Enzyme Reactivity and Inhibition

1. Enzyme reactivity - Michaelis-Menten equation

Step 1: enzyme (E) reacts with substrate (S) to form an enzyme-substrate complex (ES)

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

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

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

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

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

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

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

The rate of enzyme reaction, v, can be written as,

$$v = k_2[ES]$$

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

* When should the reaction be at its maximum?

When [S] is very large - there are many, many works (substrates) to do (react with enzyme), so the workers (enzymes) are fully working

In other words:

$$[S] \gg \frac{k_{-1}+k_2}{k_1} \text{ and } [ES] \approx [E]_{total}$$

Then:

$$v_m = k_2 [E]_{total}$$

Therefore:

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

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

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

* What should the shape of the curve look like? (Fig. 1.12)

2. Inhibition of enzyme reactions

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

<u>**Reversible inhibition models**</u>

a) competitive inhibition: E binds to I

- Master eq. for enzyme reaction:

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

- Reaction involving competitive inhibitor, I:

$$E+I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI \qquad \text{(a)}$$

- Finding solution

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

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

from (a): $k_3[E][I]=k_{-3}[EI]$

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

$$[EI]=\frac{k_3}{k_{-3}}\left(K_M\frac{[ES]}{[S]}\right)\cdot[I]=\frac{K_M}{K_I}\cdot\frac{[ES]}{[S]}\cdot[I],\ \text{where } K_I=\frac{k_{-3}}{k_3}$$

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

The rate of enzyme reaction under competitive inhibition:

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

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

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

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

- Reaction involving uncompetitive inhibitor, I:

$$ES + I \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} ESI \qquad \text{(b)}$$

- Finding solution

from (I):

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

from (b):

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

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

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

The rate of enzyme reaction under uncompetitive inhibition:

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

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

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

- Reaction involving mixed noncompetitive inhibitor, I:

$$E+I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI \qquad \text{(a)}$$

$$ES+I \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} ESI \qquad \text{(b)}$$

- Finding solution

from our previous work on competitive inhibition:

$$[EI]=\frac{K_M}{K_I}\cdot\frac{[ES]}{[S]}\cdot[I]$$

from our previous work on uncompetitive inhibition:

$$[ESI]=\frac{1}{K_I'}[ES][I]$$

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

The rate of enzyme reaction under mixed noncompetitive inhibition:

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

In many cases, $K_I \approx K_I'$ (the tendency of inhibitor binding to E and ES is the same)

Then,

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

- Effect: decreased v_m (the graph shifts down)

c) Product inhibition: the product, P, binds to ES

- Reaction for product inhibition:

$$ES + P \underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} ESP$$

Additional assumption: [ESP] does not change over time

Then, $k_5[ES][P] = k_{-5}[ESP]$

- Finding solution

$$[ESP] = \frac{1}{K_P}[ES][P], \text{ where } K_P = \frac{k_{-5}}{k_5}$$

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

The rate of enzyme reaction under product inhibition:

$$v = v_m \frac{[ES]}{[E]_{total}} = v_m \frac{[ES]}{K_M \frac{[ES]}{[S]} + \frac{1}{K_P}\frac{[ES]}{[S]}[P]} = v_m{}^* \frac{[S]}{K_M{}^* + [S]}$$

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