INHIBITED ENZYME KINETICS

Inhibited Enzyme Kinetics

- Inhibitors may bind to enzyme and reduce their activity.
- Enzyme inhibition may be reversible or irreversible.
- For reversible enzyme inhibition, there are
  - Competitive inhibition
  - Uncompetitive inhibition
  - Noncompetitive inhibition

Competitive Inhibitors (I)

- substrate analogs
- compete with substrate for the active site of the enzyme

$$\begin{array}{l} E + S \overset{K'_m}{\rightleftarrows} ES \xrightarrow{k_2} P + E \\ + \\ \mathrm{I} \\ \uparrow\!\downarrow \; K_I \\ EI \end{array}$$

Competitive Inhibitors (I)

Assuming rapid equilibrium and with the definitions of

$$v = \frac{d[P]}{dt} = k_2 [ES]$$

$$K'_m = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

$$K_I = \frac{[E][I]}{[EI]}$$

$$[E_0] = [E] + [ES] + [EI]$$

Competitive Inhibitors (I)

we then obtain, $v = \dfrac{V_m[S]}{K'_m(1+[I]/K_I)+[S]}$

$$v = \frac{V_m[S]}{K'_{m,app}+[S]} \qquad K'_{m,app} = K'_m(1+[I]/K_I)$$

When $[I] = 0, K'_{m,app} = K'_m$.

V_m remains the same as that of Michaelis-Menten equation.
$K'_{m,app}$ is greater than K'_m ➔ Net rate decreases

Competitive inhibition can be overcome by high substrate concentration

Noncompetitive Inhibitors (I)

- Not substrate analogs
- bind on site other than the active sites and reduce ezyme affinity to the substrate.

$$\begin{tikzcd}
E + S \arrow[r, shift left, "K'_m"] & ES \arrow[l, shift left] \arrow[r, "k_2"] & P + E \\
+ & + & \\
\mathrm{I} \arrow[d, shift left, "K_I"] & \mathrm{I} \arrow[d, shift left, "K_I"] & \\
EI + S \arrow[u, shift left] \arrow[r, shift left, "K'_m"] & ESI \arrow[u, shift left] \arrow[l, shift left] &
\end{tikzcd}$$

Noncompetitive Inhibitors (I)

Assuming:

- rapid equilibrium
- same equilibrium constants of inhibitor binding to E and ES → K_I
- same equilibrium constants of substrate binding to E and EI → K'_m

---

Noncompetitive Inhibitors (I)

$$\begin{tikzcd}
E + S \arrow[r, shift left, "K'_m"] & ES \arrow[l, shift left] \arrow[r, "k_2"] & P + E \\
+\ \mathrm{I} \arrow[d, shift left] & +\ \mathrm{I} \arrow[d, shift left] & \\
EI + S \arrow[u, shift left, "K_I"'] \arrow[r, shift left, "K'_m"] & ESI \arrow[l, shift left] \arrow[u, shift left, "K_I"'] &
\end{tikzcd}$$

$$v = \frac{d[P]}{dt} = k_2 [ES]$$

$$K'_m = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]} = \frac{[EI][S]}{[ESI]} \qquad K_I = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]}$$

$$[E_0] = [E] + [ES] + [EI] + [ESI]$$

Noncompetitive Inhibitors (I)

- we then obtain, $v = \dfrac{V_m[S]}{(1+[I]/K_I)(K_m^{'}+[S])}$

$$v = \frac{V_{m,app}[S]}{K_m^{'}+[S]} \qquad V_{m,app} = V_m/(1+[I]/K_I)$$

When $[I] = 0, V_{m,app} = V_m$.

K'_m remains the same as in Michaelis-Menten equation. $V_{m,app}$ is less than V_m.

High substrate concentration would NOT overcome noncompetitive inhibition ➔ other reagent need to be added to block inhibitor-enzyme binding

Uncompetitive Inhibitors (I)

- have no affinity for the enzyme itself
- bind only to the ES complex.

- Assuming rapid equilibrium,

$$\begin{tikzcd} E + S \arrow[r, shift left, "K'_m"] & ES \arrow[l, shift left] \arrow[r, "k_2"] & P + E \\ & + & \\ & \mathrm{I} \arrow[d, shift left, "K_I"] & \\ & ESI \arrow[u, shift left] & \end{tikzcd}$$

Uncompetitive Inhibitors (I)

$$v = \frac{d[P]}{dt} = k_2[ES]$$

$$K_m^{'} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

$$\begin{tikzcd}
E + S \arrow[r, leftrightarrow, "K_m'"] & ES \arrow[r, "k_2"] \arrow[d, phantom, "+"] & P + E \\
 & I \arrow[d, shift left, "K_I"] & \\
 & ESI \arrow[u, shift left] &
\end{tikzcd}$$

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

$$[E_0] = [E] + [ES] + [ESI]$$

Uncompetitive Inhibitors (I)

- we then obtain, $v = \dfrac{(V_m/(1+[I]/K_I))[S]}{(K_m^{'}/(1+[I]/K_I))+[S]}$

$$K_{m,app}^{'} = K_m^{'}/(1+[I]/K_I)$$

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

$$V_{m,app} = V_m/(1+[I]/K_I)$$

$$K_{m,app}^{'}/V_{m,app} = K_m^{'}/V_m$$

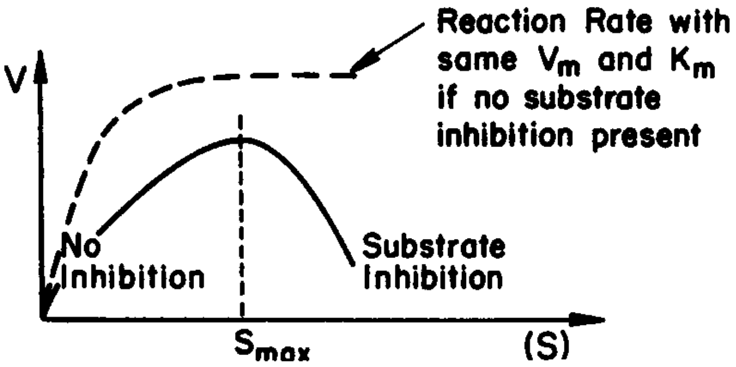
Uncompetitive Substrate Inhibitors

- high substrate concentration may cause inhibition
- bind only to the ES complex

- Assuming rapid equilibrium,

$$\begin{tikzcd} E + S \arrow[r, shift left, "K_m'"] & ES \arrow[l, shift left] \arrow[r, "k_2"] & P + E \\ & + & \\ & S \arrow[d, shift left, "K_{SI}"] & \\ & ES_2 \arrow[u, shift left] & \end{tikzcd}$$

Uncompetitive Substrate Inhibitors



Uncompetitive Substrate Inhibitors

$$v = \frac{d[P]}{dt} = k_2[ES]$$

$$\begin{tikzcd}
E + S \arrow[r, shift left, "K'_m"] & ES \arrow[l, shift left] \arrow[r, "k_2"] \arrow[d, phantom, "+"] & P + E \\
& S \arrow[d, shift left, "K_{SI}"] & \\
& ES_2 \arrow[u, shift left] &
\end{tikzcd}$$

$$K'_m = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

$$K_{SI} = \frac{[ES][S]}{[ES_2]}$$

$$[E_0] = [E] + [ES] + [ES_2]$$

Uncompetitive Substrate Inhibitors

- we then obtain, $v = \dfrac{V_m[S]}{K'_m + [S] + [S]^2 / K_{SI}}$

At low substrate concentration $[S]^2 / K_{SI} << [S]$

$$v = \frac{V_m[S]}{K'_m + [S]}$$ Michaelis-Menten Equation

At high substrate concentration $K'_m << [S]$

$$v = \frac{V_m}{1 + [S]/K_{SI}}$$

Uncompetitive Substrate Inhibitors

- The substrate concentration resulting in the maximum reaction rate can be determined by setting $dv/d[S] = 0$:

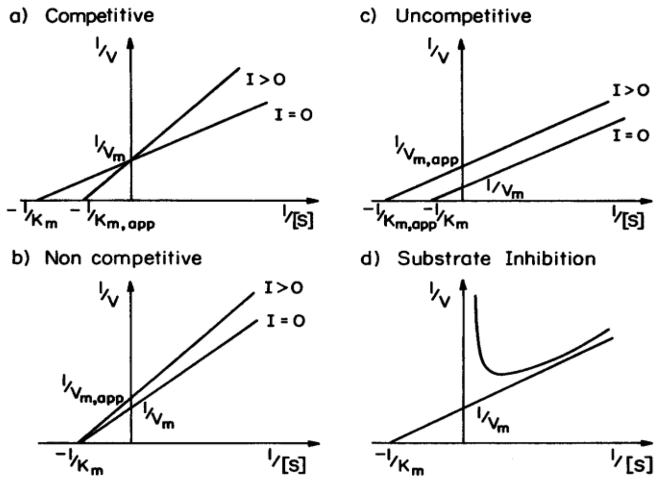
$$dv/d[S] = d\left(\frac{V_m[S]}{K_m^{'} + [S] + [S]^2 / K_{SI}}\right)/d[S] = 0$$

$$[S]_{\max} = (K_m^{'} K_{SI})^{1/2}$$

Inhibition Estimation

- Product formation rate v vs. $[S]$: **does v has a peak?**
  - YES ➔ Substrate Inhibition
    - get $[S]_{\max}$ from v vs. $[S]$ plot
    - at low substrate concentration, obtain V_m and K_m' graphically
    - calculate KI through $[S]_{\max} = (K_m^{'} K_{SI})^{1/2}$
  - NO ➔ examine the data with and without inhibitors in $1/v$ vs. $1/[S]$ plot (Lineweaver-Burk Plot).

Estimation of Inhibited Enzyme Kinetics



Estimation of Inhibited Enzyme Kinetics

- Determine the type of inhibition.
- Determine the parameters for Michaelis-Menten equation without inhibition.
- Determine the parameter of K_I for inhibited kinetics.