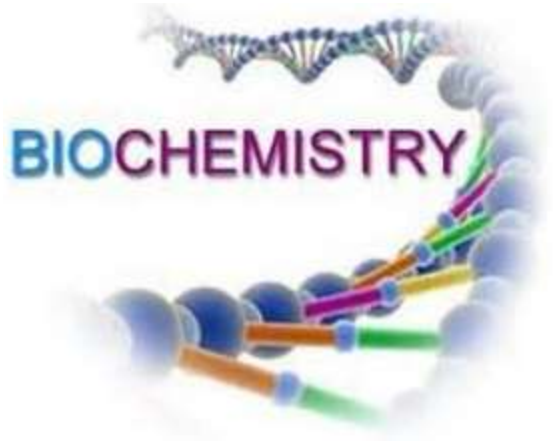
CHAPTER 6

Enzymes

6.1 An Introduction to Enzymes
6.2 How Enzymes Work
6.3 Enzyme Kinetics



6.1 An Introduction to Enzymes

■ What is enzyme（酶）?

Enzymes are catalysts (most are proteins) that catalyze chemical reactions in biological systems.

The enzyme reaction:

6.1 An Introduction to Enzymes

■ Why Enzymes?

- ✓ **Natural catalysts**
- ✓ **Speed: 10^{16} over un-catalyzed rates!**
- ✓ **Specificity: only the desired reaction occurs**
- ✓ **Permit reactions under mild conditions**

6.1 An Introduction to Enzymes

■ Chemistry composing of enzyme

- Simple proteins: Urease, protease, amylase, lipase, Rnase
- Conjugated proteins:

Holoenzyme (全酶）
- Apoenzyme (脱辅酶)
- Cofactor (辅因子)

TABLE 6–1 Some Inorganic Ions That Serve as Cofactors for Enzymes

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

6.1 An Introduction to Enzymes

- **Coenzymes function as transient carriers of specific functional groups.**

TABLE 6–2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

6.1 An Introduction to Enzymes

➢ Enzyme nomenclature and classification

■ Conventional nomenclature

1. Base on the substrate：Adding the suffix –ase to the name of the substrates (urease, protease, amylase, etc.)
2. Base on the reaction: Adding the suffix –ase to a descriptive term for the reactions they catalyze (hydrolase, transferase, kinase, phosphatase, etc.)
3. Base on the substrate and reaction: (succinate dehydrogenase, glutamate dehydrogenase, protein kinase, etc.)
4. Base on the condition and reaction: (alkaline phosphatase, acid phosphatase, etc.)
5. Base on the source: (pepsin, trypsin, etc.)
6. Base on the gene name: Being named after their gene names (Rec A, Src, etc.)

6.1 An Introduction to Enzymes

■ Systematic nomenclature

- The International Union of Biochemistry and Molecular Biology (IUBMB) maintains the classification scheme.
- Categorize in to **6** classes according to the general class of organic reactions catalyzed
- Assigned a unique number, a systematic name, a shorter common name to each enzyme

TABLE 6–3 International Classification of Enzymes

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

6.1 An Introduction to Enzymes

1. Oxidoreductases（氧化还原酶类）

Catalyzing a variety of oxidation-reduction reactions

$$AH_2 + B \rightarrow A + BH_2$$

Alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1.)

Cytochrome oxidase

L- and D-amino acid oxidase

6.1 An Introduction to Enzymes

2. Transferases（转移酶类）

Catalyzing transfer of a groups between donors and acceptors

$$A\text{-}X + B \rightarrow A + B\text{-}X$$

Hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1.)

Transaminase

Transmethylases

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3. Hydrolases（水解酶类）

Catalyzing cleavage of bonds by addition of water

$$A\text{-}B + H_2O \rightarrow AH + BOH$$

Lipase (triacylglycerol acyl hydrolase, EC 3.1.1.3.)

Choline esterase

Acid and alkaline phosphatases

Urease

6.1 An Introduction to Enzymes

4. Lysases（裂合酶类）

Catalyzing lysis of a substrate and generating a double bond (nonhydrolytic, and non-oxidative reactions)

A-B → A + B

Aldolase (ketose 1-phosphate aldehyde lysase, EC 4.1.2.7.)

Fumarase

Histidase

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5. **Isomerases**（异构酶类）

Catalyzing racemization of optical or geometric isomers

A → A'

Triose phosphate isomerase (D-glyceraldehyde 3-phosphate ketoisomerase, E.C. 5.3.1.1.)

Retinol isomerase

Phosphohexose isomerase

6.1 An Introduction to Enzymes

6. Ligases（连接酶类）

Catalyzing synthetic reactions at the expense of a high energy bond of ATP

$$\mathbf{A + B \rightarrow A\text{-}B}$$

Glutamine synthetase (L-glutamate ammonia ligase, E.C. 6.3.1.2.)

Acetyl CoA carboxylase

Succinate thiokinase

Ubiquitin ligases

6.1 An Introduction to Enzymes

- **Classification number**

E.C. Class.Subclass.Subsubclass.Individual entry

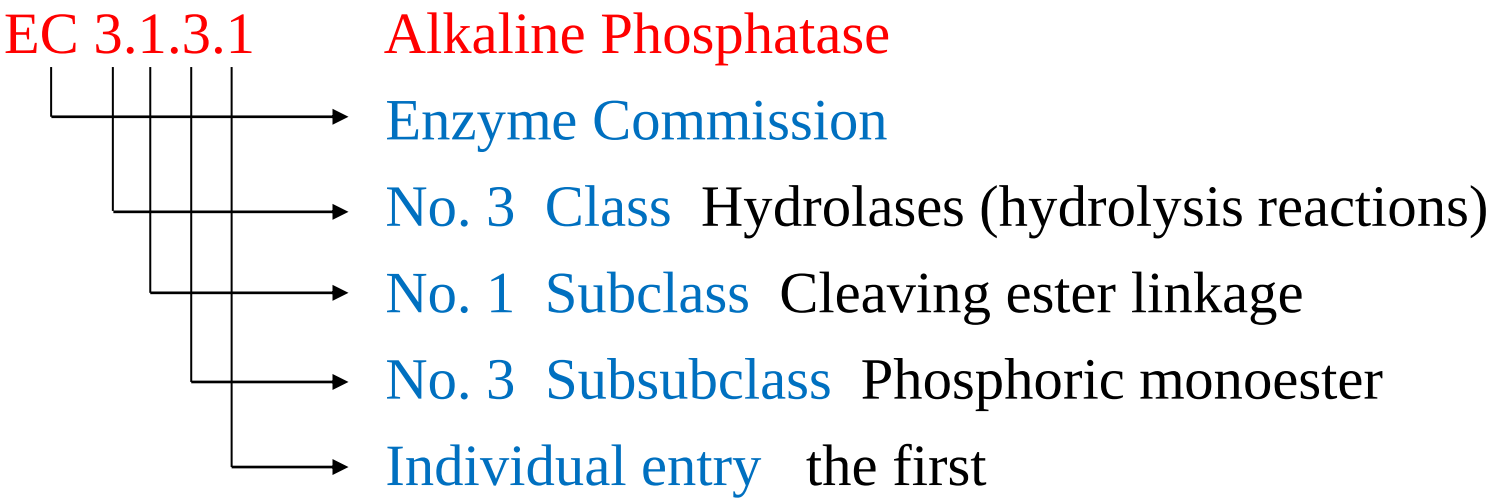
EC (Enzyme Commission)

Six Class 1、2、3、4、5、6

Subclass 1、2、3、4.....

Subsubclass 1、2、3、4.....

Individual entry 1、2、3、4...

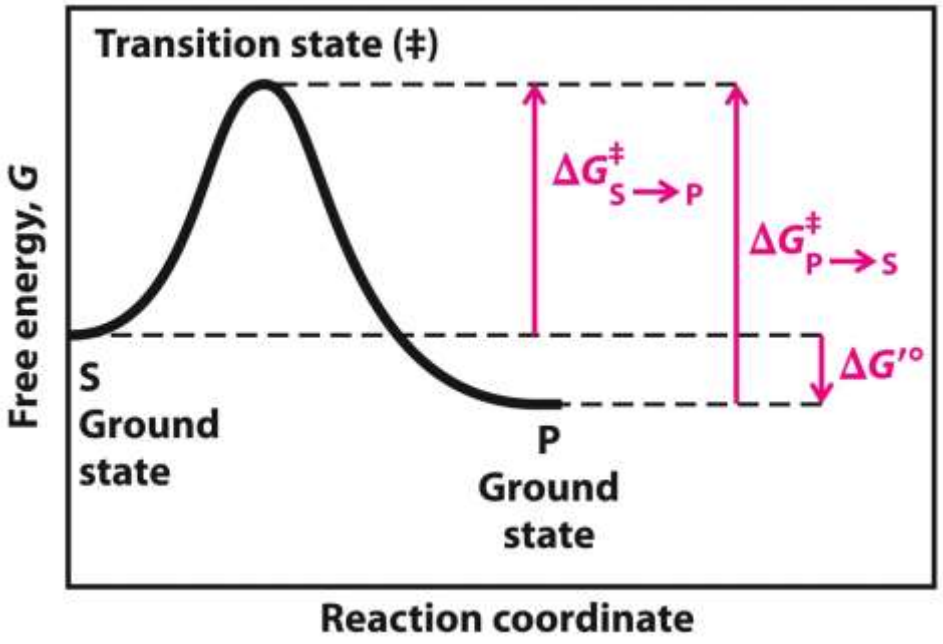


6.2 How Enzymes Work

➢ Characteristics of enzyme-catalyzed reactions

1. The common characteristics of enzymes and other catalyzers

- Accelerate chemistry reaction (thermodynamic allowable reaction）
- Reduced the activation energy
- Do not change the equilibrium constant
- Do not consume themselves



6.2 How Enzymes Work

2. Distinctive features

- High catalytic power
- High specificity
- Regulation of the enzyme activity
- Mildness of the action condition

6.2 How Enzymes Work

- **High catalytic power**

TABLE 6–5 **Some Rate Enhancements Produced by Enzymes**

Cyclophilin	10^5
Carbonic anhydrase	10^7
Triose phosphate isomerase	10^9
Carboxypeptidase A	10^{11}
Phosphoglucomutase	10^{12}
Succinyl-CoA transferase	10^{13}
Urease	10^{14}
Orotidine monophosphate decarboxylase	10^{17}

Table 6-5
Lehninger Principles of Biochemistry, Sixth Edition
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6.2 How Enzymes Work

- **High specificity**

Enzymes demonstrate the ability to distinguish different substrates.

- Structural specificity
  - absolute specificity: urease
  - relative specificity
    - group specificity
    - bond specificity
- Stereo isomer specificity
  - optical isomer specificity
  - geometrical isomer specificity

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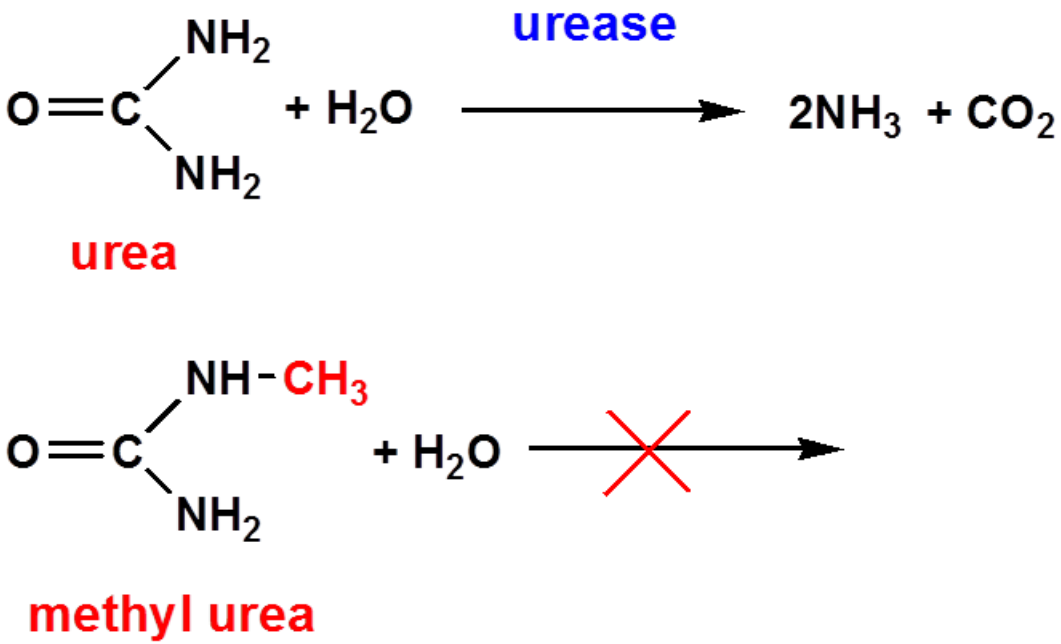
Absolute specificity: Enzymes can recognize only one type of substrate and implement their catalytic functions.



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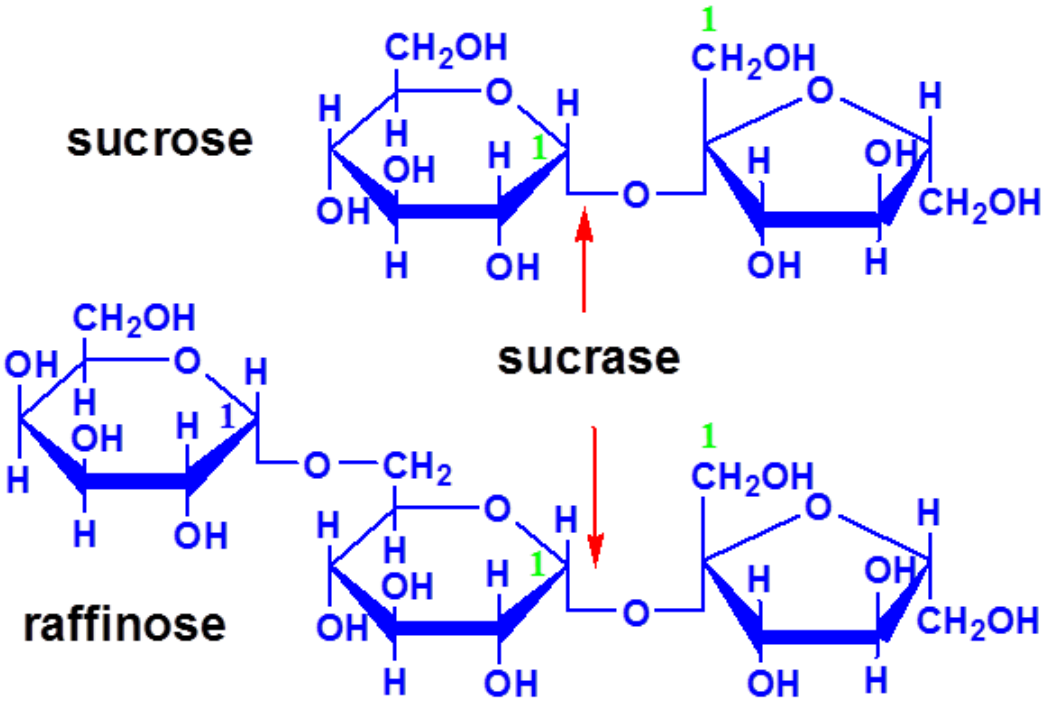
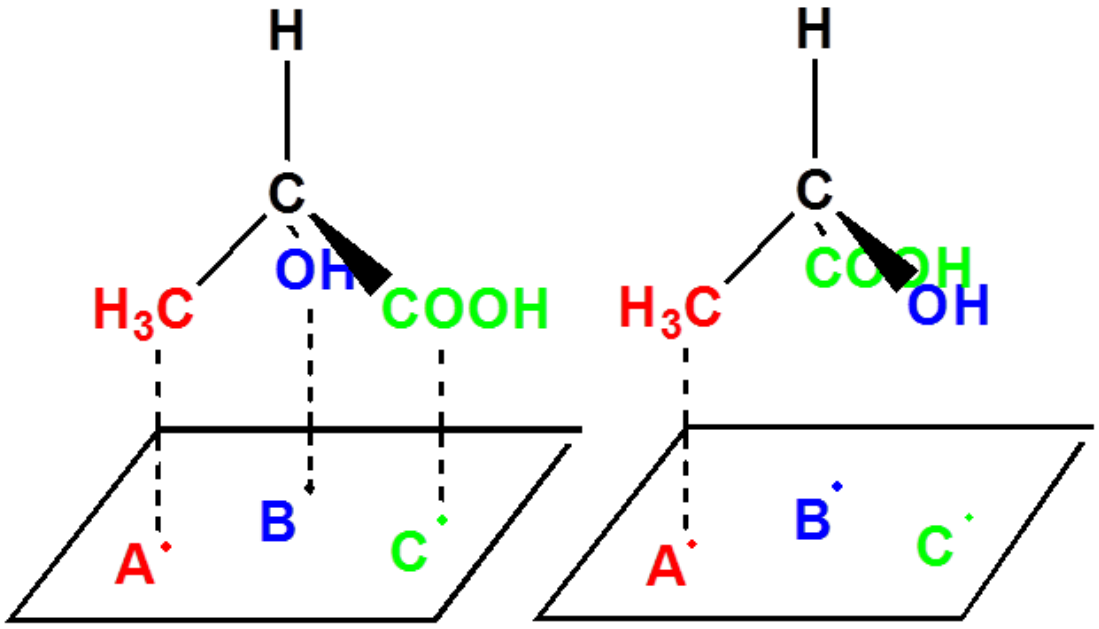
Relative specificity: Enzymes catalyze one class of substrates or one kind of chemical bond in the same type.



6.2 How Enzymes Work

Stereospecificity: The enzyme can act on only one form of isomers of the substrates



Lactate dehydrogenase can recognize only the L-form but the D-form lactate.

6.2 How Enzymes Work

Highly regulated enzymatic reaction

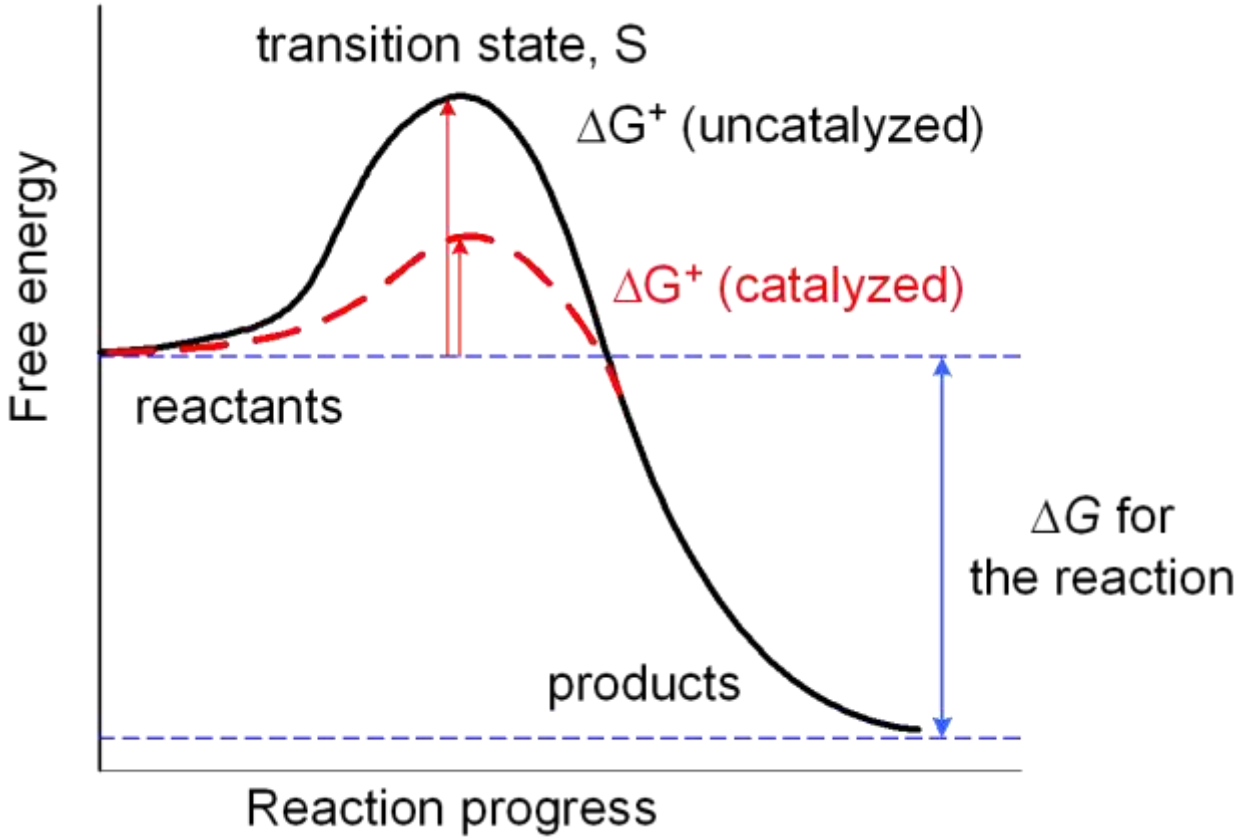
- Enzyme-catalyzed reactions can be regulated in response to the external stimuli, satisfying the needs of biological processes.
- Regulations can be accomplished through varying the enzyme quantity, adjusting the enzymatic activity, or changing the substrate concentration.

Mild action condition

Act in biological system

6.2 How Enzymes Work

- **Mechanism of enzyme-catalyzed reactions**



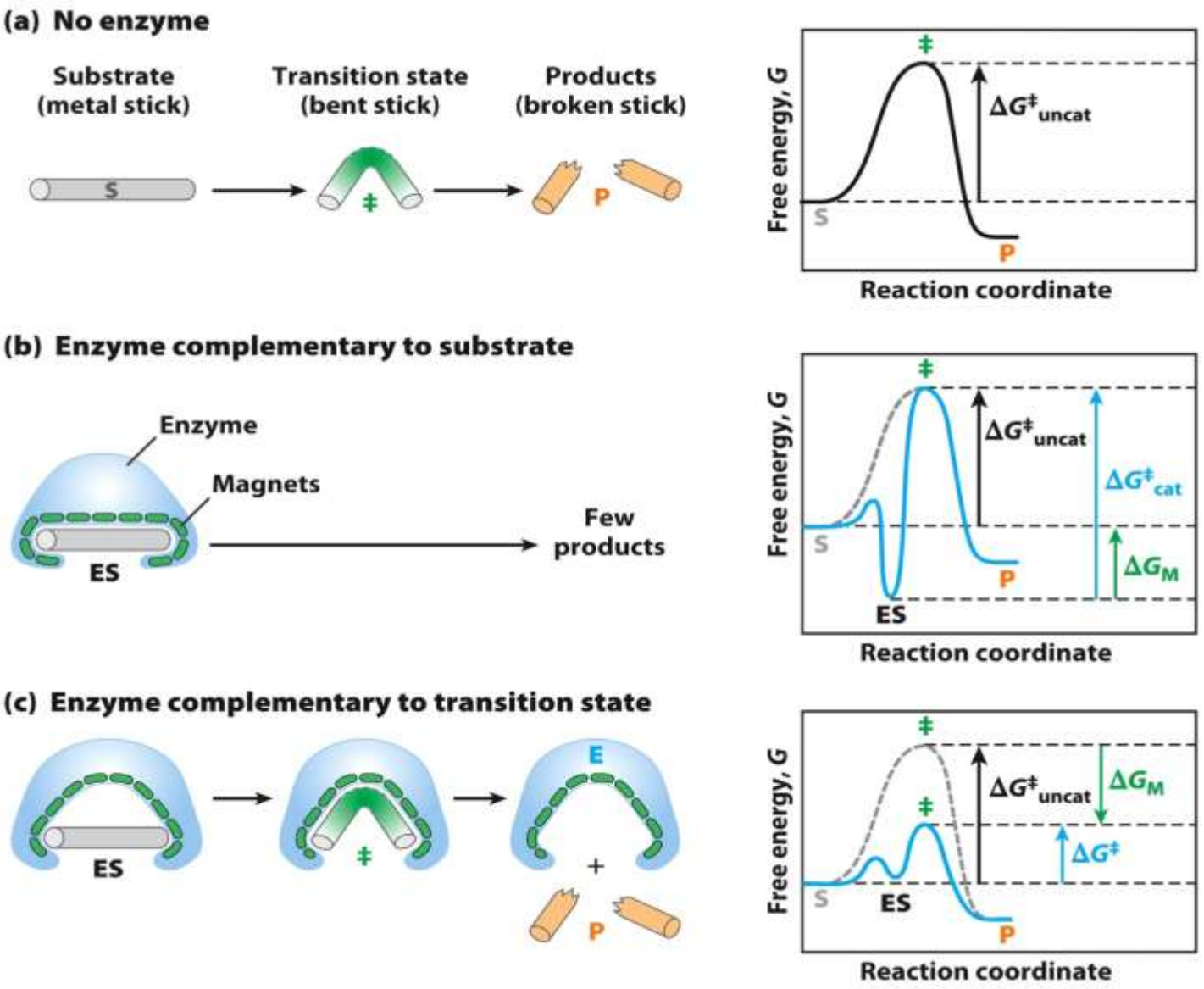
ΔG^{+}: activation energy

6.2 How Enzymes Work

Intermediate state

Forming an enzyme-substrate complex, a transition state, is a key step in the catalytic reaction.

6.2 How Enzymes Work



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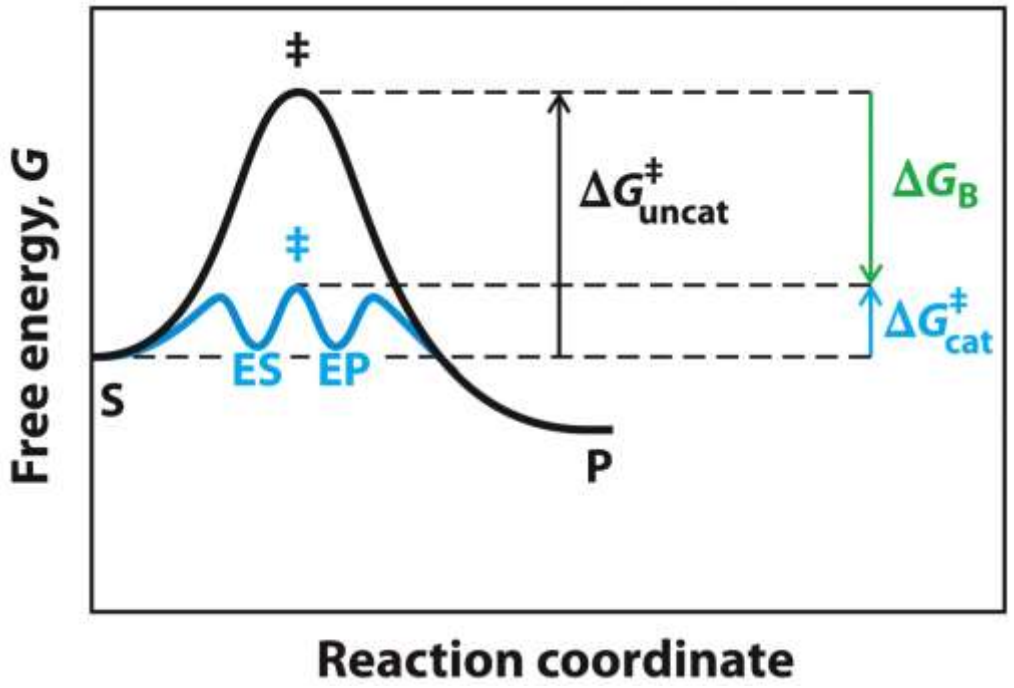
Binding energy in catalysis

The energy derived from enzyme-substrate interaction is called **binding energy**, ΔG_B.



The weak binding interactions between the enzyme and the substrate provide a substantial driving force for enzymatic catalysis.

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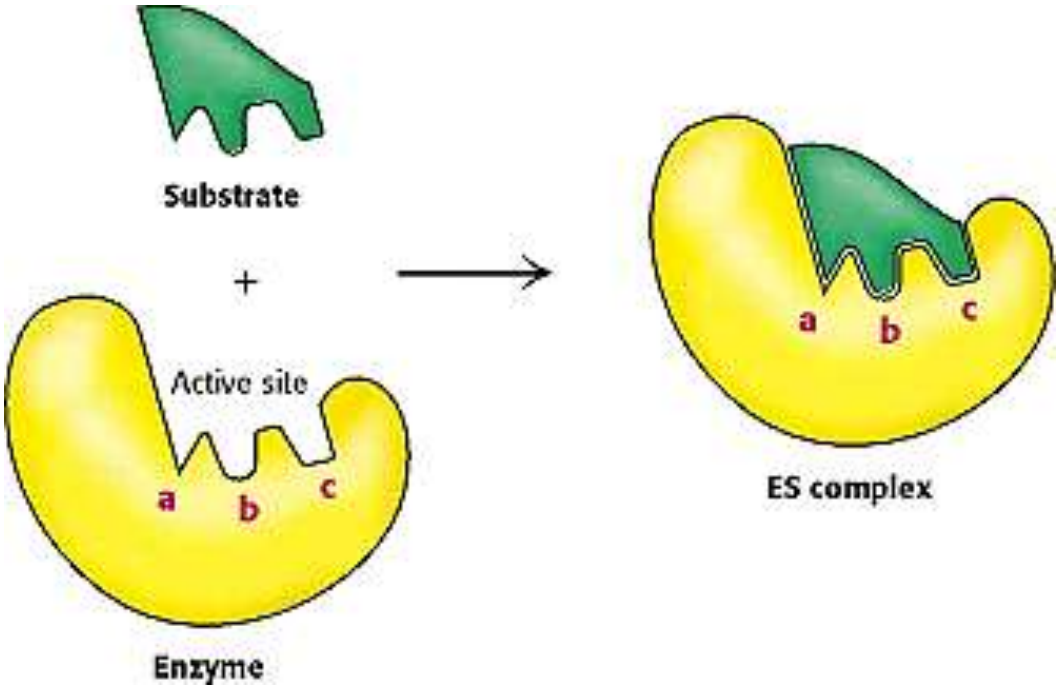
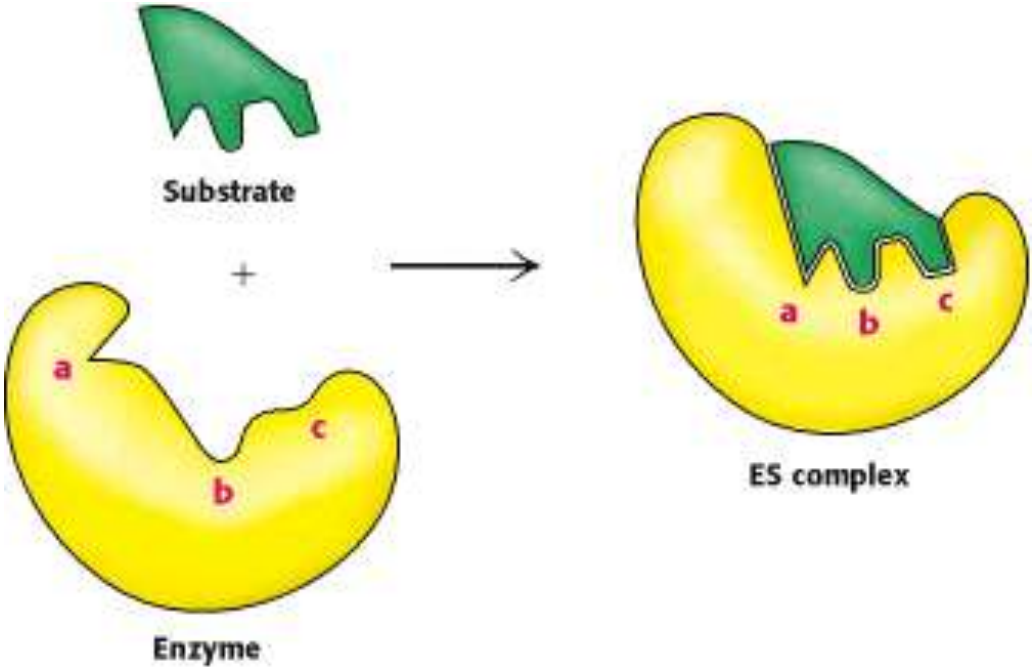
Lock-and-key model



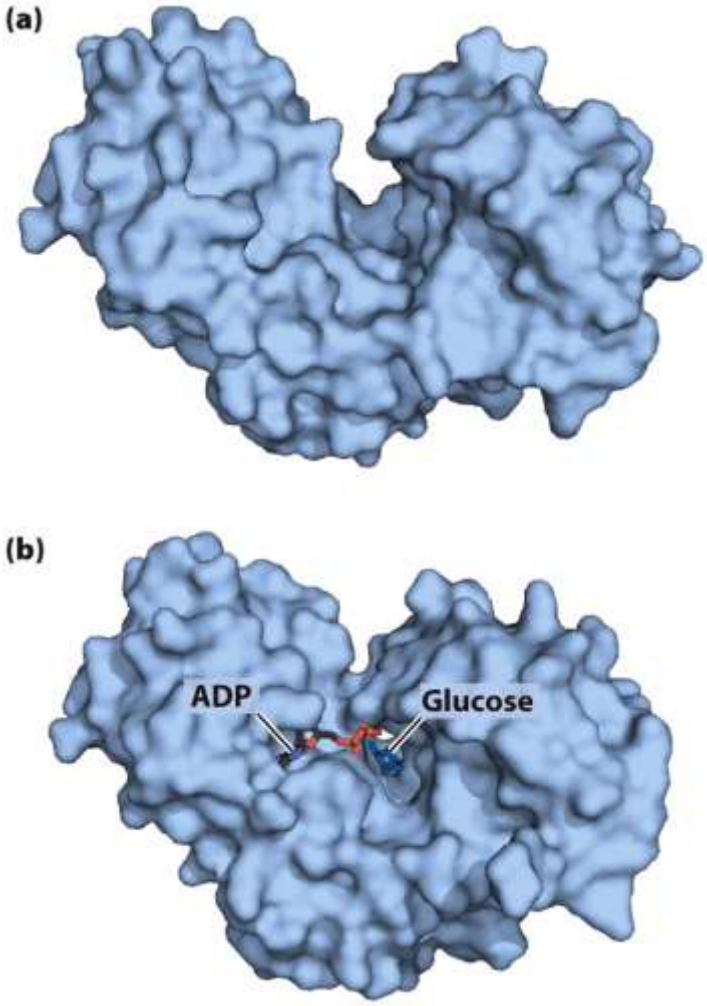
Both E and S are rigid and fixed, so they must be complementary to each other perfectly in order to have a right match.

6.2 How Enzymes Work

Induced-fit model



The binding induces conformational changes of both E and S, forcing them to get a perfect match.

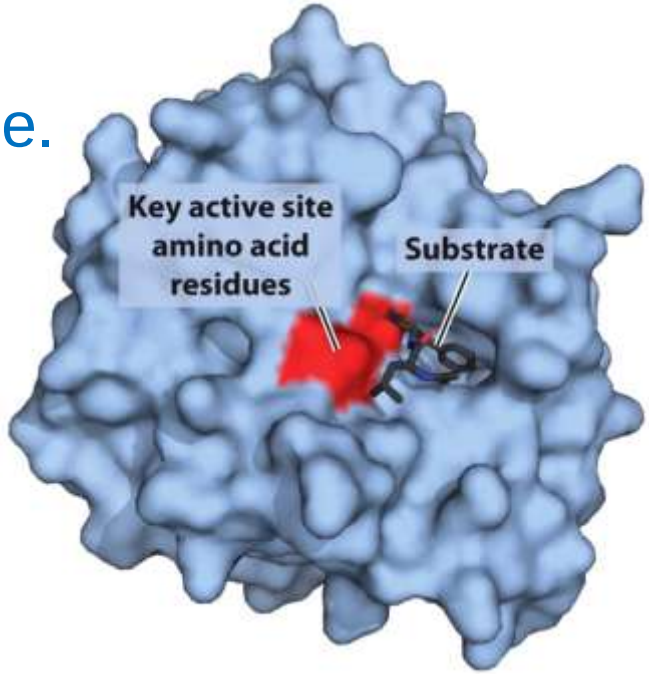


Induced fit in hexokinase

6.2 How Enzymes Work

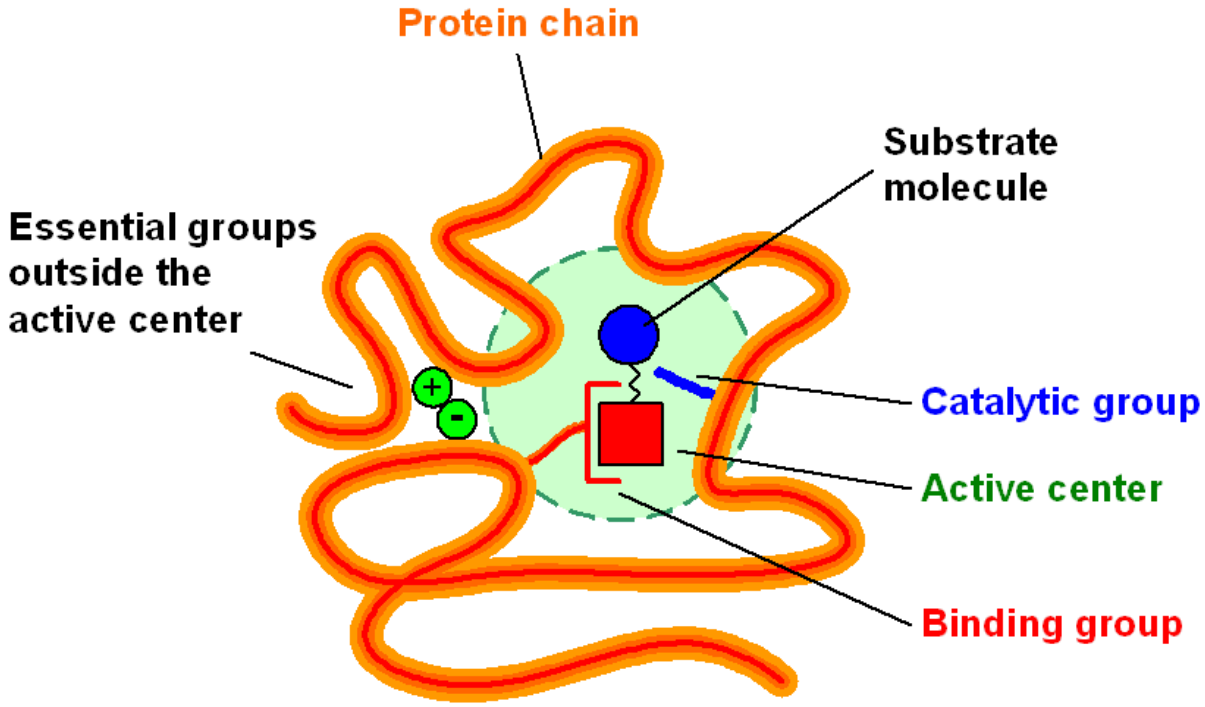
Active site

- Almost all the enzymes are proteins having well defined structures.
- Some functional groups are close enough in space to form a portion called the active site.
- Active sites look like a cleft or a crevice.
- Active sites are hydrophobic.



6.2 How Enzymes Work

- **The active center has two essential groups in general**
  1. **Binding group:** to associate with the reactants to form an enzyme-substrate complex
  2. **Catalytic group:** to catalyze the reactions and convert substrates into products



6.2 How Enzymes Work

- **The characteristics of the active center**
  - ✓ It comprises only several amino acids residues and only a small part of overall enzyme structure;
  - ✓ It's a special pocket or cleft with certain three-dimensional structure;
  - ✓ It's complementary to the structure of the substrate, but it's pliable;
  - ✓ Substrate binds to the active site through relatively weak forces;
  - ✓ The enzyme active center is more flexible than its whole conformation.

6.2 How Enzymes Work

➢ Enzymatic activity

- Enzymatic activity is a measure of the capability of an enzyme of catalyzing a chemical reaction.
- It directly affects the reaction rate.
- International unit (IU): the amount of enzyme required to convert 1 μmol of substrate to product per minute under a designated condition.
- Determination of the enzymatic activity requires proper treatment of enzymes, excess amount of substrate, optimal temperature and pH, …

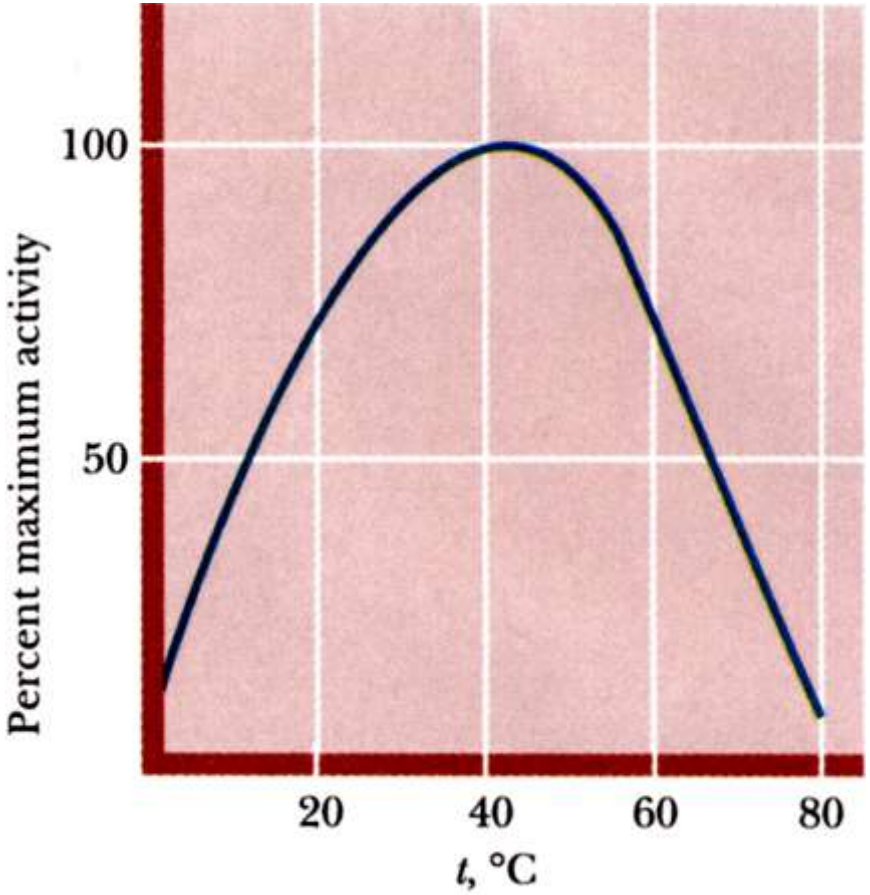
6.2 How Enzymes Work

- **Factors affecting enzyme activity**
  - Temperature
  - pH
  - Activators
  - Inhibitors
  -
  -

6.2 How Enzymes Work

■ Effect of temperature

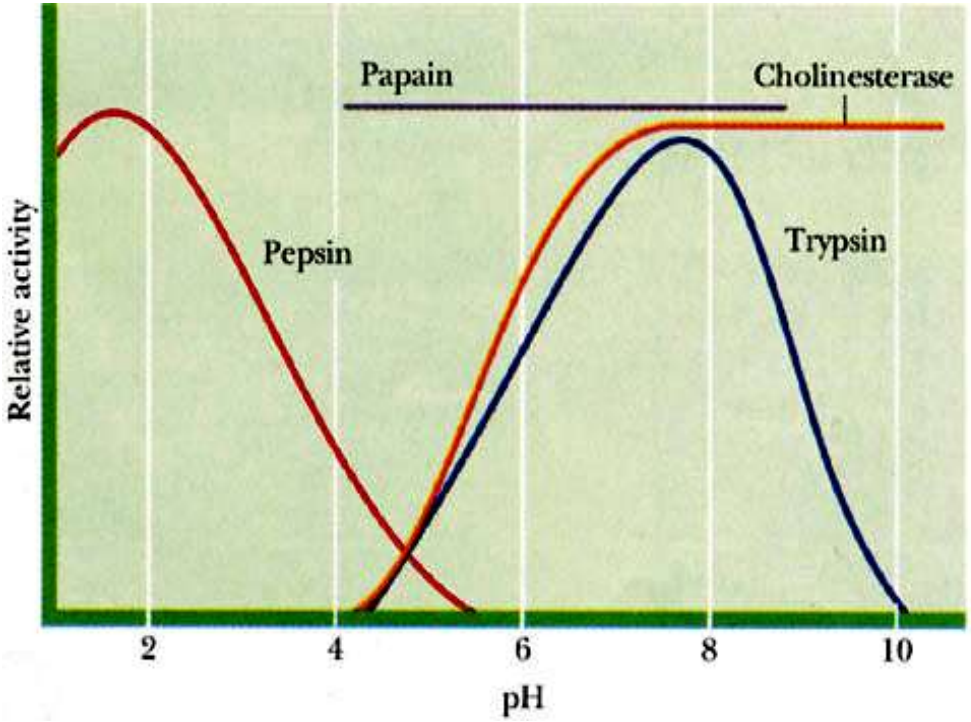
- Optimal temperature (T_o) is the characteristic T at which an enzyme has the maximal catalytic power.
- 35 ~ 40°C for warm blood species.
- Reaction rates increase by 2 folds for every 10°C rise.
- Higher T will denature the enzyme.



6.2 How Enzymes Work

Effect of pH

Optimal pH is the characteristic pH at which the enzyme has the maximal catalytic power.



Optimum pH of Some Enzymes	
Enzyme	Optimum pH
Pepsin	1.5
Catalase	7.6
Trypsin	7.7
Fumarase	7.8
Ribonuclease	7.8
Arginase	9.7

6.2 How Enzymes Work

Activator

Activators are the compounds which bind to an enzyme or an enzyme-substrate complex to enhance the enzymatic activity without being modified by the enzymes.

Inhibitors

- Inhibitors are certain molecules that can decrease the catalytic rate of an enzyme-catalyzed reaction.
- Inhibitors can be normal body metabolites and foreign substances (drugs and toxins).

6.2 How Enzymes Work

Inhibition processes

- The inhibition process can be either irreversible or reversible.
- The reversible inhibition can be competitive, non-competitive, or un-competitive.

6.2 How Enzymes Work

Regulation of enzyme

- Many biological processes take place at a specific time; at a specific location and at a specific speed.
- The catalytic capacity is the product of the enzyme concentration and their intrinsic catalytic efficiency.
- The key step of this process is to regulate either the enzymatic activity or the enzyme quantity.

6.2 How Enzymes Work

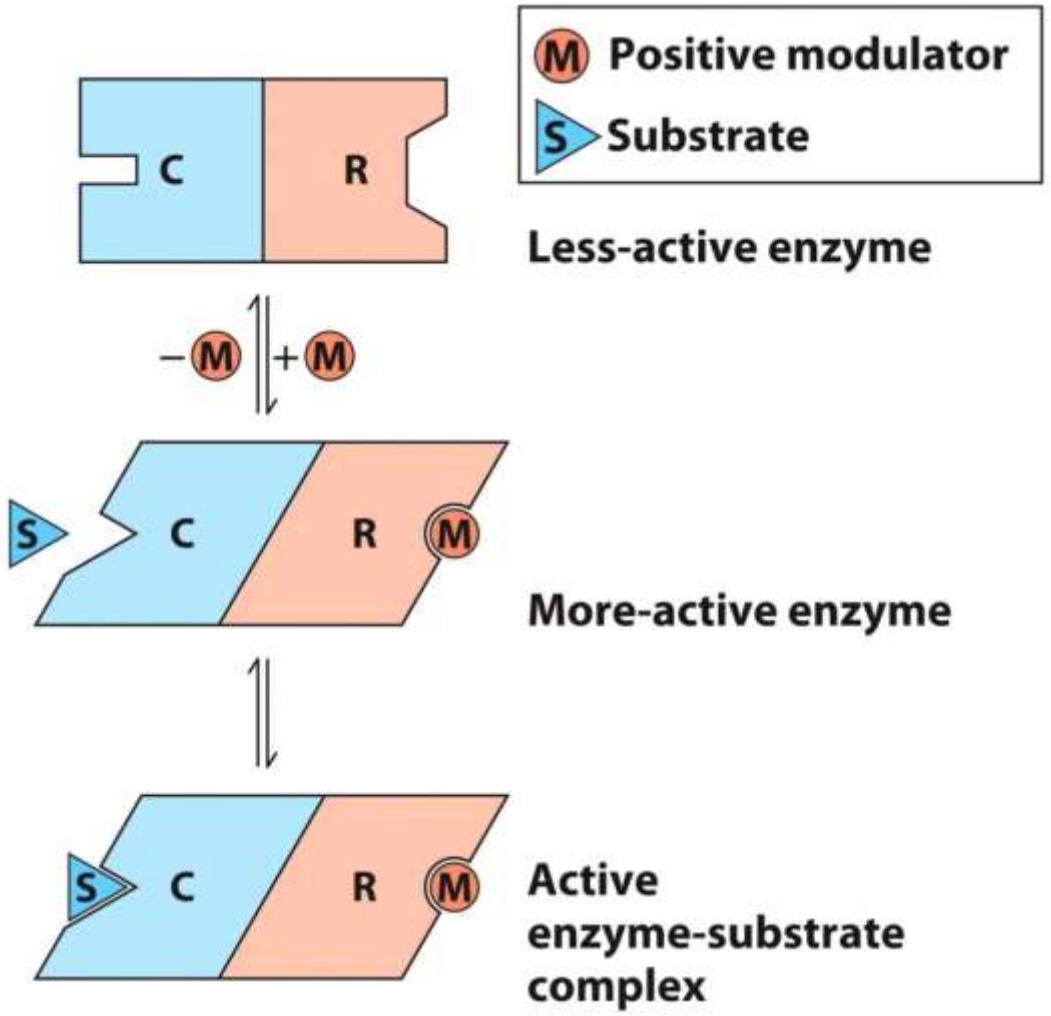
- **Reasons for regulation**
  - ✓ Maintenance of an ordered state in a timely fashion and without wasting resources
  - ✓ Conservation of energy to consume just enough nutrients
  - ✓ Rapid adjustment in response to environmental changes
  - ✓ Controlling an enzyme that catalyzes the rate-limiting reaction will regulate the entire metabolic pathway, making the biosystem control more efficient.

6.2 How Enzymes Work

■ Regulatory enzymes

Allosteric enzyme
（别构酶）

Function through reversible, noncovalent binding of regulatory compounds (allosteric modulators), which are generally small metabolites or cofactors.



6.2 How Enzymes Work

Covalent modification

Reversible

Phosphorylation

Acetylation

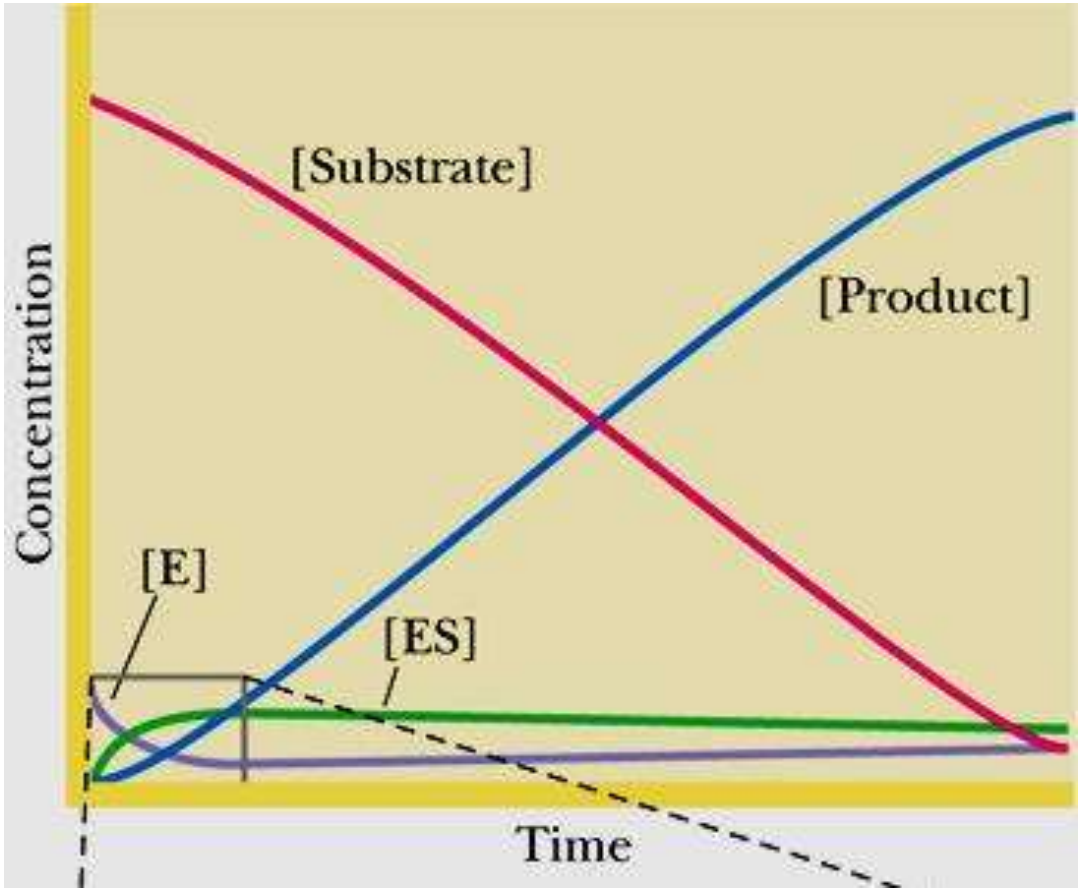
…..

Irreversible

Cleavage of enzyme precursor

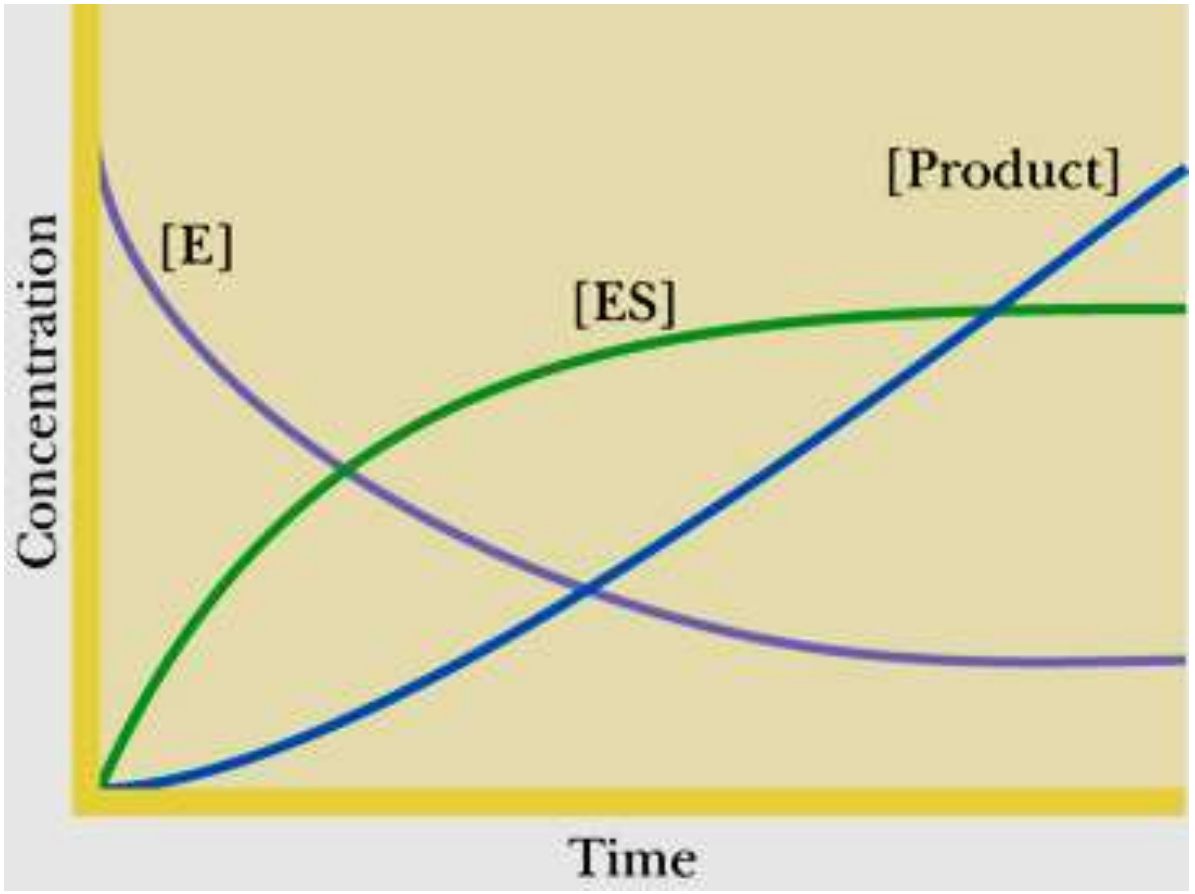
6.3 Enzyme Kinetics

- **Kinetics of enzyme-catalyzed reactions**
  - **Time course of an enzyme-catalyzed reaction**



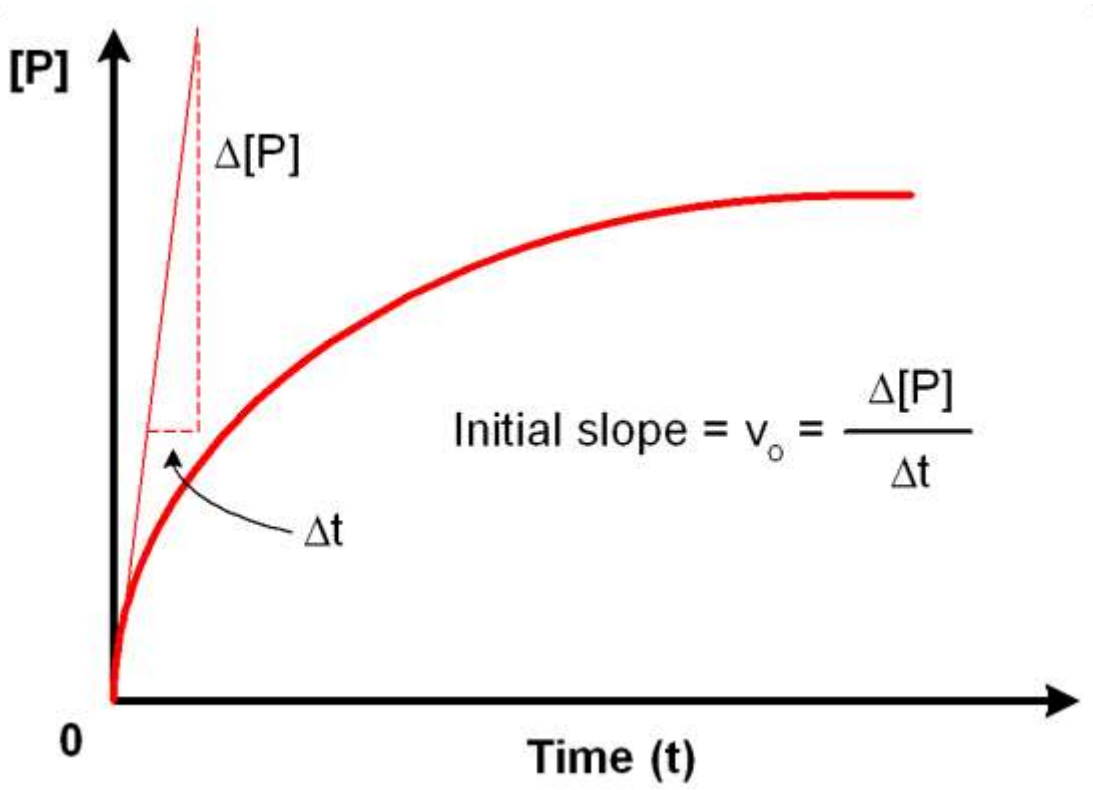
6.3 Enzyme Kinetics

- **Early stage of an enzyme-catalyzed reaction**



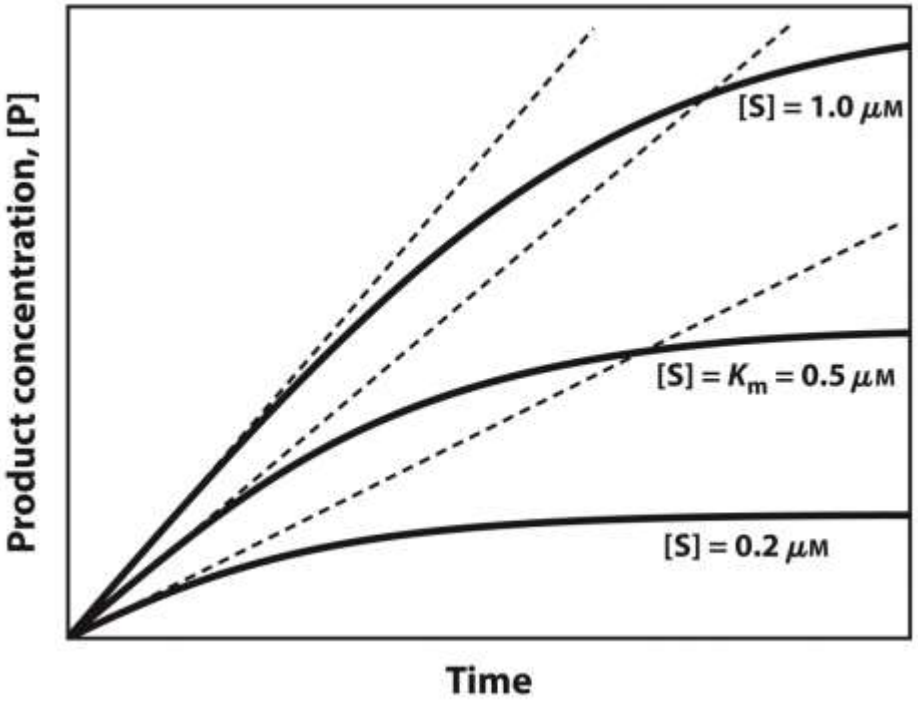
6.3 Enzyme Kinetics

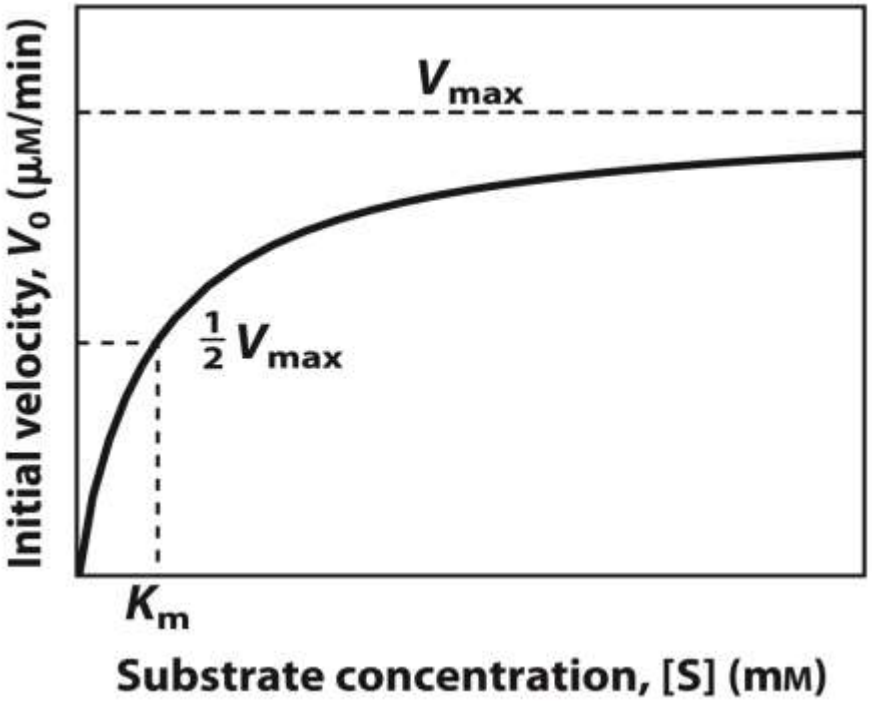
- **Reaction rate**



6.3 Enzyme Kinetics

- **Reaction velocity curve**





V_0: initial velocity (rate)
V_{max}: maximum velocity
[S]: concentration of substrate
[P]: concentration of product

6.3 Enzyme Kinetics

Intermediate state

Forming an enzyme-substrate complex, a transition state, is a key step in the catalytic reaction.

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

initial **intermediate** **final**

k_1 : rate constant for ES formation

k_{-1} : rate constant for ES dissociation

k_2 : rate constant for the product released from the active site

k_{-2} : rate constant for the reverse reaction forming EP

6.3 Enzyme Kinetics

- **Three assumptions for Michaelis-Menten equation**

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

1. **Initial velocity assumption**: the rate of any back reaction of E + P is negligible, and [P] is essentially 0.
2. **$[S]_0 >> [E]_0$, $[S] \approx [S]_0$**
3. **Equilibrium method**：The enzyme (E) and its substrate (S) associate reversibly to form an enzyme-substrate complex (ES).

6.3 Enzyme Kinetics

Steady-state assumption: The rate of formation of ES is equal to the rate of its breakdown

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

Total enzyme concentration: $[E_t] = [E] + [ES]$

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

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

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

6.3 Enzyme Kinetics

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

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

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

Define $(k_{-1} + k_2)/k_1$ as Michaelis constant K_m

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

$[ES] = [E_t][S] /(K_m + [S])$

$V_0 = k_2 [ES] = k_2[E_t][S] /(K_m + [S])$

$$V_0 = \frac{k_2[E_t][S]}{K_m + [S]}$$

$V_{max} = k_2[E_t]$

$V_0 = V_{max}[S] /(K_m + [S])$

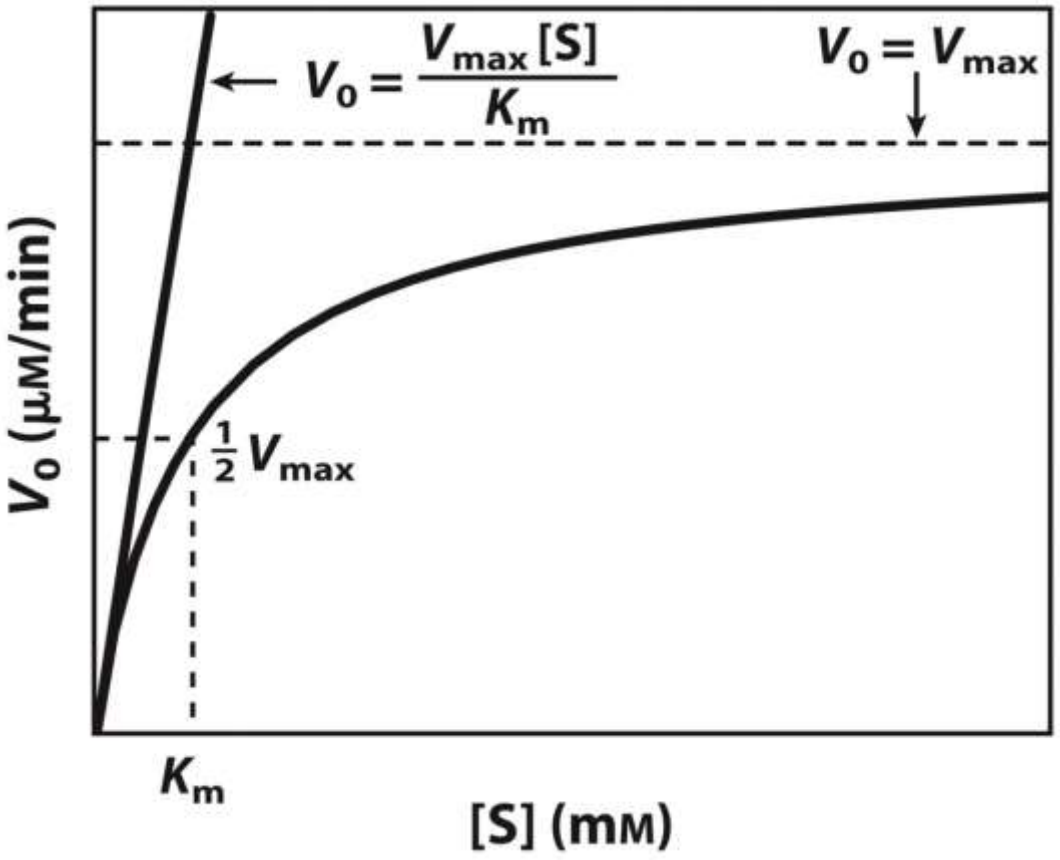
$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

6.3 Enzyme Kinetics

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

$$V_0 = \frac{V_{\max}[\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

$$K_\mathrm{m} = \frac{k_2 + k_{-1}}{k_1}$$



[S] << K_m , $V_0 \propto$ [S]

[S] >> K_m , $V_0 \approx V_{max}$

[S] = K_m , $V_0 = V_{max} / 2$

6.3 Enzyme Kinetics

■ Significance of K_m

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

- K_m is a characteristic constant of enzyme.
- The substrate concentration at which enzyme-catalyzed reaction proceeds at one-half of its maximum velocity
- K_m is independent of [E]. It is determined by the structure of E, the substrate and environmental conditions (pH, temperature, ionic strength, …)
- The value of K_m quantifies the affinity of the enzyme and the substrate under the condition of $k_2 << k_{-1}$. The larger the K_m, the smaller the affinity.

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

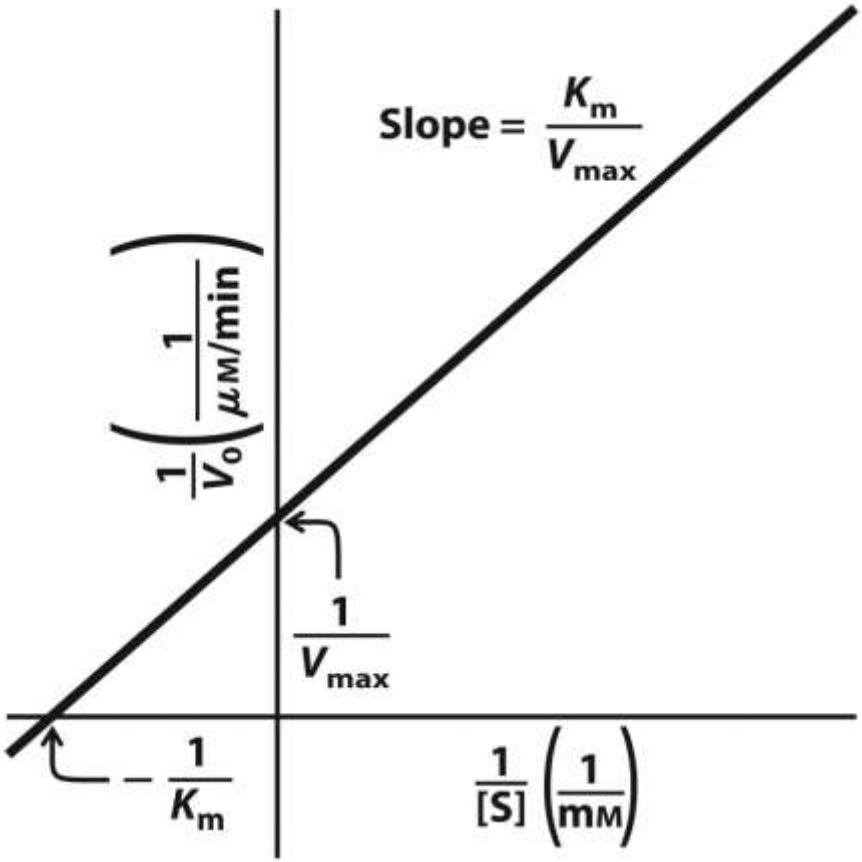
6.3 Enzyme Kinetics

■ Lineweaver-Burk plot

To determine K_m and V_{max}

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$



6.3 Enzyme Kinetics

TABLE 6–6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

Table 6-6
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The K_m can vary greatly from enzyme to enzyme, and even for different substrates of the same enzyme.

6.3 Enzyme Kinetics

■ Turnover number k_{cat}

k_{cat}: The number of substrate molecules converted into product *per enzyme molecule per unit time* when the enzyme is saturated with substrate.

It is referred to the molecular activity of the enzyme.

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

$$k_{cat} = k_2 = V_{max} / [\mathrm{E}]_0$$

$$V_0 = \frac{k_2[\mathrm{E_t}][\mathrm{S}]}{K_m + [\mathrm{S}]} \longrightarrow V_0 = \frac{k_{cat}[\mathrm{E_t}][\mathrm{S}]}{K_m + [\mathrm{S}]}$$

6.3 Enzyme Kinetics

TABLE 6–7 Turnover Number, k_{cat}, of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.5

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k_{cat} is a first-order rate constant, s^{-1}

6.3 Enzyme Kinetics

■ k_{cat}/K_m , specificity constant

The best way to compare the catalytic efficiencies of different enzymes or the turnover of different substrates by the same enzyme is to compare the ratio k_{cat}/K_m for the two reactions, which sometimes is called the **specificity constant**, is the rate constant for the conversion of E+S to E+P.

$$V_0 = \frac{k_{cat}[E_t][S]}{K_m + [S]}$$

When [S] « K_m

$$V_0 = \frac{k_{cat}}{K_m}[E_t][S]$$

V_0 in this case depends on the concentration of two reactants, $[E_t]$ and [S]; therefore this is a second-order rate equation and the constant k_{cat}/K_m is a second-order rate constant with units of $M^{-1}s^{-1}$.

6.3 Enzyme Kinetics

- **Limit of k_{cat}/K_m is the diffusion-controlled rate**

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

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

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

TABLE 6–8 Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

Enzyme	Substrate	K_{cat} (s^{-1})	K_m (M)	K_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1×10^0	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8

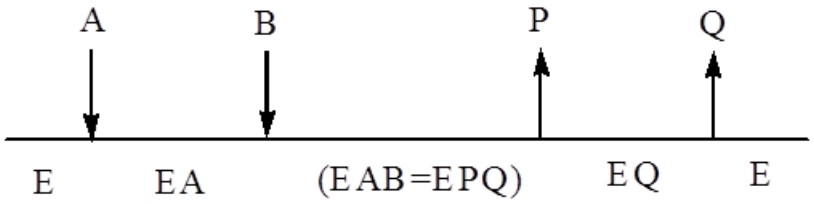
Source: Fersht, A. (1999) *Structure and Mechanism in Protein Science*, p. 166, W. H. Freeman and Company, New York.

6.3 Enzyme Kinetics

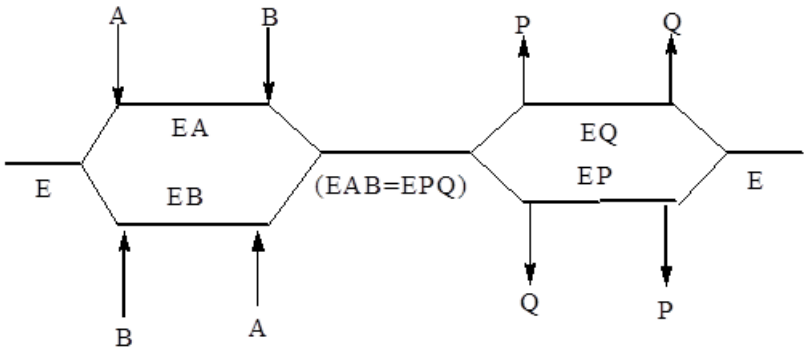
- **Enzyme-catalyzed reactions involving two substrates**

1. Form ternary complex

Ordered reaction:

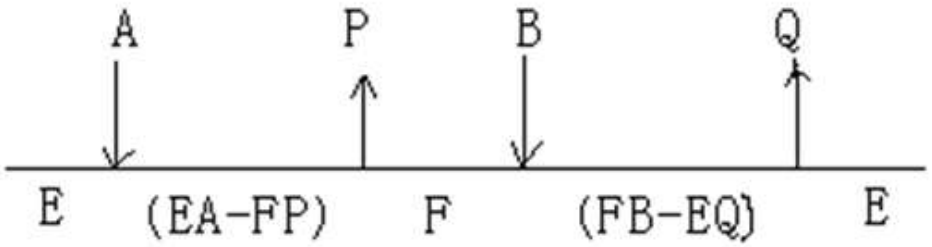


Random reaction:



2. Do not form ternary complex

Ping-Pong reaction:



King-Altman method

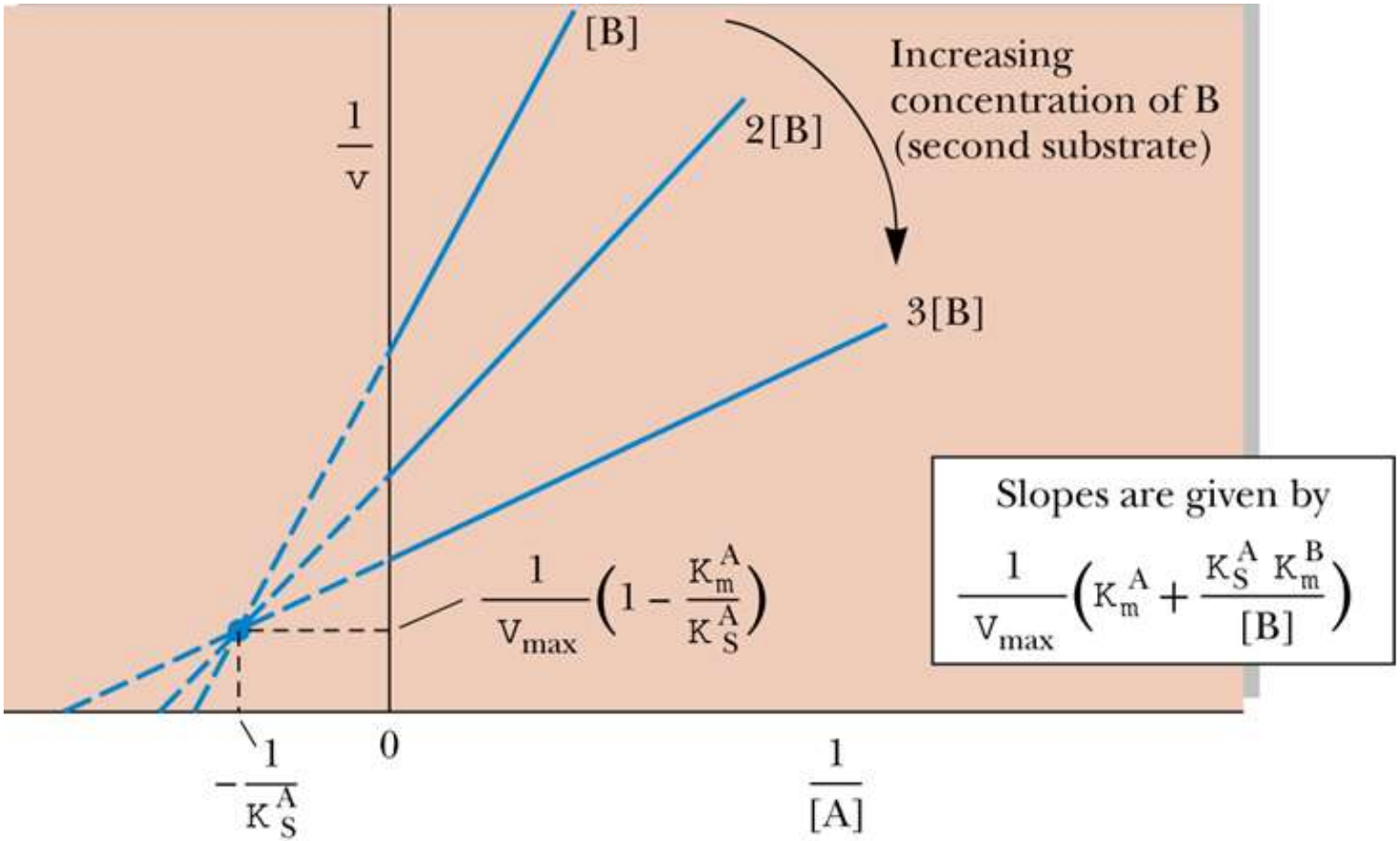
King, E. L., and Altman, C. (1956) A schematic method of deriving the rate laws for enzyme-catalyzed reactions. *J. Phys. Chem.,* **60**, 1375–1378.

6.3 Enzyme Kinetics

Ordered reaction:

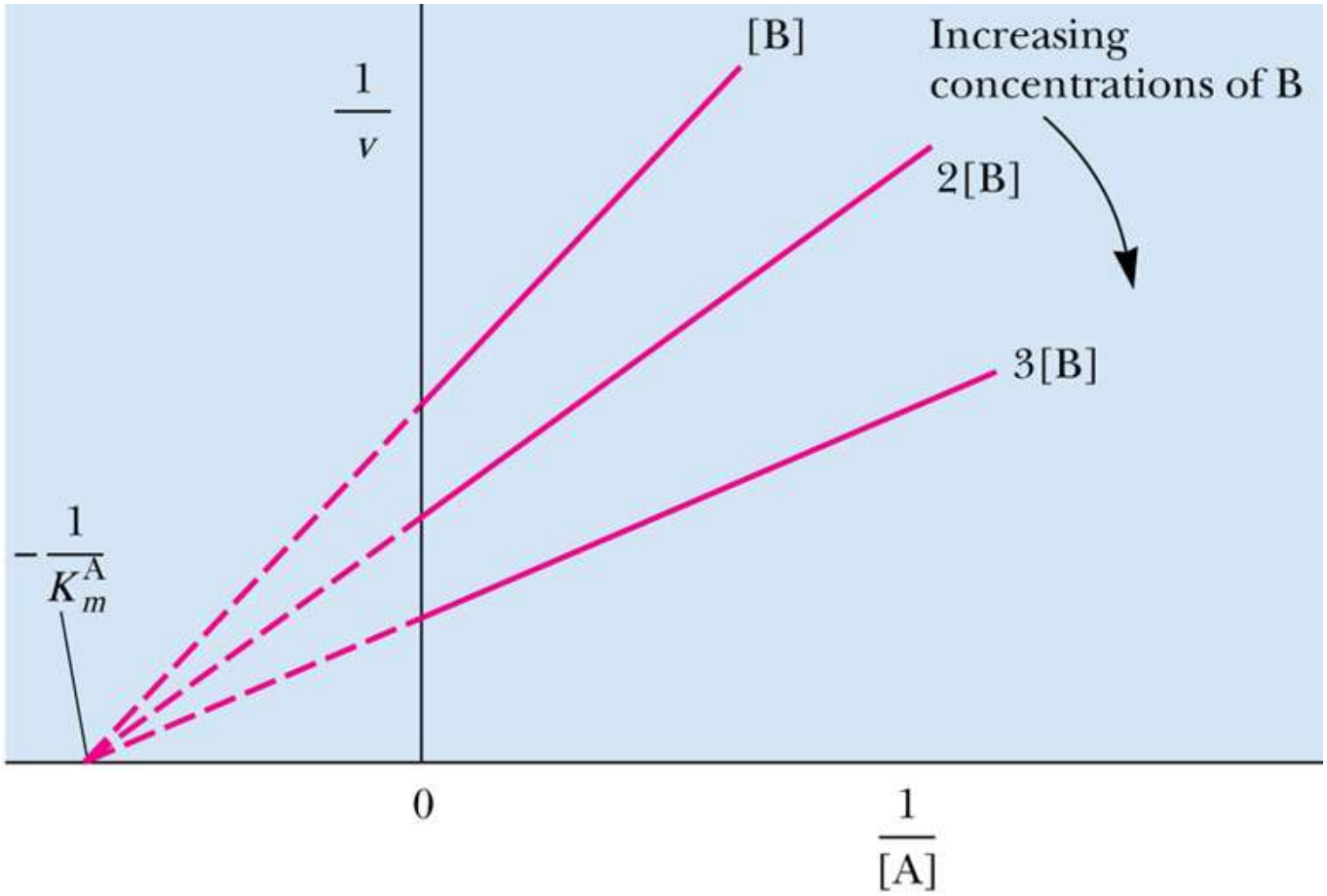
Double-reciprocal form of the rate equation:

$$\frac{1}{v} = \frac{1}{V_{max}}\left(K_m^A + \frac{K_S^A\, K_m^B}{[B]}\right)\left(\frac{1}{[A]} + \frac{1}{V_{max}}\left(1 + \frac{K_m^B}{[B]}\right)\right)$$



6.3 Enzyme Kinetics

Random reaction:



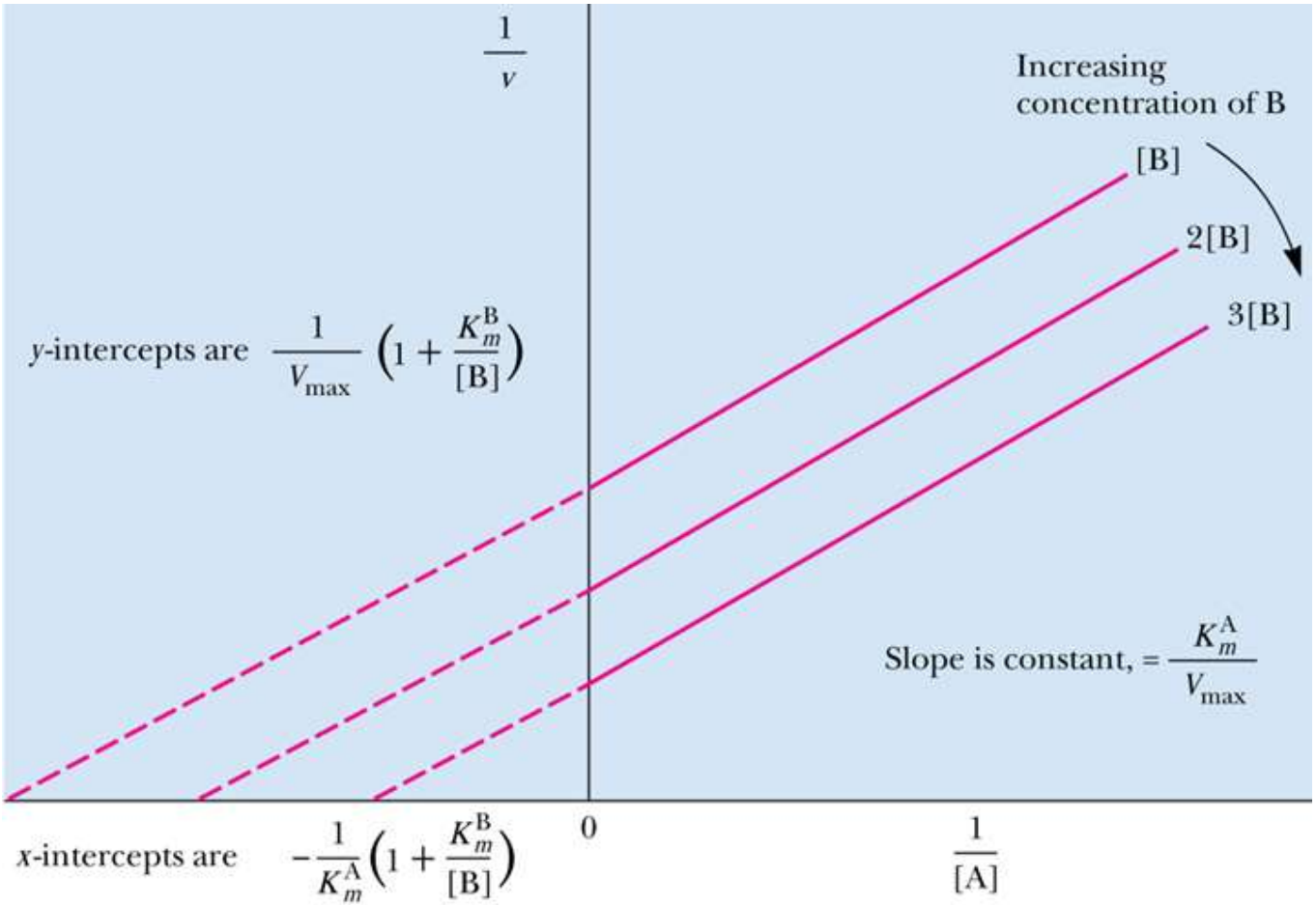
Substrate A does not affect binding of substrate B and vice versa.

6.3 Enzyme Kinetics

Ping-Pong reaction:

Double-reciprocal form of the rate equation:

$$\frac{1}{v} = \frac{K_m^A}{V_{max}}\left(\frac{1}{[A]}\right) + \left(1 + \frac{K_m^B}{[B]}\right)\left(\frac{1}{V_{max}}\right)$$



■ Inhibition of Enzyme

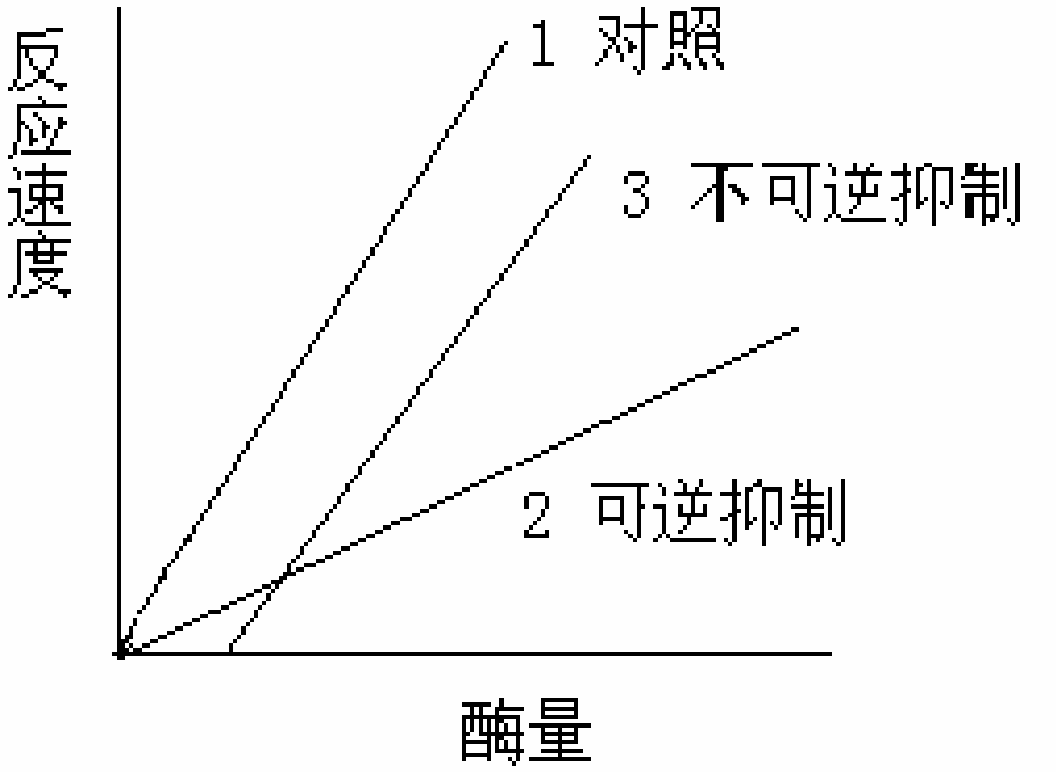
Enzyme Inhibitors

- Enzyme inhibitors are certain molecules that can decrease the catalytic rate of an enzyme-catalyzed reaction.
- Enzyme inhibitors can be normal body metabolites and foreign substances (drugs and toxins).

6.3 Enzyme Kinetics

Inhibition processes

The inhibition process can be either irreversible or reversible.



6.3 Enzyme Kinetics

Irreversible inhibition

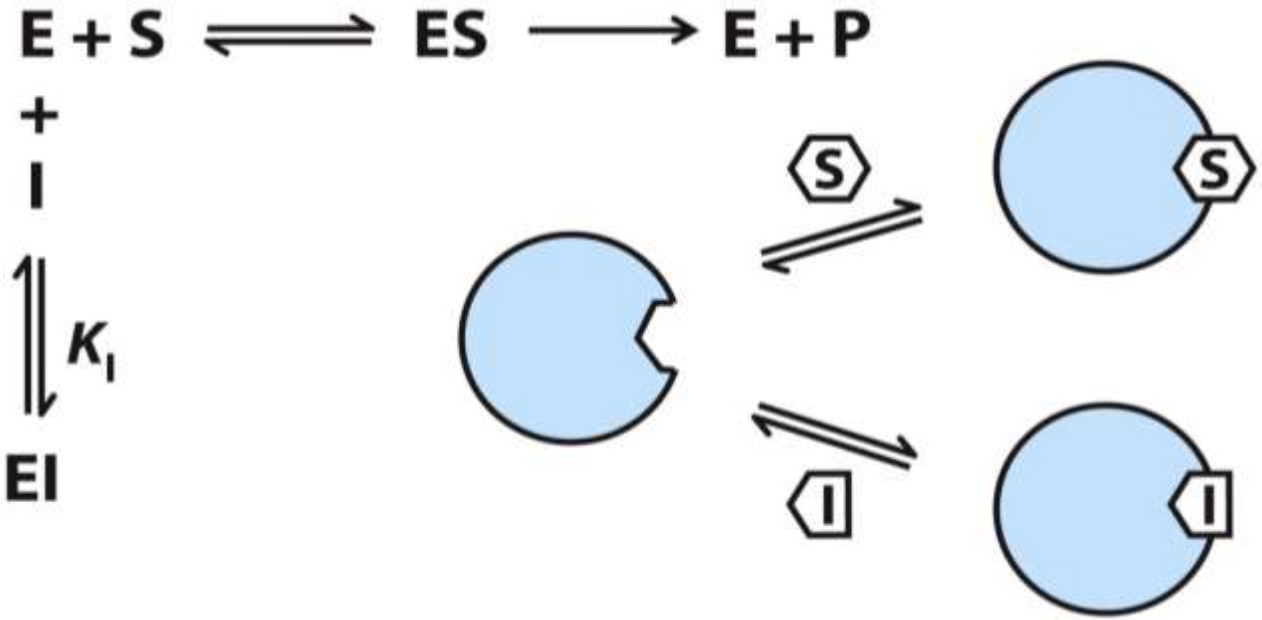
- Inhibitors are covalently bound to the essential groups of enzymes.
- Inhibitors cannot be removed with simple dialysis or super-filtration.
- Binding can cause a partial or complete loss of the enzymatic activity.

Reversible inhibition

- Inhibitors are bound to enzymes non-covalently.
- The reversible inhibition is characterized by an equilibrium between free enzymes and inhibitor-bound enzymes.
- The reversible inhibition can be competitive, un-competitive, or mixed inhibition.

6.3 Enzyme Kinetics

Competitive inhibition



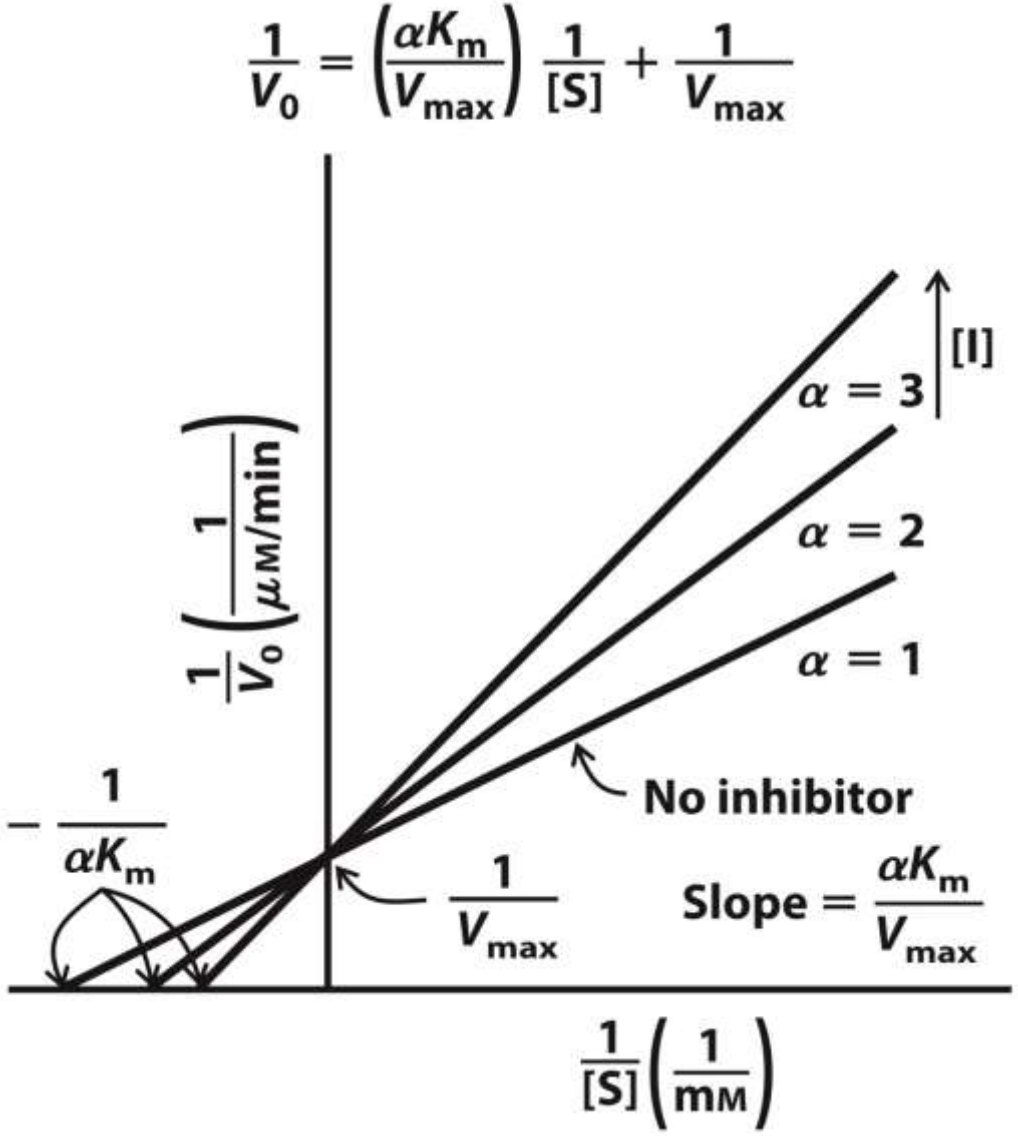
- Competitive inhibitors share the structural similarities with that of substrates.
- Competitive inhibitors compete for the active sites with the normal substrates.
- Inhibition depends on the affinity of enzymes and the ratio of [E] to [S].

6.3 Enzyme Kinetics

$$V_0 = \frac{V_{\max}[\mathrm{S}]}{\alpha K_\mathrm{m} + [\mathrm{S}]}$$

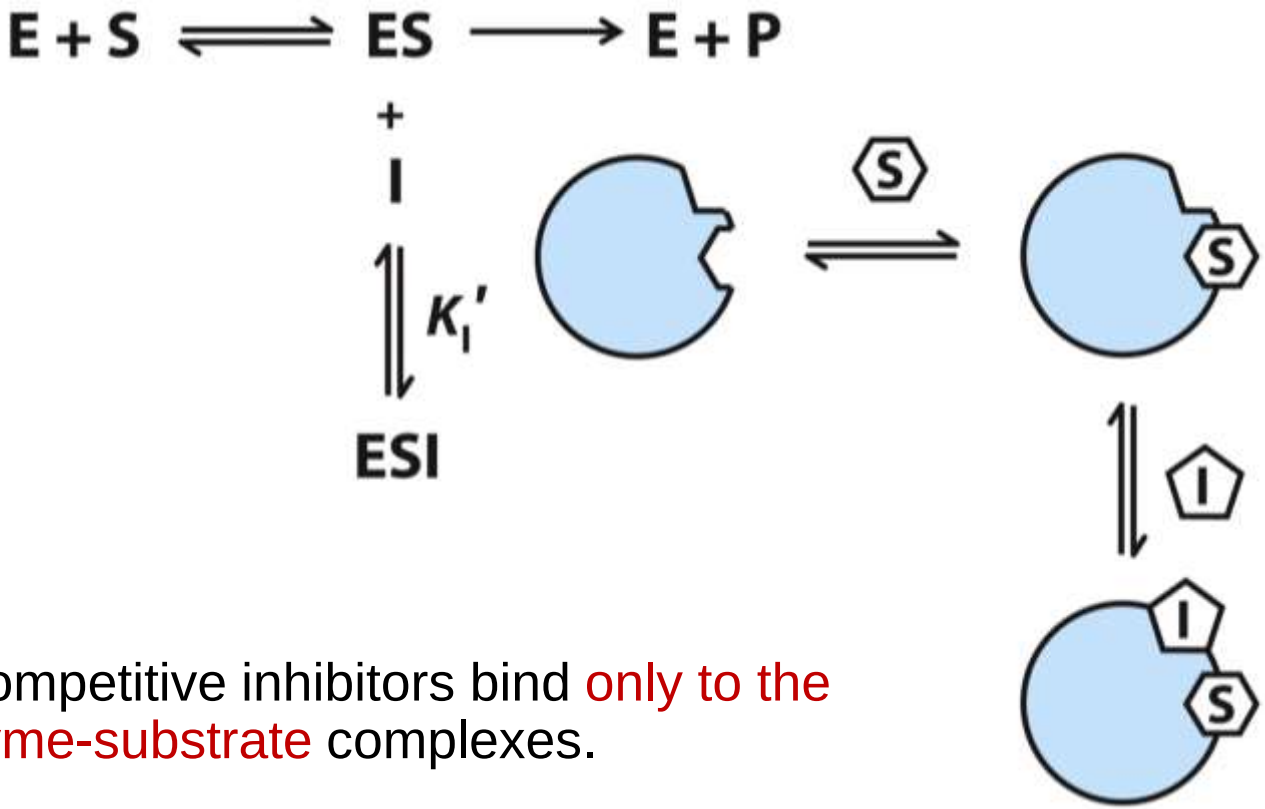
$$\alpha = 1 + \frac{[\mathrm{I}]}{K_\mathrm{I}}$$

$$K_\mathrm{I} = \frac{[\mathrm{E}][\mathrm{I}]}{[\mathrm{EI}]}$$



6.3 Enzyme Kinetics

Uncompetitive inhibition



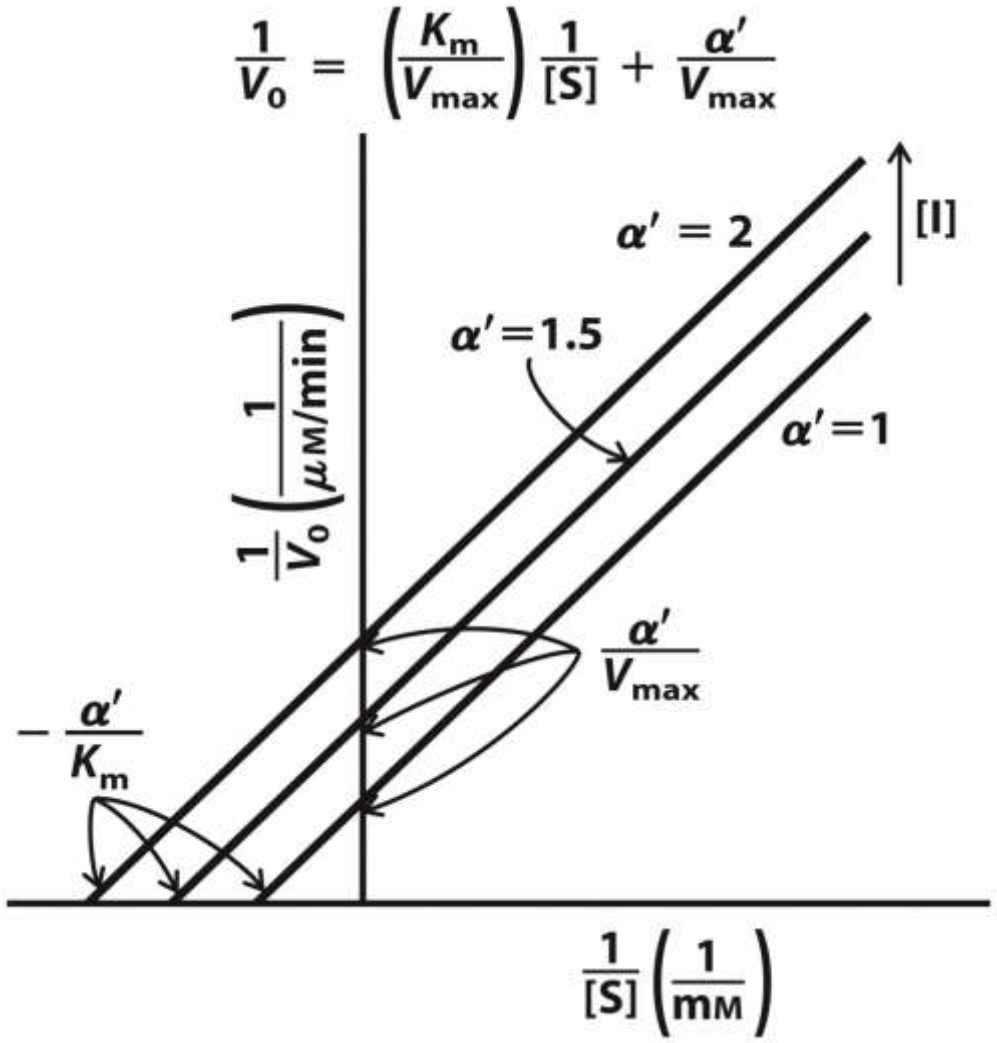
- Uncompetitive inhibitors bind only to the enzyme-substrate complexes.
- The E-I-S complexes do not proceed to form products.

6.3 Enzyme Kinetics

$$V_0 = \frac{V_{\max}[\mathrm{S}]}{K_\mathrm{m} + \alpha'[\mathrm{S}]}$$

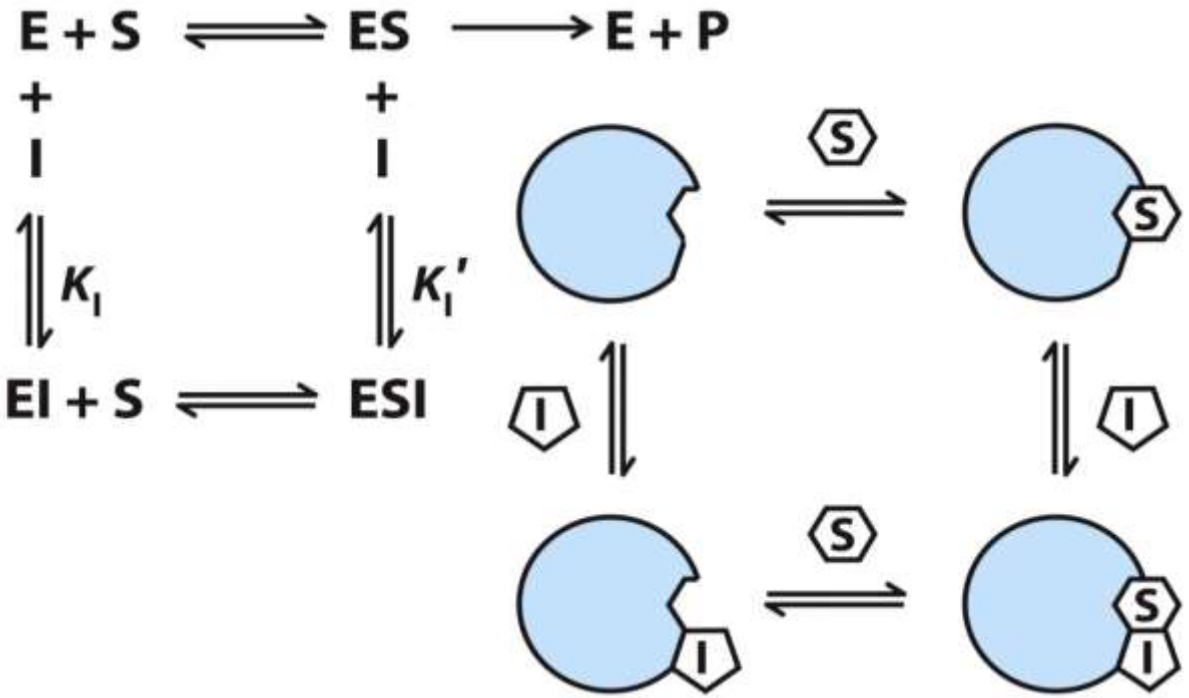
$$\alpha' = 1 + \frac{[\mathrm{I}]}{K'_\mathrm{I}}$$

$$K'_\mathrm{I} = \frac{[\mathrm{ES}][\mathrm{I}]}{[\mathrm{ESI}]}$$



6.3 Enzyme Kinetics

Mixed inhibition



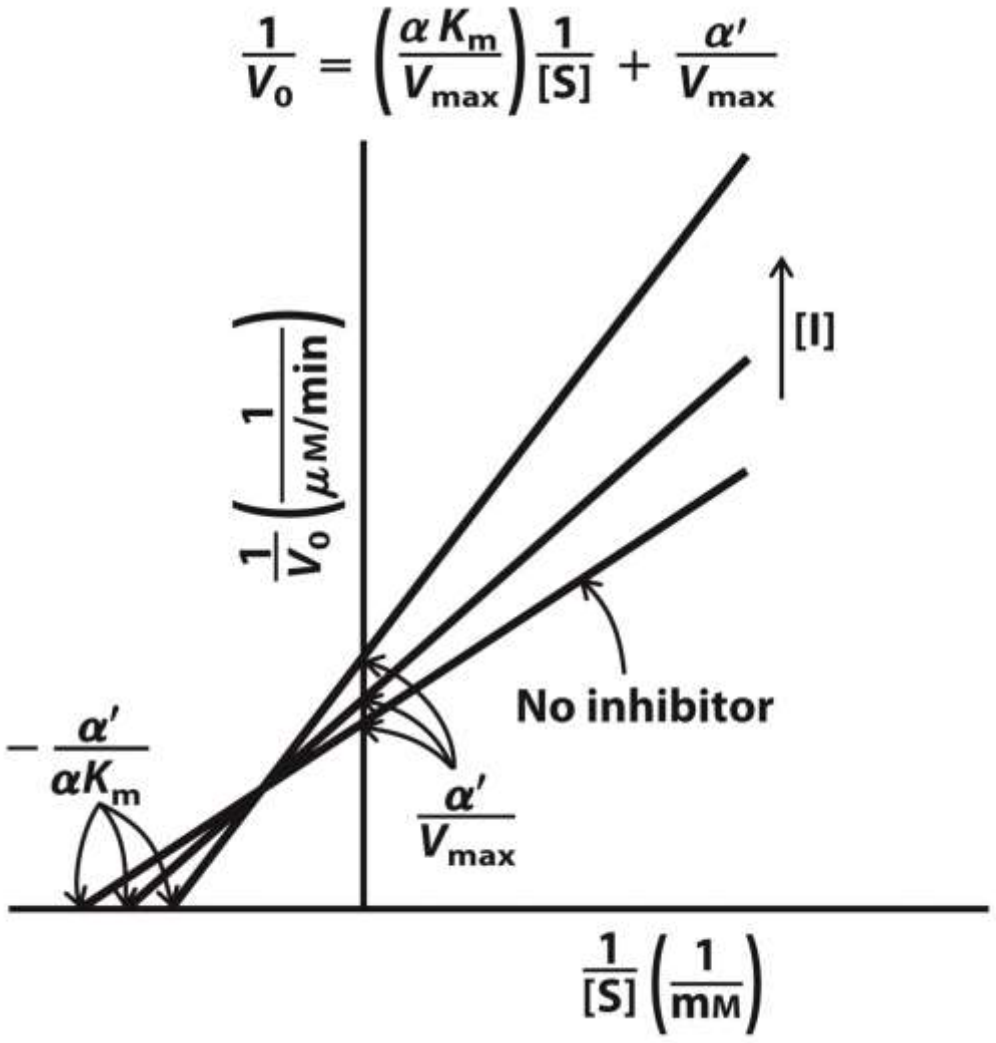
- Inhibitors bind to other sites rather than the active sites on the free enzymes or the E-S complexes.
- The E-I complex formation does not affect the binding of substrates.
- The E-I-S complexes do not proceed to form products.

6.3 Enzyme Kinetics

$$V_0 = \frac{V_{max}[S]}{\alpha K_m + \alpha'[S]}$$

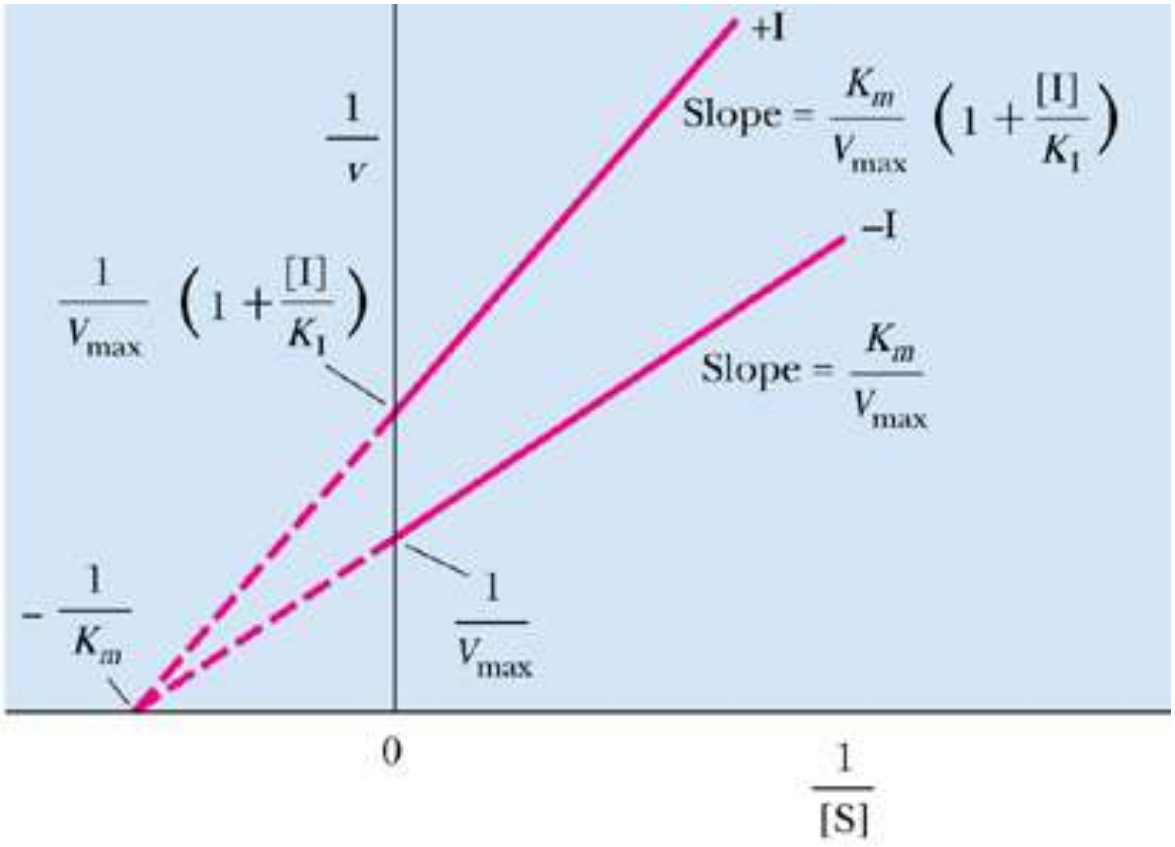
$$\alpha = 1 + \frac{[I]}{K_I} \qquad K_I = \frac{[E][I]}{[EI]}$$

$$\alpha' = 1 + \frac{[I]}{K'_I} \qquad K'_I = \frac{[ES][I]}{[ESI]}$$



6.3 Enzyme Kinetics

When $\alpha=\alpha'$, rarely encountered in experiments, classically has been defined as **noncompetitive inhibition**.



6.3 Enzyme Kinetics

TABLE 6–9 Effects of Reversible Inhibitors on Apparent V_{max} and Apparent K_m

Inhibitor type	Apparent V_{max}	Apparent K_m
None	V_{max}	K_m
Competitive	V_{max}	αK_m
Uncompetitive	V_{max}/α'	K_m/α'
Mixed	V_{max}/α'	$\alpha K_m/\alpha'$

Table 6-9
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Take home messages …

- ✓ **An Introduction to Enzymes**
  - Enzyme nomenclature and classification
- ✓ **How Enzymes Work**
  - Distinctive features of enzymes, binding energy, active site, regulatory enzyme
- ✓ **Enzymes Kinetics**
  - Michaelis-Menten equation, Significance of Km, Kcat, Inhibition of enzyme …..