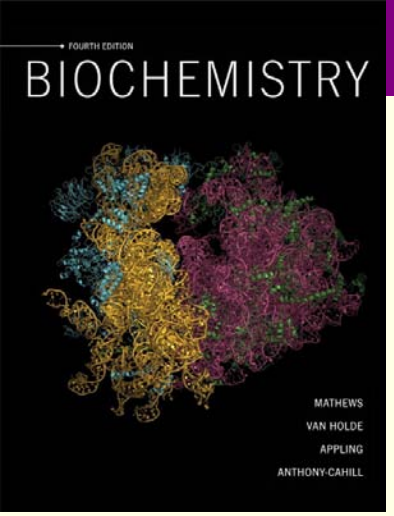


Chapter 11

Enzymes: Biological Catalysts

Significance of enzyme study:

1. Normal enzyme function is required for life maintenance

2. Medical treatment and diagnostic

3. Drug development

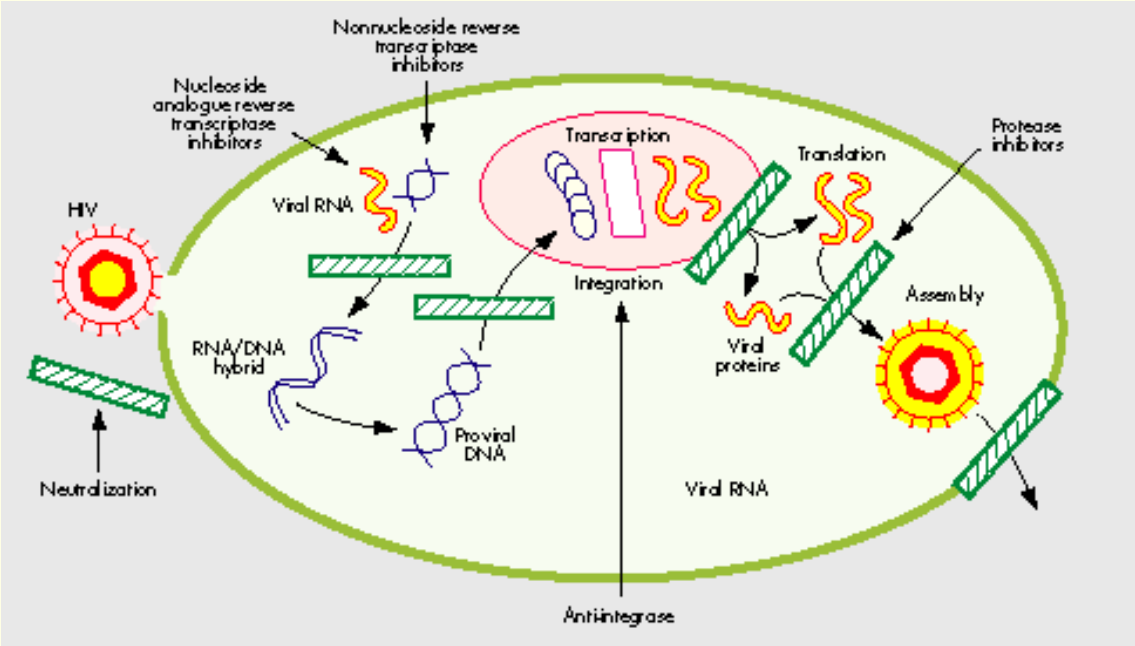
Aspartate aminotransferase (AST;*SGOT*)
Alanine aminotransferase (ALT;SGPT)

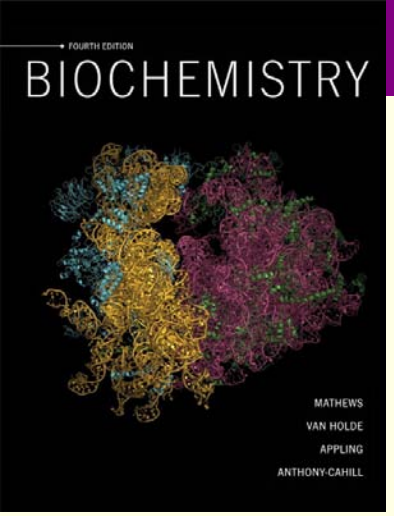
何大一博士

美國艾倫·戴蒙德艾滋病研究中心的主任

『雞尾酒療法』

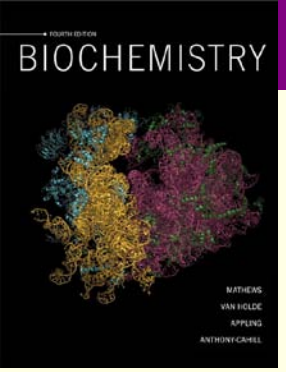
(Highly active antiretroviral therapy, HAART)，於1996年由何大一博士提出是指合併三種抗HIV病毒藥物，包括**蛋白酶抑制劑(Protease Inhibitors)** ＋**非核苷類反轉錄酶抑制劑(Non-Nucleoside Reverse Transcriptase Inhibitors)**＋**核苷類反轉錄酶抑制劑(Nucleoside Reverse Transcriptase Inhibitors)**治療，以期降低病毒量、提高免疫力、改善存活率和減少抗藥種產生。雞尾酒療法藥物一個月大約要花費三萬元新台幣，一年大約花費36萬。衛生署自1997年4月開始免費提供藥物，由指定醫院的感染科醫師負責開立處方，每位感染者及患者都可以在衛生署指定醫院獲得治療。





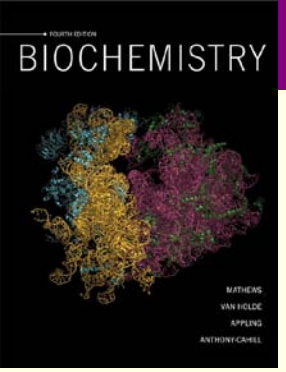
Chapter 11 Outline:

- The Role of Enzymes
- Chemical Reaction Rates and the Effects of Catalysts—A Review
- How Enzymes Act as Catalysts: Principles and Examples
- The Kinetics of Enzymatic Catalysis
- Enzyme Inhibition
- Cofactors, Vitamins, and Essential Metals
- The Diversity of Enzymatic Function
- Nonprotein Biocatalysts: Catalytic Nucleic Acids
- The Regulation of Enzyme Activity: Allosteric Enzymes
- Covalent Modifications Used to Regulate Enzyme Activity



The Role of Enzymes

- In general terms, a catalyst is a substance that increases the rate, or velocity, of a chemical reaction without itself being changed in the overall process.
- Enzymes are biological catalysts, most of which are proteins.
- For example, the protein ***trypsin*** catalyzes hydrolysis of peptide bonds in proteins and polypeptides.
- The substance that is acted on by an enzyme is called the ***substrate*** of that enzyme.



The Role of Enzymes

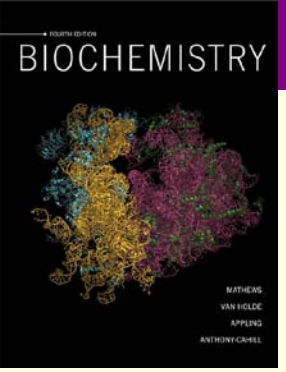
Two facts deserve emphasis:

1.Although a true catalyst *participates* in the reaction process, it is *unchanged* by the process.

•For example, after catalyzing the decomposition of an molecule, catalase is found again in exactly the same state as before, ready for another round.

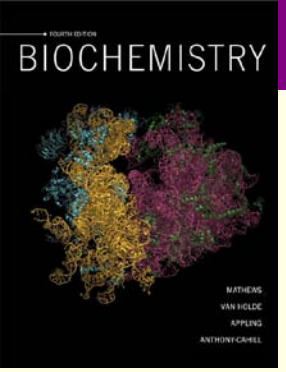
•In contrast, although hemoglobin accelerates the rate of H_2O_2 decomposition, it is oxidized in the process, from the active Fe^{2+} to the inactive Fe^{3+} form; thus, hemoglobin is not a true catalyst for this reaction.

2.Catalysts change *rates of processes but do not affect the position* of equilibrium for a reaction. A thermodynamically favorable process is not made more favorable, nor is an unfavorable process made favorable, by the presence of a catalyst. *The equilibrium state is just approached more quickly.*



Chemical Reaction Rates and the Effects of Catalysts—A Review

- A first-order reaction is one whose rate is directly proportional to the first power of the reactant concentration.
- A first-order reaction is characterized by single exponential decay of the reactant.



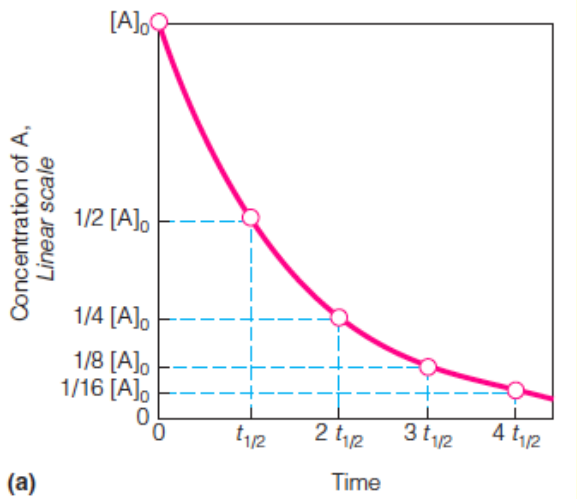
Chemical Reaction Rates and the Effects of Catalysts—A Review

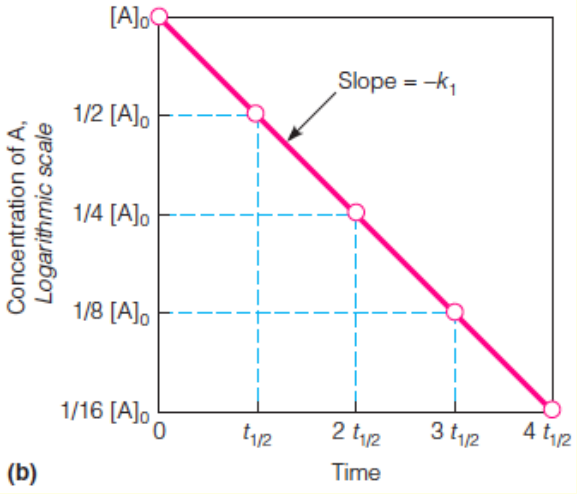
Determining the order and rate constant of an irreversible first-order reaction:

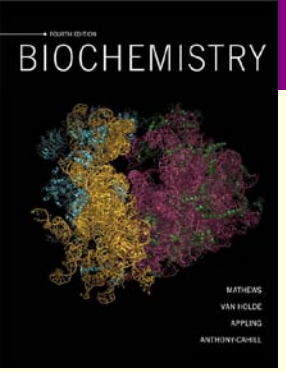
•Graphs (a) and (b) analyze the rate of a single reaction, with time expressed as multiples of the half-life ($t_{1/2}$) of the reactant. Note that for each interval of $t_{1/2}$ the reactant concentration is halved.

a) A graph of [A] versus *t* shows that the rate, defined as the slope of the curve, decreases as the reaction continues.

b) A graph of ln[A] versus t, when linear, indicates that the reaction follows the equation $[A]_t = [A]_o e^{-kt}$ and is first-order. The slope of this line (d ln[A]/d*t*) is equal to $-k_1$.

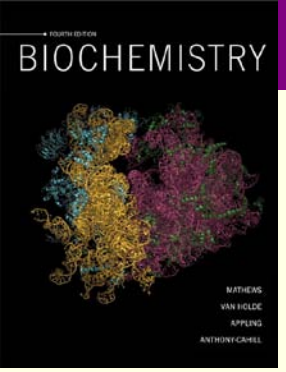






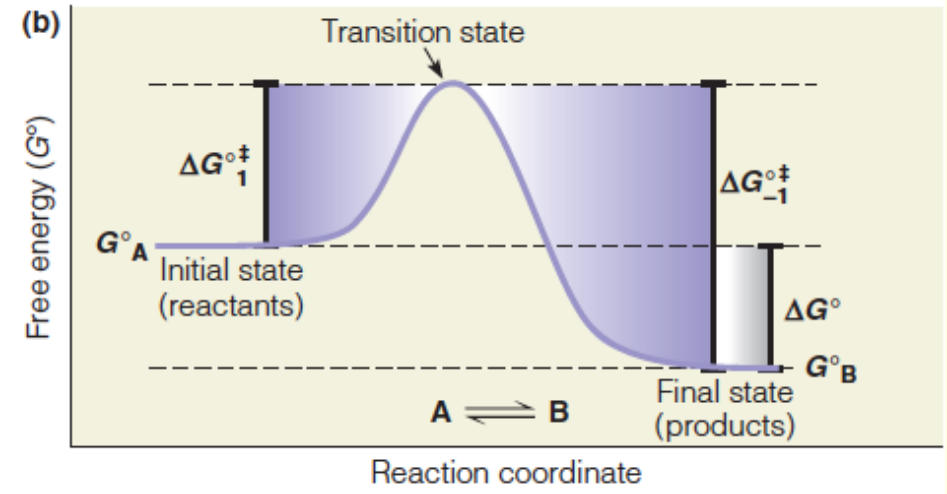
Chemical Reaction Rates and the Effects of Catalysts—A Review

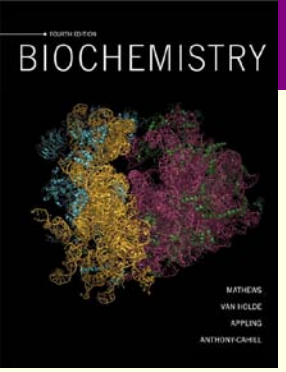
- A *first-order* rate constant has units of $(\text{time})^{-1}$, whereas a *second-order* rate constant has units of $(\text{concentration})^{-1}(\text{time})^{-1}$.
- Often, however, the analysis of complex multistep reaction schemes can be simplified by the recognition of a ***rate-limiting step***.
- The ***rate-limiting step*** is the slowest step in a multistep process.
- As such, it determines the experimentally observed rate for the entire process.



Chemical Reaction Rates and the Effects of Catalysts—A Review

- Barriers to chemical reactions occur because a reactant molecule must pass through a high-energy transition state to form products.
- This free energy barrier is called the ***activation energy***.
- Catalysts increase reaction rates by lowering the activation energy.





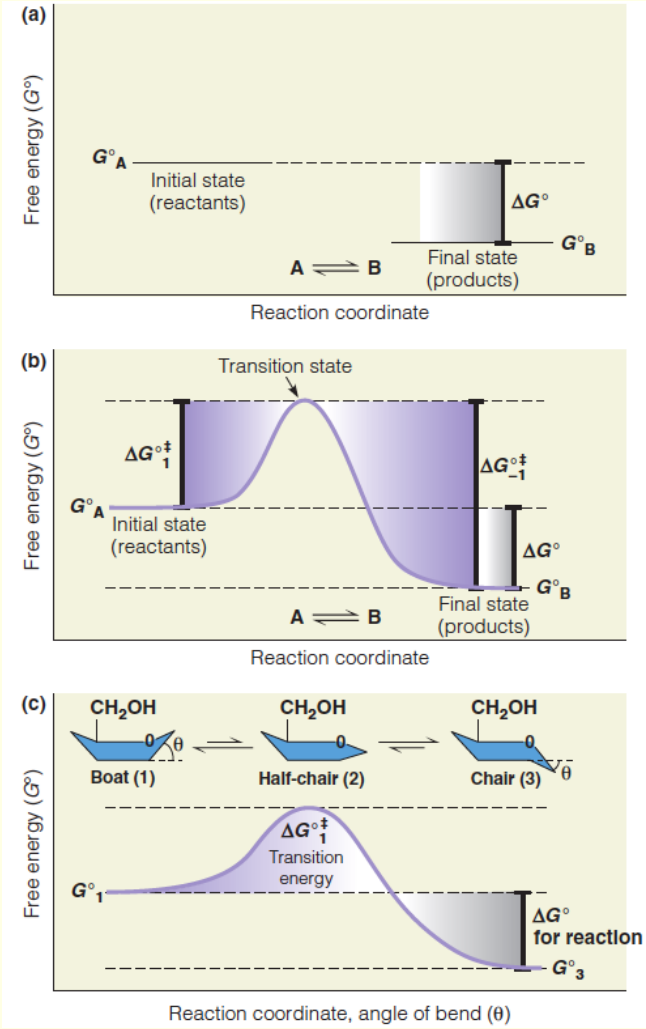
Chemical Reaction Rates and the Effects of Catalysts—A Review

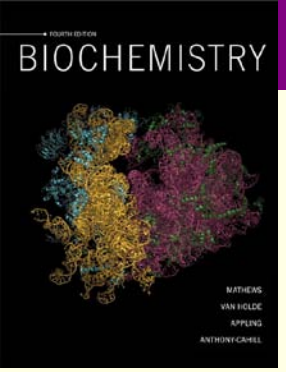
Free energy diagrams for the simple reaction A → B:

a) Only the free energy difference between the initial state and the final state is revealed.

b) Free energy diagram filled in to include the transition state through which the molecule must pass to go from A to B or *vice versa*.

c) A reasonable path for the transition of a pyranose (such as glucose) from boat (1) to chair (3) conformation. The highest energy state—the transition state—will look something like (2).





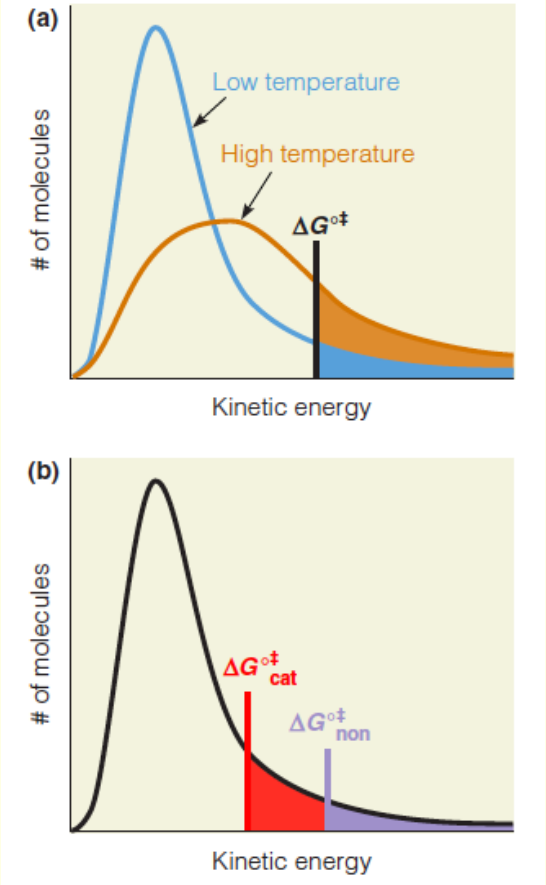
Chemical Reaction Rates and the Effects of Catalysts—A Review

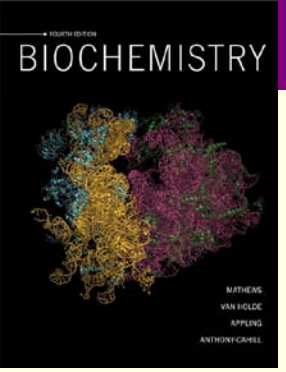
Effect of increasing temperature or lowering ΔG^{o+} on the rates of reactions:

The rates of reactions are proportional to the number of molecules with sufficient energy to overcome the activation barrier ΔG^{o+}.

(a) At higher temperature more molecules have this energy.

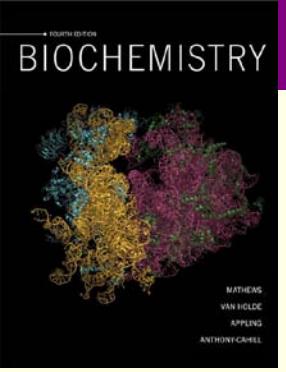
(b) Lowering the value of also increases the number of molecules with sufficient energy to attain the transition state.





Chemical Reaction Rates and the Effects of Catalysts—A Review

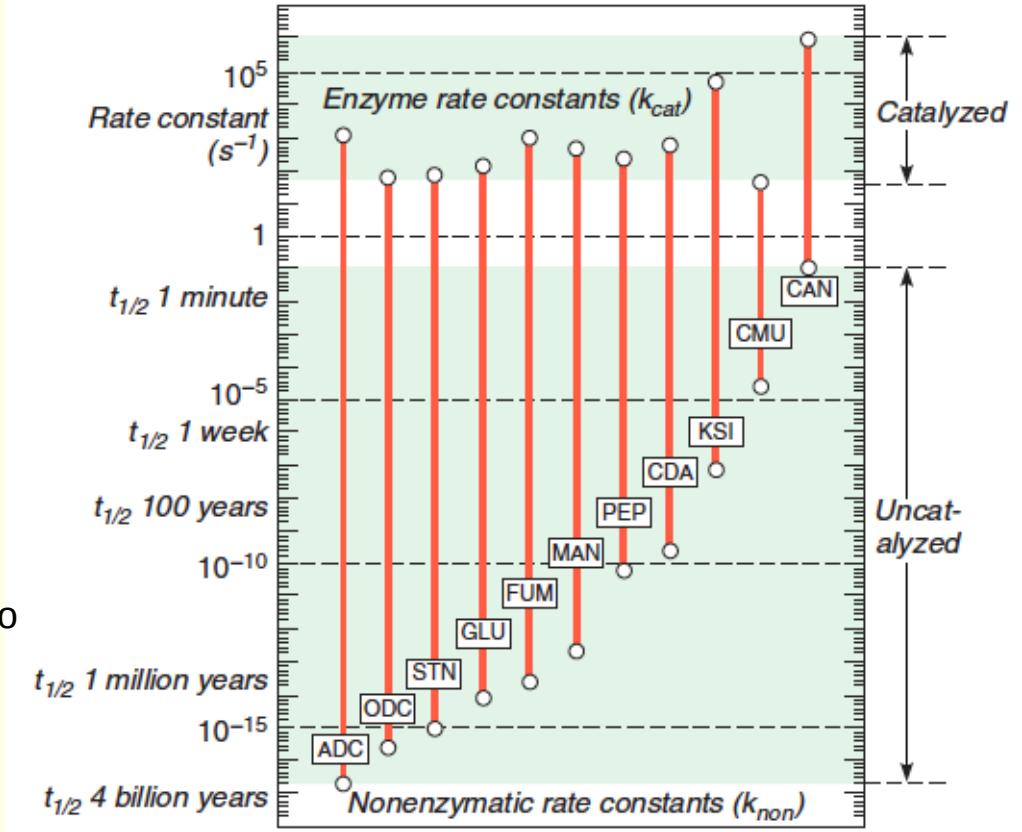
- The **rate enhancement** for an enzyme-catalyzed reaction is the ratio of the rate constants for the catalyzed (k_{cat}) and the noncatalyzed (k_{non}) reactions.
- The rate enhancement indicates how much faster the reaction occurs in the presence of the enzyme.



Chemical Reaction Rates and the Effects of Catalysts—A Review

Enzymatic rate enhancements:

- Logarithmic scale of k_{cat} and k_{non} values for some representative reactions at 25° C.
- The length of each vertical bar represents the rate enhancement achieved by the enzyme.
- ADC arginine decarboxylase; ODC orotidine 5'-phosphate decarboxylase; STN staphylococcal nuclease; GLU = sweet potato α-amylase; FUM fumarase; MAN mandelate racemase; PEP carboxypeptidase B; CDA *E. coli* cytidine deaminase; KSI ketosteroid isomerase; CMU chorismate mutase; CAN carbonic anhydrase.



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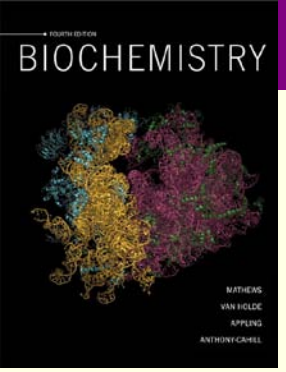


Chemical Reaction Rates and the Effects of Catalysts—A Review

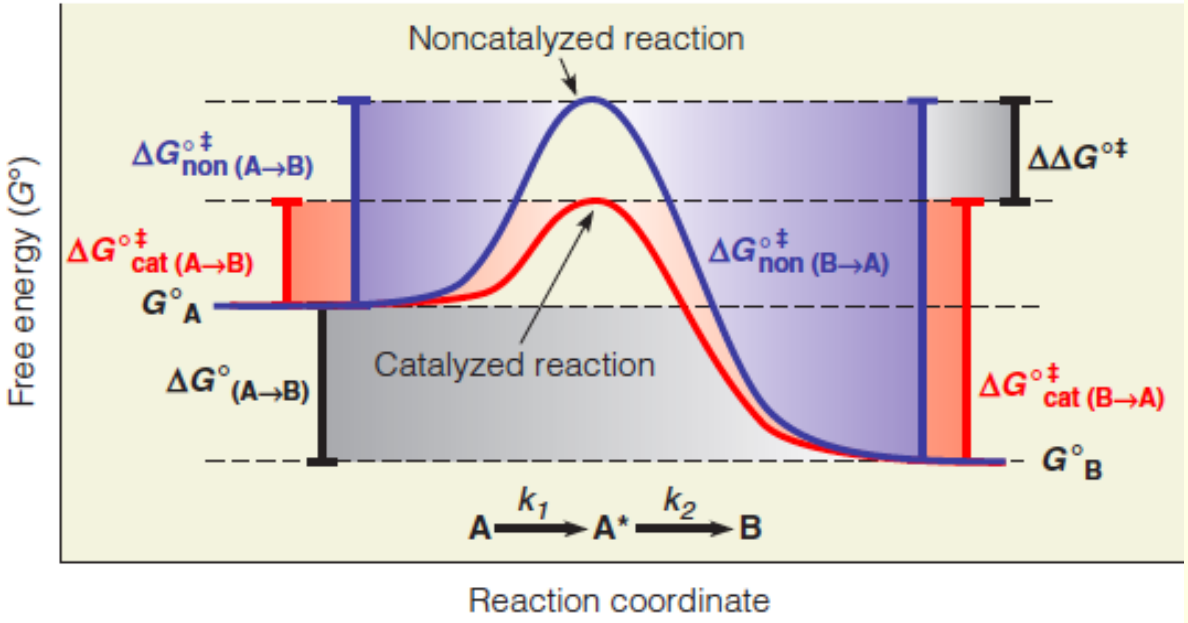
Effect of a catalyst on activation energy:

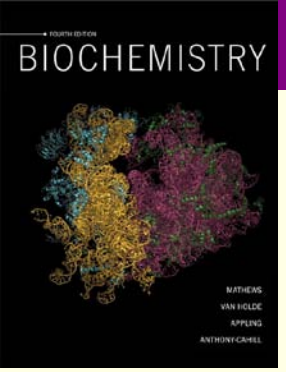
•The catalyst lowers the standard free energy of activation, ΔG^{o+}, and thereby accelerates the rate because more of the reactant molecules have the energy needed to reach this lowered transition state.

•The rate enhancement is related to $\Delta\Delta G^{o+}$.

•Note that the values of $\Delta G^{o}_{A \rightarrow B}$ for both the catalyzed and noncatalyzed reactions are the same; thus, the reaction equilibrium is not perturbed by the presence of the catalyst.

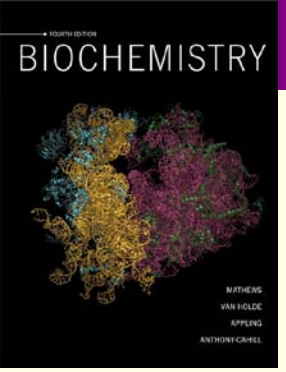
- The presence of a catalyst *increases forward and reverse rates* for a reaction, but does not affect the equilibrium composition of reactants and products.





How Enzymes Act as Catalysts: Principles and Examples

- An enzyme ***active site*** is complementary in shape, charge, and polarity to the transition state for the reaction, and to a lesser extent, its substrate.
- Enzyme-substrate ***complementarity*** is the basis for the specificity of enzyme-catalyzed reactions.

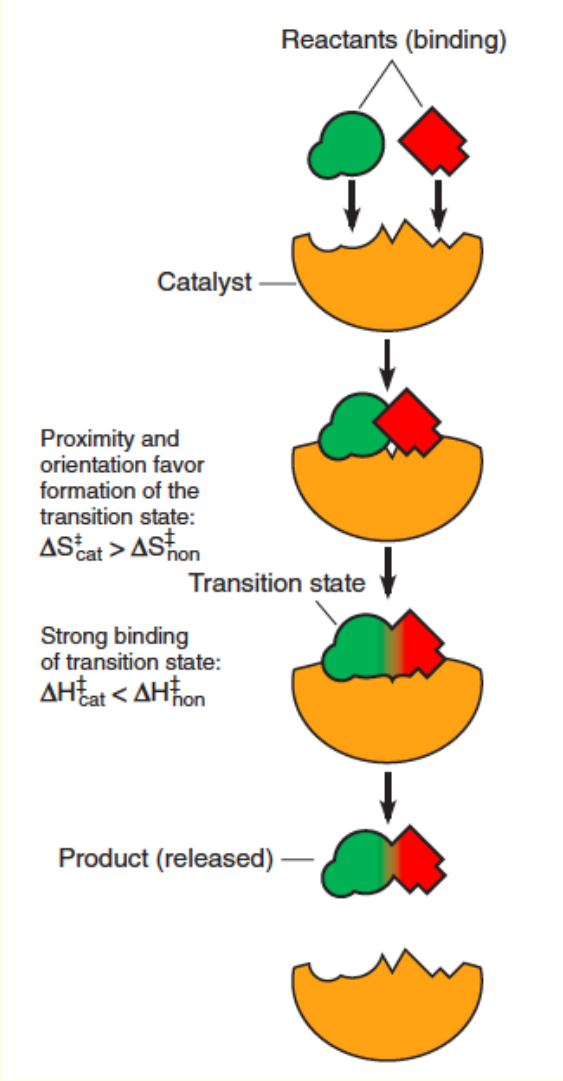


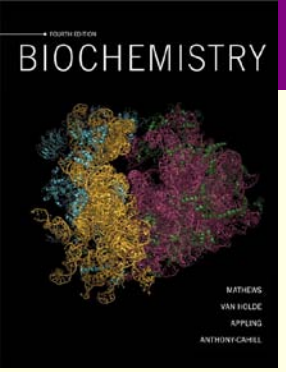
How Enzymes Act as Catalysts: Principles and Examples

Entropic and enthalpic factors in catalysis:

- In this example, two reactants are bound to sites on the catalyst which ensures their correct mutual orientation and proximity, and binds them most strongly when they are in the transition state conformation.

entropy(熵)
enthalpy(焓)



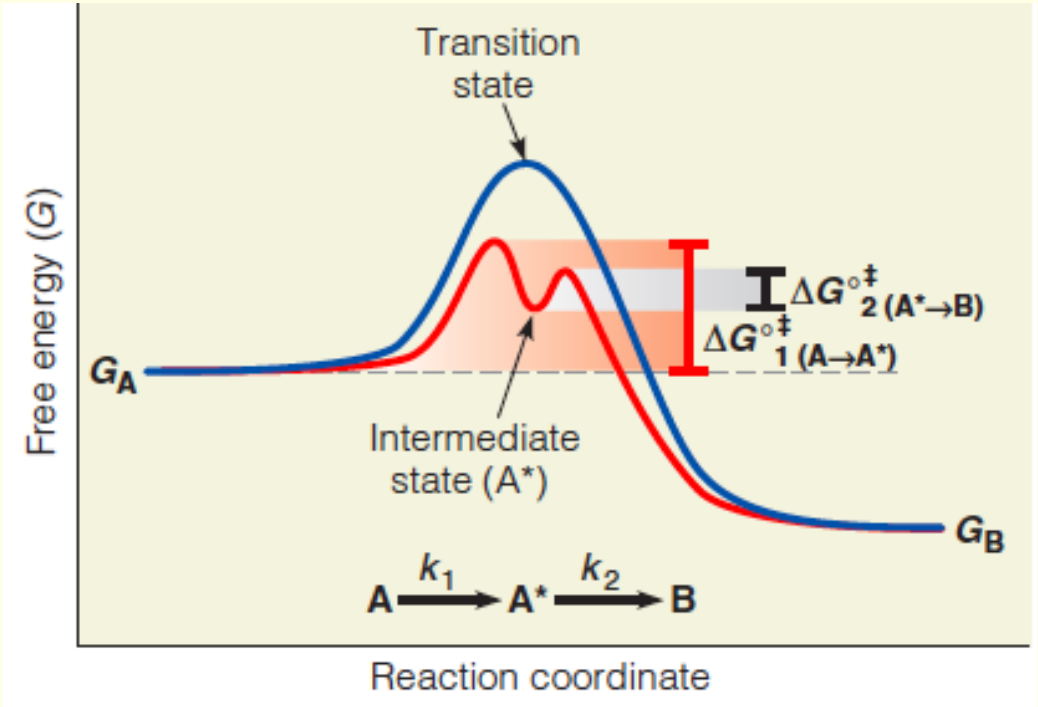


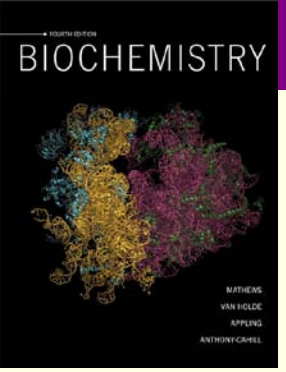
How Enzymes Act as Catalysts: Principles and Examples

Importance of intermediate states:

•An enzyme may alter the reaction pathway to one that includes one or more intermediate states that resemble the transition state but have a lower free energy.

•In the case of a single intermediate, the activation energies for formation of the intermediate state and for conversion of the intermediate to product are lower than the activation energy for the uncatalyzed reaction.





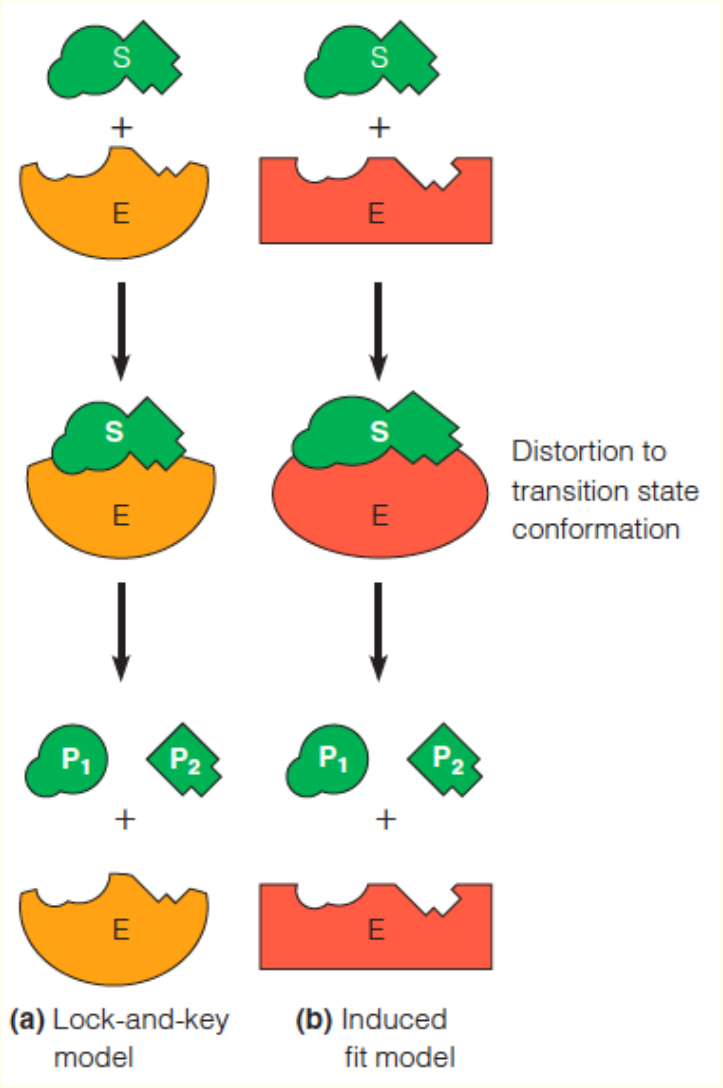
How Enzymes Act as Catalysts: Principles and Examples

Two models for enzyme–substrate interaction:

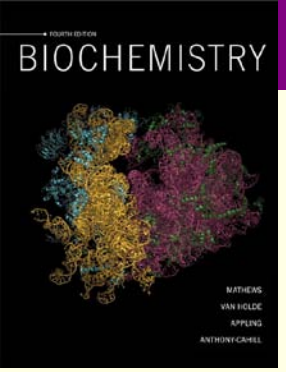
- In this example, the enzyme catalyzes a cleavage reaction.

a) The lock-and-key model. In this early model, the active site of the enzyme fits the substrate as a lock does a key.

b) The induced fit model. In this elaboration of the lock-and-key model, both enzyme and substrate are distorted on binding. The substrate is forced into a conformation approximating the transition state; the enzyme keeps the substrate under strain.



Emil Fischer (1894);
Daniel Koshland (1958)



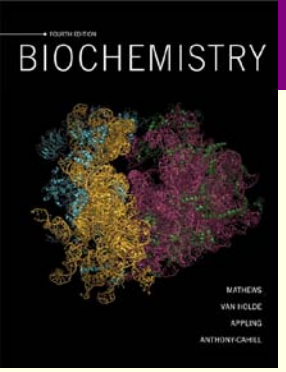
How Enzymes Act as Catalysts: Principles and Examples

Thus, an enzyme:

(1) Binds the substrate or substrates

(2) Lowers the energy of the transition state

(3) Directly promotes the catalytic event



How Enzymes Act as Catalysts: Principles and Examples

- For an enzyme (E) that catalyzes the conversion of a single substrate (S) into a single product (P), the expression for the reaction includes three steps:

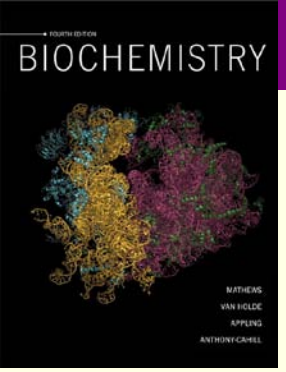
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

- ES is the ***enzyme–substrate complex***, and EP is the enzyme bound to the product.

- For many enzyme-catalyzed reactions the first step, binding of substrate, is reversible (i.e., k_1 and $k_{-1} >> k_2$).

- The second step, conversion of ES to EP, lies far to the right (i.e., $k_2 >> k_{-2}$).

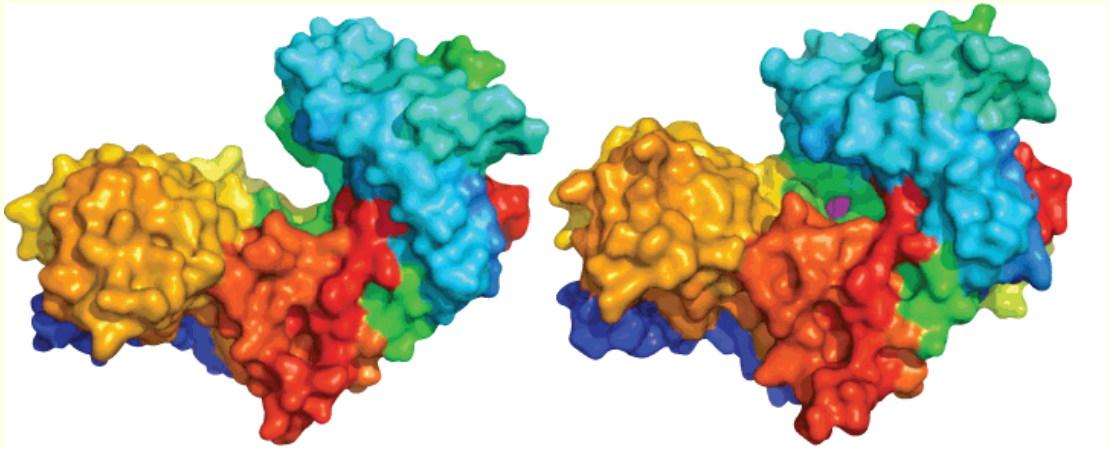
- The third step, release of product, is rapid compared to the catalytic step (i.e., $k_3 >> k_2$).

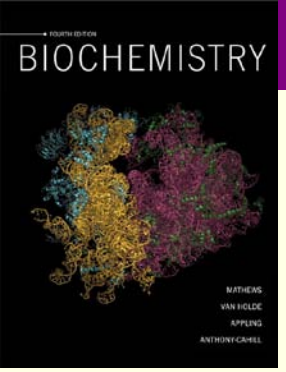


How Enzymes Act as Catalysts: Principles and Examples

The induced conformational change in hexokinase:

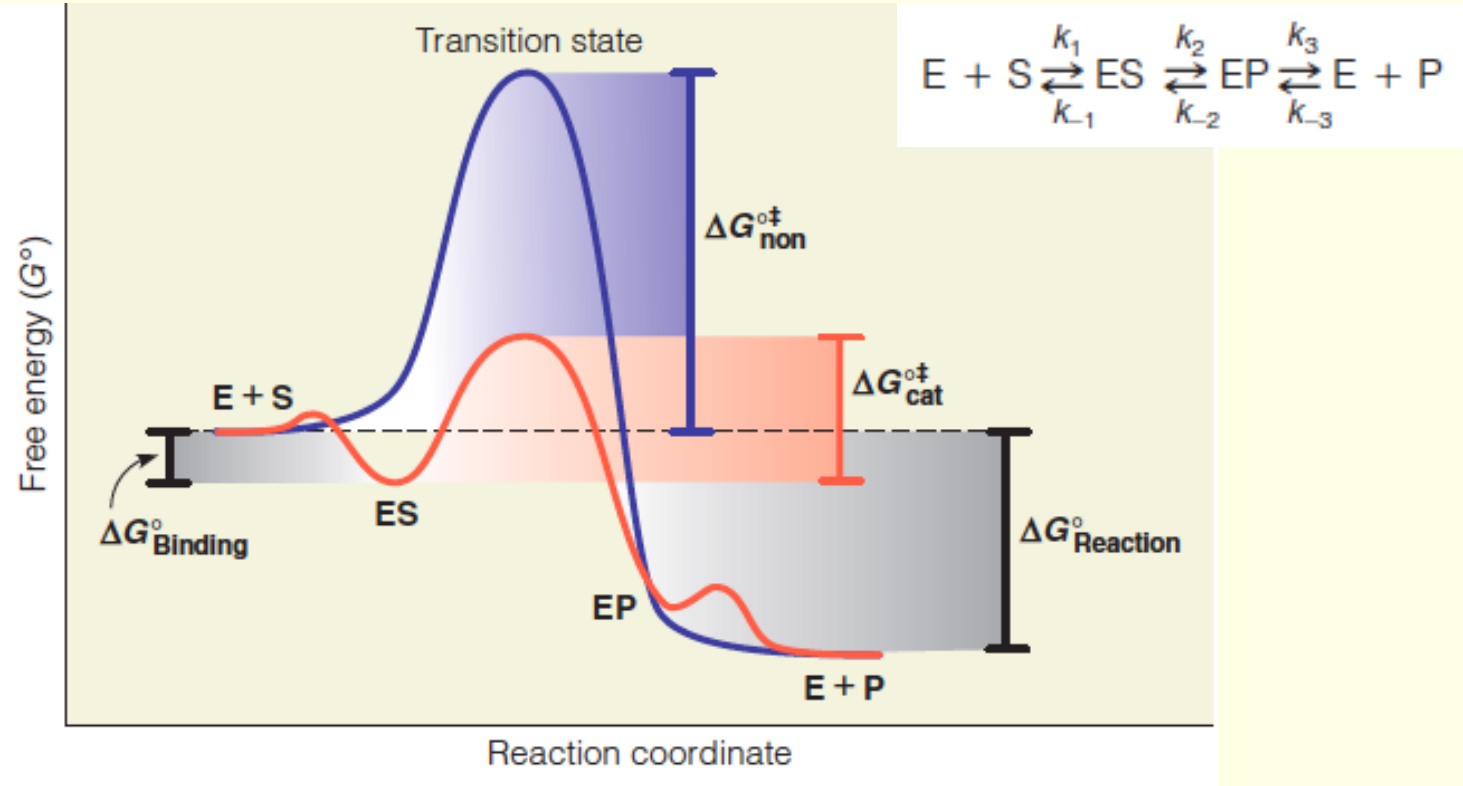
- The binding of glucose to hexokinase induces a significant conformational change in the enzyme.
- The enzyme is a single polypeptide chain, with two major domains.
- Notice how the obvious cleft between the domains (panel a) closes around the glucose molecule.

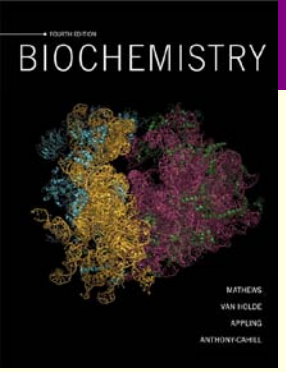




How Enzymes Act as Catalysts: Principles and Examples

Reaction coordinate diagram for a simple enzyme catalyzed reaction:

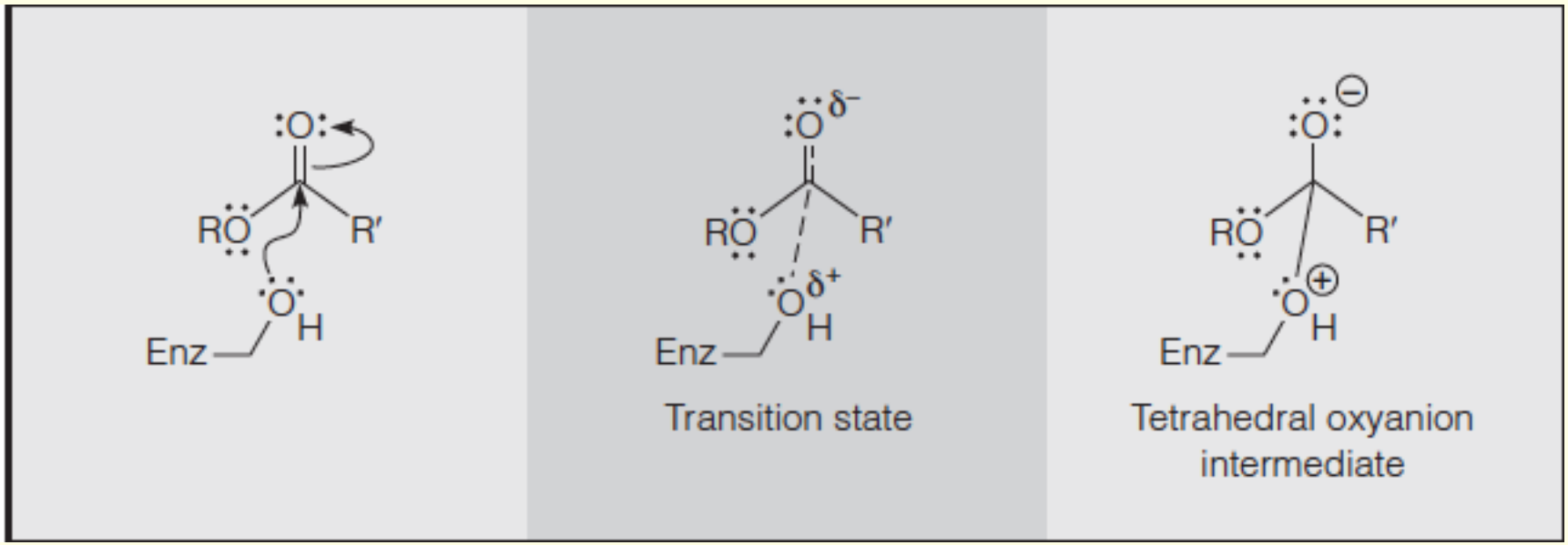


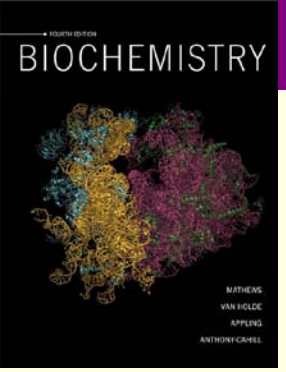


How Enzymes Act as Catalysts: Principles and Examples

Enthalpic stabilization of the transition state in an enzyme-catalyzed reaction:

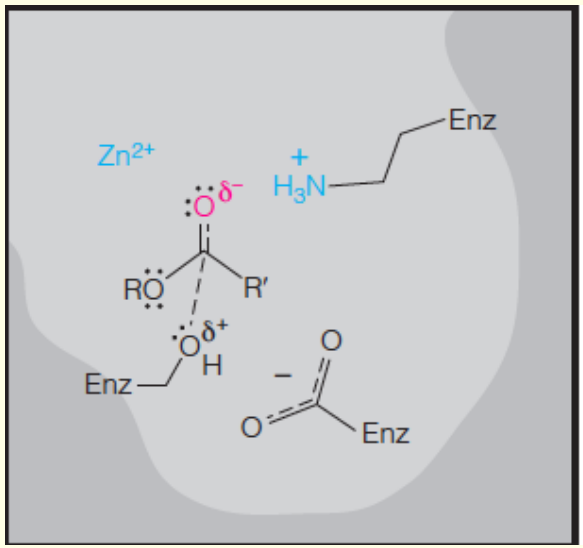
•This panel shows the transition state and tetrahedral intermediate for an enzyme-catalyzed ester cleavage.

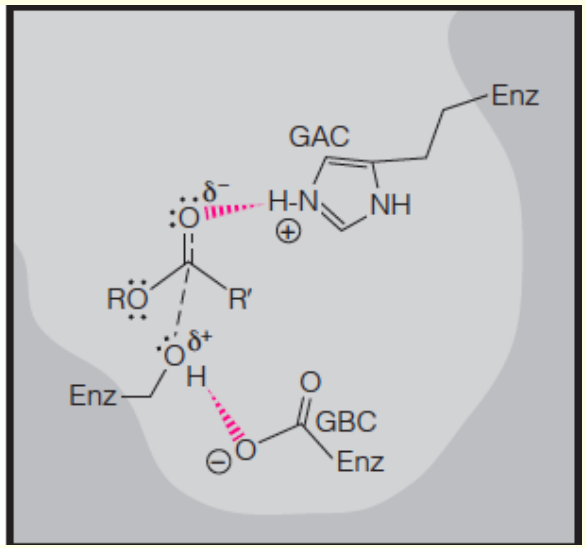


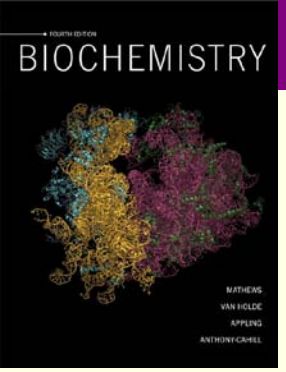


How Enzymes Act as Catalysts: Principles and Examples

- The transition state might be stabilized by electrostatic interactions with active site amino acids and/or metal ions, or it might be stabilized by general acids or bases.
- The direction of proton transfer from the proton donor to the proton acceptor is indicated by the placement of the wide end of the dashed bond near the proton acceptor.
- The GAC (General Acid Catalysis) is a proton donor and the GBC (General Base Catalysis) is a proton acceptor.

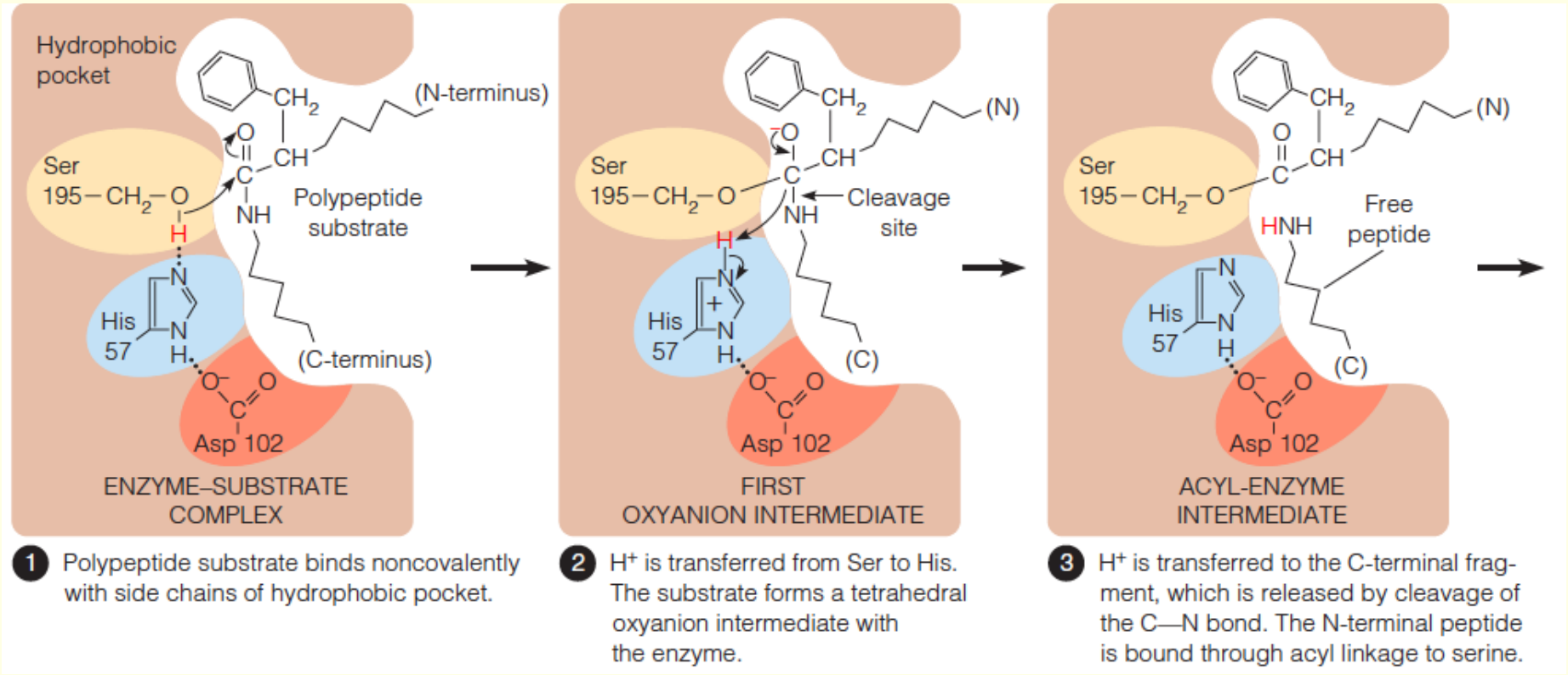




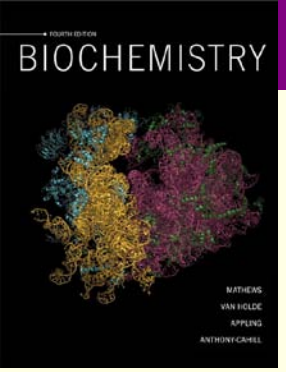


How Enzymes Act as Catalysts: Principles and Examples

Catalysis of peptide bond hydrolysis by chymotrypsin:

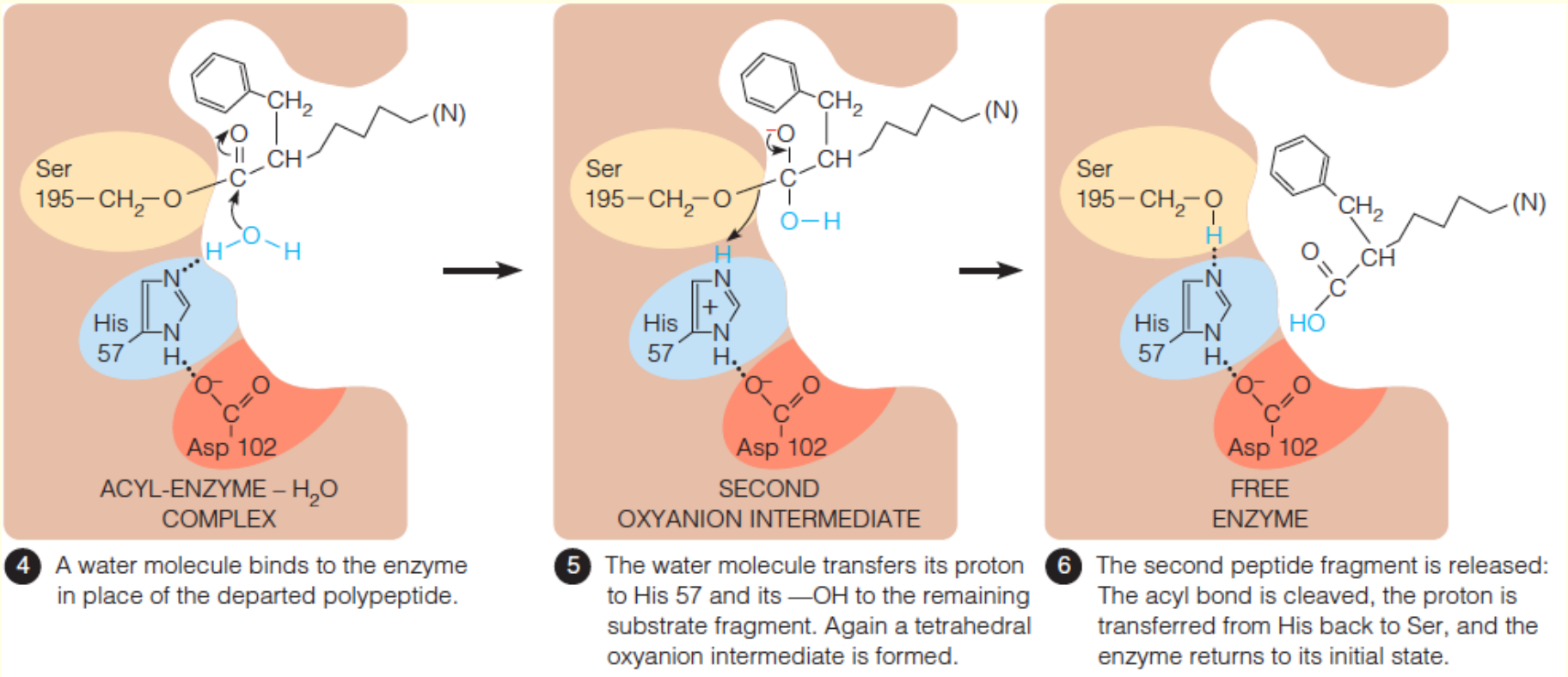


1. Polypeptide substrate binds noncovalently with side chains of hydrophobic pocket.
2. H^+ is transferred from Ser to His. The substrate forms a tetrahedral oxyanion intermediate with the enzyme.
3. H^+ is transferred to the C-terminal fragment, which is released by cleavage of the C—N bond. The N-terminal peptide is bound through acyl linkage to serine.



How Enzymes Act as Catalysts: Principles and Examples

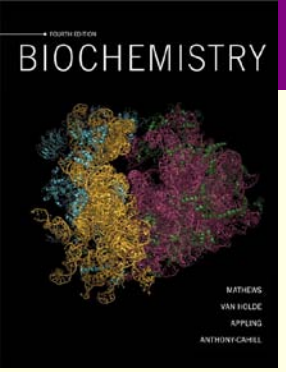
Catalysis of peptide bond hydrolysis by chymotrypsin:



4 A water molecule binds to the enzyme in place of the departed polypeptide.

5 The water molecule transfers its proton to His 57 and its —OH to the remaining substrate fragment. Again a tetrahedral oxyanion intermediate is formed.

6 The second peptide fragment is released: The acyl bond is cleaved, the proton is transferred from His back to Ser, and the enzyme returns to its initial state.



How Enzymes Act as Catalysts: Principles and Examples

The structure of chymotrypsin and the serine protease catalytic triad:

- The backbone of bovine chymotrypsin detemined by X-ray crystallopgraphy.
- Here, H57 is protonated, S195 and D102 are deprotonated.

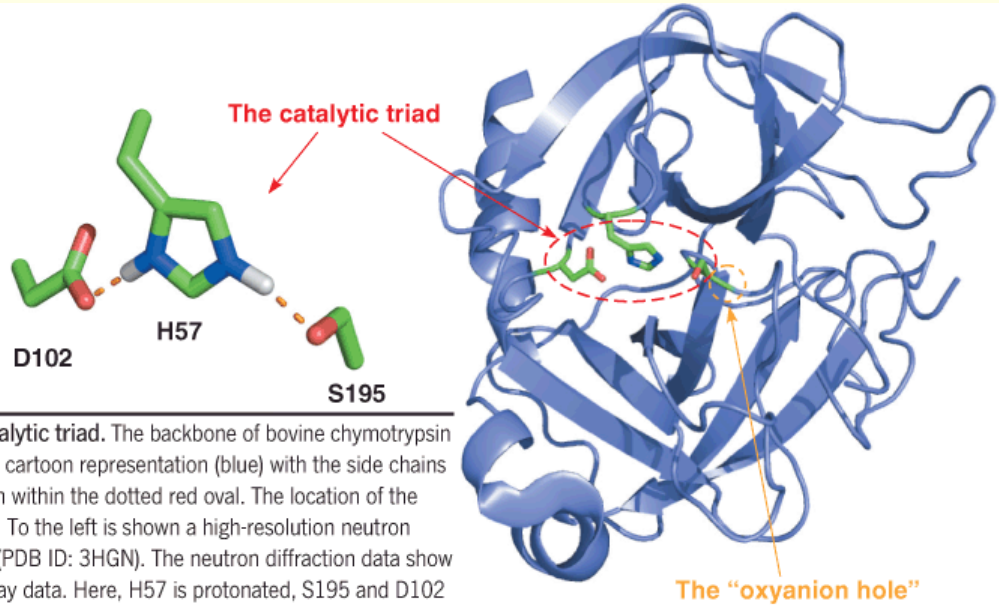
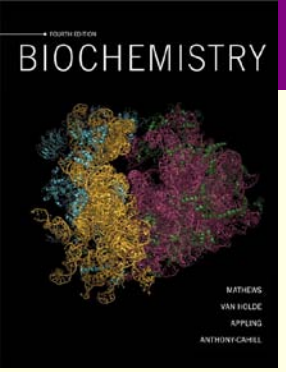


FIGURE 11.18

The structure of chymotrypsin and the serine protease catalytic triad. The backbone of bovine chymotrypsin detemined by X-ray crystallography (PDB ID: 4CHA) is shown in cartoon representation (blue) with the side chains of the catalytic triad, S195, H57, and D102, shown in stick form within the dotted red oval. The location of the oxyanion hole (see text) is indicated by the dotted orange circle. To the left is shown a high-resolution neutron diffraction structure of the catalytic triad from porcine elastase (PDB ID: 3HGN). The neutron diffraction data show the positions of H atoms with greater resolution than do the X-ray data. Here, H57 is protonated, S195 and D102 are deprotonated, and H-bonds are shown by orange dashes.

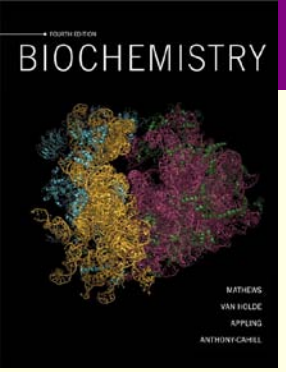


How Enzymes Act as Catalysts: Principles and Examples

TABLE 11.2 The strategies used by lysozyme and serine proteases to lower $\Delta G^{\circ\ddagger}$

Catalytic Strategy	Lysozyme	Ser Proteases
GABC	E35	H57 (catalytic triad)
Covalent	D52	S195 (catalytic triad)
Electrostatic		oxyanion hole
Other	strain of D ring	low-barrier H-bond?

- The catalysis of peptide-bond cleavage by serine proteases involves stabilization of transition states and tetrahedral intermediate states.



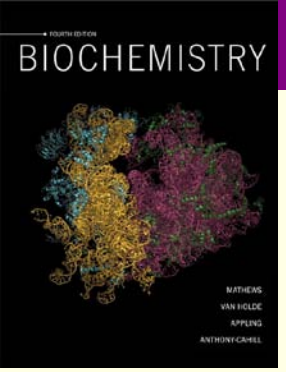
How Enzymes Act as Catalysts: Principles and Examples

Conformational Selection vs. Induced Fit:

- Induced fit implies conformational homogeneity in the unbound enzyme, which is then distorted upon substrate binding.

- Conformational selection implies that the unbound enzyme is present in multiple conformations; but, the substrate can only bind to unbound enzyme that is in the same conformation as that of the ES complex.

- Substrate binding will perturb the conformational equilibrium of the unbound enzyme, driving more E into the ES conformation by Le Chatelier's principle.



The Kinetics of Enzymatic Catalysis

- An expression for a simple reaction involving a single substrate and product was shown as:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} E + P$$

- If we analyze the initial rate of an enzyme-catalyzed reaction (i.e., before a significant concentration of P appears) and we assume that k_1, k_{-1}, and $k_3 >> k_2$, the equation above simplifies to:

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

- Where k_{cat} is the apparent rate constant for the rate-determining conversion of substrate to product.

Enzyme kinetics as an approach to understand mechanism

Enzyme kinetics --- determination of the rate of the reaction and how it changes in response to changes in experimental parameters

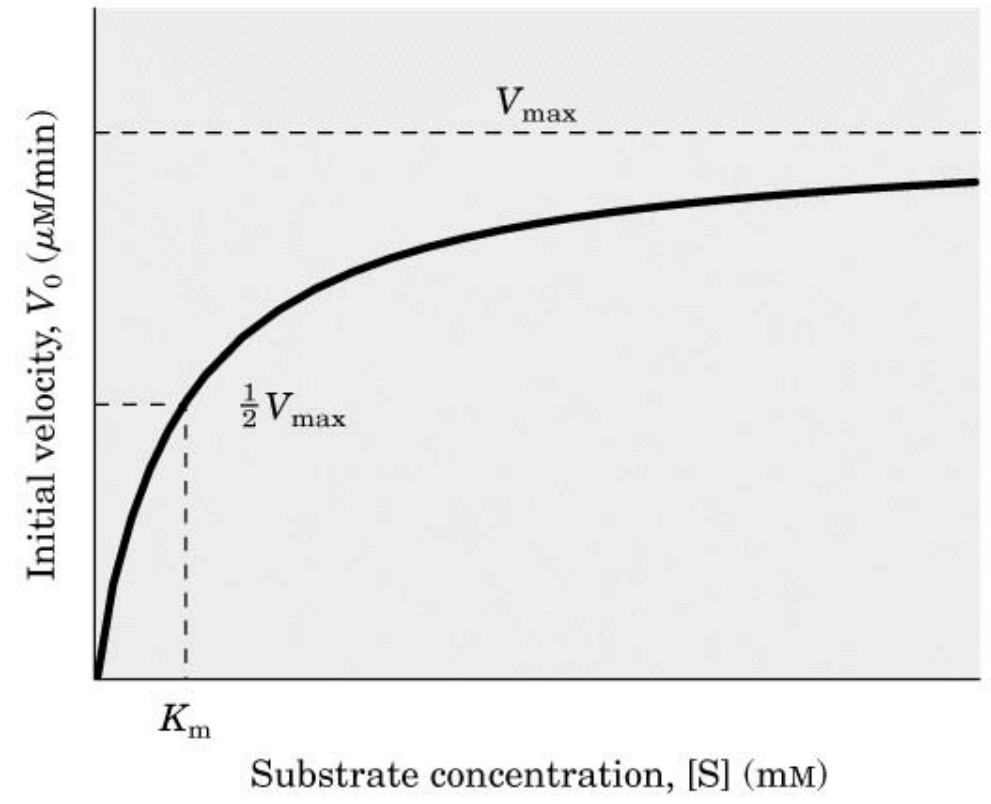


Fig. 6-11. Effect of substrate concentration on the initial velocity of an enzyme-catalyzed reaction

V_0 (initial velocity) when [S]>>[E], *t* is short

V_{max} (maximum velocity) when [S] → ∞

Leonor Michaelis
1875–1949

Maud Menten
1879–1960

* The relationship between substrate concentration and reaction rate can be expressed quantitatively *

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

$V_0 = k_2[ES]$

Rate of ES formation $= k_1([Et]-[ES])[S]$ ---- (A)

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

Steady state assumption

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

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

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

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

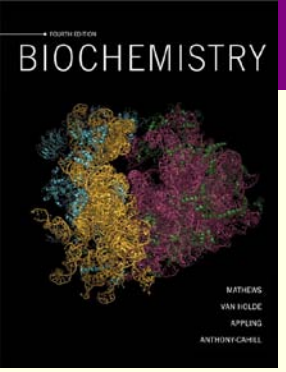
$[ES] = [Et][S] / \{[S] + (k_{-1} + k_2)/ k_1\}$

$(k_{-1} + k_2)/ k_1$ = is defined as Michaelis constant, K_m

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

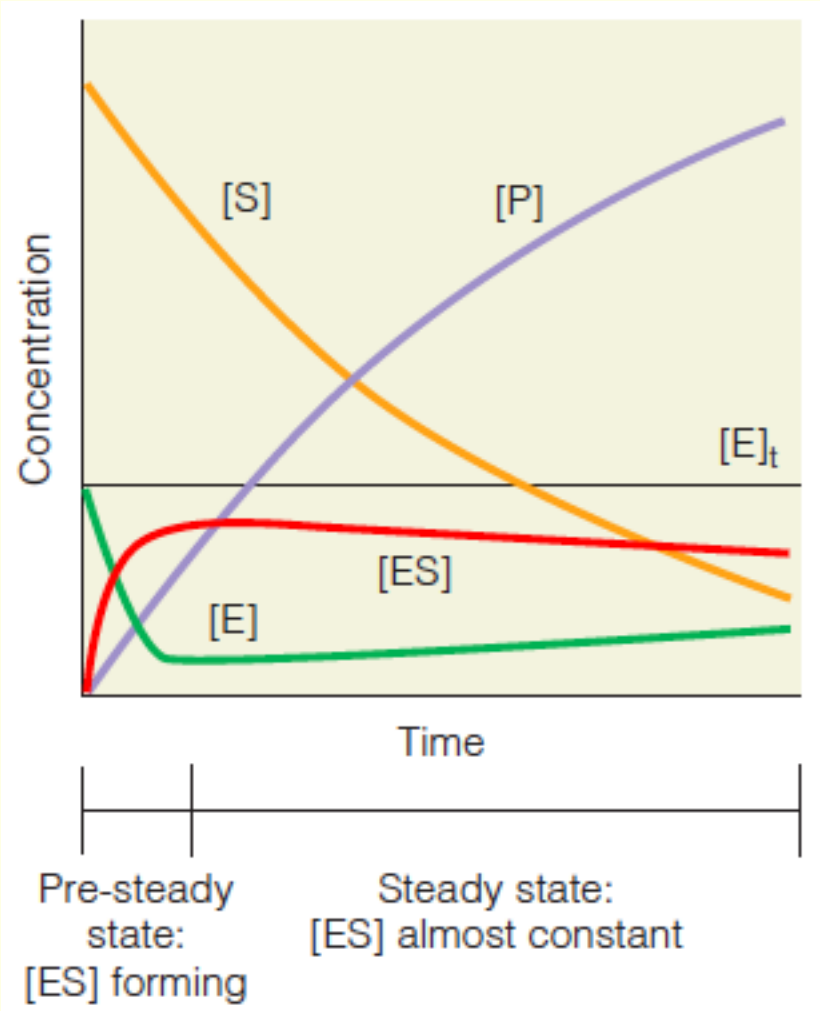
$V_0 = k_2[ES] = k_2[Et][S] / ([S] + K_m)$ ∵ $V_{max} = k_2[Et]$

$V_0 = V_{max}[S] / ([S] + K_m)$ ⋯⋯ Michaelis-Menten equation



The Kinetics of Enzymatic Catalysis

The steady state in enzyme kinetics:



Kinetic parameters are used to compare enzyme activities

$$\mathbf{Km} = (k_{-1} + k_2)/\, k_1$$

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

if $k_2 \ll k_{-1}$ $\quad \mathbf{Km} = k_{-1}/\, k_1 = \mathbf{Kd} \longrightarrow$ **Km relates to affinity**

if $k_2 \gg k_{-1}$ $\quad \mathbf{Km} = k_2/\, k_1$

if $k_2 \sim k_{-1}$

Table 6-6

K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
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

$V_{max} = k_2[Et]$

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

k_{cat}, the rate limiting of any enzyme-catalyzed reaction at saturation

$k_{cat} = V_{max} / [Et]$ (turnover number)

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	0.4

(***k*cat** measures the number of substrate molecules turned over per enzyme molecule per second)

$V_0 = V_{max}[S] / ([S] + K_m)$ ⋯⋯→ M-M equation

$k_{cat} = V_{max} / [Et]$ ⋯⋯→ Turnover number

$V_0 = k_{cat}[Et][S] / ([S] + K_m)$

when $[S] \ll K_m$ ⋯⋯ ([S] is usually low in cells)

$V_0 = k_{cat}[Et][S] / K_m$ ⋯⋯→ * (k_{cat} / K_m, specific constant) *

Table 6-8

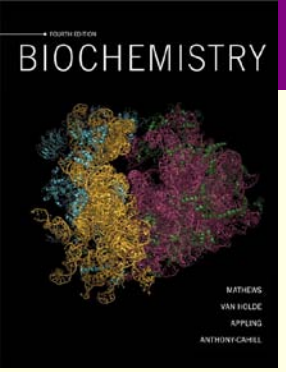
Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

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	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
Triose phosphate isomerase	Glyceraldehyde 3-phosphate	4.3×10^3	4.7×10^{-4}	2.4×10^8

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

k_{cat} / K_m has a upper limit (E and S diffuse together in aqueous solution)

~10^8 to 10^9 $M^{-1}S^{-1}$ ⟶ catalytic perfection

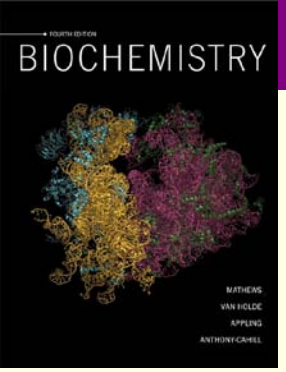


The Kinetics of Enzymatic Catalysis

TABLE 11.4 Michaelis–Menten parameters for selected enzymes, arranged in order of increasing efficiency as measured by k_{cat}/k_M

Enzyme	Reaction Catalyzed	K_M (mol/L)	$k_{cat}(s^{-1})$	k_{cat}/K_M $[(mol/L)^{-1}\,s^{-1}]$
Chymotrypsin	Ac–Phe–Ala $\xrightarrow{H_2O}$ Ac–Phe + Ala	1.5×10^{-2}	0.14	9.3
Pepsin	Phe–Gly $\xrightarrow{H_2O}$ Phe + Gly	3×10^{-4}	0.5	1.7×10^{3}
Tyrosyl-tRNA synthetase	Tyrosine + tRNA $\longrightarrow$ tyrosyl-tRNA	9×10^{-4}	7.6	8.4×10^{3}
Ribonuclease	Cytidine 2′, 3′ cyclic phosphate $\xrightarrow{H_2O}$ cytidine 3′-phosphate	7.9×10^{-3}	7.9×10^{2}	1.0×10^{5}
Carbonic anhydrase	$HCO_3^- + H^+ \longrightarrow H_2O + CO_2$	2.6×10^{-2}	4×10^{5}	1.5×10^{7}
Fumarase	Fumarate $\xrightarrow{H_2O}$ malate	5×10^{-6}	8×10^{2}	1.6×10^{8}

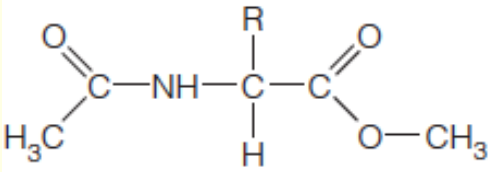
The ratio k_{cat}/K_M is a convenient measure of enzyme efficiency.



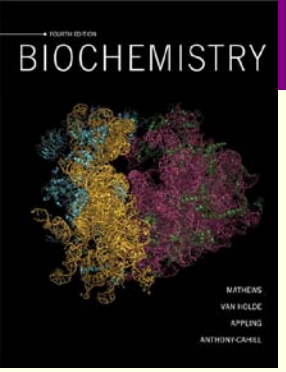
The Kinetics of Enzymatic Catalysis

TABLE 11.5 Preferences of chymotrypsin in the hydrolysis of several *N*-acetyl amino acid methyl esters, as measured by k_{cat}/k_M

Amino Acid in Ester	Amino Acid Side Chain	k_{cat}/K_M [(mol/L)$^{-1}$ s^{-1}]
Glycine	—H	1.3×10^{-1}
Norvaline	—$CH_2CH_2CH_3$	3.6×10^{2}
Norleucine	—$CH_2CH_2CH_2CH_3$	3.0×10^{3}
Phenylalanine	—CH_2— 	1.0×10^{5}



N-acetyl amino acid methyl ester



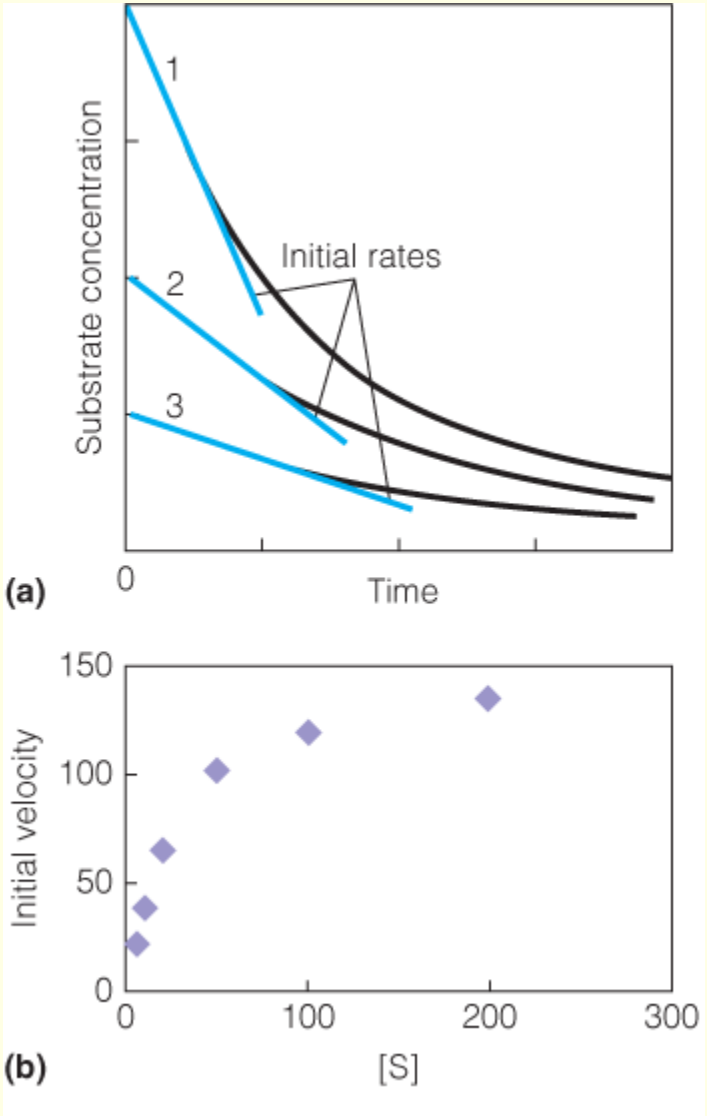
The Kinetics of Enzymatic Catalysis

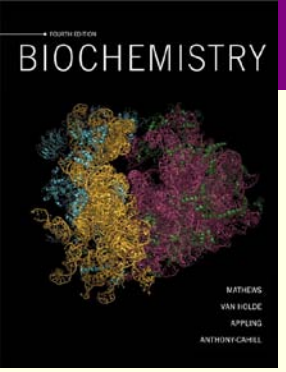
Analysis of intial rates:

a)Several reactions are performed with varying concentrations of substrate and the values of the initial rates are determined from the slopes of the curves in the early phase for each reaction.

b)Initial rate data, determined as described in panel A, are plotted as a function of the substrate concentration.

•The enzyme appears to obey the Michaelis–Menten kinetic model.





The Kinetics of Enzymatic Catalysis

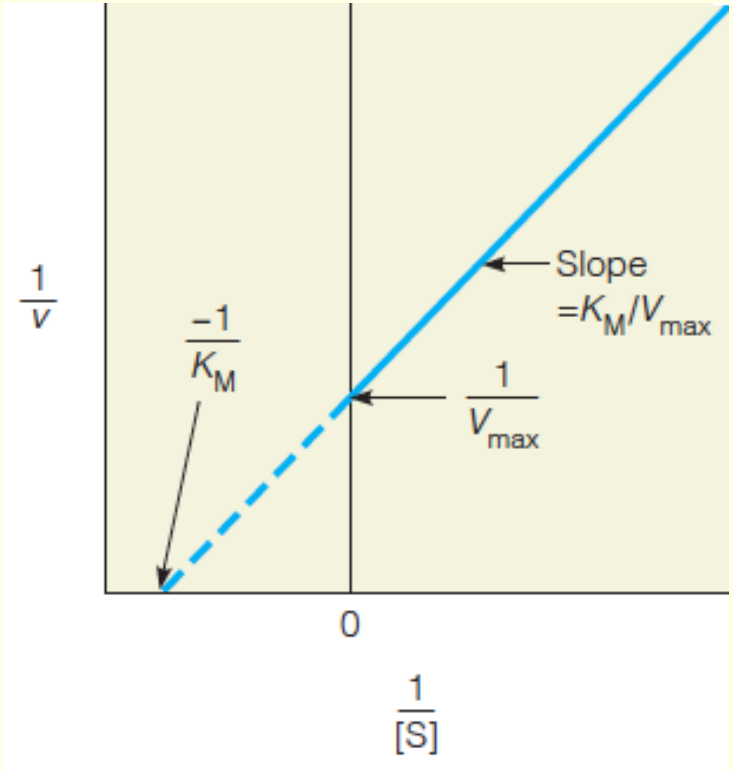
A Lineweaver–Burk plot:

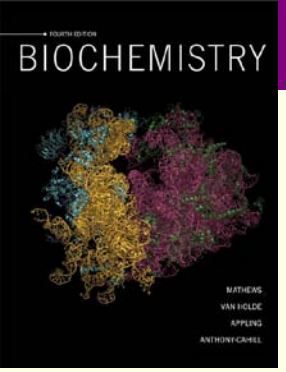
•In this double reciprocal plot, 1/v is graphed versus 1/[S].

•Note that a linear extrapolation of the data gives both V_{max} and K_M.

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

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

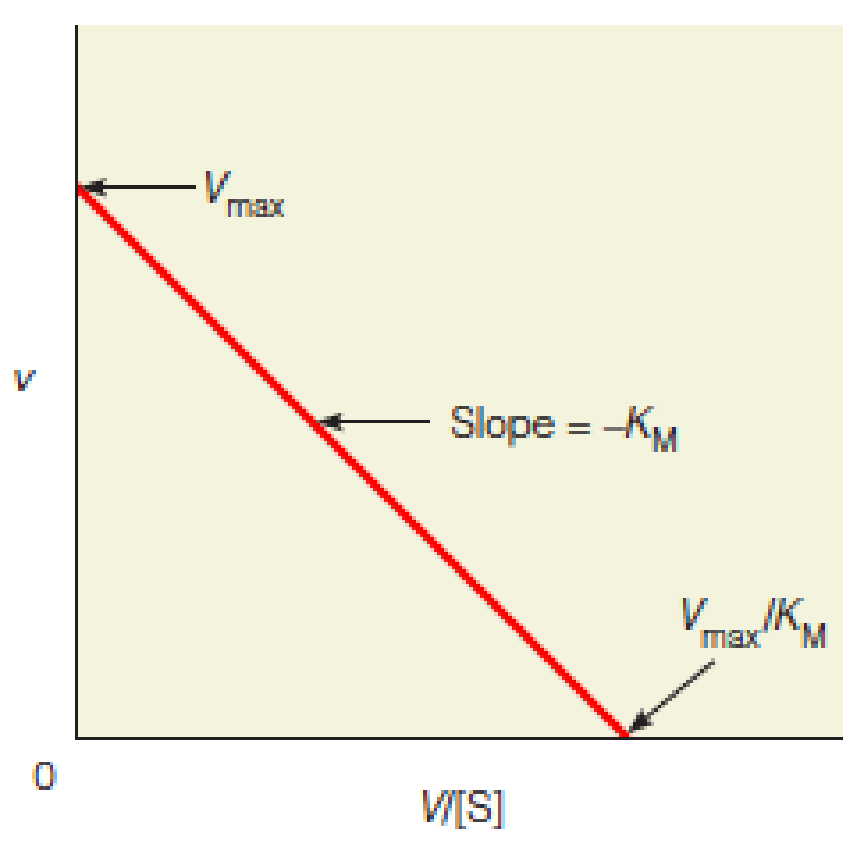


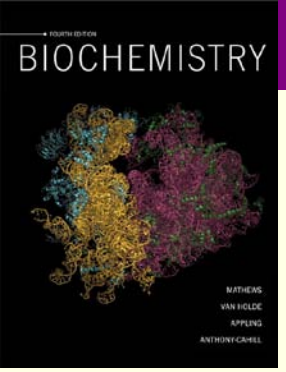


The Kinetics of Enzymatic Catalysis

An Eadie–Hofstee plot:

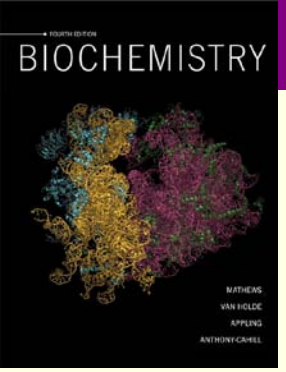
•Graphing v versus V/[S], we obtain V_{max} at (V/[S]) = 0 and K_M from the slope of the line.





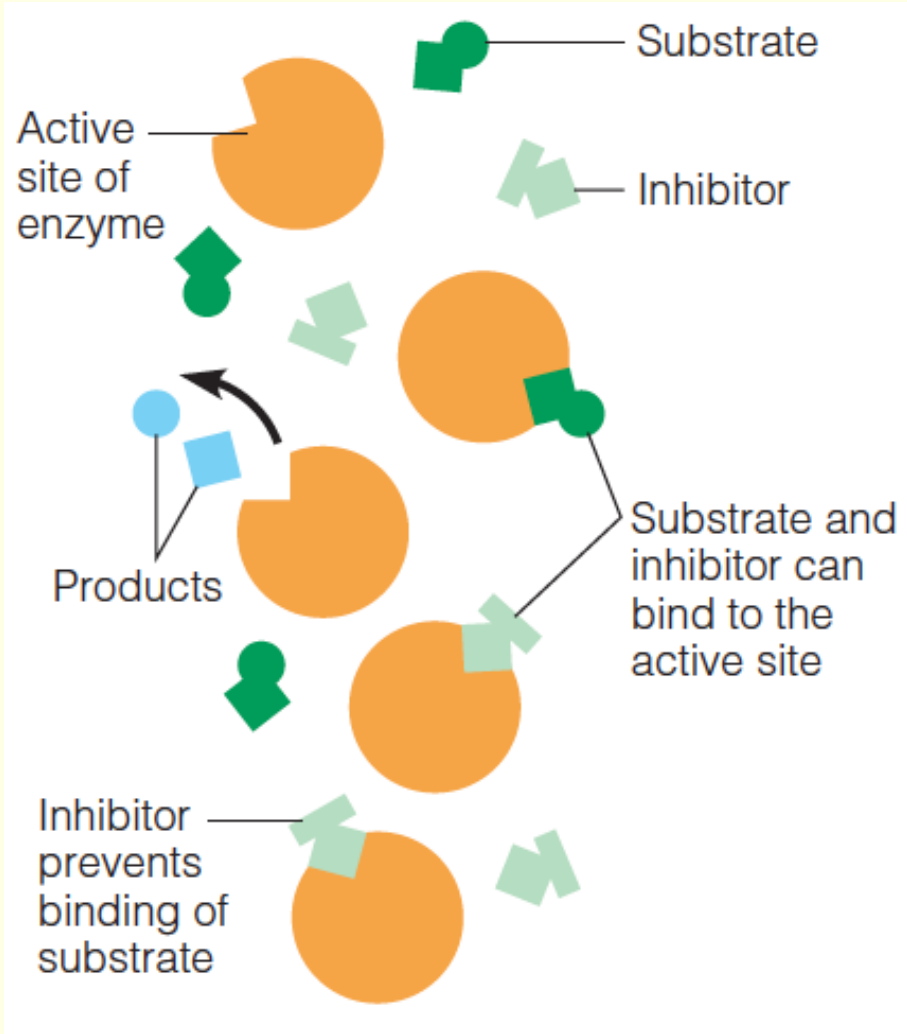
The Kinetics of Enzymatic Catalysis

- Lineweaver–Burk plots or Eadie–Hofstee plots provide convenient ways to determine K_M and k_{cat} from intial-rate data.
- The observed effects of an amino acid mutation in an enzyme active site on K_M and k_{cat} can be used to identify the role of the amino acid in substrate binding (K_M effects) and transition-state stabilization (k_{cat} effects).



Enzyme Inhibition

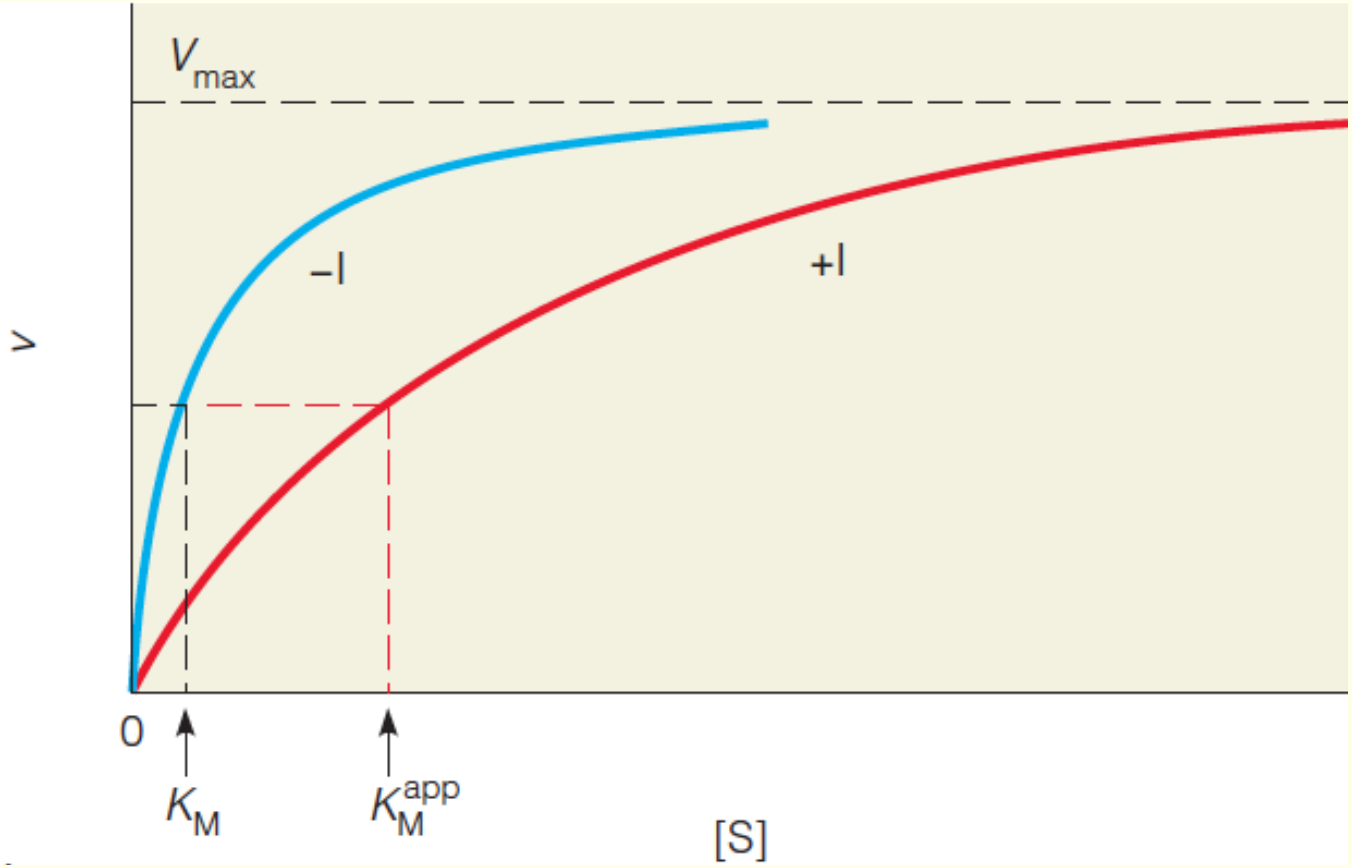
- Inhibition of enzymes can be either *reversible or irreversible*.
- A ***competitive inhibitor*** competes with substrate for the enzyme active site.
- It *increases* the apparent K_M.
- Both substrate and inhibitor can fit the active site.
- Substrate can be processed by the enzyme, but inhibitor cannot.

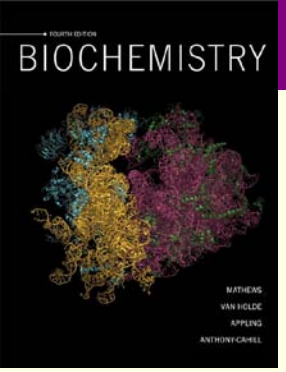


Enzyme Inhibition

Effects of competitive inhibition on enzyme kinetics:

•The effect of a competitive inhibitor (I) on reaction velocity at different substrate concentrations.

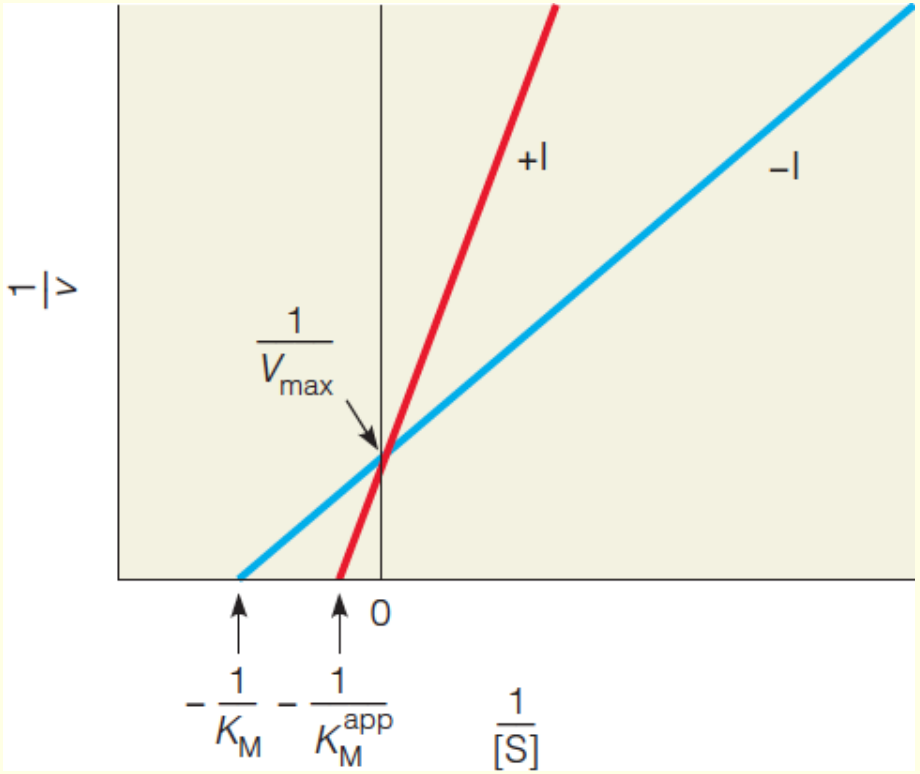


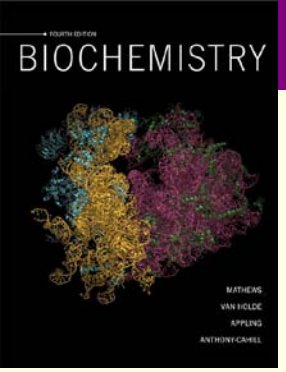


Enzyme Inhibition

Lineweaver–Burk plots of the reactions with and without a competitive inhibitor:

•The lines cross the 1/*v* axis at the same V_{max}, showing that I is a competitive inhibitor.



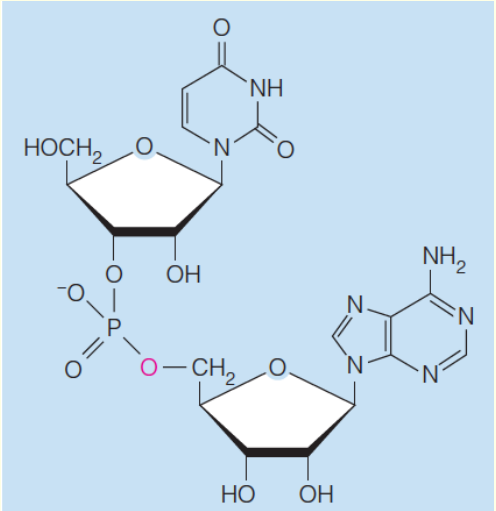


Enzyme Inhibition

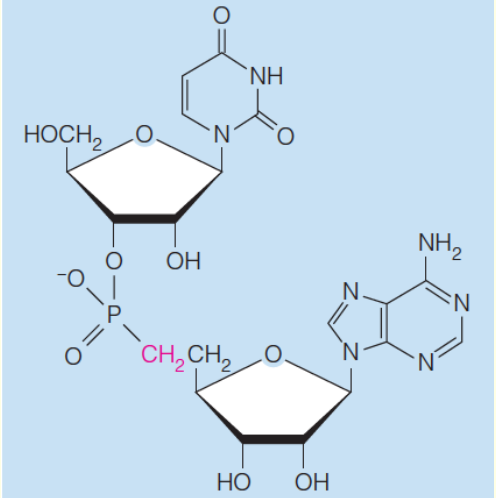
A substrate and its competitive inhibitor:

- The substrate UpA and the structurally similar molecule UpcA are competitors for the enzyme ribonuclease.

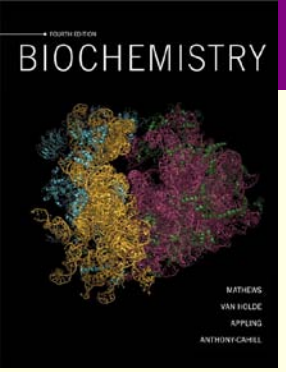
- The single difference between the substrate and the inhibitor is shown in red.



UpA: ribonuclease substrate

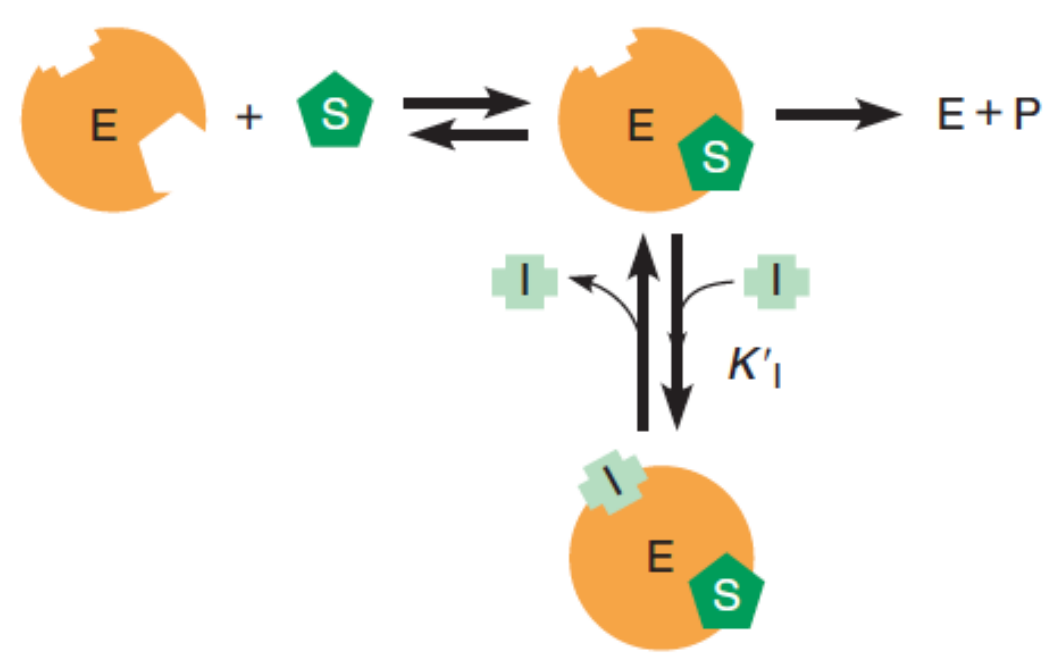


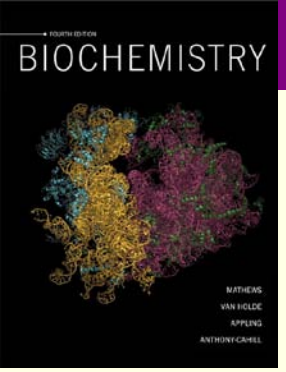
UpcA: competitive inhibitor of ribonuclease



Enzyme Inhibition

- An ***uncompetitive inhibitor*** does not compete for the active site but affects the catalytic event.
- It reduces both the apparent V_{max} and apparent K_M.
- These effects cannot be reversed by increasing [S].



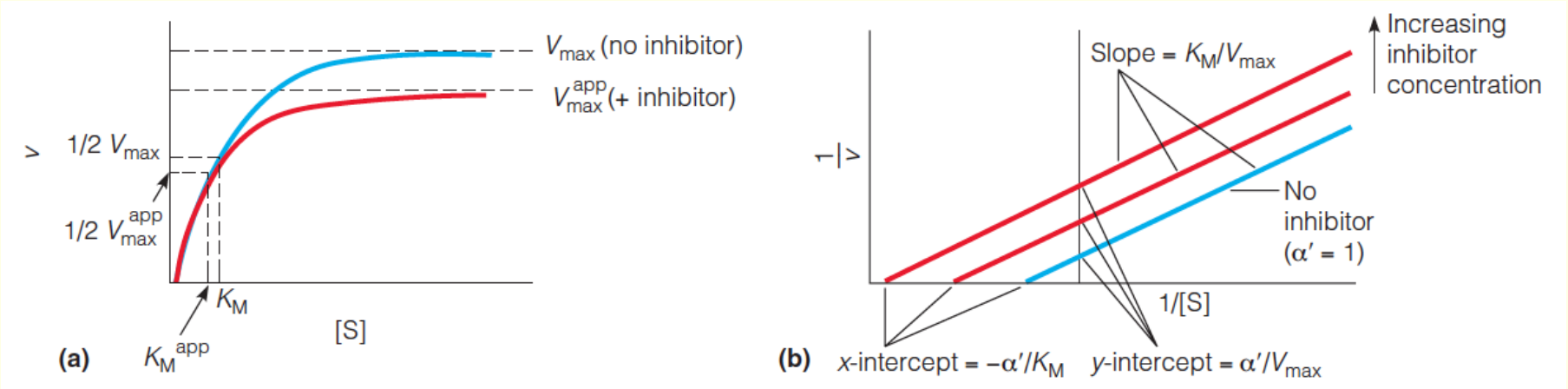


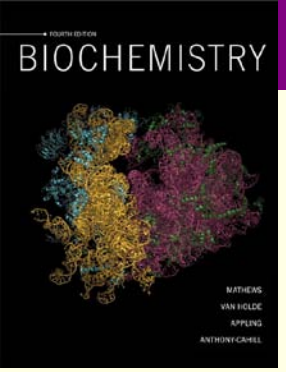
Enzyme Inhibition

Effects of uncompetitive inhibition on enzyme kinetics:

(a) The effect of an uncompetitive inhibitor (I) on reaction velocity at different substrate concentrations.
(b) Lineweaver–Burk plots of the reactions shown in (a).

•The lines are parallel and cross 1/v axis at different points, clearly distinguishing this situation from competitive inhibition.





Enzyme Inhibition

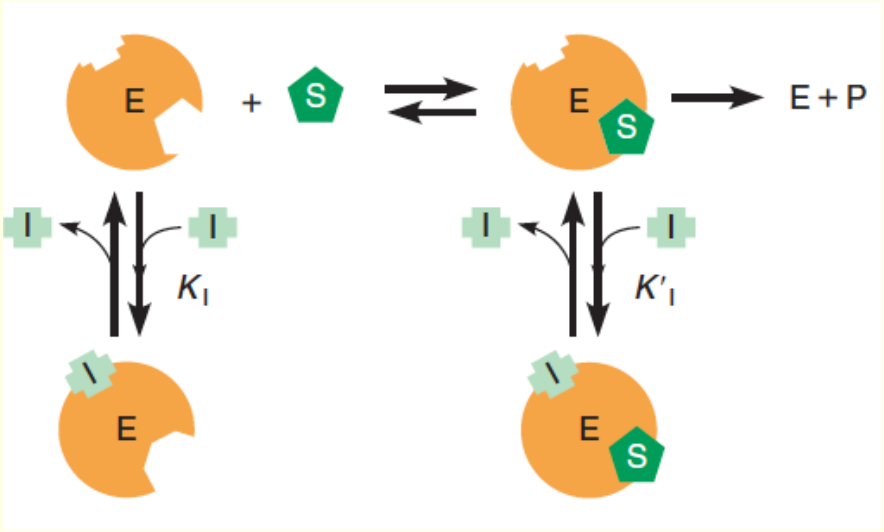
A model for mixed inhibition:

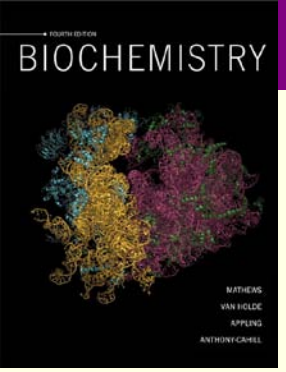
•The inhibitor binds at a site on the enzyme surface different from that of the substrate.

•In this simplified example, the inhibitor binds to both free enzyme and the ES complex.

•EI has reduced substrate binding affinity compared to free enzyme.

•The EIS complex cannot carry out the catalytic event.



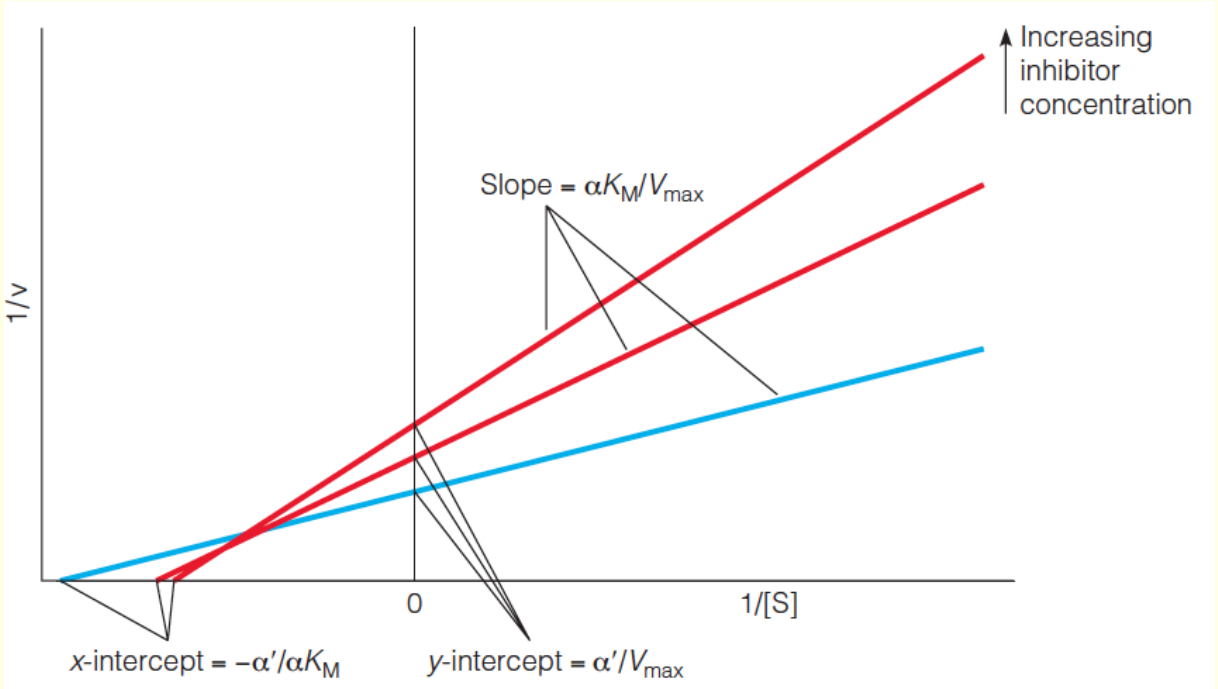


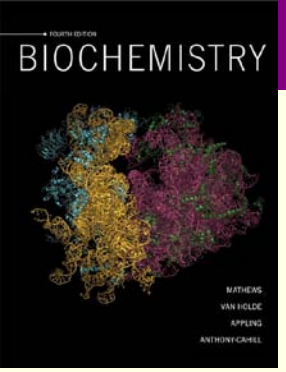
Enzyme Inhibition

Lineweaver–Burk plot for mixed inhibition kinetics:

•Vmax is decreased by a factor of and KM is increased.

•Compare this plot with those previously shown to find the features that distinguish competitive, uncompetitive, and mixed modes of inhibition.



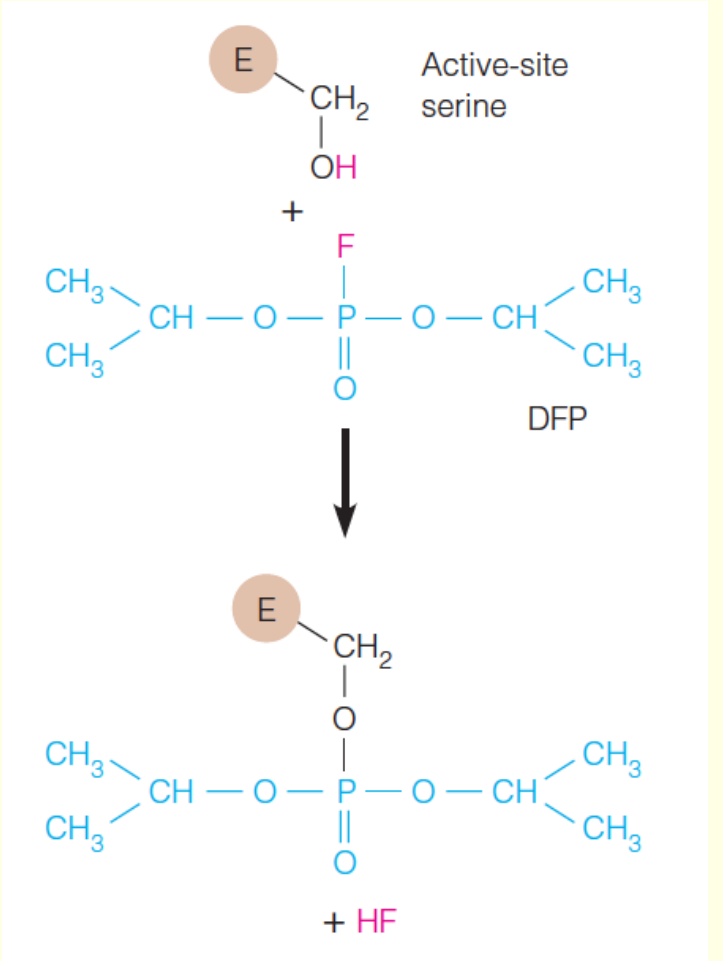


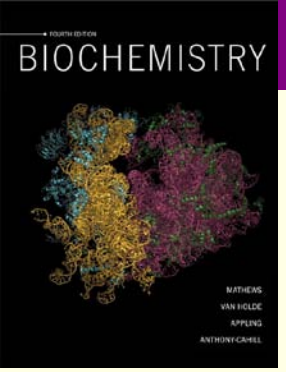
Enzyme Inhibition

- Many irreversible inhibitors bind covalently to the active sites of enzymes.

Irreversible inhibition by adduct formation:

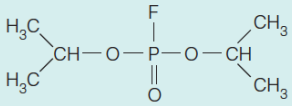
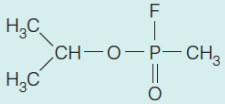
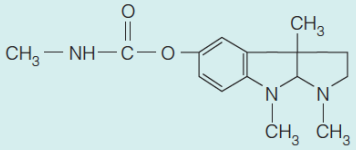
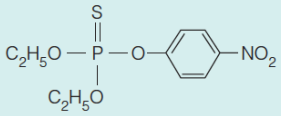
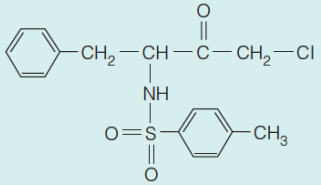
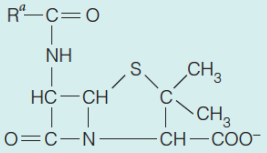
- Diisopropyl fluorophosphate (DFP) reacts with a serine group on a protein to form a covalent adduct.
- The covalent bond renders the catalytically important serine ineffective in catalysis.
- The adduct also may block substrate binding to the active site.

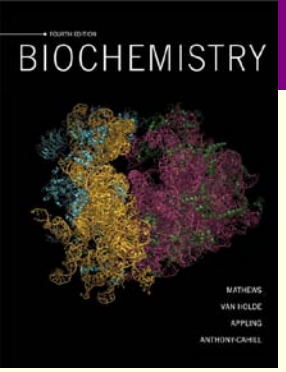




Enzyme Inhibition

TABLE 11.7 Irreversible enzyme inhibitors

Name	Formula[a]	Source	Mode of Action
Cyanide	CN^-	Bitter almonds	Reacts with enzyme metal ions (i.e., Fe, Zn, Cu); respiratory chain enzymes are primary targets (see Chapter 15)
Diisopropyl fluorophosphate (DFP, or DIFP)		Synthetic	Inhibits enzymes with active site serine, including acetylcholinesterase and serine proteases
Sarin		Synthetic (nerve gas)	Like DFP
Physostigmine		Calabar beans	Like DFP
Parathion		Synthetic (insecticide)	Like DFP, but especially inhibitory to insect acetylcholinesterase
N-Tosyl-L-phenyl-alaninechloro-methyl ketone (TPCK)		Synthetic	Reacts with His 57 of chymotrypsin
Penicillin		From *Penicillium* fungus	Inhibits enzymes in bacterial cell wall synthesis (see Chapter 9)

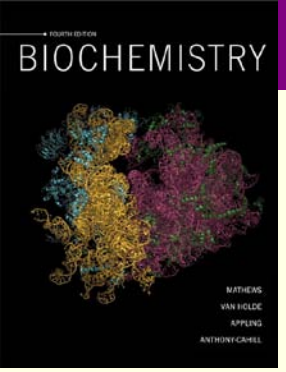


Cofactors, Vitamins, and Essential Metals

TABLE 11.8 Some important enzyme cofactors and related vitamins

Vitamin	Cofactor	Reactions Involving These Cofactors	Page Where Cofactor Is Introduced
Thiamine (vitamin B_1)	Thiamine pyrophosphate	Activation and transfer of aldehydes	536–538
Riboflavin (vitamin B_2)	Flavin mononucleotide; flavin adenine dinucleotide	Oxidation–reduction	599
Niacin	Nicotinamide adenine dinucleotide; nicotinamide adenine dinucleotide phosphate	Oxidation–reduction	446, 485, 530
Pantothenic acid	Coenzyme A	Acyl group activation and transfer	601
Pyridoxine	Pyridoxal phosphate	Various reactions involving amino acid activation	845
Biotin	Biotin	CO_2 activation and transfer	617–619
Lipoic acid	Lipoamide	Acyl group activation; oxidation–reduction	598–599
Folic acid	Tetrahydrofolate	Activation and transfer of single-carbon functional groups	848
Vitamin B_{12}	Adenosyl cobalamin; methyl cobalamin	Isomerizations and methyl group transfers	853

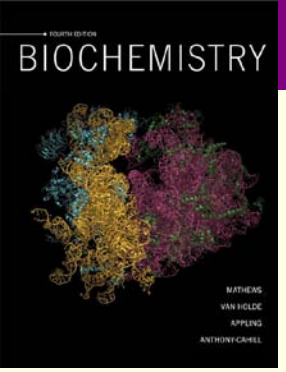
- Many essential vitamins are constituents of enzyme cofactors.



Cofactors, Vitamins, and Essential Metals

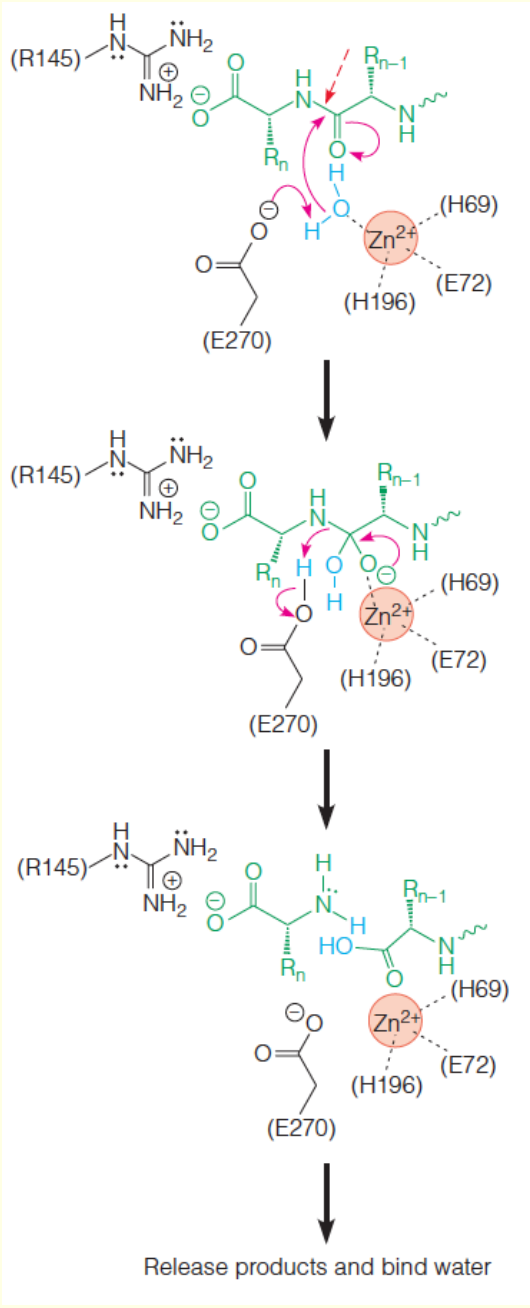
TABLE 11.9 Metals and trace elements important as enzymatic cofactors

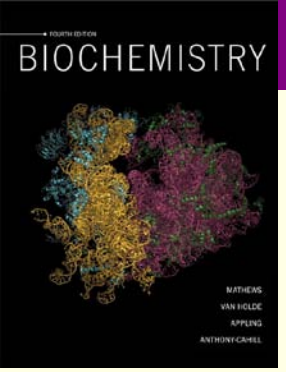
Metal	Example of Enzyme	Role of Metal
Fe	Cytochrome oxidase	Oxidation–reduction
Cu	Ascorbic acid oxidase	Oxidation–reduction
Zn	Alcohol dehydrogenase	Helps bind NAD^+
Mn	Histidine ammonialyase	Aids in catalysis by electron withdrawal
Co	Glutamate mutase	Co is part of cobalamin coenzyme
Ni	Urease	Catalytic site
Mo	Xanthine oxidase	Oxidation–reduction
V	Nitrate reductase	Oxidation–reduction
Se	Glutathione peroxidase	Replaces S in one cysteine in active site
Mg^{2+}	Many kinases	Helps bind ATP



The Diversity of Enzymatic Function

- Some enzymes require metal ions for their catalytic function, such as carboxypeptidease A.
- The zinc ion binds a water molecule and serves as an electrostatic catalyst to promote hydrolysis of the C-terminal amino acid from a peptide substrate.
- It does so by stabilizing the negative charge on the oxygen in the tetrahedral transition state.





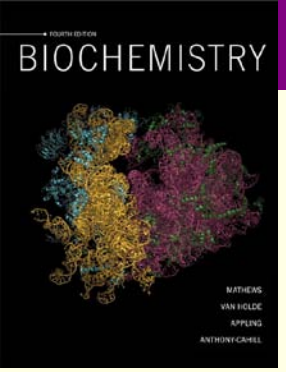
The Diversity of Enzymatic Function

A rational naming and numbering system has been devised by the Enzyme Commission of the International Union of Biochemistry and Molecular Biology (IUBMB).

Enzymes are divided into six major classes as follows:

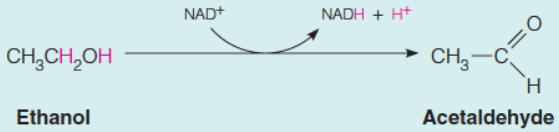
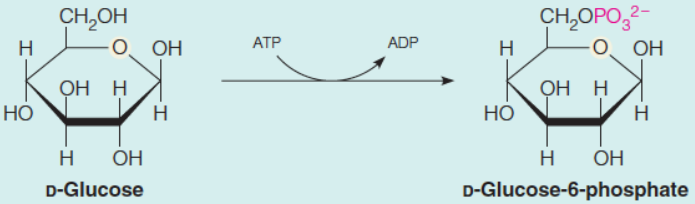
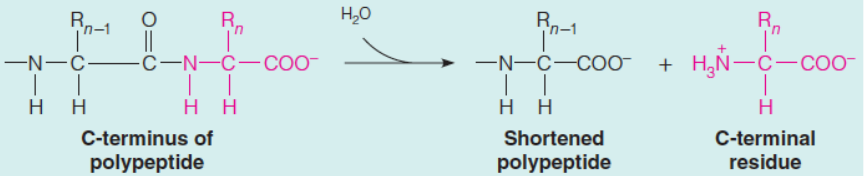
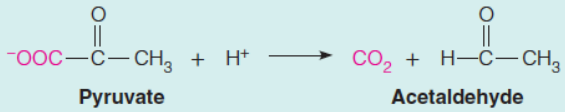
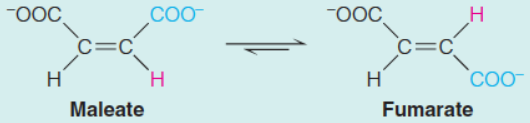
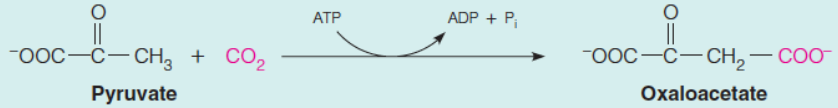
1. ***Oxidoreductases*** catalyze oxidation–reduction reactions.
2. ***Transferases*** catalyze transfer of functional groups from one molecule to another.
3. ***Hydrolases*** catalyze hydrolytic cleavage.
4. ***Lyases*** catalyze removal of a group from or addition of a group to a double bond, or other cleavages involving electron rearrangement.
5. ***Isomerases*** catalyze intramolecular rearrangement.
6. ***Ligases*** catalyze reactions in which two molecules are joined.

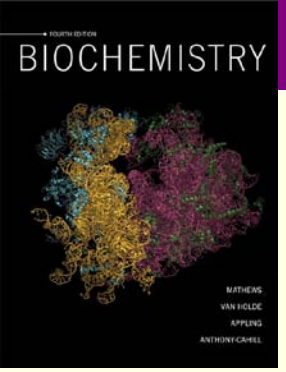
The IUBMB Enzyme Commission (EC) has given each enzyme a number with four parts, such as: EC 3.4.21.5. The first three numbers define major class, subclass, and sub-subclass, respectively.



The Diversity of Enzymatic Function

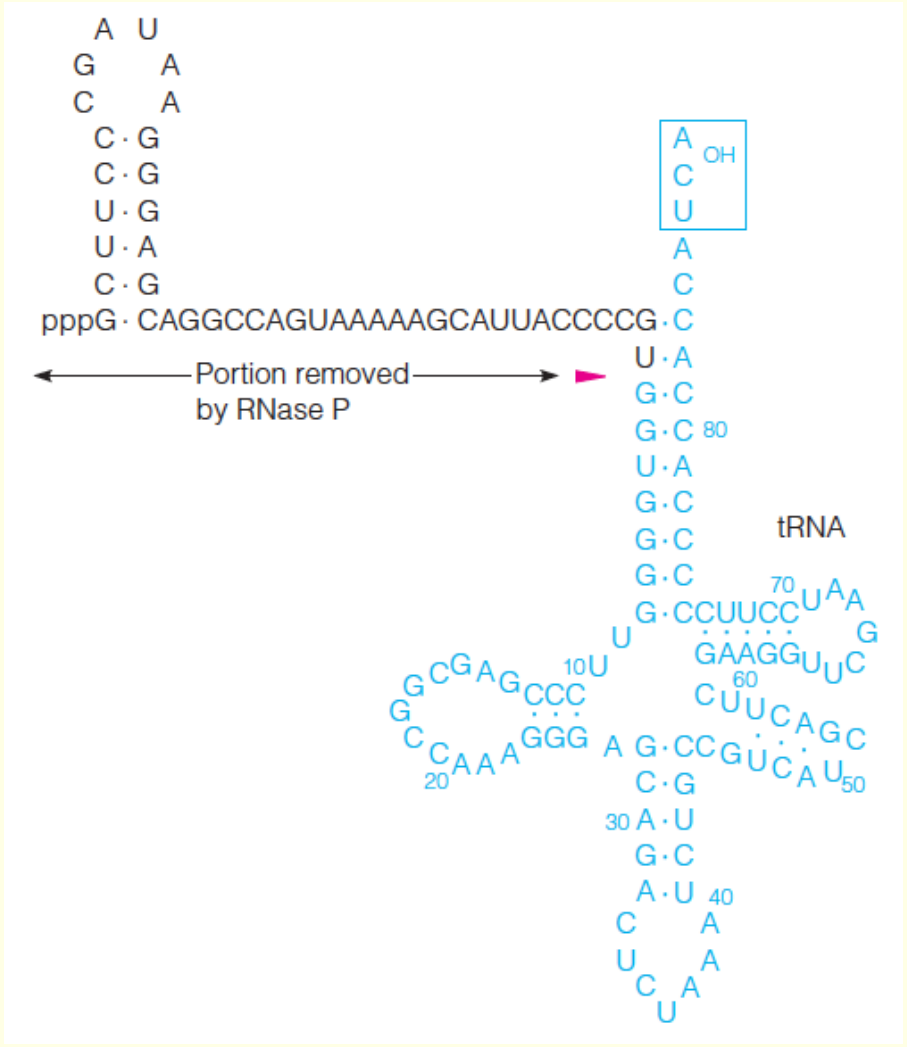
TABLE 11.10 Examples of each of the major classes of enzymes

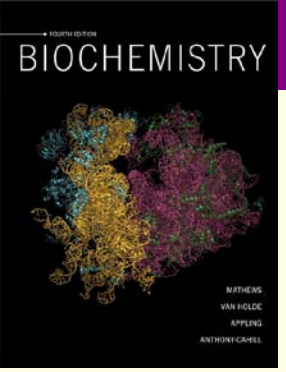
Class	Example (reaction type)	Reaction Catalyzed
1. Oxidoreductases	Alcohol dehydrogenase (EC 1.1.1.1) (oxidation with NAD^+)	
2. Transferases	Hexokinase (EC 2.7.1.2) (phosphorylation)	
3. Hydrolases	Carboxypeptidase A (EC 3.4.17.1) (peptide bond cleavage)	
4. Lyases	Pyruvate decarboxylase (EC 4.1.1.1) (decarboxylation)	
5. Isomerases	Maleate isomerase (EC 5.2.1.1) (*cis–trans* isomerization)	
6. Ligases	Pyruvate carboxylase (EC 6.4.1.1) (carboxylation)	



Nonprotein Biocatalysts: Catalytic Nucleic Acids

- ***Ribozymes***, a class of ribonucleic acids, also function as biological catalysts.
- The production of tRNA from pre-tRNA is catalyzed by an RNA–protein complex called ***ribonuclease P***.
- The RNA portion of ribonuclease P can by itself catalyze the hydrolysis of the specific phosphodiester bond indicated by the red wedge.





Nonprotein Biocatalysts: Catalytic Nucleic Acids

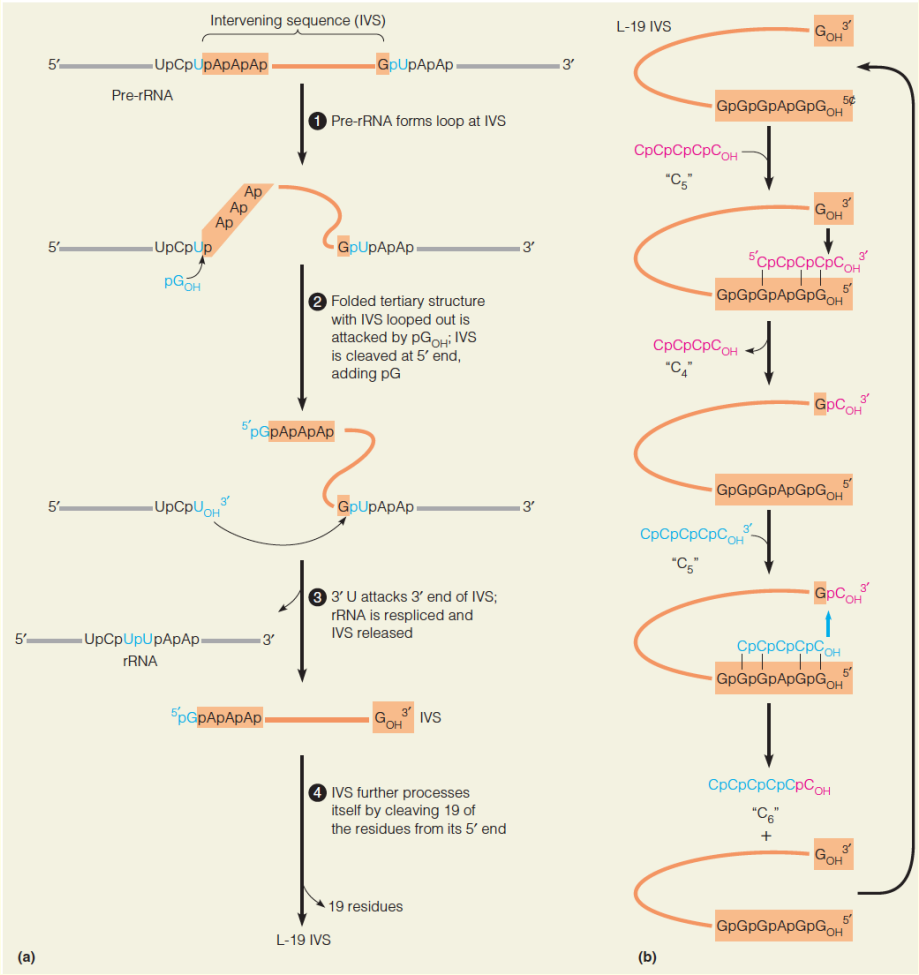
Catalysis by the intervening sequence in *Tetrahymena* preribosomal RNA:

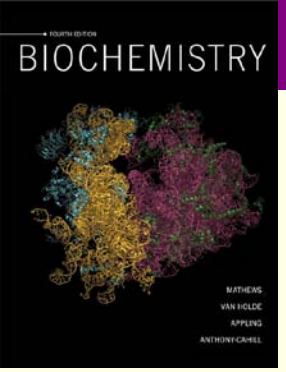
a) Self-excision and splicing of the intervening sequence (IVS). Note that a pG_{OH} is added in the reaction.

•A series of further steps reduces the IVS to L-19 IVS.

b) Conversion of $2C_5$ to $C_4 + C_6$ by L-19 IVS.

•This oligonucleotide can itself either shorten or elongate small oligonucleotides, acting here as a true ribozyme catalyst.

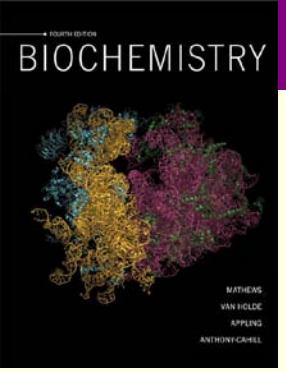




Nonprotein Biocatalysts: Catalytic Nucleic Acids

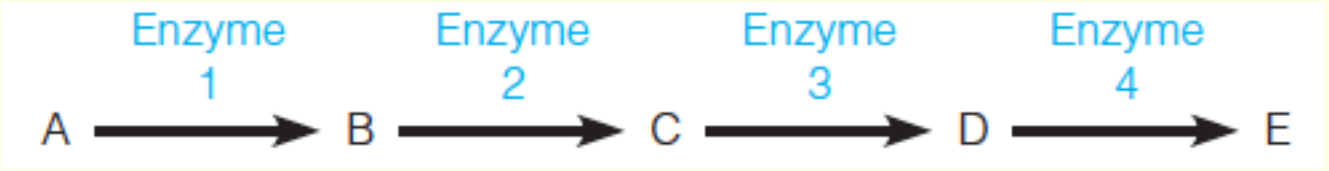
TABLE 11.11 Examples of reaction types and rate enhancements for DNAzymes

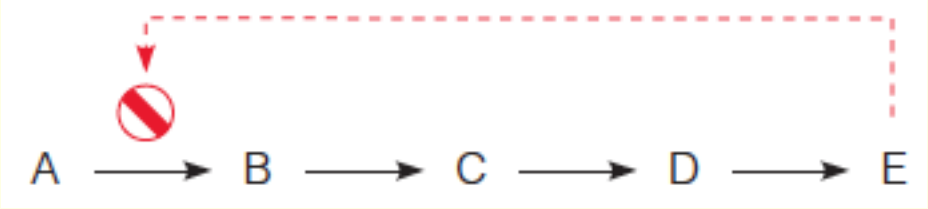
Reaction Type	k_{cat} (min^{-1})	Rate Enhancement
Various RNA transesterifications	0.007–4.3	10^5–10^8
DNA cleavage	0.05–0.2	10^7–10^8
Porphyrin metallation	1.3	10^3
DNA ligation	0.0001–0.07	10^2–10^5
Adenylylation	0.005	10^{10}
N-Glycosyl cleavage	0.2	10^6
Phosphorylation	0.012	10^9



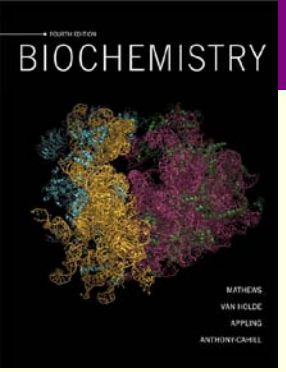
The Regulation of Enzyme Activity: Allosteric Enzymes

- Regulation of enzyme activity is essential for the efficient and ordered flow of metabolism.



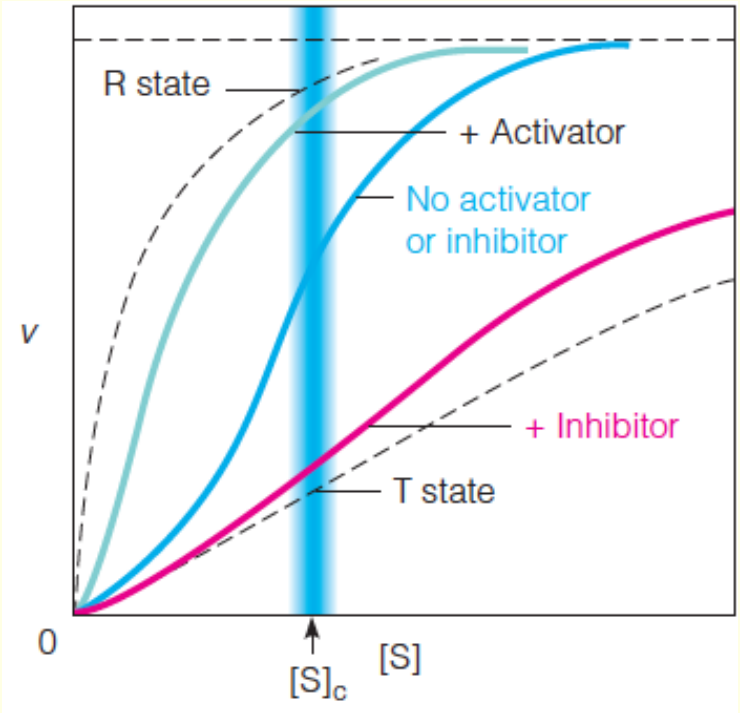


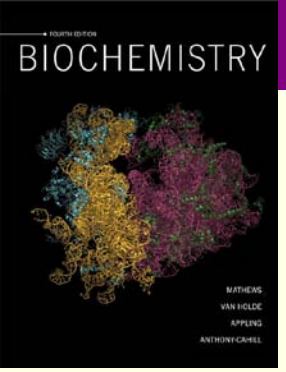
- ***Feedback control*** is important in the efficient regulation of complex metabolic pathways.



The Regulation of Enzyme Activity: Allosteric Enzymes

- Allosteric enzymes show cooperative substrate binding and can respond to a variety of inhibitors and activators.
- In the absence of activation or inhibitors, the *v* vs. [S] curve is sigmoidal.
- ***Activators*** shift the system toward the ***R state***.
- ***Inhibitors*** stabilize the ***T state***.
- $[S]_c$ represents the homeostatic concentration range for S.
- Note that effectors significantly alter the activity of the enzyme over this range of [S].





The Regulation of Enzyme Activity: Allosteric Enzymes

Control points in pyrimidine synthesis:

•The formation of ***N*-carbamoyl-L-aspartate** from carbamoyl phosphate and aspartate is the first committed step in a series of reactions that leads to synthesis of pyrimidine nucleotides, so control at or near this point is essential.

•In prokaryotes, the aspartate carbamoyltransferase (ATCase) is regulated; in most eukaryotes, regulation is on the preceding step, catalyzed by carbamoyl phosphate synthetase II.

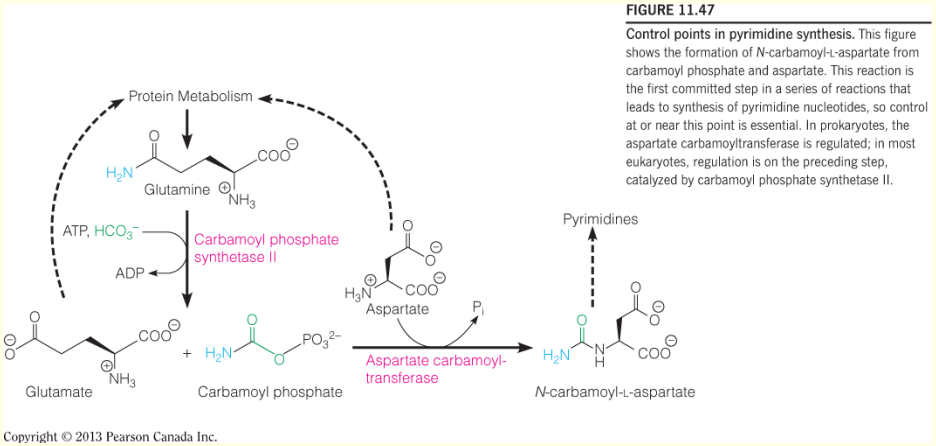
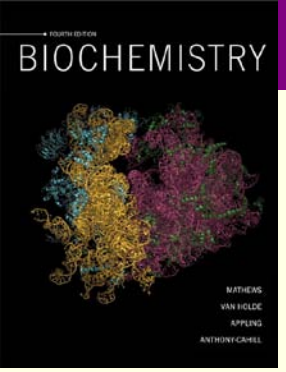


FIGURE 11.47
Control points in pyrimidine synthesis. This figure shows the formation of *N*-carbamoyl-L-aspartate from carbamoyl phosphate and aspartate. This reaction is the first committed step in a series of reactions that leads to synthesis of pyrimidine nucleotides, so control at or near this point is essential. In prokaryotes, the aspartate carbamoyltransferase is regulated; in most eukaryotes, regulation is on the preceding step, catalyzed by carbamoyl phosphate synthetase II.



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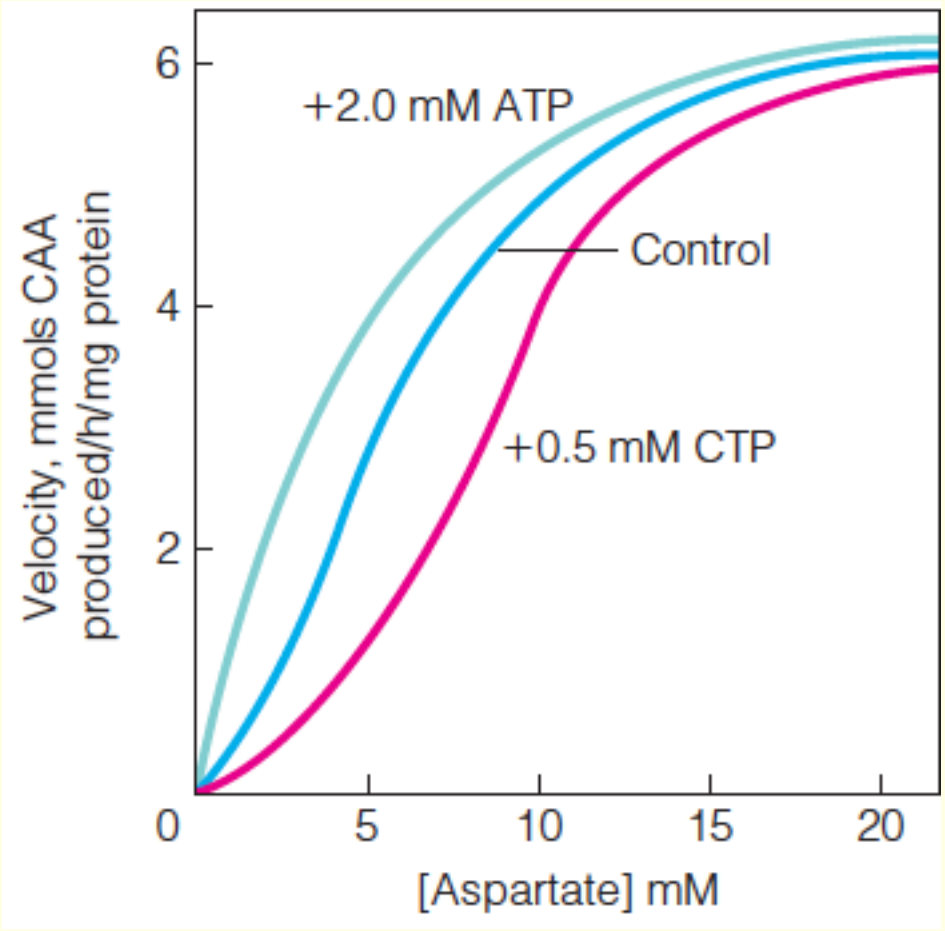
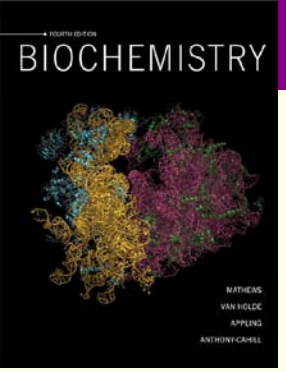
Regulation of ATCase by ATP and CTP:

- **ATP is an activator.**
- **CTP is an inhibitor.**

•The curve marked as “control” shows the behavior of the enzyme in the absence of both regulators.

•*N*-Carbamoyl-L-aspartate (CAA) is the product of the reaction.





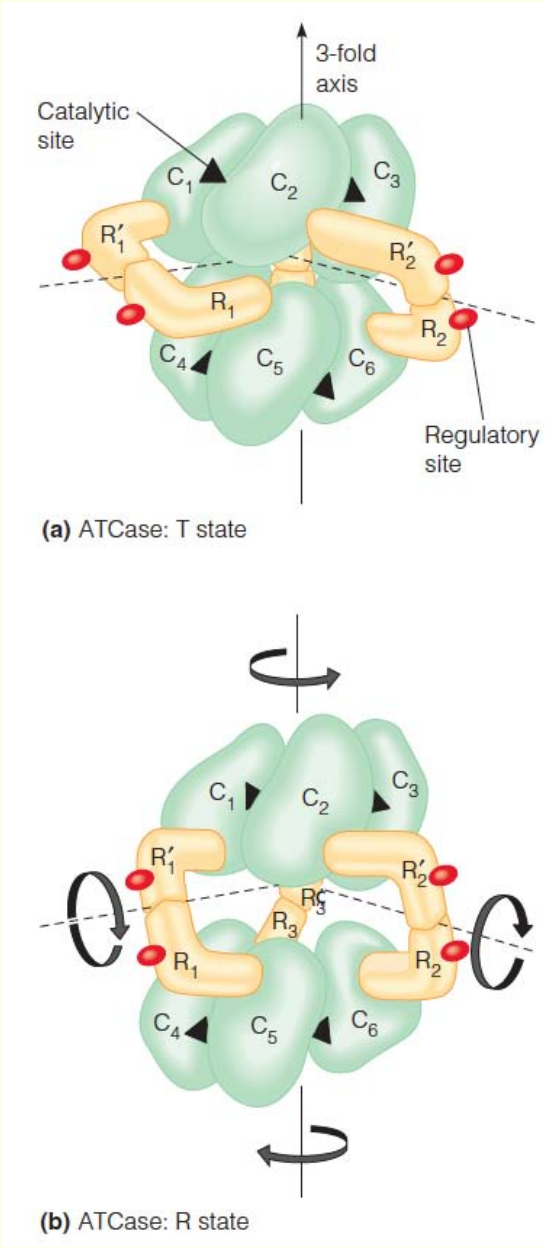
The Regulation of Enzyme Activity: Allosteric Enzymes

(a) Quaternary structure of ATCase in the T state.

- The enzyme has the six catalytic subunits (C) and six regulatory subunits (R).
- Six catalytic sites lie in or near the grooves between the catalytic subunits.
- Regulatory sites lie on the outer surfaces of the regulatory subunits.

(b) Transition of ATCase to the R state.

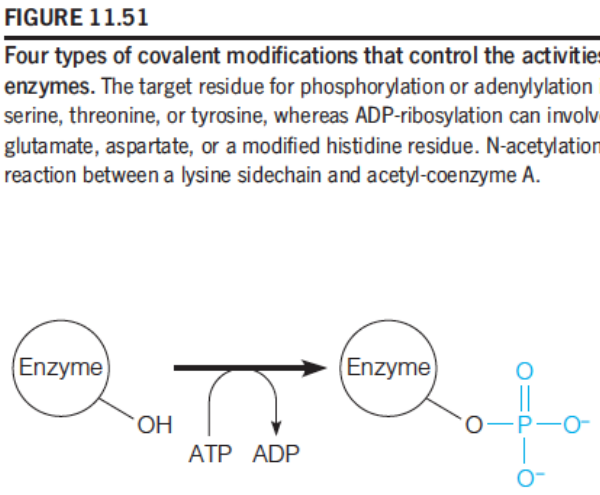
- The transition involves a rotation of the regulatory subunits, which pushes the two tiers of catalytic subunits apart and rotates them slightly about the three-fold axis



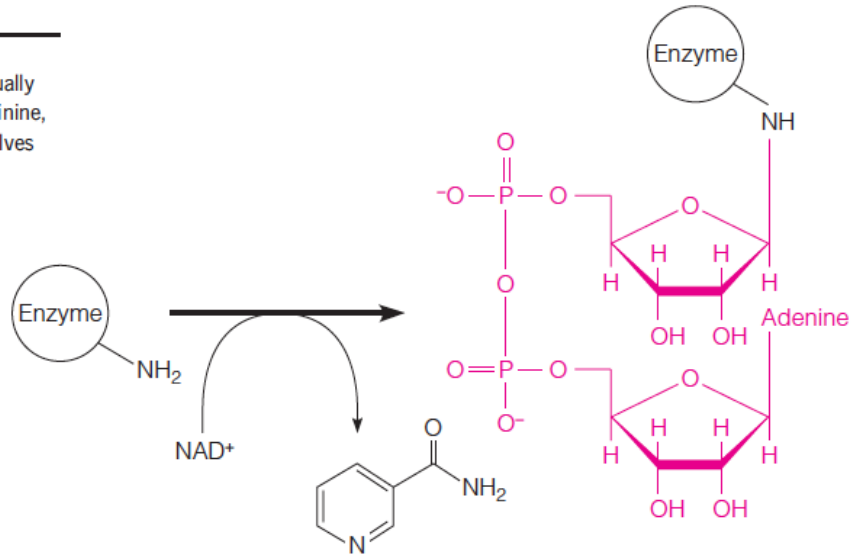
Covalent Modifications Used to Regulate Enzyme Activity

FIGURE 11.51

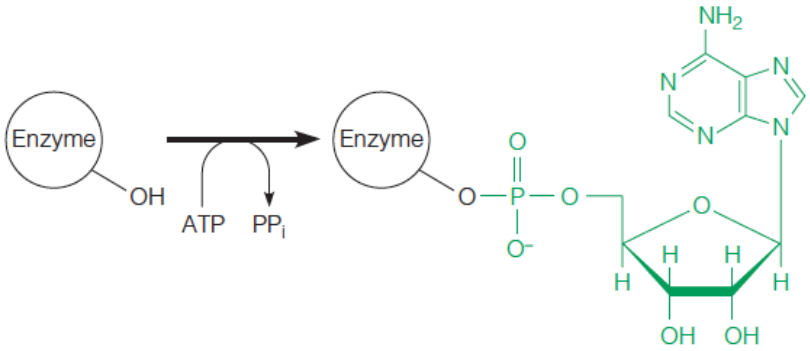
Four types of covalent modifications that control the activities of enzymes. The target residue for phosphorylation or adenylylation is usually serine, threonine, or tyrosine, whereas ADP-ribosylation can involve arginine, glutamate, aspartate, or a modified histidine residue. N-acetylation involves reaction between a lysine sidechain and acetyl-coenzyme A.



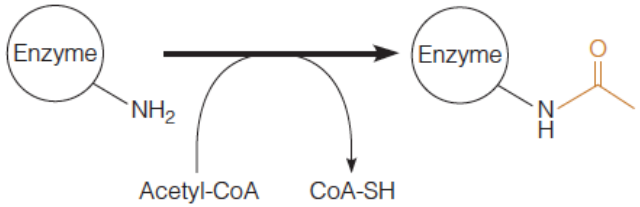
(a) Phosphorylation



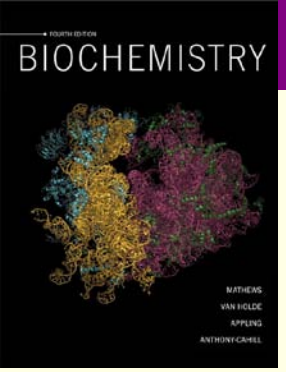
(c) ADP-ribosylation



(b) Adenylylation



(d) Acetylation

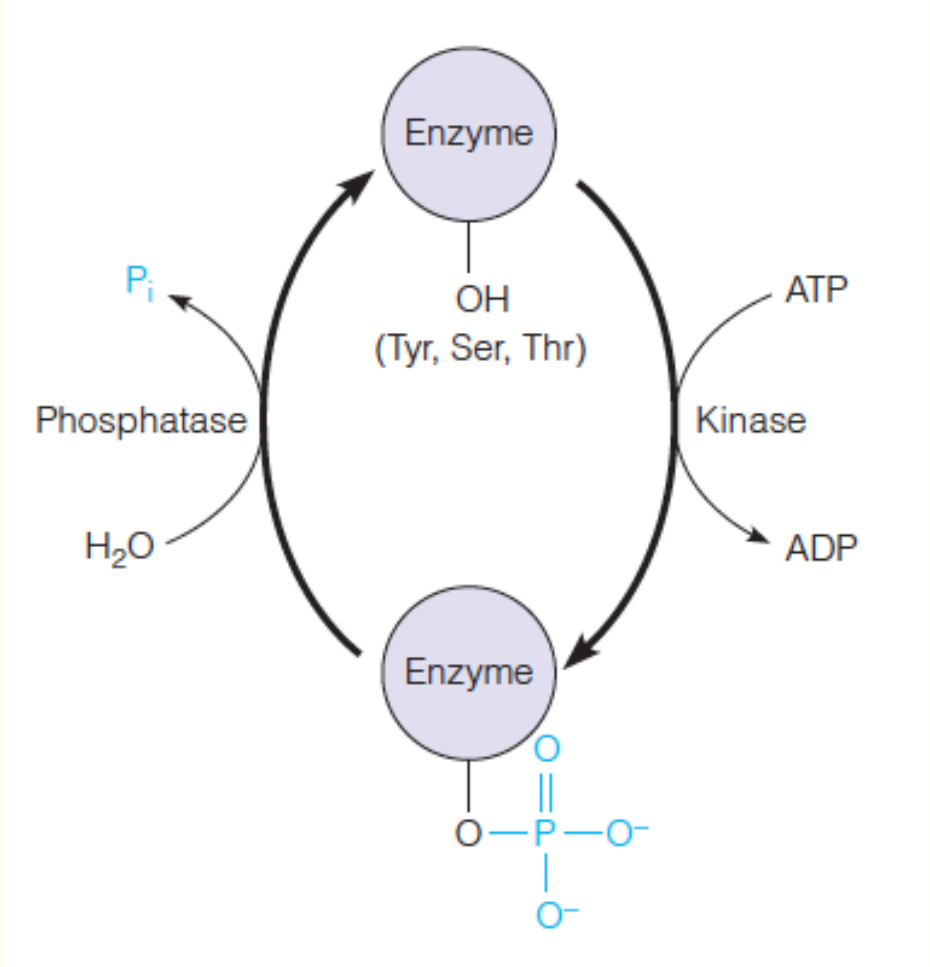


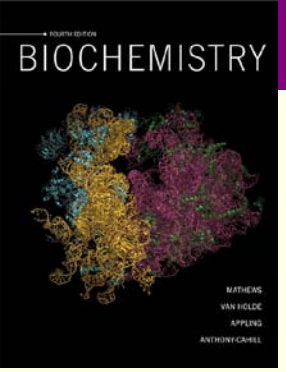
Covalent Modifications Used to Regulate Enzyme Activity

Reversible covalent modification by kinases/ phosphatases:

•The target residues for ATP-dependent phosphorylation by ***kinases*** are serine, threonine, or tyrosine.

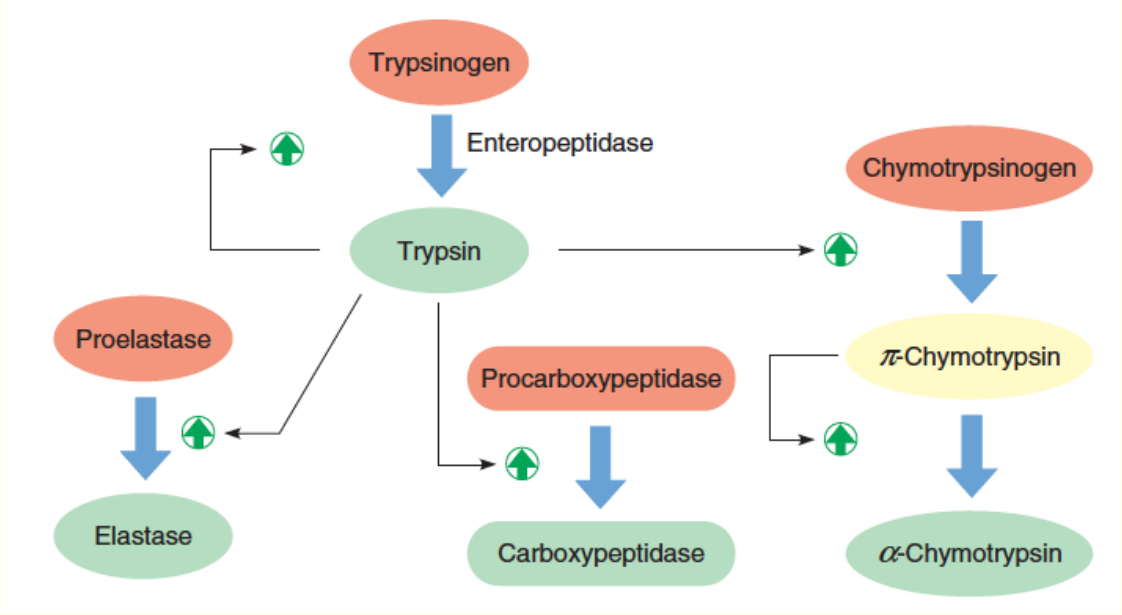
•The phosphoprotein is dephosphorylated by a ***phosphatase***-catalyzed hydrolysis reaction.

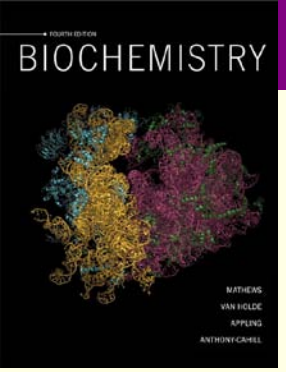




Covalent Modifications Used to Regulate Enzyme Activity

- Some enzymes, such as pancreatic proteases, are irreversibly switched on by proteolytic cleavage.
- ***Zymogens*** are activation by proteolytic cleavage.
- This schematic view shows the activation of pancreatic zymogens, molecules that become catalytically active when cleaved.





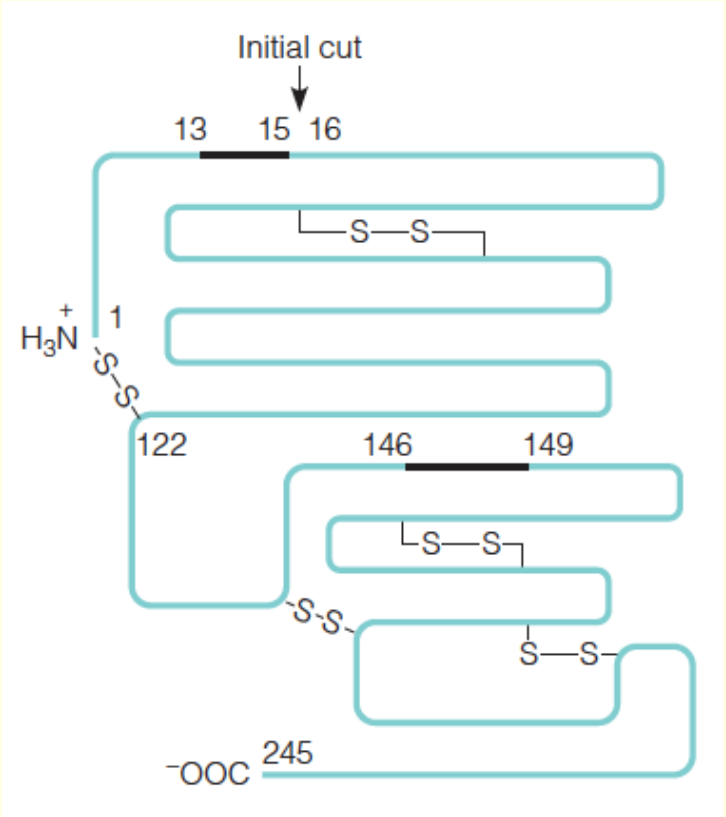
Covalent Modifications Used to Regulate Enzyme Activity

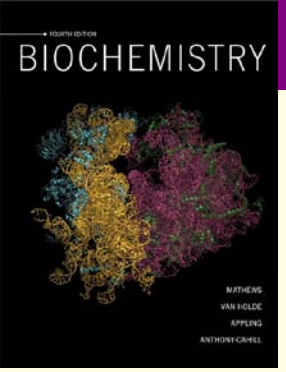
Activation of chymotrypsinogen:

•A series of cleavages produces the enzyme chymotrypsin, with the disulfide bonds continuing to hold the structure together.

•The initial cleavage between amino acids 15 and 16 (arrow) results in the formation of π-chymotrypsin.

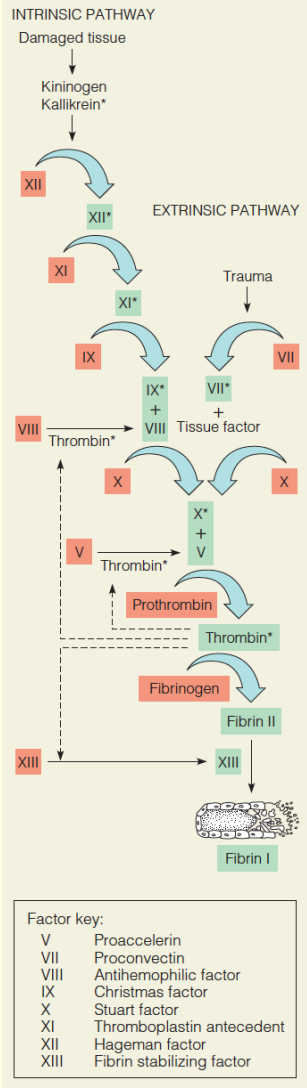
•Subsequent removal of the segments shown in black yields α-chymotrypsin.

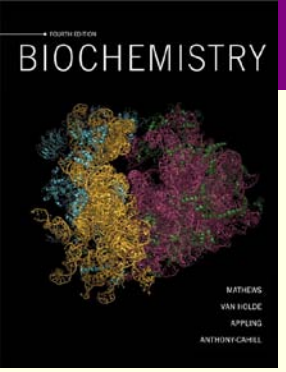




Covalent Modifications Used to Regulate Enzyme Activity

- Blood clotting involves a cascade of proteolytic activation of specific proteases, culminating in the transition of fibrinogen to fibrin.
- Each factor (protease) in the pathway can exist in an inactive form (red) or an active form (green).
- The cascade of proteolytic activations can start from exposure of blood at damaged tissue surfaces (***intrinsic pathway***) or from internal trauma to blood vessels (***extrinsic pathway***).
- The common result is activation of fibrinogen to clotting fibrin.





Covalent Modifications Used to Regulate Enzyme Activity

Formation of a blood clot:

a) Red blood cells enmeshed in the insoluble strands of a fibrin clot.

b) Electron micrograph of part of a fibrin fiber.

c) Schematic view of how fibrin monomers are thought to associate to form a fiber. Removal of fibrinopeptides A and B from fibrinogen by thrombin makes sites accessible for association with complementary sites a and b on adjacent monomers. The molecules are believed to overlap as shown because the striations seen in the fibers are 23 nm wide, exactly half the length of the fibrinogen molecule.

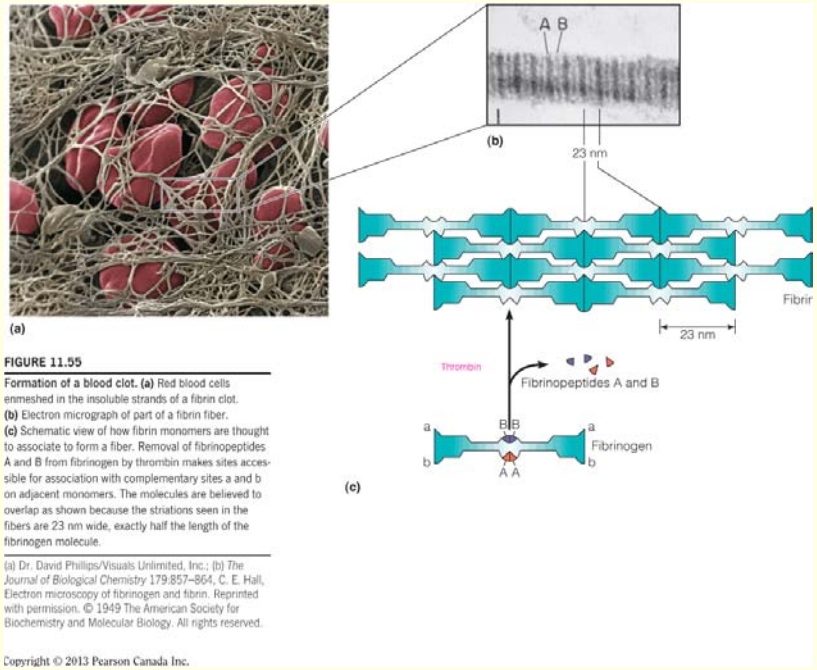
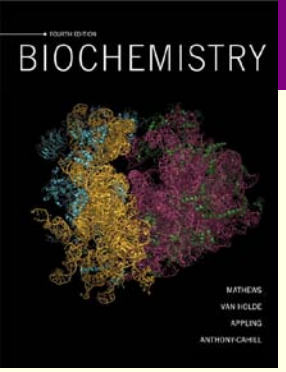


FIGURE 11.55
Formation of a blood clot. (a) Red blood cells enmeshed in the insoluble strands of a fibrin clot. **(b)** Electron micrograph of part of a fibrin fiber. **(c)** Schematic view of how fibrin monomers are thought to associate to form a fiber. Removal of fibrinopeptides A and B from fibrinogen by thrombin makes sites accessible for association with complementary sites a and b on adjacent monomers. The molecules are believed to overlap as shown because the striations seen in the fibers are 23 nm wide, exactly half the length of the fibrinogen molecule.

(a) Dr. David Phillips/Visuals Unlimited, Inc.; (b) *The Journal of Biological Chemistry* 179:857–864, C. E. Hall, Electron microscopy of fibrinogen and fibrin. Reprinted with permission. © 1949 The American Society for Biochemistry and Molecular Biology. All rights reserved.



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