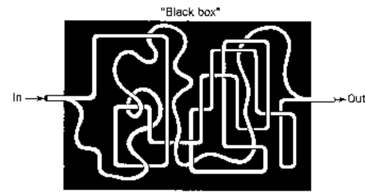
Enzyme Kinetics II

10/16/2008

Kinetic data cannot unambiguously establish a reaction mechanism.

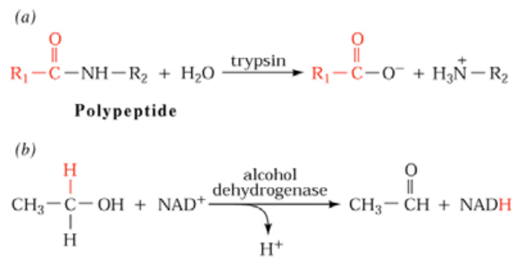


Although a phenomenological description can be obtained the nature of the reaction intermediates remain indeterminate and other independent measurements are needed.

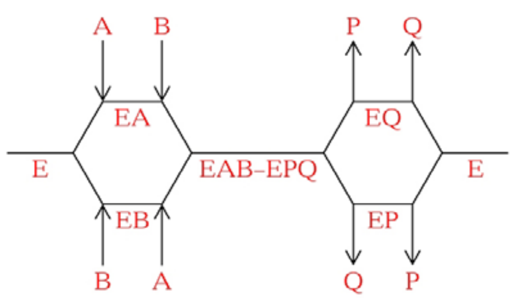
Reaction Mechanisms
A: Sequential Reactions

- All substrates must combine with enzyme before reaction can occur

Bisubstrate reactions

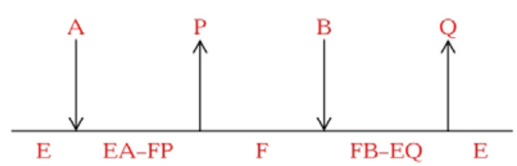


Random Bisubstrate Reactions



Ping-Pong Reactions

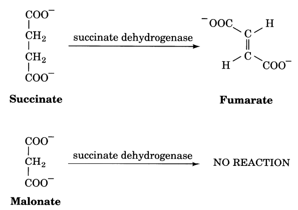
- Group transfer reactions
- One or more products released before all substrates added



Inhibition kinetics

There are three types of inhibition kinetics competitive, mixed and uncompetitive.

•Competitive- Where the inhibitor competes with the substrate.



Competitive Inhibition

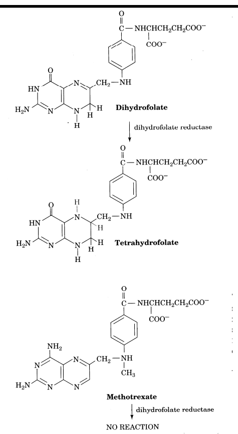
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

$$+$$

$$I$$

$$K_I \updownarrow$$

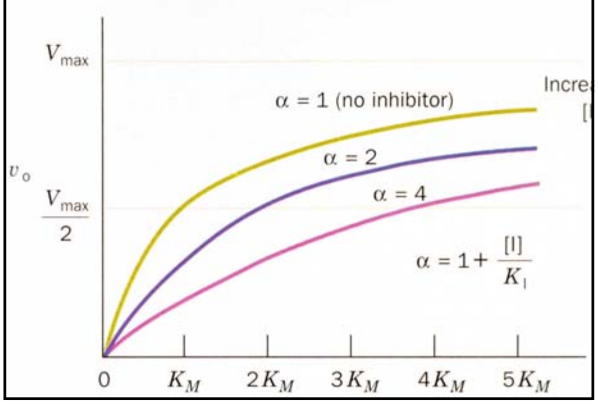
$$EI + S \longrightarrow \text{NO REACTION}$$



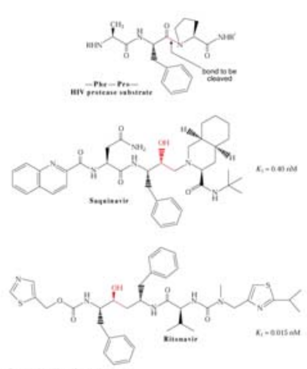
$$K_I = \frac{[E][I]}{[EI]}$$

$$v_o = \frac{V_{max}[S]}{\alpha K_M + [S]}$$

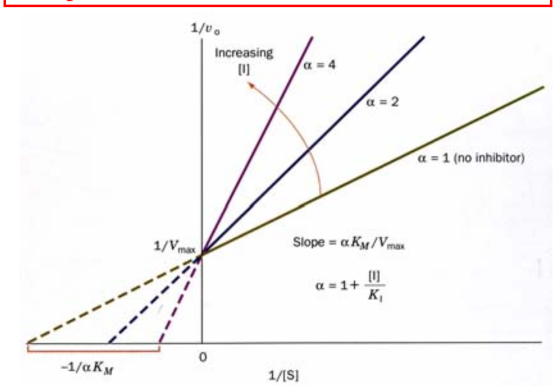
$$\alpha = \left(1 + \frac{[I]}{K_I}\right)$$



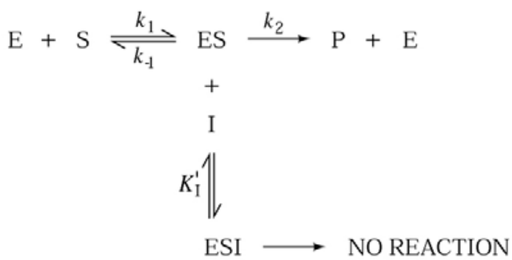
HIV protease inhibitors



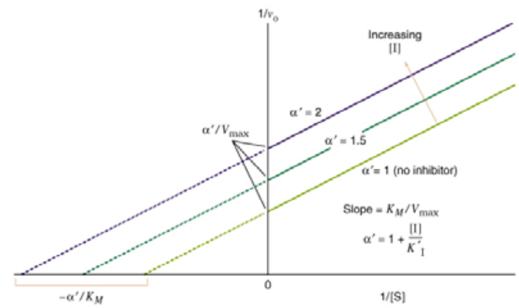
Competitive Inhibition: Lineweaver-Burke Plot



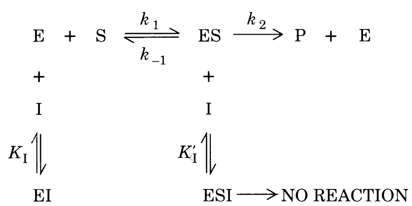
Uncompetitive Inhibition

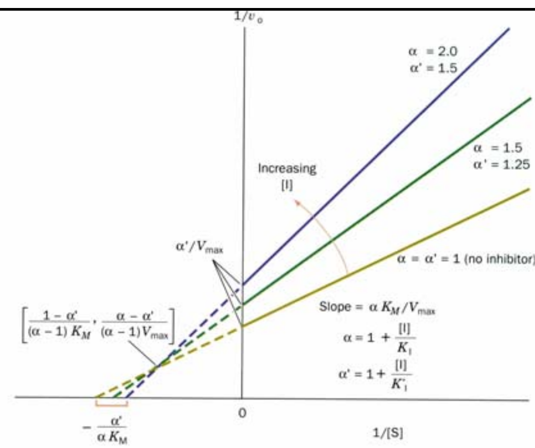


Uncompetitive Inhibition: Lineweaver-Burke Plot



Mixed inhibition





Mixed inhibition is when the inhibitor binds to the enzyme at a location distinct from the substrate binding site. The binding of the inhibitor will either alter the K_M or V_{max} or both.

$$K_I = \frac{[E][I]}{[EI]} \qquad K_I' = \frac{[ES][I]}{[ESI]}$$

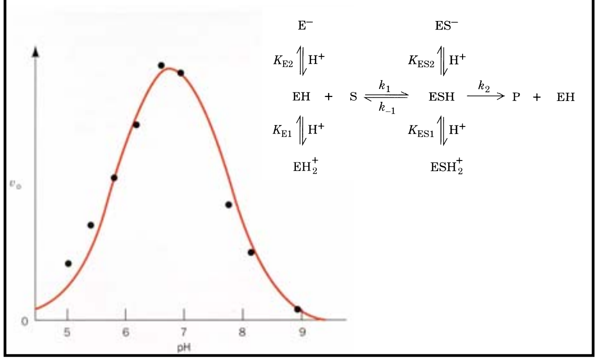
$$v_o = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]} \qquad \alpha' = \left(1 + \frac{[I]}{K_I'}\right)$$

TABLE 13-2. The Effects of Inhibitors on the Parameters of the Michaelis–Menten Equation[a]

Type of Inhibition	V_{max}^{app}	K_M^{app}
None	V_{max}	K_M
Competitive	V_{max}	αK_M
Uncompetitive	V_{max}/α'	K_M/α'
Mixed	V_{max}/α'	$\alpha K_M/\alpha'$

[a] $\alpha = 1 + \frac{[I]}{K_I}$ and $\alpha' = 1 + \frac{[I]}{K_I'}$

The effect of pH on kinetic parameters



The Michaelis–Menten equation for this model, which is derived in Appendix D, is

$$v_o = \frac{V'_{max}[S]}{K'_M + [S]} \qquad [13.47]$$

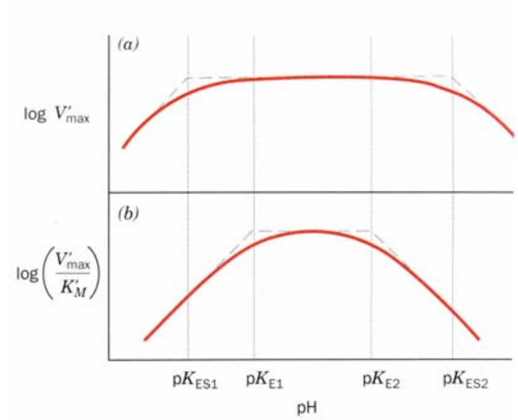
Here the apparent Michaelis–Menten parameters are defined

$$V'_{max} = V_{max}/f_2 \quad \text{and} \quad K'_M = K_M(f_1/f_2)$$

where

$$f_1 = \frac{[H^+]}{K_{E1}} + 1 + \frac{K_{E2}}{[H^+]}$$

$$f_2 = \frac{[H^+]}{K_{ES1}} + 1 + \frac{K_{ES2}}{[H^+]}$$



Lecture 17
Tuesday 10/21/08
Enzyme Kinetics / Exam II Minireview