Problem Set 26: Enzyme Inhibition — Due Dec 9, 2008

1. Consider the following modification of the Michaelis Menten mechanism:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI$$

In this mechanism, a second molecule, I, can bind to the free enzyme and prevent (or compete with) the binding of substrate.

(a) If the [S] is much greater than [I], would you expect the velocity (d[P]/dt) for this mechanism to be different than for the Michaelis Menten mechanism?

(b) Draw a Lineweaver Burk plot for this mechanism. The first line should have [I]=0, and the second should have [I] present at a reasonable concentration. Remember your answer to part (a) to decide if you are going to have a slope effect or an intercept effect or both.

2. In this question, you will derive d[P]/dt for the competitive inhibition mechanism in question 1.

(a) Write the equation for d[P]/dt

(b) Write the equation for d[ES]/dt. Apply the steady state approximation to derive an equation with [ES] on one side and [E], [S], and constants on the other side.

(c) Write an equation for d[EI]/dt. Since [EI] is also an intermediate, you can apply the steady state approximation to get an expression with [EI] on one side and [E], [I], and constants on the other side.

(d) For this mechanism, $[E] = [E]_0 - [ES] - [EI]$. Substitute the results from parts 2(b) and 2(c) into this equation and rearrange so that [E] is on one side and everything else is on the other side.

(e) Now that you have an expression for [E], you can substitute that expression back into the final equation from part 2(b) to get [ES] in terms of measurable quantities and rate constants.

(f) Take the results from 2(e) and write the final expression for d[P]/dt.

3. Rearrange the expression you derived in 2(f) for 1/v. How does a competitive inhibitor affect the slope of a Lineweaver-Burk plot? How does it affect the intercept?

4. In addition to competitive inhibition, there are two other common types of enzyme inhibition. One type is called uncompetitive inhibition. For this mechanism, the inhibitor can only bind to the ES complex and NOT to the free enzyme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$ES + I \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} ESI$$

Follow the following steps (similar, but not identical to problem 2) to derive d[P]/dt for this mechanism.

(a) Write the equation for d[P]/dt

(b) Write the equation for d[ESI]/dt. Apply the steady state approximation to derive an equation with [ESI] on one side and [ES], [I], and constants on the other side.

(c) Write the equation for d[ES]/dt. Apply the steady state approximation and your result from part (b) to derive an equation with [ES] on one side and [E], [S], and constants on the other side (it should be a relatively simple equation).

(d) For this mechanism, $[E] = [E]_0$ - [ES] - [ESI]. Substitute the results from parts (b) and (c) into this equation and rearrange so that [E] is on one side and everything else is on the other side.

(e) Now that you have an expression for [E], you can substitute that expression back into the equation from part (b) to get [ES] in terms of measurable quantities and rate constants.

(f) Take the results from part (e) and write the final expression for d[P]/dt.

5. Rearrange your final velocity equation from problem 4 into a form suitable for a Lineweaver Burk plot. How does an uncompetitive inhibitor affect the slope of a Lineweaver-Burk plot? How does it affect the intercept? Is this what you would have expected if you had applied the same arguments that you went through in problem 1?