LECTURES 8-12: Enzyme Kinetics

Problem 1

Determine the initial rate of product (P) formation from enzyme X and substrate Y that form the complex XY. The reaction kinetics is given by

$$X + Y \underset{k_{b1}}{\overset{k_{f1}}{\rightleftharpoons}} XY \underset{k_{b2}}{\overset{k_{f2}}{\rightleftharpoons}} X + P$$

Solution:

Michaelis-Menten approach : The rate of reaction is given by

$$r_p = k_{f2} C_{XY} - k_{b2} C_P C_X \tag{1.1}$$

Since enzyme is preserved $C_{X0} = C_X + C_{XY}$ (1.2)

Substituting equation (1.2) in (1.1) we get

$$r_p = \left(k_{f2} + k_{b2} C_P\right) C_{XY} - k_{b2} C_P C_{X0} \tag{1.3}$$

Assuming the first reversible reaction is in equilibrium gives

$$C_{XY} = \frac{k_{f1}}{k_{b1}} C_X C_Y \tag{1.4}$$

Substituting equation (5) in equation (3) for C_X and rearranging for C_{XY} yields

$$C_{XY} = \frac{C_{X0} C_S}{\frac{k_{b_1}}{k_{f1}} C_S} \tag{1.5}$$

Substituting equation (6) into equation (2) gives

$$r_p = \frac{k_{f2} C_{X0} \left(C_Y - \frac{k_{b2}}{k_{f2}} \frac{k_{b1}}{k_{f1}} C_P \right)}{\frac{k_{b1}}{k_{f1}} + C_S} \tag{1.6}$$

Equation (7) is in Michaelis-Menten form of equation, where

$$r_{Max} = k_{f2} C_{XO}$$

and

$$K_M = \frac{k_{b1}}{k_{f1}}$$

Problem 2

Determine the initial rate of product (P) formation from enzyme X and substrate Y that form the complex XY. The reaction kinetics is given by

$$\mathbf{X} + \mathbf{Y} \underset{k_{b1}}{\overset{k_{f1}}{\rightleftharpoons}} (\mathbf{XY})_1 \underset{k_{b2}}{\overset{k_{f2}}{\rightleftharpoons}} (\mathbf{XY})_2 \xrightarrow{k_{f3}} \mathbf{X} + \mathbf{P}$$

Solution : We can write the mass balance for the intermediates and product as:

$$\frac{d[(XY)_1]}{dt} = k_{b1}[X][Y] - (k_{f1} + k_2)[(XY)_1] \qquad (2.1)$$

$$\frac{d[(XY)_2]}{dt} = k_2[(XY)_1] - (k_{f3})[(XY)_2] \qquad (2.2)$$

$$\frac{d[P]}{dt} = (k_{f3})[(XY)_2] \qquad (2.3)$$

where $k_2 = \dfrac{k_{f2}}{k_{b2}}$

Assuming $[(XY)_1]$ and $[(XY)_2]$ are in steady state, we can eliminate $[(XY)_2]$ from equation (2.4)

$$[(XY)_1] = \frac{k_{f1}[X][Y]}{k_{b1} + k_2} \tag{2.4}$$

$$[(XY)_2] = \frac{k_2}{k_{f3}}[(XY)_1] = \frac{k_2}{k_{f3}} \bullet \frac{k_1[X][Y]}{k_{b1} + k_2} \tag{2.5}$$

Substituting equation (2.6) into equation (2.4) we get

$$r_p = \frac{d[P]}{dt} = k_{f3} \bullet \frac{k_2}{k_{f3}} \bullet \frac{k_{f1}[X][Y]}{k_{b1} + k_2} \tag{2.6}$$

Since the enzyme is preserved

$$[X_0] = [X] + [(XY)_1] + [(XY)_2] \tag{2.7}$$

Substituting for [X] in terms of $[X_0]$ in equation (2.8) we get

$$r_p = \frac{\dfrac{k_2 k_{f3}}{(k_2 + k_{f3})}[X][Y]}{\dfrac{k_{f3}}{k_2 + k_{f3}} \bullet \dfrac{k_{b1} + k_2}{k_{f1}} + [Y]} \qquad (2.8)$$

Hence the K_M and V_{max} values are reduced by the ratio $k_{f3}/(k_2 + k_{f3})$, resulting from the presence of the second intermediate $[(XY)_2]$.

Problem3

The loading of O_2 to Hb (hemoglobin) follows a cooperative binding and is given by

$$Hb + nO_2 \underset{K_2}{\overset{K_1}{\rightleftharpoons}} Hb(O_2)_n$$

Develop a graphical method for determination of the coefficient 'n' from measurements of Hb $(O_2)_n$

Solution:

Total hemoglobin concentration is given by

$$[Hb]_T = [Hb] + [Hb(O_2)_n] = \text{Constant}$$

$$\frac{[Hb(O_2)_n]}{[Hb]_T} = \frac{[Hb(O_2)_n]}{[Hb] + [Hb(O_2)_n]} \qquad (3.1)$$

Given reaction is

$$Hb + nO_2 \longrightarrow Hb(O_2)_n$$

Assuming steady state, therefore

$$K_1[Hb][O_2]^n = K_2[Hb(O_2)_n] \tag{3.2}$$

From equation (3.1) and equation (3.2) we get

$$\frac{[Hb(O_2)_n]}{[Hb]_T} = \frac{\frac{K_1}{K_2}[Hb][O_2]^n}{[Hb] + \frac{K_1}{K_2}[Hb][O_2]^n} \tag{3.3}$$

As concentration is directly proportional to pressure, we can write equation (3.3) as

$$\frac{\left[\mathrm{Hb}(\mathrm{O}_2)_n\right]}{\left[\mathrm{Hb}\right]_T} = \frac{\frac{K_1}{K_2}\alpha^n P_{O_2}^n}{1+\frac{K_1}{K_2}\alpha^n P_{O_2}^n} \tag{3.4}$$

After the algebraic manipulations

$$\frac{\left[\mathrm{Hb}(\mathrm{O}_2)_n\right]}{\left[\mathrm{Hb}\right]_T} = \frac{P_{O_2}^n}{\frac{K_1}{K_2\alpha^n}+P_{O_2}^n} \tag{3.5}$$

Now, binding of oxygen to hemoglobin in terms of partial pressure of oxygen is given by Hill Equation.

Hill Equation:

$$S_{O_2} = \frac{P_{O_2}^n}{P_{50}^n + P_{O_2}^n} \tag{3.6}$$

Therefore

$$S_{O_2} = \frac{[Hb(O_2)_n]}{[Hb]_T} \tag{3.7}$$

From equation (3.6) and equation (3.7)

$$S_{O_2} = \frac{P_{O_2}^n}{\frac{K_1}{K_2\alpha^n} + P_{O_2}^n} \tag{3.8}$$

Rearranging we get

$$\left\{\frac{S_{O_2}}{1 - S_{O_2}}\right\} = \frac{K_1}{K_2\alpha^n} P_{O_2}^n \tag{3.9}$$

Taking log on both side, we get

$$\ln\left\{\frac{S_{O_2}}{1 - S_{O_2}}\right\} = \ln\left(\frac{K_1}{K_2\alpha^n}\right) + n \ln P_{O_2} \tag{3.10}$$

Thus a logarithmic plot of $\left\{\frac{S_{O_2}}{1-S_{O_2}}\right\}$ versus P_{O2} will give a straight line of slope 'n'

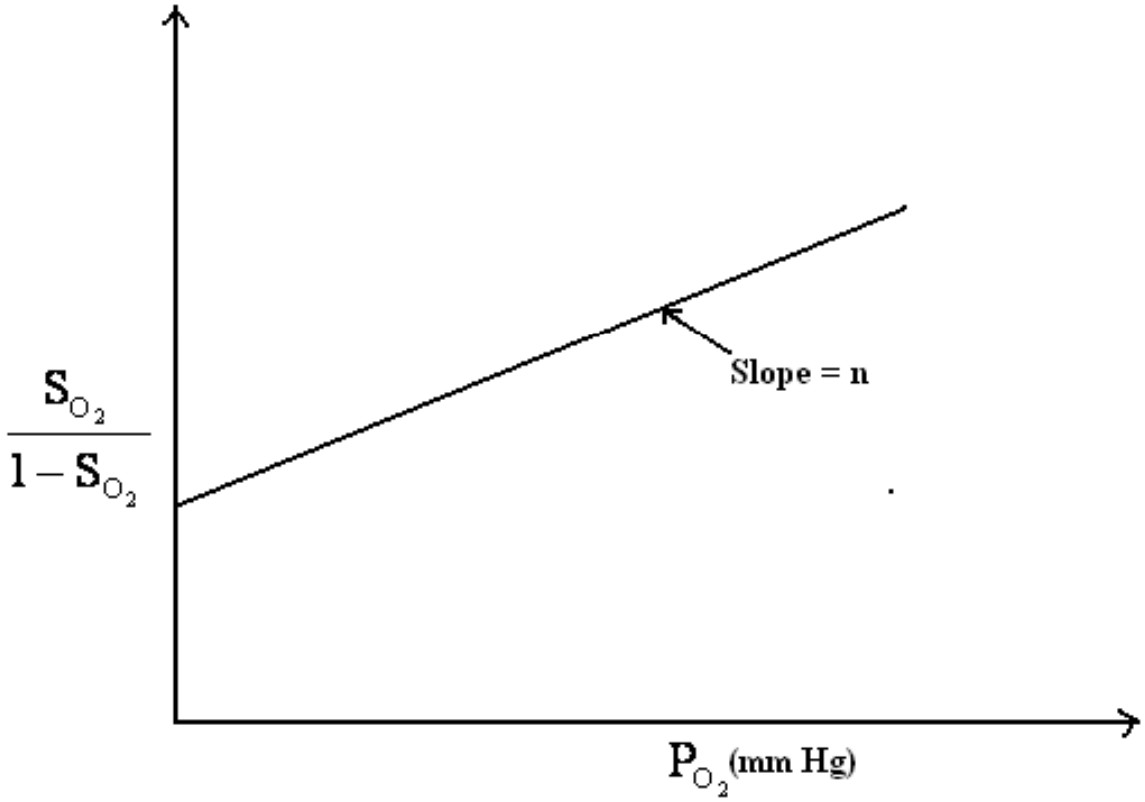

Fig. 3.1 Binding of oxygen to hemoglobin versus Partial pressure of oxygen

Problem 4

An inhibitor Y is added to the enzymatic reaction at a level of 1.2 gm/L. Their data were obtained for K_M=10 gm/L.

Rate	Substrate
1.8	20
1.3	9.8
0.98	6.7
0.8	5.1
0.67	4.2
0.6	3.2
0.45	2.6

a. Identify the type of inhibition (i.e. Competitive, Non-competitive or Uncompetitive)

b. Find the K_i (Inhibitor kinetic constant)

Solution: Lets us assume that the given enzymatic reaction is inhibited by competitive inhibition. For an instance of competitive inhibition the Lineweaver-Burke equation is

$$\frac{1}{V} = \frac{K_M}{V_{Max}} \frac{1}{[S]} \left[1 + \frac{[I]}{K_i} \right] + \frac{1}{V_{Max}} \qquad (4.1)$$

So we plot $\frac{1}{V}$ versus $\frac{1}{S}$ to get the values of V_{Max} and K_i

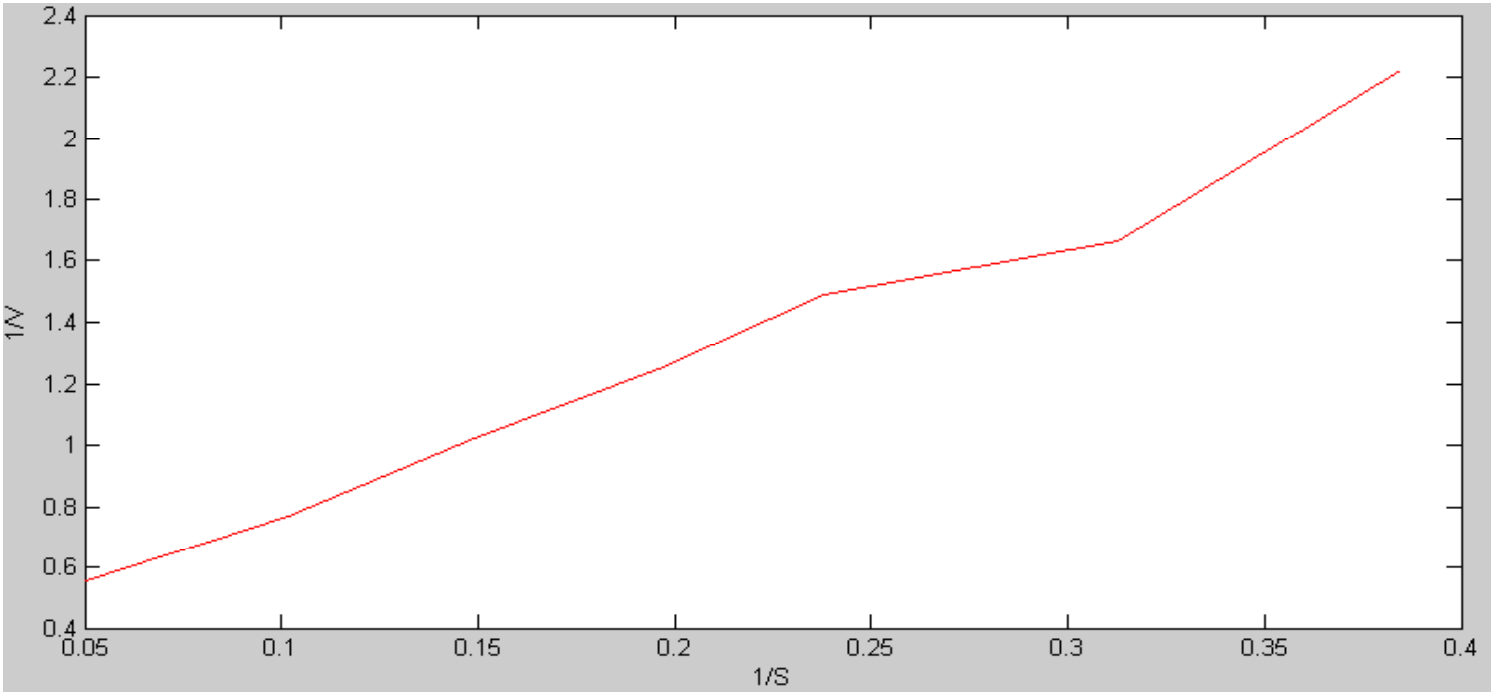

Fig. 4.1. 1/V versus 1/S plot

From fig.4.1 we get V_{max}=1.8181. Slope = 2.33826, therefore

$$\frac{1}{V_{Max}}\left[1+\frac{[I]}{K_i}\right]=2.3383$$

Hence inhibitor constant K_i = -2.087

For Competitive inhibition, K_i is the dissociation constant for the enzyme –inhibitor complex. A lower value of K_i denotes a stronger inhibition. So as the value of K_i (-2.087) is negative our assumption was correct and it is a competitive inhibition.

It is important to note that competitive inhibition can be overcome by adding additional substrate.