Allosteric Enzymes

3.3.4. Models for More Complex Enzyme Kinetics

3.3.4.1. Allosteric enzymes. Some enzymes have more than one substrate binding site. The binding of one substrate to the enzyme facilitates binding of other substrate molecules. This behavior is known as *allostery* or *cooperative binding*, and regulatory enzymes show this behavior. The rate expression in this case is

$$v = -\frac{d[S]}{dt} = \frac{V_m[S]^n}{K''_m + [S]^n} \tag{3.18}$$

where n = cooperativity coefficient and $n > 1$ indicates positive cooperativity. Figure 3.8 compares Michaelis–Menten kinetics with allosteric enzyme kinetics, indicating a sigmoidal shape of υ –[S] plot for allosteric enzymes.

The cooperativity coefficient can be determined by rearranging eq. 3.18 as

$$\ln\frac{v}{V_m - v} = n\ln[S] - \ln K''_m \tag{3.19}$$

and by plotting $\ln v/(V_m - v)$ versus $\ln[S]$ as depicted in Fig. 3.9.

Allosteric enzyme kinetics

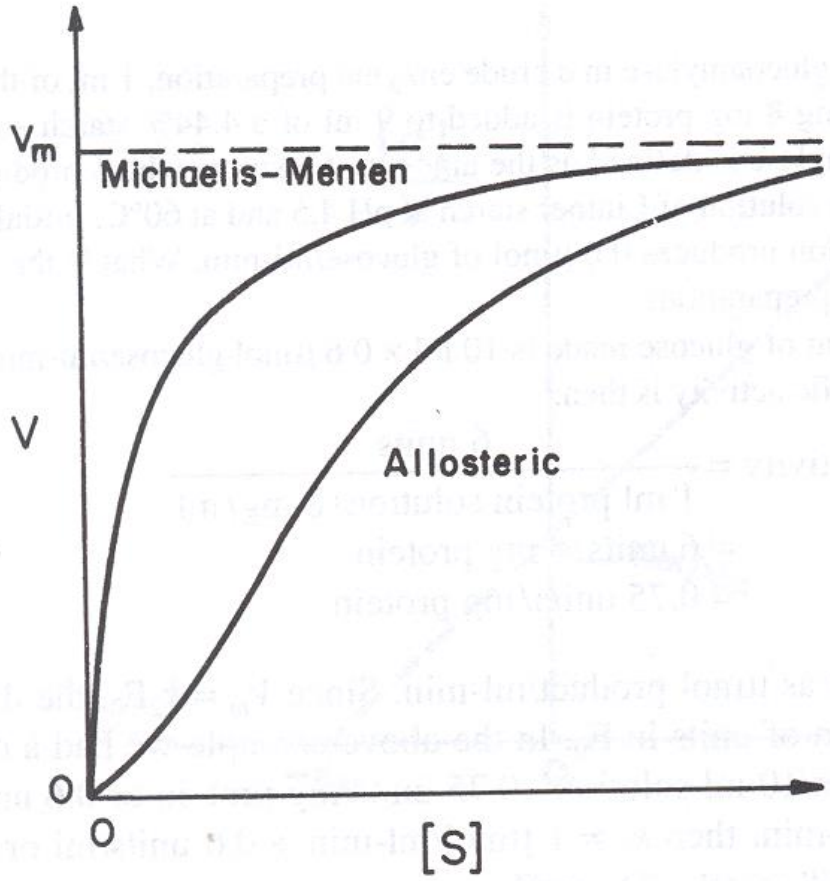


Figure 3.8. Comparison of Michaelis–Menten and allosteric enzyme kinetics.

Determination of cooperativity coefficient

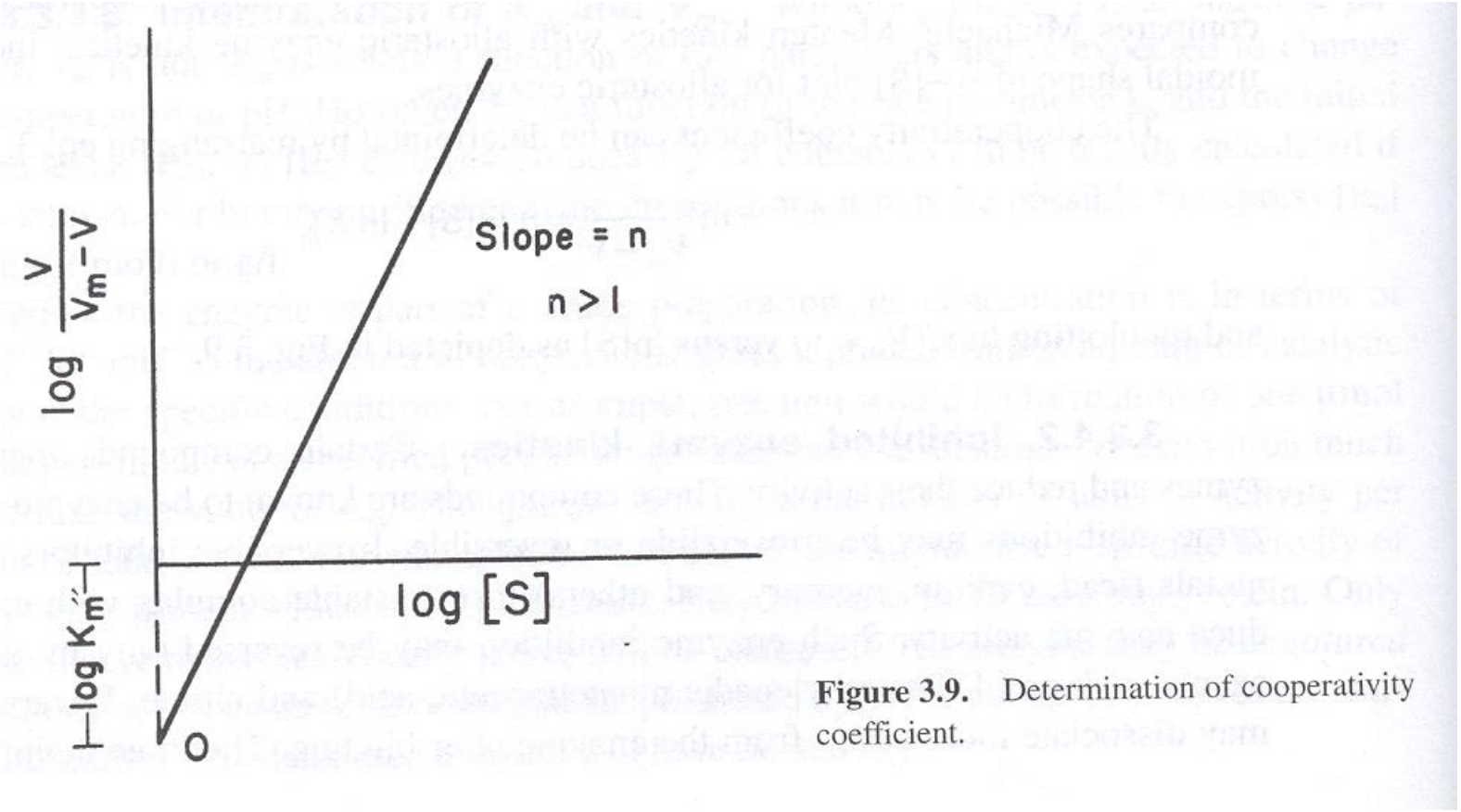


Figure 3.9. Determination of cooperativity coefficient.

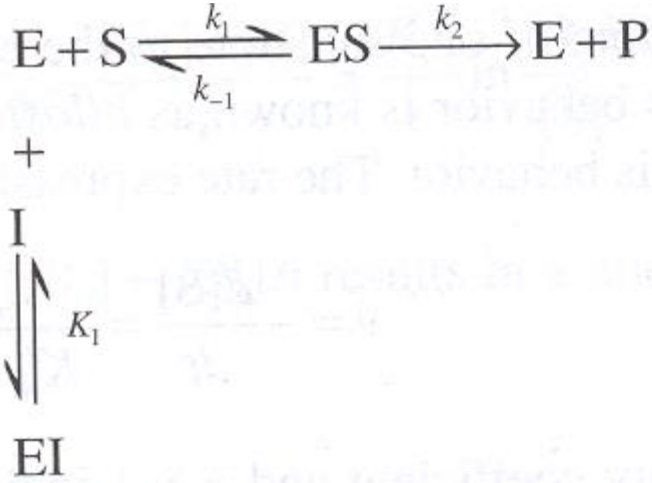
Inhibited Enzyme Kinetics

- Inhibitor
  - Inhibitor binds to enzymes and reduces their activity.
- Irreversible inhibitor
  - Heavy metals form a stable complex with enzymes.
  - Pd, Cd, Hg, and others
  - May be reversed only by using chelating agents such as EDTA and citrate.
- Reversible inhibitor
  - Dissociate more easily from the enzyme

Reversible Enzyme Inhibition

- Competitive inhibition
- Noncompetitive inhibition
- Uncompetitive inhibition
- Substrate inhibition

Competitive Inhibition



(3.20)

Assuming rapid equilibrium and with the definition of

$$K'_m = \frac{[E][S]}{[ES]}, \qquad K_I = \frac{[E][I]}{[EI]}$$

$$[E_0] = [E] + [ES] + [EI] \quad \text{and} \quad v = k_2[ES]$$

Assuming rapid equilibrium and with the definition of

$$K'_m = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]}, \qquad K_I = \frac{[\mathrm{E}][\mathrm{I}]}{[\mathrm{EI}]}$$

$$[\mathrm{E}_0] = [\mathrm{E}] + [\mathrm{ES}] + [\mathrm{EI}] \quad \text{and} \quad v = k_2[\mathrm{ES}]$$

we can develop the following equation for the rate of enzymatic conversion:

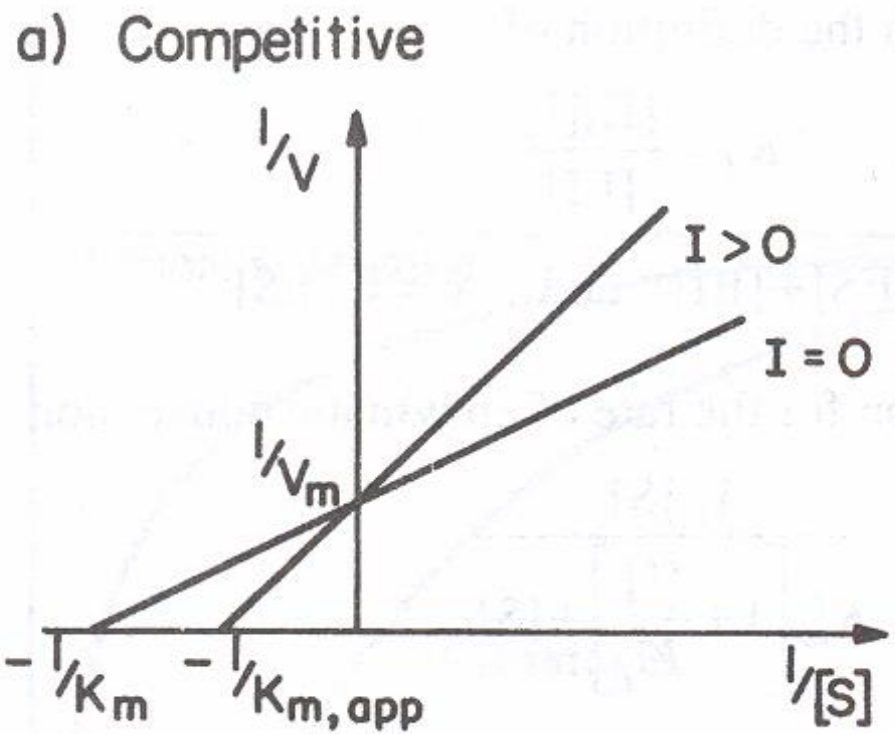
$$v = \frac{V_m[\mathrm{S}]}{K'_m\left[1 + \frac{[\mathrm{I}]}{K_I}\right] + [\mathrm{S}]}$$

or

$$v = \frac{V_m[\mathrm{S}]}{K'_{m,\,\mathrm{app}} + [\mathrm{S}]}$$

where $K'_{m,\mathrm{app}} = K'_m\left(1 + \frac{[\mathrm{I}]}{K_1}\right)$.

L-B Plot



$$v = \frac{V_m[S]}{K'_m\left[1+\frac{[I]}{K_I}\right]+[S]}$$

Noncompetitive Inhibition

$$\begin{array}{ccc} E + S & \underset{K'_m}{\rightleftharpoons} & ES \xrightarrow[k_2]{} E + P \\ + & & + \\ I & & I \\ K_I \Downarrow\Uparrow & & \Downarrow\Uparrow \\ EI + S & \underset{K'_m}{\rightleftharpoons} & ESI \end{array} \qquad (3.24)$$

$$K'_m = \frac{[E][S]}{[ES]} = \frac{[EI][S]}{[ESI]}, \qquad K_I = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]} \qquad (3.25)$$

$$[E_0] = [E] + [ES] + [EI] + [ESI] \text{ and } v = k_2[ES]$$

$$K_m' = \frac{[E][S]}{[ES]} = \frac{[EI][S]}{[ESI]}, \qquad K_I = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]} \tag{3.25}$$

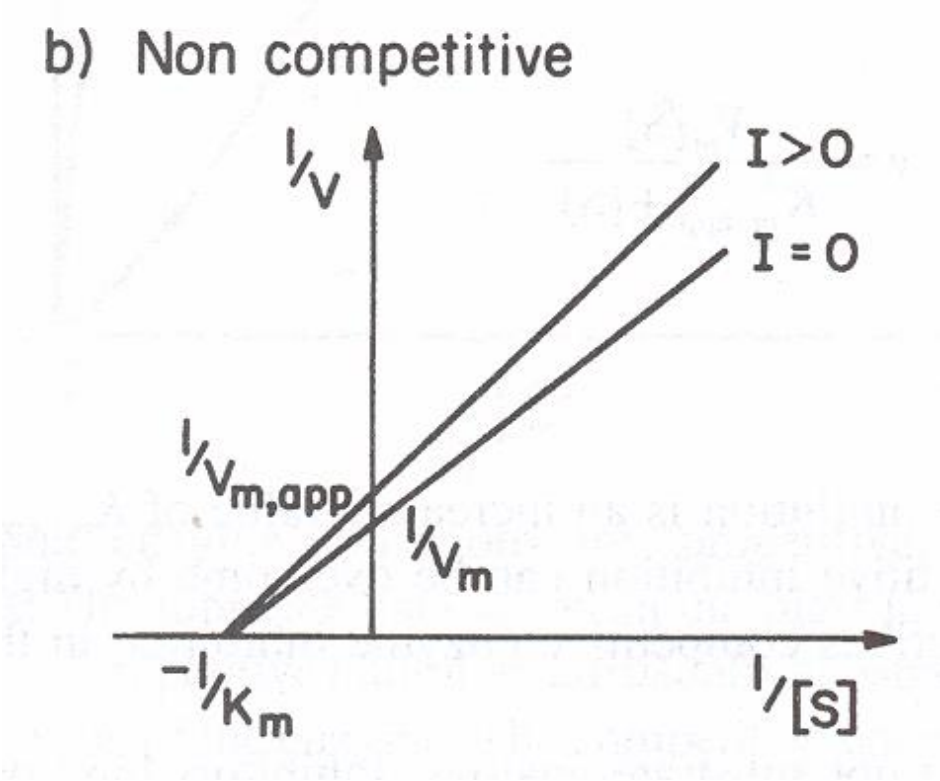
$$[E_0] = [E] + [ES] + [EI] + [ESI] \text{ and } v = k_2[ES]$$

$$v = \frac{V_m}{\left(1 + \frac{[I]}{K_1}\right)\left(1 + \frac{K_m'}{[S]}\right)} \tag{3.26}$$

$$v = \frac{V_{m,\text{app}}}{\left(1 + \frac{K_m'}{[S]}\right)} \tag{3.27}$$

where $V_{m,\text{app}} = \dfrac{V_m}{\left(1 + \dfrac{[I]}{K_1}\right)}$

L-B Plot



$$v = \frac{V_{m,\text{app}}}{\left(1 + \frac{K'_m}{[S]}\right)}$$

where $V_{m,\text{app}} = \frac{V_m}{\left(1 + \frac{[I]}{K_1}\right)}$

Uncompetitive Inhibition

$$\begin{array}{ccccc} \mathrm{E}+\mathrm{S} & \underset{}{\overset{K'_m}{\rightleftharpoons}} & \mathrm{ES} & \xrightarrow{k_2} & \mathrm{E}+\mathrm{P} \\ & & + & & \\ & & \mathrm{I} & & \\ & & \Big\Updownarrow K_1 & & \\ & & \mathrm{ESI} & & \end{array} \tag{3.28}$$

$$K'_m = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]}, \quad K_1 = \frac{[\mathrm{ES}][\mathrm{I}]}{[\mathrm{ESI}]} \tag{3.29}$$

$$[\mathrm{E}_0] = [\mathrm{E}] + [\mathrm{ES}] + [\mathrm{ESI}] \text{ and } v = k_2[\mathrm{ES}]$$

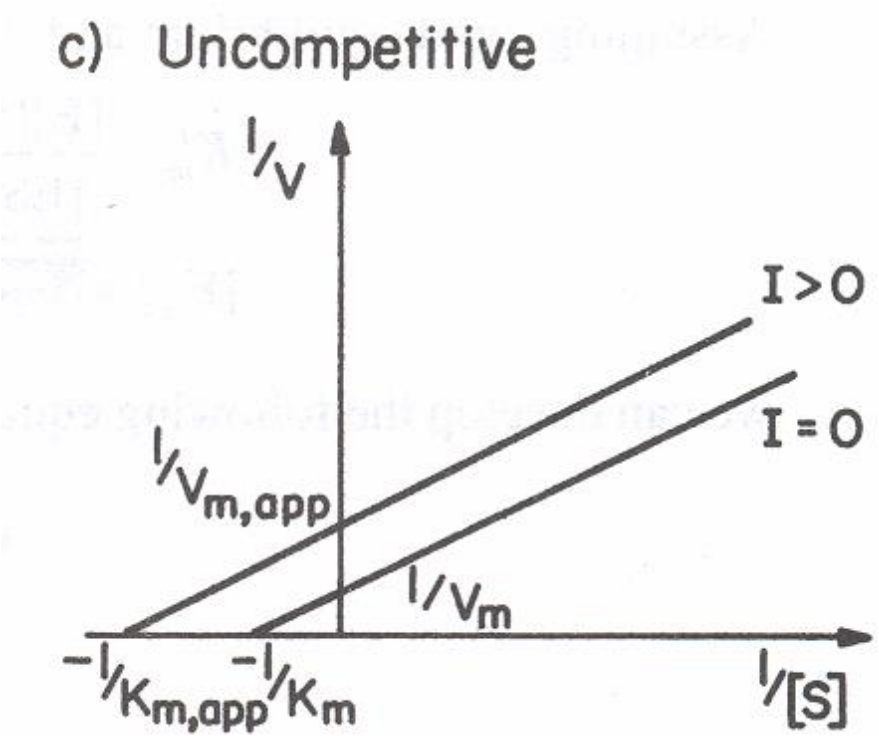
$$K'_m = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]}, \quad K_1 = \frac{[\mathrm{ES}][\mathrm{I}]}{[\mathrm{ESI}]} \tag{3.29}$$

$$[\mathrm{E}_0] = [\mathrm{E}] + [\mathrm{ES}] + [\mathrm{ESI}] \text{ and } v = k_2[\mathrm{ES}]$$

$$v = \frac{\dfrac{V_m}{\left(1 + \dfrac{[\mathrm{I}]}{K_1}\right)}[\mathrm{S}]}{\dfrac{K'_m}{\left(1 + \dfrac{[\mathrm{I}]}{K_1}\right)} + [\mathrm{S}]} \tag{3.30}$$

$$v = \frac{V_{m,\mathrm{app}}[\mathrm{S}]}{K'_{m,\mathrm{app}} + [\mathrm{S}]} \tag{3.31}$$

L-B Plot



$$v = \frac{\dfrac{V_m}{\left(1+\dfrac{[I]}{K_1}\right)}[S]}{\dfrac{K'_m}{\left(1+\dfrac{[I]}{K_1}\right)}+[S]}$$

$$v = \frac{V_{m,app}[S]}{K'_{m,app}+[S]}$$

Substrate Inhibition

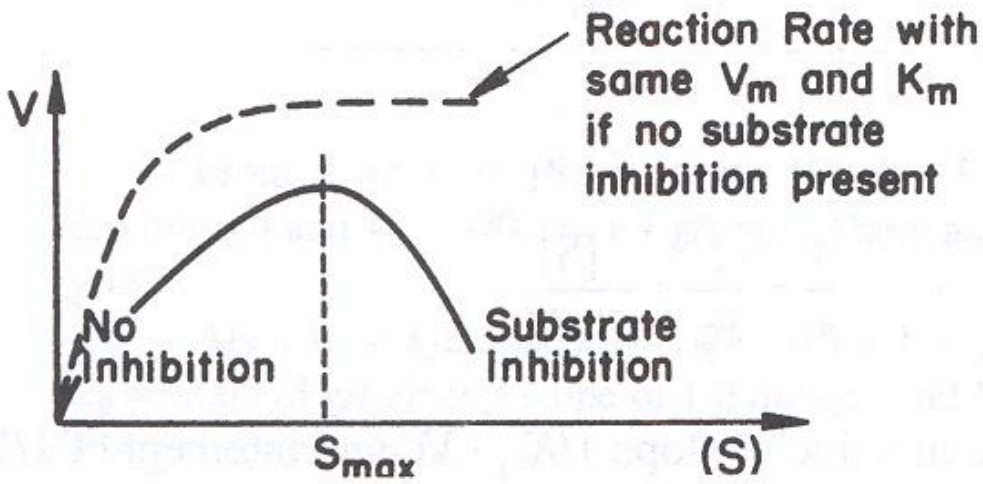
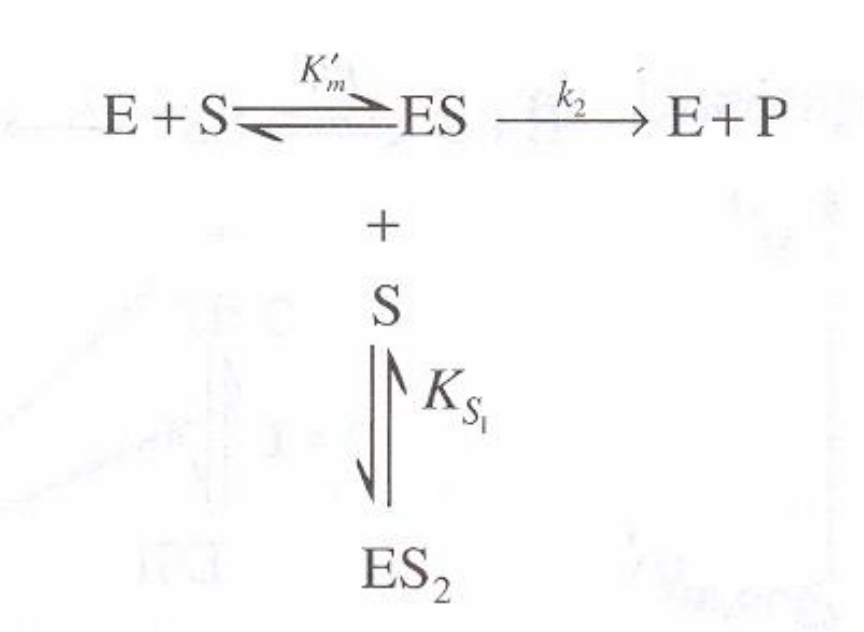


Figure 3.11. Comparison of substrate-inhibited and uninhibited enzymatic reactions.

Substrate Inhibition



$$K_{S_1} = \frac{[S][ES]}{[ES_2]}, \quad K'_m = \frac{[S][E]}{[ES]}$$

$$K_{S_1} = \frac{[S][ES]}{[ES_2]}, \quad K'_m = \frac{[S][E]}{[ES]}$$

$$v = \frac{V_m[S]}{K'_m + [S] + \dfrac{[S]^2}{K_{S_1}}}$$

At low substrate concentrations, $[S]^2/K_{S_1} << 1$, and inhibition effect is not observed. The rate is

$$v = \frac{V_m}{\left[1 + \dfrac{K'_m}{[S]}\right]} \tag{3.35}$$

or

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K'_m}{V_m}\frac{1}{[S]} \tag{3.36}$$

A plot of $1/v$ versus $1/[S]$ results in a line of slope K'_m/V_m and intercept of $1/V_m$.

$$v = \frac{V_m[S]}{K'_m + [S] + \frac{[S]^2}{K_{S_1}}}$$

At high substrate concentrations, $K'_m/[S] \ll 1$, and inhibition is dominant. The rate in this case is

$$v = \frac{V_m}{\left(1 + \frac{[S]}{K_{S_1}}\right)} \tag{3.37}$$

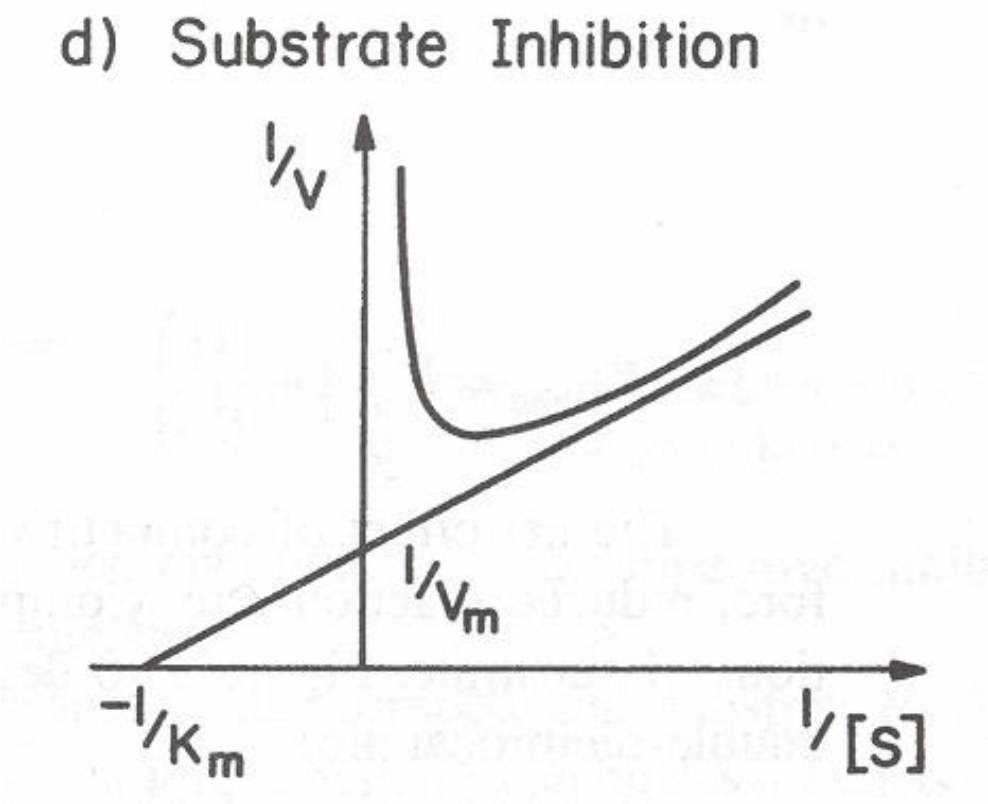
$$\frac{1}{v} = \frac{1}{V_m} + \frac{[S]}{K_{S_1} V_m} \tag{3.38}$$

A plot of $1/v$ versus [S] results in a line of slope $1/K_{s_1} \cdot V_m$ and intercept of $1/V_m$.

The substrate concentration resulting in the maximum reaction rate can be determined by setting $dv/d[S] = 0$. The $[S]_{max}$ is given by

$$[S]_{max} = \sqrt{K'_m K_{S_1}}$$

L-B Plot



L-B Plot

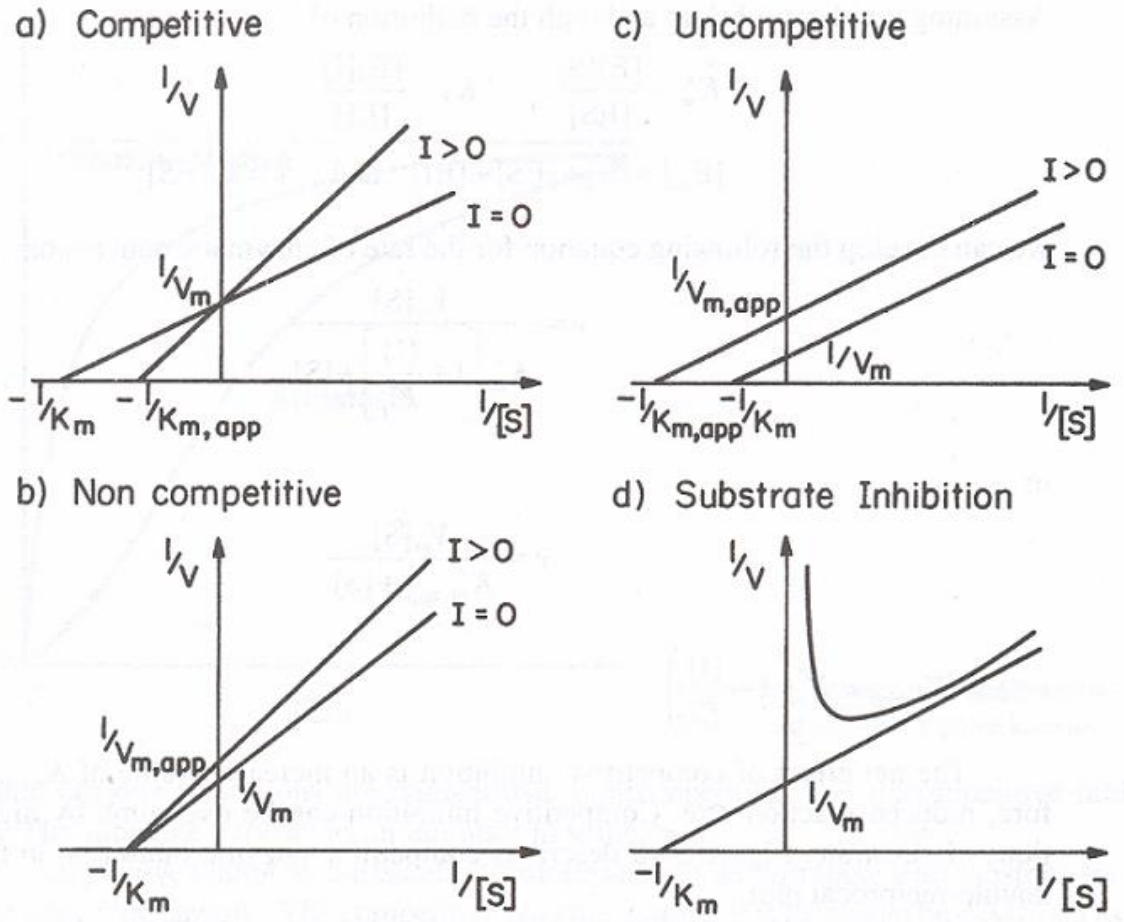
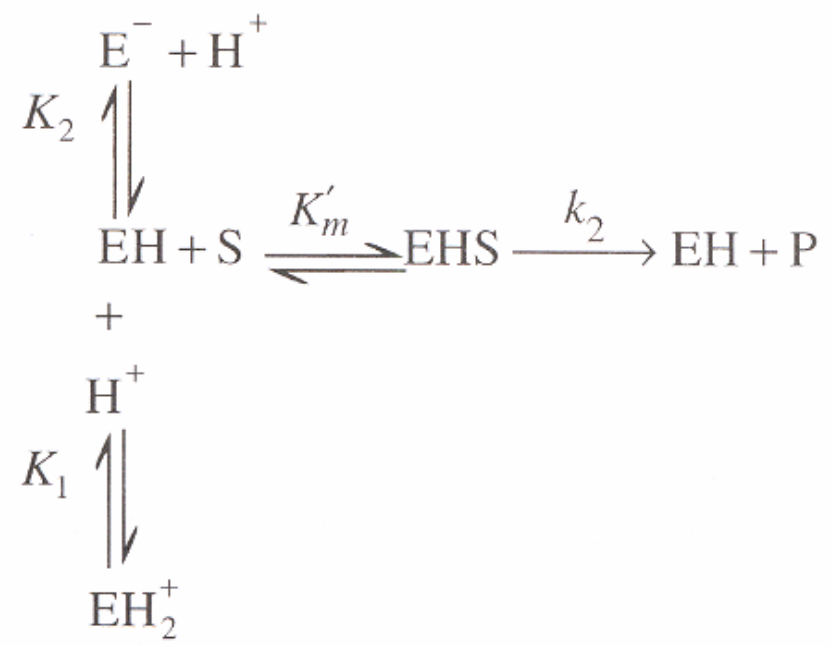


Figure 3.10. Different forms of inhibited enzyme kinetics.

Effect of pH

3.3.5.1. pH effects. Certain enzymes have ionic groups on their active sites, and these ionic groups must be in a suitable form (acid or base) to function. Variations in the pH of the medium result in changes in the ionic form of the active site and changes in the activity of the enzyme and hence the reaction rate. Changes in pH may also alter the three-dimensional shape of the enzyme. For these reasons, enzymes are only active over a certain pH range. The pH of the medium may affect the maximum reaction rate, K_m, and the stability of the enzyme. In some cases, the substrate may contain ionic groups, and the pH of the medium affects the affinity of the substrate to the enzyme.

Effect of pH



$$K_m' = \frac{[EH][S]}{[EHS]}$$

$$K_1 = \frac{[EH][H^+]}{[EH_2^+]}$$

$$K_2 = \frac{[E^-][H^+]}{[EH]}$$

$$[E_0] = [E^-] + [EH] + [EH_2^+] + [EHS],$$

$$v = k_2[EHS]$$

Effect of pH

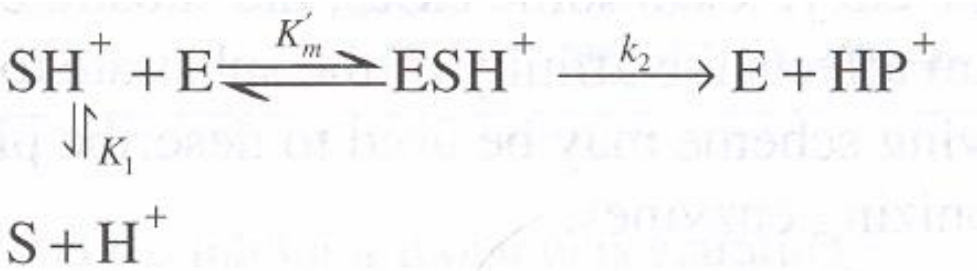
$$v = \frac{V_m[S]}{K_m'\left[1 + \frac{K_2}{[H^+]} + \frac{[H^+]}{K_1}\right] + [S]}$$

$$v = \frac{V_m[S]}{K_{m,app}' + [S]}$$

where $K_{m,app}' = K_m'\left[1 + \frac{K_2}{[H^+]} + \frac{[H^+]}{K_1}\right]$

Effect of pH

For the case of ionizing substrate,



$$v = \frac{V_m[S]}{K_m' \frac{K_1}{[H^+]} + [S]}$$

Effect of pH

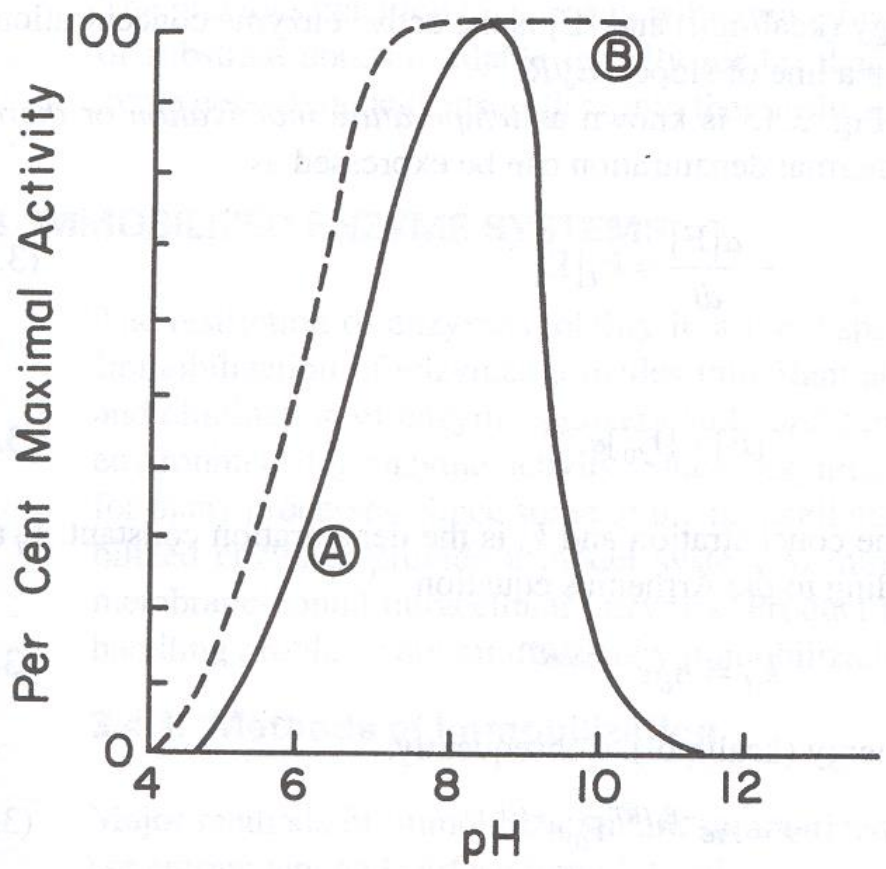
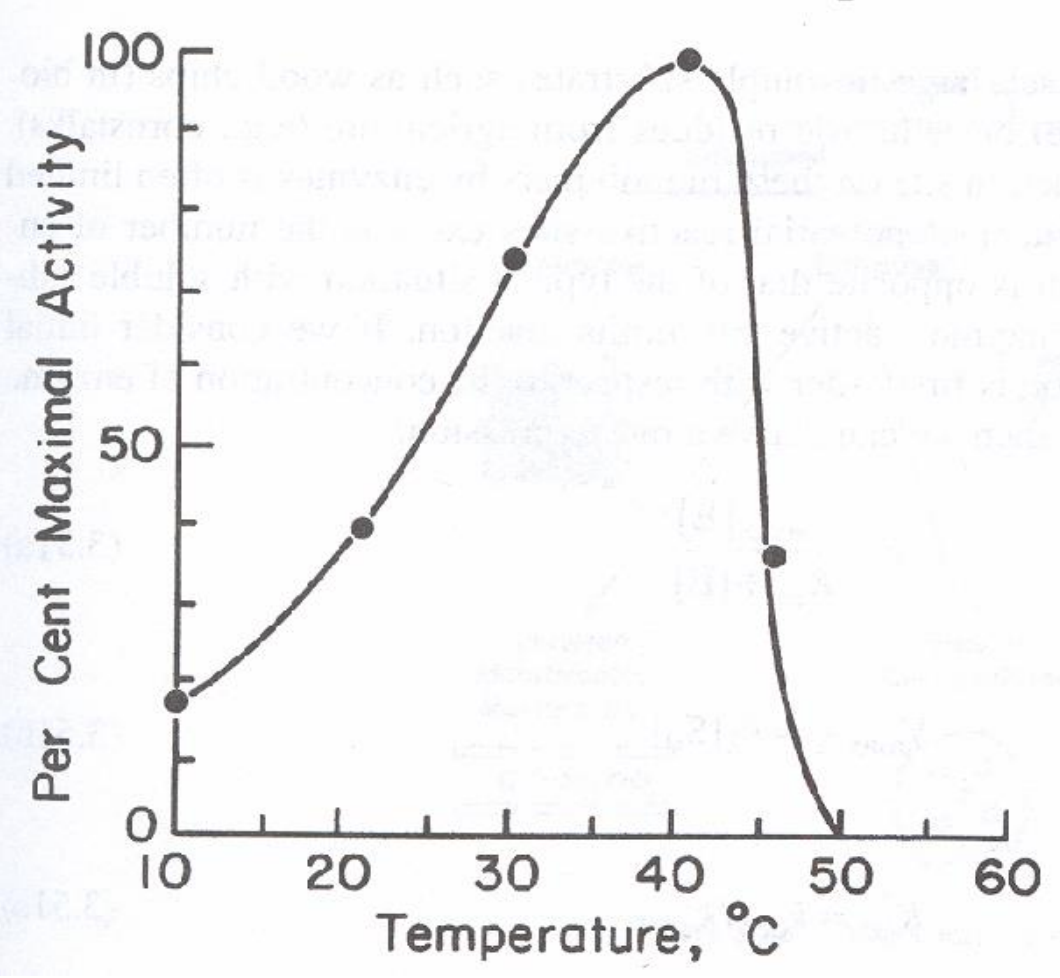


Figure 3.14. The pH-activity profiles of two enzymes. (A) approximate activity for trypsin; (B) approximate activity for cholinesterase.

Effect of Temperature



Effect of Temperature

$$v = k_2[E]$$

Temperature Activation

$$k_2 = Ae^{-E_a/RT}$$

Temperature Deactivation

$$-\frac{d[\mathrm{E}]}{dt} = k_d[\mathrm{E}]$$

$$[\mathrm{E}] = [\mathrm{E}_0]e^{-k_d t}$$

where $k_d = A_d e^{-E_d/RT}$

$$v = Ae^{-E_a/RT}\mathrm{E}_0 e^{-k_d t}$$

Insoluble Substrates

$$v = \frac{V_{max,S}[E]}{K_{eq} + [E]}$$

where $V_{max,S} = k_2[S_0]$

$$K'_{eq} = k_{des} / k_{ads}$$