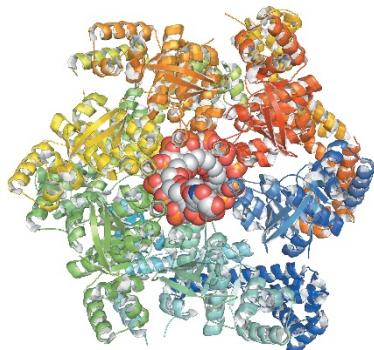


biochemistry



Reginald H. Garrett | Charles M. Grisham  
SIXTH EDITION

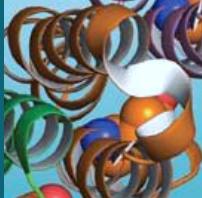
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Charles M. Grisham

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# Chapter 14

## Mechanisms of Enzyme Action

# Outline



- Magnitudes of enzyme-induced rate accelerations
- The central role of transition-state stabilization in enzyme catalysis
- Transition-state analogs act as competitive inhibitors and make good drugs
- Other factors contributing to catalysis
  - Near-attack conformations (NACs)
  - ES complex destabilization
  - Unstable covalent intermediates
  - General acid-base catalysis
  - Low-barrier H-bonds (LBHB)
  - Metal catalysis
- Example mechanisms for some typical enzymes

# 14.1 What Are the Magnitudes of Enzyme-Induced Rate Accelerations?



**TABLE 14.1** A Comparison of Enzyme-Catalyzed Reactions and Their Uncatalyzed Counterparts

Reaction	Enzyme	Uncatalyzed Rate, $v_u$ (sec $^{-1}$ )	Catalyzed Rate, $v_e$ (sec $^{-1}$ )	$v_e/v_u$
Fructose-1,6-bisP $\longrightarrow$ fructose-6-P + P <sub>i</sub>	Fructose-1,6-bisphosphatase	$2 \times 10^{-20}$	21	$1.05 \times 10^{21}$
(Glucose) <sub>n</sub> + H <sub>2</sub> O $\longrightarrow$ (glucose) <sub>n-2</sub> + maltose	$\beta$ -amylase	$1.9 \times 10^{-15}$	$1.4 \times 10^3$	$7.2 \times 10^{17}$
DNA, RNA cleavage	Staphylococcal nuclease	$7 \times 10^{-16}$	95	$1.4 \times 10^{17}$
CH <sub>3</sub> —O—PO <sub>3</sub> <sup>2-</sup> + H <sub>2</sub> O $\longrightarrow$ CH <sub>3</sub> OH + HPO <sub>4</sub> <sup>2-</sup>	Alkaline phosphatase	$1 \times 10^{-15}$	14	$1.4 \times 10^{16}$
$\text{H}_2\text{N}-\overset{\text{O}}{\underset{\text{  }}{\text{C}}}-\text{NH}_2 + 2 \text{H}_2\text{O} + \text{H}^+ \longrightarrow 2 \text{NH}_4^+ + \text{HCO}_3^-$	Urease	$3 \times 10^{-10}$	$3 \times 10^4$	$1 \times 10^{14}$
$\text{R}-\overset{\text{O}}{\underset{\text{  }}{\text{C}}}-\text{O}-\text{CH}_2\text{CH}_3 + \text{H}_2\text{O} \longrightarrow \text{RCOOH} + \text{HOCH}_2\text{CH}_3$	Chymotrypsin	$1 \times 10^{-10}$	$1 \times 10^2$	$1 \times 10^{12}$
Glucose + ATP $\longrightarrow$ Glucose-6-P + ADP	Hexokinase	$<1 \times 10^{-13}$	$1.3 \times 10^{-3}$	$>1.3 \times 10^{10}$
CH <sub>3</sub> CH <sub>2</sub> OH + NAD <sup>+</sup> $\longrightarrow$ $\text{CH}_3\overset{\text{O}}{\underset{\text{  }}{\text{C}}}\text{H} + \text{NADH} + \text{H}^+$	Alcohol dehydrogenase	$<6 \times 10^{-12}$	$2.7 \times 10^{-5}$	$>4.5 \times 10^6$
CO <sub>2</sub> + H <sub>2</sub> O $\longrightarrow$ HCO <sub>3</sub> <sup>-</sup> + H <sup>+</sup>	Carbonic anhydrase	$10^{-2}$	$10^5$	$1 \times 10^7$
Creatine + ATP $\longrightarrow$ Cr-P + ADP	Creatine kinase	$<3 \times 10^{-9}$	$4 \times 10^{-5}$	$>1.33 \times 10^4$

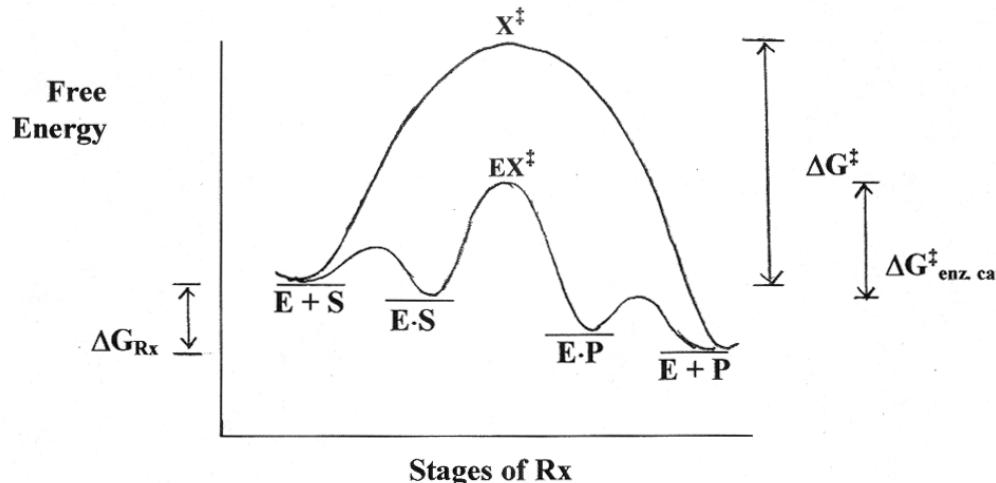
# ENZYME MECHANISMS

How do enzymes work?

By speeding attainment of  $K_{eq}$ , typically from  $10^7$  to  $10^{14}$  times faster.

$K_{eq}$  is not changed!     $\Delta G_{Rx}$  is not changed!

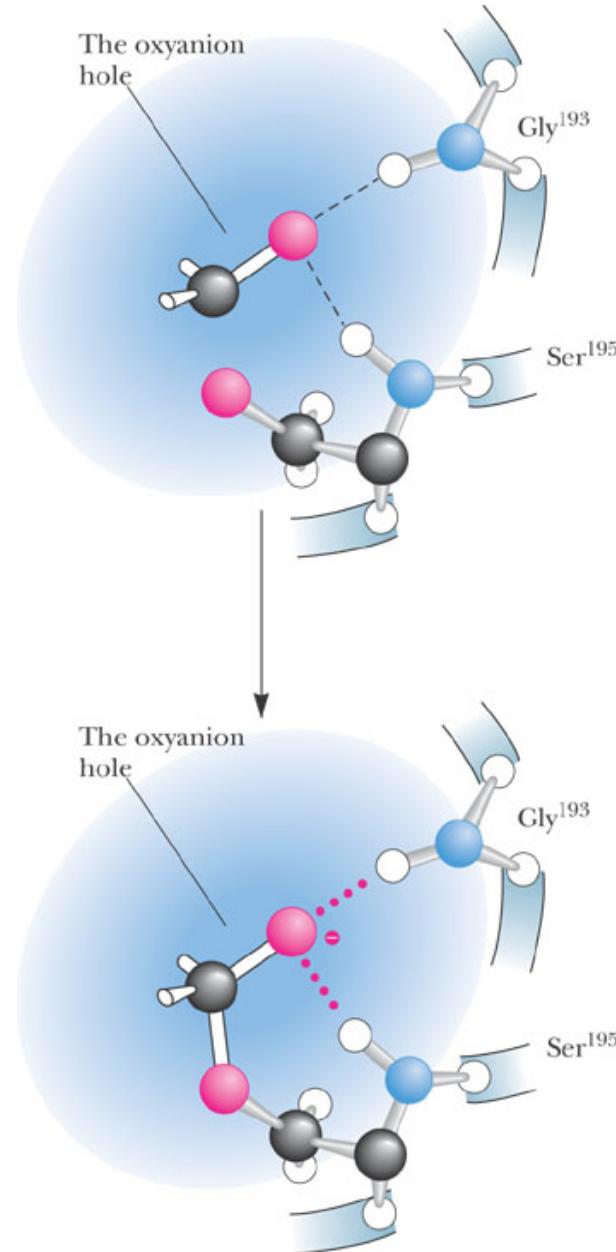
But activation energy  $\Delta G^\ddagger$  is lowered.



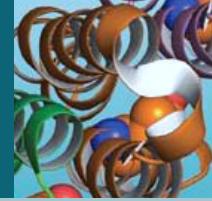
Reasons for catalysis by enzymes

1. Stabilization of transition state intermediates
2. Proximity and orientation of substrates
3. Entropy loss in ES formation
4. Destabilization of ES due to:
  - a. geometric strain
  - b. electronic strain
  - c. desolvation of substrate

The “ oxyanion hole” of chymotrypsin stabilizes the tetrahedral oxyanion transition states of the mechanism in Figure 14.21.



# 14.4 How Tightly Do Transition-State Analogs Bind to the Active Site?



## (a) Yeast aldolase reaction

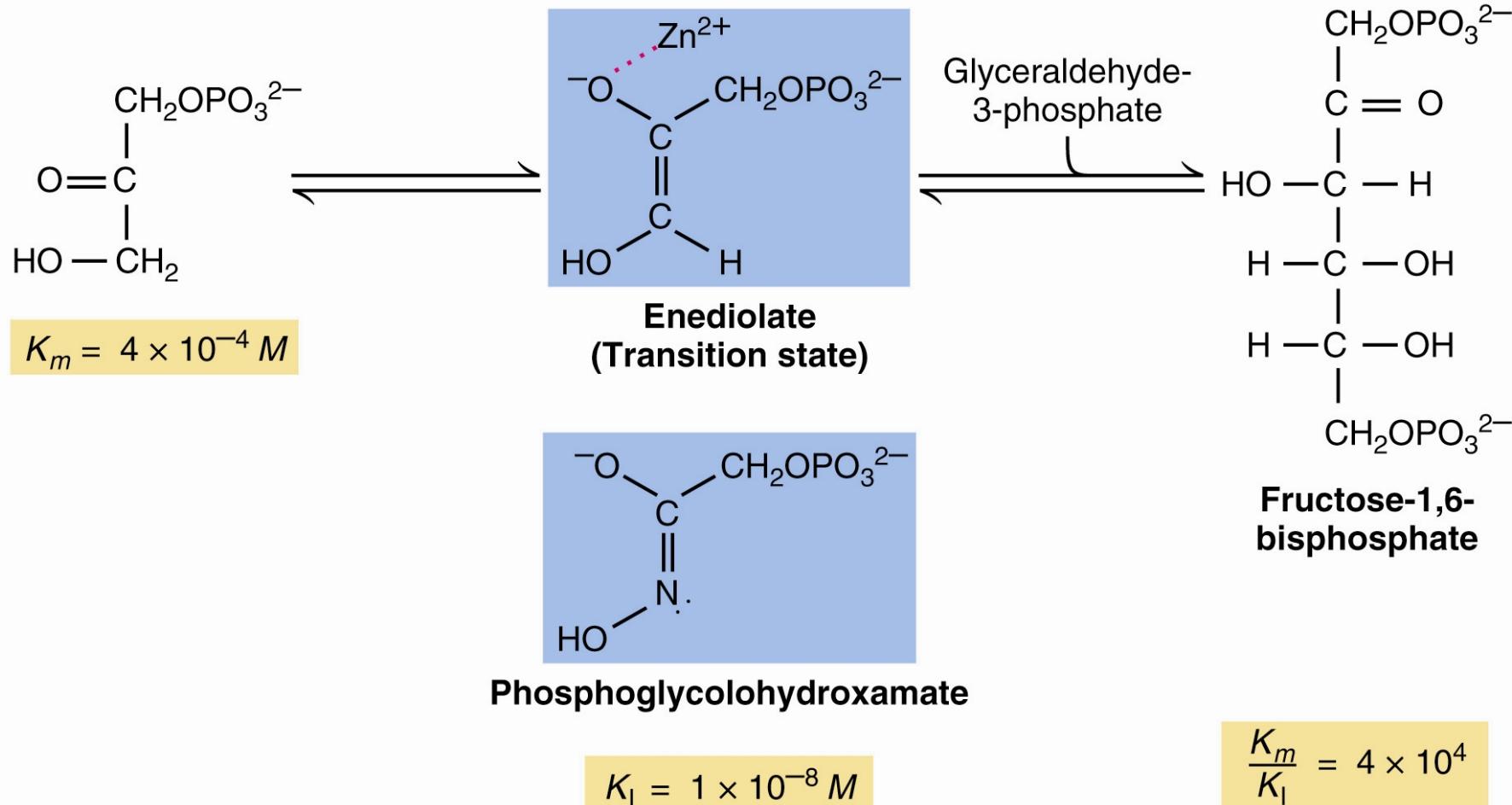
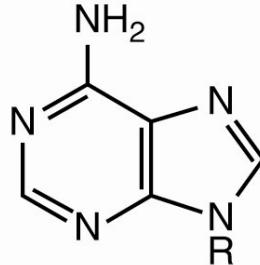


Figure 14.6 (a) Phosphoglycolohydroxamate is an analog of the enediolate transition state of the yeast aldolase reaction.

# 14.4 How Tightly Do Transition-State Analogs Bind to the Active Site?



(b) Calf intestinal adenosine deaminase reaction

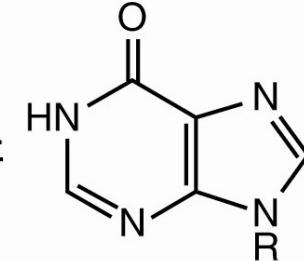


Adenosine

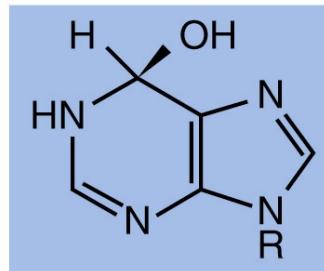
$$K_m = 3 \times 10^{-5} M$$



Transition state



Inosine



Hydrated form of  
purine ribonucleoside

$$K_I = 3 \times 10^{-13} M$$

$$\frac{K_m}{K_I} = 1 \times 10^8$$

(b) Purine riboside inhibits adenosine deaminase. The hydrated form is an analog of the transition state of the reaction.

# Transition-State Analogs Make Our World Better

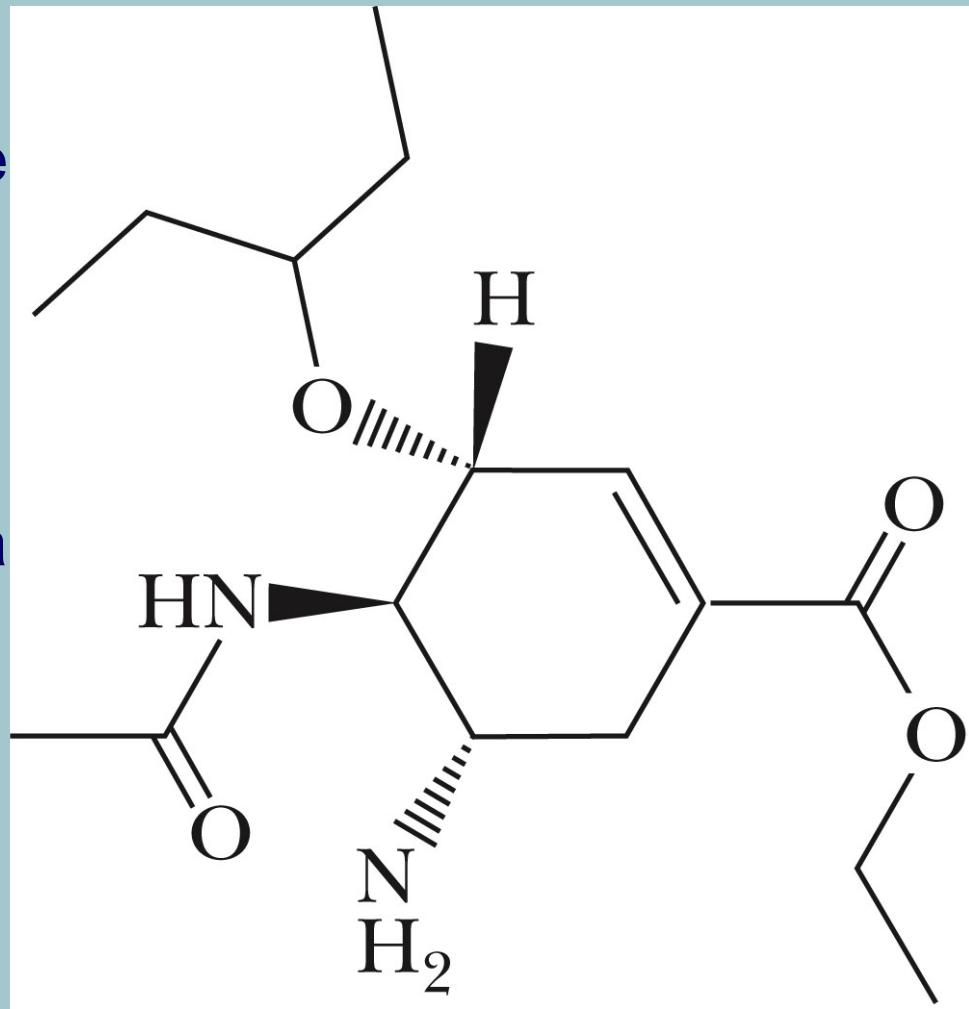


- Enzymes are often targets for drugs and other beneficial agents
- Transition state analogs often make ideal enzyme inhibitors
- Enalapril and Aliskiren lower blood pressure
- Statins lower serum cholesterol
- Protease inhibitors are AIDS drugs
- Juvenile hormone esterase is a pesticide target
- Tamiflu is a viral neuraminidase inhibitor

# Tamiflu is a Viral Neuraminidase Inhibitor

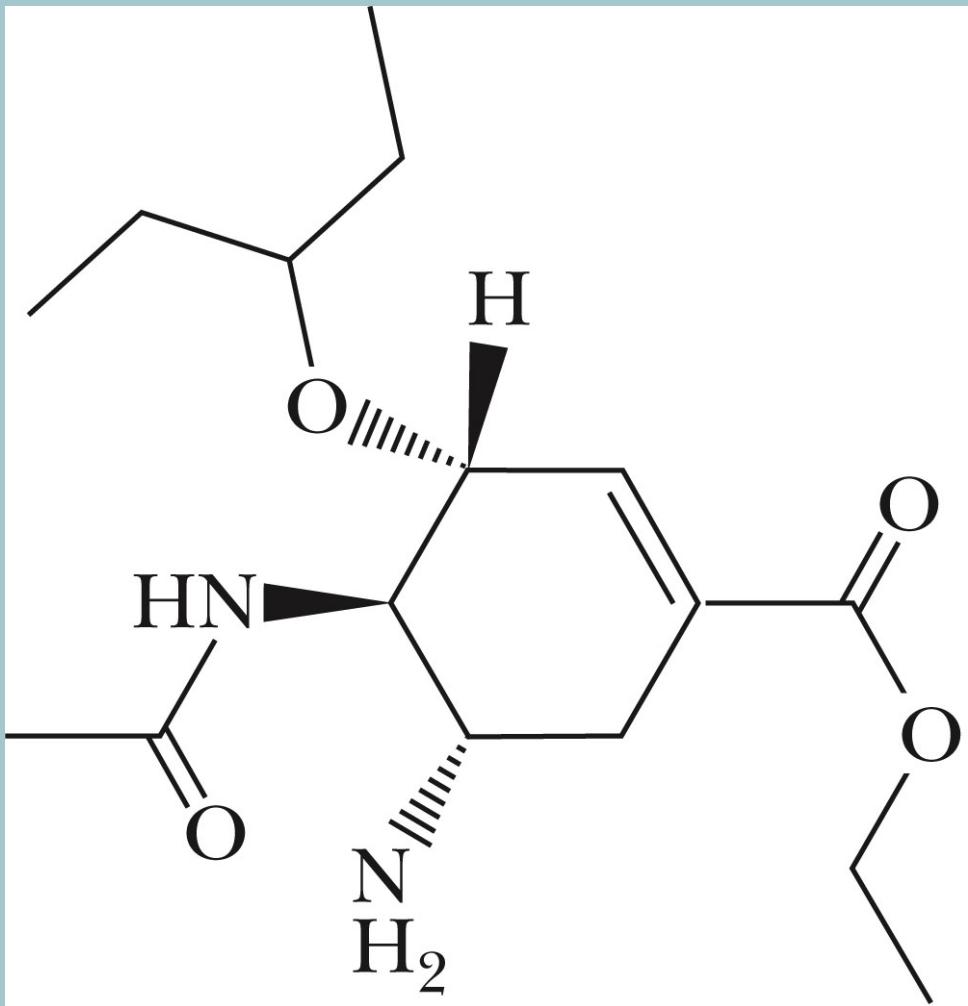
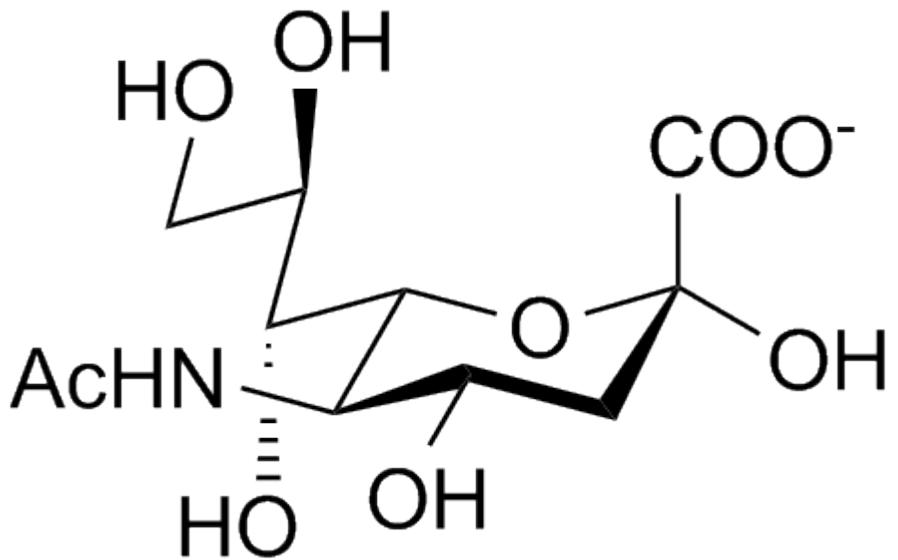
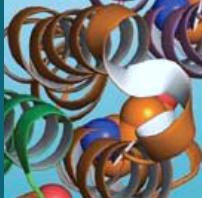


- Influenza is a serious illness that affects 5% to 15% of the earth's population each year and results in up to 500,000 deaths annually.
- Neuraminidase is a major glycoprotein on the influenza virus membrane envelope that is essential for viral replication and infectivity.
- Tamiflu is a neuraminidase inhibitor and antiviral agent based on the transition state of the neuraminidase reaction.



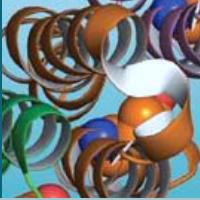
Tamiflu

# Neuraminic acid, aka sialic acid



Tamiflu

# How many other drug targets might there be?



- The human genome contains approximately 20,000 genes
- How many might be targets for drug therapy?
- More than 3000 experimental drugs are presently under study and testing
- These and many future drugs will be designed as transition-state analog inhibitors
- See the DrugBank:

<http://www.drugbank.ca/>

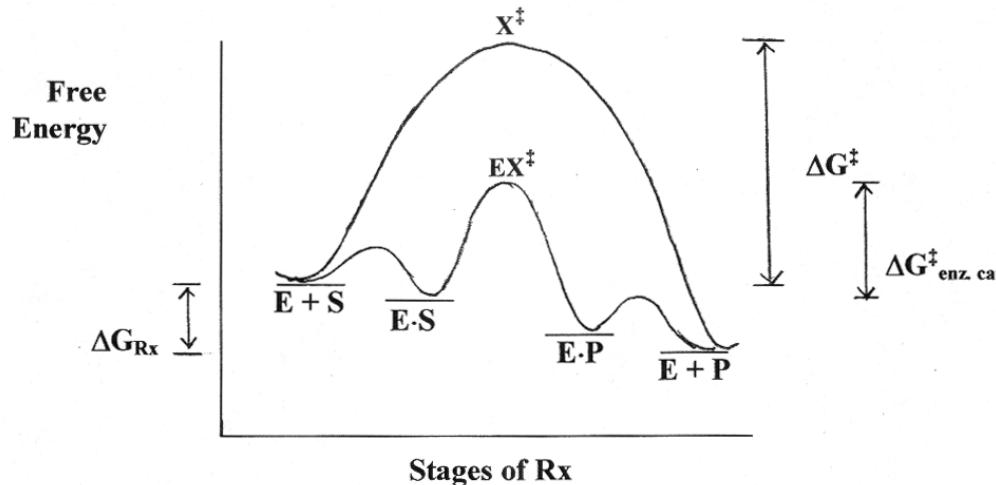
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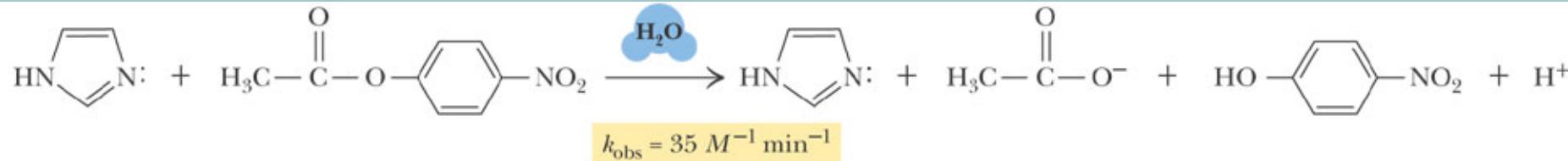
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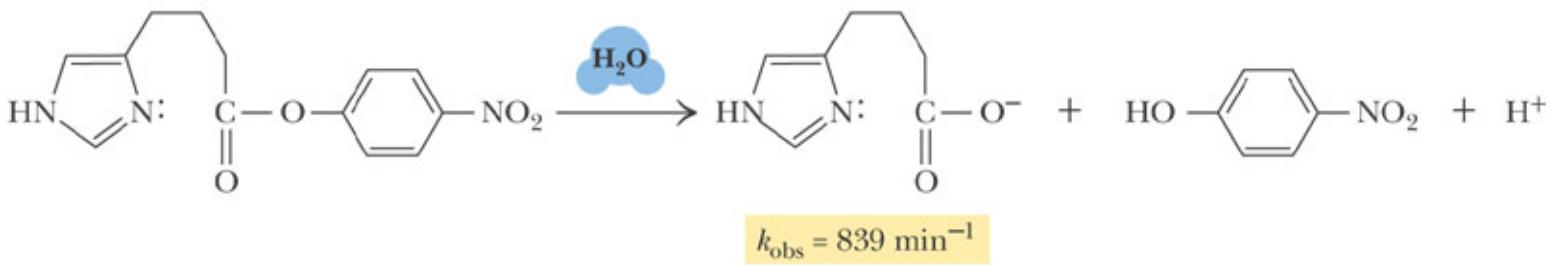
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2. Proximity and orientation of substrates
3. Entropy loss in ES formation
4. Destabilization of ES due to:
  - a. geometric strain
  - b. electronic strain
  - c. desolvation of substrate

(a)



(b)



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An example of proximity effects in catalysis. (a) The imidazole-catalyzed hydrolysis of *p*-nitrophenylacetate is slow, but (b) the corresponding intramolecular reaction is 24-fold faster (assuming [imidazole] = 1 M in [a]).

Reaction	Rate const. ( $M^{-1} \text{ sec}^{-1}$ )	Ratio
	$5.9 \times 10^{-6}$	
	$1.5 \times 10^6$	$2.5 \times 10^{11}$

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Orientation effects in intramolecular reactions can be dramatic. Steric crowding by methyl groups provides a rate acceleration of  $2.5 \times 10^{11}$  for the lower reaction compared to the upper reaction. (Adapted from Milstien, S., and Cohen, L.A., 1972. *Stereopopulation control I. Rate enhancements in the lactonization of o-hydroxyhydrocinnamic acid*. Journal of the American Chemical Society 94:9158-9165.)

# Enzymes facilitate formation of near-attack complexes



- X-ray crystal structure studies and computer modeling have shown that the reacting atoms and catalytic groups are precisely positioned for their roles
- Such preorganization selects substrate conformations in which *the reacting atoms are in van der Waals contact and at an angle resembling the bond to be formed in the transition state*
- Thomas Bruice has termed such arrangements **near-attack conformations (NACs)**
- NACs are precursors to reaction transition states

# Enzymes facilitate formation of near-attack complexes

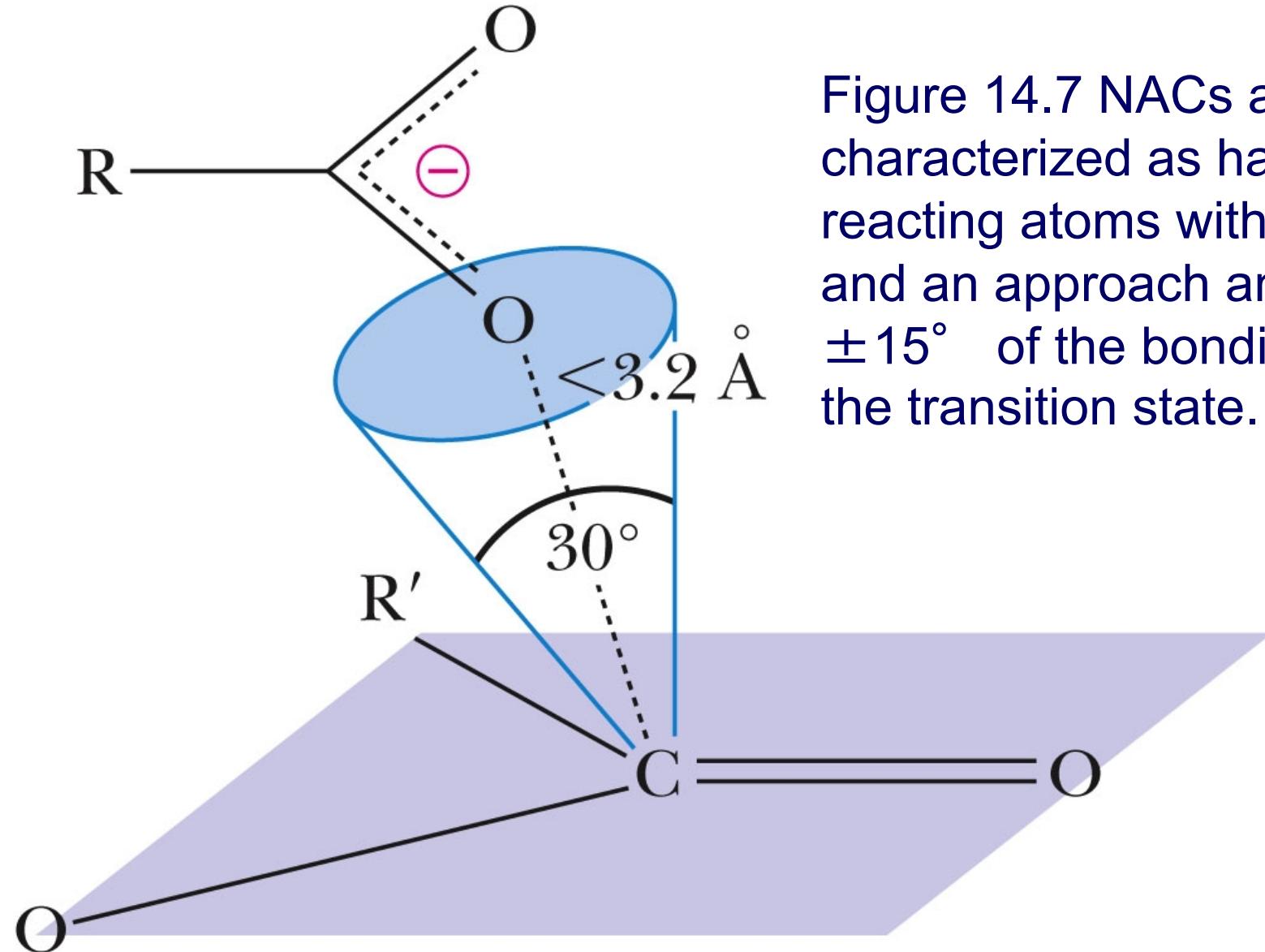
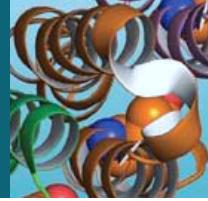


Figure 14.7 NACs are characterized as having reacting atoms within  $3.2 \text{ \AA}$  and an approach angle of  $\pm 15^\circ$  of the bonding angle in the transition state.

# Protein Motions Are Essential to Enzyme Catalysis



- Proteins are constantly moving – bonds vibrate, side chains bend and rotate, backbone loops wiggle and sway, and whole domains move as a unit
- Enzymes depend on such motions to provoke and direct catalytic events
- Protein motions support catalysis in several ways.  
Active site conformation changes can:
  - Assist substrate binding
  - Bring catalytic groups into position
  - Induce formation of NACs
  - Assist in bond making and bond breaking

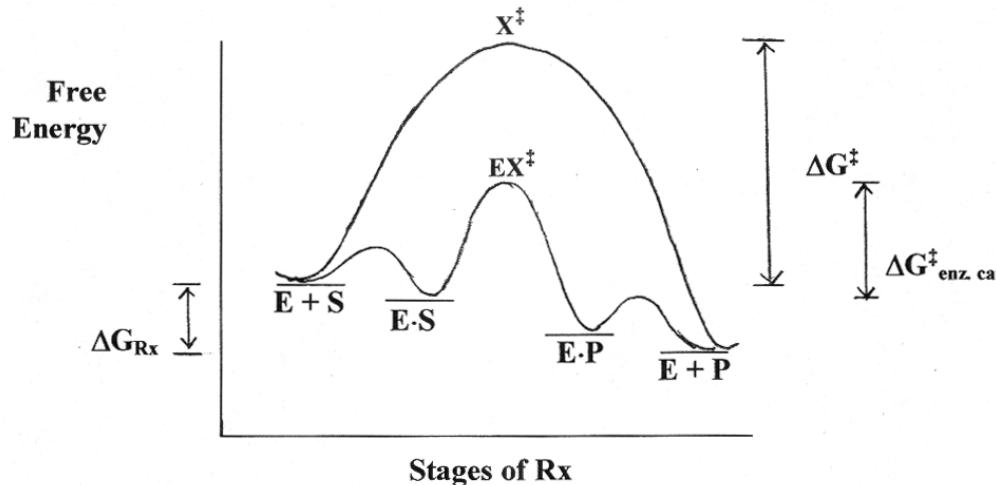
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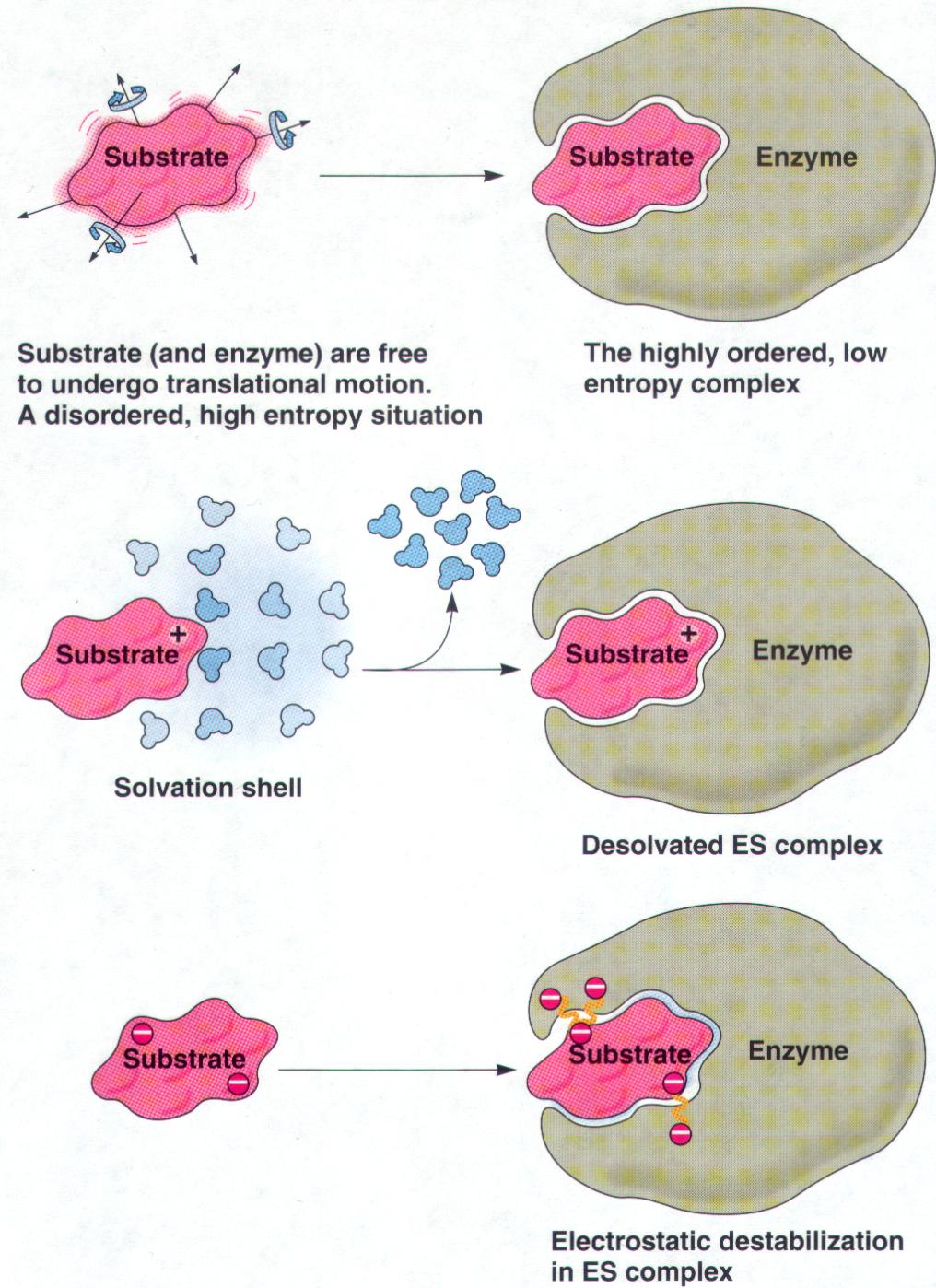
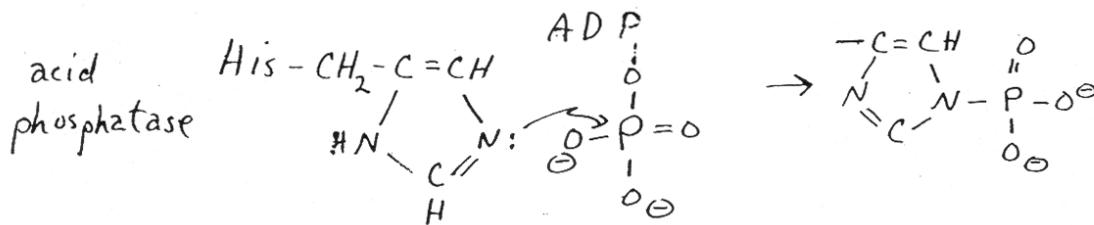
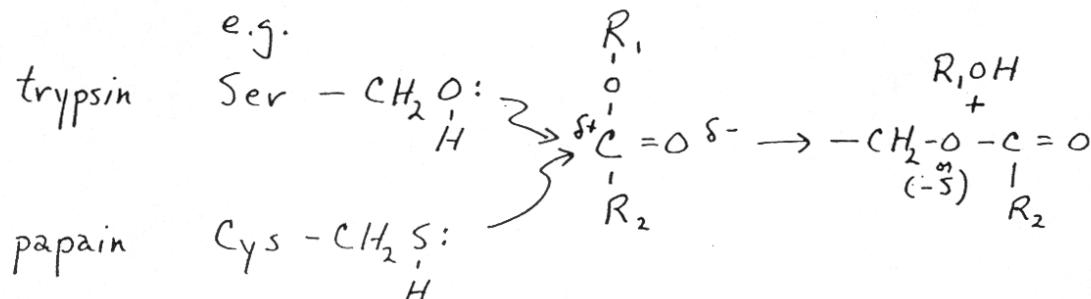


Fig. 14-4

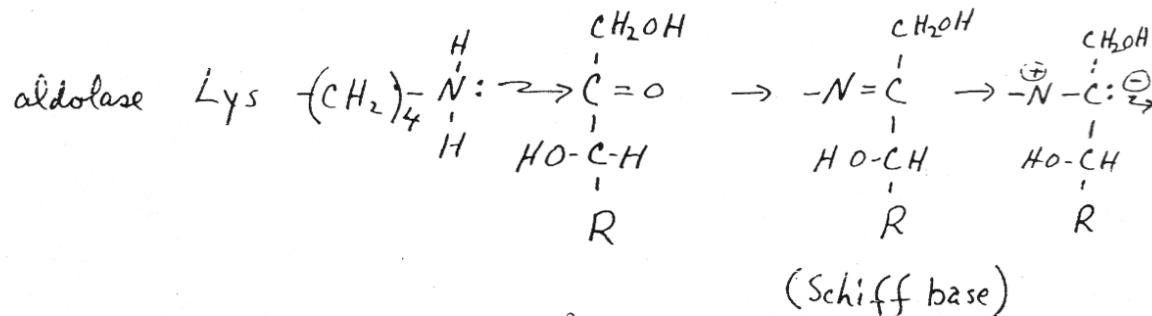
5. Formation of unstable covalent intermediate

- a. with side chains of reactive standard amino acids
- b. with prosthetic groups, i. e., coenzymes: pyridoxol phosphate, thiamine pyrophosphate, etc.

Examples of Unstable Covalent Intermediates with std. amino acids



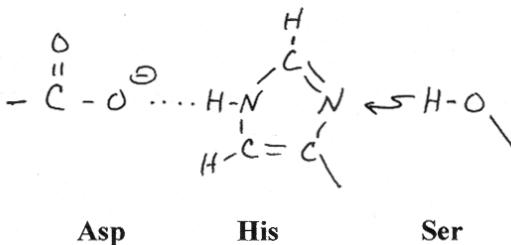
(but alkaline phosphatase has Ser-PO<sub>4</sub> intermediate)



**6. General acid and/or general base catalysis via proton donation or acceptance**

- a. By standard amino acid side chains, typically the acid and conjugate-base forms of Glu, Asp, or His, but possibly others.**

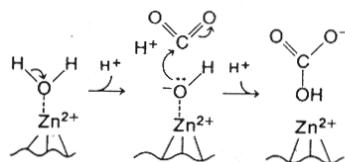
For example, in serine proteases:



This triad was once called a "Charge Relay System" but Asp doesn't accept H<sup>+</sup>. The H<sup>+</sup> transfer stops at His.

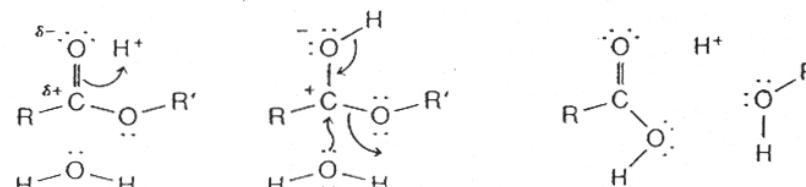
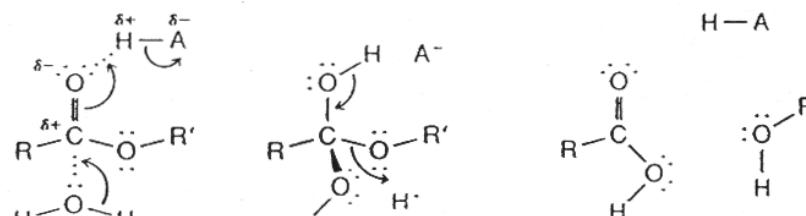
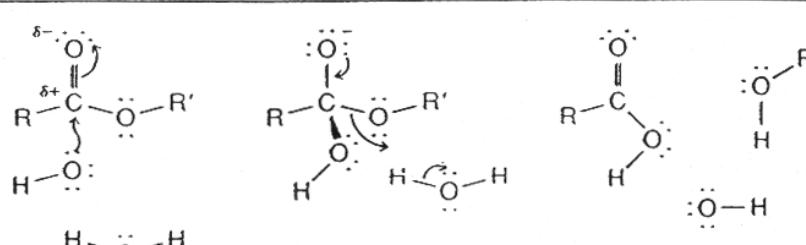
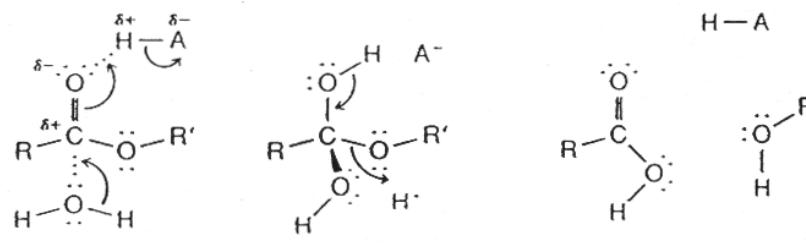
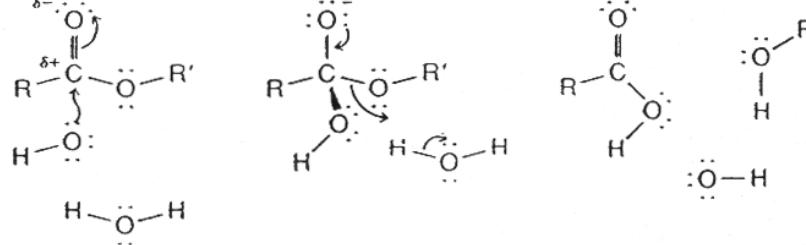
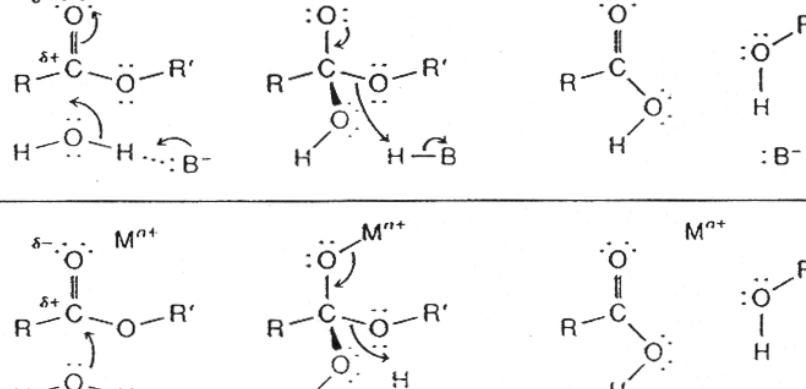
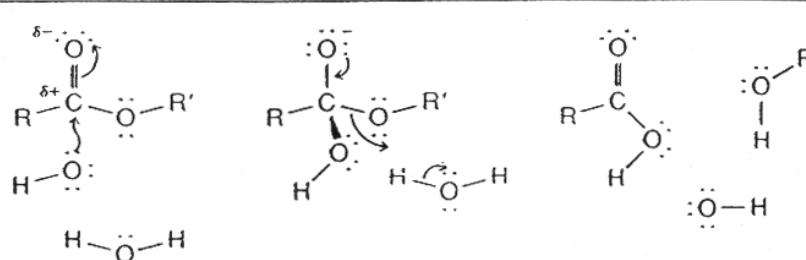
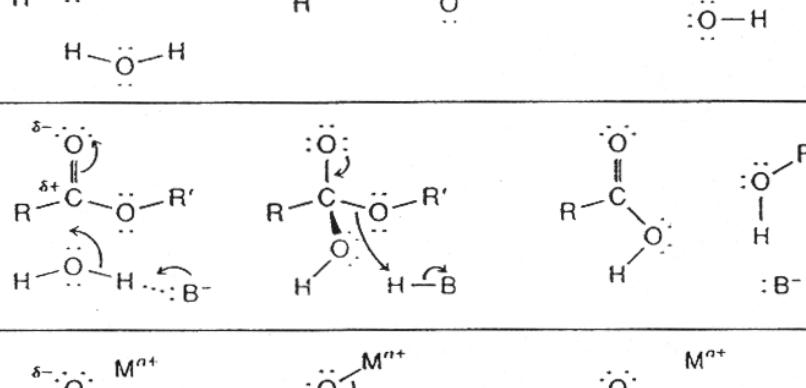
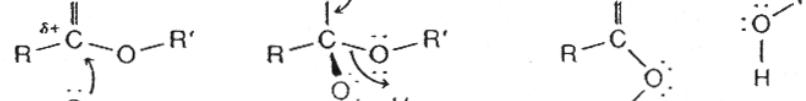
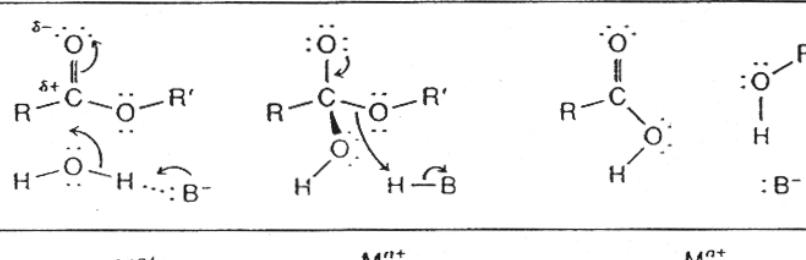
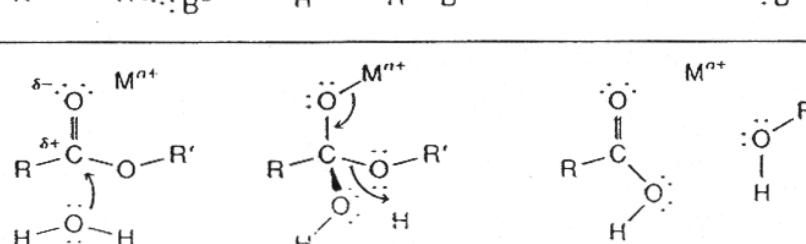
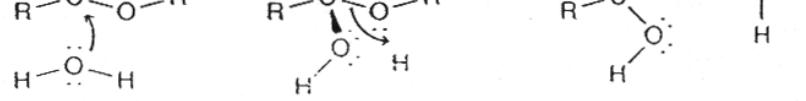
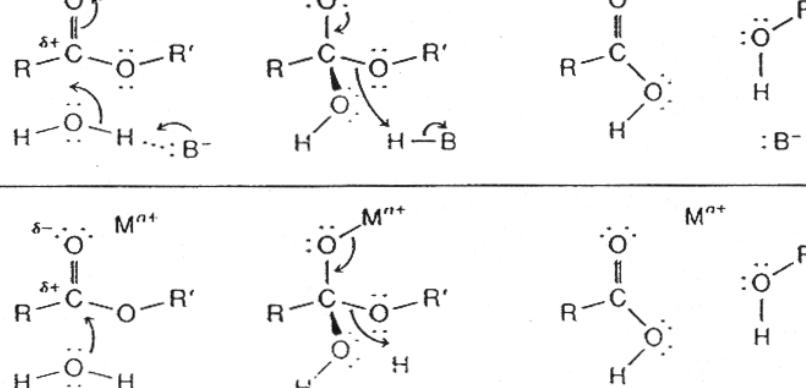
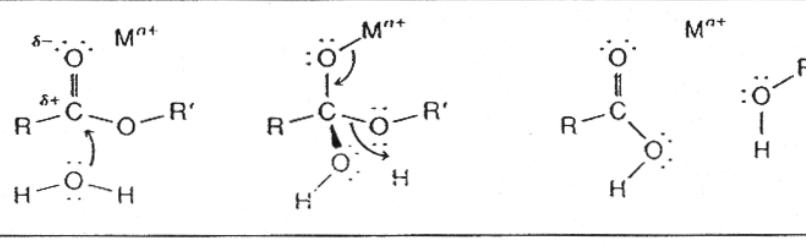
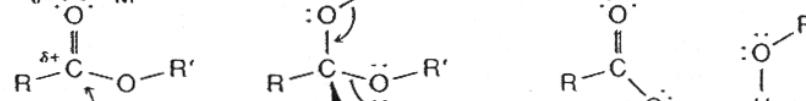
- b. Indirectly via a type of metal ion catalysis, e.g., Zn<sup>++</sup> bound OH<sup>-</sup>**
- Zn<sup>++</sup> often is chelated with 3 side chains (e.g., His) and in the 4th position it can be chelated with H<sub>2</sub>O to generate OH<sup>-</sup> at pH7 to serve as a nucleophile

**In carbonic anhydrase**



**7. Metal ion catalysis**

- a. as above via general base catalysis**
- b. via electronic strain due to ability to polarize bonds as shown on p. 4**

	Reactants	Transition state	Products
(a) Acid catalysis			
(b) General acid catalysis			
(c) Hydroxide catalysis			
(d) General base catalysis			
(e) Metal ion catalysis			

# Shifts in pKa for Residues in Active Sites Create Catalytic Agents



From Creighton, Proteins (1993)

**Table 9.1** Anomalous pK<sub>a</sub> Values of Ionizing Groups in Enzyme Active Sites

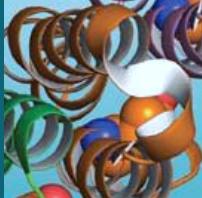
Enzyme	Ionizing group	Observed pK <sub>a</sub>	Normal pK <sub>a</sub> <sup>a</sup>
Acetoacetate decarboxylase <sup>b</sup>	Lys	6.0	10.4–11.1
Carboxypeptidase A <sup>c</sup>	Glu 27	7.0	4.3–4.5
α-Chymotrypsin <sup>d</sup>	α-NH <sub>2</sub>	10.0	6.8–8.0
Lysozyme <sup>e</sup>	Glu 35	6.5	4.3–4.5
Papain <sup>f</sup>	His 159	8.5	6.0–7.0
	Cys 25	3.3	9.0–9.5
Pepsin <sup>g</sup>	Asp 32	1.5	3.9–4.0
Rhodanese <sup>h</sup>	Cys 247	6.5	9.0–9.5

# Low-Barrier Hydrogen Bonds (LBHBs)

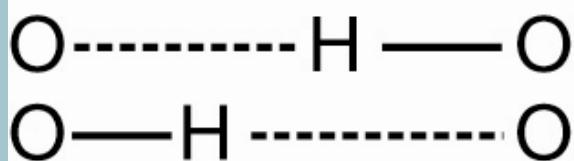


- The typical H-bond strength is 10-30 kJ/mol, and the O-O separation is typically 0.28 nm
- As distance between heteroatoms becomes smaller (<0.25 nm), H bonds become stronger
- Stabilization energies can approach 60 kJ/mol in solution
- $pK_a$  values of the two electronegative atoms must be similar to form an LBHB
- Energy released in forming an LBHB can assist catalysis

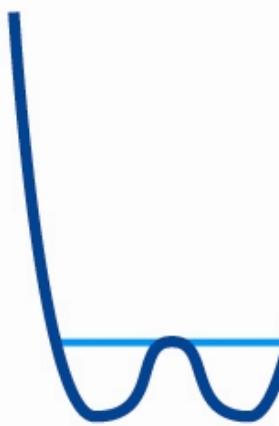
# Low-Barrier Hydrogen Bonds (LBHBs)



(a)



(b)



(c)

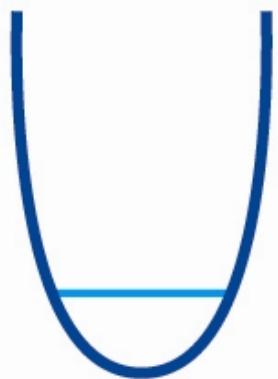
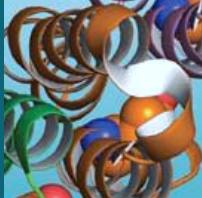


Figure 14.13 Energy diagrams for conventional H bonds (a), and low-barrier hydrogen bonds (b and c). In (c), the O-O distance is 0.23 to 0.24 nm, and bond order for each O-H interaction is 0.5.

# How Do Active-Site Residues Interact to Support Catalysis?



- About half of the amino acids in an active site engage directly in catalytic effects in enzyme active sites
- Other residues may function in secondary roles in the active site:
  - Raising or lowering catalytic residue  $pK_a$  values
  - Orienting catalytic residues
  - Stabilizing charge
  - Transferring protons via hydrogen tunneling (in LBHB)

# The Serine Proteases: *trypsin, chymotrypsin, elastase, thrombin, subtilisin, plasmin, TPA*



- Serine proteases are homologous, but locations of the three crucial residues (the triad Asp, His, Ser) differ somewhat
- Enzymologists agree, however, to number them always as His-57, Asp-102, Ser-195
- **The Mechanism: A mixture of covalent and general acid-base catalysis**
- Asp-102 functions to orient His-57 via charge interaction
- His-57 acts as a general acid and base
- Ser-195 forms a covalent bond with peptide to be cleaved
- Covalent bond formation turns a trigonal C into a tetrahedral C
- The tetrahedral oxyanion intermediate is stabilized by N-Hs of Gly-193 and Ser-195

# Serine Protease Binding Pockets are Adapted to Particular Substrates

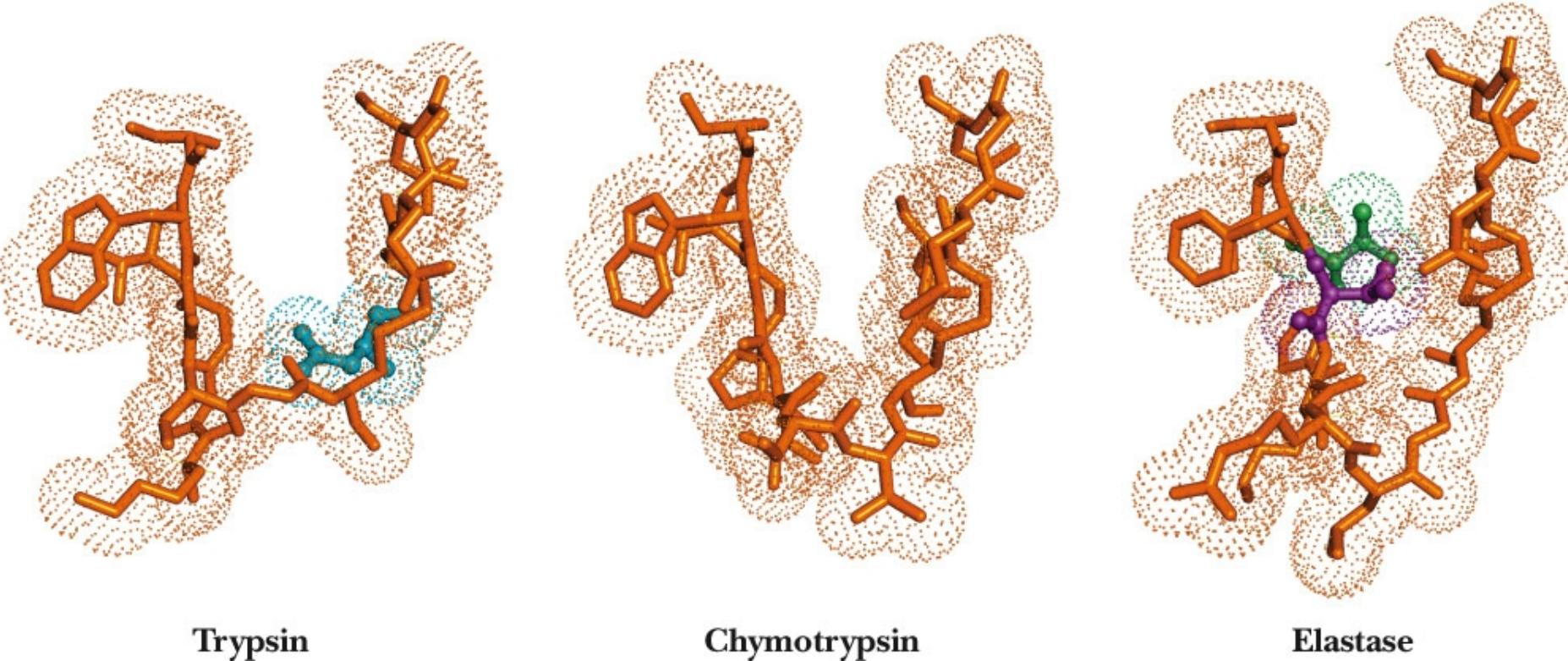
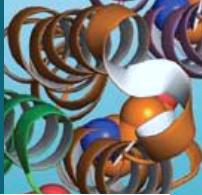
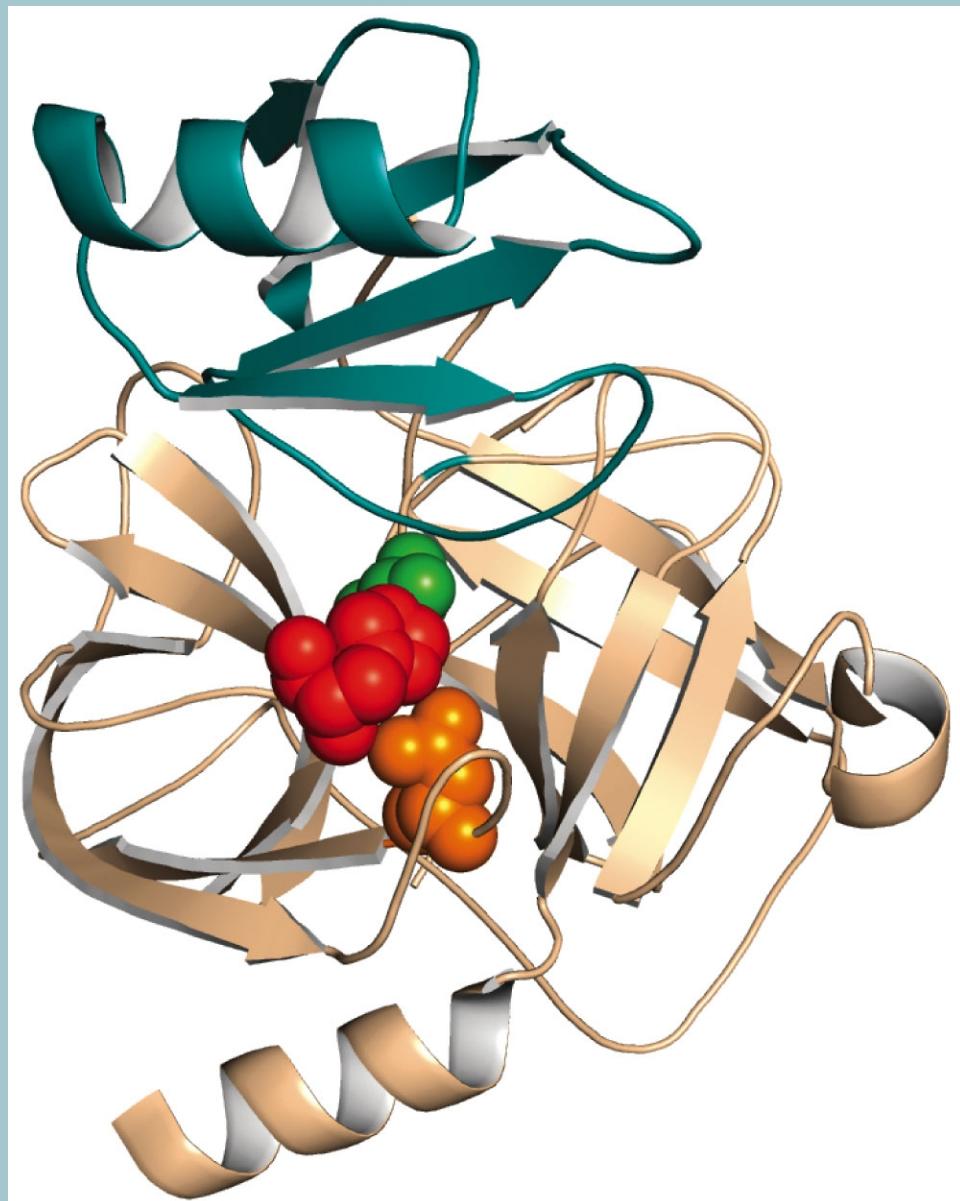


Figure 14.18 The substrate-binding pockets of trypsin, chymotrypsin, and elastase. Asp<sup>189</sup> (aqua) coordinates Arg and Lys residues of substrates in the trypsin pocket. Val<sup>216</sup> (purple) and Thr<sup>226</sup> (green) make the elastase pocket shallow and able to accommodate only small, nonbulky residues. The chymotrypsin pocket is hydrophobic.

# The Catalytic Triad of the Serine Proteases



Figure 14.16 Structure of chymotrypsin (white) in a complex with eglin C (blue ribbon structure), a target substrate. His<sup>57</sup> (red) is flanked by Asp<sup>102</sup> (gold) and Ser<sup>195</sup> (green). The catalytic site is filled by a peptide segment of eglin. Note how close Ser<sup>195</sup> is to the peptide that would be cleaved in the reaction.



# The Catalytic Triad of the Serine Proteases

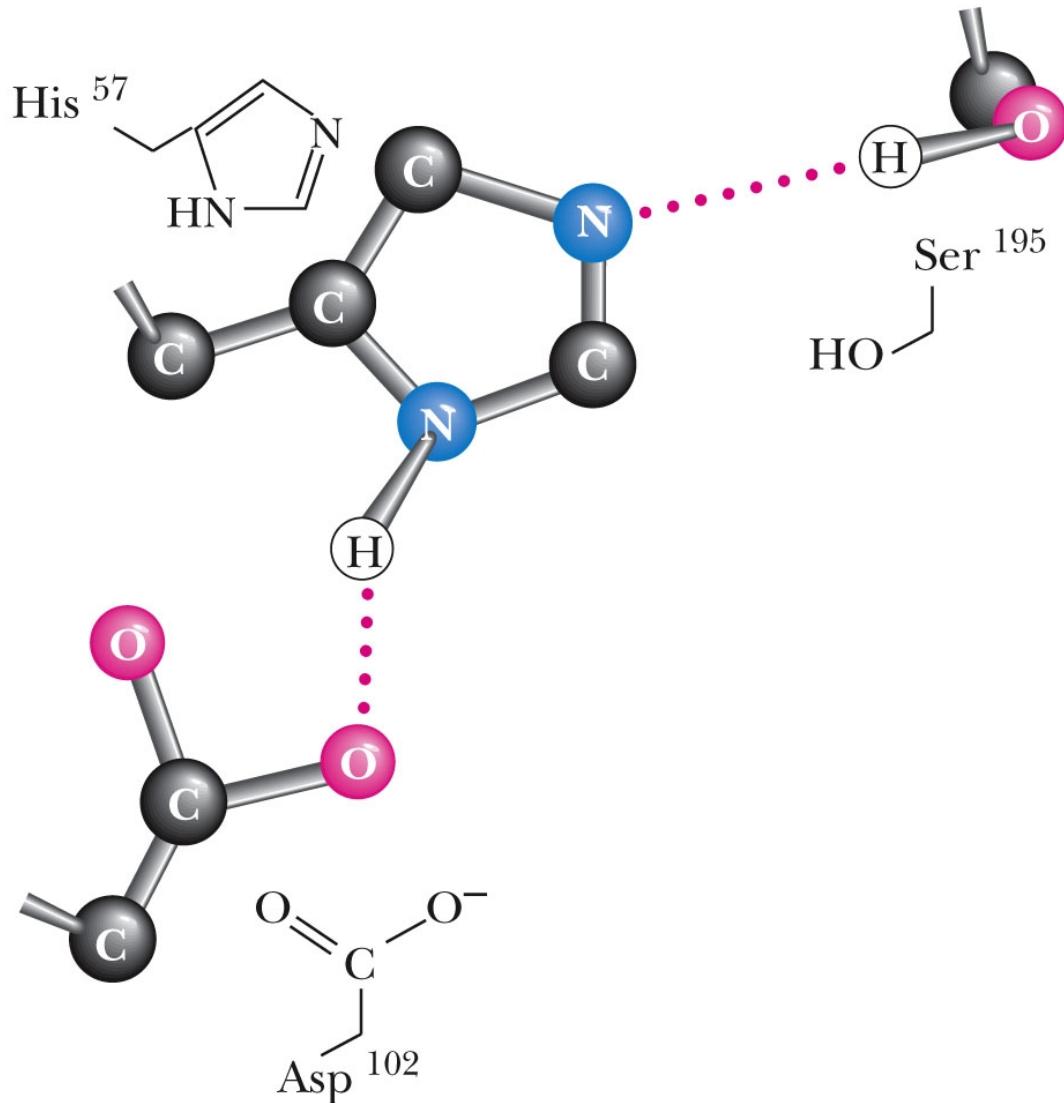
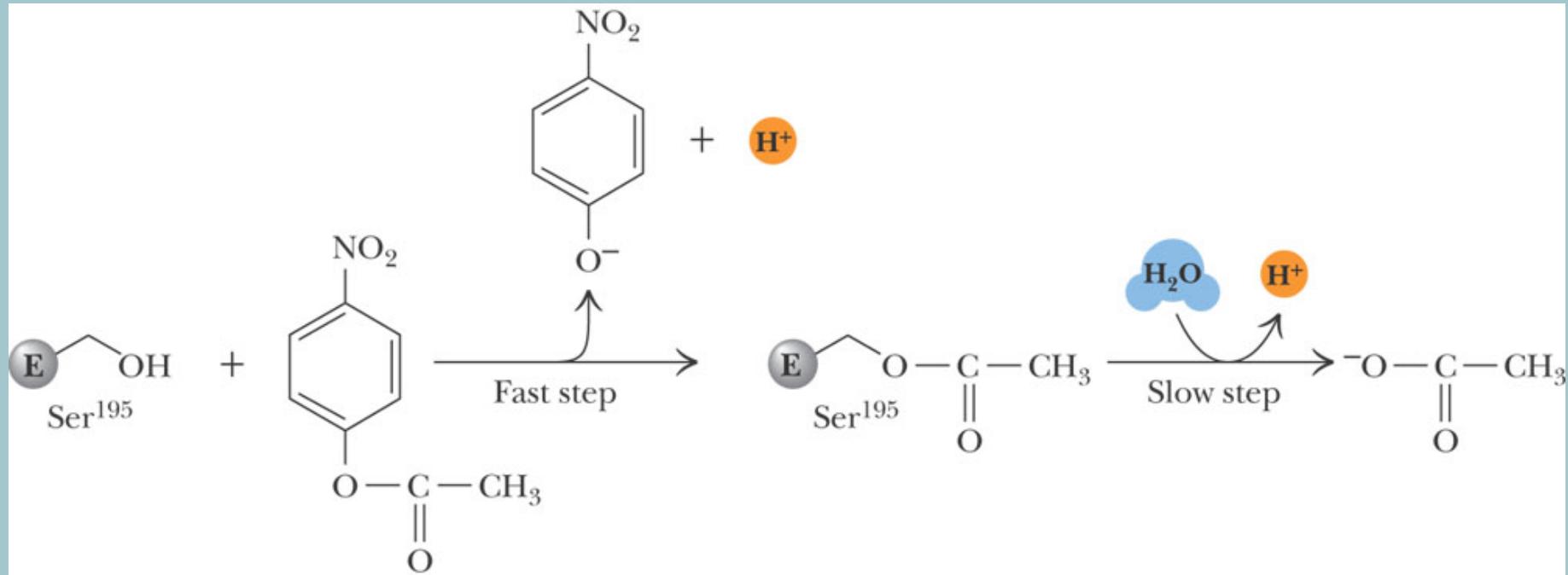


Figure 14.17 The catalytic triad at the active site of chymotrypsin (and the other serine proteases).

Figure 14.20 Cleavage of a peptide bond by a serine protease involves two steps:

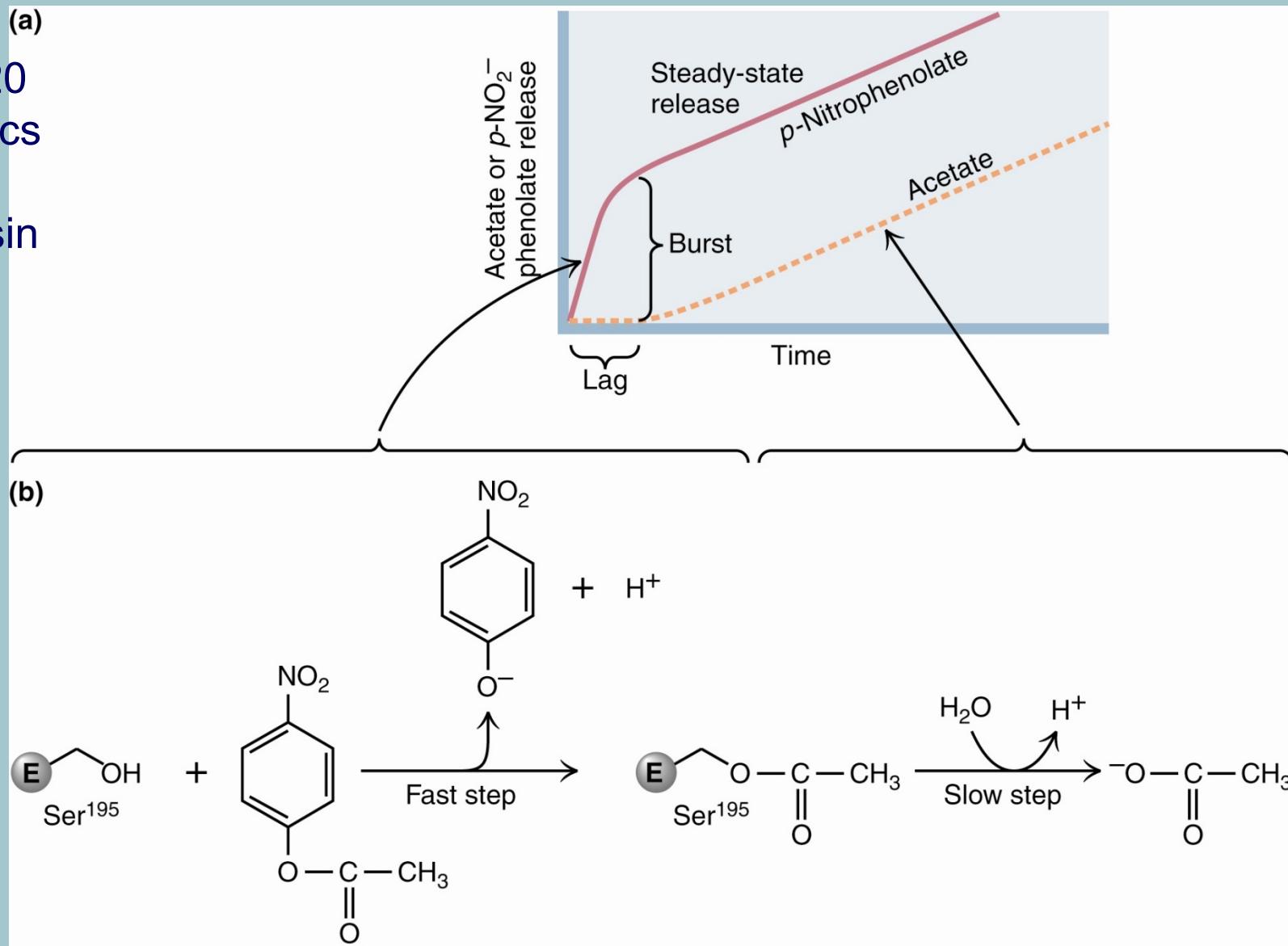
- 1) Rapid formation of the acyl-enzyme intermediate with release of the first product followed by
- 2) Slow release of the second product by hydrolysis of the acyl-enzyme intermediate.



# Serine Proteases Display Burst Kinetics



Figure 14.20  
Burst kinetics  
in the  
chymotrypsin  
reaction.



**Figure 14.21**  
**A detailed mechanism for the chymotrypsin reaction. Note the low-barrier hydrogen bond (LBHB) in (c) and (g).**

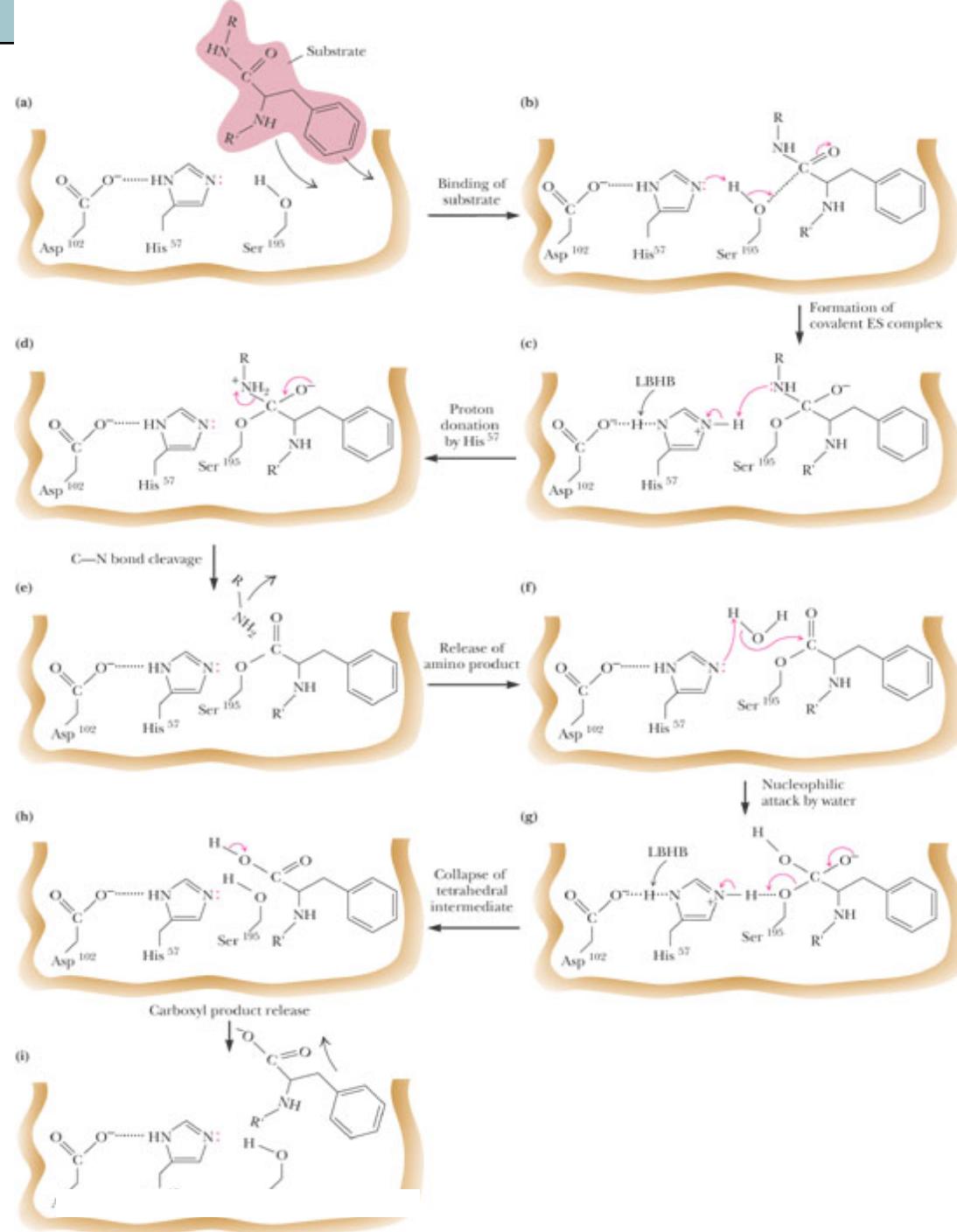
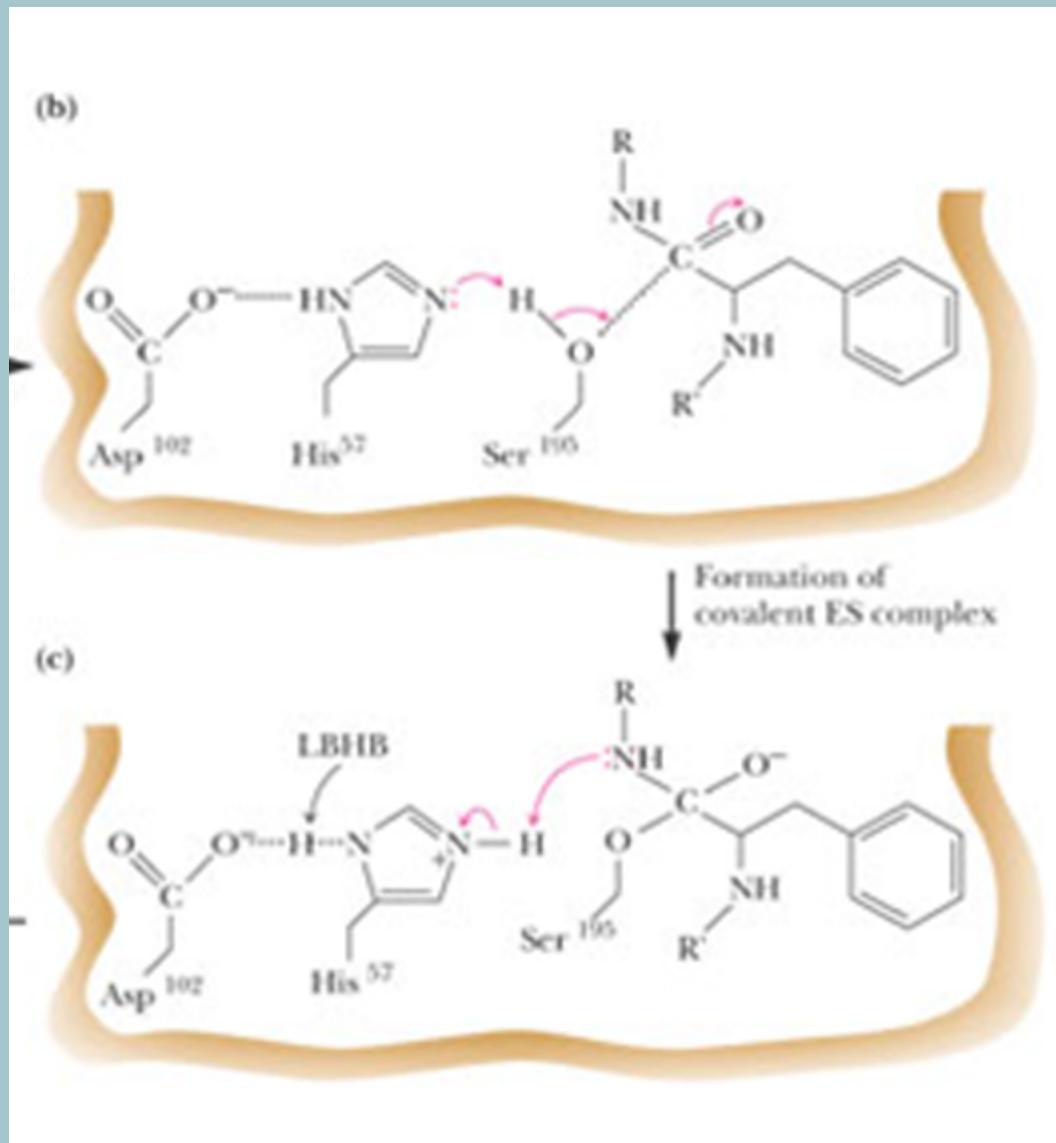


Figure 14.21  
A detailed mechanism for the chymotrypsin reaction. Note the low-barrier hydrogen bond (LBHB) in (c) and (g).



# Aspartic proteases play many roles in humans



**TABLE 14.3**

Some Representative Aspartic Proteases

Name	Source	Function
Pepsin*	Stomach	Digestion of dietary protein
Chymosin†	Stomach	Digestion of dietary protein
Cathepsin D	Spleen, liver, and many other animal tissues	Lysosomal digestion of proteins
Renin‡	Kidney	Conversion of angiotensinogen to angiotensin I; regulation of blood pressure
HIV-protease§	AIDS virus	Processing of AIDS virus proteins

# Aspartic Protease Mechanism



- All involve two Asp residues at the active site
- These two Asp residues work together as general acid-base catalysts
- Molecular dynamics simulations indicate that aspartic proteases employ low-barrier hydrogen bonds (LBHBs) in their mechanism

# A Mechanism for the Aspartic Proteases

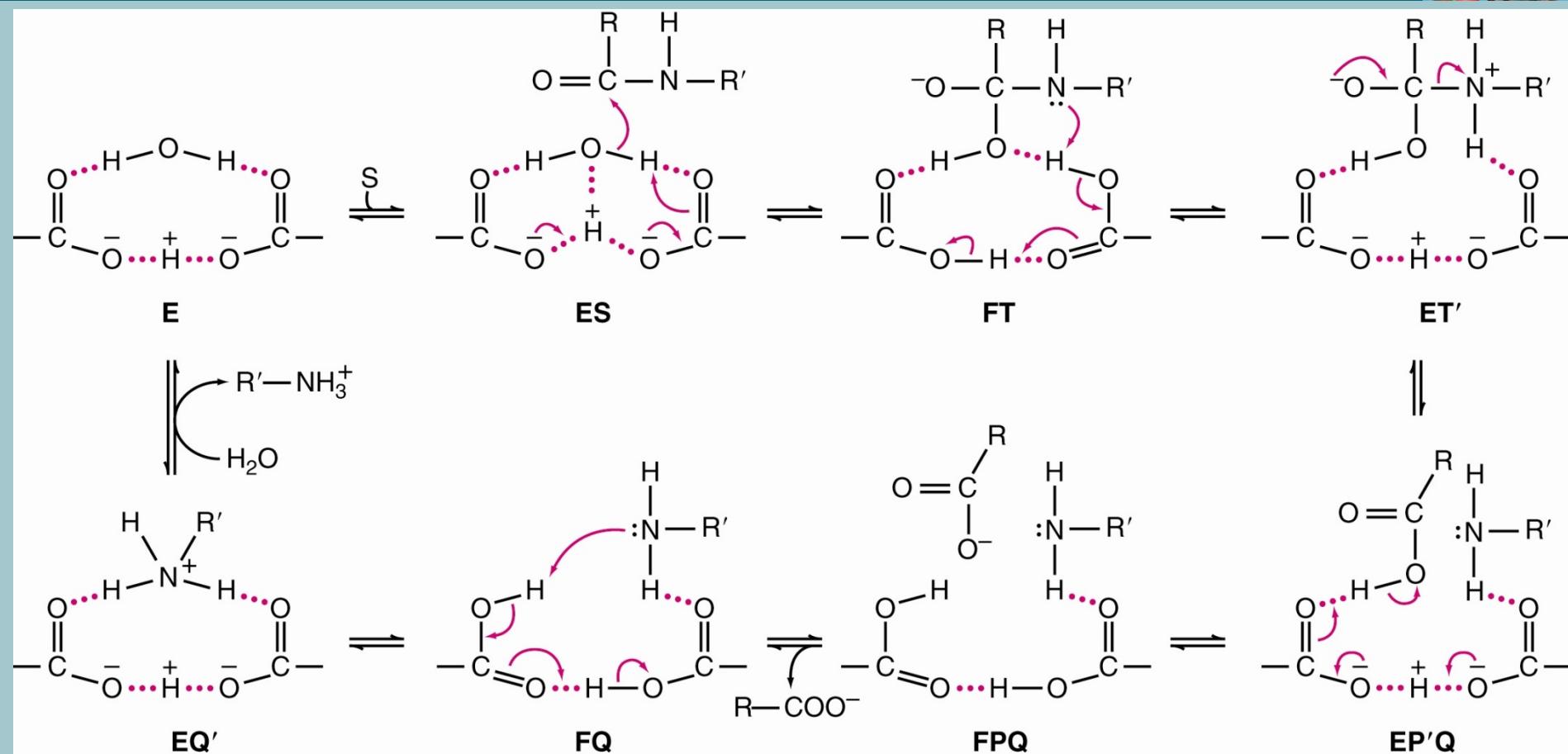


Figure 14.24 Mechanism for the aspartic proteases. LBHBs play a role in states E, ES, ET', EQ', and EP'Q.

# Proteolytic cleavage pattern for the HIV genome

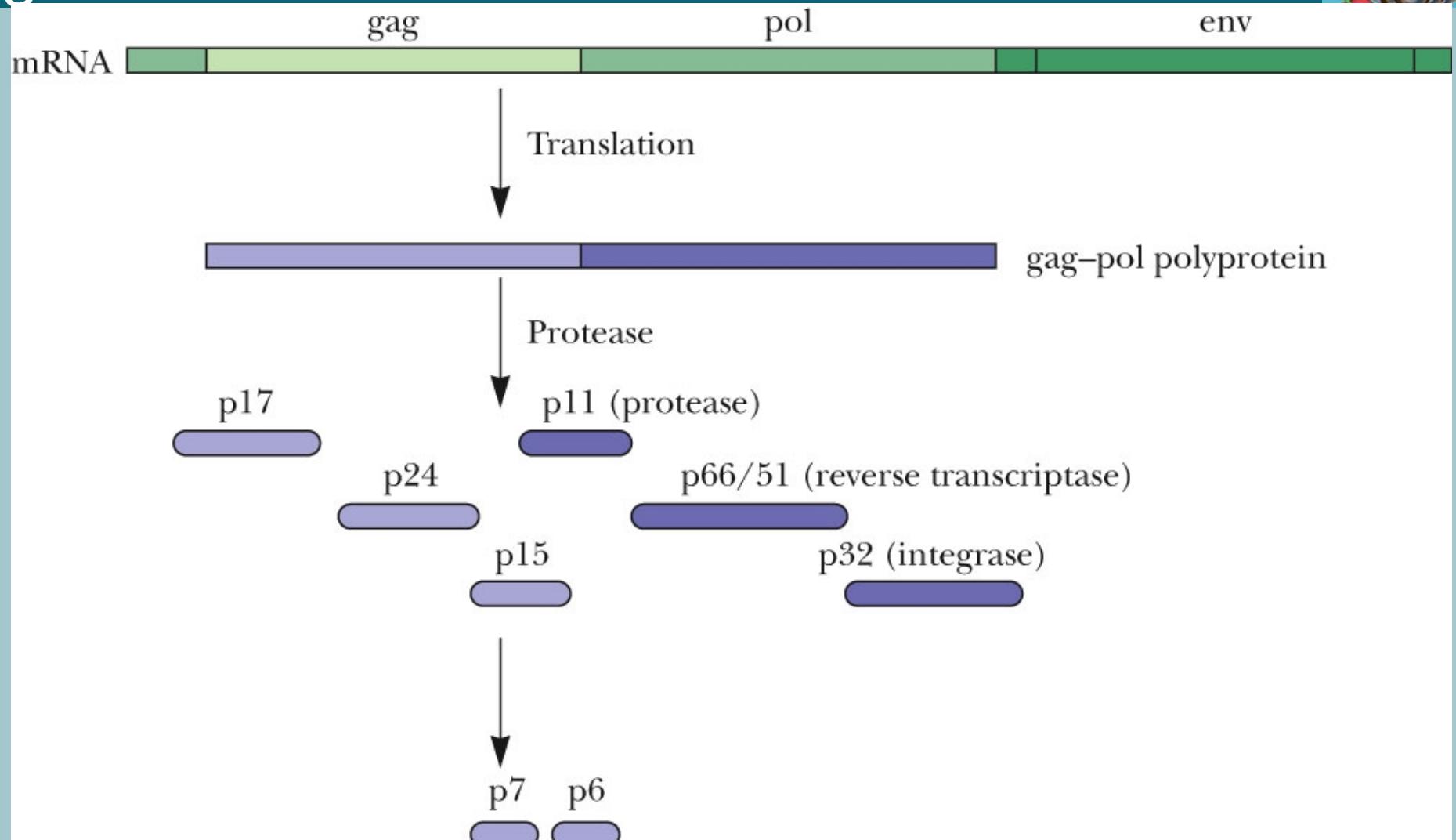


Figure 14.26 HIV mRNA provides the genetic information for synthesis of a polyprotein. Cleavage yields the active products.

# Protease Inhibitors Block the Active Site of HIV-1 Protease

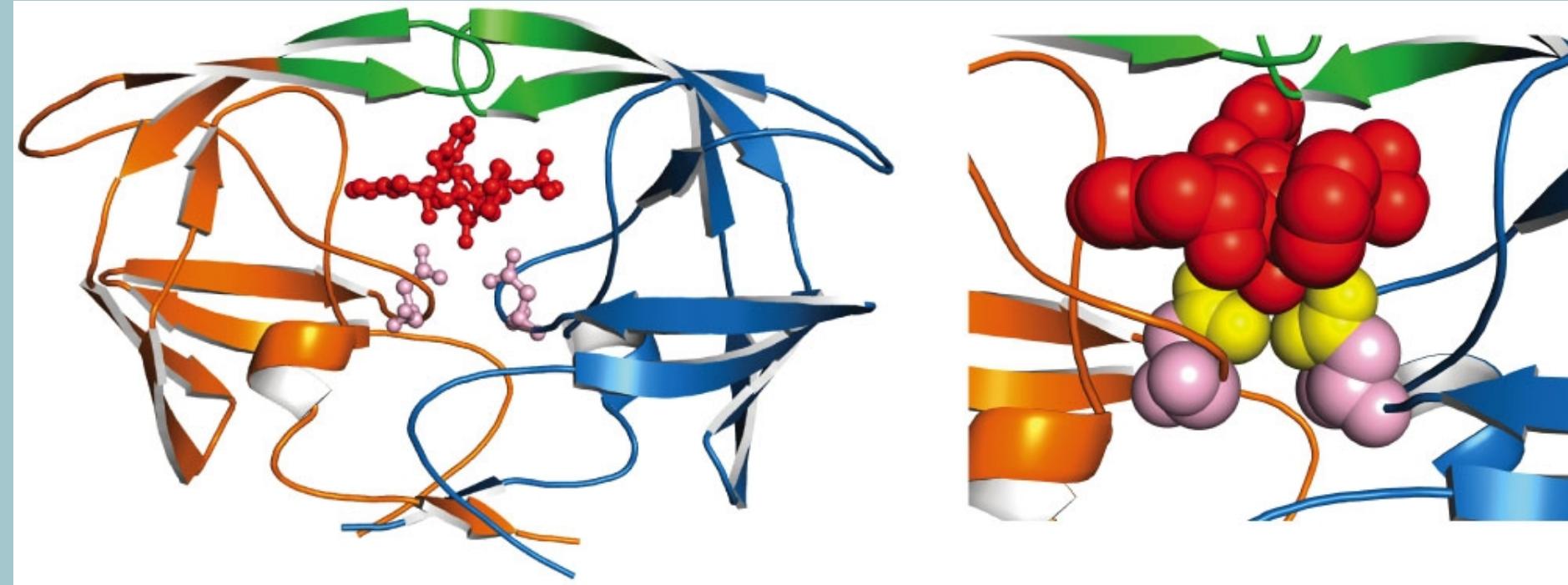
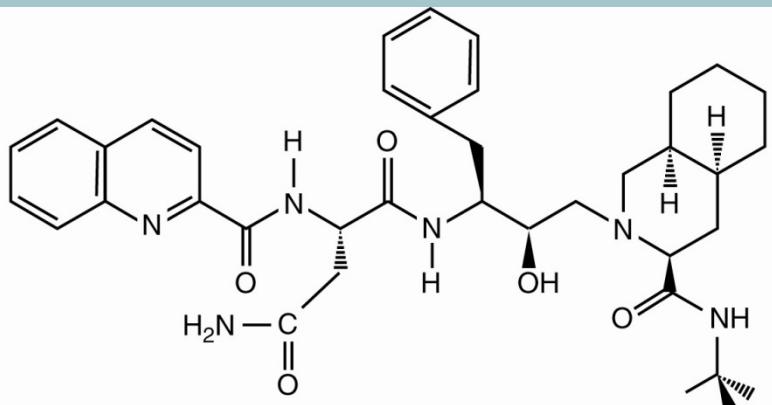
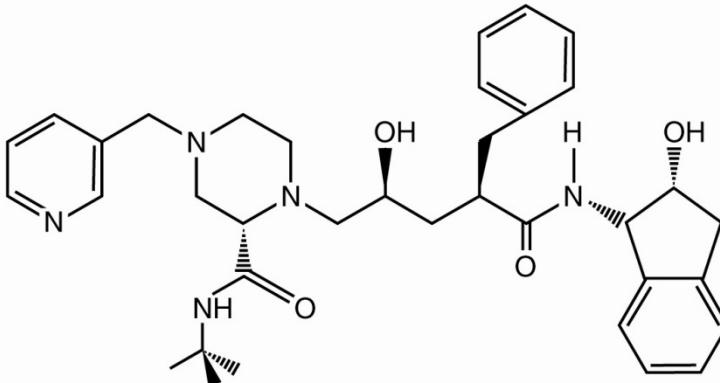


Figure 14.27 HIV-1 protease complexed with the inhibitor Crixivan (red) made by Merck. The “flaps” that cover the active site are green; the catalytic active site Asp residues are violet.

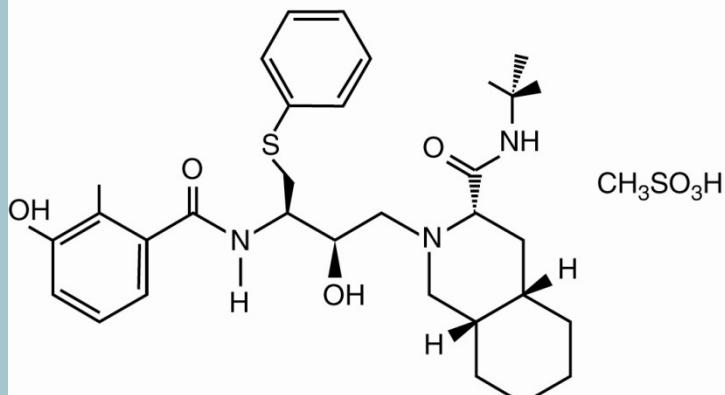
# Protease Inhibitors Give Life to AIDS Patients



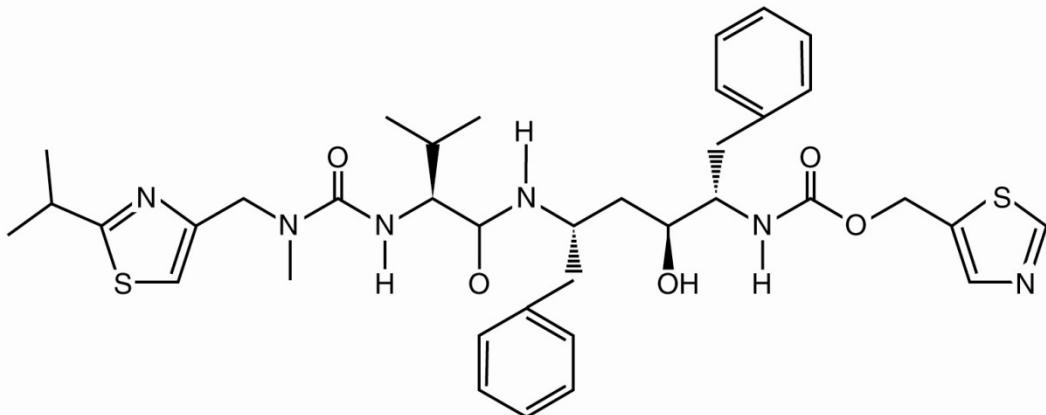
### **Invirase (saquinavir)**



### Crixivan (indinavir)



#### **Viracept (nelfinavir mesylate)**



#### Norvir (ritonavir)

G & G, p.504

# Protease inhibitor drugs used by AIDS Patients