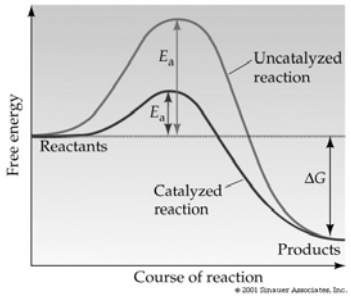
Proteins Act As Catalysts



Properties of Enzymes

- **Catalyst** - speeds up attainment of reaction equilibrium
- **Enzymatic reactions** - 10^3 to 10^{17} faster than the corresponding uncatalyzed reactions
- **Substrates** - highly specific reactants for enzymes

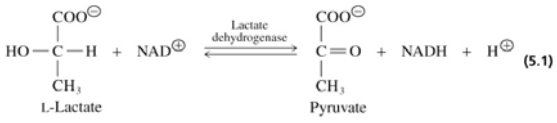
Properties of enzymes (continued)

- **Stereospecificity** - many enzymes act upon only one stereoisomer of a substrate
- **Reaction specificity** - enzyme product yields are essentially 100% (there is no formation of wasteful byproducts)
- **Active site** - where enzyme reactions take place

The Six Classes of Enzymes

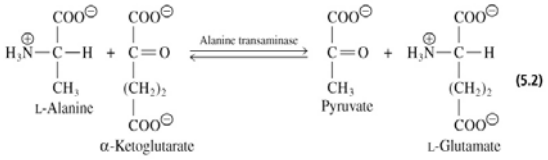
1. Oxidoreductases (**dehydrogenases**)

- Catalyze oxidation-reduction reactions



2. Transferases

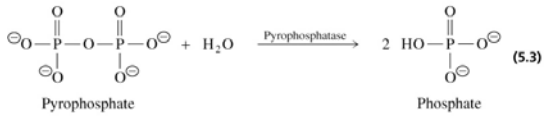
• Catalyze group transfer reactions



(5.2)

3. Hydrolases

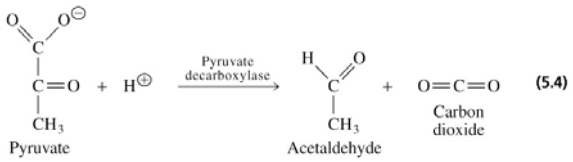
• Catalyze hydrolysis reactions where water is the acceptor of the transferred group



(5.3)

4. Lyases

• Catalyze lysis of a substrate, generating a double bond in a nonhydrolytic, nonoxidative elimination (**Synthases** catalyze the addition to a double bond, the reverse reaction of a lyase)



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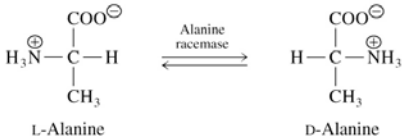
5. Isomerases

• Catalyze isomerization reactions



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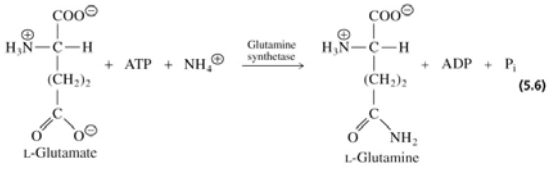
6. Ligases (synthetases)

- Catalyze ligation, or joining of two substrates
- Require chemical energy (e.g. ATP)



What is Rate?

How fast <u>something</u> happens

The units are the change of your "something" divided by change in time

.For example: the rate of traveling in a car

$$\text{Rate} = \frac{\Delta \text{ miles}}{\Delta \text{ time}}$$

What are the units of rate when driving a car?

<u>Chemical Kinetics</u> is similar except the "change in something" is change in concentration over change in time.

$$\text{Rate} = \frac{\Delta\,[\text{concentration}]}{\Delta\ \text{time}}$$

Another way to write it is……

$$\text{Rate} = \frac{d[\text{concentration}]}{dt}$$

**** remember if the concentration are reactants, there is a negative sign in front of the change in concentration to make overall rate positive.**

Effect of Concentration on Reaction Rates

The best way to describe how concentration of reactants affect rates is to use a rate law

Consider the reaction:

Rate constant $\quad A + B \rightarrow C$

$$\text{Rate} = k[A]^x[B]^y$$

x and y are NOT necessarily from the stoichiometry in the reaction

Consider a Zero Order Reaction

A ⟶ B

Rate = -d[A]/dt **Rate = k**

-d[A]/dt = k

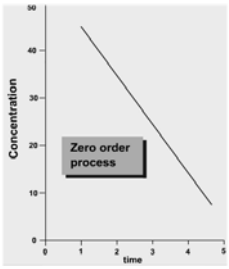
Separate variables

d[A] = -kt

Take the integral of both sides

Zero Order Reaction

$[A] = [A]_0 - kt$



Consider a First Order Reaction

A ⟶ B

Rate = -d[A]/dt **Rate = k[A]**

-d[A]/dt = k[A]

Separate variables

d[A]/[A] = -kt

Take the integral of both sides

First Order Reaction

A ⟶ B

$\ln [A] = \ln [A]_0 - kt$

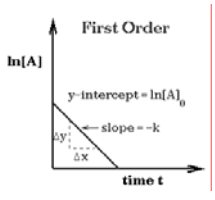
$$-\frac{dA}{dt} = k_1 A$$

$$\int_{A_0}^{A} \frac{dA}{A} = -k_1 \int_0^t dt$$

$$\ln A\Big|_{A_0}^{A} = -k_1 t\Big|_0^t$$

$\ln A - \ln A_0 = -k_1 t$ which gives

2. $\ln A = \ln A_0 - k_1 t$ or $A = A_0 e^{-k_1 t}$



Consider a Second Order Reaction

$A^2 \longrightarrow B$

Rate = -d[A]/dt **Rate = k[A]^2**

-d[A]/dt = k[A]^2

Separate variables

d[A]/[A]^2 = -kt

Take the integral of both sides

Second Order Reaction

$A^2 \longrightarrow B$

1/[A] = 1/[A]$_o$ + kt

$$-\frac{dA}{dt} = k_2 A^2$$

$$\int_{A_0}^{A} \frac{dA}{A^2} = \int_{A_0}^{A} A^{-2} dA = -k_2 \int_0^t dt$$

$$\left.\frac{A^{n+1}}{n+1}\right|_{A_0}^{A} = \left.\frac{A^{-1}}{-1}\right|_{A_0}^{A} = -k_2 t \Big|_0^t$$

$$-\frac{1}{A} - \left(-\frac{1}{A_0}\right) = -k_2 t$$

$$\frac{1}{A} = \frac{1}{A_0} + k_2 t$$

Chemical Kinetics

- Experiments examine the amount of **product** (**P**) formed per unit of time (**Δ[P] / Δ t**)
- **Velocity** (***v***) - the rate of a reaction (varies with reactant concentration)
- **Rate constant** (***k***) - indicates the speed or efficiency of a reaction

First order rate equation

- Rate for <u>nonenzymatic</u> conversion of substrate (S) to product (P) in a first order reaction: (*k* is expressed in reciprocal time units (s^{-1}))

d[P] / dt = *v* = *k*[S]

Second order reaction

- For reactions: $S_1 + S_2 \longrightarrow P_1 + P_2$
- Rate is determined by the concentration of both substrates
- Rate equation: $v = k[S_1]^1[S_2]^1$

Pseudo first order reaction

- If the concentration of one reactant is so high that it remains essentially constant, reaction becomes zero order with respect to that reactant
- Overall reaction is then pseudo first-order

$$v = k[S_1]^1[S_2]^0 = k'[S_1]^1$$

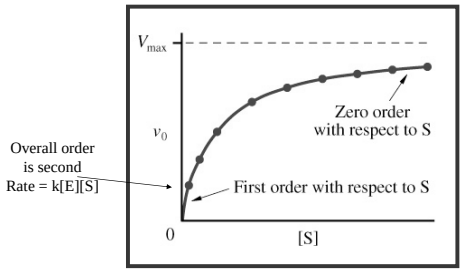
Enzyme Kinetics

- **Enzyme-substrate complex** (**ES**) - complex formed when specific substrates fit into the enzyme active site

$$E + S \longrightarrow ES \longrightarrow E + P$$

- When [S] >> [E], every enzyme binds a molecule of substrate (enzyme is **saturated** with substrate)
- Under these conditions the rate depends only upon [E], and the reaction is **pseudo-first order**

Rate Data for a Typical Enzyme Catalyzed Reaction



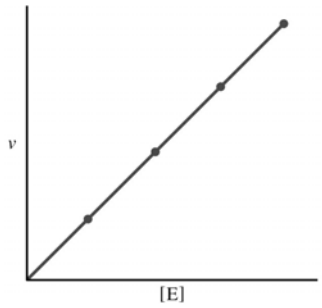
Saturation kinetics – when the [S] gets high, the rate no longer depends on [S]

Observables

- **Maximum velocity (V_{max})** is reached when an enzyme is saturated with substrate (high [S])
- At high [S] the reaction rate is independent of [S] (zero order with respect to S)
- At low [S] reaction is first order with respect to S
- The shape of a v_o versus [S] curve is a rectangular hyperbola, indicating saturation of the enzyme active site as [S] increases

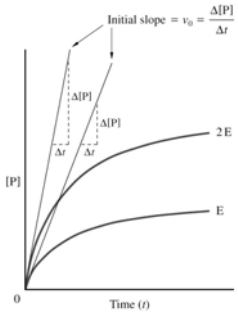
Effect of enzyme concentration [E] on velocity (v)

- Fixed, saturating [S]
- Pseudo-first order enzyme-catalyzed reaction

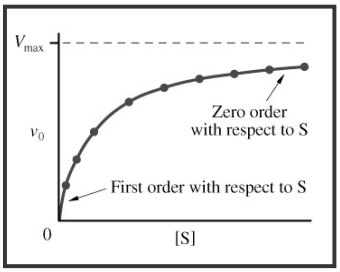


Progress curve for an enzyme-catalyzed reaction

- The initial velocity (v_o) is the slope of the initial linear portion of the curve
- Rate of the reaction doubles when twice as much enzyme is used



How Do You Explain the Data?



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

- k_1 and k_{-1} represent rapid noncovalent association /dissociation of substrate from enzyme active site
- k_2 = rate constant for formation of product from ES

Which of these reactions is the rate-determining step?

Rate = $k_1[E][S]$ Rate = $k_{-1}[ES]$

Rate = $k_2[ES]$

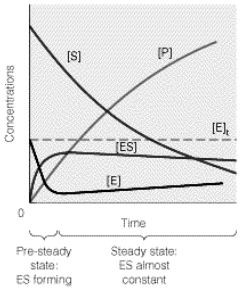
Because it is a chemical change instead of nonconvalent interaction like substrate binding

Since formation of product is the rate determining step, there is a period of time that the rate of formation of ES equals the rate of decomposition

This is called Steady State Approximation

[ES] = constant

Rate of ES formation = Rate of ES decomposition



What is the Rate Law for the Formation of ES?

Rate = $k_1[E][S]$

(the back reaction of E + P is negligible)

What is the Rate Law for the Decomposition of ES?

Rate = $k_{-1}[ES] + k_2[ES]$

Therefore……

$$k_1[E][S] = k_{-1}[ES] + k_2[ES]$$

[E] is free enzyme which is equal to E_T –ES

Substitute in

$$k_1(E_T\text{-}ES)[S] = k_{-1}[ES] + k_2[ES]$$

Collect all the rate constants to one side

$$\underbrace{\frac{k_{-1} + k_2}{k_1}}_{K_M} = \frac{(E_T - [ES])[S]}{[ES]}$$

K_M **Michaelis Constant**

$$K_M = \frac{(E_T - [ES])[S]}{[ES]}$$

Solve for ES

$$[ES] = \frac{E_T\,[S]}{K_M + [S]}$$

Express [ES] in terms that we can measure

Remember the rate determining step: $v = k_2\,[ES]$

$\therefore [ES] = v/k_2$

Substitute $v = \frac{k_2\,E_T[S]}{K_M + [S]}$

$$v = \frac{k_2\,E_T[S]}{K_M + [S]}$$

When the substrate concentration is much larger than [E], the enzyme will be saturated with substrate and virtually all the enzyme will be present as ES thus leading to the attainment of the maximum velocity.

$\therefore Vmax = k_2\,E_T$

Substitute $v = \underbrace{\frac{Vmax[S]}{K_M + [S]}}$

Michaelis-Menton Equation

Does this equation agree with observed behavior of an enzyme?

Condition A

[S] is much less than k_m

[S] << K_M

…only a small percentage of the overall enzyme is present in a combined form with the substrate.

Under these conditions:

$$v = \frac{Vmax[S]}{K_M + [S]}$$ Since [S] is small $$v \cong \frac{Vmax[S]}{K_M}$$

∴ Initial velocity (v_o) depends on [S]. This is first order kinetics with respect to S.

Does this equation agree with observed behavior of an enzyme?

Condition C

[S] is much greater than K_M

[S] >> K_M

…we have no dependence of v on substrate concentration (zero order) and v attains the maximal velocity (Vmax).

Under these conditions:

$$v = \frac{Vmax[S]}{K_M + [S]}$$ Since [S] is large $$v \cong \frac{Vmax[S]}{[S]}$$

$$v = Vmax$$

Does this equation agree with observed behavior of an enzyme?

Condition B

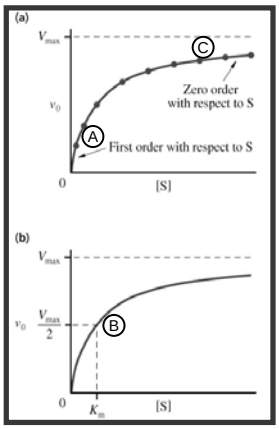
[S] = K_M

Under these conditions:

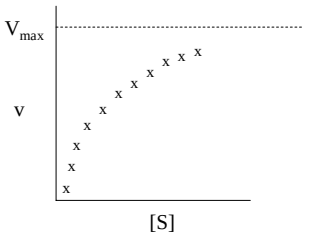
$$v = \frac{Vmax[S]}{K_M + [S]}$$ Since [S] = K_M $$v = \frac{Vmax[S]}{[S] + [S]}$$

$$v = \frac{Vmax[S]}{2[S]} = \frac{Vmax}{2}$$

The equation works!



Problem: Often one cannot read Vmax directly from experimental data



Use a Lineweaver Burk Equation- a transformation of Michaelis Menton equation.

Another name for it is a Double Reciprocal Plot

$$v = \frac{Vmax[S]}{K_M + [S]}$$

Take the reciprocal and separate into a y = mx + b

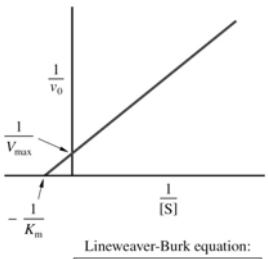
$$\frac{1}{v} = \frac{K_M}{Vmax} \cdot \frac{1}{[S]} + \frac{1}{Vmax}$$

y = m x + b

Measurement of K_M and V_{max}

The double-reciprocal **Lineweaver-Burk plot** is a linear transformation of the Michaelis-Menten plot

($1/v_o$ versus $1/[S]$)



Lineweaver-Burk equation:

$$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Smallest 1/v value = largest V value

The Meanings of K_M

- K_M = [S] when v_o = 1/2 V_{max}
- $K_M \cong k_{-1} / k_1 = K_s$ (the enzyme-substrate dissociation constant) when k_{cat} << either k_1 or k_{-1}
- The lower the value of K_M, the tighter the substrate binding
- K_M can be a measure of the affinity of E for S

$$K_M = \frac{(E_T - [ES])[S]}{[ES]} \qquad \frac{\text{Free enzyme}}{\text{Bound enzyme}}$$

The Meanings of K_M

1. K_M = [S] when $v_o = 1/2\ V_{max}$

K_M is the concentration of substrate at which half of the active sites are filled.

2. $K_M = \dfrac{k_{-1} + k_2}{k_1}$ **Suppose $k_2 << k_{-1}$**

$K_M \cong \dfrac{k_{-1}}{k_1}$ } **Dissociation Constant for ES complex**

$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$ $\quad k_1[E][S] = k_{-1}[ES]$

$\dfrac{[E][S]}{[ES]} = \dfrac{k_{-1}}{k_1}$ ↓ K_M, tighter binding of substrate to enzyme.

If K_M is a dissociation constant, then it is a measure of the affinity of E for S.

In general, we can't use K_M or V_{max} to compare one enzyme to another.

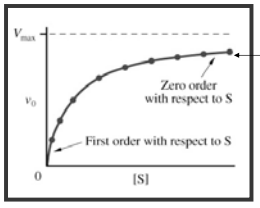
So, how can we compare the activity of one enzyme to another?

Turnover number – maximum of product molecules formed per active site per unit of time when enzyme is fully saturated with substrate.

k_{cat} = turnover number

$$k_{cat} = \frac{\text{molecules of product/unit time}}{\text{enzyme molecule}} = \frac{V_{max}}{E_T}$$

$$\therefore V_{max} = k_{cat}(E_T)$$



1st order for ES → E + P (max substrate)

Rate limiting step

$k_{cat} = k_2$

$\therefore V_{max} = k_{cat}(E_T)$

This is usually for simple one-substrate enzymes.

Most enzymes are not normally saturated with substrate.

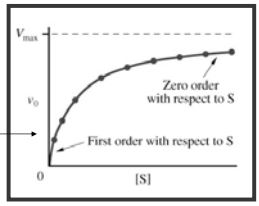
$$v = \frac{V_{max}[S]}{K_M + [S]} = \frac{k_{cat} E_T[S]}{K_M + [S]}$$

If [S] is low, the K_M >> [S] so

$$V = \frac{k_{cat} E_T[S]}{K_M}$$

$\dfrac{k_{cat}}{K_M}$ **is the second order rate constant for the reaction of E + S to E + P.**

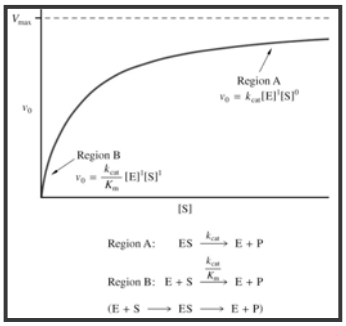
Low concentration of substrate…overall second order.



$\dfrac{k_{cat}}{K_M}$ is used to compare the activity of one enzyme to another.

When [S] << K_M, the enzymatic velocity depends on the value of k_{cat}/K_M and on [S].

Meanings of k_{cat} and k_{cat}/K_M



What are the units of k_{cat}/K_M?

Examples of catalytic constants

Enzyme	$k_{cat}(s^{-1})$
Papain	10
Ribonuclease	10^2
Carboxypeptidase	10^2
Trypsin	10^2 (to 10^3)
Acetylcholinesterase	10^3
Kinases	10^3
Dehydrogenases	10^3
Transaminases	10^3
Carbonic anhydrase	10^6
Superoxide dismutase	10^6
Catalase	10^7

Values of k_{cat}/K_M

- k_{cat}/K_M can approach rate of encounter of two uncharged molecules in solution (10^8 to $10^9 M^{-1}s^{-1}$)
- k_{cat}/K_M is also a measure of enzyme <u>specificity</u> for different substrates (**specificity constant**)
- **rate acceleration** = $\mathbf{k_{cat}/k_n}$

  (k_n = rate constant in the absence of enzyme)

Are there any upper limits for k_{cat}/K_M?

Assume $k_{cat} = k_2$

$$\frac{k_{cat}}{K_M} = \frac{k_2 k_1}{k_2 + k_{-1}}$$

Can never be greater than 1

$$\frac{k_{cat}}{K_M} \leq k_1$$

Upper limit – diffusion limit – reaction can never be faster than the time it takes for S to diffuse to an E, ≅ 10^9 M^{-1} s^{-1}.

TABLE 5.2 Rate accelerations of some enzymes

	Nonenzymatic rate constant (k_n in s^{-1})	Enzymatic rate constant (k_{cat} in s^{-1})	Rate acceleration (k_{cat}/k_n)
Carbonic anhydrase	10^{-1}	10^{6}	8×10^{6}
Chymotrypsin	4×10^{-9}	4×10^{-2}	10^{7}
Lysozyme	3×10^{-9}	5×10^{-1}	2×10^{8}
Triose phosphate isomerase	4×10^{-6}	4×10^{3}	10^{9}
Fumarase	2×10^{-8}	2×10^{3}	10^{11}
β-Amylase	3×10^{-9}	10^{3}	3×10^{11}
Adenosine deaminase	2×10^{-10}	4×10^{2}	2×10^{12}
Urease	3×10^{-10}	3×10^{4}	10^{14}
Mandelate racemase	3×10^{-13}	5×10^{2}	1.7×10^{15}
Alkaline phosphatase	10^{-15}	10^{2}	10^{17}
Orotidine 5′-phosphate decarboxylase	3×10^{-16}	4×10	10^{17}

Factors that Affect Enzyme Activity

- **Temperature**
- **pH**
- **Inhibitors**

Temperature

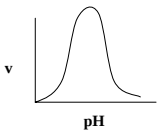
In general, a 10°C increase in T doubles the rate

pH

Each enzyme has its own range of pH in which it will work.

Two good examples are the enzymes pepsin and catalase.

Pepsin only works between pH 1 - pH 4 (acidic)
Catalase only works between pH 7 - pH 11



Reversible Enzyme Inhibition

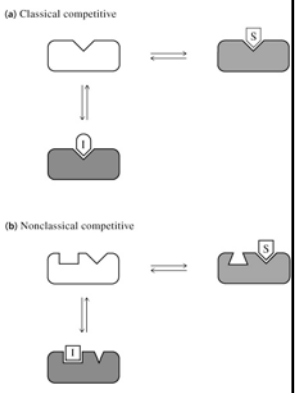
- **Inhibitor** (**I**) binds to an enzyme and prevents formation of ES complex or breakdown to E + P
- **Inhibition constant** ($\mathbf{K_I}$) is a dissociation constant

  EI $\rightleftharpoons$ E + I
- There are three basic types of inhibition: **Competitive**, **Uncompetitive** and **Noncompetitive**
- These can be distinguished experimentally by their effects on the enzyme kinetic patterns

Reversible enzyme inhibitors

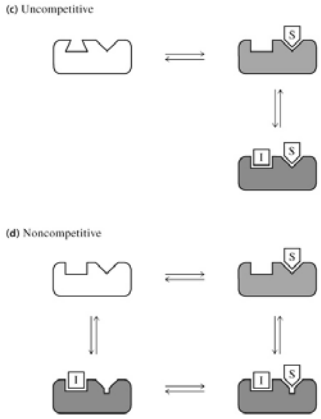
(a) **<u>Competitive</u>.** S and I bind to same site on E

(b) **<u>Nonclassical competitive</u>.** Binding of S at active site prevents binding of I at separate site. Binding of I at separate site prevents S binding at active site.



(c) **<u>Uncompetitive</u>.** I binds only to ES (inactivates E)

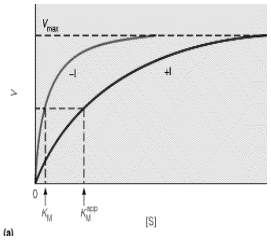
(d) **<u>Noncompetitive</u>.** I binds to either E or ES to inactivate the enzyme



A. Competitive Inhibition

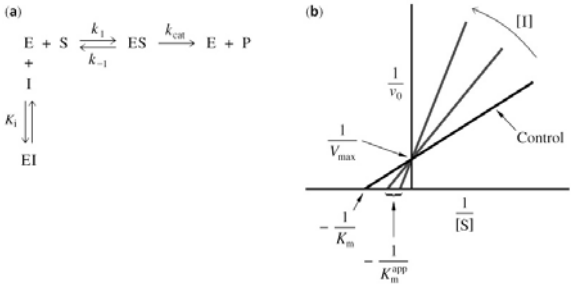
- **Inhibitor binds only to <u>free</u> <u>enzyme</u> (E) not (ES)**
- **Substrate cannot bind when I is bound at active site (S and I "compete" for the enzyme active site)**
- **V_{max} is the same with or without I (high S can still saturate the enzyme even in the presence of I)**
- **Apparent K_M (K_M^{app}) measured in the presence of I is larger than K_M (measured in absence of I)**
- **Competitive inhibitors usually <u>resemble</u> <u>the</u> <u>substrate</u>**

Competitive Inhibition

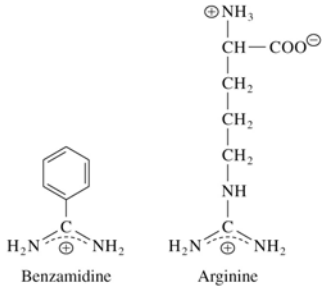


Same V_{max}
K_M increases

Competitive inhibition. (a) Kinetic scheme. (b) Lineweaver-Burk plot



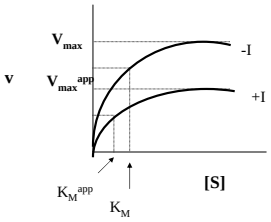
Benzamidine competes with arginine for binding to trypsin



B. Uncompetitive Inhibition

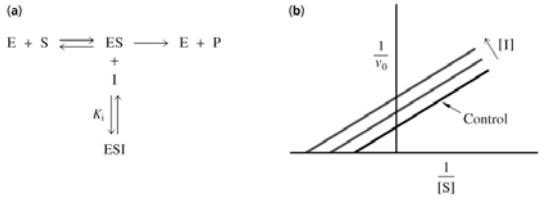
- Uncompetitive inhibitors bind to ES <u>not</u> to free E
- V_{max} decreased by conversion of some E to ESI
- K_M (K_M^{app}) is also decreased
- Lines on double-reciprocal plots are parallel
- This type of inhibition usually only occurs in multisubstrate reactions

Uncompetitive Inhibition



V_{max} decreases
K_M decreases

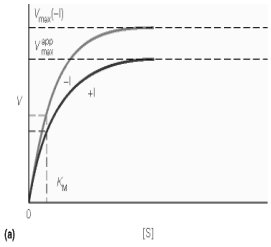
Uncompetitive inhibition



C. Noncompetitive Inhibition

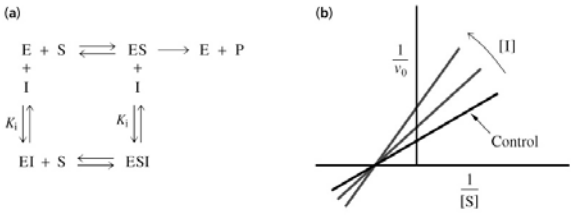
- Noncompetitive inhibitors bind to <u>both</u> E and ES
- Inhibitors do not bind at the same site as S
- V_{max} decreases
- K_m does not change
- Inhibition cannot be overcome by addition of S
- Lines on double-reciprocal plot intersect on x axis

Noncompetitive Inhibition



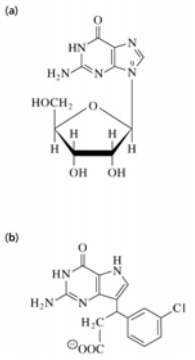
V_{max} decreases
K_M stays the same

Noncompetitive inhibition



D. Uses of Enzyme Inhibition

Comparison of a substrate (a) and a designed inhibitor (b) for the enzyme purine nucleoside phosphorylase



Irreversible Enzyme Inhibition

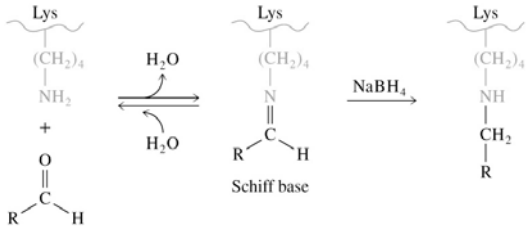
- **Irreversible inhibitors form <u>stable</u> <u>covalent</u> <u>bonds</u> with the enzyme (e.g. alkylation or acylation of an active site side chain)**
- **There are many naturally-occurring and synthetic irreversible inhibitors**
- **These inhibitors can be used to identify the amino acid residues at enzyme active sites**
- **Incubation of I with enzyme results in loss of activity**

Affinity labels for studying enzyme active sites

- **Affinity labels** are active-site directed reagents
- They are <u>irreversible</u> inhibitors
- Affinity labels resemble substrates, but contain reactive groups to interact covalently with the enzyme

Covalent complex with lysine residues

- Reduction of a Schiff base forms a stable substituted enzyme



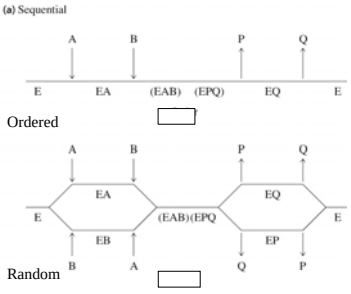
Inhibition of serine protease with DFP

- **Diisopropyl fluorophosphate (DFP) is an organic phosphate that inactivates serine proteases**
- **DFP reacts with the active site serine (Ser-195) of chymotrypsin to form DFP-chymotrypsin**
- **Such organophosphorous inhibitors are used as insecticides or for enzyme research**
- **These inhibitors are toxic because they inhibit acetylcholinesterase (a serine protease that hydrolyzes the neurotransmitter acetylcholine)**

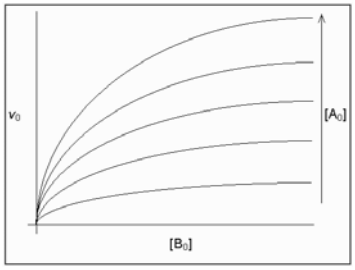
Kinetics of Multisubstrate Reactions

Single Displacement

(a) Sequential (ordered or random)



***K*m for substrate B and *V*max are not true constants unless [A] is saturating - they are dependant on [A].**



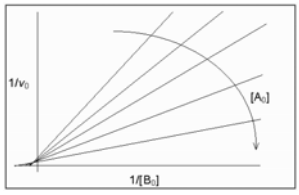
a) When [B] is variable and [A] is constant.

$$\frac{1}{v_0} = \frac{1}{[B_0]}\left[\frac{K_b}{V_{max}} + \frac{K_{ia}K_b}{[A_0]V_{max}}\right] + \left[\frac{1}{V_{max}} \cdot \; 1 + \frac{K_a}{[A_0]}\right]$$

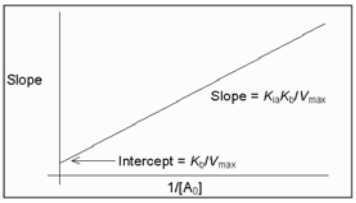
b) When [A] is variable and [B] is constant.

$$\frac{1}{v_0} = \frac{1}{[A_0]}\frac{K_a}{V_{max}} + \frac{K_{ia}K_b}{[B_0]V_{max}} + \frac{1}{V_{max}} \; 1 + \frac{K_b}{[B_0]}$$

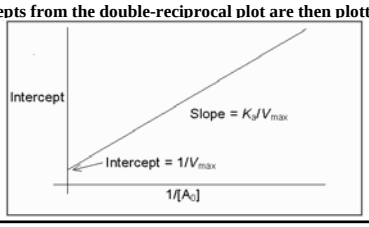
The data are plotted on a double-reciprocal plot:



The slopes from the double reciprocal plot are then plotted against $1/[A_0]$:

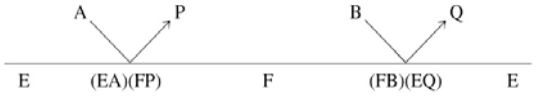


The y-intercepts from the double-reciprocal plot are then plotted against $1/[A_0]$:



(b) Double Displacement

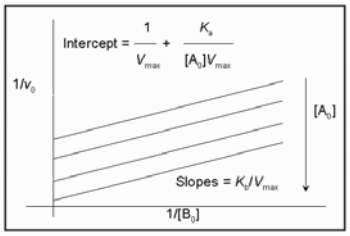
(b) Ping-pong



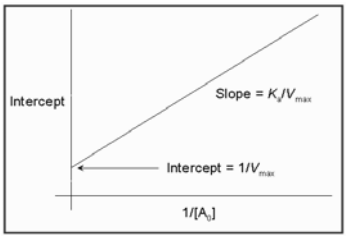
Experimental data can be plotted according to the linear form:

$$\frac{1}{v_0} = \frac{1}{V_{max}} + \frac{K_a}{[A_0]V_{max}} + \frac{K_b}{[B_0]V_{max}}$$

Ping-pong mechanisms can be distinguished from sequential mechanisms by the Lineweaver-Burk plot:



The y-intercepts are then plotted against $1/[A_0]$:

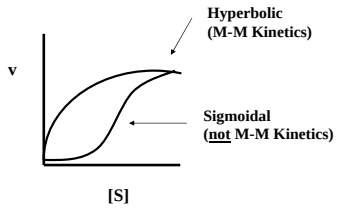


Regulatory Enzymes
(group of enzymes which do not exhibit MM Kinetics)

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \ldots$$

- **Often E_1 is a regulatory enzyme (1st committed step in a metabolic sequence of reactions)**
- **Often multimeric**
- **Often bind some metabolite other than S at a place other than the active site which will affect activity (allosterism)**
- **Often exhibit sigmodial kinetics**

Sigmoidal vs Hyperbolic



The best way to understand the difference between hyperbolic (M-M) and sigmoidal (non M-M) is to study the difference between Myoglobin and Hemoglobin

Protein component of Mb and Hb is globin

- Myoglobin is composed of 8 α helices
- Heme prosthetic group binds oxygen
- **His-93** is complexed to the iron atom, and **His-64** forms a hydrogen bond with oxygen
- Interior of Mb almost all hydrophobic amino acids
- Heme occupies a hydrophobic cleft formed by three a helices and two loops

Hemoglobin (Hb)

- Hb is an $\alpha_2\beta_2$ tetramer (2 α globin subunits, 2 β globin subunits)
- Each globin subunit is similar in structure to myoglobin
- Each subunit has a heme group
- The α chain has 7 α helices, β chain has 8 α helices

Myoglobin vs Hemoglobin

- Single polypeptide chain
- 153 amino acids
- Typical globular protein
- In muscle
- Contains One Heme
- Stores oxygen
- Follows M-M

- 4 polypeptide chains 2 α 141 AA; 2 β 145 AA
- In Red Blood Cells
- Contains Four Heme Groups
- Carries oxygen
- Does not follow M-M

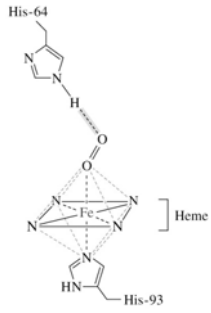
Oxygen Binding to Mb and Hb

A. Oxygen Binds Reversibly to Heme

- **Oxymyoglobin** - oxygen bearing myoglobin
- **Deoxymyoglobin** - oxygen-free myoglobin
- In oxymyoglobin, six ligands are coordinated to the ferrous ion in octahedral symmetry
- Oxygen is coordinated between the iron and the imidazole sidechain of His-64

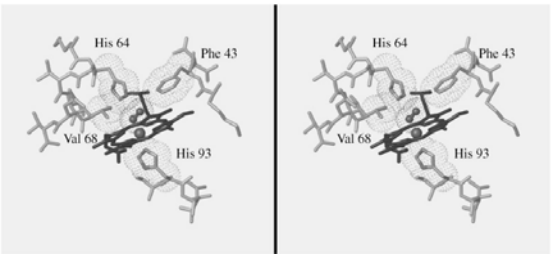
Oxygen-binding site of whale oxymyoglobin

- Octahedral geometry of coordination complex (six ligands around iron)
- His-93 (proximal histidine) liganded to Fe
- His-64 (distal histidine)



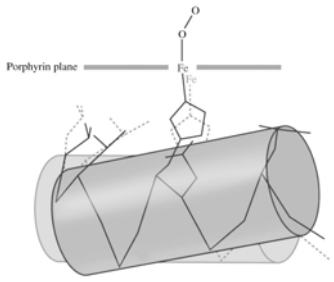
Oxygen-binding in myoglobin OxyMb

- O_2 (green), Fe(II) orange



Conformational changes in a hemoglobin chain induced by oxygenation

- Oxygen binding to Fe pulls the His toward ring plane
- Helix with His shifts position, disrupting some ion pairs between subunits (blue to red position)



Oxygen-Binding Curves of Myoglobin and Hemoglobin

- Curves show <u>reversible</u> <u>binding</u> of O_2 to Mb and Hb
- <u>Fractional</u> <u>saturation</u> (Y) is plotted versus the partial pressure of oxygen, pO_2 (oxygen concentration)
- The shape of the Hb curve shows a **positive cooperativity** in the binding of 4 O_2 molecules (i.e. the O_2 affinity of Hb <u>increases</u> as each O_2 molecule is bound)

O_2 binding curves (continued)

- Mb-O_2 binding curve is <u>hyperbolic</u>, indicating a single equilibrium constant for binding O_2
- Hb-O_2 binding curve is <u>sigmoidal</u>, and reflects the binding of 4 molecules of O_2, one per each heme group

Easy to Explain Hyperbolic Binding Curve of Mb

Simple Equilibrium Binding

$Mb + O_2 \leftrightarrow MbO_2$ $\quad K_f = \dfrac{[MbO_2]}{[Mb]\,[O_2]}$

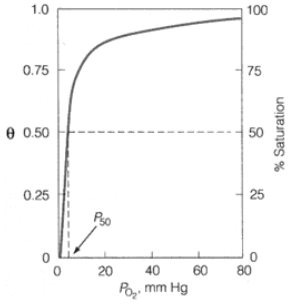
$$Y = \frac{[MbO_2]}{[Mb] + [MbO_2]}$$

$$Y = \frac{Kf\,[Mb][O_2]}{[Mb] + Kf\,[Mb][O_2]}$$

$$Y = \frac{Kf\,[O_2]}{1 + Kf\,[O_2]} \qquad Y = \frac{[O_2]}{K_d + [O_2]}$$

← This is the form for a hyperbolic curve

$K_d = P_{50}$ $\qquad Y = \dfrac{[O_2]}{P_{50} + [O_2]}$



Hemoglobin unlike Myoglobin has Sequential Interaction

This means that the binding sites depend on each other through some kind of interaction

A = Hb; B = O_2

$A + B \leftrightarrow AB$ $\qquad K_f$

$AB + O_2 \leftrightarrow AB_2$ $\qquad aK_f$ $\quad a > 1$

$AB_2 + O_2 \leftrightarrow AB_3$ $\qquad abK_f$ $\quad b > 1$

$AB_3 + O_2 \leftrightarrow AB_4$ $\qquad abcK_f$ $\quad c > 1$

Take this model to its logical conclusion which gives an equation useful for analyzing sigmoidal binding data.

All or nothing! $\qquad A + 4B \leftrightarrow AB_4 \qquad K' = \dfrac{[AB_4]}{[A][B]^4}$

A = Hb; B = O_2

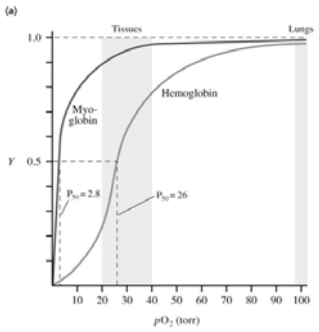
$$Y = \frac{[AB_4]}{[A] + [AB_4]}$$

$$Y = \frac{[B]^4}{Kd^4 + [B]^4}$$

← This is the form for a sigmoidal curve

Oxygen-binding curves

(a) Comparison of O_2-binding to Mb and Hb



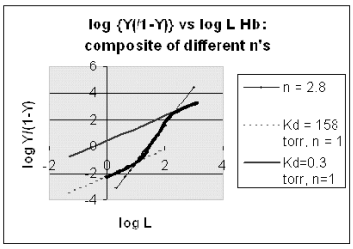
Hill Equation

$$Y = \frac{P_{O2}^{\,n}}{P_{50}^{\,n} + P_{O2}^{\,n}}$$

Again, Kd = P_{50}

$$\ln\left(\frac{Y}{1-Y}\right) = -n \ln P_{50} + n \ln P_{O2}$$

Hill Plot



The Hill Coefficient

n_H = maximum slope

n_H = 1 hyperbolic binding (noncooperative)

n_H > 1 positive cooperativity (filling one binding site increases the affinity of other binding sites)

n_H < 1 negative cooperativity

n_H = n (where n = 4) complete cooperativity for Hb

For hemoglobin, n_H =2.8

How does positive cooperativity work?

Conformational change on binding

Regulation of Enzyme Activity

- **Regulatory enzymes** - activity can be reversibly modulated by effectors
- Such enzymes are usually found at the first unique step in a metabolic pathway (the first "committed" step)
- Regulation at this step conserves material and energy and prevents accumulation of intermediates

Two Methods of Regulation

(1) Noncovalent allosteric regulation

(2) Covalent modification

- **Allosteric enzymes** have a second **regulatory site** (allosteric site) distinct from the active site
- Allosteric inhibitors or activators bind to this site and regulate enzyme activity via conformational changes

Hemoglobin is an Allosteric Protein

- Oxygen binding and release from Hb are regulated by **allosteric interactions**
- **Allosteric effectors** (**modulators**) bind to a protein at a site separate from the functional binding site (may be activators or inhibitors)
- The activity of an **allosteric protein** is regulated by allosteric effectors

Two conformations of hemoglobin: T and R

- **Active** (**R state**) and **inactive** (**T state**) forms are in rapid equilibrium in allosteric proteins
- Binding of substrates and allosteric activators stabilize the R state and shift the equilibrium in the R direction
- Allosteric inhibitors stabilize the T state and shift the equilibrium in the T direction

Equilibrium between different conformations of protein in aqueous solution

$$R \leftrightarrow T$$

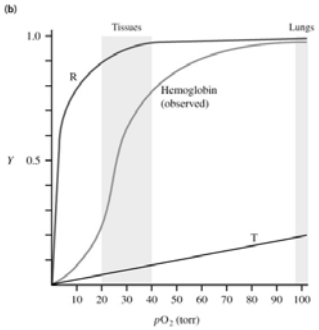
Relaxed
High Affinity

Tense
Low Affinity

Positive cooperativity – O_2 binds preferentially to R form and shifts the equilibrium to R side

(b) Oxygen-binding curves

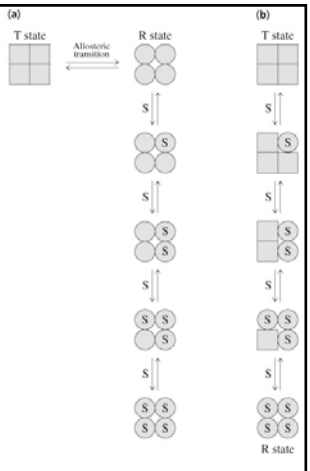
(b) Binding of the R (high-affinity) and T (low affinity) forms of Hb



Two models

(a) <u>Concerted</u> <u>model</u>: subunits either all T state or all R state

(b) <u>Sequential</u> <u>model</u>: Mixture of T subunits and R subunits is possible. Binding of S converts only that subunit from T to R

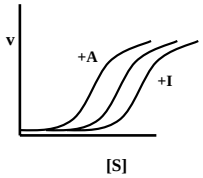


Concerted Theory

Symmetry-driven Theory

The enzyme has two forms –all Ⓡ form or all Ⓣ form

The binding to inhibitors and activators will bind to either the T or R state, respectively.

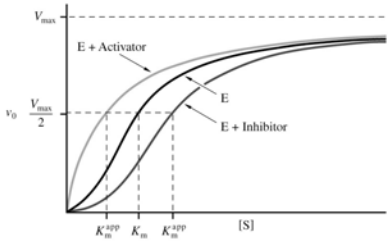


A – Activator
I - Inhibitor

- **Substrate binds to shift equilibrium to R form**
- **Inhibitors preferentially bind to the lower affinity form (T state) and shifts equilibrium to T form**
- **Activators preferentially bind to the higher affinity form (R state) and shifts equilibrium to R form**

Role of cooperativity of binding in regulation

- **Addition of modulators alters enzyme activity**
- **Activators can lower K_M, inhibitors can raise K_M**



Sequential Theory

Ligand-induced Theory

The enzyme has two forms – R form and T form but it <u>differs from concerted theory in allowing the existence of both high- and low-affinity subunits in an oligomeric molecule with fractional saturation.</u>

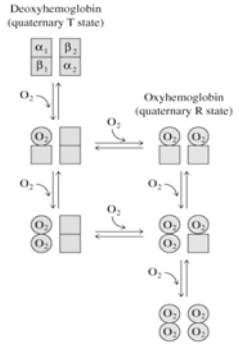
Hill Equation - Quantify the cooperativity

Allosteric Effects on Hemoglobin

- **Oxygen Binding**
- **2,3-Bisphosphoglycerate binding**
- **pH (Bohr Effect)**

Conformational changes during O_2 binding to hemoglobin

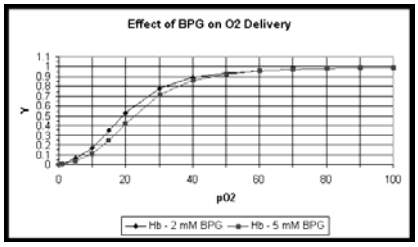
- **Oxygen binding to Hb has aspects of both the sequential and concerted models**



2,3-Bisphosphoglycerate Binding

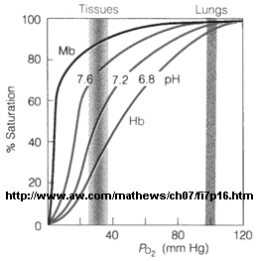
- **BPG binds to deoxy form of Hb**
- **Binds to a site other than where O_2 binds**
- **BPG causes the T form to predominate**
- **Therefore, <u>lowers</u> oxygen affinity**

2,3-Bisphosphoglycerate Binding



This is an adaptive response, requiring several days at high altitude. The production of excess BPG, although it reduces the oxygen affinity, it makes the protein more efficient at delivering oxygen to the tissues at high altitudes.

The Bohr Effect



Lower pH → decrease Hb affinity for O_2

Thus <u>more</u> oxygen is delivered to tissues at a lower pH even when the pO2 remains unchanged

$Hb{\cdot}4O_2 + nH^+ \leftrightarrow Hb{\cdot}nH^+ + 4O_2$

When a tissue is more active, the amount of carbon dioxide produced will be increased (P_{CO2} is higher). Carbon dioxide reacts with water as shown in the following equation:

$CO_2 + H_2O$ <---------> $H^+ + HCO_3^-$