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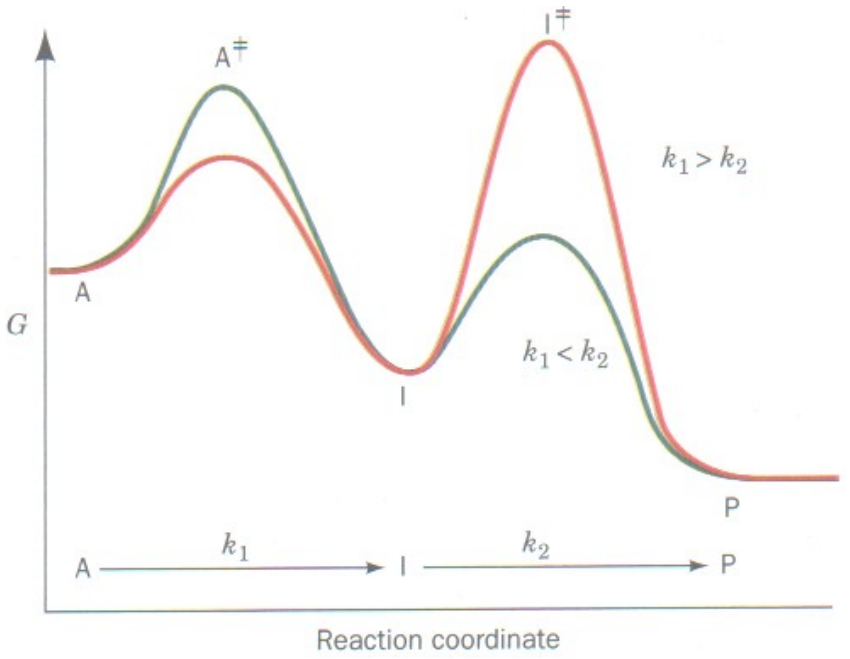
Chapter 14: Rates of Enyzmatic Reactions

Voet & Voet: Pages 472-493



Initial Velocity

Steady State assumption: Allows reaction rate equation (in term of [ES]) to be rewritten in terms that are experimentally measurable

$$0 = d\frac{[ES]}{dt} = k_1[E_T - E][S] - k_{-1}[ES] - k_2[ES]$$

reaction rate is generally measured for a short duration after reaction start (initial velocity, v_o)

$$v = d\frac{[P]}{dt} = k_2[ES] = k_2\frac{([E_T][S])}{(K_M + [S])}$$

ie. before significant amounts of substrate (10%) have been converted to product

Minimizes possible back reactions, product inhibition and other phenomenon

The maximum velocity of the enzyme catalyzed reaction occurs when $[E_{total}] = [ES]$

$$V_{max} = k_2[E_T]$$

Result is the Michaelis-Menten equation; the basic equation of enzymology

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

K_M – Michaelis constant

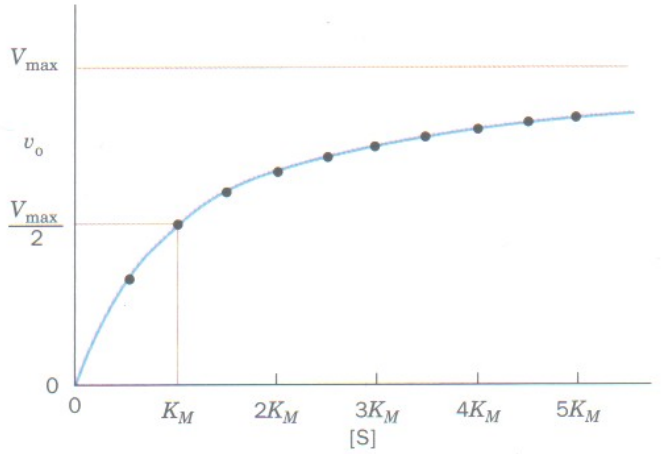
Plots of initial velocity vs. substrate concentration yield hyperbolic curves for Michaelis-Menten reactions

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

K_M is the substrate concentration at which the reaction velocity is ½ maximal

Proof: when K_M = [S], the Michaelis-Menten equation reduces to $v_o = V_{max}/2$

small K_M is associated with efficient catalysis at low [S]



For $k_2 <<< k_{-1}$, K_M is a measure of substrate affinity

Restatement of equilibrium assumption

Analysis of Kinetic Data

Several methods exist for determining the kinetic constants in the Michaelis-Menten equation

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

(1) v_o vs [S] plots require extrapolation of V_{max}

(2) Lineweaver-Burk (double reciprocal) plot: $1/v_o$ vs $1/[S]$

$$\frac{1}{v_o} = \frac{(K_M + [S])}{(V_{max}[S])}$$

Better estimate of V_{max} ... but most measurements are near the y axis and small errors can skew calculation of constants

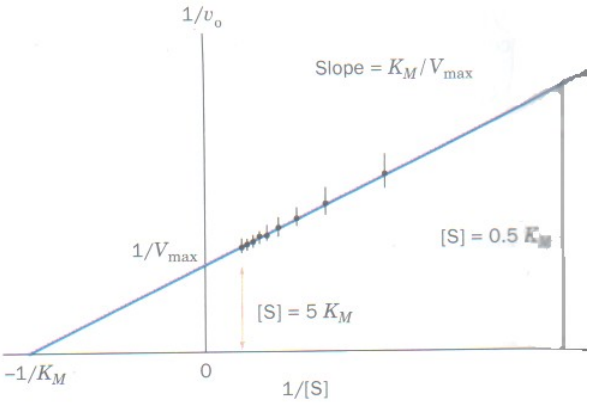
(3) Eadie-Hofstee (double reciprocal × V_{max}) plot: v_o vs $v_o/[S]$

$$\frac{1}{v_o} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

Improved version of Lineweaver-Burk that is less sensitive to small errors

(4) Regression methods and initial velocity plots (Best)

Computational methods overcome extrapolation problem



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Modified Michaelis Menten

Michaelis-Menten equation can be modified to consider the back reaction from product to substrate

- **This treats [ES] as a reaction intermediate and may be considered a one intermediate Michaelis-Menten equation**

$$E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \underset{k_{-2}}{\overset{k_2}{\Leftrightarrow}} E + P$$

Much more complicated but still contains terms that are measurable and solvable

- **$V_{f,max}$ and $V_{r,max}$ are the maximal velocities of the forward and backward reaction**
- **$K_{f,s}$ and $K_{r,p}$ are the Michaelis constants for the forward and backward reaction**

$$v = \frac{\left(\frac{(V_{(f,max)}[S])}{K_{(s,M)}} - \frac{(V_{(r,max)}[P])}{K_{(p,M)}}\right)}{\left(1 + \frac{[S]}{K_{(s,M)}} + \frac{[P]}{K_{(p,M)}}\right)}$$

Not responsible for deriving or reproducing equation but must recognize various symbols

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Haldane Relationship

For modified Michaelis-Menten equations that consider back reactions, the Haldane Relationship can be utilized to determine K_{eq} and (sometimes) other kinetic constants

- **at equilibria, v = 0 and the rates of formation and breakdown of product are constant**
- **modified Michaelis-Menten equation is still valid at equilibria (v = 0) and can be reduced to the Haldane Relationship**

Haldane Relation shows the kinetic parameters for an enzyme catalyzed reversible reaction are related by the equilibrium constant

$$K_{eq} = \frac{[P]_{eq}}{[S]_{eq}} = \frac{(V_{(max,f)} K_{(M,p)})}{(V_{(max,r)} K_{(M,s)})}$$

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Steady State Limitations

Steady-State (modified Michaelis-Menten) Kinetic Analysis of a reaction cannot unambiguously establish its mechanism

- **Can experimentally determine up to 4 kinetic parameters (V_{max} and K_M for forward and reverse reaction)**

Modified Michaelis-Menten equations with 2 or more reaction intermediates have the same form (but more kinetic parameters) as the one intermediate case

- **Cannot determine these addition terms using steady state kinetic approaches as we only have 4 known quantities**

In general, Steady State approaches can only monitor reaction steps that consume or produce the substrate or product

- **Kinetic data not compatible with a given mechanism can be used to reject that mechanism**

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Inhibition

Inhibitors are compounds that bind to enzyme in such a way that the activity of the enzyme is reduced

(1) many inhibitors are substances that resemble the enzyme's natural substrate but react slowly or not at all

(2) inhibitors are commonly used to investigate the nature of the substrate-binding site and to elucidate enzyme mechanism

(3) Most clinical drugs are enzyme inhibitors produced synthetically or isolated from natural sources

recently the first rationally 'designed' drugs have been utilized

Large portion of pharmacology is medicinal use of inhibitors

must consider many issues over and above binding

dosage, solubility, side effects, cost, *etc*

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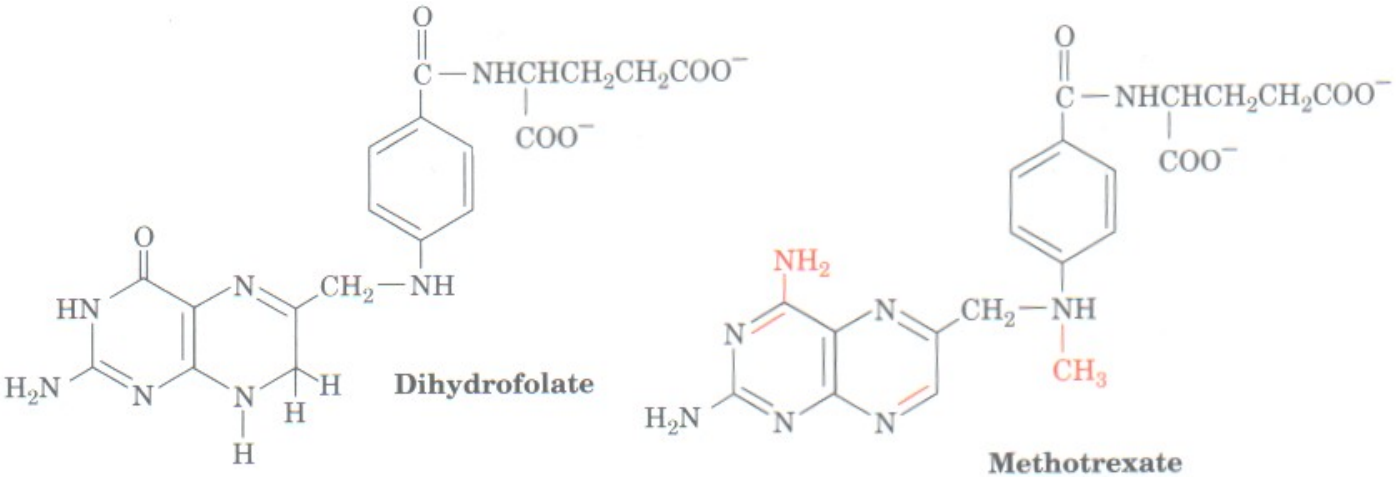
Example: methotrexate

Enzyme inhibitor used as chemotherapeutic agent

- dihydrofolate reductase catalyzes an essential step in the synthesis of dTMP
- methotrexate binds to and inhibits dihydrofolate reductase preventing dTMP biosynthesis
  - <u>preferentially</u> kills actively dividing cells (*eg* cancer cells)

Methotrexate is structurally related to the normal substrate and binds in the enzyme active site in a conformation similar but not identical to the substrate



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Types of Inhibition

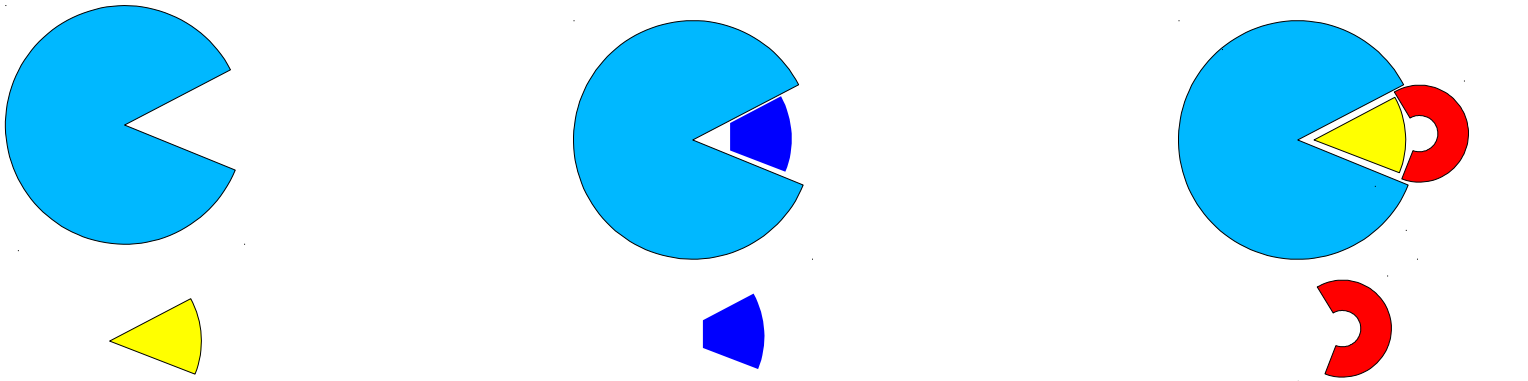
Enzyme inhibitors have a variety of mechanisms

Consider 3 simple inhibitor mechanisms

(1) Competitive inhibition – inhibitor competes with normal substrate for enzymatic binding site

(2) Uncompetitive inhibition – inhibitor only binds to the enzyme substrate complex

(3) Mixed inhibition – inhibitor binds both to the substrate binding site of the enzyme and to the enzyme substrate complex (both competitive and uncompetitive)



substrate

competitive inhibitor

uncompetitive inhibitor

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Competitive Inhibition

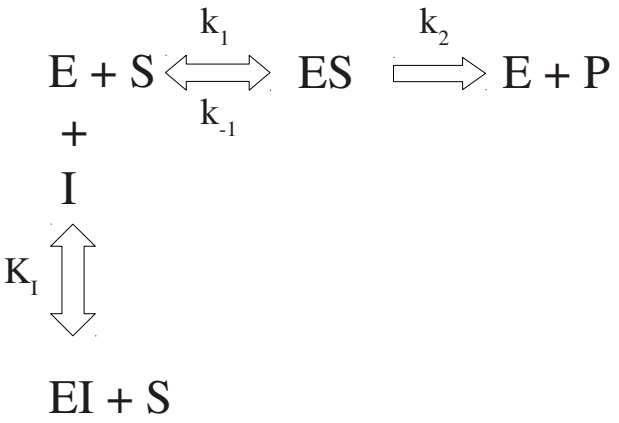
(1) Competitive inhibition – inhibitor competes with normal substrate for enzymatic binding site

Inhibitor structurally resembles the substrate

General chemical reaction for competitive inhibition

assumes inhibitor (I) binds reversibly and is in rapid equilibrium so that K_I = [E][I]/[EI]

assumes EI is inactive (no reaction)



Competitive inhibitors reduce the [E] available for substrate binding

Competitive Inhibition equation

Michaelis-Menten equation must reflect

$E_T = E + ES + EI$

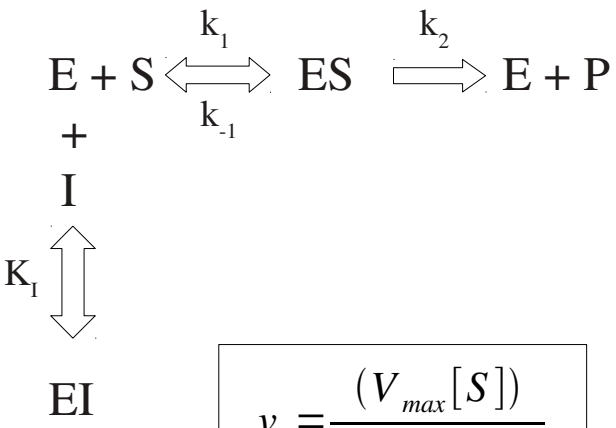
Derivation requires developing expressions for [E] and [EI]

Ultimately yields a Michaelis-Menten equation with the K_M modulated by a factor, α

α, depends upon the ratio of [I] and K_I

inhibition affects K_M by reducing the concentration of the enzyme [E]

inhibition does not affect V_{max} as it can be reversed a higher substrate concentrations



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

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

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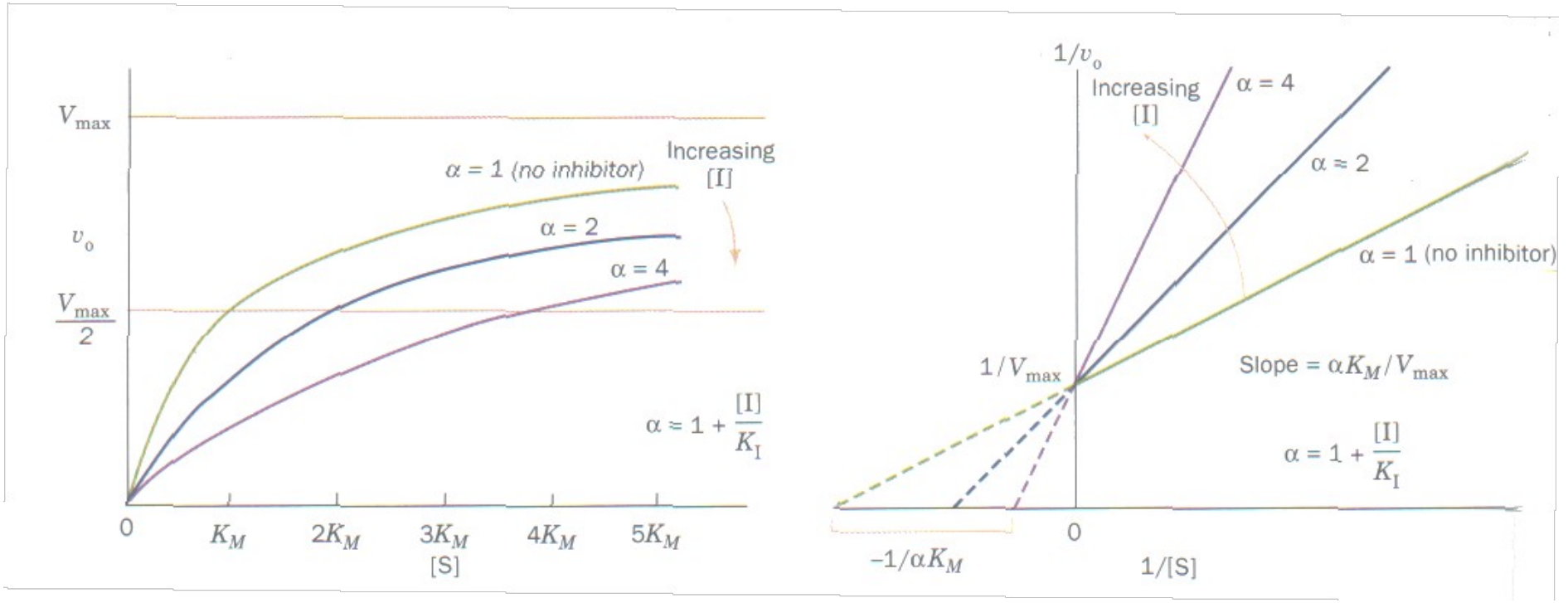
Competitive Inhibition plot

Initial reaction rates and double-reciprocal plots for a reaction in the presence of varying [I]

- **increases the K_M and causes K_M dependent parameters (slope and x-intercept) to vary accordingly**
- **does not affect V_{max} dependent y-intercept**

$$\frac{1}{v_o} = (\alpha \frac{K_M}{V_{max}})(\frac{1}{[S]}) + \frac{1}{V_{max}}$$



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Uncompetitive Inhibition

(2) Uncompetitive inhibition – inhibitor binds to the enzyme-substrate complex and not the free enzyme

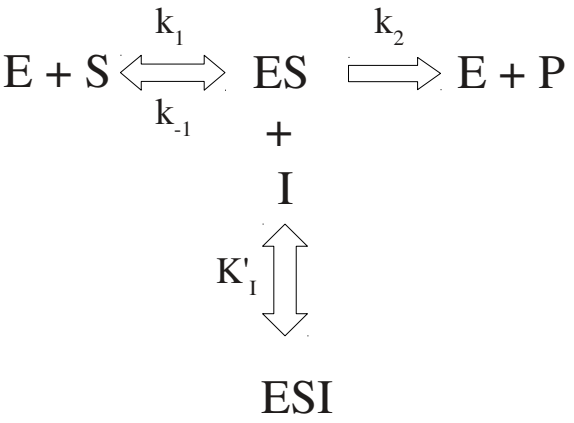
Uncompetitive inhibitors bind and change the active site conformation in such a way the reaction cannot be completed

General chemical reaction for uncompetitive inhibition

assumes inhibitor (I) binds reversibly and is in rapid equilibrium so that $K'_I = [ES][I]/[ESI]$

assumes ESI is inactive (no reaction)

Uncompetitive inhibitors reduce the [ES] available for conversion to product and thus affect V_{max}



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

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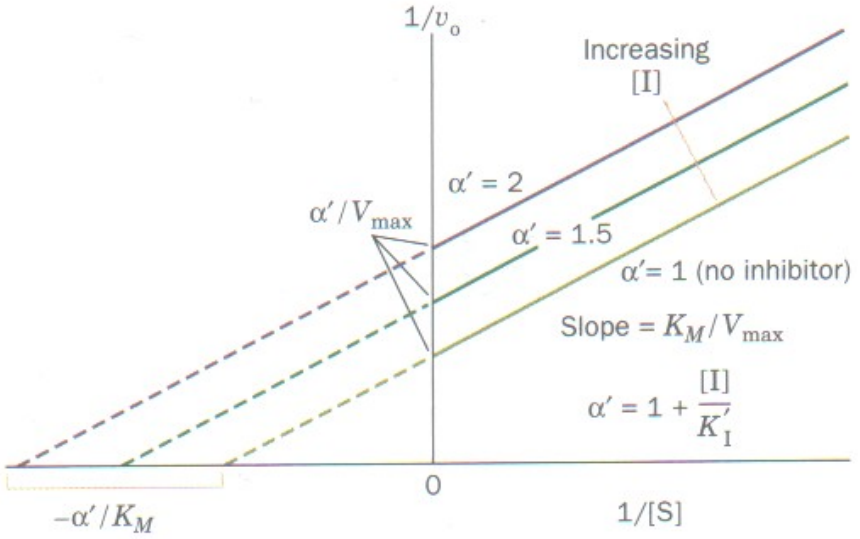
Uncompetitive Inhibition plot

Double-reciprocal plots for a reaction in the presence of varying [I]

- **affects intercept terms**
- **does not affect slope term (K_M /V_{max})**

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



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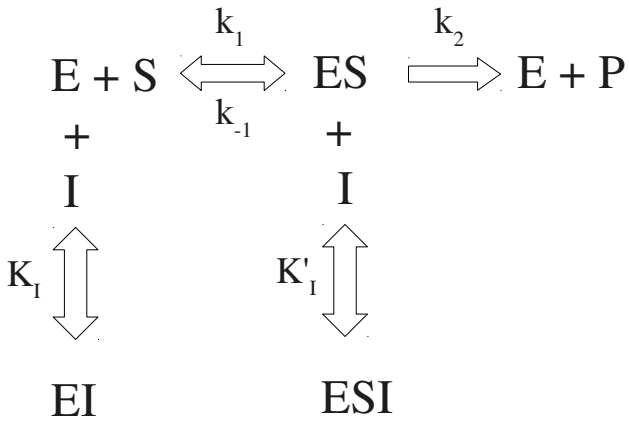
Mixed Inhibition

(3) Mixed inhibition – inhibitor binds to the free enzyme and the enzyme-substrate complex

Mixed inhibitors bind sites that participate in both substrate binding and catalysis

General chemical reaction for mixed inhibition



assumes inhibitor binding is in equilibrium and each step has its own dissociation constant

K_I = [E][I]/[EI]

K'_I = [ES][I]/[ESI]

Mixed inhibitors have properties of both competitive and uncompetitive inhibitors

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

$$\alpha = (1 + \frac{[I]}{K_I}) \qquad \alpha' = (1 + \frac{[I]}{(K_I')})$$

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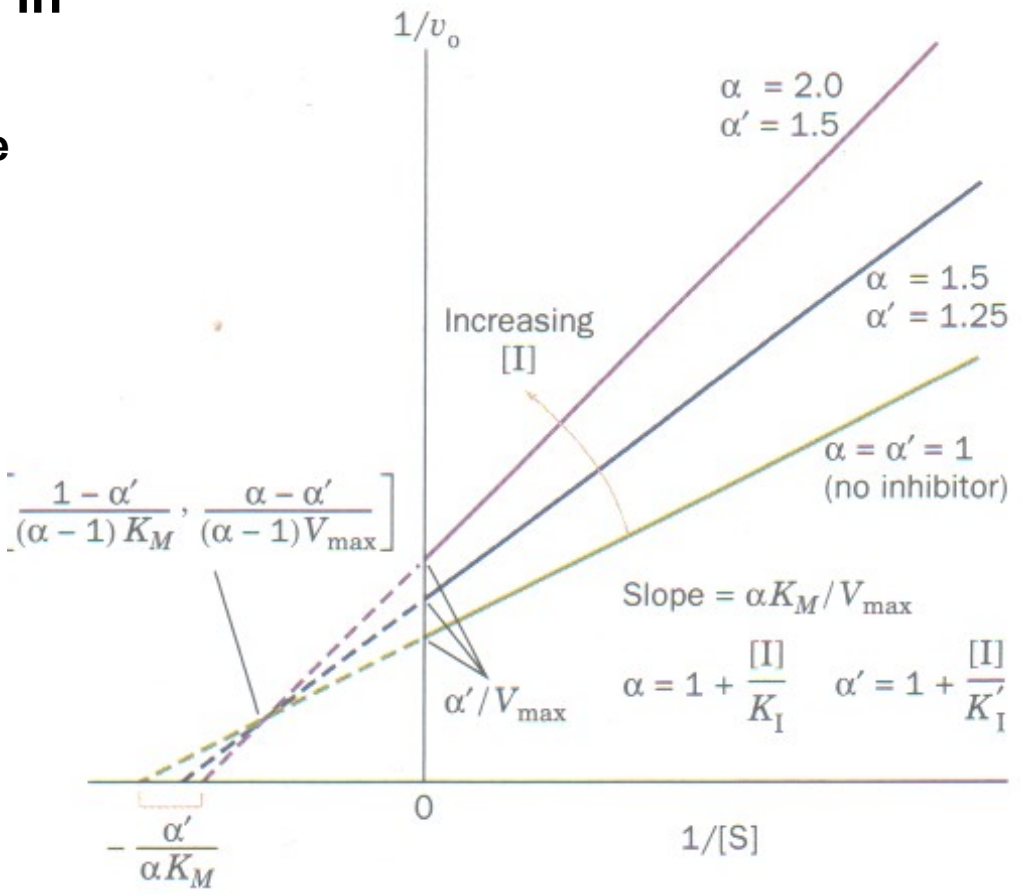
Mixed Inhibition Plots

Double-reciprocal plots for a reaction in the presence of varying [I]

- **affects both intercepts like uncompetitive**
- **affects slope like competitive**

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



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Summary of Inhibition

The values of K_M and V_{max} measured in the presence of inhibitor are referred to as the apparent K_M (K_m^{app}) and the apparent V_{max} (V_{max}^{app})

This would represent the case where you have not accounted for the inhibitor in your initial rate or double-reciprocal plot

The effects of inhibitors on K_m^{app} and the apparent V_{max}^{app} can be used to identify the type of inhibition

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

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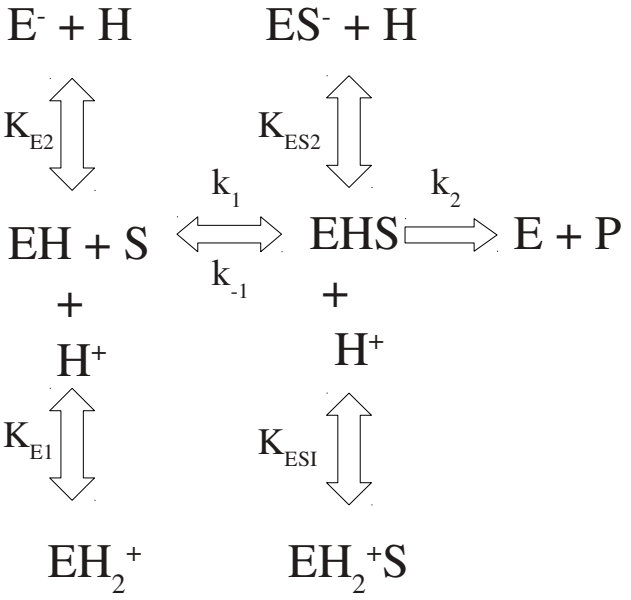
Effects of pH

Enzymes are pH sensitive and are usually only active in a narrow pH range (usually 5-9) due to:

- **(1) pH sensitivity of substrate binding**
- **(2) reduced catalytic efficiency of the enzyme**
- **(3) ionization of substrate**
- **(4) protein structural changes (usually at pH extremes)**

Kinetic analysis as a function of pH provides information about the nature of functionally (catalytically) important residues

- **general chemical equation for enzyme with two ionizable groups**
- **assumes only EH and ESH are active**



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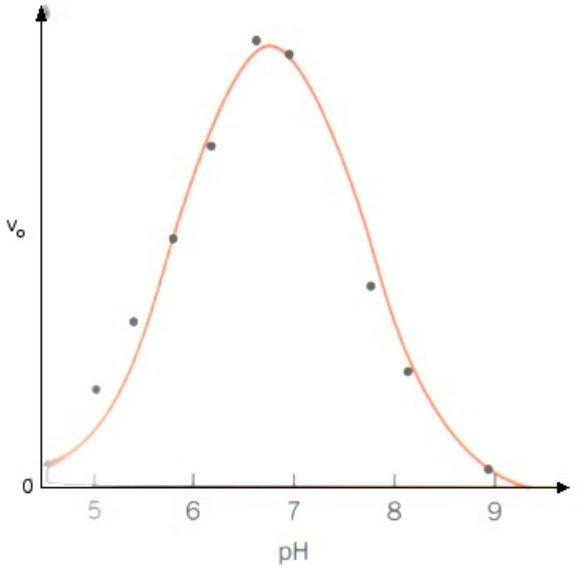
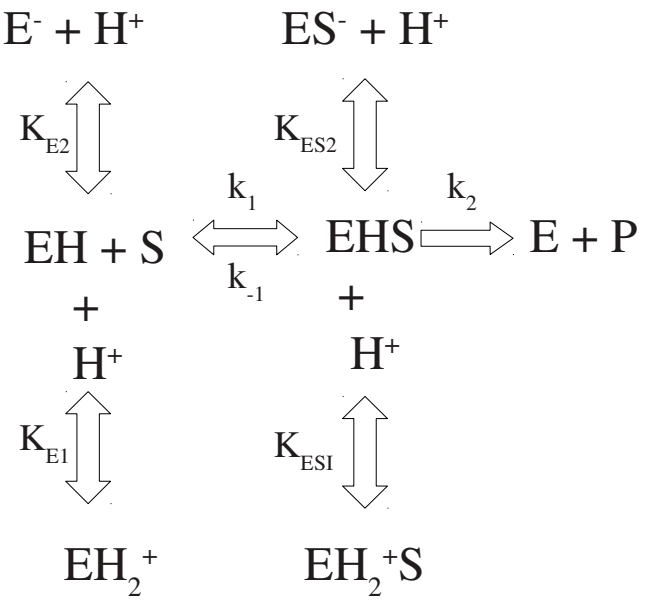
Kinetic equations and pH

At each pH, the enzyme behaves according to the simple Michaelis-Menten equation

- **the pH dependence of f_1 and f_2 causes v_o to vary in a bell-shaped manner**
- **f_1 and f_2 modulate the amount of EH and EHS at a given pH**





$$v_o = \frac{(V_{max}'[S])}{(K_M' + [S])}$$

$$K_M' = K_M\left(\frac{f_1}{f_2}\right) \qquad V_{max}' = \frac{V_{max}}{f_2}$$

$$f_1 = \frac{[H^{+1}]}{K_{E1}} + 1 + \frac{K_{E2}}{[H^{+1}]} \qquad f_2 = \frac{[H^{+1}]}{K_{ES1}} + 1 + \frac{K_{ES2}}{[H^{+1}]}$$

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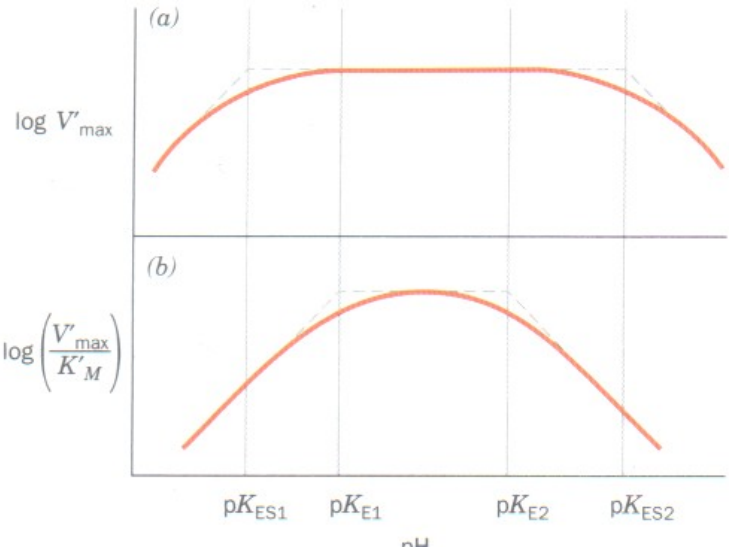
Evaluation of Ionization constants

The ionization constants in our pH dependent general chemical reaction can be determined by plotting log V'_{max} vs pH and log (V'_{max} /K'_M) vs pH

- log V'_{max} vs pH provides information about K_{ES1} and K_{ES2} or the final step of the reaction
- log (V'_{max} /K'_M) vs pH provides information about K_{E1} and K_{E2} or the first step of the reaction

Knowing the ionization constant of a catalytic residue gives a clue to its identity

- a pK_{E1} of 4 would suggest a carboxylate group such as an Asp or Glu contains
- the experimentally determined pKs may deviate considerably from the pK_a of equivalent free amino acids due to the local environment



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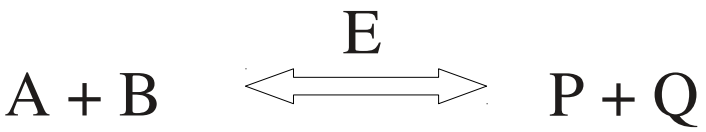
Bisubstrate Reactions

~60 % of all enzymatic reaction are bisubstrate reactions that require two substrates and yield two products



Almost all bisubstrate reactions fall into two groups:

- ***transferase reactions*** **– one functional group is transferred between the substrates**
- ***redox reactions*** **– reducing equivalents are transferred between the substrates**

While there are many possible bisubstrate reaction mechanism, only a few types commonly occur

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Reaction Terminology

Clelland Nomenclature

Substrates are A, B, C, D *in the order they add to the enzyme*

Products are P, Q, R, S, T *in the order they leave the enzyme*

Stable **enzyme forms** are E, F, G

stable enzyme forms are defined as those incapable of converting to another stable form by itself

Number of reactants and products in a given reaction are specified by the terms *uni*, *bi, ter* and *quad*

eg. Reaction with 1 substrate and two products - Uni Bi reaction

Bisubstrate reactions with two products - Bi Bi reaction

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Bisubstrate Reaction Types

Two major mechanism for bisubstrate reactions

(1) Sequential Reactions – all substrates must combine with the enzyme before the reaction can occur and products can be released

Single step conversion of substrate to product gives rise to alternate name, single displacement reaction

Subdivided into Ordered and Random

- **Ordered Sequential mechanism require each substrate to bind and each product to be released in particular order**
- **Random Sequential mechanism allow substrate to bind and product to be released in any order**

(2) Ping-Pong Reactions – one or more products are released before all substrates have combined with the enzyme

- **also referred to as double displacement reaction**

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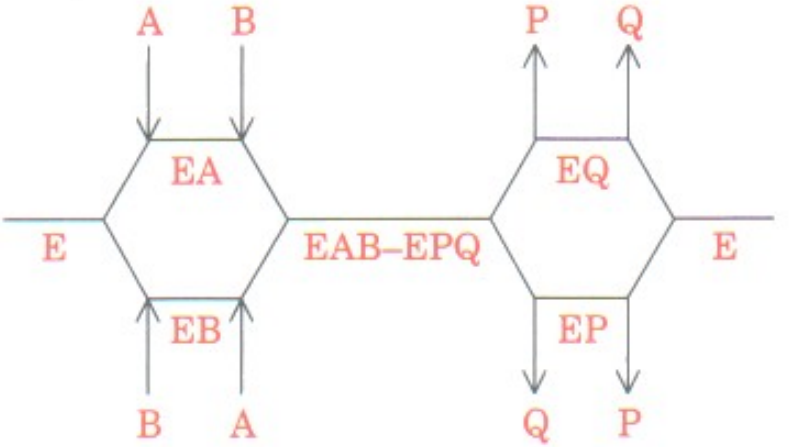
Bisubstrate Reaction Types

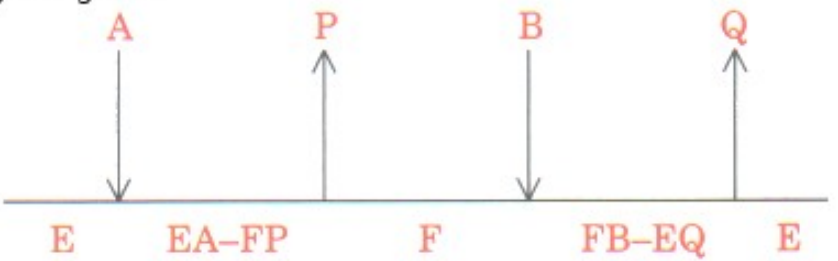
Shorthand notation for various reaction types (Clelland)

Ordered Sequential Bi Bi

Random Sequential Bi Bi



Ping Pong Bi Bi



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Bisubstrate Reaction Rates

As one would expect, the various bisubstrate mechanism can be distinguished using initial rates kinetics

somewhat more complex for bisubstrate than unisubstrate

Ordered Bi Bi Reaction rate equation

$$\frac{1}{v_o} = \frac{1}{V_{max}} + \frac{K_M^A}{(V_{max}[A])} + \frac{K_M^B}{(V_{max}[B])} + \frac{(K_M^A K_M^B)}{(V_{max}[A][B])}$$

Ping-Pong Reaction rate equation

$$\frac{1}{v_o} = \frac{K_M^A}{(V_{max}[A])} + \frac{K_M^B}{(V_{max}[B])} + \frac{1}{V_{max}}$$

where K^A_M and K^B_M are the [A] and [B] required for half maximal activity.

V_{max} is maximal velocity when both A and B are saturating