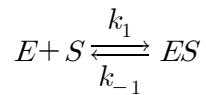


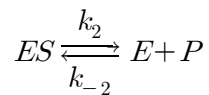
## Enzyme Reactivity and Inhibition

### 1. Enzyme reactivity - Michaelis-Menten equation

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



Step 2: the enzyme-substrate complex breaks down to form free enzyme and products (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,  $k_1[E][S] = k_{-1}[ES] + k_2[ES]$

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

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

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

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

$$\begin{aligned} v &= k_2[ES] \\ &= \frac{k_2[S][E]_{total}}{[S] + \left(\frac{k_{-1} + k_2}{k_1}\right)} \end{aligned}$$

- \* 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:

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

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

$K_M$  = 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)

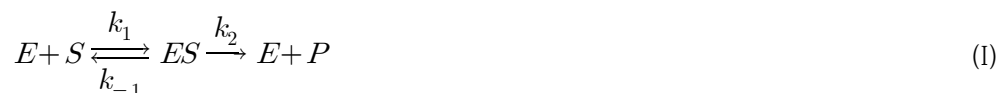
## 2. Inhibition of enzyme reactions

- 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

### Reversible inhibition models

a) competitive inhibition: E binds to I

- Master eq. for enzyme reaction:



- Reaction involving competitive inhibitor, I:



- Finding solution

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

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

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:



- Finding solution

from (1):

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

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:



- 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)

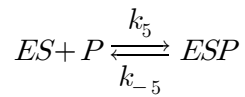
Then,

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

- Effect: decreased  $v_m$  (the graph shifts down)

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

- Reaction for product inhibition:



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)