Enzyme Kinetics

Enzymes are important biochemical catalysts that are vital to life. The properties of enzymes can be controlled through the use of inhibitors. In this problem we will investigate the kinetics of these reactions.

The uninhibited enzyme-substrate reaction can be modelled with a two-step mechanism:

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

$$ES \xrightarrow{k_2} P$$

where **E** is the enzyme, **S** is the substrate, and **P** is the product.

1. By using the steady-state approximation, show that the rate of production of **P** is given by

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

where $K_M = (k_{-1} + k_2) \cdot k_1^{-1}$, and $V_{max} = k_2[E]_T$ where $[E]_T$ is the total enzyme concentration.

2. What is the reaction order in substrate at low and high substrate concentrations?

Low:

High:

Competitive Inhibition involves an inhibitor **I** that reacts with the enzyme **E** to form an unreactive enzyme-inhibitor complex **EI:**

$$\mathrm{E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES}$$

$$\mathrm{E + I \overset{K_I}{\rightleftharpoons} EI}$$

$$\mathrm{ES \xrightarrow{k_2} P}$$

3. Assuming pre-equilibrium with the enzyme-inhibitor complex, show that the rate of production of **P** is given by

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

where K_M and V_{max} are defined as before, and $\alpha = 1 + [I] \cdot K_I$.

Uncompetitive inhibition involves an inhibitor **I** binding instead to the enzyme substrate complex, and not the free enzyme:

$$\mathrm{E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES}$$

$$\mathrm{ES \xrightarrow{k_2} P}$$

$$\mathrm{ES + I \overset{K_I{}'}{\rightleftharpoons} ESI}$$

4. Assuming pre-equilibrium with the enzyme-inhibitor complex, show that the rate of production of **P** is given by

$$Rate = \frac{V_{max}[S]}{K_M + \alpha'[S]}$$

where K_M and V_{max} are defined as before, and $\alpha' = 1 + [I] \cdot (K_I')^{-1}$.

Finally, mixed inhibitors (also called noncompetitive inhibitors) are inhibitors **I** that react with both the enzyme and the enzyme-substrate complexes to form complexes **ES** and **ESI** respectively with equilibrium constants K_I and K_I'.

5. Assuming pre-equilibrium with the enzyme-inhibitor complexes, show that the rate of production of **P** is given by

$$Rate = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]}$$

where K_M, V_{max}, α and α' are defined as before.