University of Washington
Department of Chemistry
Chemistry 457
Spring Quarter 2013

Lecture 30: Inhibition
5 June 2013

A. Lineweaver-Burk Plots

- We learned that a common scheme for biological enzyme function is based on the mechanism $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$, which yields the Michaelis-Menten rate equation:

$$rate = \frac{d[P]}{dt} = \frac{V_{\max}[S]}{K_M + [S]} \tag{30.1}$$

where $K_M = \frac{k_{-1} + k_2}{k_1}$ and $V_{\max} = k_2[E]_0$.

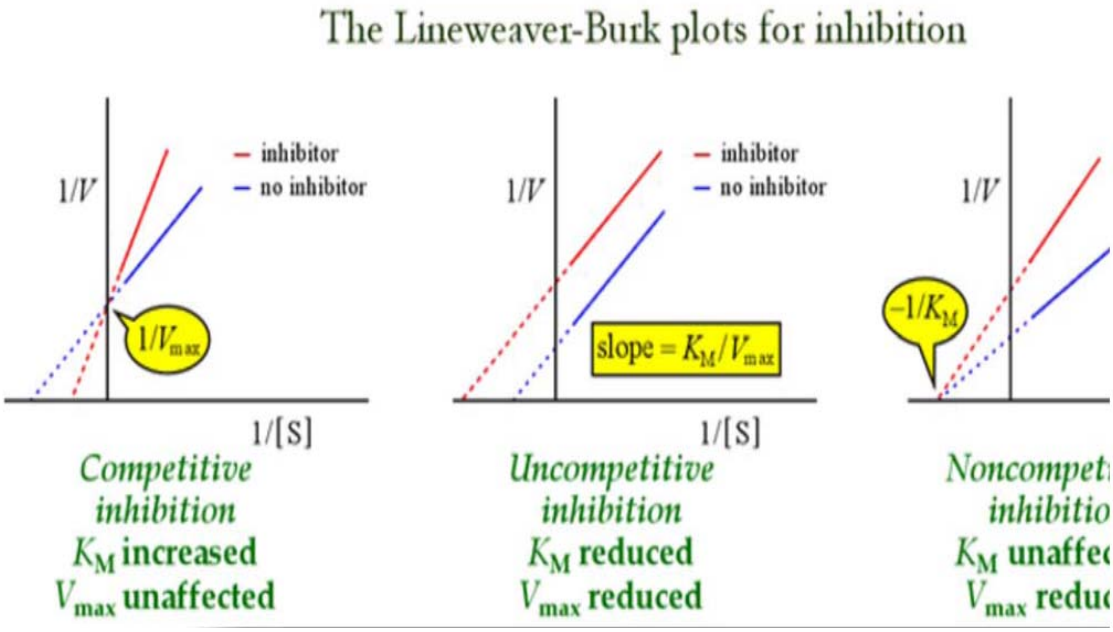
- Equation 30.1 can be linearized by taking the inverse of both sides of equation 30.1:

$$\frac{1}{rate} = \frac{K_M + [S]}{V_{\max}[S]} = \frac{K_M}{V_{\max}[S]} + \frac{1}{V_{\max}} \tag{30.2}$$

  - Equation 30.2 means that an inverse plot (i.e. 1/rate plotted as a function of 1/[S]) is a straight like with slope $\frac{K_M}{V_{\max}}$ and a y-intercept of $\frac{1}{V_{\max}}$.

    Equation 30.2 is called the Lineweaver-Burk equation. Double inverse plots are extremely useful for observing the effects of inhibitors, See Figure 30.1.

Figure 30.1



B. Competitive Inhibition

- It is assumed that the inhibitor I binds to the same site on the enzyme as the substrate. Therefore a competitive inhibitor often resembles structurally the substrate. The mechanism is:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$$
$$E + I \rightleftharpoons EI \qquad (30.3)$$
$$ES \xrightarrow{k_2} E + P$$

- The same steady state approximation is applied to ES which yields

$$[ES] = \frac{k_1}{k_2 + k_{-1}}[E][S] = \frac{[E][S]}{K_M} \qquad (30.4)$$

- The rate is as before

$$rate = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2}{K_M}[E][S] \qquad (30.5)$$

- As before, [E] and [ES] cannot be measured. [S] and [I] are measureables. So we have to eliminate [E] and {ES} from the rate equations. To do this we again start with E conservation:

$$[E]_0 = [E] + [ES] + [EI]$$
$$= [E] + \frac{[E][S]}{K_M} + \frac{[E][I]}{K_{EI}} \qquad (30.6)$$
$$\text{where } K_{EI} = \frac{[E][I]}{[EI]}$$

- Then rearrange 30.6:

$$[E] = \frac{[E]_0}{1 + \frac{[S]}{K_M} + \frac{[I]}{K_{EI}}} \qquad (30.7)$$

- Put equation 30.7 into the rate equation to obtain

$$rate = \frac{k_2}{K_M}[E][S] = \frac{k_2[E]_0[S]}{[S] + K_M\left(1 + \frac{[I]}{K_{EI}}\right)} \qquad (30.8)$$

- The Lineweaver-Burke equation now has the form:

$$\frac{1}{rate} = \frac{[S] + K_M\left(1 + \frac{[I]}{K_{EI}}\right)}{k_2[E]_0[S]} = \frac{K_M}{k_2[E]_0}\left(1 + \frac{[I]}{K_{EI}}\right)\frac{1}{[S]} + \frac{1}{k_2[E]_0} \qquad (30.9)$$
$$= \frac{K_M}{V_{\max}}\left(1 + \frac{[I]}{K_{EI}}\right)\frac{1}{[S]} + \frac{1}{V_{\max}}$$

- Equation 30.9 means that for competitive inhibition the intercept of the Lineweaver-Burke plot remains the same but the slope is increased by $\frac{[I]}{K_{EI}}$. See Figure 30.1.For the Lineweaver-Burke equation 30.9 it is clear that as [S] greatly exceeds [I] the rate will increase.

C. Noncompetitive Inhibition

- In non-competitive inhibition, the inhibitors binds the enzyme on a different site than the substrate and thus a non-competitive inhibitor need not resemble the substrate. The mechanism is

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$
$$E + I \overset{K_{EI}}{\rightleftharpoons} EI \qquad (30.10)$$
$$ES + I \overset{K_{ESI}}{\rightleftharpoons} ESI$$

- The rate is given by as usual by

$$rate = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2}{K_M}[E][S] \qquad (30.11)$$

- We proceed as usual to eliminate all [E], [ES], [EI], and [ESI] terms.This is based on the same conservation principle:

$$\begin{aligned}[E]_0 &= [E] + [ES] + [EI] + [ESI] \\ &= [E] + \frac{[E][S]}{K_M} + \frac{[E][I]}{K_{EI}} + \frac{[ES][I]}{K_{ESI}} \\ &= [E] + \frac{[E][S]}{K_M} + \frac{[E][I]}{K_{EI}} + \frac{[E][S][I]}{K_M K_{ESI}} \qquad (30.12) \\ \therefore [E] &= \frac{[E]_0}{1 + \frac{[S]}{K_M} + \frac{[I]}{K_{EI}} + \frac{[S][I]}{K_M K_{ESI}}}\end{aligned}$$

- We now substitute equation 30.12 into rate equation 30.11

$$\begin{aligned}rate &= \frac{k_2}{K_M}[E][S] = \frac{k_2}{K_M}[S]\frac{[E]_0}{1 + \frac{[S]}{K_M} + \frac{[I]}{K_{EI}} + \frac{[S][I]}{K_M K_{ESI}}} \\ &= \frac{k_2[E]_0}{1 + \frac{K_M}{[S]} + \frac{K_M[I]}{K_{EI}[S]} + \frac{[I]}{K_{ESI}}} = \frac{V_{\max}}{1 + \frac{[I]}{K_{ESI}} + \frac{K_M}{[S]}\left(1 + \frac{[I]}{K_{EI}}\right)}\end{aligned} \qquad (30.13)$$

- It is easiest to deal with the inverse plot:

$$\frac{1}{rate} = \frac{1}{V_{\max}} + \frac{[I]}{V_{\max}K_{ESI}} + \frac{K_M}{V_{\max}[S]}\left(1 + \frac{[I]}{K_{EI}}\right) \qquad (30.14)$$

- Equation 30.14 is the Lineweaver-Burk equation for non-competitive inhibition. A common and reasonable assumption is that $K_{EI} = K_{ESI}$ and we obtain a simpler expression

$$\begin{aligned} \frac{1}{rate} &= \frac{1}{V_{\max}} + \frac{[I]}{V_{\max} K_{EI}} + \frac{K_M}{V_{\max}[S]}\left(1 + \frac{[I]}{K_{EI}}\right) \\ &= \left(1 + \frac{[I]}{K_{EI}}\right)\left(\frac{1}{V_{\max}} + \frac{K_M}{V_{\max}[S]}\right) \end{aligned} \qquad (30.15)$$

- In this type of inhibition the slope and y-intercept change with [I]. The x intercept which corresponds to $\frac{1}{rate} = 0$ occurs for $[S] = -K_M$.