CHEM 361A - Lecture 11 Activity

Equilibrium

In-Class

1. The interaction of ligands with proteins and with specific receptor sites on membrane surfaces is one of the most extensively studied biochemical phenomena. In this question we will apply principles of equilibrium to examine the binding of ligands and metal ions to macromolecules in solution.

   (a) The simplest case is where one site of macromolecule, P, binds to one molecule or ion of a ligand, L, can be expressed as:

   $$P + L \rightleftharpoons PL$$

   $$K_a = \frac{[PL]}{[P][L]}$$

   Write an equilibrium expression for this association reaction. Express the equilibrium constant as K_a.

   (b) Write an equilibrium expression for the dissociation reaction. Express the equilibrium constant as K_d.

   $$PL \rightleftharpoons L + P$$

   $$\boxed{K_d = \frac{[P][L]}{[PL]}}$$

   (c) Describe how tightly bound the complex PL is when K_d is very large and very small.

   (d) What is the relationship between K_a and K_d?

   $$K_a K_d = 1$$

   (e) The fractional saturation of sites is quantified as

   $$\begin{aligned} Y &= \frac{\text{concentration of L bound to P}}{\text{total concentration of all forms of P}} \\ &= \frac{[PL]}{[P] + [PL]} \end{aligned} \tag{1}$$

   Solve the equilibrium expression found in 1b for [PL] and substitute into equation 1 and simplify. You should get an expression solely in terms of [L] and K_d.

   (f) If there were n equivalent binding sites per macromolecule, the expression found in 1e would instead be

   $$\boxed{Y = \frac{n[L]}{[L] + K}} \tag{2}$$

   where for n equivalent sites

   $$K = (K_{d1}K_{d2}\ldots K_{dn})^{\frac{1}{n}}$$

   i. A Hughes-Klotz plot is when you plot $\frac{1}{Y}$ as a function of $\frac{1}{[L]}$. Write an expression using equation 2 of $\frac{1}{Y}$ as a function of $\frac{1}{[L]}$. What is the slope and the y-intercept?

   $$\frac{1}{Y} = \frac{[L] + K}{n[L]}$$

   $$\frac{1}{Y} = \frac{1}{n} + \frac{K}{n[L]}$$

ii. A Scatchard plot (`https://en.wikipedia.org/wiki/Scatchard_equation`) is when you plot $\frac{Y}{[L]}$ as a function of Y. Write an expression using equation 2 of $\frac{Y}{[L]}$ as a function of Y. What is the slope and the y-intercept?

(g) The calcium ion binds to a certain protein to form a 1:1 complex. The corresponding data is documented in Table 1. Fit the data in Table 1 and determine the dissociation constant, K_d, of the Ca^{2+}-protein complex. For this experiment, the protein concentration was kept at 96 μM for each run. (1μM $= 1 \times 10^{-6}$M).

Table 1: Ca^{2+}-protein complex data

Total Ca^{2+} (μM)	60	120	180	240	480
Ca^{2+} bound to protein (μM)	31.2	51.2	63.4	70.8	83.4

2. The standard state in biochemistry is slightly different than in Physical Chemistry. In biochemistry the hydrogen-ion concentration for the standard state is 10^{-7} M, because the physiological pH is about 7. Because of this change in the standard state of the concentration of H^+, we will employ a slightly different notation for the standard change in Gibbs Free Energy ($\Delta G^{\ominus\prime}$). In practice, this means for a process such as

$$\mathrm{A} + \mathrm{xH}^+ \rightleftharpoons \mathrm{B} + \mathrm{C}$$

the Gibbs Free Energy change using the biochemists standard state is

$$\Delta_r G = \Delta_r G^{\ominus\prime} + RT \ln \frac{([B]/1M)([C]/1M)}{([A]/1M)([H^+]/1 \times 10^{-7}M)^x}$$

(a) NAD^+ and NADH are the oxidized and reduced forms of nicotinamide adenine dinucleotide. For the oxidation of NADH

$$\mathrm{NADH} + \mathrm{H}^+ \rightleftharpoons \mathrm{NAD}^+ + \mathrm{H}_2\text{; where } \Delta_r G^{\ominus} = -21.8 \text{ kJ mol}^{-1} \text{ at 298 K.}$$

Find the equilibrium constant K using the Physical Chemist standard state at 298 K.

(b) If the $\Delta_r G$ is the same regardless of the standard state used ($\Delta_r G^{\ominus}$ or $\Delta_r G^{\ominus\prime}$), determine the difference between the two standards states.

(c) Determine $\Delta_r G^{\ominus\prime}$ for the reaction in question 2a. What do you notice in terms of the spontaneity of the reaction?

(d) Determine K′, the equilibrium constant according to the Biochemist Standard State, at 298 K.

Homework

3. Many chemical and biological reactions are not spontaneous ($\Delta G > 0$). However, we have seen that in certain cases, these reactions become spontaneous when they are coupled with a spontaneous process. For example, the conversion of glucose to glucose-6-phosphate is not spontaneous unless it is coupled with the hydrolysis of ATP.

   From the following reactions at 298 K:

$$\text{fumarate}^{2-} + \text{NH}_4{}^{+} \rightleftharpoons \text{asparatate}^{-};\ \Delta_r G^{\ominus\prime} = -36.7 \text{ kJ mol}^{-1}$$
$$\text{fumarate}^{2-} + \text{H}_2\text{O} \rightleftharpoons \text{malate}^{2-};\ \Delta_r G^{\ominus\prime} = -2.9 \text{ kJ mol}^{-1}$$

   For the following reaction

$$\text{malate}^{2-} + \text{NH}_4{}^{+} \rightleftharpoons \text{aspartate}^{-} + \text{H}_2\text{O}$$

   calculate

   (a) $\Delta_r G^{\ominus\prime}$ (-33.8 kJ mol^{-1})

   (b) K' (8.4×10^5)

   (c) If the reaction is spontaneous at standard Biochemical conditions, was it necessary for each individual step to be spontaneous? Why?

$$[PL] = \frac{[P][L]}{K_d}$$

$$Y = \frac{[PL]}{[P] + [PL]}$$

$$Y = \frac{\dfrac{\cancel{[P]}[L]}{\cancel{K_d}}}{\dfrac{\cancel{[P]}}{K_d} + \dfrac{\cancel{[P]}[L]}{\cancel{K_d}}} \cdot \frac{\cancel{K_d}}{\cancel{K_d}}$$

$$Y = \frac{[L]}{K_d + [L]}$$