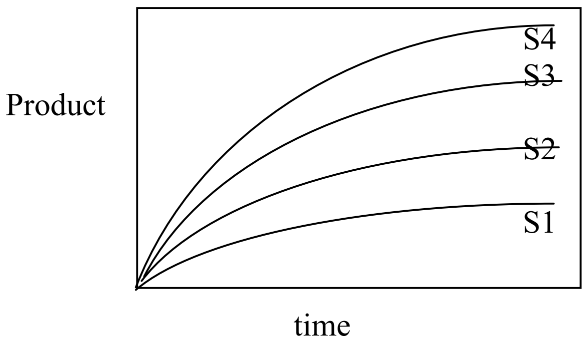
Lecture 10

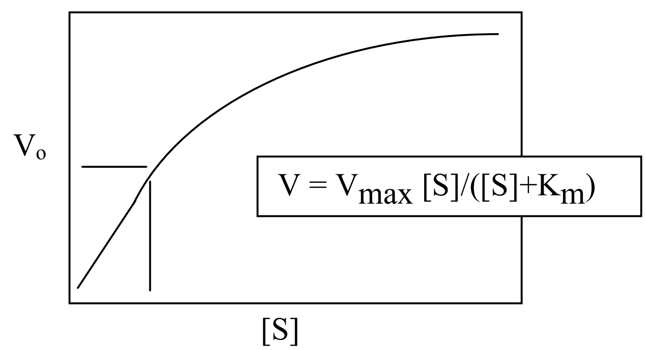
Enzyme inhibition kinetics

Review getting and analyzing data:

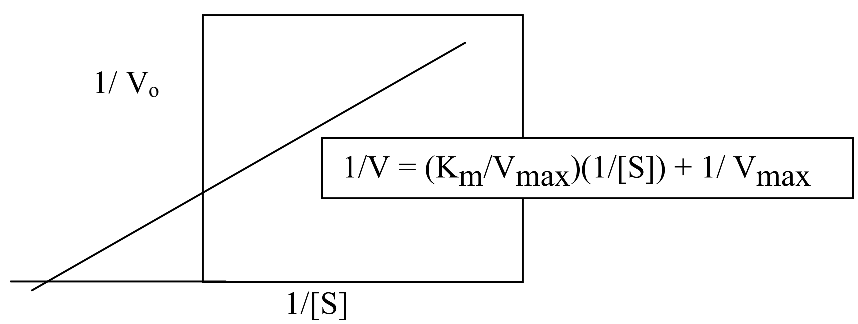
Product vs time for increasing substrate concentrations



Initial velocity vs substrate conc.



Lineweaver-Burke:



Inhibition

Issue: changing the rate of enzyme activity in the cell (why?)

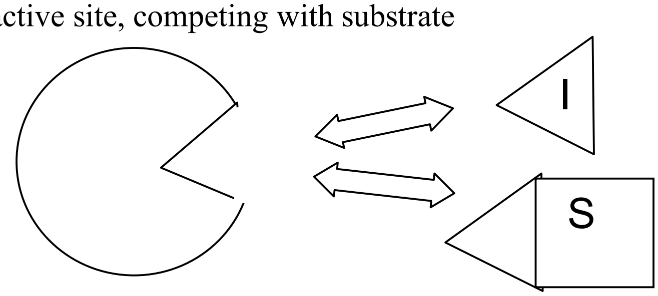
- Understand normal control of enzyme activity
- Analogs for crystalography
- Inhibitory drugs

Reversible inhibition: different types of mechanisms distinguishable by kinetics

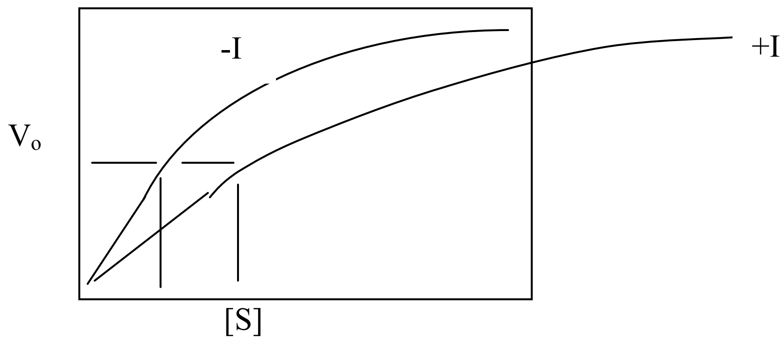
- competitive
- non-competitive
- uncompetitive

Competitive inhibition

Inhibitor binds to the active site, competing with substrate



For a fixed concentration of inhibitor and increasing substrate, expect the maximum to be the same, K_m to increase



Equations:

$E + S \rightleftharpoons ES \rightarrow E + P$ $\qquad K_m \sim [E][S]/[ES]$

$E + I \rightleftharpoons EI$ $\qquad K_I = [E][I]/[EI]$

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

See p. 399 (G and G) for derivation of modified Michaelis-Menten equation:

$V = V_{max}[S]/([S] + K_m (1 + [I]/K_I))$ $\qquad$ define $K_{m, apparent} = K_m (1 + [I]/K_I)$

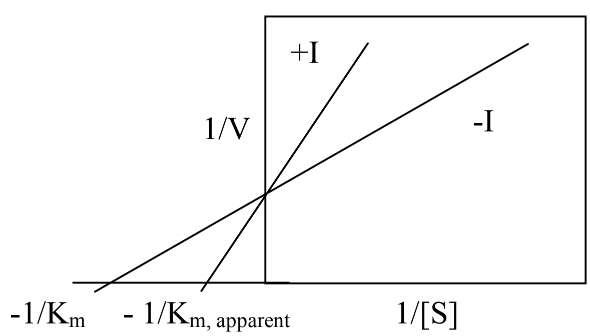
Note the effect of $1+[I]/K_I$ on K_m:

as [I] increases, $K_{m, apparent} = K_m (1 + [I]/K_I)$ increases; at $[I] = K_I$, $K_{m, apparent} = 2 \times K_m$
(reduced "affinity" for S)
as [S] increases, $[S] >> K_m (1 + [I]/K_I)$, and $V \rightarrow V_{max}$

Lineweaver-Burke formulation: again replace K_m with $K_m (1 + [I]/K_I)$

$1/V = \{K_m (1 + [I]/K_I)/V_{max}\}(1/[S]) + 1/V_{max}$

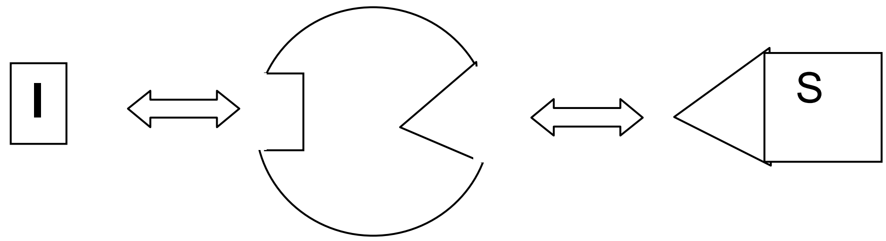
as [I] increases, slope increases but the y intercept is unchanged



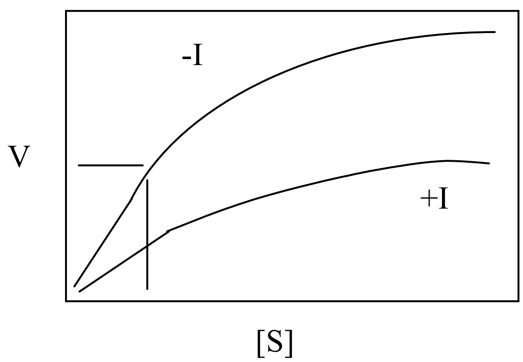
How calculate K_I? If $K_{m, apparent} = K_m (1 + [I]/K_I)$, then
$K_I = K_m [I]/(K_{m,apparent} - K_m)$

Non-competitive inhibition

Inhibitor and substrate bind to different sites



Expect a lower V_{max}, the same K_m

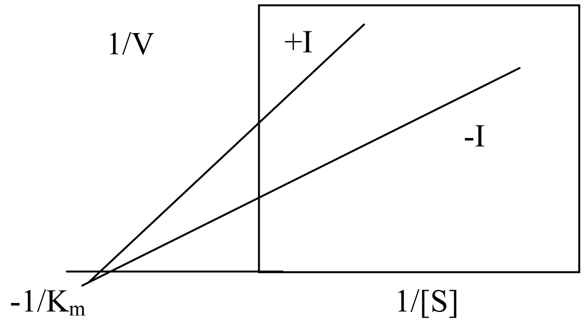


$E + S \rightleftharpoons ES \rightarrow E + P$
$E + I \rightleftharpoons EI$
$EI + S \rightleftharpoons EIS$
$ES + I \rightleftharpoons EIS$

$K_m \sim [E][S]/[ES]$; $K_i = [E][I]/[EI] = [ES][I]/[ESI]$

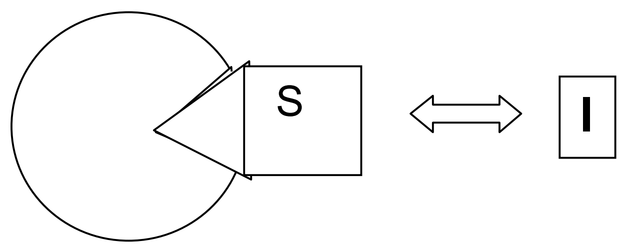
$V = \frac{(V_{max}/(1 + [I]/K_i))[S]}{([S] + K_m)}$ define $V_{max,\ apparent} = V_{max}/(1 + [I]/K_I)$

as [I] increases, $V_{max,\ apparent} = V_{max}/(1 + [I]/K_I)$ decreases; at $[I] = K_I$, $V_{max,\ apparent} = ½\ V_{max}$



<u>Uncompetitive inhibition</u>

Inhibitor binds only to ES



$E + S \rightleftharpoons ES \rightarrow E + P$
$ES + I \rightleftharpoons EIS$

Expect (?) a lower V_{max} and a lower K_m (!)

$$V = \frac{(V_{max}/(1 + [I]/K_i))[S]}{(K_m/(1 + [I]/K_i)) + [S]}$$

$V_{max,\ apparent} = V_{max}/(1 + [I]/K_i)$; $K_{m,\ apparent} = K_m/(1 + [I]/K_i)$