Homework (May 5)

10.12 An enzyme has a K_M value of $2.8 \times 10^{-5}\ M$ and a V_{max} value of $53\ \mu M\ \text{min}^{-1}$. Calculate the value of v_0 if $[S] = 3.7 \times 10^{-4}\ M$ and $[I] = 4.8 \times 10^{-4}\ M$ for **(a)** a competitive inhibitor, **(b)** a noncompetitive inhibitor, and **(c)** an uncompetitive inhibitor. ($K_I = 1.7 \times 10^{-5}\ M$ for all three cases.)

(a) For a competitive inhibitor, from Equation 10.17,

$$
\begin{aligned}
v_0 &= \frac{V_{max}[S]}{K_M\left(1 + \frac{[I]}{K_I}\right) + [S]} \\
&= \frac{(53\ \mu M\ \text{min}^{-1})\,(3.7 \times 10^{-4}\ M)}{(2.8 \times 10^{-5}\ M)\left(1 + \frac{4.8\times10^{-4}\ M}{1.7\times10^{-5}\ M}\right) + 3.7 \times 10^{-4}\ M} \\
&= 16.5\ \mu M\ \text{min}^{-1} \\
&= 16\ \mu M\ \text{min}^{-1}
\end{aligned}
$$

(b) For a noncompetitive inhibitor, Equation 10.19 gives,

$$v_0 = \frac{\frac{V_{max}}{\left(1+\frac{[I]}{K_I}\right)}[S]}{K_M + [S]}$$

$$= \frac{\frac{53\ \mu M\ min^{-1}}{\left(1+\frac{4.8\times 10^{-4}\ M}{1.7\times 10^{-5}\ M}\right)}\left(3.7 \times 10^{-4}\ M\right)}{2.8 \times 10^{-5}\ M + 3.7 \times 10^{-4}\ M}$$

$$= 1.69\ \mu M\ min^{-1}$$

$$= 1.7\ \mu M\ min^{-1}$$

(c) For an uncompetitive inhibitor, Equation 10.22 is appropriate,

$$v_0 = \frac{\frac{V_{max}}{\left(1+\frac{[I]}{K_I}\right)}[S]}{\frac{K_M}{\left(1+\frac{[I]}{K_I}\right)} + [S]}$$

$$= \frac{\frac{53\ \mu M\ min^{-1}}{\left(1+\frac{4.8\times 10^{-4}\ M}{1.7\times 10^{-5}\ M}\right)}\left(3.7 \times 10^{-4}\ M\right)}{\frac{2.8\times 10^{-5}\ M}{\left(1+\frac{4.8\times 10^{-4}\ M}{1.7\times 10^{-5}\ M}\right)} + 3.7 \times 10^{-4}\ M}$$

$$= 1.81\ \mu M\ min^{-1}$$

$$= 1.8\ \mu M\ min^{-1}$$

10.13 The degree of inhibition i is given by $i\% = (1 - \alpha)\ 100\%$, where $\alpha = (v_0)_{inhibition}/v_0$. Calculate the percent inhibition for each of the three cases in Problem 10.12.

First v_0 in the absence of inhibitor must be found.

$$v_0 = \frac{V_{max}[S]}{K_M + [S]}$$

$$= \frac{\left(53\ \mu M\ min^{-1}\right)\left(3.7 \times 10^{-4}\ M\right)}{2.8 \times 10^{-5}\ M + 3.7 \times 10^{-4}\ M}$$

$$= 49.3\ \mu M\ min^{-1}$$

(a)

$$\alpha = \frac{16.5\ \mu M\ min^{-1}}{49.3\ \mu M\ min^{-1}} = 0.335$$

percent inhibition = (1 − 0.335) (100%) = 67%

(b)

$$\alpha = \frac{1.69\ \mu M\ min^{-1}}{49.3\ \mu M\ min^{-1}} = 3.43 \times 10^{-2}$$

percent inhibition $= \left(1 - 3.43 \times 10^{-2}\right)(100\%) = 96.7\%$

(c)

$$\alpha = \frac{1.81\ \mu M\ \text{min}^{-1}}{49.3\ \mu M\ \text{min}^{-1}} = 3.67 \times 10^{-2}$$

$$\text{percent inhibition} = \left(1 - 3.67 \times 10^{-2}\right)(100\%) = 96.3\%$$

10.14 An enzyme-catalyzed reaction ($K_M = 2.7 \times 10^{-3}\ M$) is inhibited by a competitive inhibitor I ($K_I = 3.1 \times 10^{-5}\ M$). Suppose that the substrate concentration is $3.6 \times 10^{-4}\ M$. How much of the inhibitor is needed for 65% inhibition? How much does the substrate concentration have to be increased to reduce the inhibition to 25%?

Expressions for the initial rate in the absence and presence of a competitive inhibitor are given by Equations 10.10 and 10.17, respectively. Dividing the former by the latter gives

$$\frac{v_0}{(v_0)_{\text{inhibition}}} = \frac{K_M\left(1 + \frac{[I]}{K_I}\right) + [S]}{K_M + [S]}$$

$$= 1 + \frac{K_M[I]}{(K_M + [S])K_I}$$

This can be solved for [I].

$$[I] = K_I\left(\frac{v_0}{(v_0)_{\text{inhibition}}} - 1\right)\left(1 + \frac{[S]}{K_M}\right)$$

It can also be solved for [S].

$$[S] = K_M\left(\frac{[I]}{K_I\left(\frac{v_0}{(v_0)_{\text{inhibition}}} - 1\right)} - 1\right)$$

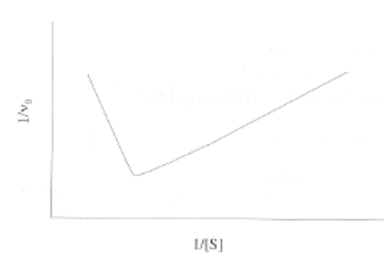
The expression for [I] is used in answering the first part of the question. For 65% inhibition, $(v_0)_{\text{inhibition}} = (1 - 0.65)v_0 = 0.35v_0$, and

$$[I] = \left(3.1 \times 10^{-5}\ M\right)\left(\frac{1}{0.35} - 1\right)\left(1 + \frac{3.6 \times 10^{-4}\ M}{2.7 \times 10^{-3}\ M}\right) = 6.52 \times 10^{-5}\ M = 6.5 \times 10^{-5}\ M$$

To reduce the inhibition to 25%, where $(v_0)_{\text{inhibition}} = 0.75v_0$, at this concentration of inhibitor, use the expression for [S] to find the required substrate concentration.

$$[S] = \left(2.7 \times 10^{-3}\ M\right)\left[\frac{6.52 \times 10^{-5}\ M}{\left(3.1 \times 10^{-5}\ M\right)\left(\frac{1}{0.75} - 1\right)} - 1\right] = 1.4 \times 10^{-2}\ M$$

10.32



The plot shows that at high substrate concentration (low values of 1/[S]), the initial rate of the reaction decreases ($1/v_0$ increases). Thus, the substrate must act as an inhibitor to the enzyme.