

BCMB 3100 – Chapter 5 Lecture

- Six Classes (IUBMB)
- Kinetics
- Michaelis-Menten Equation
- V_o , K_m , V_{max} , K_{cat}
- Lineweaver-Burk Plot

Six major groups of enzymes (see pages 130-132)

_____ : (oxidation-reduction reactions)

_____ : (group transfer reactions)

_____ : (hydrolysis)

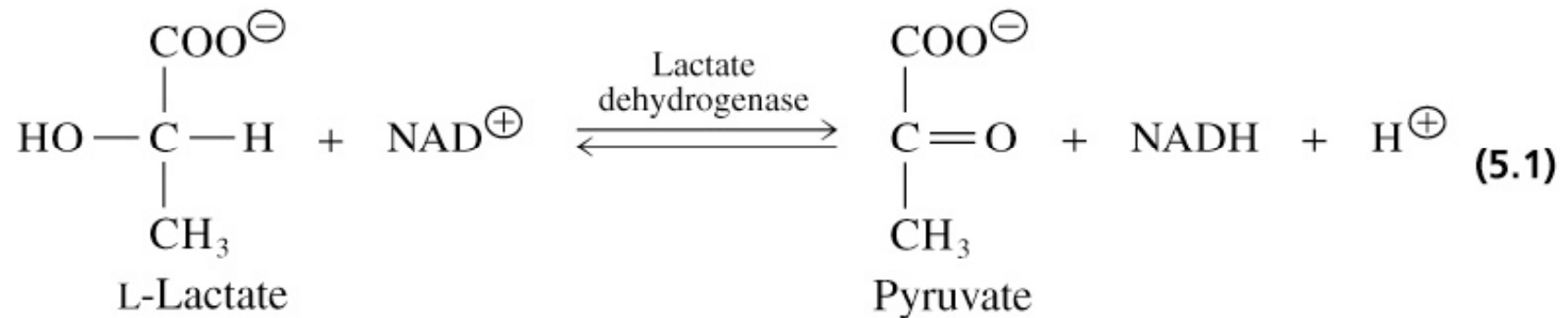
_____ : (nonhydrolytic and nonoxidative lysis → double bond)

_____ : (isomerization)

_____ : (ligation reaction requiring energy from NTP (nucleoside triphosphate))

1. Oxidoreductases (dehydrogenases)

Catalyze oxidation-reduction reactions



Oxidation can be identified as:

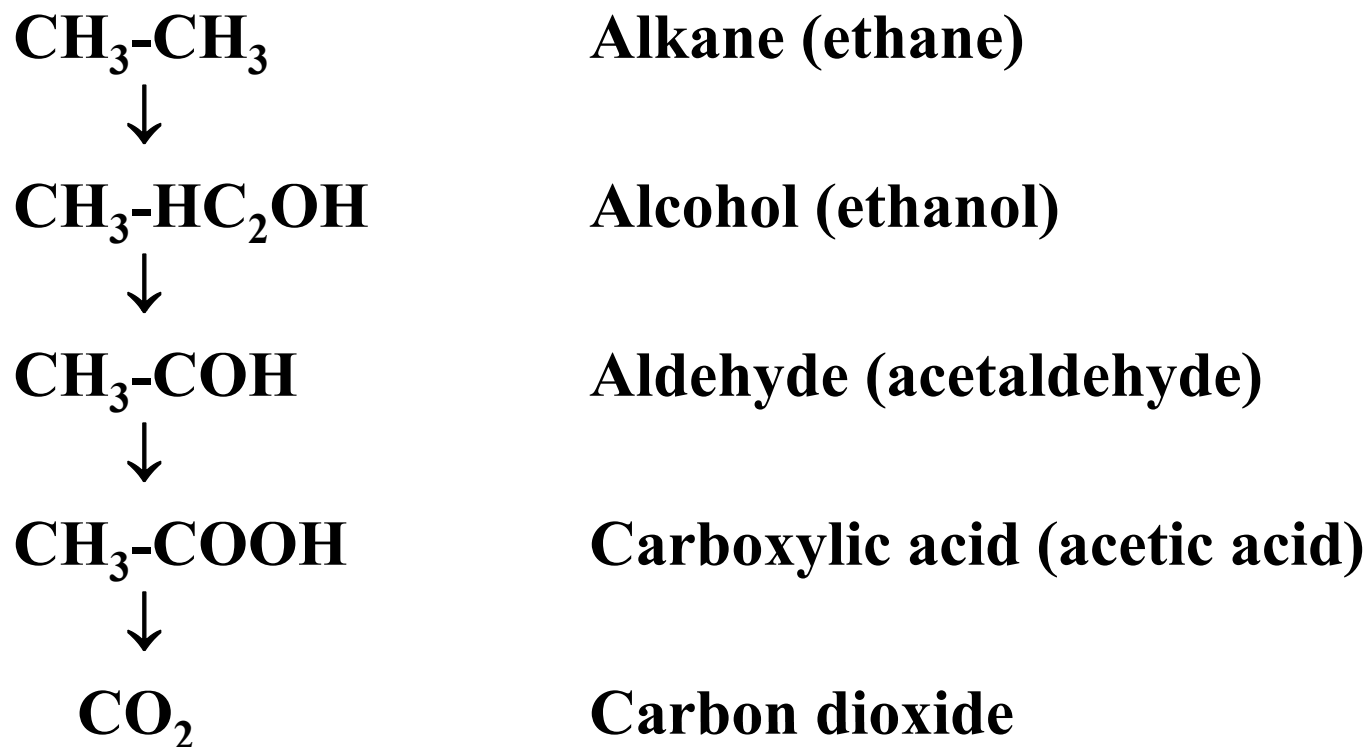
- * Addition of oxygen
- * Removal of hydrogen
- * Increase in valency of metal ion (e.g. $\text{Fe}^{+2} \rightarrow \text{Fe}^{+3}$)

See also pg 199 (Box 7.2) and pg 200 Fig. 7.9

Other examples: pg 336, etc.

The Oxidation States of Carbon

Most reduced

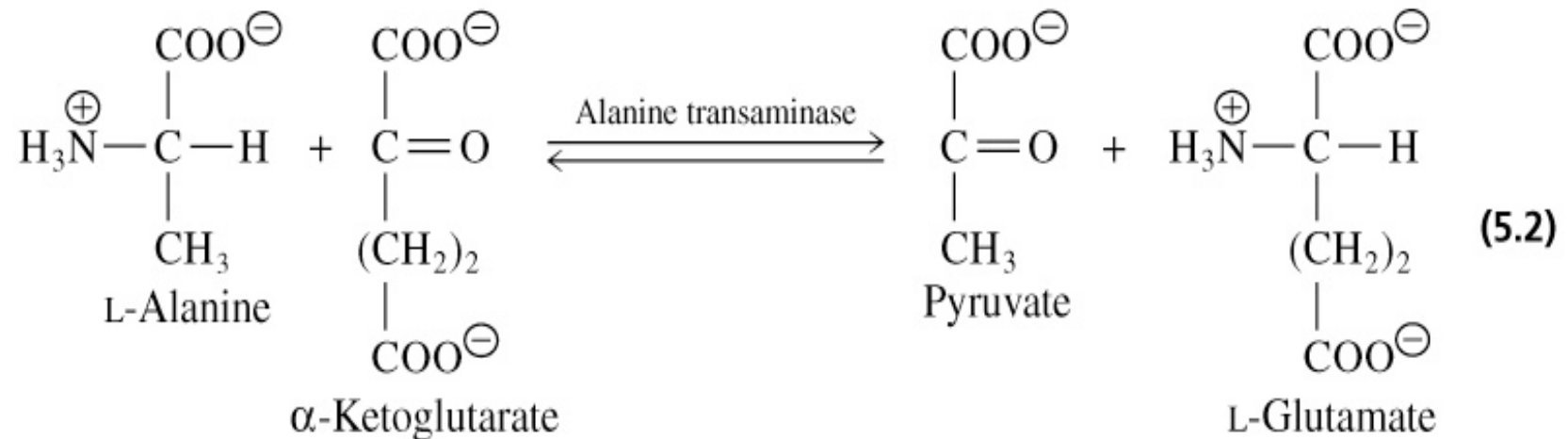


Most oxidized

Each of the arrows indicates an oxidation reaction. All except the last reaction are oxidations brought about by dehydrogenation.

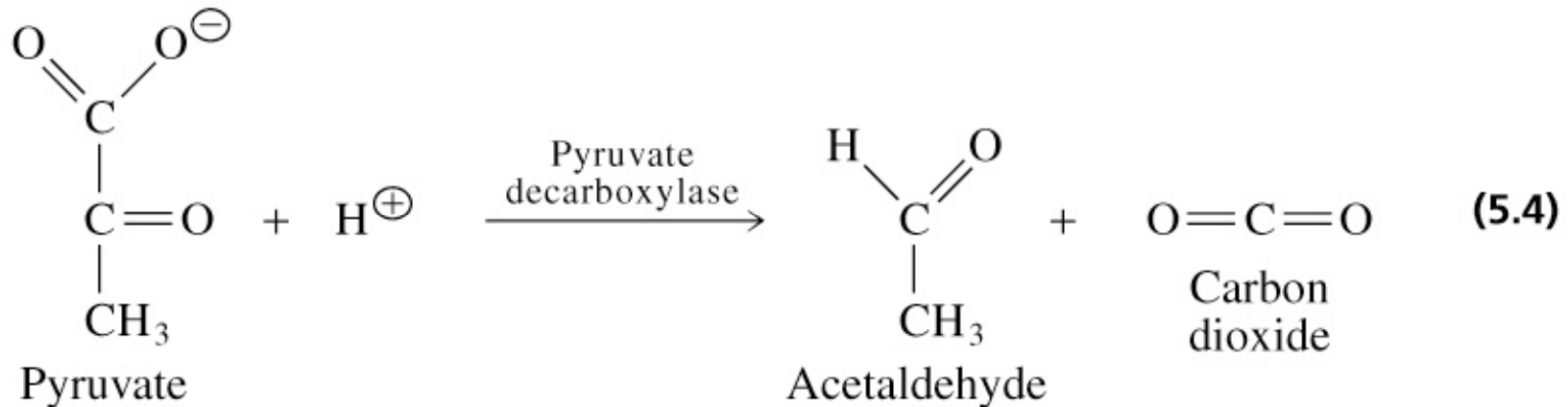
2. Transferases

- Catalyze group transfer reactions



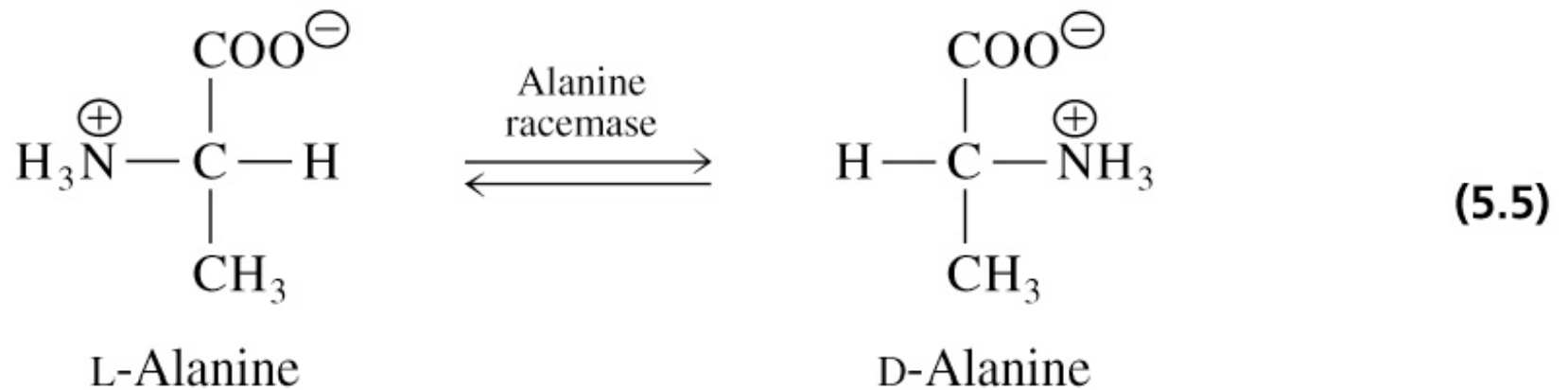
4. Lyases

- Catalyze lysis of a substrate, generating a double bond in a nonhydrolytic, nonoxidative elimination (i.e. nonhydrolytic & nonoxidative lysis that yields a double bond)



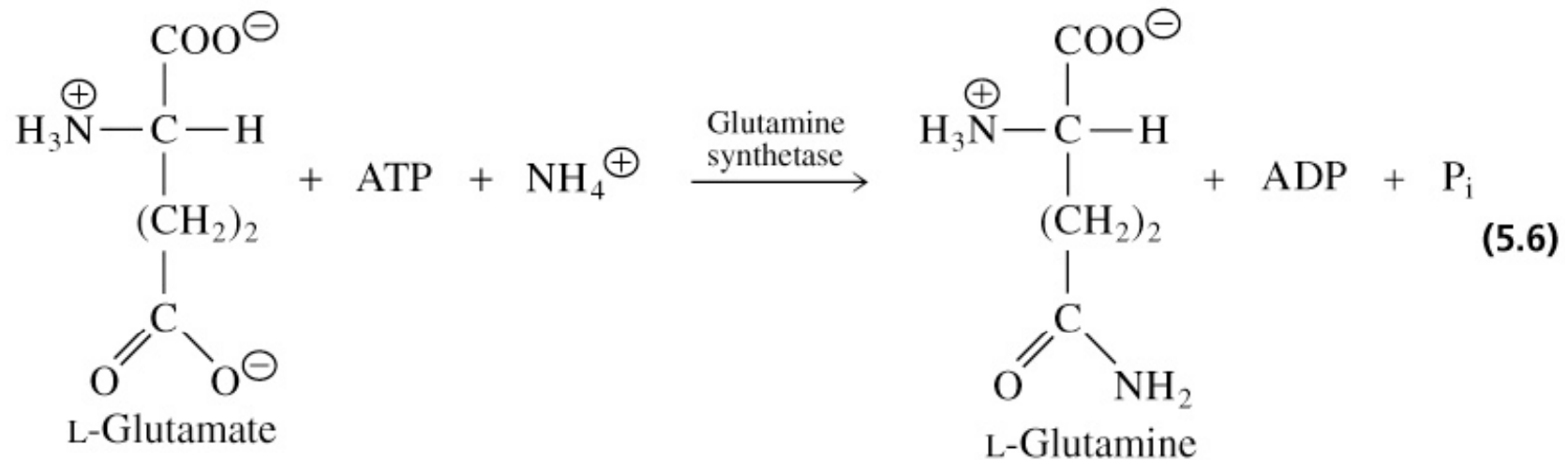
5. Isomerases

- Catalyze isomerization reactions



6. Ligases (synthetases)

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



Challenge of the Weekend, September 4, 2009

Find a minimum of five examples of enzymes and their reactions for each of the 6 classes of enzymes. (You should be able to find all or most of these in your book)

Label an individual page with one of each of the names of the 6 classes of enzymes. On each page for that particular class of enzymes, give a list of at least 5 full reactions, including substrate(s), products and enzyme name. A completed assignment will be six pages with a total of 30 reactions.

For credit, these must be turned in before the start of Breakout Session on Tuesday, September 7.

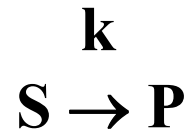
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- **Enzymes**
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- **Lineweaver-Burk Plot**

Principles of Kinetics

(for non-enzyme-catalyzed reactions) (1)

first -order reaction



$$\frac{\Delta[\text{P}]}{\Delta t} = v = k[\text{S}] \quad \text{rate equation}$$

v = velocity, P = product, S = substrate,

k = rate constant (s⁻¹)

Principles of Kinetics

(for non-enzyme-catalyzed reactions) (2)

second-order reaction (bimolecular)



k = rate constant $M^{-1} s^{-1}$

pseudo first-order reaction

If concentration of a reactant is so high that it remains constant during reaction = zero-order

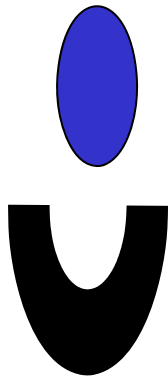
$$v = k[S_1]^1[S_2]^0 = k[S_1]^1$$

Important for enzyme assays

Emil Fischer 1894

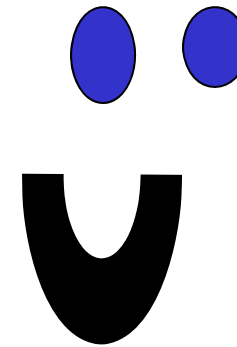
model for enzyme:substrate interactions

Substrate



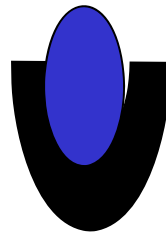
Enzyme

Product



Enzyme

ES complex



Enzyme Kinetics

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



- When $[S] \gg [E]$, 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**

Enzyme kinetics: study of the rates of enzyme-catalyzed reactions

Why study enzyme kinetics? - enzyme kinetics gives information about:

enzyme catalysis

enzyme mechanisms

regulation of enzyme activity (i.e. inhibitors & activators)

basis of enzyme assays

The kinetics of an enzyme are affected by:

[enzyme]

[substrate]

pH & temperature

coenzymes, activators, inhibitors

Enzyme Kinetics



Fig 5.2 Effect of enzyme concentration [E] on velocity (v)

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

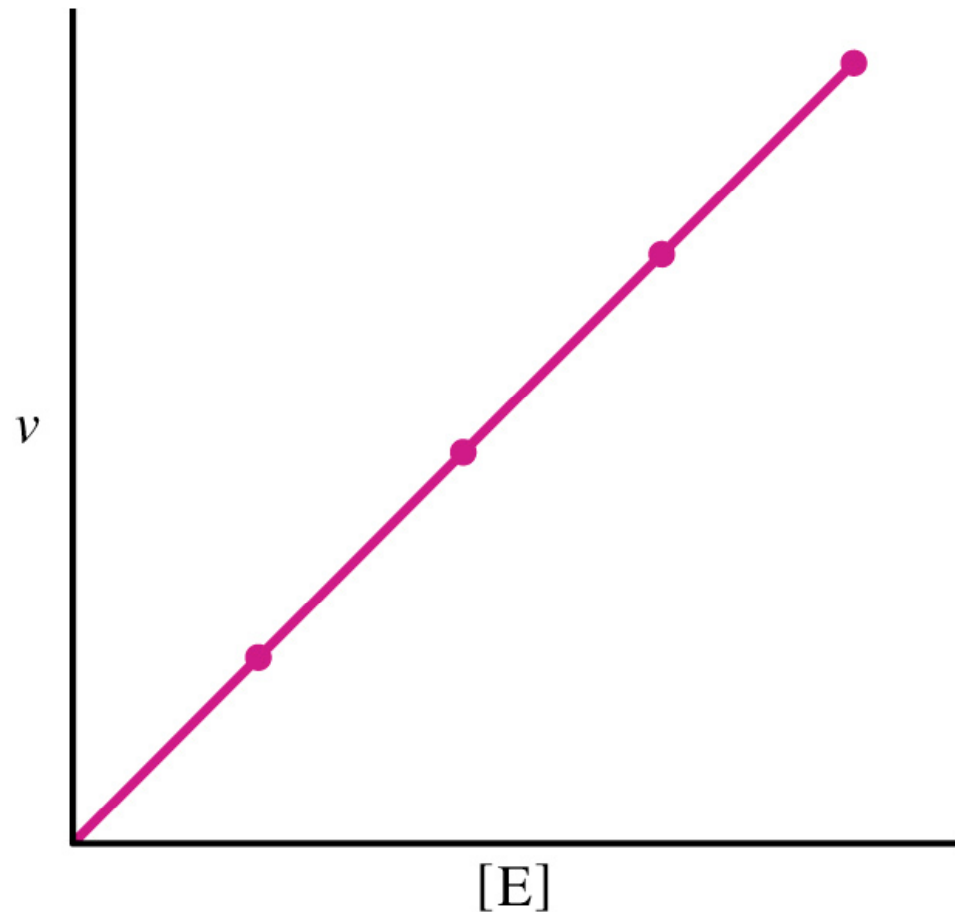


Fig 5.3 Progress curve for an enzyme-catalyzed reaction

- The initial velocity (v_0) is the slope of the initial of the curve
- Rate of the reaction doubles when twice as much enzyme is used

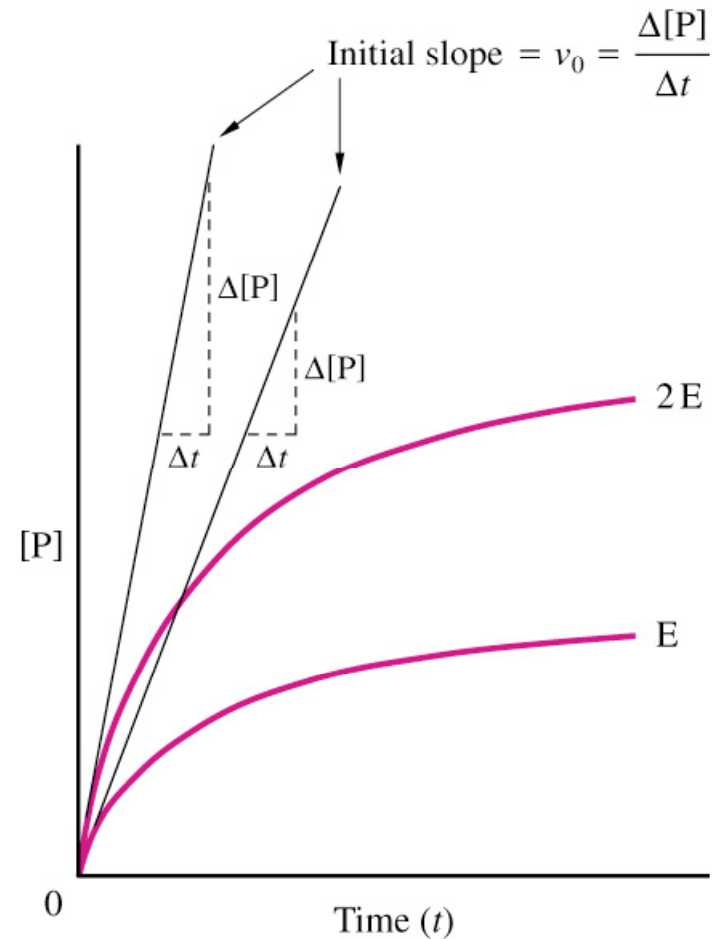
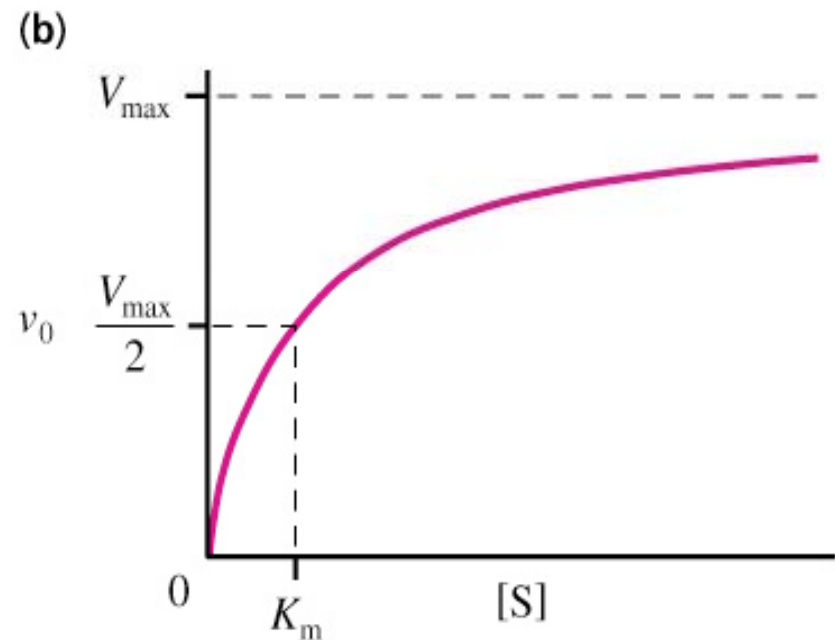
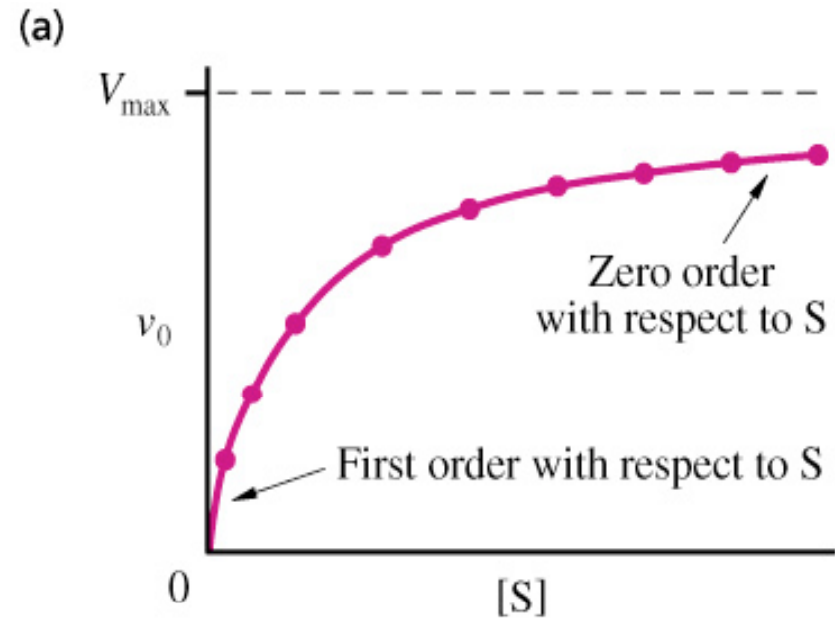


Fig 5.4 Plots of initial velocity (v_0) versus [S]

- (a) Each v_0 vs [S] point is from one kinetic run
- (b) Michaelis constant (K_m) equals the concentration of substrate needed for 1/2 maximum velocity



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Michaelis-Menten Kinetics apply to many enzymes

(1913) Leonor Michaelis & Maud Menten proposed simple model for enzyme kinetics



Assumption #1: (no product reverts to S, true in initial stage of reaction, $k_{-2} = 0$) (initial velocity = V_0)



$$(k_2 = k_{\text{cat}})$$



K_{cat} = catalytic constant = turnover number

number of catalytic events per second per enzyme

Goal: expression that relates rate of catalysis to concentration of S and E and rate constants for individual steps.

$$v = k_2[\text{ES}]$$



[ES] can be described by [E] & [S] by considering individual rate constants

rate of formation of ES = $k_1[E][S]$

rate of breakdown of ES = $(k_{-1} + k_2) [ES]$

Assumption #2: Steady state conditions (i.e. concentration of ES stays the same while concentration of E and S changes). Occurs when formation of ES = breakdown of ES.

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

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

$$[\text{ES}] = [\text{E}][\text{S}] / (\text{k}_{-1} + \text{k}_2) / \text{k}_1$$

Define constant K_m (Michaelis constant)

$$K_m = (\text{k}_{-1} + \text{k}_2) / \text{k}_1$$

Therefore $[\text{ES}] = [\text{E}] [\text{S}] / K_m$

Solve for $[\text{ES}] \dots\dots\dots$

(see your text. You do NOT need to know how to solve for $[\text{ES}]$)



Velocity of an enzyme-catalyzed reaction (depends upon rate of conversion of ES to E + P)

$$v_o = k_2[ES]$$

Recall $k_2 = k_{cat}$

Vmax occurs when [S] much > than Km so

$$\mathbf{Vmax = k_2[E_T]}$$

The Michaelis-Menten equation

- Equation describes v_o versus $[S]$ plots
- K_m is the _____

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

$$V_o = V_{\max}[S] / K_m + [S]$$

At low [S], $[S] \lll K_m$ & $V_o = V_{\max}[S] / K_m$

thus the rate is proportional to [S]

At high [S], $[S] \gggg K_m$ & $V = V_{\max}$

Thus rate is independent of [S]

When $[S] = K_m$ $V = 1/2 V_{\max}$

Thus $K_m = [S]$ at which reaction is half its maximal value

Turnover number: number of S molecules converted into P by E per second when E is fully saturated with S.

$$\text{Turnover number} = k_2 = k_{\text{cat}} = \text{s}^{-1}$$

Turnover number can be calculated from $V_{\text{max}} = k_2[\text{ET}]$

Turnover number of most enzymes is from 1 to 10,000 per sec

Under physiological conditions enzymes are not normally saturated with S.

[S]/K_m is usually between 0.01 and 1

(K_m usually 10⁻¹ to 10⁻⁷M)

How do you characterize enzyme kinetics under these low [S]?

$$V = [E][S] k_2 / K_m$$

When [S] <<<<< K_m [E] ≅ [E_T] so

$$V = [E_T][S] k_2 / K_m$$

Thus, V depends on value of [S] & k₂ / K_m

k₂ / K_m is limited by k₁ which is limited < 10⁹M⁻¹ s⁻¹

(due to limits of diffusion). A few enzymes catalyze reactions at this upper physical rate = diffusion controlled reactions.

Fig 5.4 K_m and physiological substrate concentrations

- K_m values for enzymes are typically just above $[S]$, so that the enzyme rate is sensitive to small changes in $[S]$

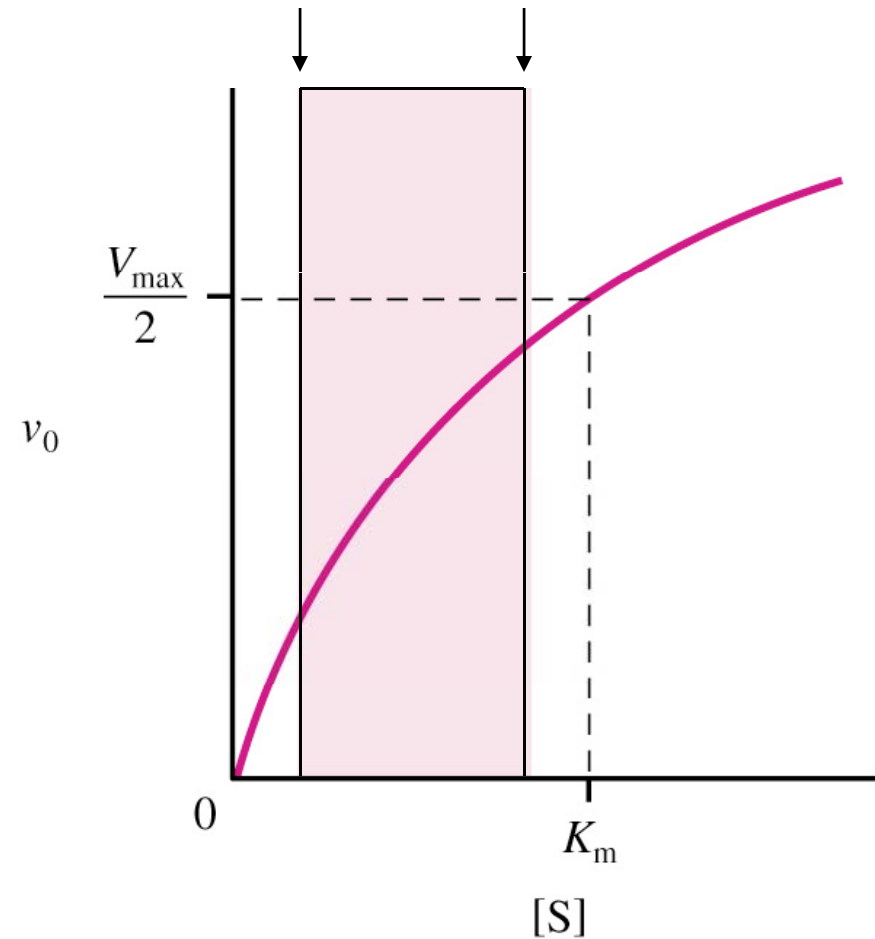


Fig 5.5 Meanings of k_{cat} and k_{cat}/K_m

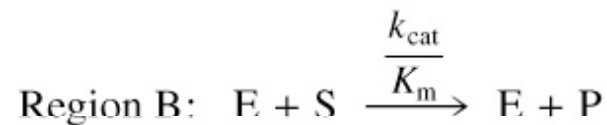
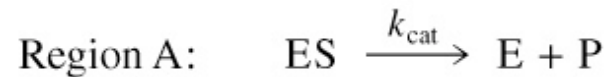
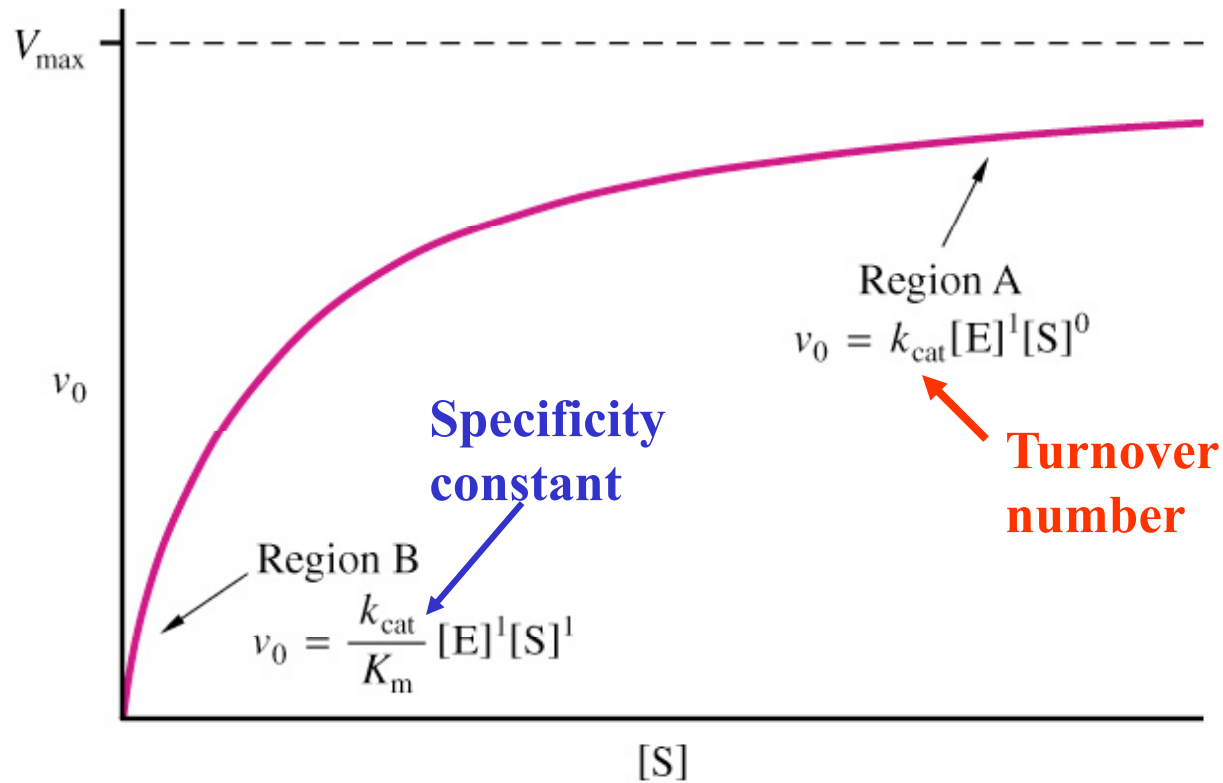


Table 5.1 Examples of catalytic constants

Enzyme	$k_{\text{cat}}(\text{s}^{-1})$
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 $10^9\text{M}^{-1}\text{s}^{-1}$)
- k_{cat}/K_m is also a measure of enzyme specificity for different substrates (**specificity constant**)
- **rate acceleration** = k_{cat}/k_n
(k_n = rate constant in the absence of enzyme)

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}

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V_{max} and K_m can be determined by measuring the rate of the reaction at different [S] if an enzyme operates by Michaelis-Menten kinetics

Transformation of the Michaelis-Menten equation (i.e. taking the reciprocal of both sides)

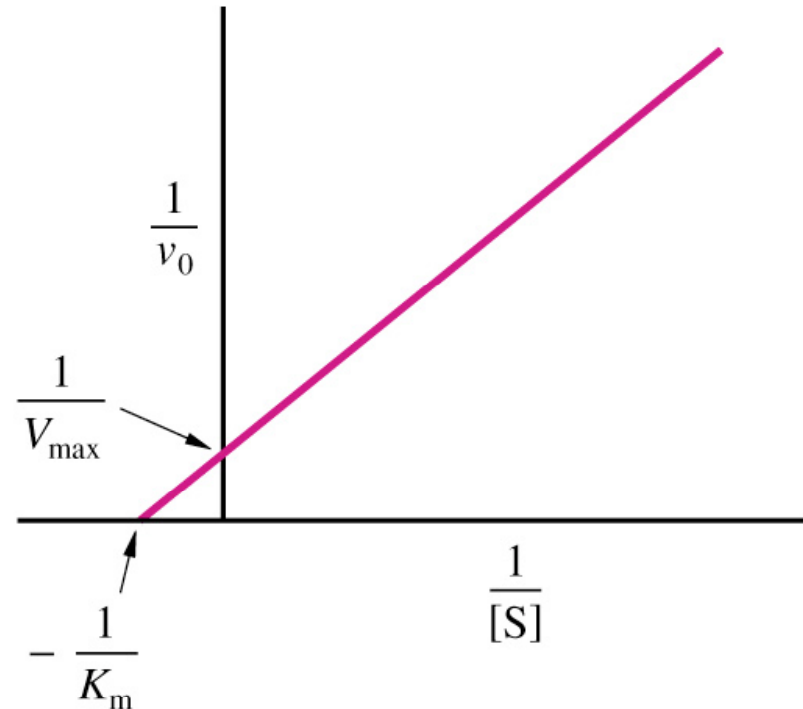
$$1/V = 1/V_{\max} + (K_m / V_{\max} \times 1/[S])$$

Recall $y = a + bx$ $b = \text{slope}$, $a = y \text{ intercept}$

A plot of $1/V$ versus $1/[S]$ is a **Lineweaver-Burk plot (a straight line plot)**

Measurement of K_m and V_{max}

Fig 5.6 The double-reciprocal **Lineweaver-Burk plot** is a linear transformation of the Michaelis-Menten plot ($1/v_0$ versus $1/[S]$)



Lineweaver-Burk equation:

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

**K_m and V_{max} can be determined from the
Lineweaver-Burk plot**

**K_m depends on enzyme, pH, temperature
and ionic strength of environment**

The fraction of enzyme sites filled = f_{ES}

$$f_{ES} = V/V_{max}$$

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- **Lineweaver-Burk Plot**
- **Kinetics of Multisubstrate Reactions**

Kinetics of Multisubstrate Reactions

Cleland notation for bi-substrate reactions

Kinetic Mechanisms

Fig 5.7 Notations for bisubstrate reactions

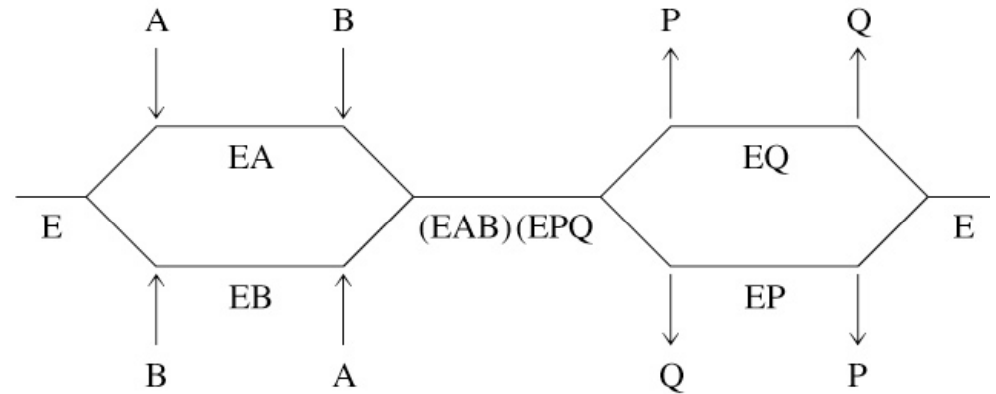
(a) Sequential (ordered or random)

(a) Sequential

Sequential reactions



Ordered



Random

Fig. 5.7 (b) Bisubstrate reactions

Ping-pong reaction

(b) Ping-pong



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- **Michaelis-Menten Equation**
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- **Lineweaver-Burk Plot**
- **Kinetics of Multisubstrate Reactions**
- **Reversible Inhibition**

Reversible Enzyme Inhibition

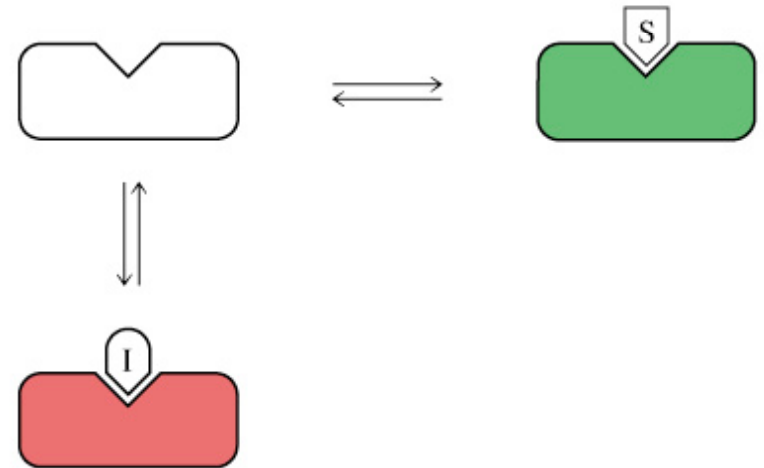
- Three types of reversible inhibition: **Competitive**, **Uncompetitive** and **Noncompetitive**

Fig 5.8 Reversible enzyme inhibitors

(a) Classical competitive. S and I bind to same site on E

(b) **Nonclassical competitive**. 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.

(a) Classical competitive



(b) Nonclassical competitive

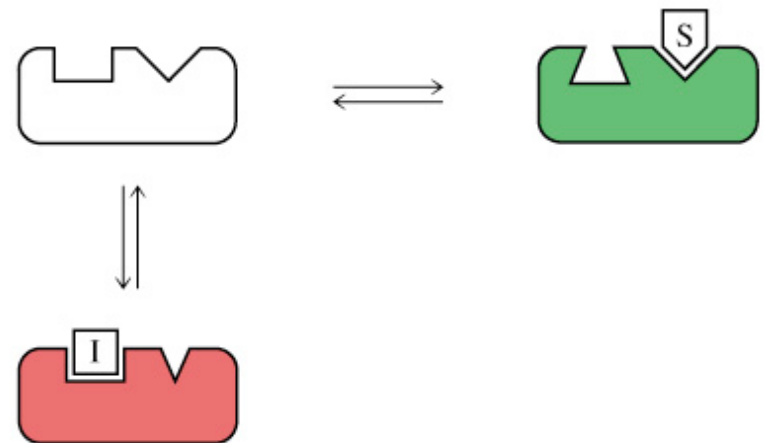
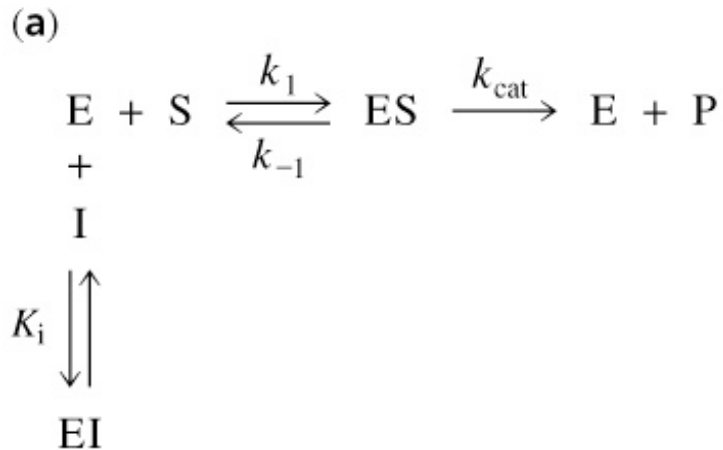


Fig 5.9 Competitive inhibition.

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



Apparent is increased
 stays the same

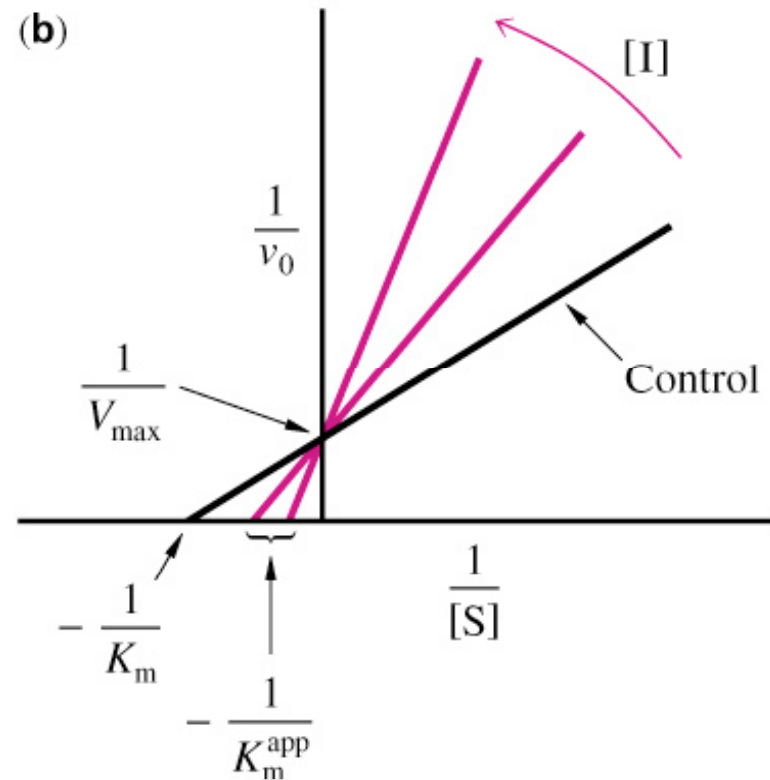
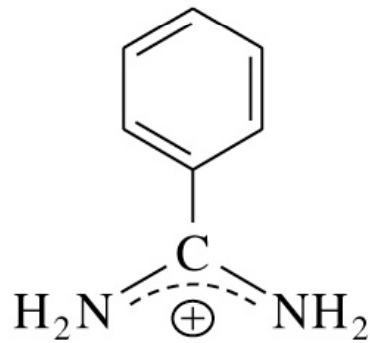
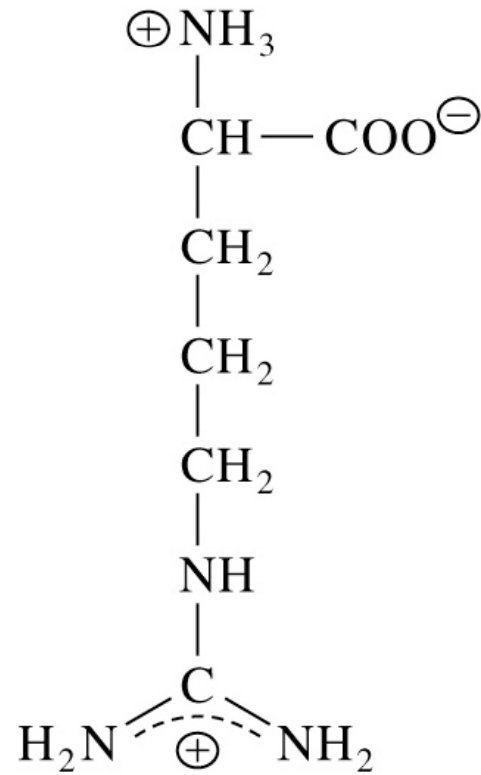


Fig 5.10 Benzamidine competes with arginine for binding to trypsin

Example of a competitive inhibitor



Benzamidine

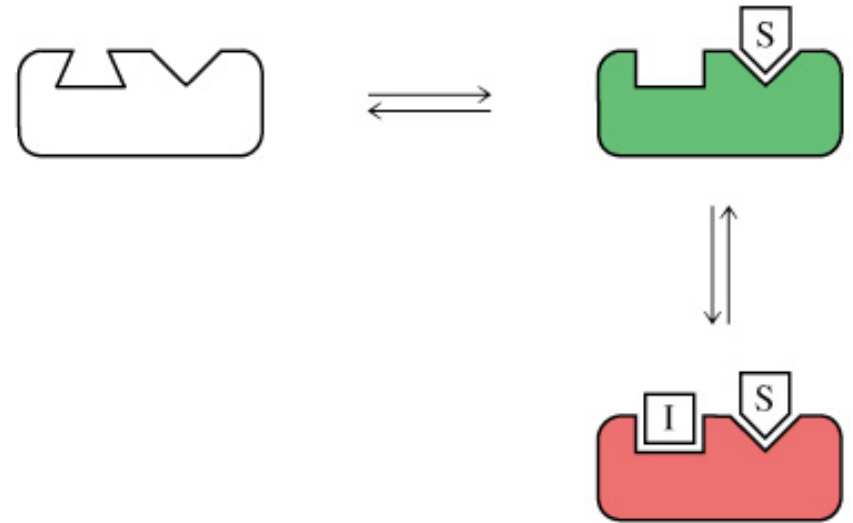


Arginine

Fig 5.8 (cont)

(c) _____
I binds only to ES
(inactivates E)

(c) Uncompetitive



(d) _____
I binds to either E or
ES to inactivate the
enzyme

(d) Noncompetitive

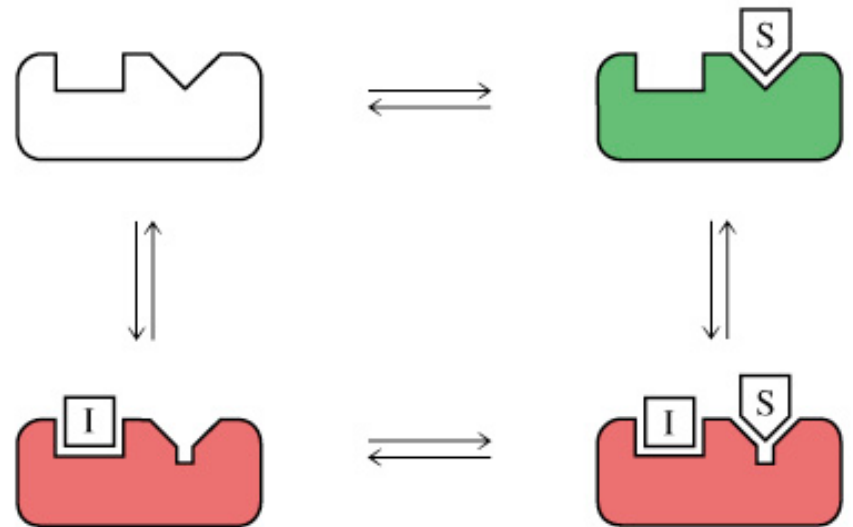
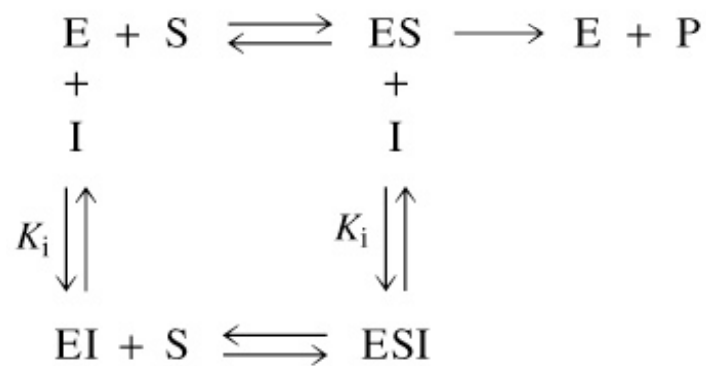
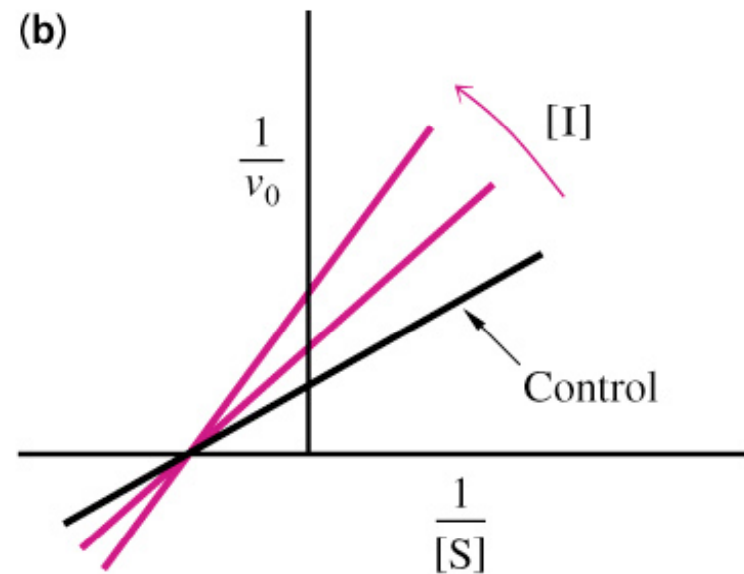


Fig 5.12 Noncompetitive inhibition

(a)



(b)



Apparent _____ stays the same

_____ is decreased

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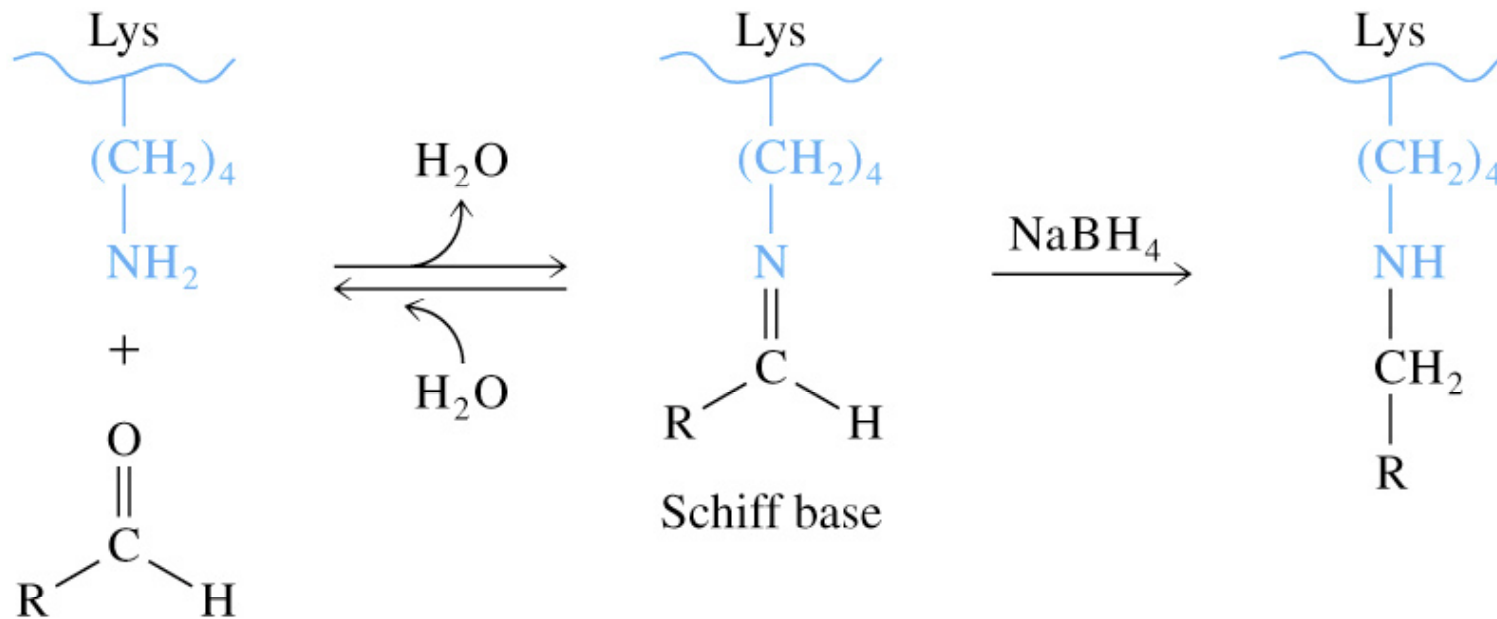
- **Michaelis-Menten Equation**
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- **Reversible Inhibition**
- *Irreversible Inhibition*

Irreversible Enzyme Inhibition

- Irreversible inhibitors form stable covalent bonds with the enzyme
- Irreversible inhibitors can be used to identify the amino acid residues at enzyme active sites
- Incubation of inhibitor with enzyme results in loss of activity

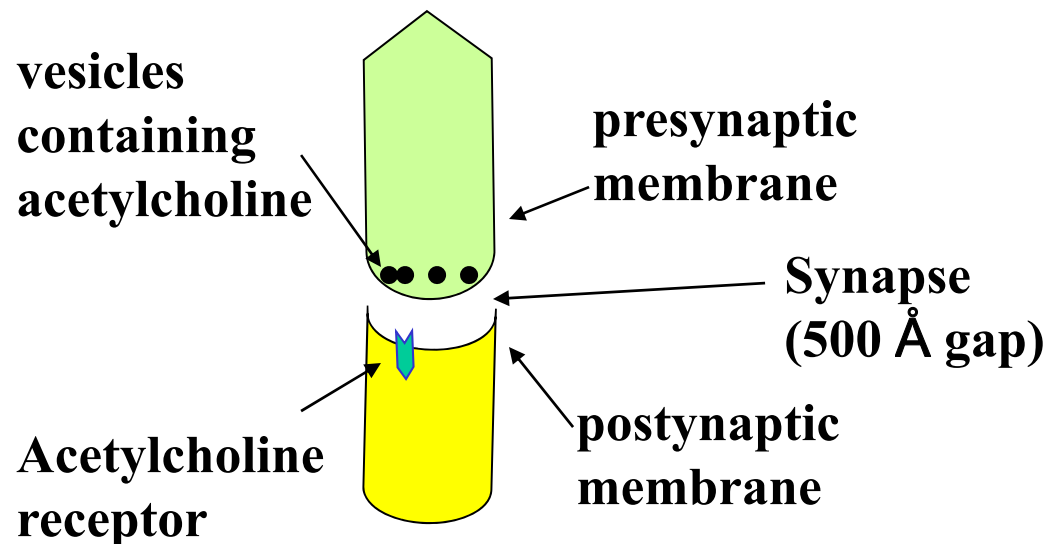
Fig 5.14 Covalent complex with lysine residues

- Reduction of a Schiff base forms a stable substituted enzyme



Example of irreversible inhibition

Inhibition of acetylcholine esterase (enzyme that hydrolyses the neurotransmitter acetylcholine & restores polarization of a nerve).

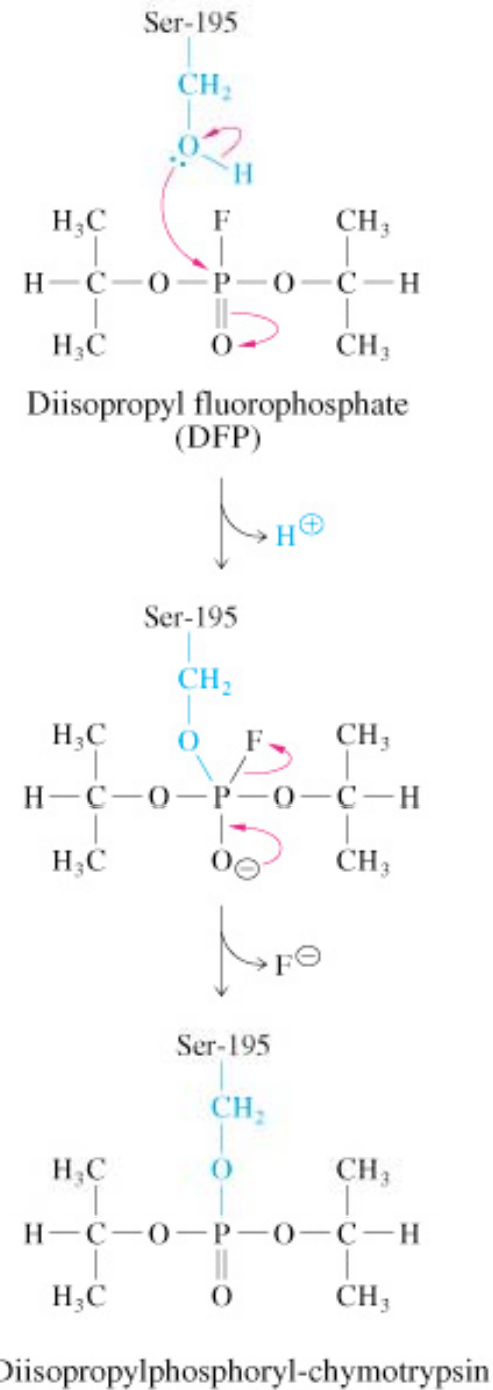


Nerve gases are organic phosphate compounds (e.g. DFP) that form stable phosphoryl-enzymes complexes by reacting with the OH of the active-site Ser. They kill by causing respiratory paralysis.

Fig 5.15

- Reaction of DFP with Ser-195 of chymotrypsin

DFP = diisopropyl fluorophosphate



Affinity labels for studying enzyme active sites

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

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- **Lineweaver-Burk Plot**
- **Kinetics of Multisubstrate Reactions**
- **Reversible Inhibition**
- **Irreversible Inhibition**
- *Regulatory Enzymes*

General Features of Regulatory Enzymes (1)

1. **Regulatory compounds (_____)** bind regulatory sites separate from catalytic sites. They can be activators or inhibitors.
2. **Allosteric modulators** bind noncovalently, may alter K_m or V_{max} , and are **NOT** chemically altered by the enzyme they regulate
3. **Catalytic and regulatory sites are different**

General Features of Regulatory Enzymes (2)

4. v_0 vs [S] curve for regulatory enzymes is often sigmoidal, suggesting cooperativity of S binding

5. Most regulatory enzymes have 4° structure. Subunits may be identical or different

Levels of enzyme regulation

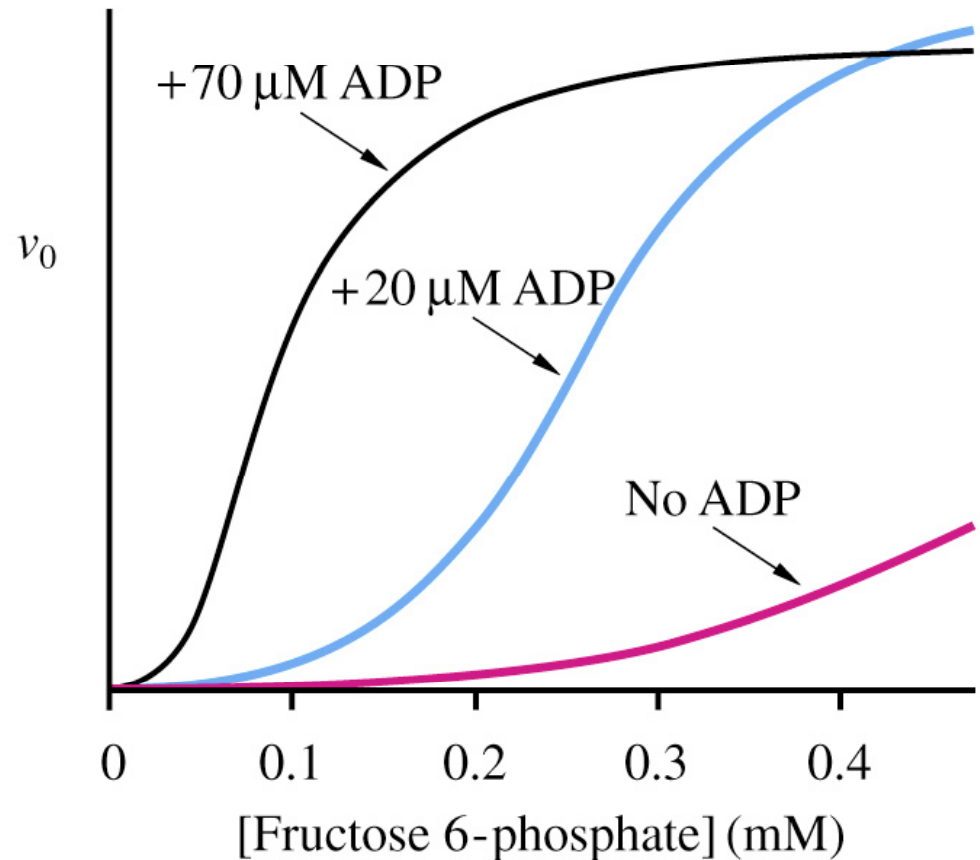
1. allosteric R \rightarrow T transition (fastest)

2. covalent modification of enzyme (e.g. phosphorylation)

3. regulation of enzyme synthesis or degradation (slowest)

Plots of initial velocity versus F6P for PFK-1 (phosphofructoskinase-1)

- ADP is an allosteric activator of PFK-1 and lowers the apparent K_m without affecting V_{max}
- For a given F6P concentration the v_o is larger in the presence of ADP



See Fig. 5.21 and pgs. 149-150

Example of regulation of enzyme activity by covalent modification of enzyme (e.g. phosphorylation)

Fig 5.25 Pyruvate dehydrogenase regulation

- **Phosphorylation** stabilizes the **inactive state** (red)
- **Dephosphorylation** stabilizes the **active state** (green)

