Enzyme reactivity: Michaelis-Menten equation
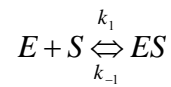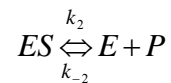
Step 1: enzyme (E) reacts with substrate (S) to form an enzyme-substrate complex (ES):

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

Step 2: the enzyme-substrate complex breaks down to form free enzyme and products (P):

$$ES \underset{k_{-2}}{\overset{k_2}{\Leftrightarrow}} E + P$$

Assumptions: 1) Step 2 is essentially irreversible (the rate of ES formation from E and P is very small)

2) [ES] does not change over time (pseudo-steady state)

Then, $k_1[S][E] = k_{-1}[ES] + k_2[ES]$

$$[E]_{total} = [E] + [ES]$$

$$k_1[S]([E]_{total} - [ES]) = (k_{-1} + k_2)[ES]$$

$$[ES] = \frac{k_1[S][E]_{total}}{k_{-1} + k_2 + k_1[S]} = \frac{[S][E]_{total}}{[S] + \left(\dfrac{k_{-1} + k_2}{k_1}\right)}$$

The rate of enzyme reaction,

$$v = k_2[ES]$$

$$= \frac{k_2[S][E]_{total}}{[S] + \left(\dfrac{k_{-1} + k_2}{k_1}\right)}$$

* When should the reaction be at its maximum?

When [S] is very large: $[S] \gg \dfrac{k_{-1} + k_2}{k_1}$ and $[ES] \approx [E]_{total}$

Then, $\quad v_m = k_2 [E]_{total}$

$$v = \dfrac{v_m [S]}{K_M + [S]}$$

where $\quad K_M = \dfrac{k_{-1} + k_2}{k_1}$

K_M = Michaelis constant, the concentration at which the velocity of the reaction reaches half the maximum velocity

The shape of the curve: see Fig [1.12]

Inhibition of enzyme reactions

Inhibition: chemical agents can reduce the activity of an enzyme by binding to it
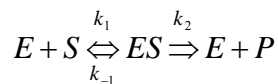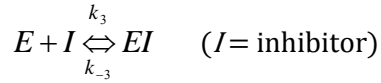
Reversible vs. irreversible

Reversible inhibition: an inhibitor binds to enzymes with non-covalent interactions; the effect of enzyme inhibition disappears when the inhibitor is removed

Irreversible inhibition: an inhibitor binds to enzymes with covalent interactions; the enzymes are made permanently inactive

Reversible inhibition models

1) competitive inhibition: E binds to I

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

$$E + I \underset{k_{-3}}{\overset{k_3}{\Leftrightarrow}} EI \qquad (I = \text{inhibitor})$$

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

$$k_3[E][I] = k_{-3}[EI]$$

$$[EI] = \frac{k_3}{k_{-3}}[E][I]$$

$$k_1[S][E] = (k_{-1} + k_2)[ES]$$

$$[EI] = \frac{k_3}{k_{-3}}\left[K_M \frac{[ES]}{[S]} \right] \cdot [I] = \frac{K_M}{K_I} \frac{[ES]}{[S]} \cdot [I] \qquad \text{where } K_I = k_{-3}/k_3$$
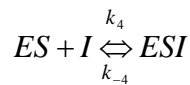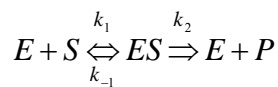
$$[E]_{total} = [E] + [EI] + [ES]$$

The rate of enzyme reaction,

$$\frac{v}{v_m} = \frac{[ES]}{[E]_{total}} = \frac{[ES]}{K_M \dfrac{[ES]}{[S]} + \dfrac{K_M}{K_I} \cdot \dfrac{[ES]}{[S]}[I] + +[ES]} = \frac{[S]}{K_M {}^* + [S]}$$

Effect: increased K_M (the graph shifts to the right)

2) noncompetitive inhibition: I binds to both ES and E or to ES only

2-1) uncompetitive inhibition: I binds to ES only

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

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

$$[E] = K_M \frac{[ES]}{[S]}$$

$$k_4[ES][I] = k_{-4}[ESI]$$

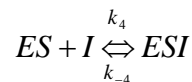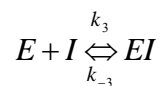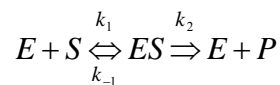$$[ESI] = \frac{k_4}{k_{-4}}[ES][I] = \frac{1}{K_I{}'}[ES][I] \qquad \text{where } K_I{}' = k_{-4}/k_4$$

$$[E]_{total} = [E] + [ESI] + [ES]$$

The rate of enzyme reaction,

$$\frac{v}{v_m} = \frac{[ES]}{[E]_{total}} = \frac{[ES]}{K_M \frac{[ES]}{[S]} + \frac{1}{K_I{}'}[ES][I] + [ES]} = \frac{\frac{1}{(1+[I]/K_I{}')}[S]}{\frac{1}{(1+[I]/K_I{}')}K_M + [S]} = v_m * \frac{[S]}{K_M * + [S]}$$

Effect: decreased v_m and K_M (the graph shifts down and to the left)

2-2) mixed noncompetitive inhibition: I binds to both ES and E – more common

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

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

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

$$[E] = K_M \frac{[ES]}{[S]}$$

$$[EI] = \frac{k_3}{k_{-3}}[E][I] = \frac{K_M}{K_I}\frac{[ES]}{[S]} \cdot [I]$$

$$[ESI] = \frac{k_4}{k_{-4}}[ES][I] = \frac{1}{K_I{}'}[ES][I]$$

$$[E]_{total} = [E] + [EI] + [ESI] + [ES]$$

The rate of enzyme reaction,

$$\frac{v}{v_m} = \frac{[ES]}{[E]_{total}} = \frac{[ES]}{K_M \dfrac{[ES]}{[S]} + \dfrac{K_M}{K_I}\dfrac{[ES]}{[S]}\cdot[I] + \dfrac{1}{K_I{}'}[ES][I] + [ES]}$$

In many cases, KI ≈ KI' (the tendency of inhibitor binding to E and ES is the same)

Then,

$$\frac{v}{v_m} = \frac{1}{1 + \dfrac{[I]}{K_I}} \cdot \frac{[S]}{K_M + [S]}$$

effect: decreased v_m (the graph shifts down)