 8.314\times10^{-3}(\text{kJ/mol K})\times(273+25)\ \text{K}\times\ln10^{-7.0}$$

$$\Delta G^\circ = -30.5 + 39.9 = 9.4\ \text{kJ/mol}$$

6. For the process A ⇌ B, K_{eq}(AB) is 0.02 at 37ºC. For the process B ⇌ C, K_{eq}(BC) is 1000 at 37ºC.
a. Determine K_{eq}(AC), the equilibrium constant for the overall process A ⇌ C, from K_{eq}(AB) and K_{eq}(BC).
b. Determine standard state free energy changes for all three processes, and use ΔG°(AC) to determine K_{eq}(AC). Make sure that this value agrees with that determined in part a, of this problem.

Answer:

For A ⇌ B and B ⇌ C,

$$K_{eq}(AB) = \frac{[B]_{eq}}{[A]_{eq}},\ \text{and}\ K_{eq}(BC) = \frac{[C]_{eq}}{[B]_{eq}}$$

By solving for $[B]_{eq}$ in the above two equations we find:

$$[B]_{eq} = K_{eq}(AB)\times[A]_{eq} = \frac{[C]_{eq}}{K_{eq}(BC)}$$

This equation can be rearranged to give:

$$\frac{[C]_{eq}}{[A]_{eq}} = K_{eq}(AC) = K_{eq}(AB)\times K_{eq}(BC) = 0.02\times1000\ \text{or,}$$

$$K_{eq}(AC) = 20$$

The standard free energy change is calculated as follows:

$$\Delta G^{\circ}(AB) = -RT\ln K_{eq}(AB) = -8.314\,(J/mol\,K) \times 310\,K \times \ln 0.02$$

$$\Delta G^{\circ}(AB) = 10.1\ kJ/mol$$

$$\Delta G^{\circ}(BC) = -RT\ln K_{eq}(BC) = -8.314\,(J/mol K) \times 310\,K \times \ln 1000$$

$$\Delta G^{\circ}(BC) = -17.8\ kJ/mol$$

$$\Delta G^{\circ}(AC) = -RT\ln K_{eq}(AC) = -8.314\,(J/mol\,K) \times 310\,K \times \ln 20$$

$$\Delta G^{\circ}(AC) = -7.72\,kJ/mol$$

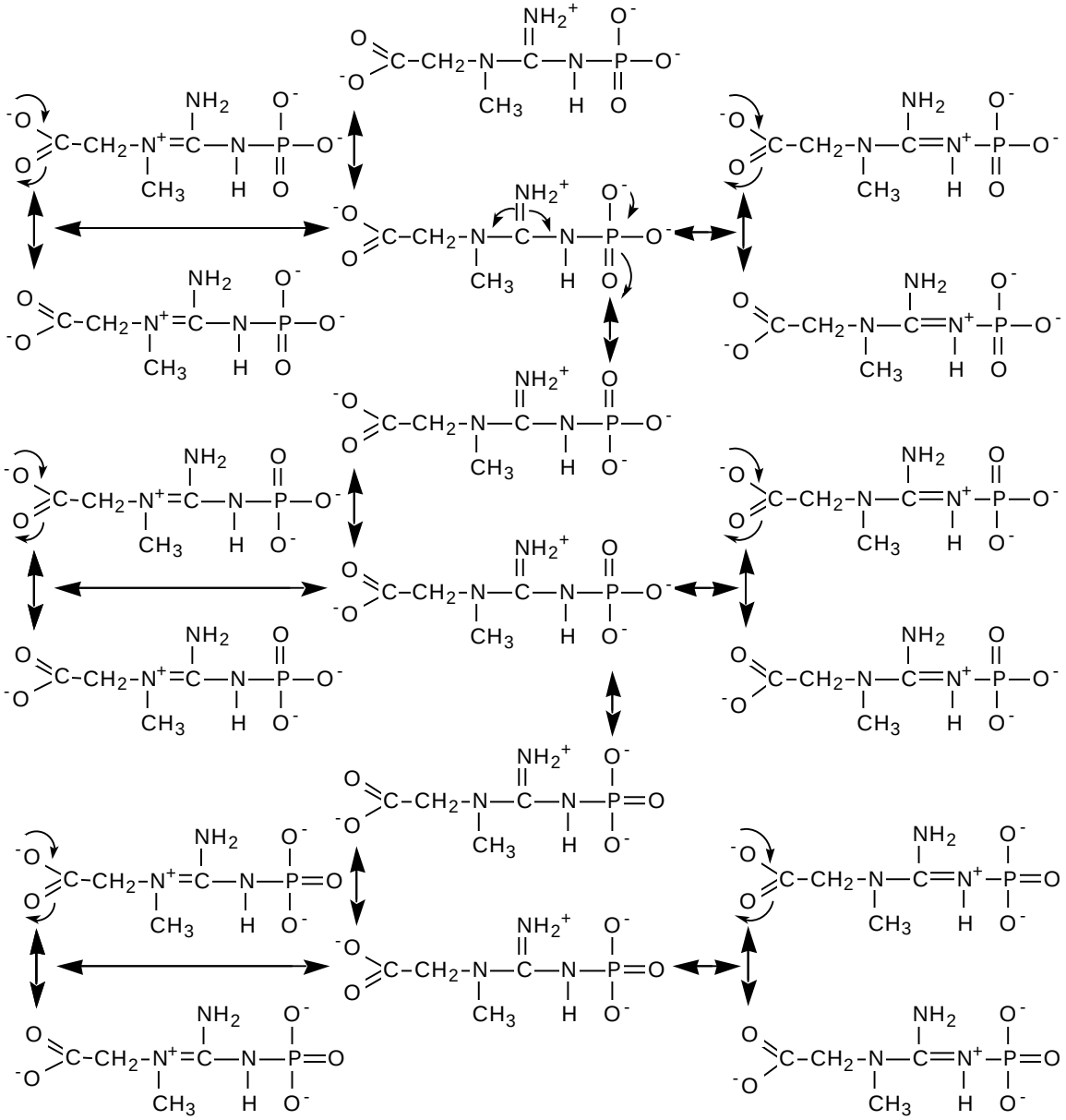
or

$$\Delta G^{\circ}(AC) = \Delta G^{\circ}(AB) + \Delta G^{\circ}(BC)$$

$$\Delta G^{\circ}(AC) = 10.1\,kJ/mol - 17.8\,kJ/mol = 7.70\,kJ/mol$$

7. Draw all possible resonance structures for creatine phosphate and discuss their possible effects on resonance stabilization of the molecule.

Answer: Creatine phosphate



8. Write the equilibrium constant, K_{eq}, for the hydrolysis of creatine phosphate and calculate a value for K_{eq} at 25°C from the value of $\bullet G^{o\prime}$ in Table 3.3.

Answer: For the reaction:

$$\text{creatine phosphate} + H_2O \;\mathrm{D}\; \text{creatine} + P_i,\ {}^{2}G^{o\prime} = -43.3\,kJ/mol$$

$$K_{eq} = \frac{[\text{creatine}]_{eq}[P_i]_{eq}}{[\text{creatine phosphate}]_{eq}[H_2O]_{eq}}$$

From ${}^{2}G^{o\prime} = -RT\ln K_{eq}$, we can write:

$$K_{eq} = e^{\frac{-{}^{2}G^{o\prime}}{RT}} = e^{\frac{-(-43.3\times10^{3})}{8.314\times298}}$$

$$K_{eq} = 3.89\times10^{7}$$

9. Imagine that creatine phosphate, rather than ATP, is the universal energy carrier molecule in the human body. Repeat the calculation presented in section 3.8,

calculating the weight of creatine phosphate that would need to be consumed each day by a typical adult human if creatine phosphate could not be recycled. If recycling of creatine phosphate were possible, and if the typical adult human body contained 20 grams of creatine phosphate, how many times would each creatine phosphate molecule need to be turned over or recycled each day? Repeat the calculation assuming that glycerol-3-phosphate is the universal energy carrier, and that the body contains 20 grams of glycerol-3-phosphate.

Answer: The calculation presented in section 3.8 determined the number of moles of ATP that must be hydrolyzed under cellular conditions to provide 5,860 kJ of energy. Under standard conditions, ATP hydrolysis yields 35.7 kJ/mol (see Table 3.3) whereas under cellular conditions the value is approximately 50 kJ/mol. In order to repeat the calculation using creatine phosphate, we must estimate the free energy of hydrolysis under cellular conditions. Alternatively, we can assume that the same number of moles of creatine phosphate must be hydrolyzed as ATP. The energy of hydrolysis of creatine phosphate is larger (i.e., more negative) than that of ATP. So, hydrolysis of an equivalent molar amount of creatine phosphate will release considerably more energy. Let us try both solutions.

First, let us assume that an equivalent number of moles of creatine phosphate is hydrolyzed. (The reason for considering this is that metabolic energy is in effect quantized as high-energy bonds.) From section 3.8 we see that 117 moles of ATP are required. An equal number of moles of creatine phosphate weighs:

$$(117 \text{ moles}) \times 180(\text{g/mol}) = 21{,}060\text{g}$$

And, the turnover of creatine phosphate is

$$\frac{21{,}060\text{g}}{20\text{g}} = 1{,}052 \text{ times}$$

To calculate the free energy of hydrolysis of creatine phosphate under cellular conditions, let us assume that in resting muscle, creatine phosphate is approximately 20 mM, the concentration of P_i is approximately 5 mM (see Problem 10), and approximately 10% of creatine phosphate or 2 mM is as creatine. Using these values and the standard free energy of hydrolysis of creatine phosphate (from Table 3.3) we calculate the energy of hydrolysis under cellular conditions as follows:

$$\Delta G = \Delta G^\circ + RT\ln\frac{[\text{creatine}][P_i]}{[\text{creatine phosphate}]}$$

$$\Delta G = -43.3(\text{kJ/mol}) + 8.314\times10^{-3}(\text{kJ/mol K}) \times (273+37)\text{K} \times \ln\frac{(2\times10^{-3})(5\times10^{-3})}{20\times10^{-3}}$$

$$\Delta G = -62.9\text{kJ/mol}$$

The number of moles of creatine phosphate is given by

$$\frac{5860 \text{ kJ}}{62.9\text{kJ/mol}} = 93.2 \text{ moles}$$

The molecular weight of creatine phosphate is 180. Therefore

$93.2 \text{ moles} \times 180(\text{g/mole}) = 16{,}780$ g is required.

The turnover of creating phosphate is $\frac{16{,}780\text{g}}{20\text{g}} = 839$ times.

The solution to the problem using glycerol-3-phosphate is slightly more complicated. From Table 3.3 we see that the standard free energy of hydrolysis of the compound is only - 9.2 kJ/mol, considerably lower than the - 35.7 kJ/mol listed for ATP hydrolysis. So, we cannot simply assume that an equivalent number of moles of glycerol-3-phosphate will substitute for ATP hydrolysis as we did for creatine phosphate above because hydrolysis of an equivalent number of moles of glycerol-3-phosphate will not supply sufficient energy. To solve the problem, we must estimate the energy of hydrolysis of glycerol-3-phosphate under cellular conditions as we did for creatine phosphate hydrolysis above. Let us assume that $[P_i]$ = 5 mM, and that the ratio of [glycerol]:[glycerol-3-phosphate] is 1:10. Under these conditions,

$$\Delta G = -9.2(\text{kJ/mol}) + 8.314\times10^{-3}(\text{kJ/mol K})\times(273+37)\ \text{K}\times\ln\frac{5\times10^{-3}}{10}$$

$$\Delta G = -28.8\text{kJ/mol}$$

The number of moles of glycerol-3-phosphate is given by

$$\frac{5860\ \text{kJ}}{28.8\text{kJ/mol}} = 203\ \text{moles}$$

The molecular weight of glycerol-3-phosphate is 172.08. Therefore

$203\ \text{moles}\times172.08(\text{g/mole}) = 35{,}013\text{g}$ is required.

The turnover of glycerol-3-phosphate is $\frac{35{,}013\text{g}}{20\text{g}} = 1{,}751$ times.

If we use only the standard free energy, we require:

$$\frac{5860\ \text{kJ}}{9.2\text{kJ/mol}} = 637\ \text{moles or}\ 637\ \text{moles}\times172.08(\text{g/mole}) = 109{,}615\text{g}$$

The turnover of creating phosphate is $\frac{109{,}615\text{g}}{20\text{g}} = 5{,}480$ times.

10. Calculate the free energy of hydrolysis of ATP in a rat liver cell in which the ATP, ADP, and P_i concentrations are 3.4, 1.3, and 4.8 mM, respectively.

Answer: For [ATP] = 3.4 mM, [ADP] = 1.3 mM, and $[P_i]$ = 4.8 mM, calculate the ΔG of hydrolysis of ATP.

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

$$\Delta G = -30.5\text{kJ/mol (from Table 3.3)} + 8.314\times10^{-3}\times(293+25)\ \text{K}\times\ln\frac{(1.3\times10^{-3})(4.8\times10^{-3})}{3.4\times10^{-3}}$$

$$\Delta G = -46.1\ \text{kJ/mol}$$

At 37ºC, $\Delta G = -46.7$ kJ/mol

11. Hexokinase catalyzes the phosphorylation of glucose from ATP, yielding glucose-6-P and ADP. Using the values of Table 3.3, calculate the standard-state free energy change and equilibrium constant for the hexokinase reaction.

Answer:

Hexokinase catalyzes the following reaction :

glucose + ATP D glucose-6-P + ADP

This reaction may be broken d own into the following two reactions:

glucose + P_i D glucose-6-P + H_2O (1) and,

ATP + H_2O D ADP + P_i (2)

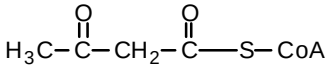
From Table 3.3, we find that $\Delta G^{\circ\prime}$ = -13.9 kJ/mol for glucose-6-P hydrolysis.
Thus, the reverse reaction, namely reaction (1), must have $\Delta G^{\circ\prime}$ = +13.9 kJ/mol.

From Table 3.3, we also find that ATP hydrolysis has $\Delta G^{\circ\prime}$ = - 30.5 kJ/mol.
The overall $\Delta G^{\circ\prime}$ for phosphoryl transfer from ATP to glucose is:

$$\Delta G^{\circ\prime} = +13.9 + (-30.5) = -16.6\text{kJ/mol and,}$$

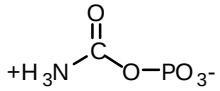
$$K_{eq} = e^{-\frac{\Delta G^{\circ\prime}}{RT}} = e^{-\frac{(16.6\times10^{3})}{8.314\times310}} = 626.9$$

12. Would you expect the free energy of hydrolysis of acetoacetyl-coenzyme A (see diagram) to be greater than, equal to, or less than that of acetyl-coenzyme A? Provide a chemical rationale for your answer.



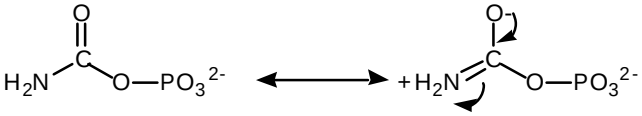
Answer: Hydrolysis of acetyl-coenzyme A produces free coenzyme A and acetate whereas hydrolysis of acetoacetyl-coenzyme A releases acetoacetate and coenzyme A. Acetate is relatively stable; however, acetoacetate is unstable and will break down to acetone and CO_2. Thus, the instability of one of the products of hydrolysis of acetoacetyl-coenzyme A, namely acetoacetate, will make the reverse reaction (i.e., production of acetoacetyl-coenzyme A) unlikely. Furthermore, the terminal acetyl group of acetoacetyl-coenzyme A is electron-withdrawing in nature and will destabilize the thiol ester bond of acetoacetyl-CoA. Thus, the free energy of hydrolysis of acetoacetyl-coenzyme A is expected to be greater than that of acetyl-coenzyme A and in fact, the free energy of hydrolysis is -43.9 kJ/mol for acetoacetyl-CoA and -31.5 kJ/mol for acetyl-CoA.

13. Consider carbamoylphosphate, a precursor in the biosynthesis of pyrimidines:



Based on the discussion of high energy phosphates in this chapter, would you expect carbamoyl phosphate to possess a high free energy of hydrolysis? Provide a chemical rationale for your answer.

Answer: Is carbamoyl phosphate destabilized due to electrostatic repulsion? The carbonyl oxygen will develop a partial-negative charge causing charge-repulsion with the negatively charged phosphate. So, charge destabilization exists. Are the products stabilized by resonance or by ionization? Without regard to resonance states of the phosphate group, there are two possible resonance structures for carbamoyl phosphate:



However, we expect that the carbonyl-carbon must pass through a positively charged intermediate and in doing so, affect phosphate resonance. Thus, the products are resonance stabilized. Finally, are there entropy factors? The products of hydrolysis are phosphate and carbamic acid. Carbamic acid is unstable and decomposes to CO_2 and NH_3 unless stabilized as a salt by interacting with a cation. With these considerations in mind, we expect carbamoyl phosphate to be unstable and therefore a high energy compound. The free energy of hydrolysis of carbamoyl phosphate is -51.5 kJ/mol, whereas for acetyl phosphate it is only -43.3 kJ/mol.

14. Consider the data in Figures 3.4 and 3.5. Is the denaturation of chymotrypsinogen spontaneous at 58ºC? And what is the temperature at which the native and denatured forms of chymotrypsinogen are in equilibrium?

Answer: Figure 3.4 is a plot of $\bullet G^{o}$ versus temperature and from it we see that at 58ºC, $\bullet G^{o}$ is approximately –3 kJ/mol. Thus, we expect denaturation to be spontaneous at this temperature if we start with a solution of chymotrypsinogen in its native conformation. The data presented in Figure 3.5 do not address the question of spontaneity of the reaction at 58ºC. The $\bullet S^{o}$ is positive at this temperature, suggesting that disorder in the denatured state is greater than that in the native state. This is consistent with an unfolding process but not predictive of the process occurring.

To calculate the temperature at which the process is at equilibrium we must find the temperature at which $K_{eq} = 1$ and $\bullet G^{o} = 0$. We can use the data used to construct Figure 3.3 to make this calculation. From Figure 3.3 we see that a plot of $R\ln K_{eq}$ versus 1000/T is linear and with a slope of magnitude 533 kJ/mol. A linear plot implies the following:

$$R \ln K_{eq} = -533 \times \frac{1000}{T} + b$$

We can solve the equation for b, the x-intercept, and can evaluate b by using any one data point in Figure 3.3 that is in the linear portion of the plot. Once b is evaluated, we can substitute it

into the above equation and find T at which K_{eq} = 1.0. Remembering that T is in degrees Kelvin, we find that the protein is in equilibrium at T = 56.6ºC.

15. Consider Tables 3.1 and 3.2, as well as the discussion of Table 3.2 in the text, and discuss the meaning of a positive ΔC_p in Table 3.1.

Answer: In each case of protein denaturation presented in Table 3.1, we see that ΔC_p is positive. The corresponding ΔG^o values in each case are also positive, implying that the native state is favored over the denatured state i.e., denaturation is unfavorable for these proteins in the conditions specified. Table 3.2 presents us with a reaction also accompanied by a positive ΔC_p, namely, transfer of toluene into water. This process is unfavorable because hydrophobic toluene will order water molecules due to its inability to hydrogen bond with water. A similar process occurs when proteins denature: their hydrophobic cores become exposed to water.

Questions for Self Study

1. True of False.
   a. The internal energy of an isolated system is conserved. _____
   b. A closed system can exchange matter but not energy with the surroundings. ____
   c. An open system includes a system and its surroundings. _____
   d. An open system can exchange matter with another open system. _____
   e. The internal energy of an open system is always constant. _____

2. The first law of thermodynamics states that there are only two ways to change the internal energy of any system. What are they?

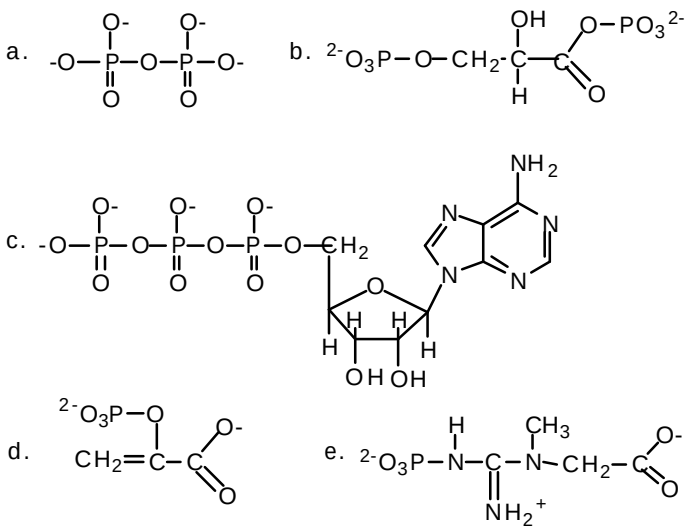
3. Enthalpy, H, is defined as H = E + PV and $\Delta E = q + w$. Under what conditions is $\Delta H = q$?

4. Define the terms in the following expression: $S = k \ln W$.

5. Match the items in the two columns.

a. $\Delta G = \Delta H - T\Delta S$	1. Reaction spontaneous as written.
b. $\Delta G = \Delta G^\circ + RT \ln ([P]/[R])$	2. Used to determine standard Gibbs free energy change.
c. $\Delta G^\circ = 0$	3. Reaction unfavorable.
d. $\Delta G = 0$	4. Used to calculate amount of free energy released when reaction proceeds to equilibrium.
e. $\Delta G > 0$	5. Definition of change in Gibbs free energy.
f. $\Delta G < 0$	6. System at equilibrium.
g. $\Delta G^\circ = - RT \ln K_{eq}$	7. $K_{eq} = 1$.

6. The compounds shown below include ATP, pyrophosphate, phosphoenolpyruvate, creatine phosphate, and 1,3-bisphosphoglycerate. They are all examples of high-energy compounds. Identify each, locate the high-energy bond and list the products of hydrolysis of this bond.



7. What are the three chemical reasons for the large negative energy of hydrolysis of phosphoric acid anhydride linkage for compounds such at ATP, ADP, and pyrophosphate?

8. Creatine phosphate is an example of a guanidinium phosphate, a high-energy phosphate compound. This compound is abundant in muscle tissue. What is its function?

9. During protein synthesis amino acids are joined in amide linkage on the ribosome. The substrates for this reaction are not free amino acids but rather amino acids attached via their carboxyl groups to the 3' hydroxyl group of tRNAs. What kind of bond is formed between the amino acid and the tRNA? Is this a high-energy bond? Given the fact that aminoacyl-tRNA formation is accompanied by hydrolysis of ATP to AMP and PP_i and that PP_i is subsequently hydrolyzed to 2 P_i, how many high-energy phosphates are consumed to produce an aminoacyl-tRNA?

10. Although the standard free energy of hydrolysis of ATP is around -30 kJ/mol the cellular free energy change is even more negative. What factors contribute to this?

Answers

1. a./T; b./F; c./F; d./T; e./F.

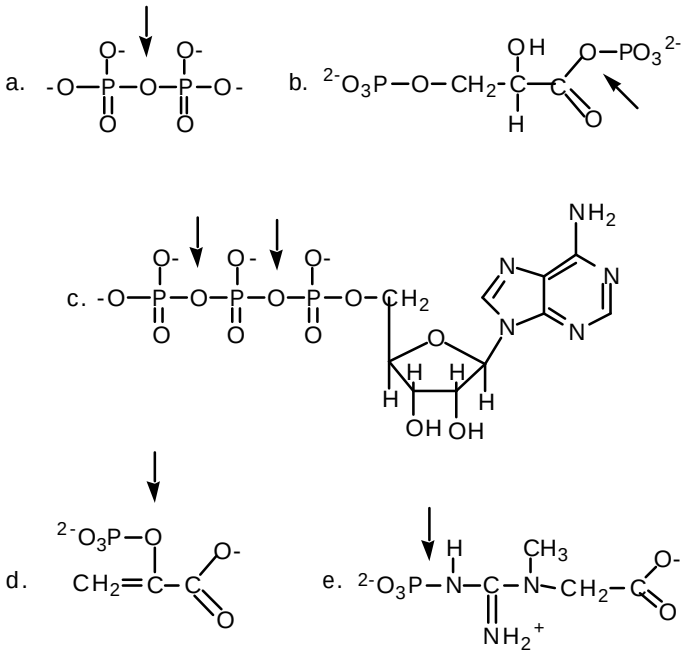
2. Energy flow in the form of heat or work.

3. In general, $\bullet H = \bullet E + P\bullet V + V\bullet P = q + w + P\bullet V + V\bullet P$. When the pressure of the system remains constant (i.e., $\bullet P = 0$) and work is limited to only mechanical work (i.e., $w = -P\bullet V$) then $\bullet H = q$.

4. S is the entropy, k is Boltzmann's constant, ln W is the natural logarithm of the number of ways, W, of arranging the components of a system.

5. a./5; b./4; c./7; d./6; e./3; f./1; g./2.

6. a./pyrophosphate, hydrolysis products 2 P_i. b./1,3-bisphosphoglycerate, hydrolysis products 3-phosphoglycerate and P_i. c./ATP, hydrolysis products either ADP and P_i or AMP and pyrophosphate. d./phosphoenolpyruvate, hydrolysis products pyruvate and P_i. e./creatine phosphate, hydrolysis products creatine and P_i. The location of high-energy bonds is shown below.



7. Bond strain due to electrostatic repulsion, stabilization of products by ionization and resonance, and entropy factors.

8. Creatine phosphate is used to replenish supplies of ATP by transferring phosphate to ADP in a reaction catalyzed by creatine kinase.

9. Amino acid ester bonds in aminoacyl-tRNAs are high-energy bonds produced at the expense of two high-energy phosphate bonds.

10. The presence of divalent and monovalent cations and the maintenance of low levels of ADP and P_i and high levels of ATP are responsible for the large negative free energy change of ATP under cellular conditions.

Additional Problems

1. Show that $\Delta G^\circ = -RT \ln K_{eq}$.

2. The term ΔG° may be evaluated by measuring ΔG for a reaction in which reactants and products start out at 1 M concentration. The ΔG measured in this case is the standard-state free energy and is equal ΔG°. Prove this.

3. If a particular reaction is allowed to reach equilibrium, is the concentration of product ever dependent on the initial concentrations of reactant and product?

4. Confusion reigns supreme when work and energy expenditure are discussed. Define the term work. Are work and energy expenditure synonymous?

5. There are two statements about spontaneity of reactions: $\Delta S > 0$, and $\Delta G < 0$. Justify that these statements are in fact true descriptions of spontaneity.

6. DNA ligase catalyzes formation of a phosphodiester bond between a 5'-phosphate and a 3'-hydroxyl group on the ends of two DNAs to be joined. Many ligases use hydrolysis of ATP to drive phosphodiester bond synthesis; however, the E. coli ligase uses NAD^+ as a high-energy

compound. Explain why NAD^+ is considered a high-energy compound. What type of reaction is required to release energy from NAD^+ and what are the products?

7. You find yourself in the laboratory, late at night, working on an important assay and you discover that the last of the ATP stock is used up. After frantically searching everywhere, you find a 10 mg bottle of 2'-deoxyadenosine 5'-triphosphate. Is this a high-energy compound? Will it substitute for ATP in your assay?

8. Stock solutions of ATP are usually adjusted to around neutral pH to stabilize them. Why?

9. Intense muscle activity depletes ATP and creatine phosphate stores and produces ADP, creatine, AMP, and P_i. ADP is produced by ATP hydrolysis catalyzed by myosin, a component of the contractile apparatus of muscle. Creatine is a product of creatine kinase activity, and AMP is produced by adenylate kinase from two ADPs. Given this information, explain how high-energy phosphate compounds in muscle are interconnected.

10. The standard free energy of hydrolysis of glucose-1-phosphate is -21 kJ/mol whereas it is only -13.9 kJ/mol for glucose-6-phosphate. Provide an explanation for this difference.

Abbreviated Answers

1. At equilibrium $\bullet G = 0$ and the concentration of reactants and products are at their equilibrium values. Using

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

$$\Delta G = 0 = \Delta G^{\circ} + RT \ln \frac{[C_{eq}][D_{eq}]}{[A_{eq}][B_{eq}]}$$

or, solving for ΔG° we find that :

$$\Delta G^{\circ} = -RT \ln \frac{[C_{eq}][D_{eq}]}{[A_{eq}][B_{eq}]} = -RT \ln K_{eq}$$

2. Using

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

$$\Delta G = \Delta G^{\circ} + RT \ln \frac{1M \times 1M}{1M \times 1M} = \Delta G^{\circ} + RT \ln 1$$

But, $\ln 1 = 0$, and

$$\Delta G = \Delta G^{\circ}$$

3. The equilibrium constant is independent of the initial concentrations of reactants and products but the equilibrium constant is the ratio of the product of the concentration of products to the product of the concentration of reactants. This ratio is independent of initial concentrations. Clearly, the absolute amount of product depends on the initial concentration of reactant.

4. The first definition of work found in The Random House College Dictionary, Revised Edition is "exertion or effort directed to produce or accomplish something...". This is close to the thermodynamic definition of work but not quite right. We can modify the definition by replacing "exertion or effort" with "energy" an important modification because it introduces a term that can be quantitated. The rest of the definition is acceptable; however, an important addendum is necessary. Work is not all of the energy expended to produce or accomplish something. Rather, it is only that portion of expended energy that is equal to the amount of energy released when the something that was accomplished is allowed to return to its original state.

5. Entropy is a measure of disorder and for a reaction to be spontaneous $\Delta S > 0$ or since $S_f - S_i > 0$, $S_f > S_i$. A spontaneous reaction results in an increase in disorder.

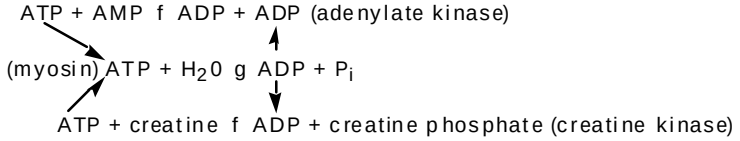
Gibbs free energy is the amount of energy available to do work at constant pressure and temperature. Using G as a criterion for spontaneity requires that $\Delta G < 0$ or since $G_f - G_i < 0$, $G_f < G_i$. The amount of energy available to do work decreases for a spontaneous reaction.

6. NAD^+ contains a single high-energy phosphoric anhydride linkage between AMP and nicotinamide monophosphate nucleotide. Hydrolysis will release AMP and nicotinamide nucleoside monophosphate.

7. 2'-Deoxyadenosine 5'-triphosphate or dATP contains two high-energy phosphoric anhydride bonds. However, if the assays being performed are enzymatic assays (as opposed to some chemical assay), then it is out-of-the-question to even think about substituting dATP for ATP. ATP is a ribonucleotide; dATP is a deoxyribonucleotide. dATP is used for synthesis of DNA and little else.

8. At first thought it would might seem reasonable to make the ATP solution slightly acidic. This will neutralize the negatively-charged phosphates and should lead to stabilization of the phosphoric anhydride linkages. The problem with this idea is that the N-glycosidic linkage is acid labile. This bond is more stable in alkaline solution but the anhydride bonds are readily cleaved by hydroxide attack.

9. The key here is to recognize that kinases are enzymes that transfer the γ-phosphate of ATP to a target molecule. Thus, creatine kinase phosphorylates creatine and adenylate kinase phosphorylates AMP. The following scheme outlines how ATP, ADP, AMP, creatine phosphate, creatine, and P_i are related.



10. Phosphate is an electron-withdrawing group that is attached to quite different carbons in glucose-6-phosphate versus glucose-1-phosphate. In the latter, carbon-1 is already bonded to an electronegative oxygen in the pyranose form.

Summary

Thermodynamics - a collection of laws and principles describing the flows and interchanges of heat, energy and matter - can provide important insights into metabolism and bioenergetics. Thermodynamics distinguishes between closed systems, which cannot exchange matter or energy with the surroundings; isolated systems, which may exchange heat, but not matter, with the surroundings; and open systems, which may exchange matter, energy or both with the surroundings.

The first law of thermodynamics states that the total energy of an isolated system is conserved. E, the internal energy function, which is equal to the sum of heat absorbed and work done on the system, is a useful state function, which keeps track of energy transfers in such systems. In constant pressure processes, it is often more convenient to use the enthalpy function ($H = E + PV$) for analysis of heat and energy exchange. For biochemical processes, in which pressure is usually constant and volume changes are small, enthalpy (H) and internal energy (E) are often essentially equal. Enthalpy changes for biochemical processes can often be determined from a plot of $R(\ln K_{eq})$ versus $1/T$ (a van't Hoff plot).

Entropy is a measure of disorder or randomness in the system. The second law of thermodynamics states that systems tend to proceed from ordered (low entropy) states to disordered (high entropy) states. The entropy change for any reversible process is simply the heat transferred divided by the temperature at which the transfer occurs: $dS_{rev} = dq/T$.

The third law of thermodynamics states that the entropy of any crystalline, perfectly ordered substance must approach zero as the temperature approaches 0° K, and at 0° K, entropy is exactly zero. The concept of entropy thus invokes the notion of heat capacity - the amount of heat any substance can store as the temperature of that substance is raised by one degree K. Absolute entropies may be calculated for any substance if the heat capacity can be evaluated at temperatures from 0° K to the temperature of interest, but entropy changes are usually much more important in biochemical systems.

The free energy function is defined as G = H - TS and, for any process at constant pressure and temperature (i.e., most biochemical processes), •G = •H - T•S. If •G is negative, the process is exergonic and will proceed spontaneously in the forward direction. If •G is positive, the reaction or process is endergonic and will proceed spontaneously in the reverse direction. The sign and value of •G, however, do not allow one to determine how fast a process will proceed. It is convenient to define a standard state for processes of interest, so that the thermodynamic parameters of different processes may be compared. The standard state for reactions in solution is 1 M concentration for all reactants and products. The free energy change for a process A + B D C + D at concentrations other than standard state is given by:

$$\bullet G = \bullet G^\circ + RT \ln([C][D]/[A][B])$$

and the standard state free energy change is related to the equilibrium constant for the reaction by $\bullet G^\circ = -RT \ln K_{eq}$. This states that the equilibrium established for a reaction in solution is a function of the standard state free energy change for the process. In essence, •G° is another way of writing an equilibrium constant. Moreover, the entropy change for a process may be determined if the enthalpy change and free energy change calculated from knowledge of the equilibrium constant and its dependence on temperature are known.

The standard state convention of 1 M concentrations becomes awkward for reactions in which hydrogen ions are produced or consumed. Biochemists circumvent this problem by defining a modified standard state of 1 M concentration for all species except H^+, for which the standard state is 1×10^{-7} M or pH 7.

Thermodynamic parameters may provide insights about a process. As an example, consider heat capacity changes: Positive values indicate increase freedom of movement whereas negative values indicate less freedom of motion.

Many processes in living things must run against their thermodynamic potential, i.e., in the direction of positive •G. These processes are driven in the thermodynamically unfavorable direction via coupling with highly favorable processes. Coupled processes are vitally important in intermediary metabolism, oxidative phosphorylation, membrane transport and many other processes essential to life.

There is a hierarchy of energetics among organisms: certain organisms capture solar energy directly, whereas others derive their energy from this group in subsequent chemical processes. Once captured in chemical form, energy can be released in controlled exergonic reactions to drive life processes. High energy phosphate anhydrides and reduced coenzymes mediate the flow of energy from exergonic reactions to the energy-requiring processes of life. These molecules are transient forms of stored energy, rapidly carrying energy from point to point in the organism. One of the most important high energy phosphates is ATP, which acts as an intermediate energy shuttle molecule. The free energy of hydrolysis of ATP (for hydrolysis to ADP and phosphate) is less than that of PEP, cyclic-AMP, 1,3-BPG, creatine phosphate, acetyl phosphate and pyrophosphate, but is greater than that of the lower energy phosphate esters, such as glycerol-3-phosphate and the sugar phosphates. Group transfer potential - the free energy change that occurs upon hydrolysis (i.e., transfer of a chemical group to water) - is a convenient parameter for quantitating the energetics of such processes. The phosphoryl group transfer potentials for high energy phosphates range from -31.9 kJoule/mole for UDP-glucose to -35.7 kJoule/mole for ATP to -62.2 kJoule/mole for PEP.

The large negative •G°' values for the hydrolysis of phosphoric acid anhydrides (such as ATP, GTP, ADP, GDP, sugar nucleotides and pyrophosphate) may be ascribed to 1) destabilization of the reactant due to bond strain caused by electrostatic repulsion, 2) stabilization of the products by ionization and resonance, and 3) increases in entropy upon product formation. Mixed anhydrides of phosphoric and carboxylic acids - known as acyl phosphates - are also energy-rich. Other classes of high energy species include enol phosphates, such as PEP, guanidinium phosphates, such as creatine phosphate, cyclic nucleotides, such as 3',5'-cyclic AMP, amino acid esters, such as aminoacyl-tRNA and thiol esters, such as coenzyme A. Other biochemically

important high energy molecules include the pyridine nucleotides (NADH, NADPH), sugar nucleotides (UDP-glucose) and S-adenosyl methionine, which is involved in the transfer of methyl groups in many metabolic processes.

Though the hydrolysis of ATP and other high energy phosphates are often portrayed as simple processes, these reactions are far more complex in real biological systems. ATP, ADP and similar molecules can exist in several different ionization states, and each of these individual species can bind divalent and monovalent metal ions, so that metal ion complexes must also be considered. For example, ATP has five dissociable protons. The adenine ring amino group exhibits a pK_a of 4.06, whereas the last proton to dissociate from the triphosphate chain possesses a pK_a of 6.95. Thus, at pH 7, ATP is a mixture of ATP^{4-} and $HATP^{3-}$. If equilibrium constants for the hydrolysis reactions are re-defined in terms of total concentrations of the ionized species, then such equilibria can be considered quantitatively, and fractions of each ionic species in solution can be determined. The effects of metal ion binding equilibria on the hydrolysis equilibria can be quantitated in a similar fashion.

The free energy changes for hydrolysis of high energy phosphates such as ATP are also functions of concentration. In the environment of the typical cell, where ATP, ADP and phosphate are usually 5 mM or less, the free energy change for ATP hydrolysis is substantially larger in magnitude than the standard state value of -30.5 kJoule/mole.

High energy molecules such as ATP are rapidly recycled in most biological environments. The typical 70 kg human body contains only about 50 grams of ATP/ADP. Each of these ATP molecules must be recycled nearly 2,000 times each day to meet the energy needs of the body.