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Most Enzymes are Proteins

Cofactor

Coenzyme

Prosthetic group

Holoenzyme

Apoenzyme

Apoprotein

TABLE 6–1	Some Inorganic Ions That Serve as Cofactors for Enzymes
Ions	Enzymes
Cu²⁺	Cytochrome oxidase
Fe²⁺ or Fe³⁺	Cytochrome oxidase, catalase, peroxidase
K⁺	Pyruvate kinase
Mg²⁺	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn²⁺	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni²⁺	Urease
Se	Glutathione peroxidase
Zn²⁺	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Table 6-1

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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
Biotin	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)

Note: The structures and modes of action of these coenzymes are described in Part II.

Table 6-2

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Nomenclature


Remember the five basic reactions in biochemistry

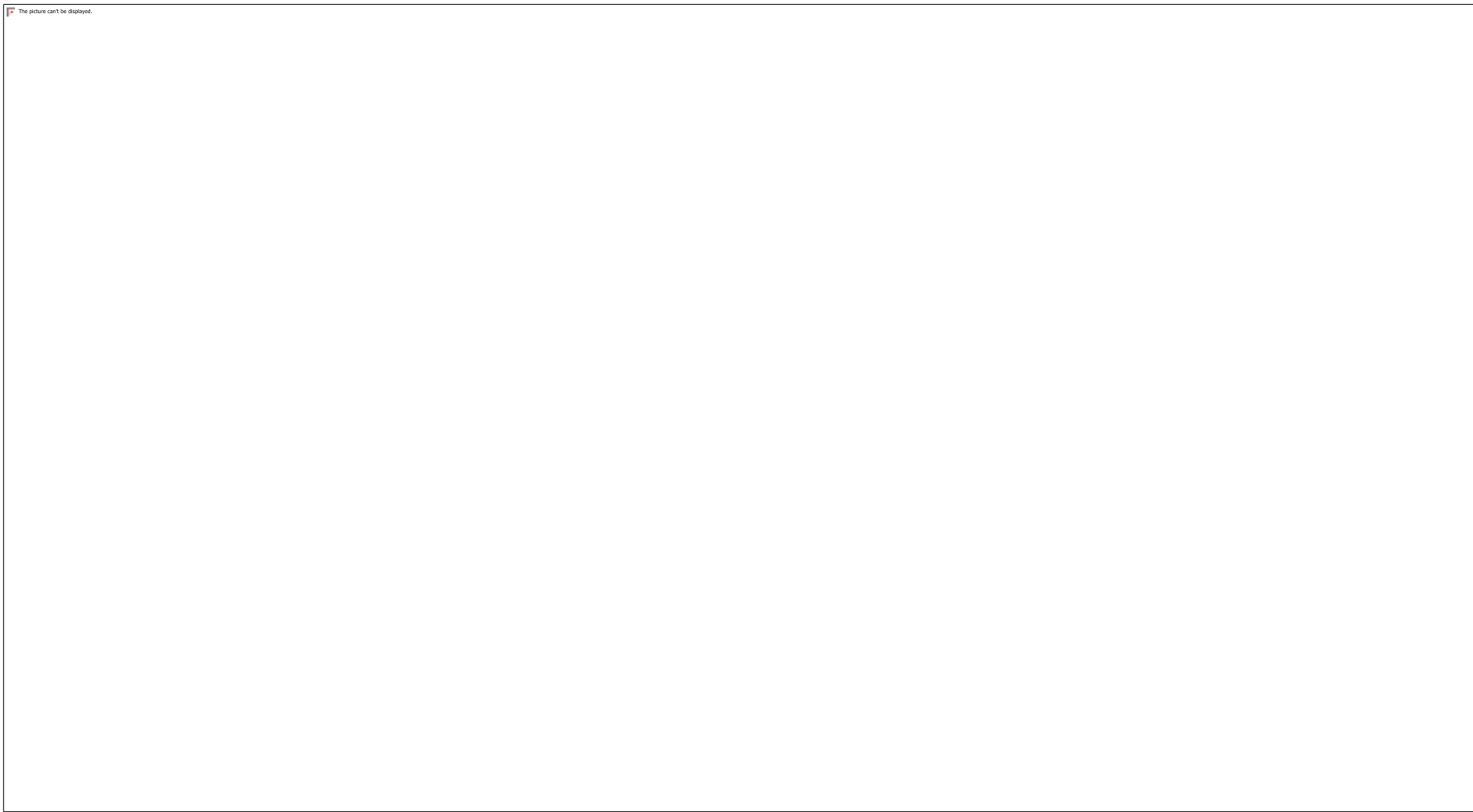
TABLE 6–3		International Classification of Enzymes
Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
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


Table 6-3


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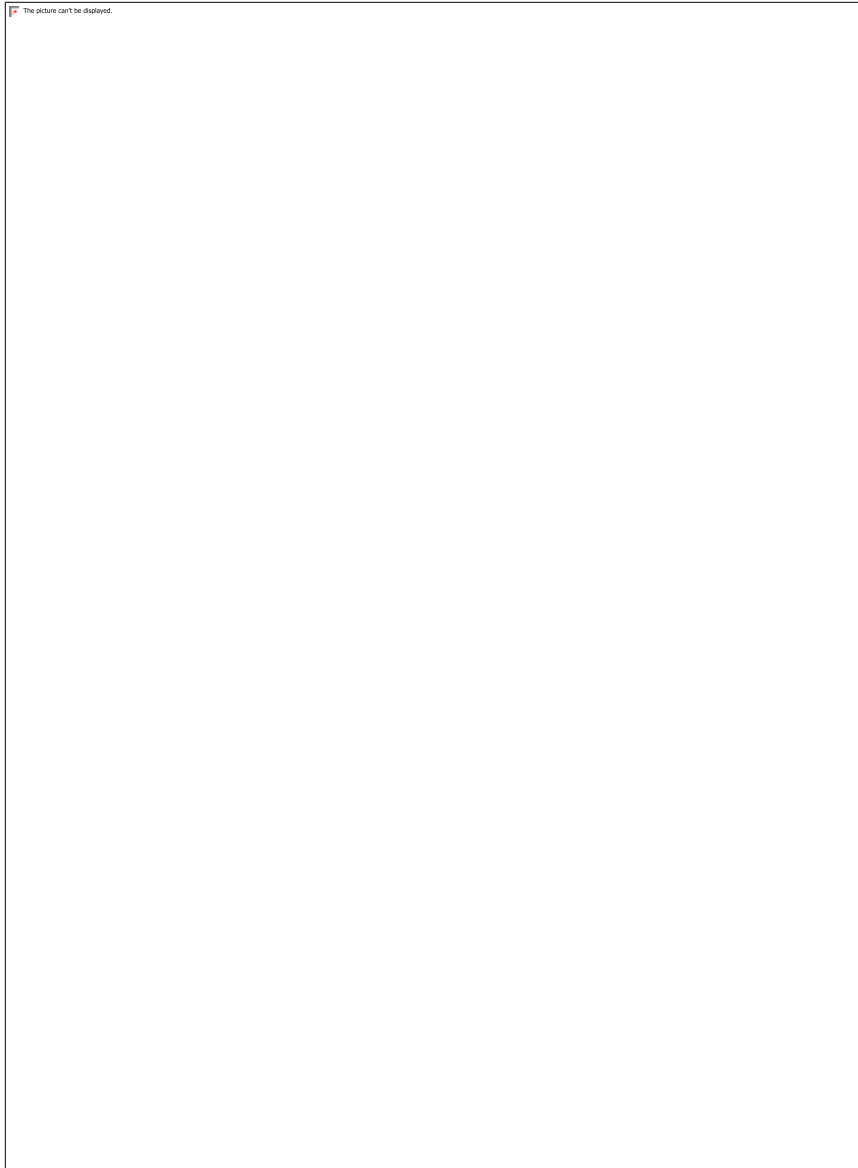
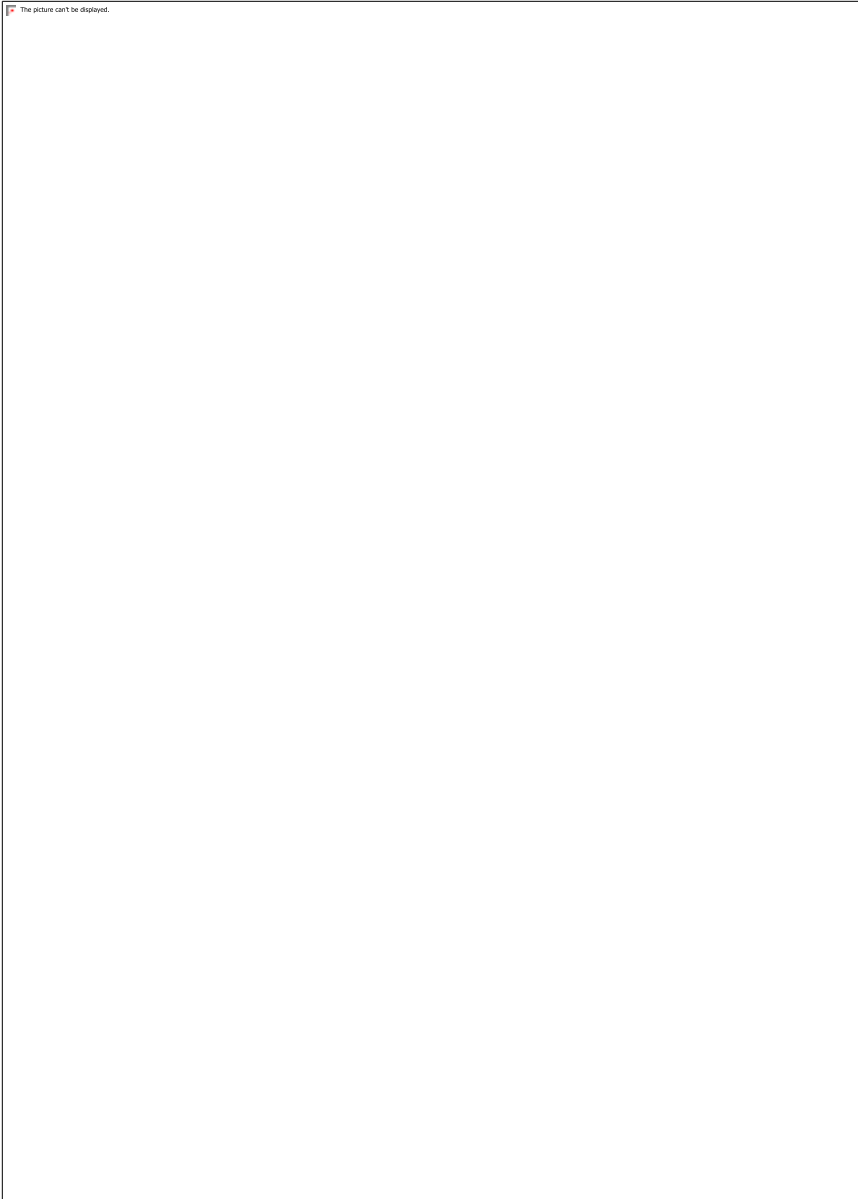



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EC 1.1.1.1

Common name: alcohol dehydrogenase

Reaction: an alcohol + NAD⁺ = an aldehyde or ketone + NADH + H⁺

Other name(s): aldehyde reductase; ADH; alcohol dehydrogenase (NAD); aliphatic alcohol dehydrogenase; ethanol dehydrogenase; NAD-dependent alcohol dehydrogenase; NAD-specific aromatic alcohol dehydrogenase; NADH-alcohol dehydrogenase; NADH-aldehyde dehydrogenase; primary alcohol dehydrogenase; yeast alcohol dehydrogenase

Systematic name: alcohol:NAD⁺ oxidoreductase

Comments: A zinc protein. Acts on primary or secondary alcohols or hemiacetals; the animal, but not the yeast, enzyme acts also on cyclic secondary alcohols.

CAS registry number: 9031-72-5

References:

1. Brändén, G.-I., Jörnvall, H., Eklund, H. and Furugren, B. Alcohol dehydrogenase. In: Boyer, P.D. (Ed.), *The Enzymes*, 3rd ed., vol. 11, Academic Press, New York, 1975, p. 103-190.
2. Jörnvall, H. Differences between alcohol dehydrogenases. Structural properties and evolutionary aspects. *Eur. J. Biochem.* 72 (1977) 443-452. [Medline UI: [77115786](#)]
3. Negelein, E. and Wulff, H.-J. Diphosphopyridinproteid ackohol, acetaldehyd. *Biochem. Z.* 293 (1937) 351-389.
4. Sund, H. and Theorell, H. Alcohol dehydrogenase. In: Boyer, P.D., Lardy, H. and Myrbäck, K. (Eds.), *The Enzymes*, 2nd ed., vol. 7, Academic Press, New York, 1963, p. 25-83.
5. Theorell, H. Kinetics and equilibria in the liver alcohol dehydrogenase system. *Adv. Enzymol. Relat. Subj. Biochem.* 20 (1958) 31-49.

[EC 1.1.1.1 created 1961]

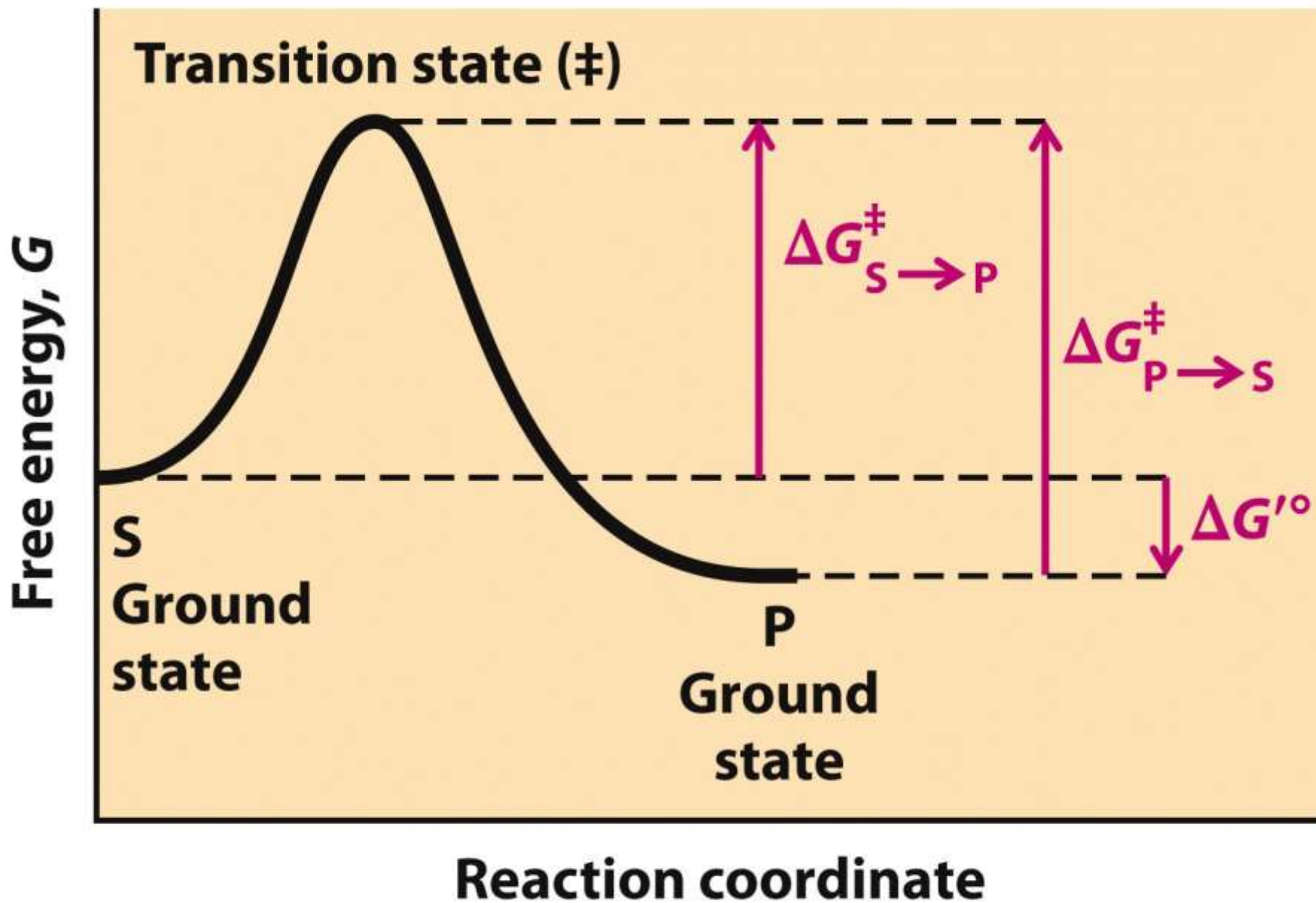


Figure 6-2

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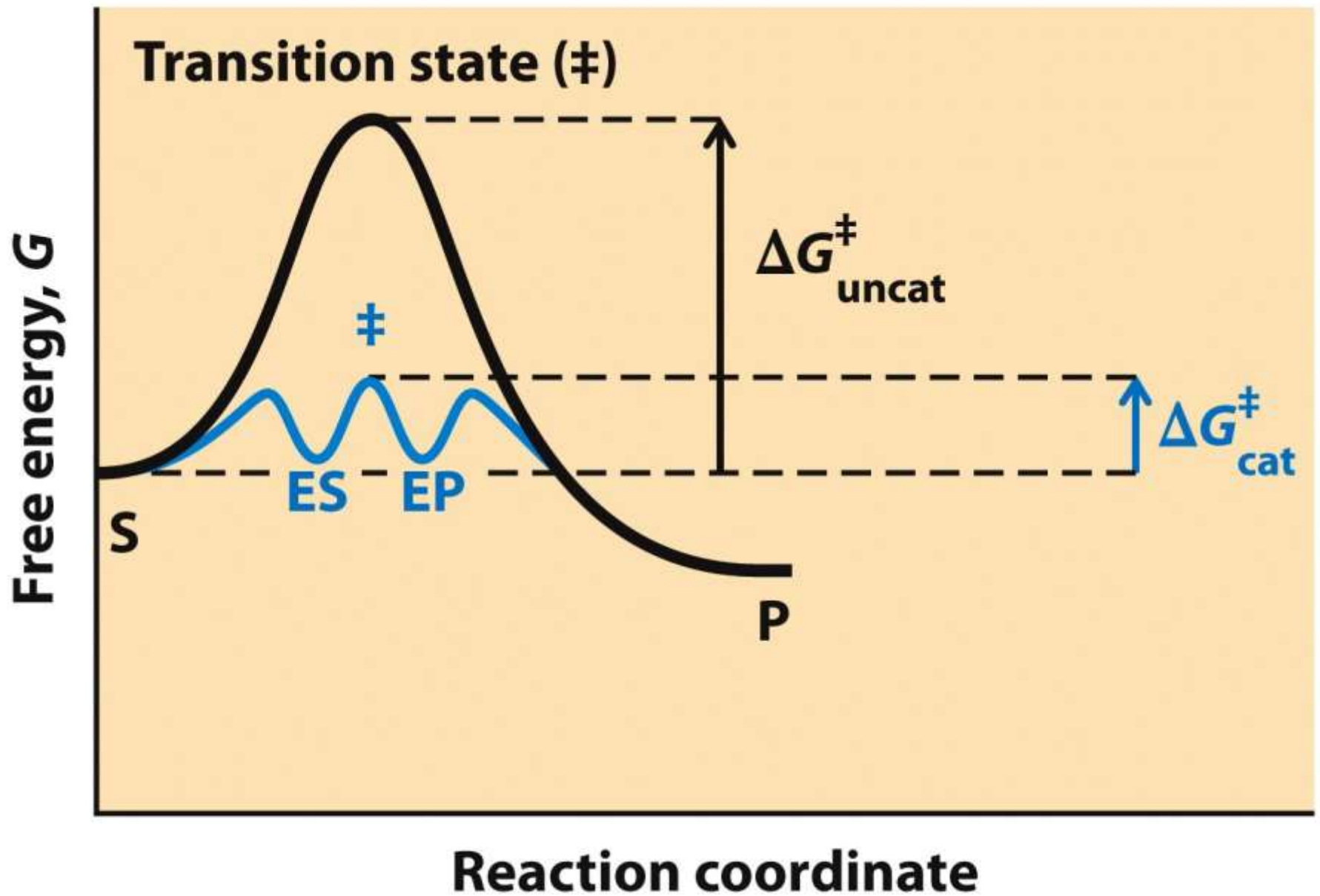


Figure 6-3

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Ground state

Standard free-energy change

Biochemical standard free-energy change

Transition state

Activation energy

Reaction intermediate

Rate-limiting step

Rate-determining step

Equilibrium constant

Rate constant

Rate equation

Binding energy

TABLE 6–4**Relationship between K'_{eq} and $\Delta G'^{\circ}$**

K'_{eq}	$\Delta G'^{\circ}$ (kJ/mol)
10^{-6}	34.2
10^{-5}	28.5
10^{-4}	22.8
10^{-3}	17.1
10^{-2}	11.4
10^{-1}	5.7
1	0.0
10^1	–5.7
10^2	–11.4
10^3	–17.1

Note: The relationship is calculated from $\Delta G'^{\circ} = -RT \ln K'_{eq}$ (Eqn 6–3).

Table 6-4*Lehninger Principles of Biochemistry, Fifth Edition*

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

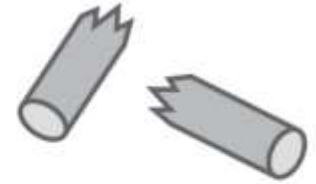
Cyclophilin	10^5
Carbonic anhydrase	10^7
Triose phosphate isomerase	10^9
Carboxypeptidase A	10^{11}
Phosphoglucomutase	10^{12}
Succinyl-CoA transferase	10^{13}
Urease	10^{14}
Orotidine monophosphate decarboxylase	10^{17}

Table 6-5*Lehninger Principles of Biochemistry, Fifth Edition*

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Weak interactions optimized in the
transition state

No enzyme



**Substrate
(metal stick)**

**Transition state
(bent stick)**

**Products
(broken stick)**

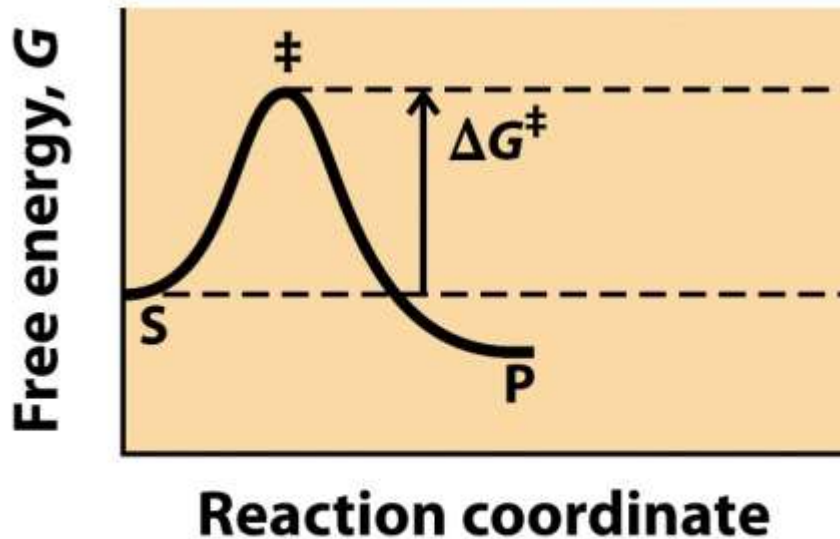


Figure 6-5a

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Enzyme complementary to substrate

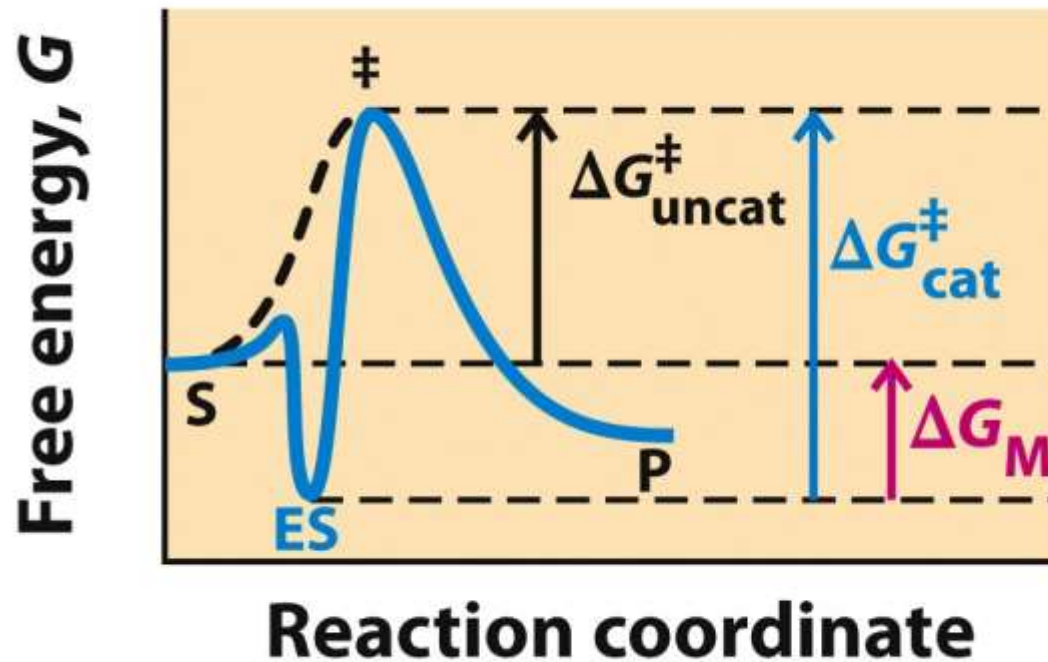
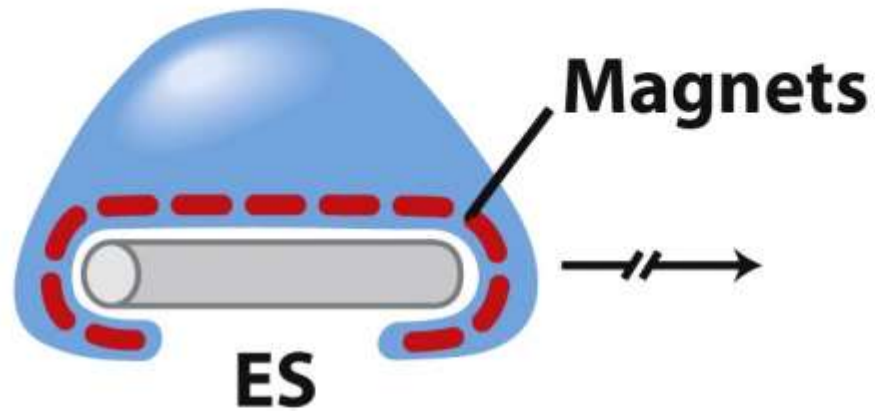


Figure 6-5b
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Enzyme complementary to transition state

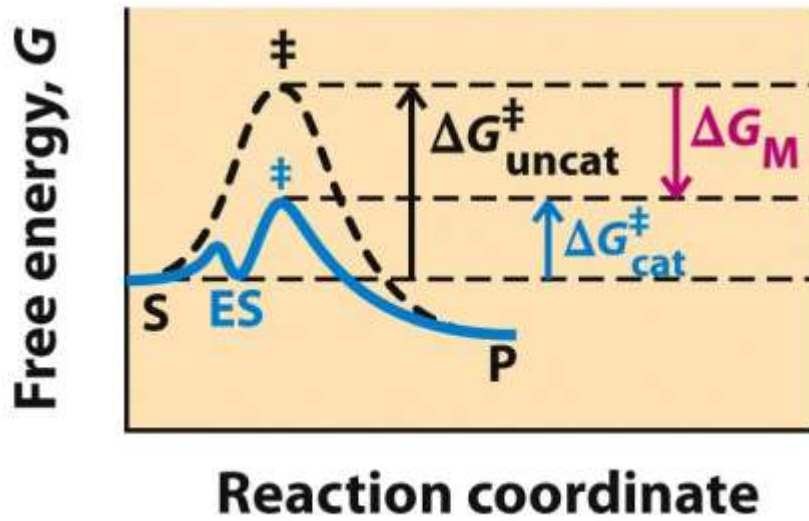
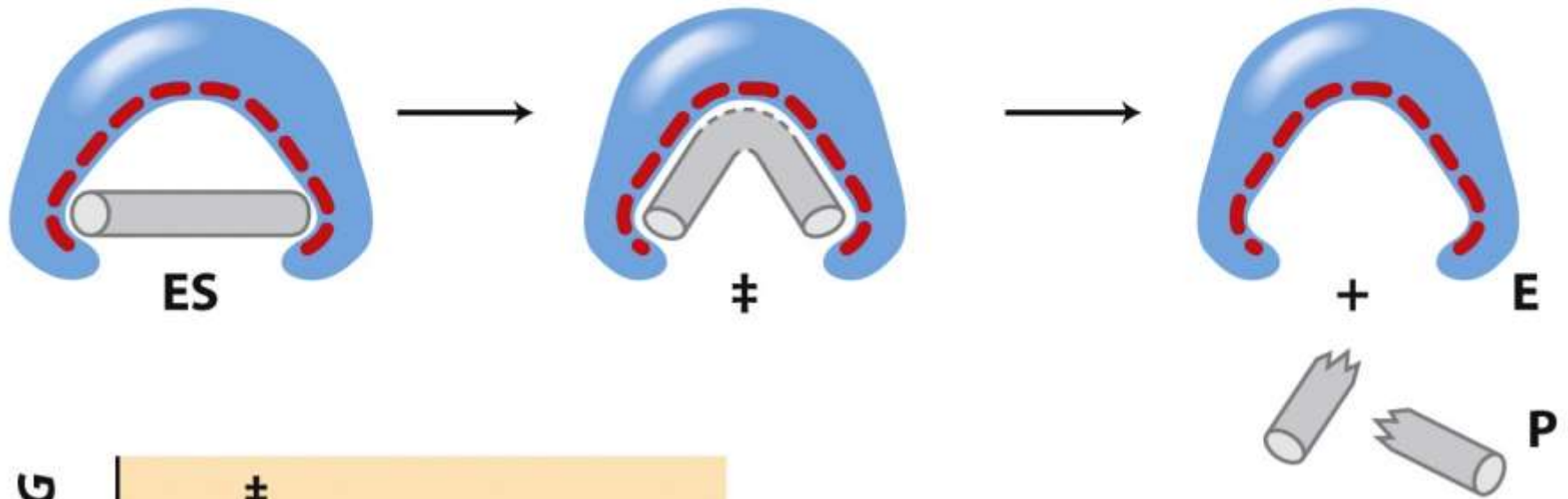


Figure 6-5c

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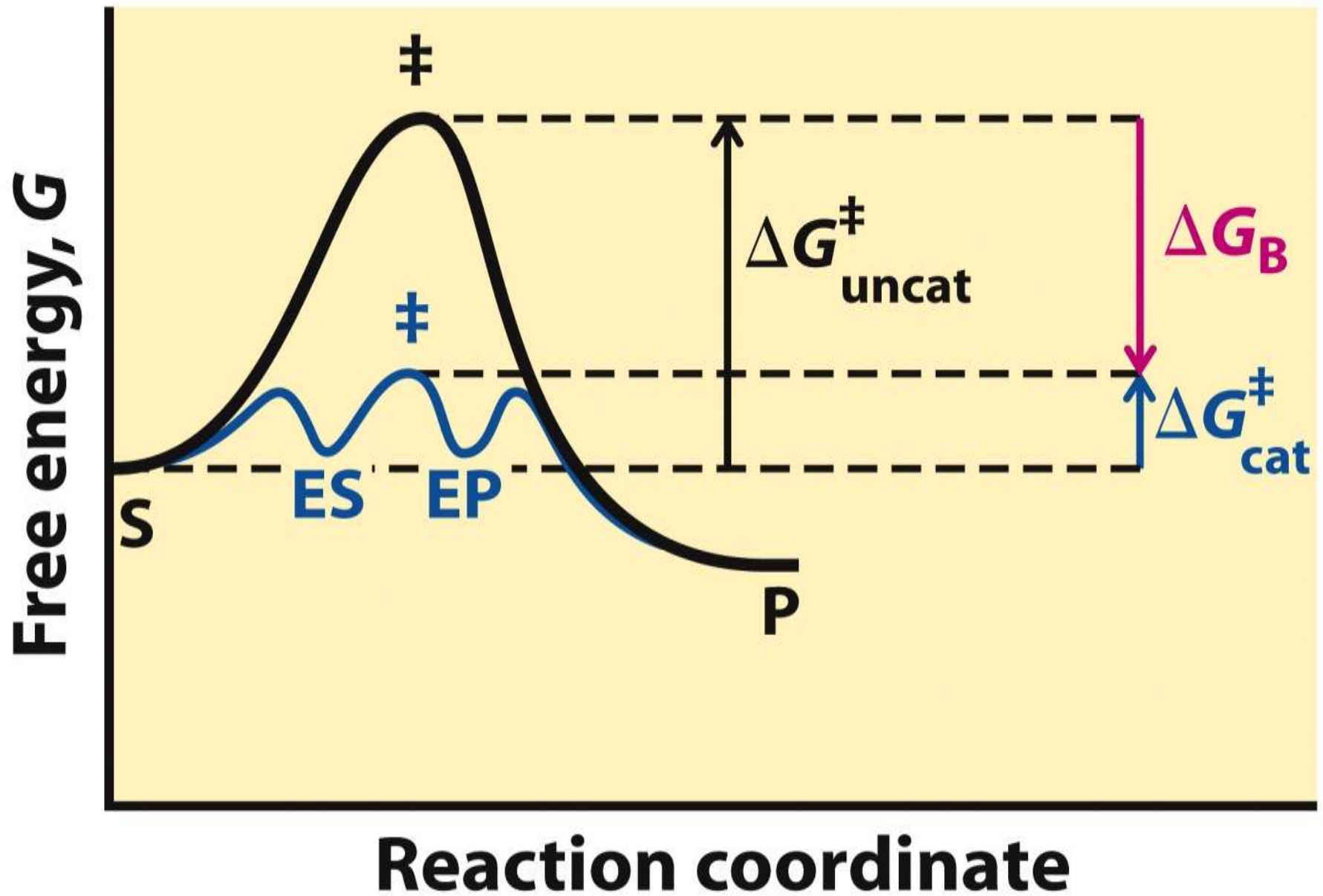


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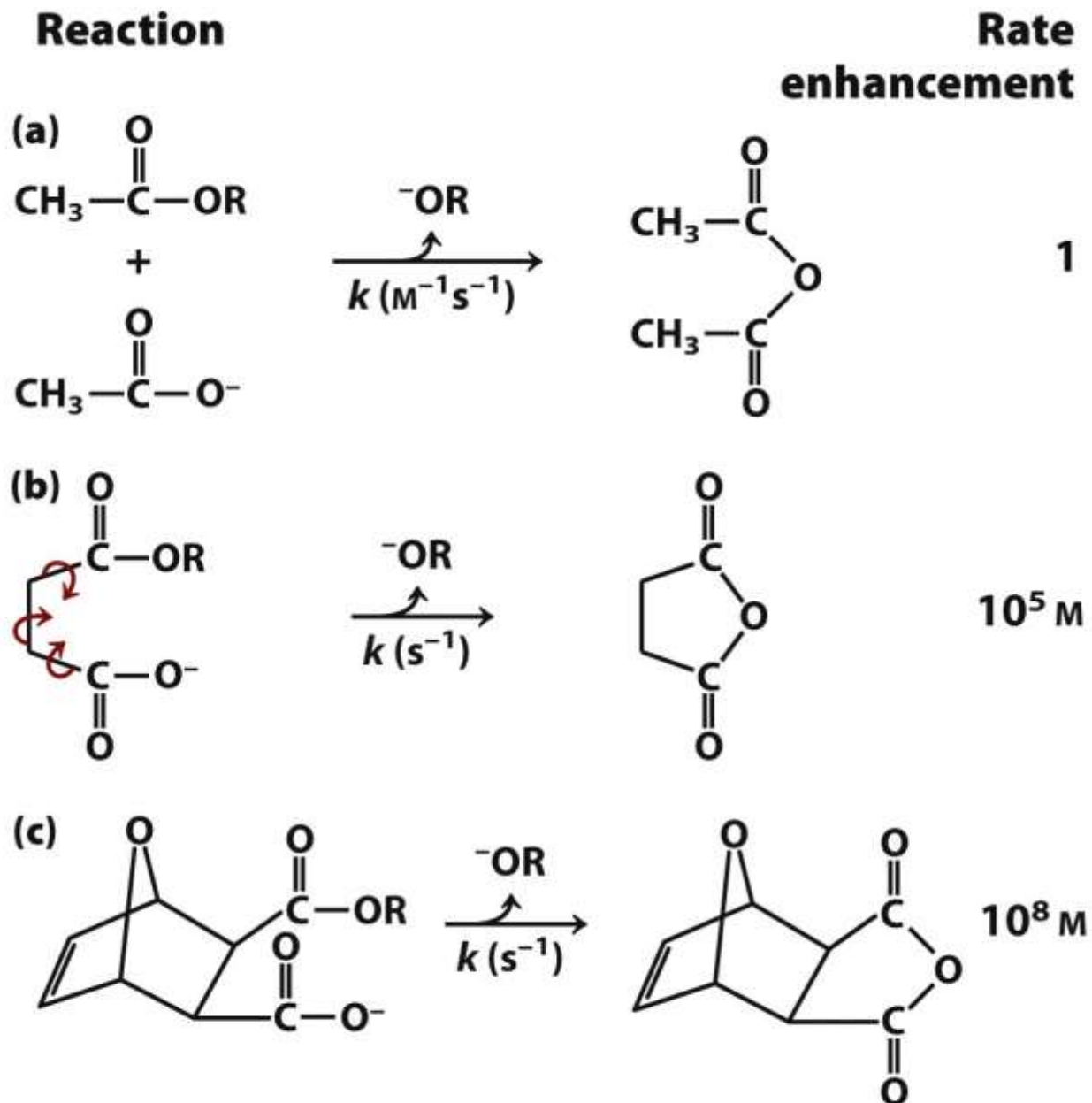


Figure 6-7

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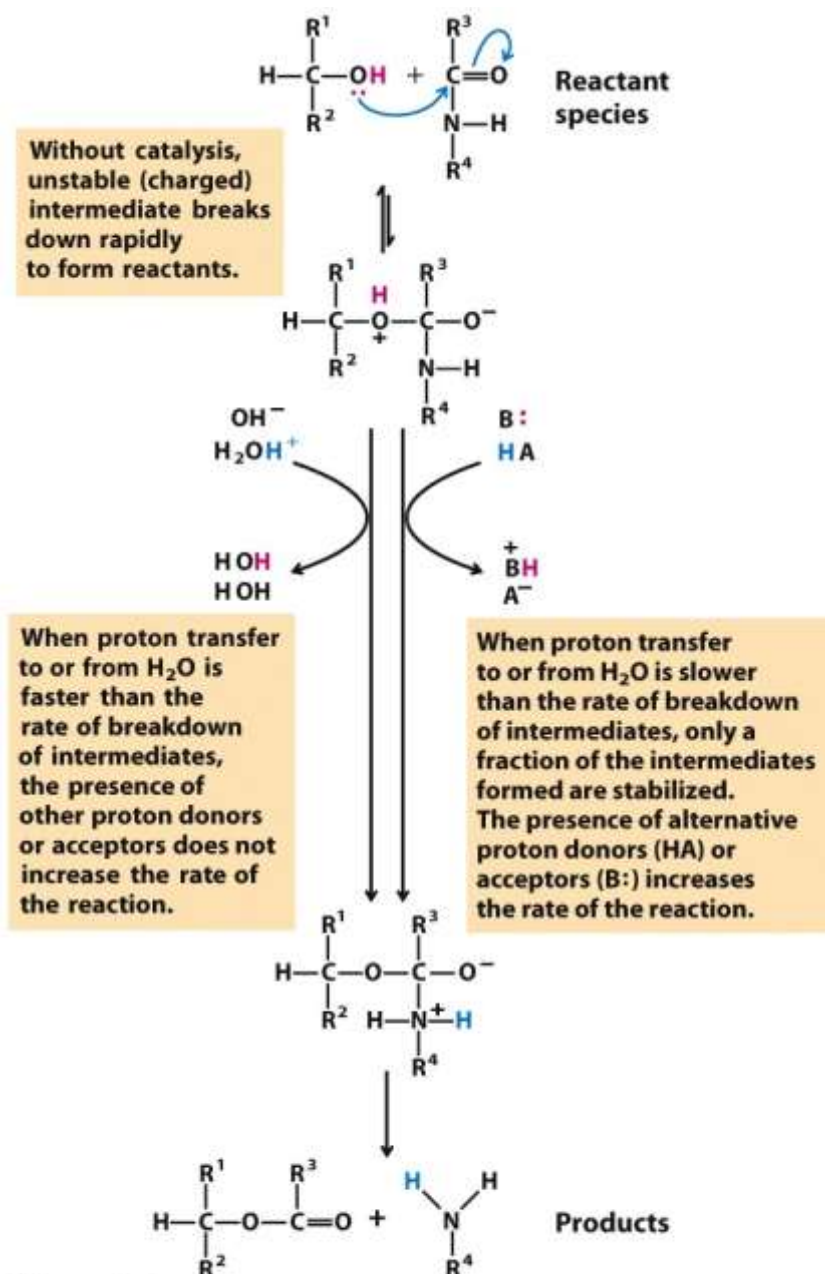


Figure 6-8

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Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	$\text{R}-\text{COOH}$	$\text{R}-\text{COO}^-$
Lys, Arg	$\text{R}-\overset{\text{H}}{\underset{\text{H}}{\overset{+}{\text{N}}}}$	$\text{R}-\ddot{\text{N}}\text{H}_2$
Cys	$\text{R}-\text{SH}$	$\text{R}-\text{S}^-$
His	$ \begin{array}{c} \text{R}-\text{C}=\text{CH} \\ \diagup \quad \diagdown \\ \text{HN} \quad \text{N}^+\text{H} \\ \diagdown \quad \diagup \\ \text{C} \\ \text{H} \end{array} $	$ \begin{array}{c} \text{R}-\text{C}=\text{CH} \\ \diagup \quad \diagdown \\ \text{HN} \quad \text{N}: \\ \diagdown \quad \diagup \\ \text{C} \\ \text{H} \end{array} $
Ser	$\text{R}-\text{OH}$	$\text{R}-\text{O}^-$
Tyr	$ \text{R}-\text{C}_6\text{H}_4-\text{OH} $	$ \text{R}-\text{C}_6\text{H}_4-\text{O}^- $

Figure 6-9

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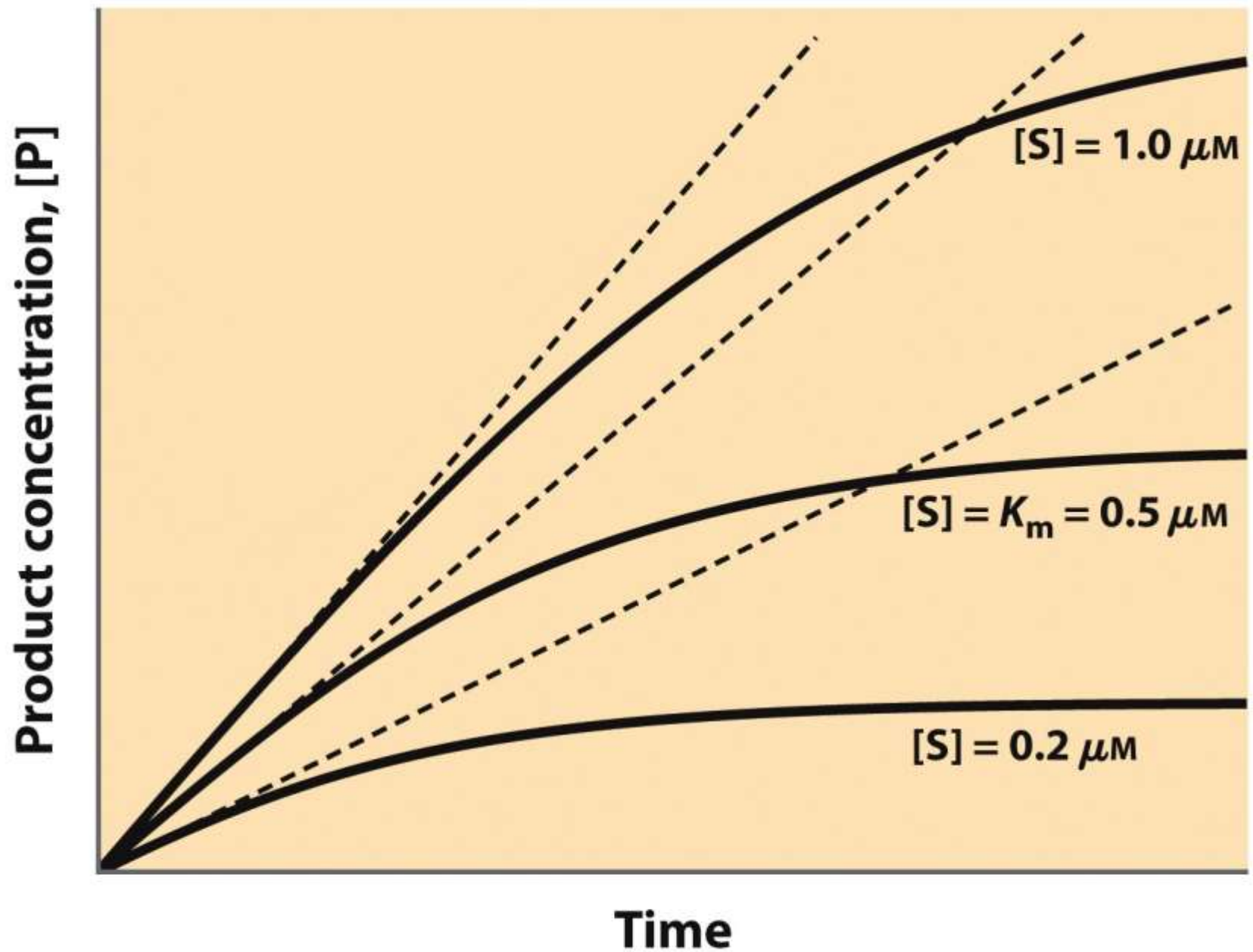
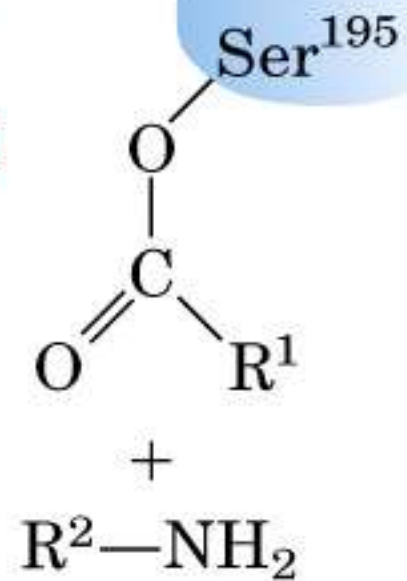
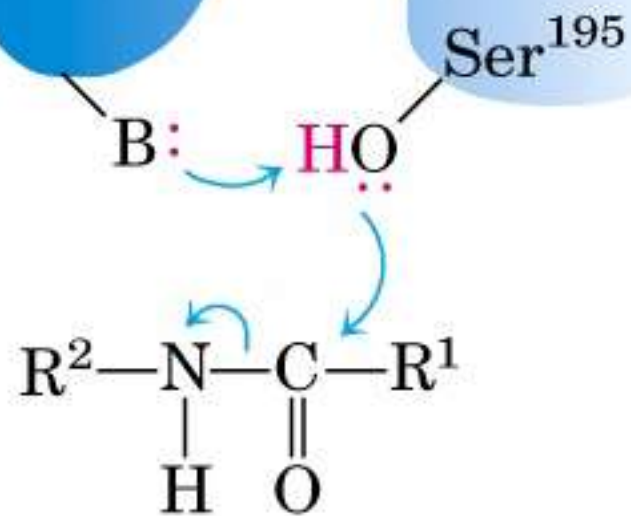


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Chymotrypsin



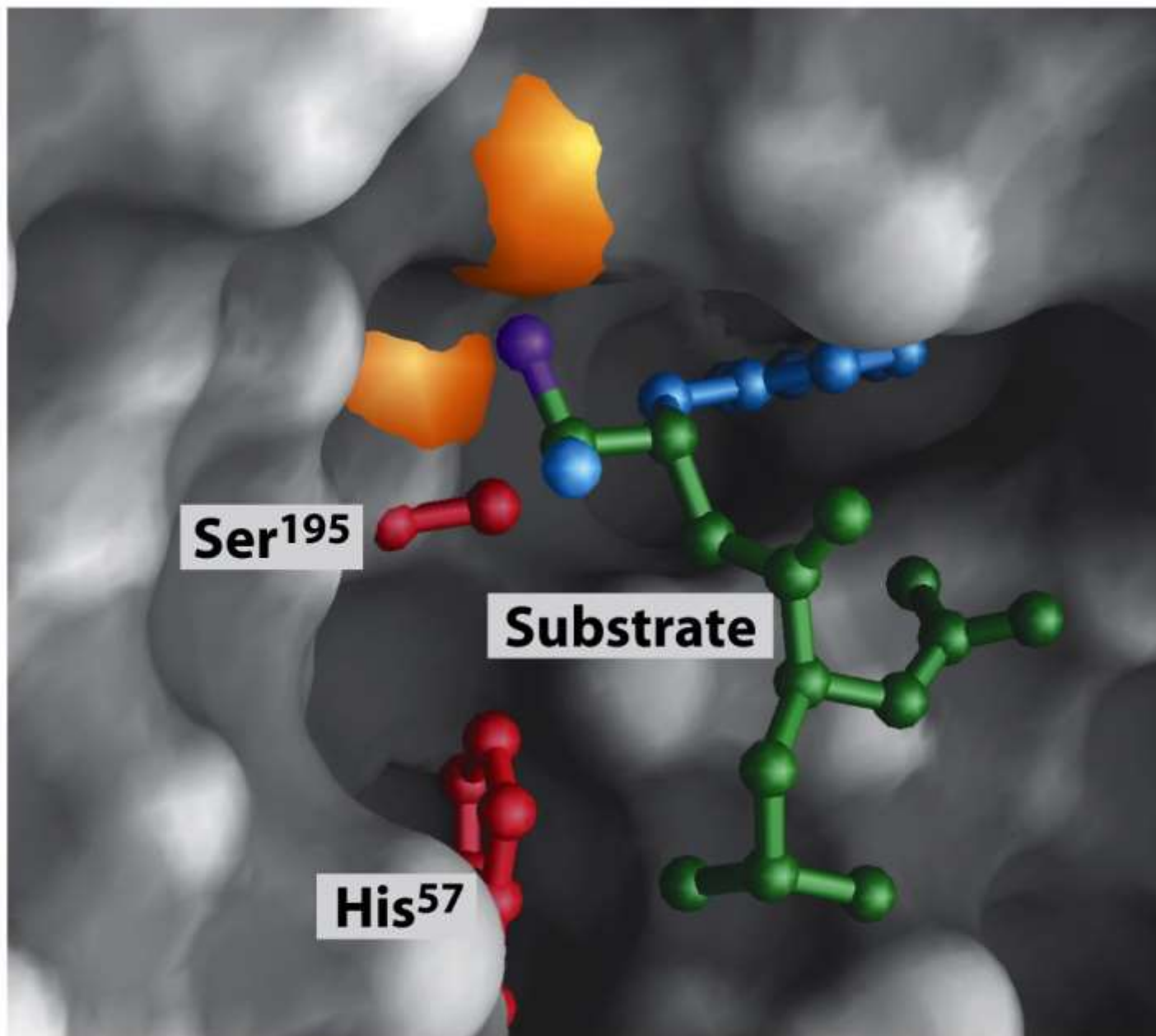


Figure 6-18d

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Enzyme Kinetics

Enzyme kinetics

Initial rate (or initial velocity)

Maximum velocity

Pre-steady state

Steady state

Steady-state kinetics

Steady-state assumption

Michaelis constant

Michaelis-Menten equation

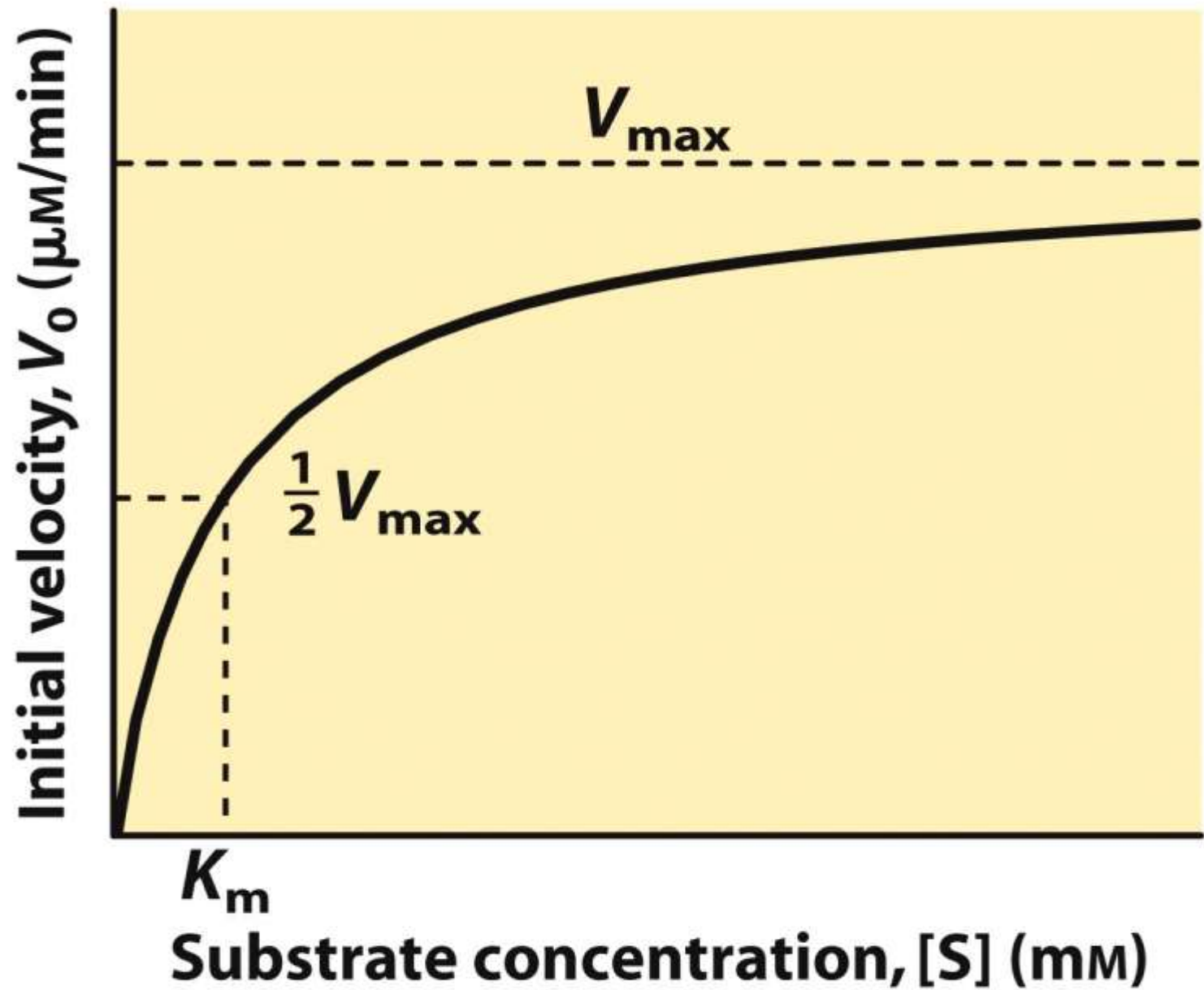


Figure 6-11

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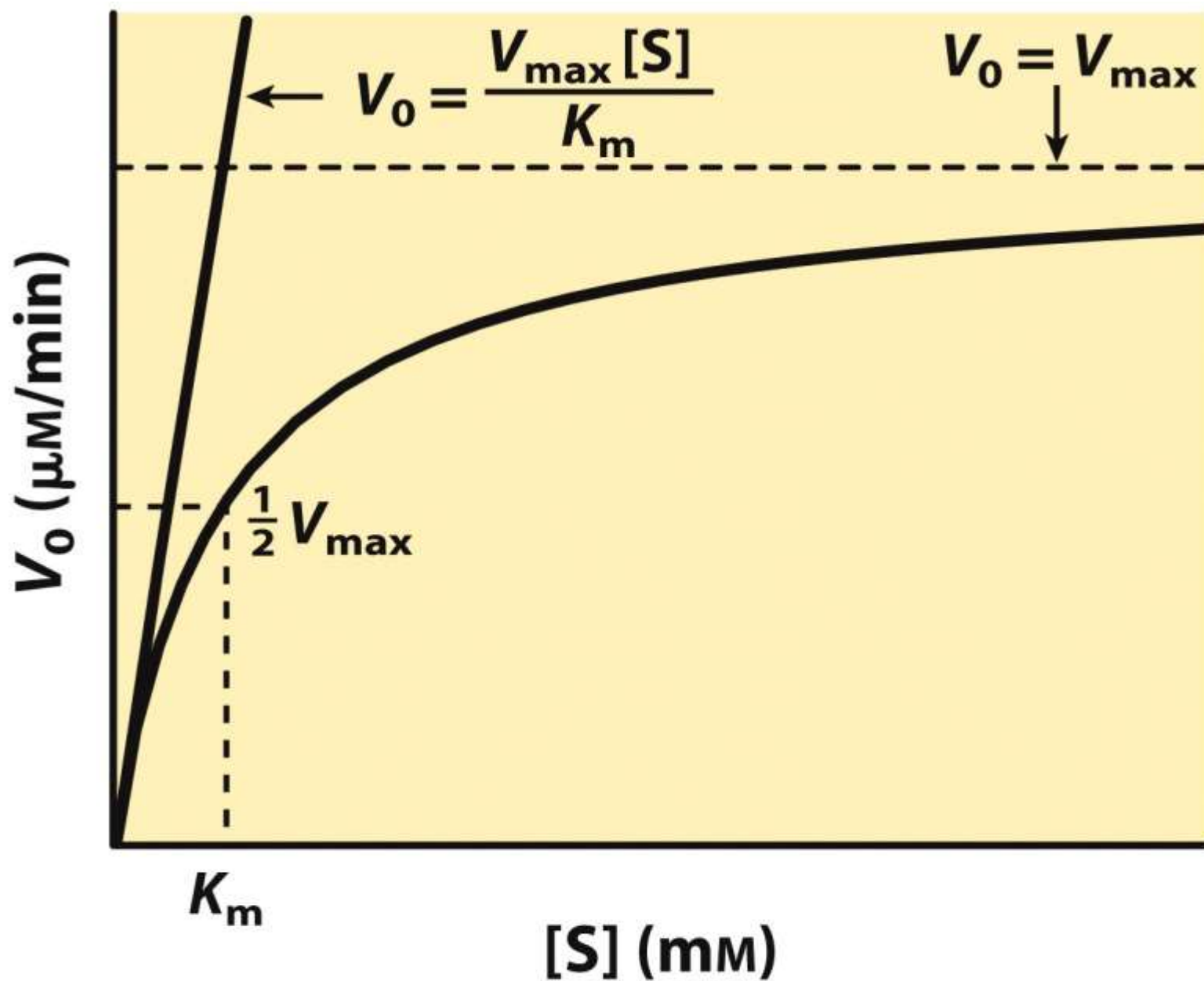


Figure 6-12

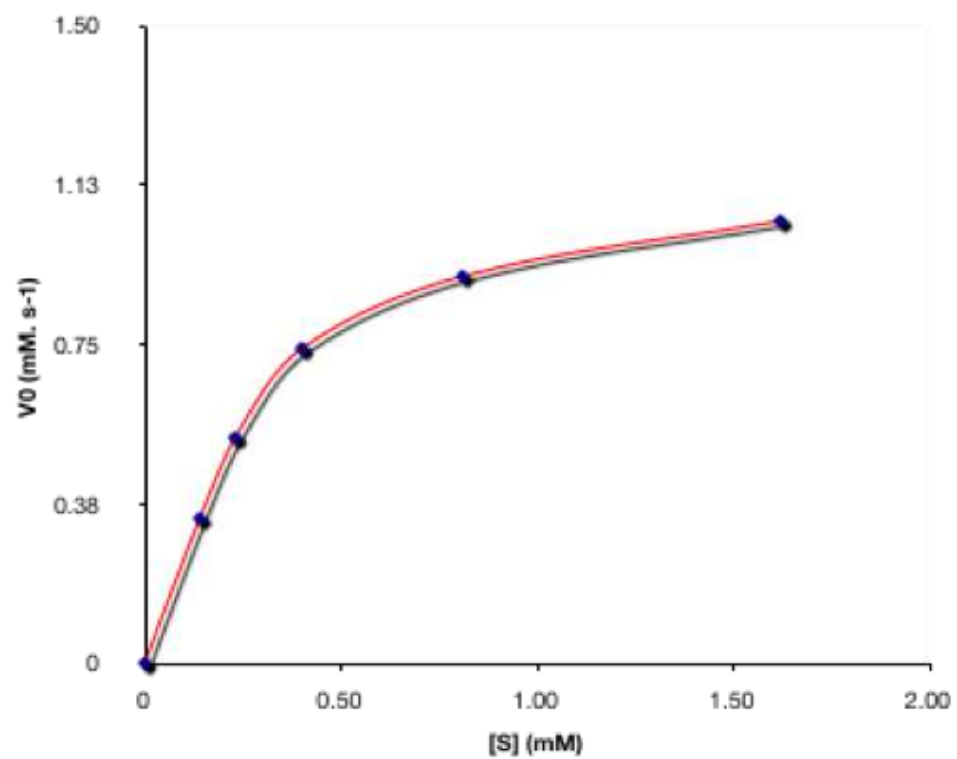
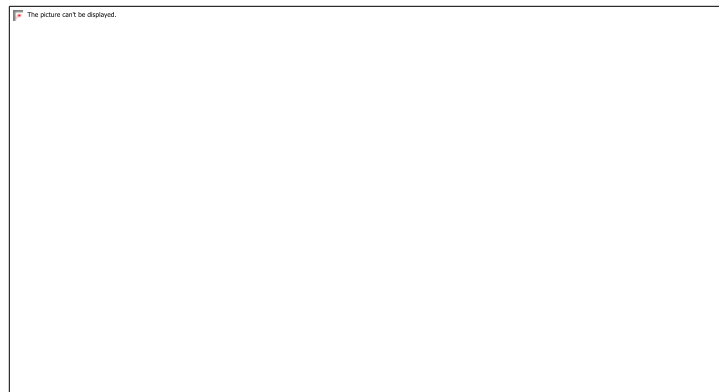
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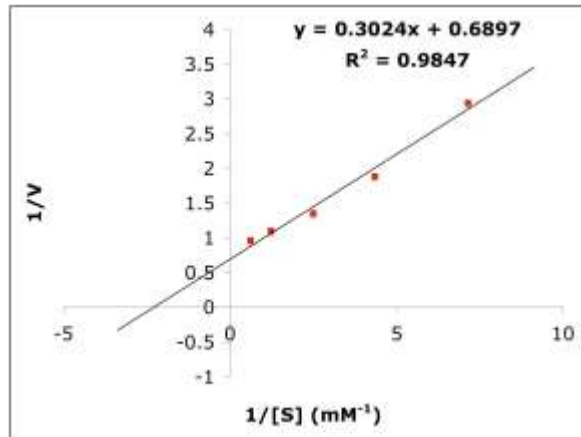
Double-reciprocal plot

Dissociation constant

Turnover number



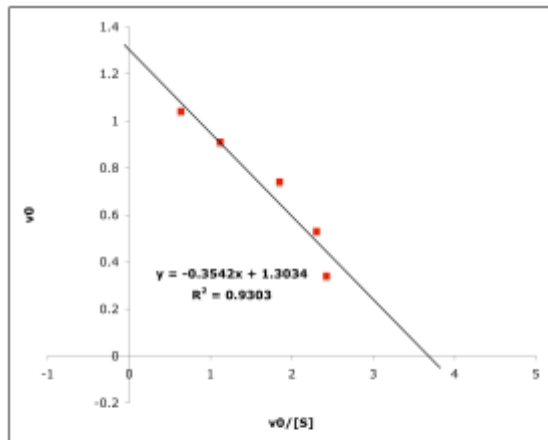
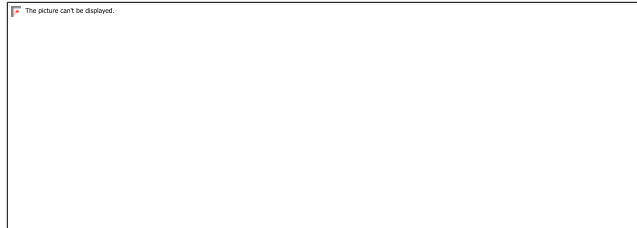
Lineweaver-Burk Plot



$$V_{\max} = 1/0.6897 = 1.45$$

$$K_m = -1/(-2.28) = 0.44 \text{ (M)}$$

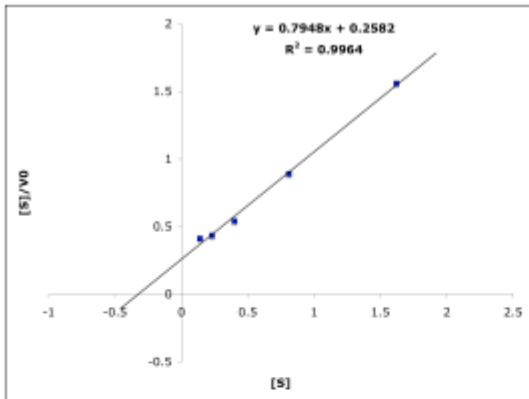
Eadie-Hofstee Plot



$$V_{\max} = 1.30$$

$$K_m = 0.354$$

Haynes-Woolf Plot



$$K_m = 0.32$$

$$V_{\max} = 0.80$$

Eisenthal-Cornish-Bowden Direct Plot

Direct Linear Plot

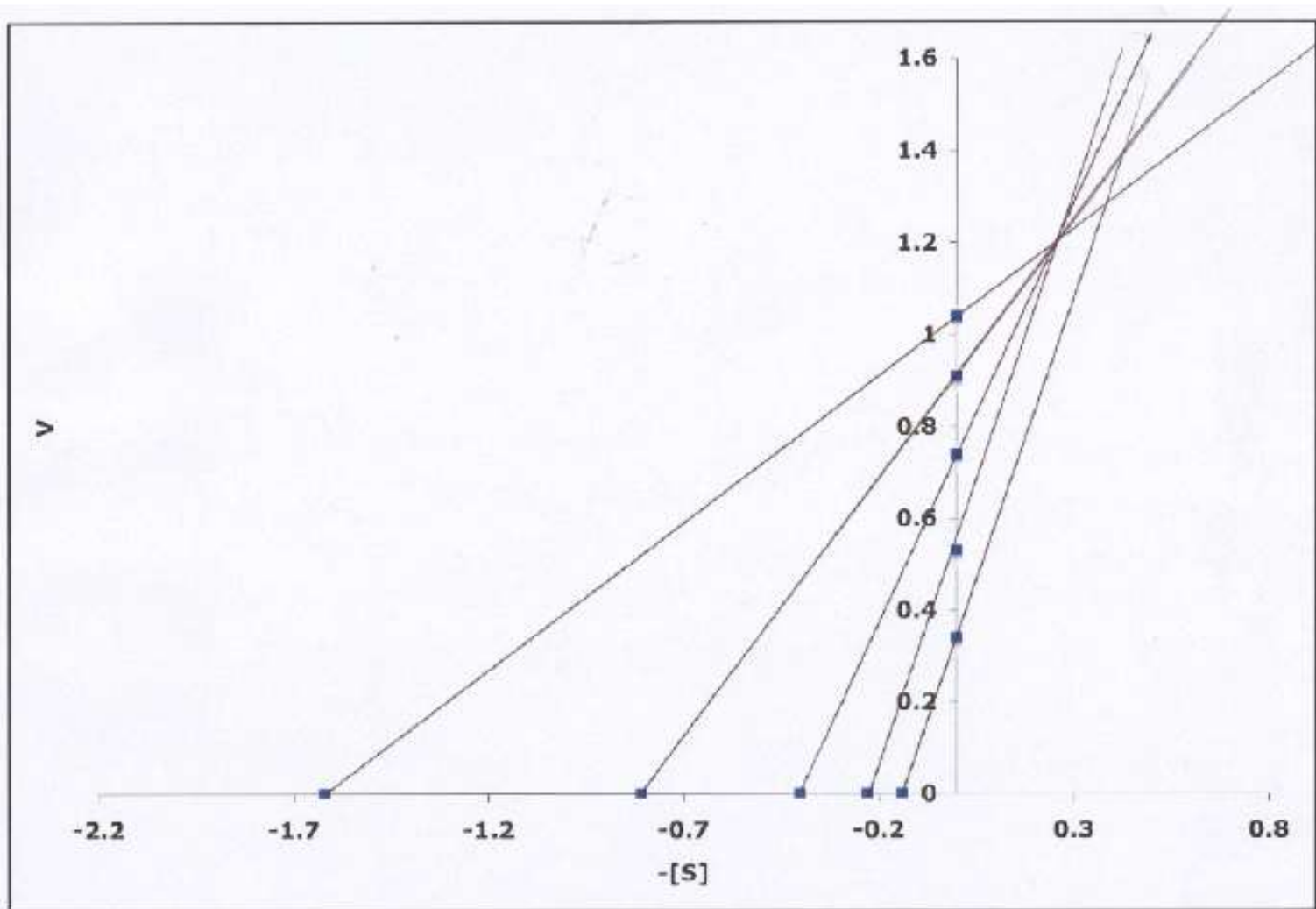


TABLE 6–6		K_m for Some Enzymes and Substrates
Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO₃[−]	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

Table 6-6

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TABLE 6–7		Turnover Numbers, 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	

Table 6-7

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TABLE 6–8		Enzymes for Which $k_{\text{cat}}/K_{\text{m}}$ Is Close to the Diffusion-Controlled Limit (10^8 to $10^9 \text{ M}^{-1}\text{s}^{-1}$)		
Enzyme	Substrate	k_{cat} (s^{-1})	K_{m} (M)	$k_{\text{cat}}/K_{\text{m}}$ ($\text{M}^{-1}\text{s}^{-1}$)
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.

Table 6-8

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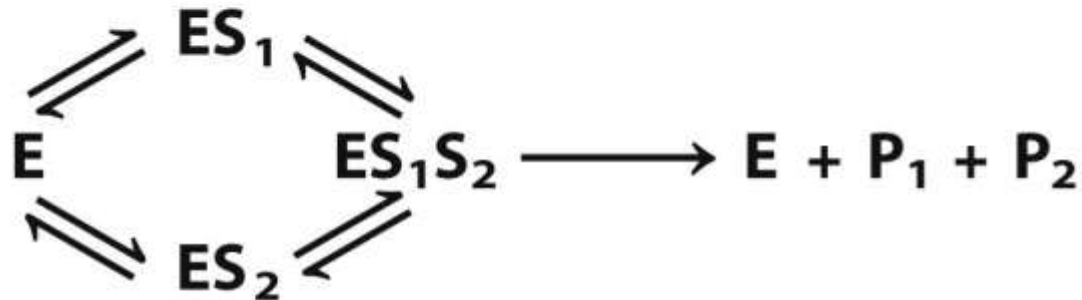
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More complex systems

More complex systems

(a) Enzyme reaction involving a ternary complex

Random order



Ordered



(b) Enzyme reaction in which no ternary complex is formed



Figure 6-13

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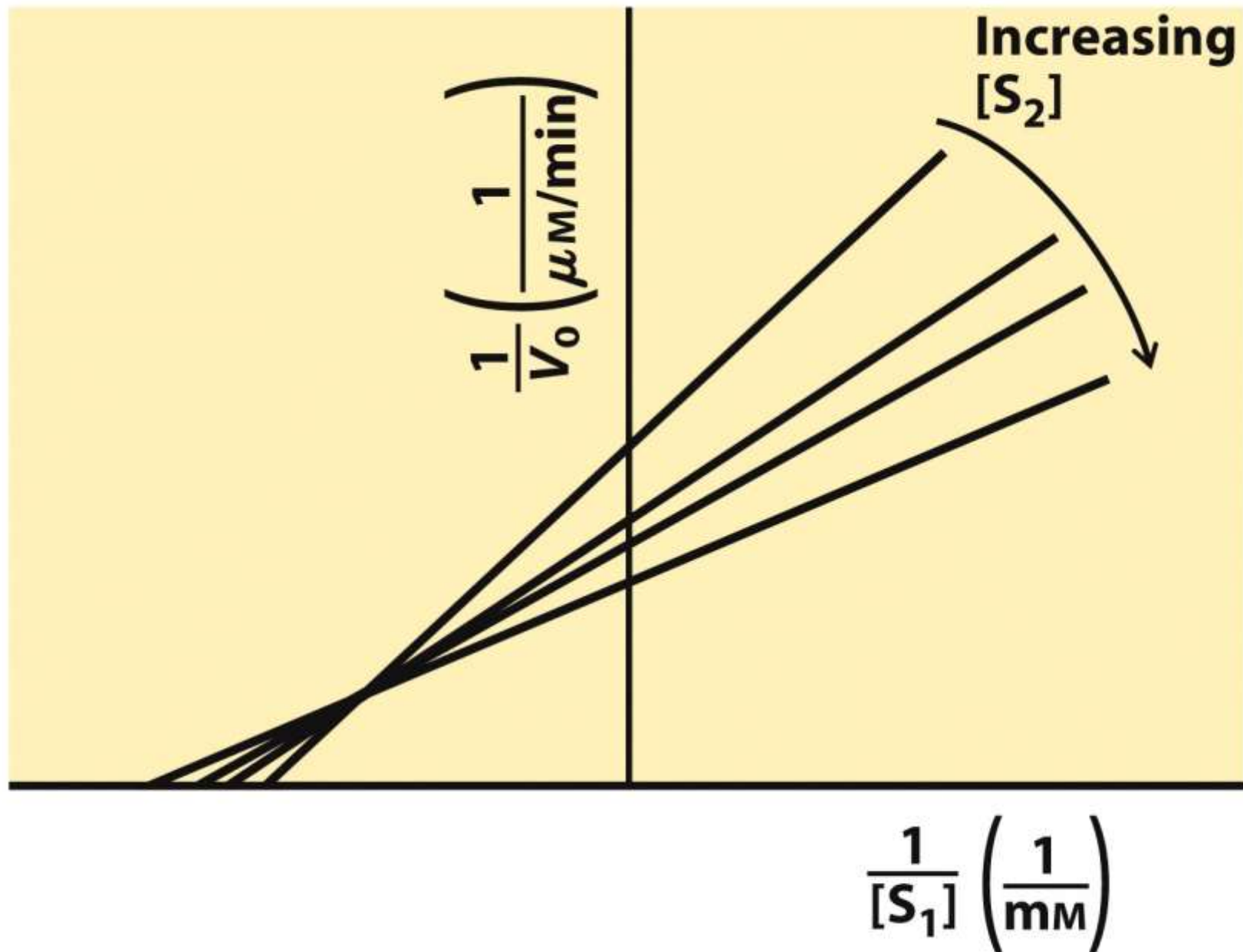


Figure 6-14a

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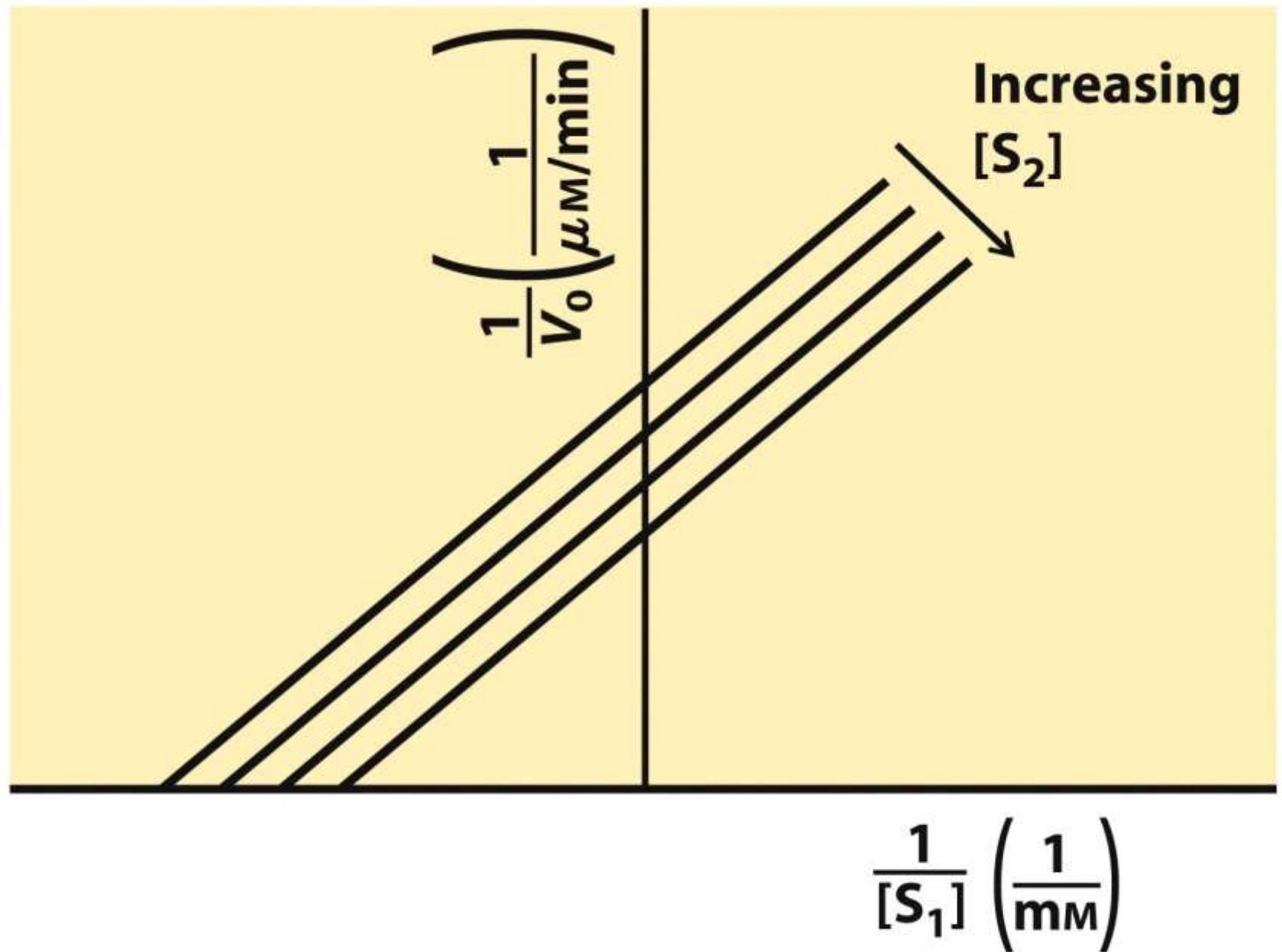


Figure 6-14b

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Reversible Inhibition

Reversible inhibition is a type of enzyme inhibition where the inhibitor binds to the enzyme in a reversible manner, allowing the enzyme to regain its activity once the inhibitor is removed.

There are two main types of reversible inhibition: competitive inhibition and non-competitive inhibition.

Competitive Inhibition: In competitive inhibition, the inhibitor binds to the active site of the enzyme, preventing the substrate from binding. This type of inhibition is reversible, and the enzyme's activity can be restored by increasing the concentration of the substrate.

Non-competitive Inhibition: In non-competitive inhibition, the inhibitor binds to a site on the enzyme other than the active site, causing a change in the enzyme's shape and preventing the substrate from binding. This type of inhibition is also reversible, and the enzyme's activity can be restored by increasing the concentration of the substrate.

Reversible inhibition is important in many biological processes, such as the regulation of enzyme activity and the control of metabolic pathways.

Understanding reversible inhibition is crucial for developing drugs and understanding the mechanisms of various diseases.

Competitive inhibition

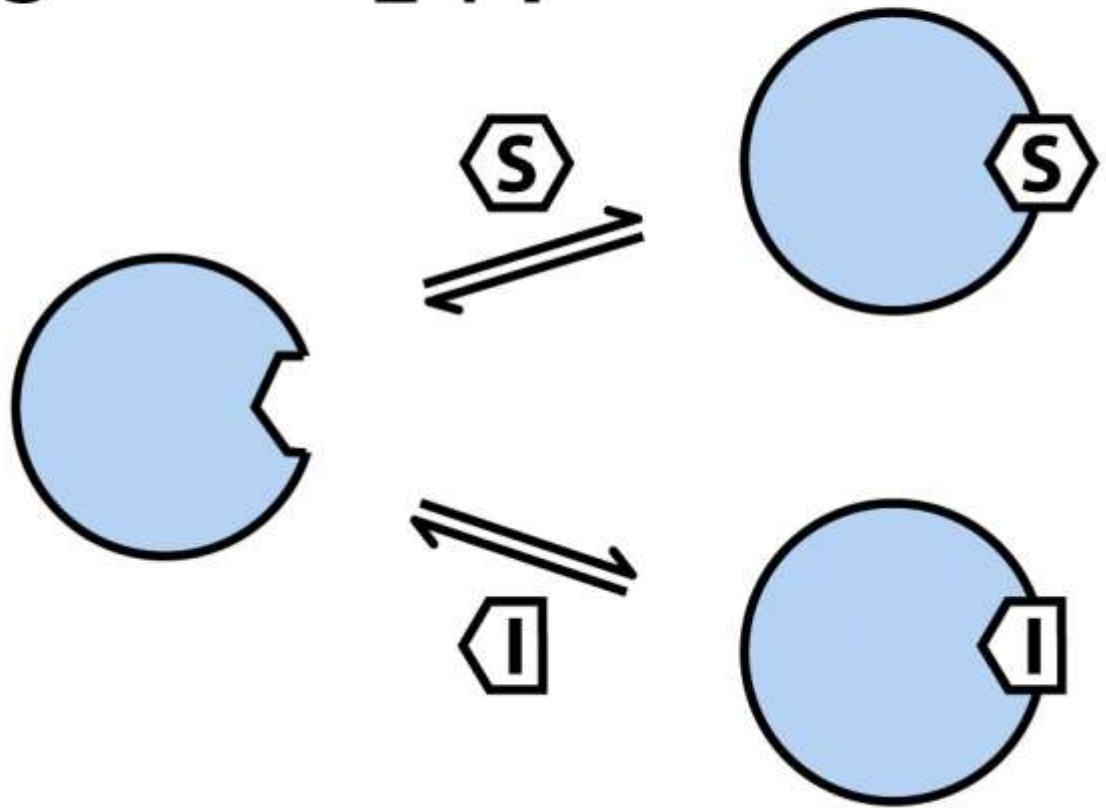


Figure 6-15a

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Uncompetitive inhibition

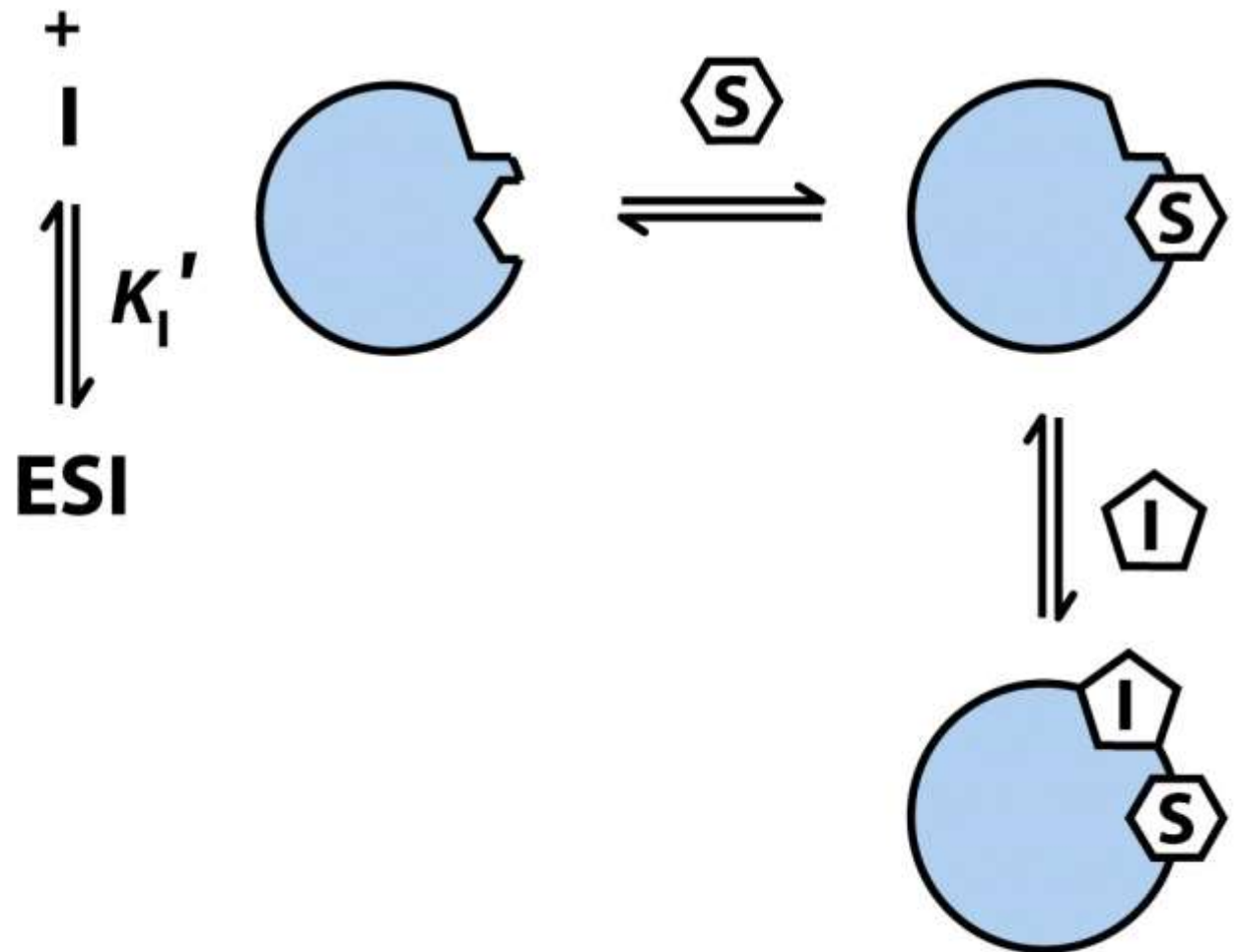


Figure 6-15b

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Mixed inhibition

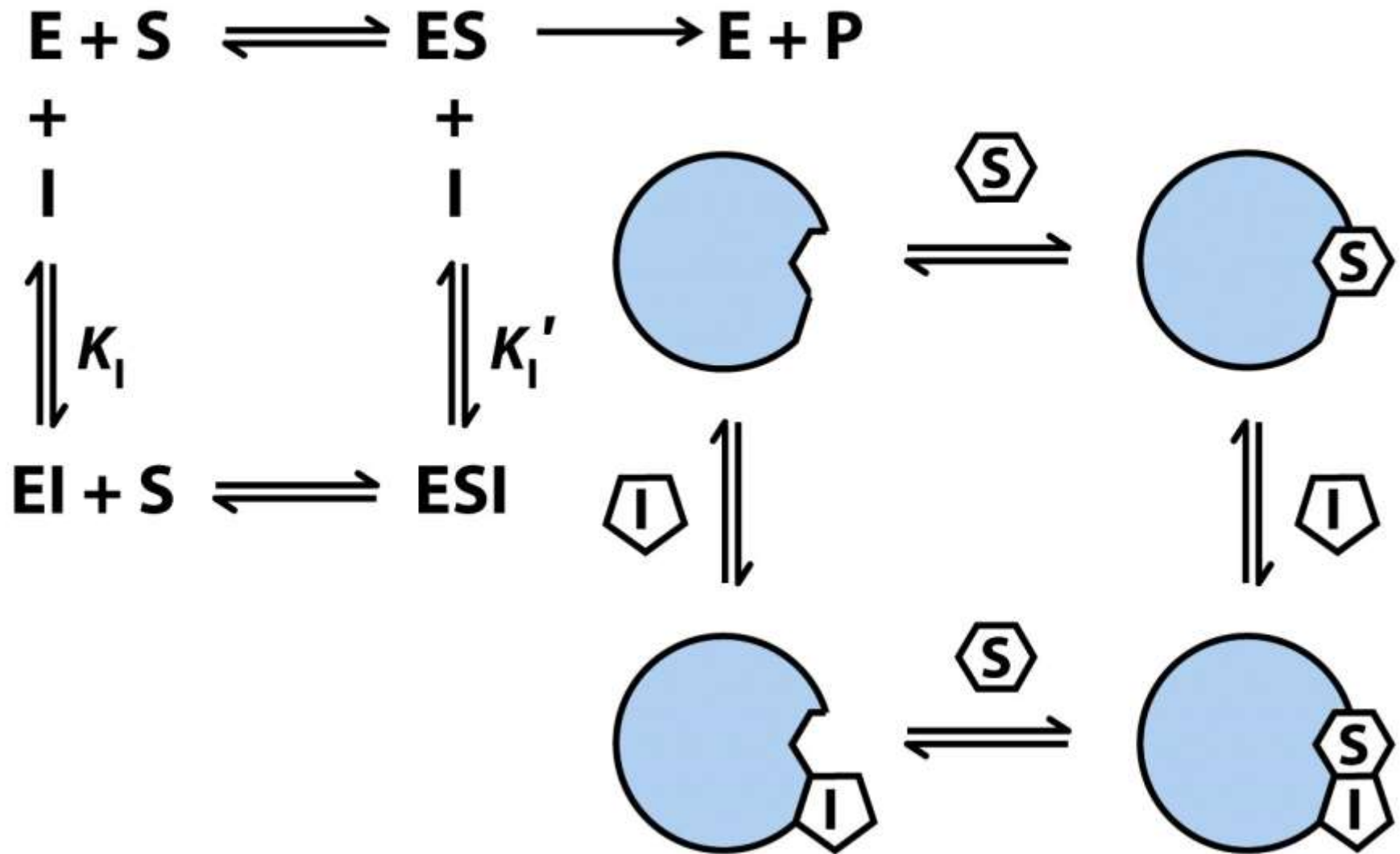
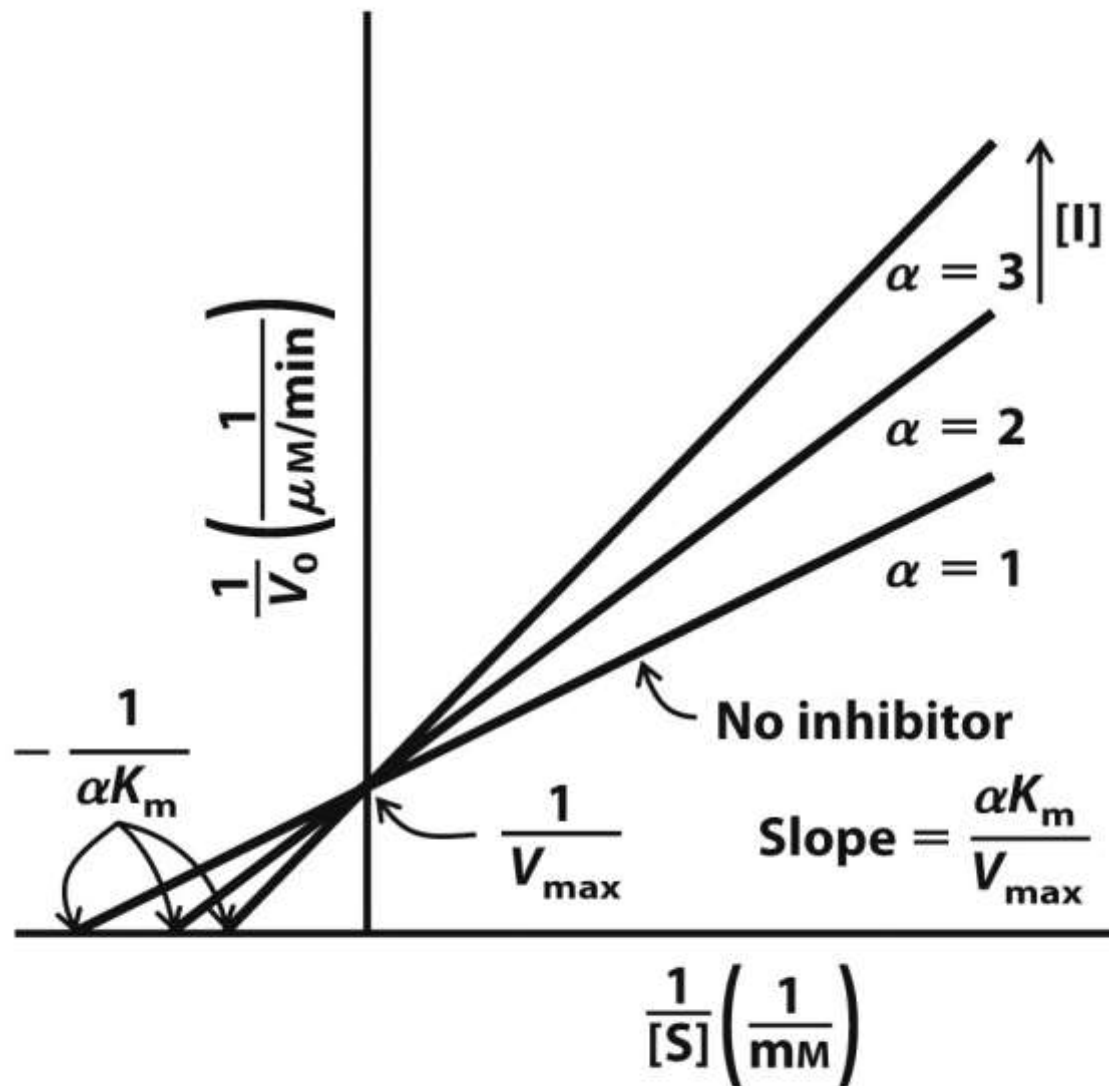


Figure 6-15c
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$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{\max}} \right) \frac{1}{[S]} + \frac{1}{V_{\max}}$$

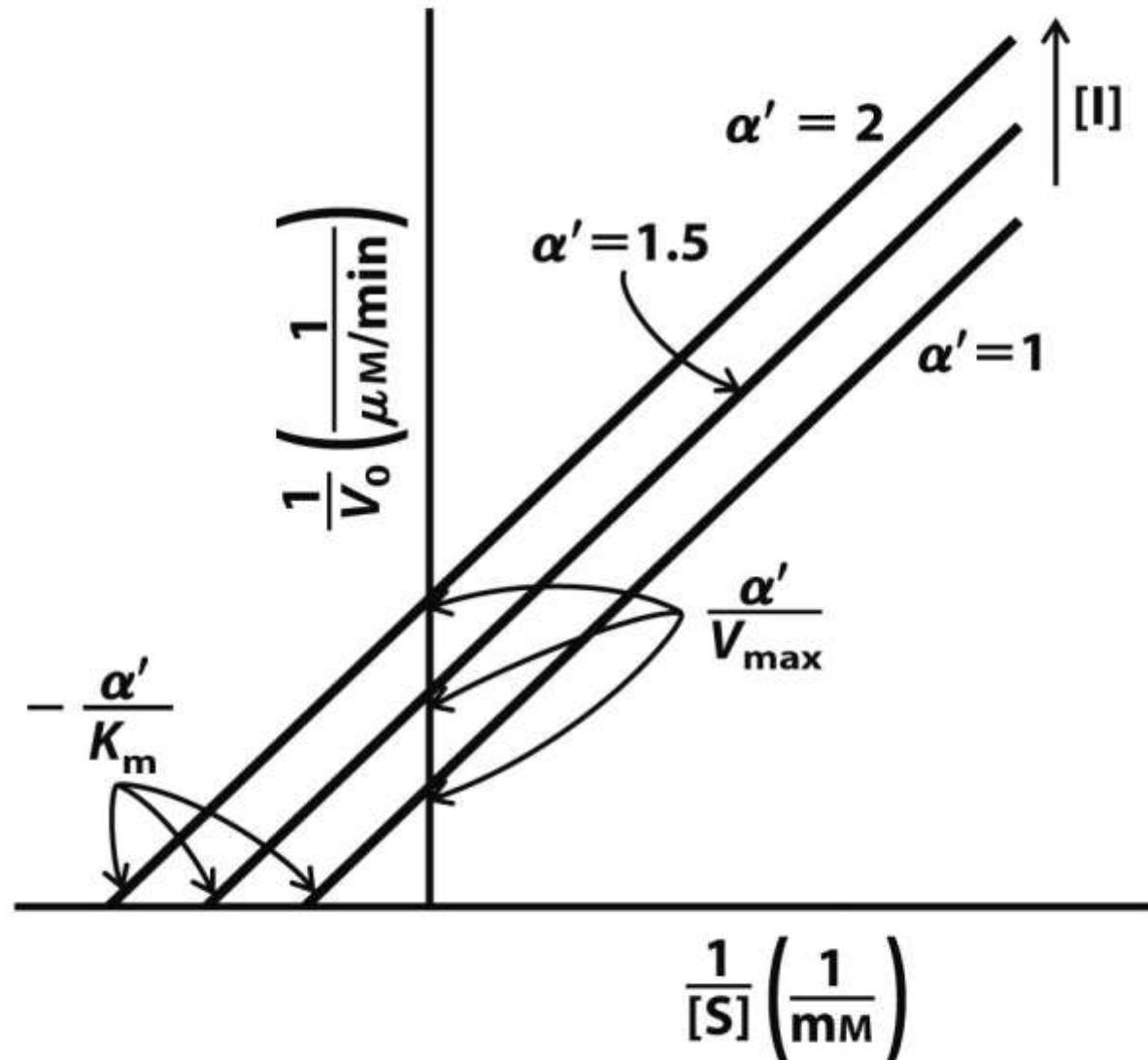


Box 6-2 figure 1

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$$\frac{1}{V_0} = \left(\frac{K_m}{V_{\max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{\max}}$$

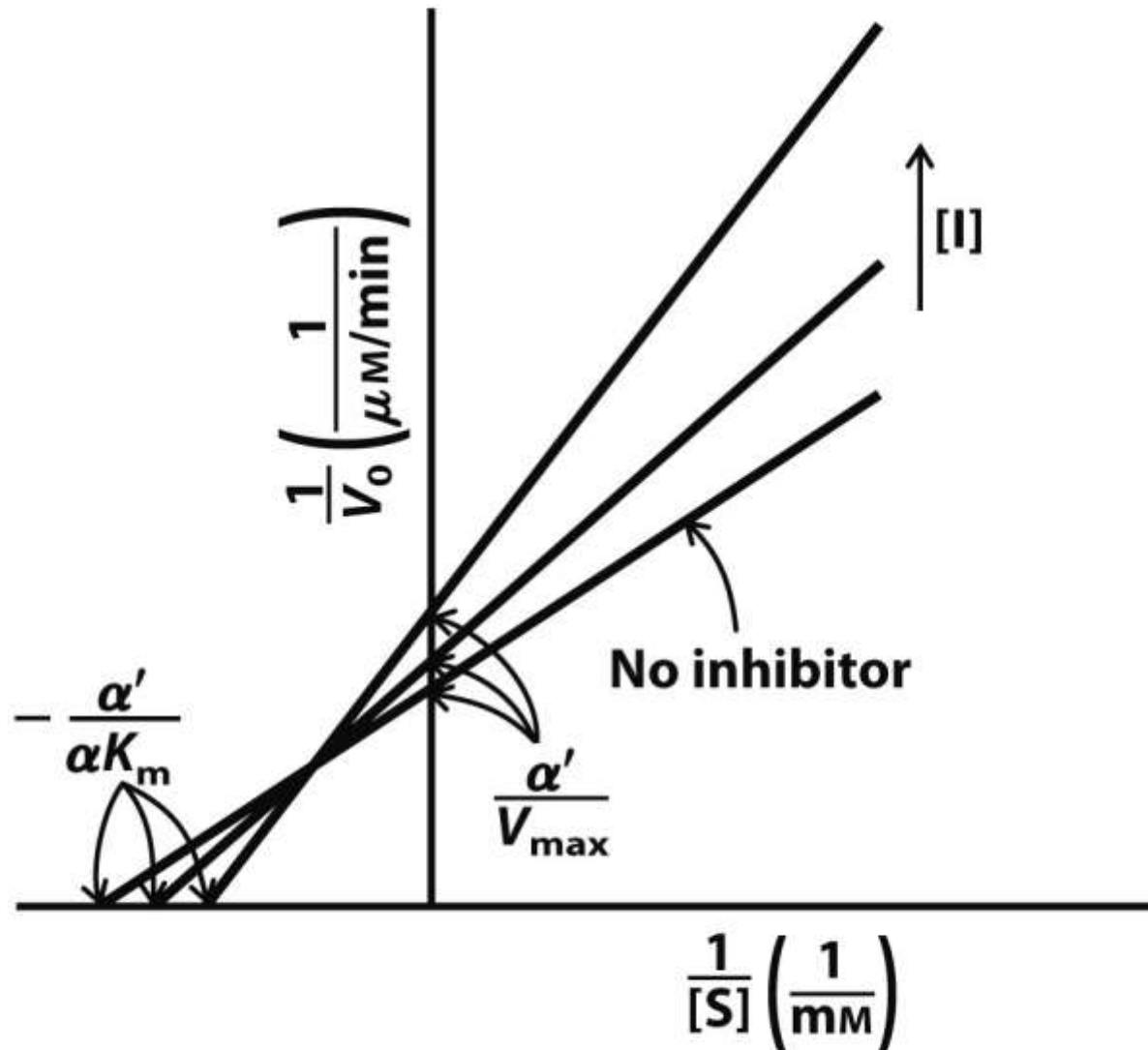


Box 6-2 figure 2

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$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{\max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{\max}}$$



Box 6-2 figure 3

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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*Lehninger Principles of Biochemistry, Fifth Edition*

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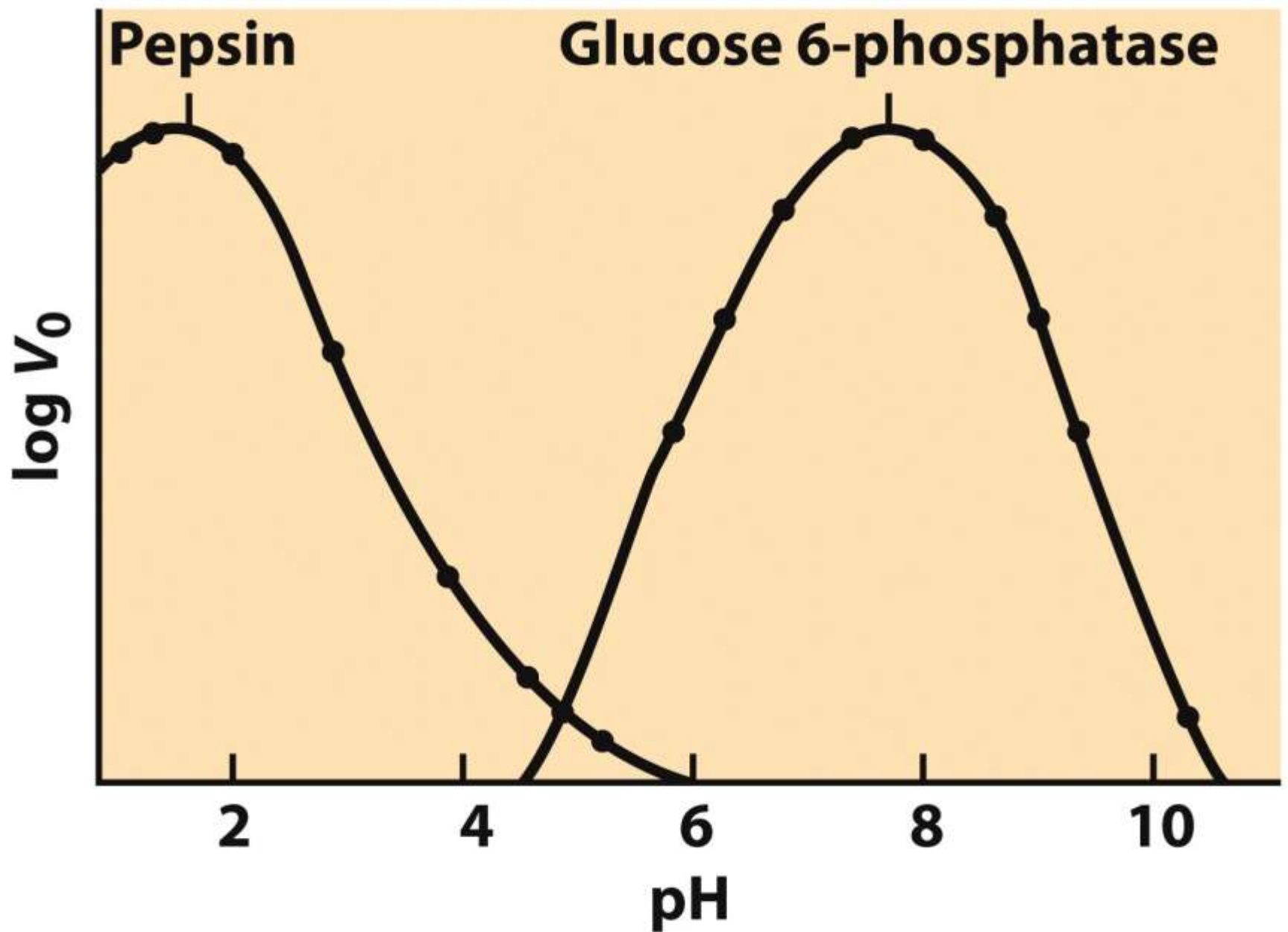


Figure 6-17
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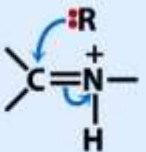
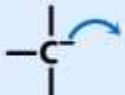
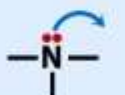
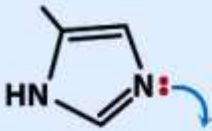
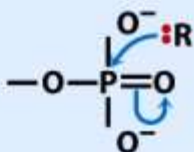
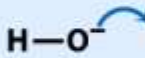

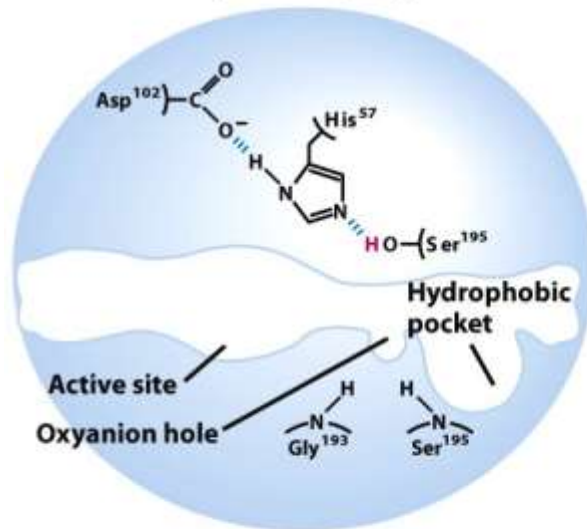
Nucleophiles	Electrophiles
 <p>Negatively charged oxygen (as in an unprotonated hydroxyl group or an ionized carboxylic acid)</p>	 <p>Carbon atom of a carbonyl group (the more electronegative oxygen of the carbonyl group pulls electrons away from the carbon)</p>
 <p>Negatively charged sulfhydryl</p>	 <p>Protonated imine group (activated for nucleophilic attack at the carbon by protonation of the imine)</p>
 <p>Carbanion</p>	 <p>Uncharged amine group</p>
 <p>Imidazole</p>	 <p>Phosphorus of a phosphate group</p>
 <p>Hydroxide ion</p>	 <p>Proton</p>

Figure 6-21 part 1

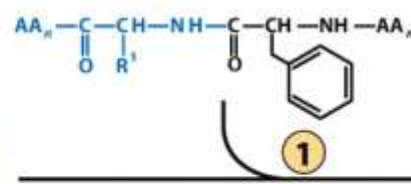
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Chymotrypsin (free enzyme)



Substrate (a polypeptide)



When substrate binds, the side chain of the residue adjacent to the peptide bond to be cleaved nestles in a hydrophobic pocket on the enzyme, positioning the peptide bond for attack.

Interaction of Ser¹⁹⁵ and His⁵⁷ generates a strongly nucleophilic alkoxide ion on Ser¹⁹⁵; the ion attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme. This is accompanied by formation of a short-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bonding in the oxyanion hole.



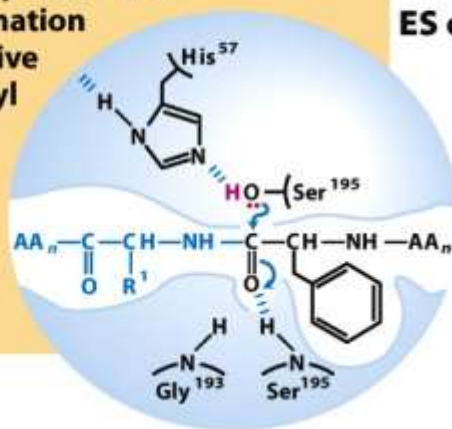
ES complex

Figure 6-21 part 2a

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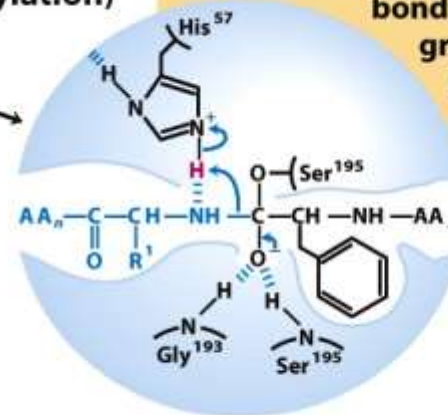
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Interaction of Ser¹⁹⁵ and His⁵⁷ generates a strongly nucleophilic alkoxide ion on Ser¹⁹⁵; the ion attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme. This is accompanied by formation of a short-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bonding in the oxyanion hole.



Short-lived intermediate* (acylation)

2



Instability of the negative charge on the substrate carbonyl oxygen leads to collapse of the tetrahedral intermediate; re-formation of a double bond with carbon displaces the bond between carbon and the amino group of the peptide linkage, breaking the peptide bond. The amino leaving group is protonated by His⁵⁷, facilitating its displacement.

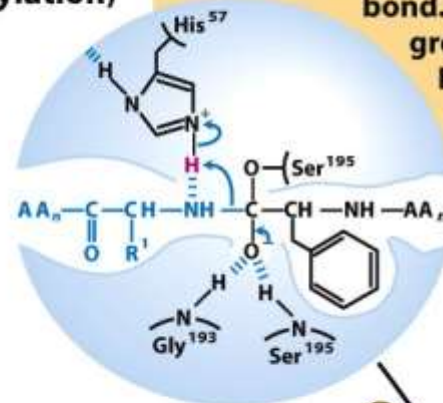
Figure 6-21 part 2b

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**Short-lived
intermediate*
(acylation)**

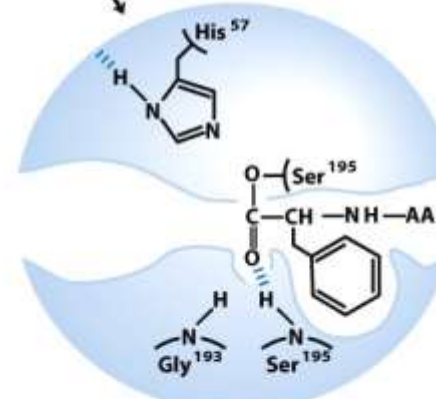
Instability of the negative charge on the substrate carbonyl oxygen leads to collapse of the tetrahedral intermediate; re-formation of a double bond with carbon displaces the bond between carbon and the amino group of the peptide linkage, breaking the peptide bond. The amino leaving group is protonated by His⁵⁷, facilitating its displacement.



Product 1



3



**Acyl-enzyme
intermediate**

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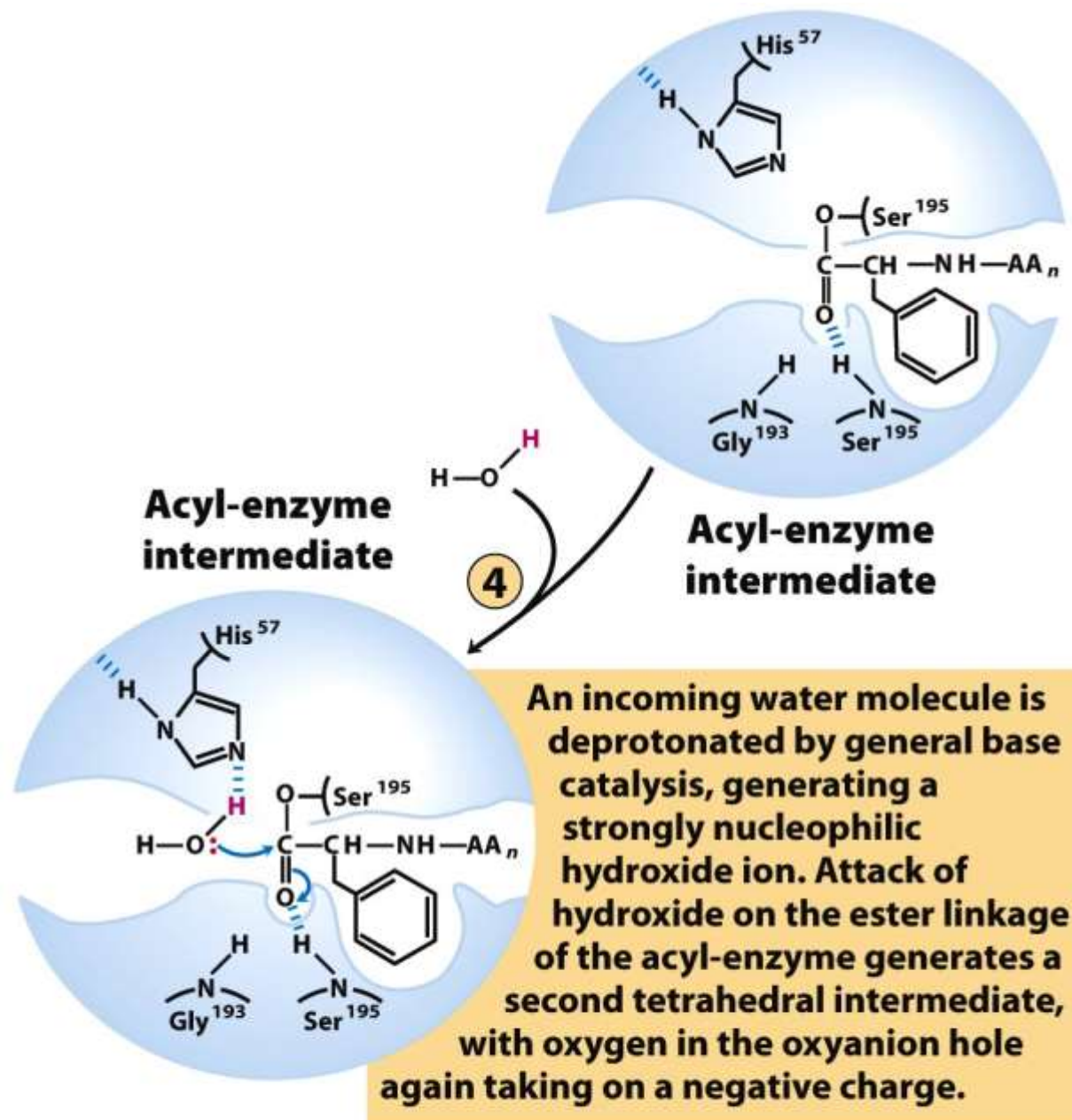
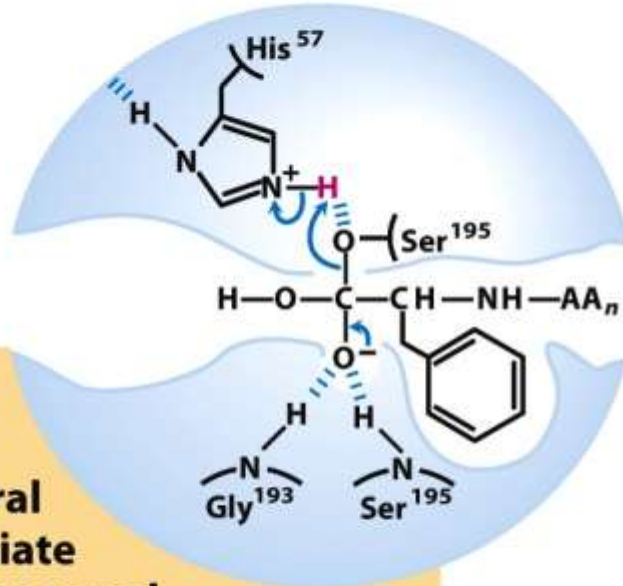


Figure 6-21 part 2d

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**Short-lived
intermediate*
(deacylation)**



**Collapse
of the
tetrahedral
intermediate
forms the second
product, a carboxylate anion,
and displaces Ser¹⁹⁵.**

5

**Acyl-enzyme
intermediate**

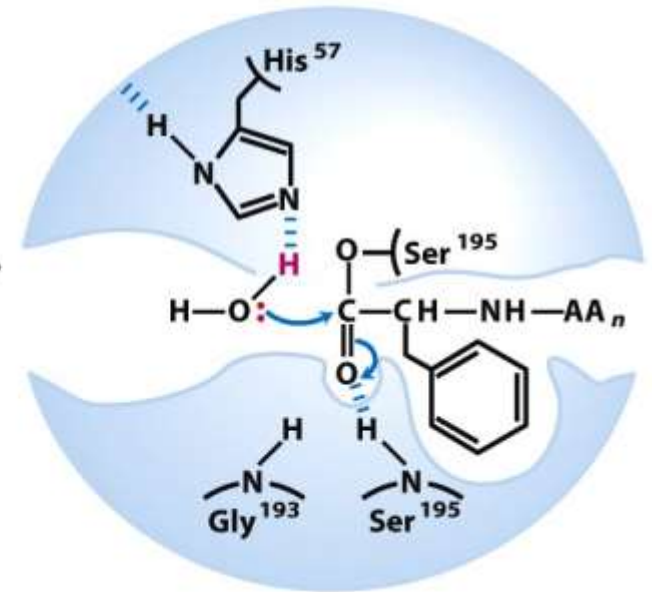
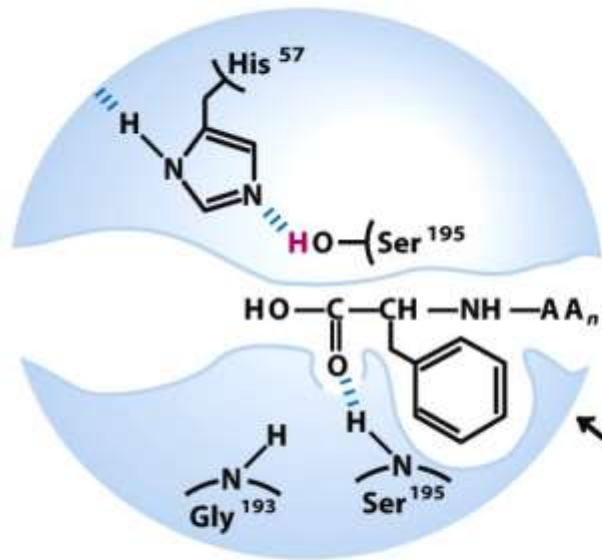


Figure 6-21 part 2e

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Enzyme-product 2 complex



Short-lived intermediate* (deacylation)

6

Collapse of the tetrahedral intermediate forms the second product, a carboxylate anion, and displaces Ser¹⁹⁵.

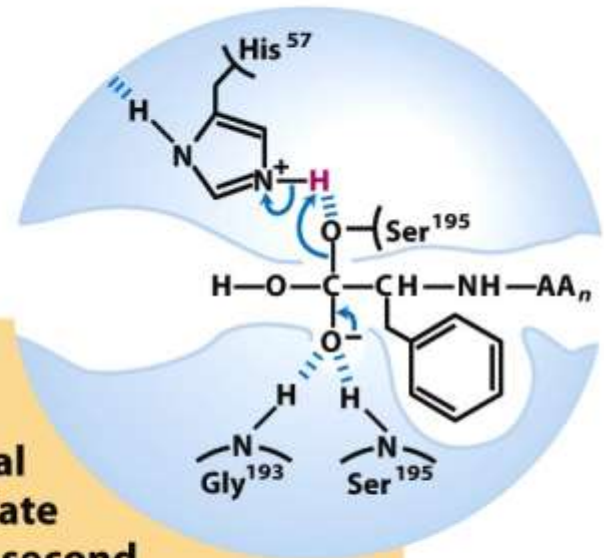


Figure 6-21 part 2f

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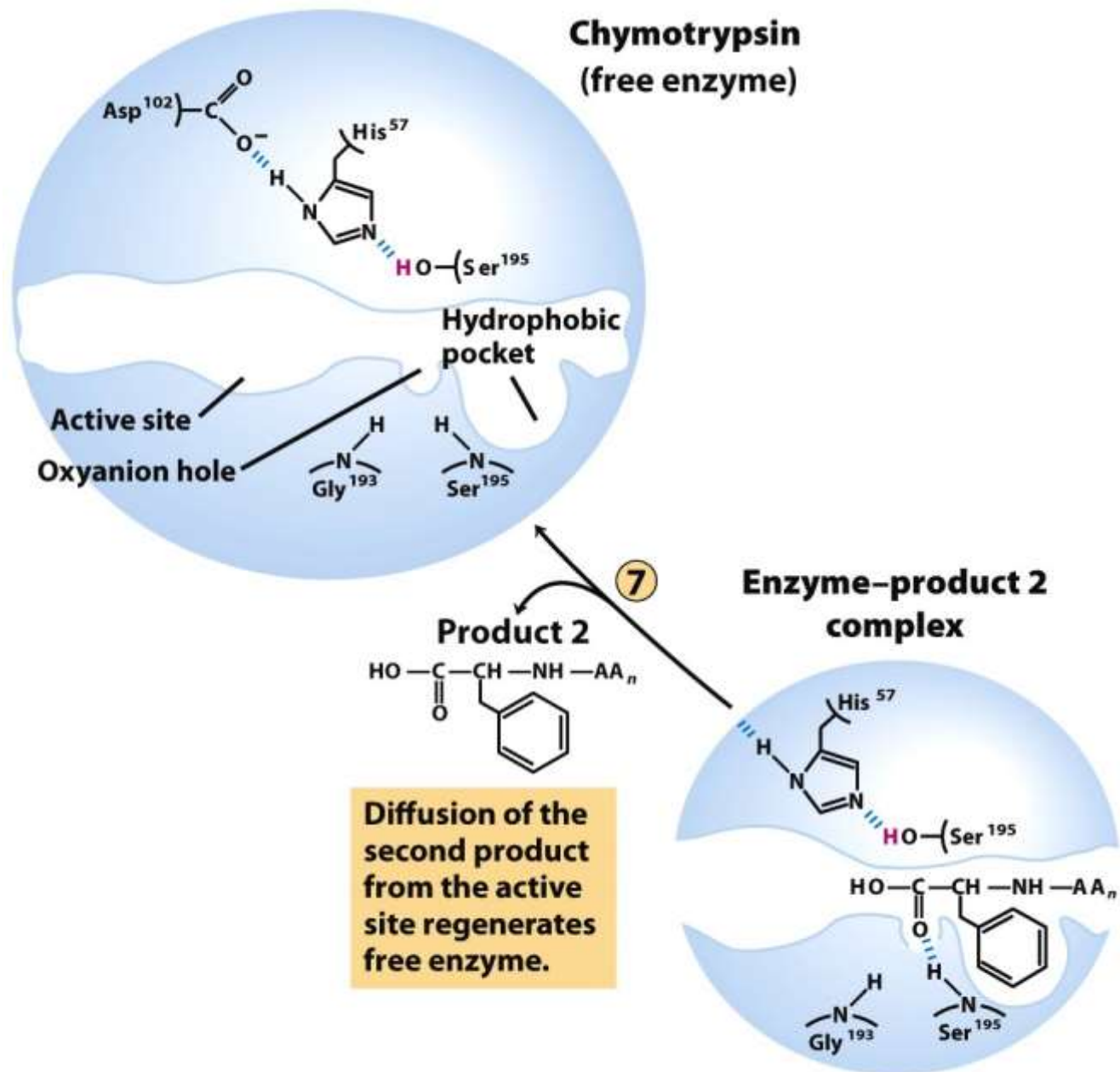
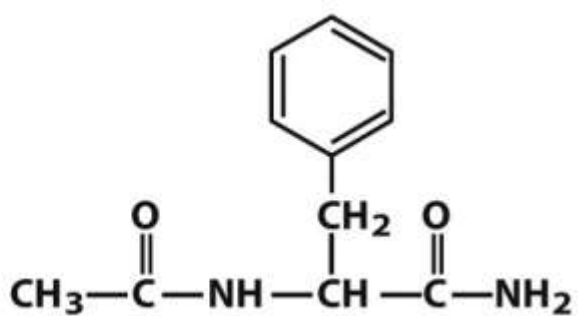
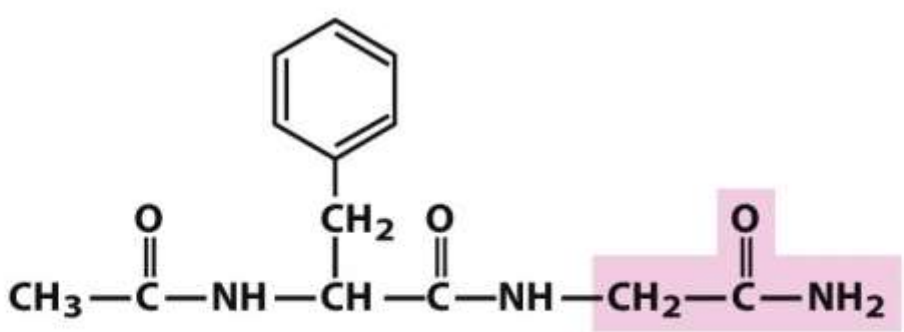
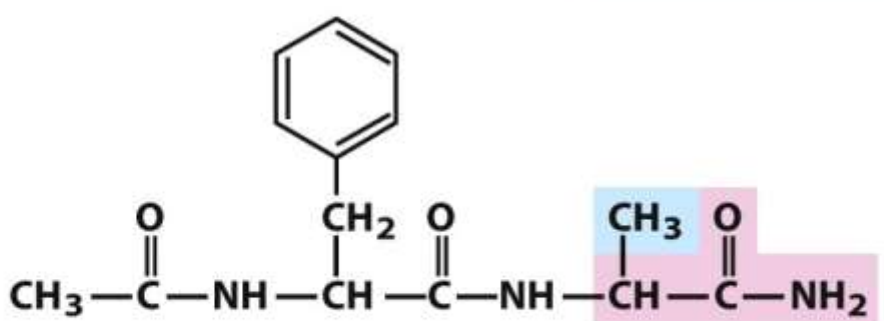


Figure 6-21 part 2g
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		k_{cat} (s^{-1})	K_{m} (mM)	$k_{\text{cat}}/K_{\text{m}}$ ($\text{M}^{-1} \text{s}^{-1}$)
Substrate A	 $\text{CH}_3-\text{C}(=\text{O})-\text{NH}-\text{CH}(\text{CH}_2\text{Ph})-\text{C}(=\text{O})-\text{NH}_2$	0.06	31	2
Substrate B	 $\text{CH}_3-\text{C}(=\text{O})-\text{NH}-\text{CH}(\text{CH}_2\text{Ph})-\text{C}(=\text{O})-\text{NH}-\text{CH}_2-\text{C}(=\text{O})-\text{NH}_2$	0.14	15	10
Substrate C	 $\text{CH}_3-\text{C}(=\text{O})-\text{NH}-\text{CH}(\text{CH}_2\text{Ph})-\text{C}(=\text{O})-\text{NH}-\text{CH}(\text{CH}_3)-\text{C}(=\text{O})-\text{NH}_2$	2.8	25	114

Box 6-3 figure 1

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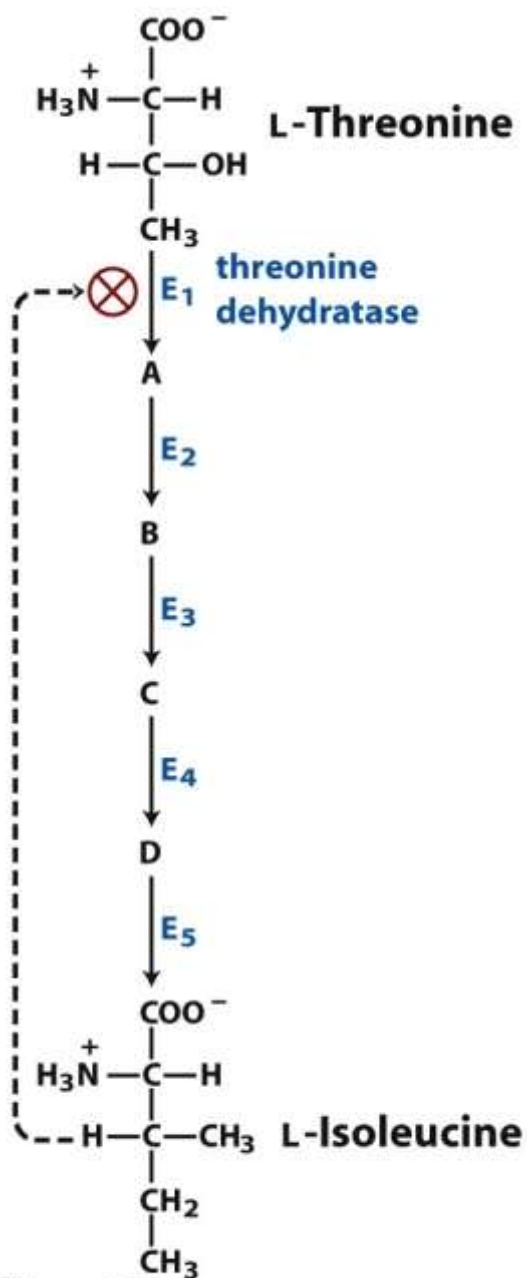


Figure 6-33

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