

Chemistry 331

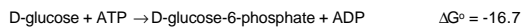
Lecture 19 Applications

NC State University

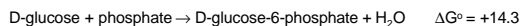
Thermodynamics of glycolysis

Reaction	ΔG° (kJ/mol)
D-glucose + ATP \rightarrow D-glucose-6-phosphate + ADP	$\Delta G^\circ = -16.7$
D-glucose-6-phosphate \rightarrow D-fructose-6-phosphate	$\Delta G^\circ = +1.7$
D-fructose-6-phosphate + ATP \rightarrow D-fructose-1,6-diphosphate + ADP	$\Delta G^\circ = -14.2$
D-fructose-1,6-diphosphate \rightarrow glyceraldehyde-3-phosphate + dihydroxyacetone phosphate	$\Delta G^\circ = +23.8$
dihydroxyacetone phosphate \rightarrow glyceraldehyde-3-phosphate	$\Delta G^\circ = +7.5$
glyceraldehyde-3-phosphate + phosphate + NAD $^+$ \rightarrow 1,3-diphosphoglycerate + NADH + H $^+$	$\Delta G^\circ = +6.3$
1,3-diphosphoglycerate + ADP \rightarrow 3-phosphoglycerate + ATP	$\Delta G^\circ = -18.8$
3-phosphoglycerate \rightarrow 2-phosphoglycerate	$\Delta G^\circ = +4.6$
2-phosphoglycerate \rightarrow 2-phosphoenolpyruvate + H $_2$ O	$\Delta G^\circ = +1.7$
2-phosphoenolpyruvate + ADP \rightarrow pyruvate + ATP	$\Delta G^\circ = -31.4$
pyruvate + NADH + H $^+$ \rightarrow lactate + NAD $^+$	$\Delta G^\circ = -25.1$
pyruvate \rightarrow acetaldehyde + CO $_2$	$\Delta G^\circ = -19.8$
acetaldehyde + NADH + H $^+$ \rightarrow ethanol + NAD $^+$	$\Delta G^\circ = -23.7$

Phosphorylation of glucose



The reaction can be decomposed into two reactions.



The sum of the two reactions results in an overall negative free energy change under standard conditions. In this manner the strongly spontaneous hydrolysis of ATP is coupled to the otherwise unspontaneous glucose phosphorylation. This reaction is typical of the role played by ATP in the cell.

Note that the values for ΔG° assume concentrations of 1 M. Clearly, the concentrations in the cell are often quite different from the standard state and this will have profound consequences for the direction of spontaneous change.

The role of enzymes

All of the reactions in the glycolytic pathway are catalyzed by enzymes. For example, the reaction considered on the previous slide is catalyzed by hexokinase. The role of the enzyme is to speed up the reaction, but the enzyme does not change thermodynamics of the process. The role of enzymes is the same as that of any catalyst. Catalysts affect the kinetics of the reaction, but not the thermodynamics. We will consider the role of catalysts in the second half of the course.

Notice that ΔG° for certain steps is positive. For example, D-glucose-6-phosphate \rightarrow D-fructose-6-phosphate $\Delta G^\circ = +1.7$ is catalyzed by phosphoglucose isomerase.

The equilibrium constant for this process is $K = \exp\{-\Delta G^\circ/RT\} = \exp\{-1700/8.31/310\} \sim 0.5$. Under standard conditions there will be only a small concentration of D-fructose-6-phosphate.

Question

Given that $K \sim 0.5$ for the reaction
D-glucose-6-phosphate \rightarrow D-fructose-6-phosphate

Calculate the concentration of D-fructose-6-phosphate at equilibrium under standard conditions.

- A. 1 M
- B. 0.5 M
- C. 0.333 M
- D. 0.15 M

Question

Given that $K \sim 0.5$ for the reaction
D-glucose-6-phosphate \rightarrow D-fructose-6-phosphate

Calculate the concentration of D-fructose-6-phosphate at equilibrium under standard conditions.

- A. 1 M

- B. 0.5 M

- C. 0.333 M

- D. 0.15 M

$$K = \frac{x}{1-x}, \quad K(1-x) = x, \quad K = (1+K)x$$
$$x = \frac{K}{1+K} = \frac{0.5}{1.5} = 0.333$$

Intracellular conditions are not equilibrium conditions

If the subsequent step in a series of reactions is highly spontaneous this will tend to deplete the product for the previous reaction. Thus, more of the product will tend to be formed by L'Chateliers principle.

We can observe this quantitatively by considering the value of Q, the reaction quotient. Since,

$$\Delta G = \Delta G^\circ + RT \ln Q$$

the value of ΔG may not be zero. In other words, the coupled series of reactions in the cell are not at equilibrium but rather they proceed under steady state conditions where the concentrations are not at 1 M, but are poised so that the overall effect on a series of reactions is to produce a net spontaneous change.

Sample Problem in Metabolism

The enzyme aldolase catalyzes the conversion of fructose 1,6-diphosphate (FDP) to dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP). Under physiological conditions the concentrations of these species in red blood cells (erythrocytes) are $[FDP] = 35 \mu\text{M}$, $[DHAP] = 130 \mu\text{M}$ and $[GAP] = 15 \mu\text{M}$. Will the conversion occur spontaneously under these conditions?

Solution: The standard free energy change for the reaction is
 $FDP \rightarrow DHAP + GAP \quad \Delta G^\circ = +23.8 \text{ kJ}$

and

$$Q = \frac{[DHAP][GAP]}{[FDP]} = \frac{(130 \times 10^{-6})(15 \times 10^{-6})}{(35 \times 10^{-6})} = 5.57 \times 10^{-5}$$

$$\Delta G = \Delta G^\circ + RT \ln Q = 23800 \text{ J/mol} + (8.31 \text{ J/mol}\cdot\text{K})(310 \text{ K}) \ln(5.57 \times 10^{-5}) = -1434 \text{ J/mol or } -1.43 \text{ kJ/mol}$$

The reaction will occur spontaneously under the conditions of the cell.

Thermodynamics of DNA hybridization

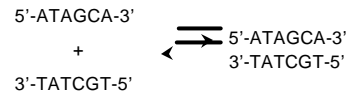
A combination of spectroscopy and calorimetry was used to determine the free energies of melting of short oligonucleotides. Based on these measurements the free energy of a helix can be determined based on 10 sets of nearest-neighbor pairs shown on the next slide.

In addition to these values we need to know the free energy of the initiation (i.e. the first base pair). The overall free energy is then calculated from:

$$\Delta G^\circ = \Delta G^\circ (\text{initiation}) + \sum \Delta G^\circ (\text{nearest neighbors})$$

Sample problem

Determine the melt temperature for the oligonucleotide



Solution:

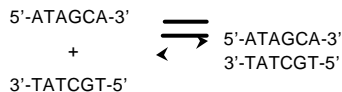
$$\Delta G^\circ = \Delta G^\circ (\text{initiation}) + \sum \Delta G^\circ (\text{nearest neighbors})$$

$$\begin{aligned} &= \Delta G^\circ \{GC \text{ init}\} + \Delta G^\circ \left\{ \begin{array}{c} AT \\ TA \end{array} \right\} + \Delta G^\circ \left\{ \begin{array}{c} TA \\ AT \end{array} \right\} + \Delta G^\circ \left\{ \begin{array}{c} AG \\ TC \end{array} \right\} + \Delta G^\circ \left\{ \begin{array}{c} GC \\ CG \end{array} \right\} + \Delta G^\circ \left\{ \begin{array}{c} CA \\ GT \end{array} \right\} \\ &= 20.9 \quad -6.3 \quad -3.8 \quad -6.7 \quad -13.0 \quad -6.9 \\ &= -16.8 \text{ kJ} \end{aligned}$$

Note that we use GC initiation if there is a single GC base pair. Only use AT initiation of the strands are all A and T.

Sample problem

Determine the melt temperature for the oligonucleotide



Solution (cont;d):

$$\Delta G^\circ = -16.8 \text{ kJ}$$

Notice that the free energy of initiation is positive. Initiation is unfavorable because of the entropy that must be overcome to bring the chains together. To calculate the melt temperature we need the enthalpy of reaction as well.

$$\begin{aligned} \Delta H^\circ &= \Delta H^\circ \left\{ \begin{array}{c} AT \\ TA \end{array} \right\} + \Delta H^\circ \left\{ \begin{array}{c} TA \\ AT \end{array} \right\} + \Delta H^\circ \left\{ \begin{array}{c} AG \\ TC \end{array} \right\} + \Delta H^\circ \left\{ \begin{array}{c} GC \\ CG \end{array} \right\} + \Delta H^\circ \left\{ \begin{array}{c} CA \\ GT \end{array} \right\} \\ &= -36.0 \quad -25.1 \quad -32.6 \quad -46.4 \quad -24.3 \\ &= -164.4 \text{ kJ} \end{aligned}$$

Sample problem

Given that $\Delta G^\circ = -16.8 \text{ kJ/mol}$ and $\Delta H^\circ = -164.4 \text{ kJ/mol}$ for the hexamer, determine the melt temperature.

- A. 42 °C
- B. 48 °C
- C. 52 °C
- D. 58 °C

Sample problem

Given that $\Delta G^\circ = -16.8 \text{ kJ/mol}$ and $\Delta H^\circ = -164.4 \text{ kJ/mol}$ for the hexamer, determine the melt temperature.

A. 42 °C

B. 48 °C

C. 52 °C

D. 58 °C

$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T = (-164.4 + 16.8)/298 \times 1000 = -495.3 \text{ J/mol-K}$$

The melt temperature occurs when $\Delta G^\circ = 0$.

$$T = \Delta H^\circ/\Delta S^\circ = -164,400/(-495.3) = 331 \text{ K} = 58 \text{ °C}$$

Henry's law constant and the solubility of gases

Henry's law constants in H ₂ O (atm x 10 ³)	
He	131
N ₂	86
CO	57
O ₂	43
Ar	40
CO ₂	1.6

Problem: Divers get the bends if bubbles of N₂ form in their blood because they rise too rapidly. Calculate the molarity of N₂ in water (i.e. blood) at sea level and 100 m below sea level.

$$\text{At sea level: } a_{\text{N}_2} = P_{\text{N}_2}/k_{\text{H,N}_2} = 0.8 \text{ atm}/86 \times 10^3 \text{ atm} = 9.3 \times 10^{-6}$$

$$c_{\text{N}_2} = 55.6 a_{\text{N}_2} = 5 \times 10^{-4} \text{ mol/L}$$

$$\text{At 100 m: } a_{\text{N}_2} = P_{\text{N}_2}/k_{\text{H,N}_2} = 9.8 \text{ atm}/86 \times 10^3 \text{ atm} = 1.1 \times 10^{-4}$$

$$c_{\text{N}_2} = 55.6 a_{\text{N}_2} = 6 \times 10^{-3} \text{ mol/L}$$

Question

Henry's law constants in H ₂ O (atm x 10 ³)	
He	131
N ₂	86
CO	57
O ₂	43
Ar	40
CO ₂	1.6

A species of fish requires a concentration of O₂ > 100 μM. A marine biologist is trying to determine the depth profile for O₂ in sea water. The first step is to calculate the concentration of O₂ in sea water at sea level.

As an assistant you perform the calculation and find that the O₂ concentration is:

A. 250 μM

B. 430 μM

C. 760 μM

D. 4300 μM

Question

Henry's law constants in H ₂ O (atm x 10 ³)	
He	131
N ₂	86
CO	57
O ₂	43
Ar	40
CO ₂	1.6

Most fish require a concentration of O₂ that is greater than 100 μM. A marine biologist is trying to determine the depth profile for O₂ in sea water. The first step is to calculate the concentration of O₂ in sea water at sea level.

As an assistant you perform the calculation and find that the O₂ concentration is:

A. 250 μM

B. 430 μM

C. 760 μM

D. 4300 μM

$$a_{\text{O}_2} = P_{\text{O}_2}/k_{\text{H,O}_2} = 0.2 \text{ atm}/43 \times 10^3 \text{ atm} = 4.7 \times 10^{-6}$$

$$c_{\text{O}_2} = 55.6 a_{\text{O}_2} = 2.5 \times 10^{-4} \text{ mol/L}$$

Use of osmotic pressure to determine molar mass

The van't Hoff equation can be modified to form used for the determination of molar mass by osmometry.

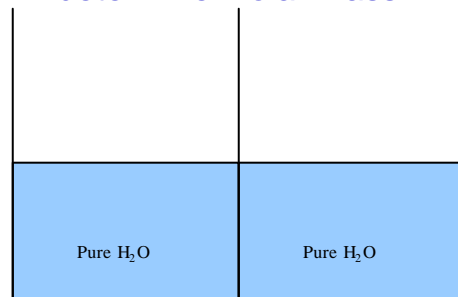
$$\Pi = cRT$$

$$\Pi = \frac{w}{M}RT$$

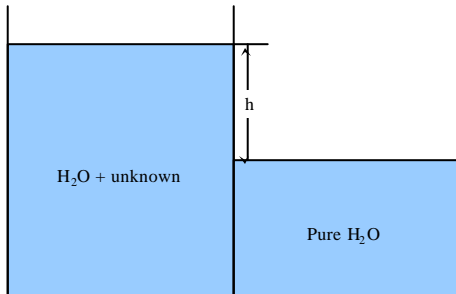
Here we related to the concentration c in moles/liter to the concentration w in grams/liter and the molar mass M in grams/mole.

The experimental configuration uses the measurement of height as an estimate of the osmotic pressure. The equation $\Pi = \rho gh$ is used ($h = \Pi/\rho g$).

Use of osmotic pressure to determine molar mass



Use of osmotic pressure to determine molar mass



Use of osmotic pressure to determine molar mass

A sample of 1.5 mg. of a protein of unknown molar mass is added to an osmometer. The solution volume is 1 mL. The solution height increases by 10 cm. The measurement temperature is 298 K. What is the molar mass of the protein?

- A. 37,900
- B. 39,700
- C. 79,300
- D. 97,300

Use of osmotic pressure to determine molar mass

A sample of 1.5 mg. of a protein of unknown molar mass is added to an osmometer. The solution volume is 1 mL. The solution height increases by 10 cm. The measurement temperature is 298 K. What is the molar mass of the protein?

- A. 37,900

$$M = \frac{wRT}{\Pi} = \frac{wRT}{rgh} = \frac{(1.5 \text{ kg/m}^3)(8.31 \text{ J/mol}\cdot\text{K})(298 \text{ K})}{(1000 \text{ kg/m}^3)(9.8 \text{ m/s}^2)(0.01 \text{ m})}$$

$$= 37.9 \text{ kg/mol}$$

$$= 37,900 \text{ g/mol}$$

- B. 39,700
- C. 79,300
- D. 97,300