CHAPTER 9

CATALYTIC STRATEGIES USED BY ENZYMES

1. Covalent catalysis
   (reactive group in active site)

2. General acid-base catalysis
   (proton donor or acceptor)

3. Metal ion catalysis
   (metal ion involved in catalysis)

4. Catalysis by approximation
   (two distinct substrates bind to active site)

CLASSES OF PROTEASES

1. Serine proteases (e.g. chymotrypsin)

2. Cysteine (or thiol or sulfhydryl) proteases
   (e.g. papain)

3. Aspartyl (or carboxyl or acid) proteases
   (e.g. pepsin)

4. Metalloproteases (or zinc proteases)
   (e.g. carboxypeptidase A)

TABLE 14-4. A SELECTION OF SERINE PROTEASES

Enzyme	Source	Function
Trypsin	Pancreas	Digestion of proteins
Chymotrypsin	Pancreas	Digestion of proteins
Elastase	Pancreas	Digestion of proteins
Thrombin	Vertebrate serum	Blood clotting
Plasmin	Vertebrate serum	Dissolution of blood clots
Kallikrein	Blood and tissues	Control of blood flow
Complement C1	Serum	Cell lysis in the immune response
Acrosomal protease	Sperm acrosome	Penetration of ovum
Lysosomal protease	Animal cells	Cell protein turnover
Cocoonase	Moth larvae	Dissolution of cocoon after metamorphosis
α-Lytic protease	*Bacillus sorangium*	Possibly digestion
Proteases A and B	*Streptomyces griseus*	Possibly digestion
Subtilisin	*Bacillus subtilus*	Possibly digestion

Source: Stroud, R.M., *Sci. Am.* **231**(1): 86 (1974).

CHYMOTRYPSIN

Main features:

- Mammalian digestive enzyme
- It hydrolyzes peptide bonds on the carboxyl side of aromatic and hydrophobic residues, such as methionine, and ester bonds
- Member of the serine protease family
- 25 kDa protein
- It consists of three polypeptide chains connected by two interchain disulfide bonds
- Dimensions: 51 x 40 x 40 Å
- Secondary structure: mainly antiparallel β- pleated sheet, little α helix

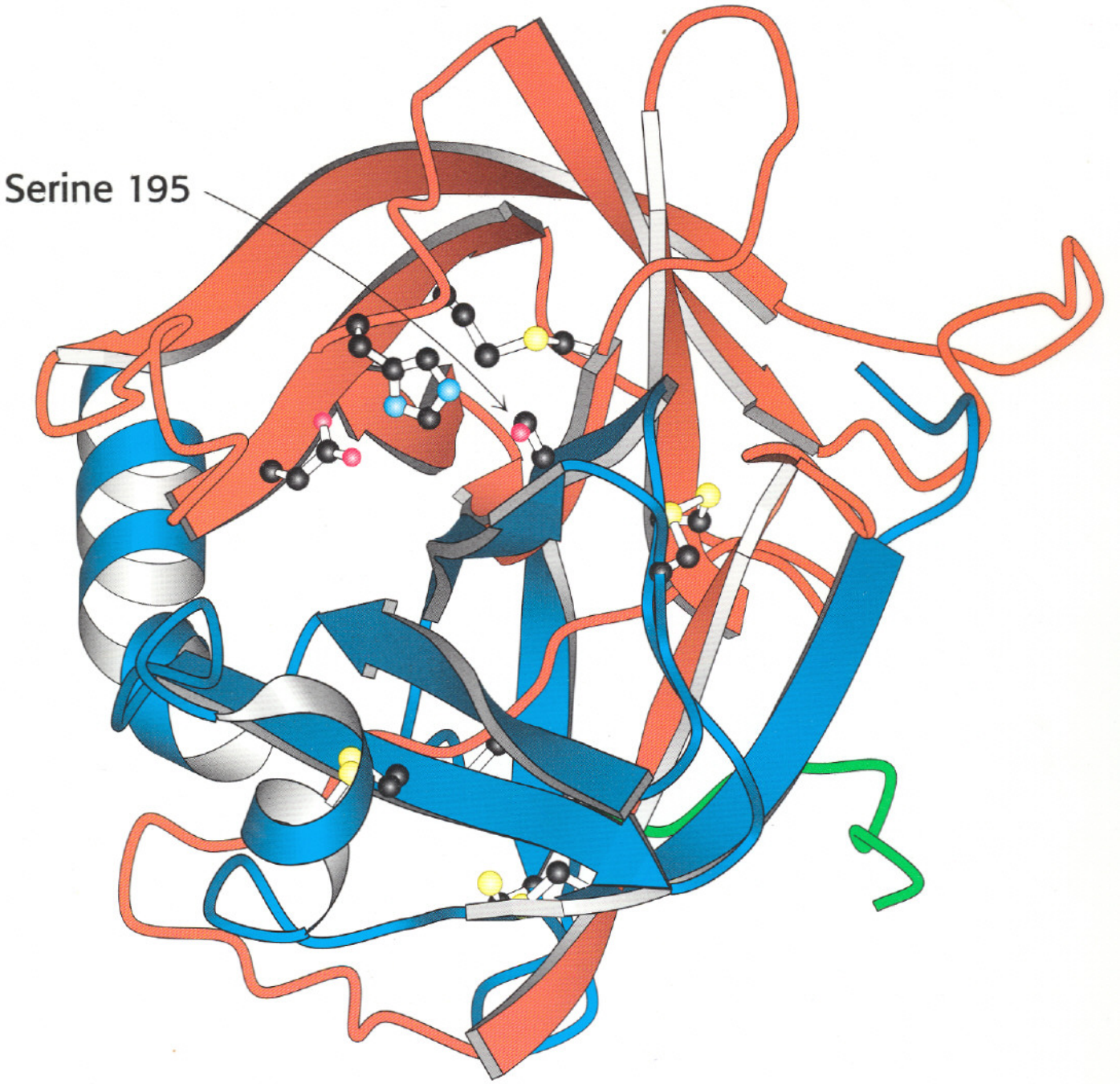


Figure 9-6
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.

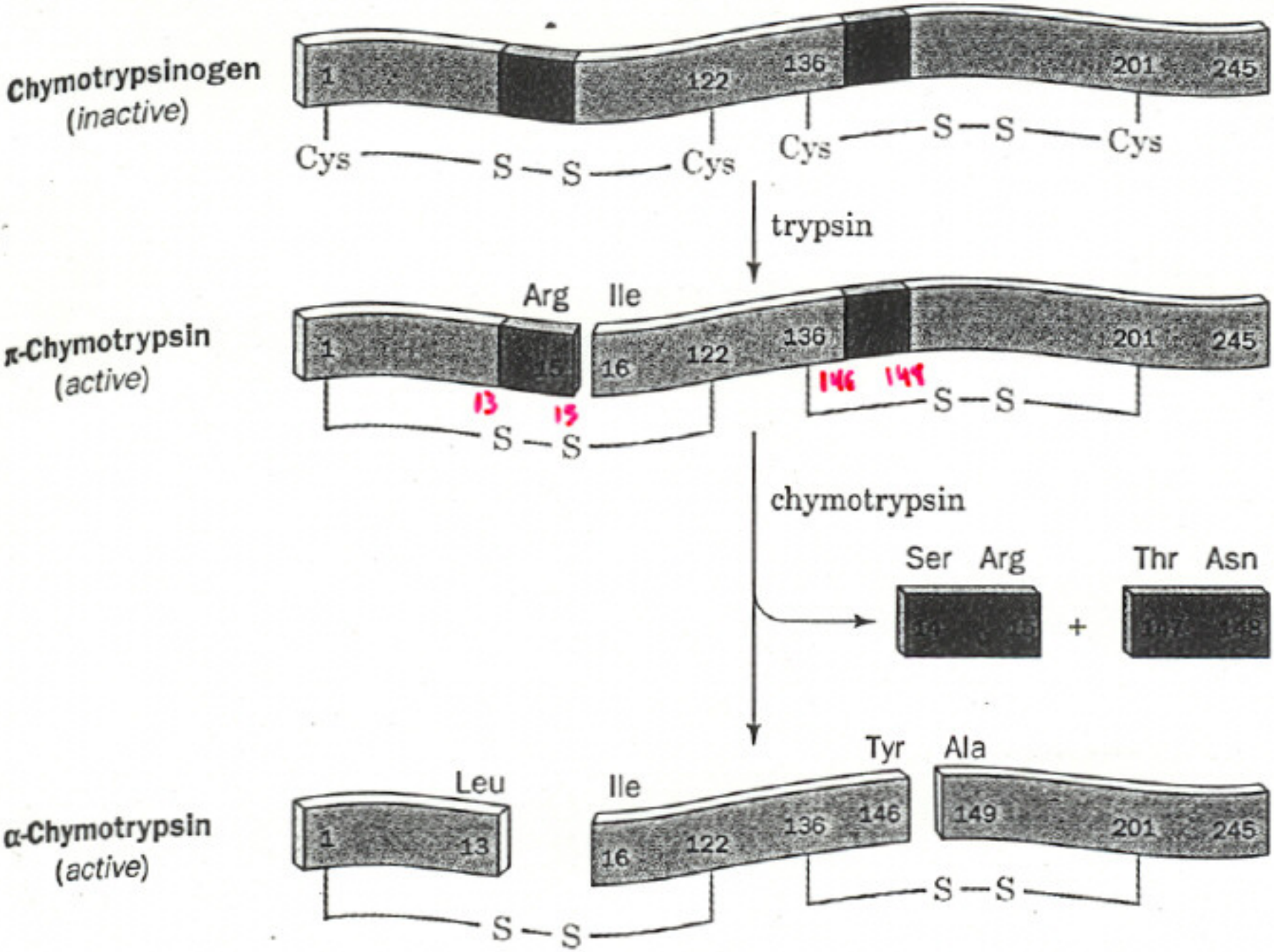
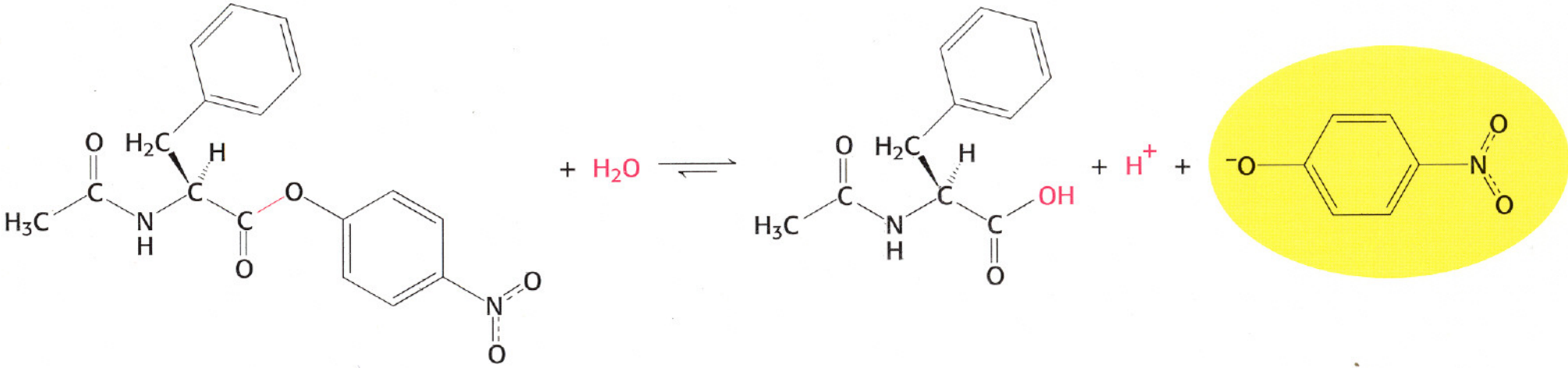


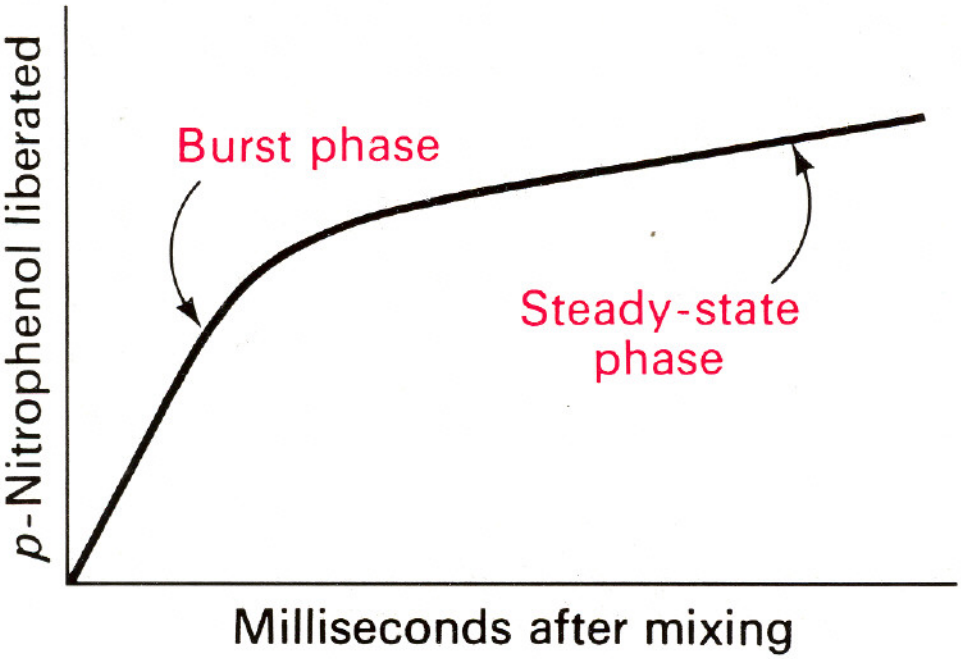
Figure 11-30. **The activation of chymotrypsinogen by proteolytic cleavage.** Both π- and α-chymotrypsin are enzymatically active. ● See Kinemage Exercise 10-4.

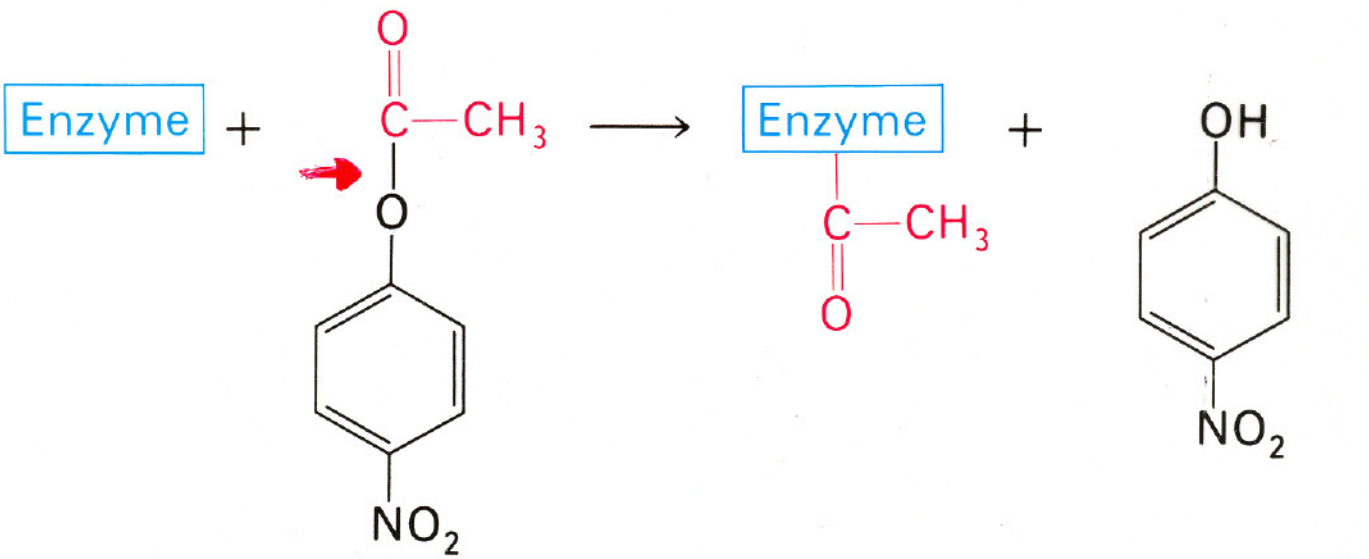


***N*-Acetyl-L-phenylalanine *p*-nitrophenyl ester**

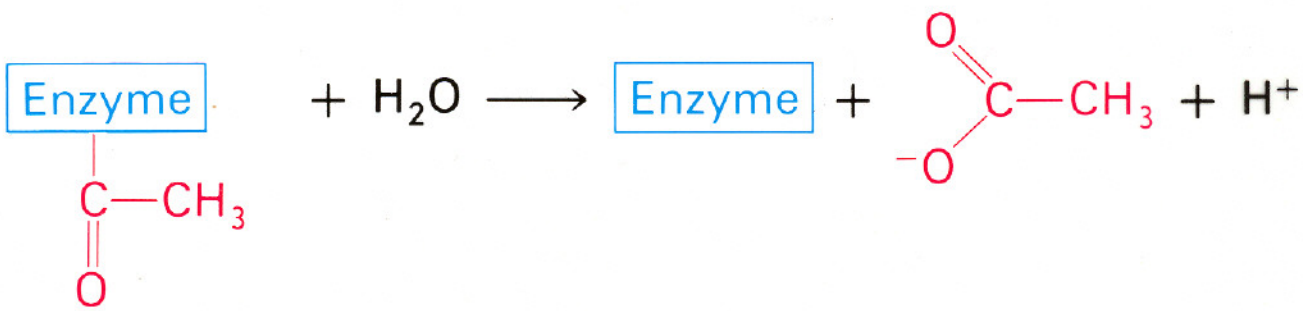
***p*-Nitrophenolate**

Figure 9-3
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.





p-Nitrophenyl acetate | **Acetyl-enzyme intermediate** | **p-Nitrophenol**



Acetyl-enzyme intermediate | **Acetate**

Figures 9-29, 9-30, and 9-31, page 223

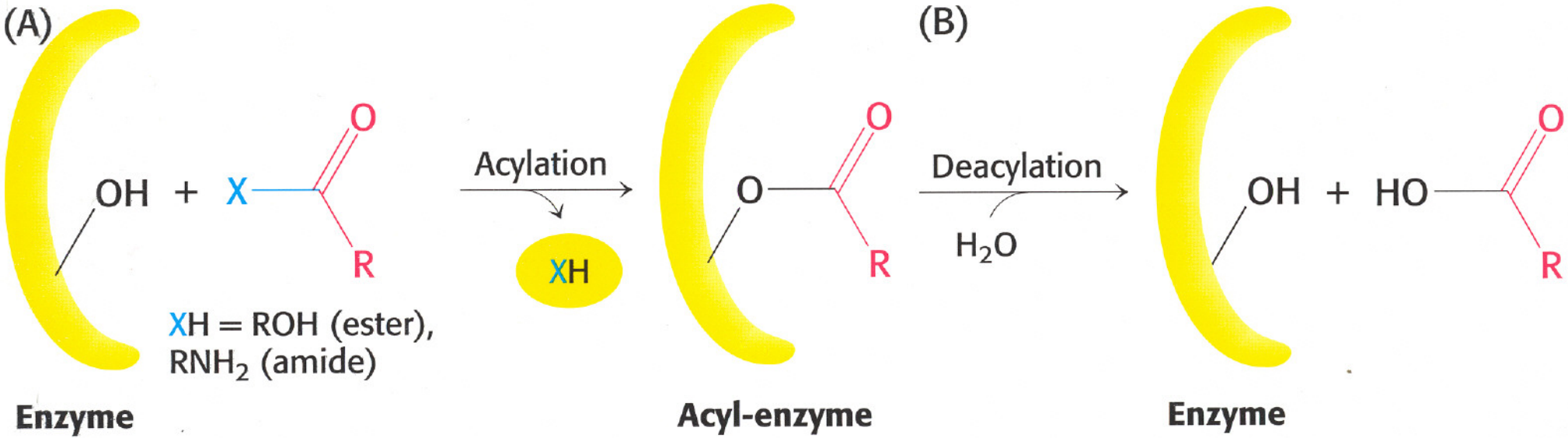


Figure 9-5
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.

- It is synthesized as a single- chain inactive precursor called chymotrypsinogen

Catalytic triad:

Serine 195, Histidine 57, Aspartate 102

Steps of the Catalytic Mechanism:

1. Hydrolysis of the peptide bond starts with an attack by the oxygen atom of the hydroxyl group of Ser-195 on the carbonyl carbon atom of the susceptible peptide bond

2. Formation of a transient tetrahedral intermediate

3. Transfer of a proton from Ser- 195 to His- 57

4. Deacylation

Acylation stage

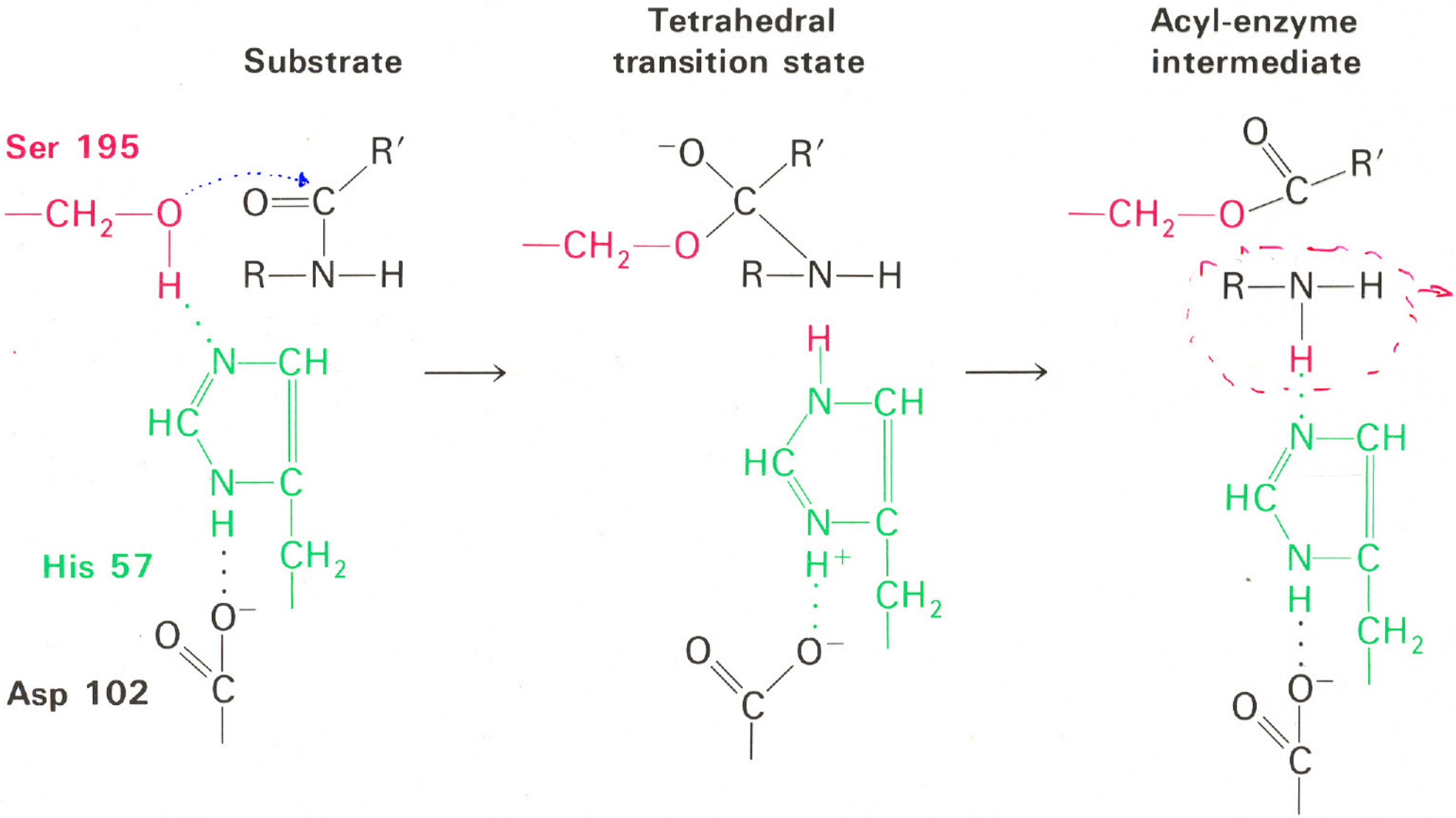
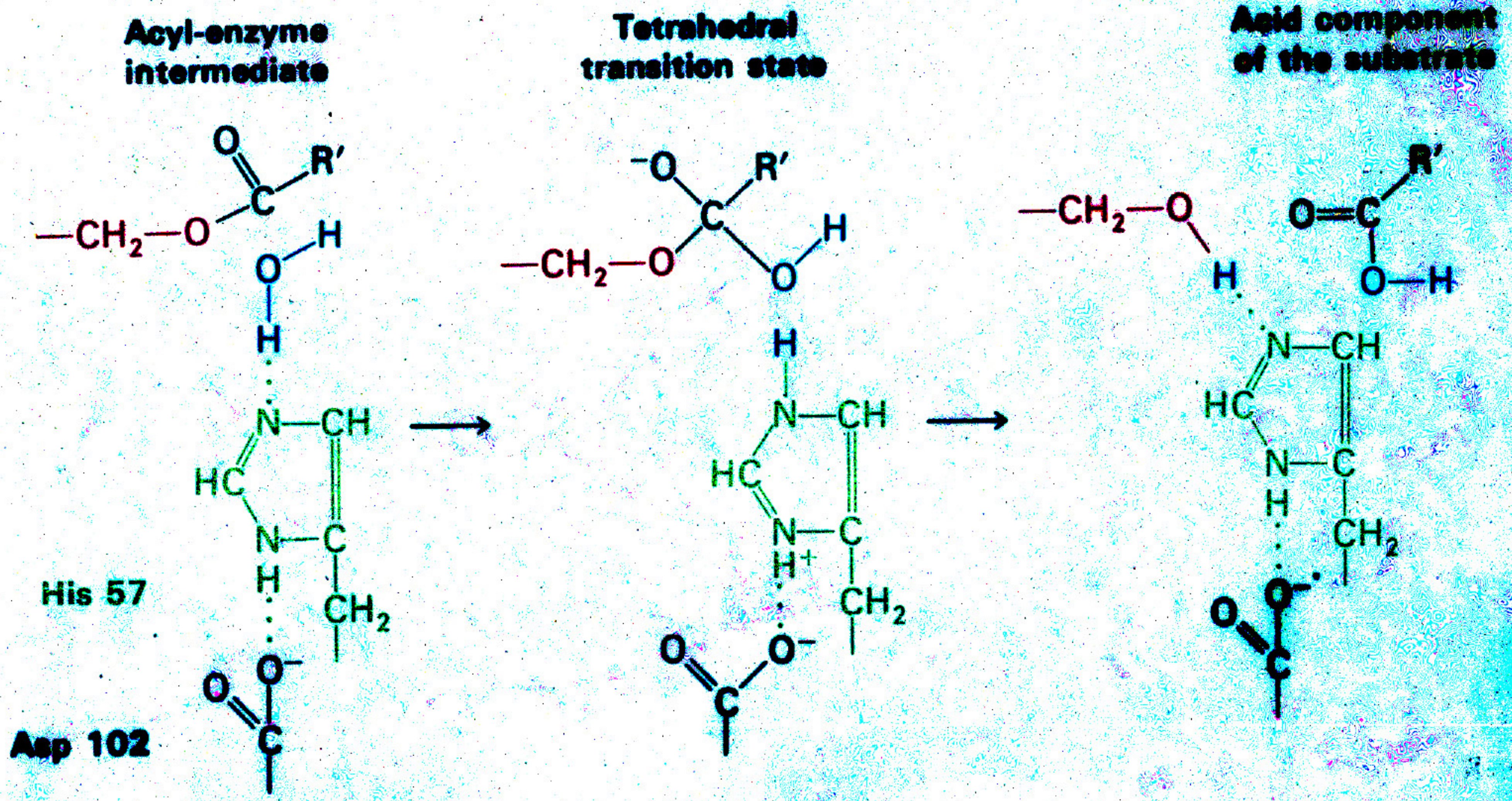


Figure 9-37, page 226

deacylation stage



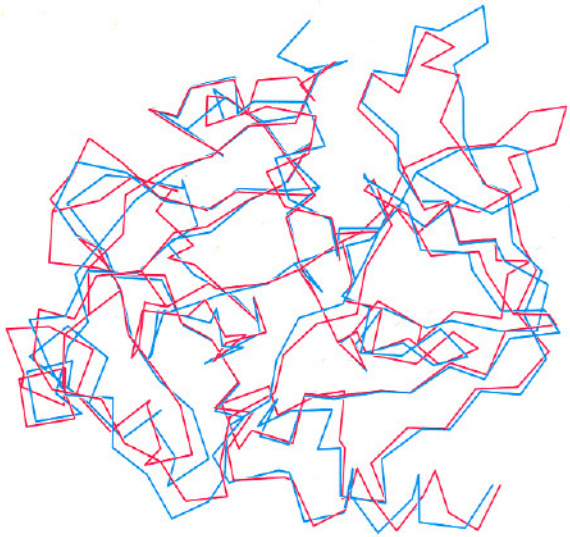
T-34

Set I

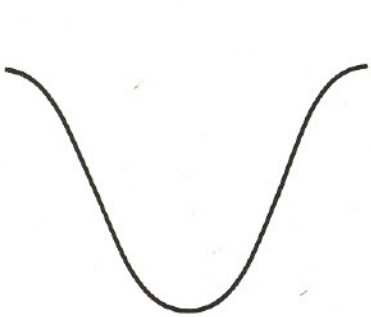
Trypsin & Elastase:

Trypsin and elastase are like chymotrypsin in many respects:

- About 40% of the amino acid sequences of these three enzymes are identical
- Their 3- D structures are very similar
- A serine- histidine- aspartate catalytic triad is present in all three
- The serine residue of the catalytic triad is modified by fluorophosphates
- The amino acid sequence around this serine is the same in all three enzymes: Gly- Asp- Ser- Gly- Gly- Pro
- All three enzymes have identical catalytic mechanisms

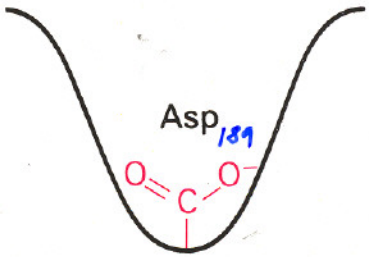


- chymotrypsin
- elastase



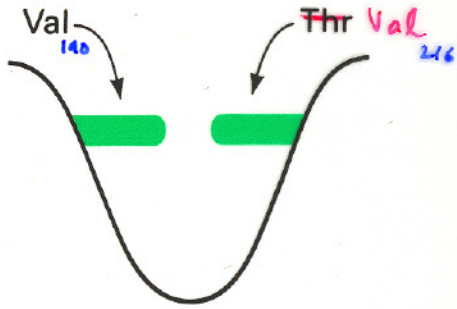
Chymotrypsin

Specificity: Phe, Trp, Tyr
Met, His, Asn, Leu



Trypsin

(Ser 189 → Asp)
Specificity:
Lys, Arg



Elastase

(Gly → Val
Gly → [redacted] Val)
Specificity:
Ala, Gly, Ser,
Val

Figures 9-39 and 9-40, page 227

Stryer: *Biochemistry,* Fourth Edition

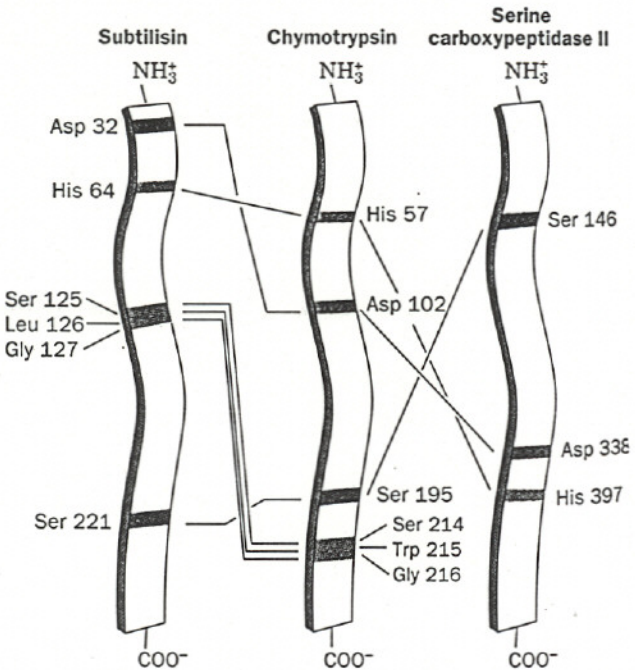


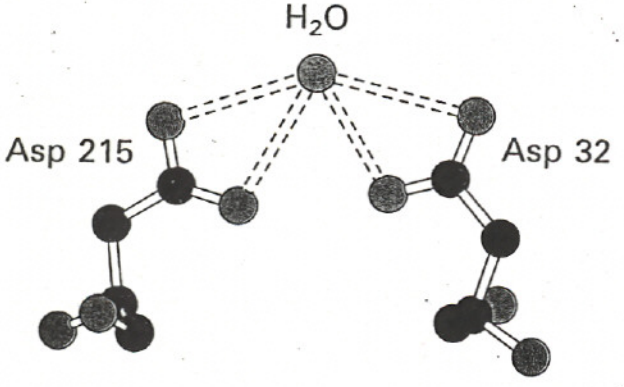
FIGURE 14-22. A diagram indicating the relative positions of the active site residues in the primary structures of subtilisin (*left*), chymotrypsin (*middle*), and serine carboxypeptidase II (*right*). The catalytic triad consists of Ser 221, His 64, and Asp 32 in subtilisin and of Ser 146, His 397, and Asp 338 in serine carboxypeptidase II. The peptide backbones of Ser 214, Trp 215, and Gly 216 in chymotrypsin, and their counterparts in subtilisin, participate in substrate-binding interactions. [After Robertus, J.D., Alden, R.A., Birktoft, J.J., Kraut, J., Powers, J.C., and Wilcox, P.E., *Biochemistry* **11**, 2449 (1972).]

Pepsin:

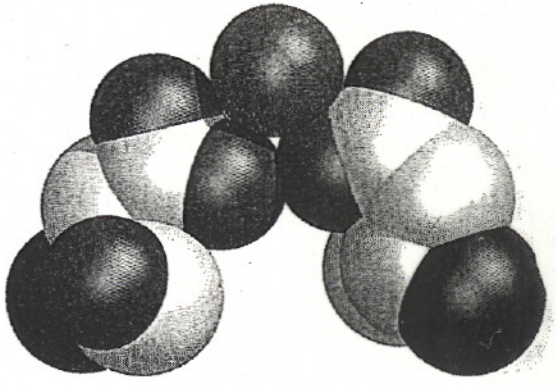
- Aspartyl protease
- 35-kDa- single- chain protein
- Optimum pH for activity: 2- 3
- The active site contains a water molecule flanked by two aspartates
- It consists of two structurally similar lobes

The two aspartates, one on each lobe, play a dual role in catalysis:

1. They activate the water molecule positioned between them
2. They serve as protons acceptors and donors



A



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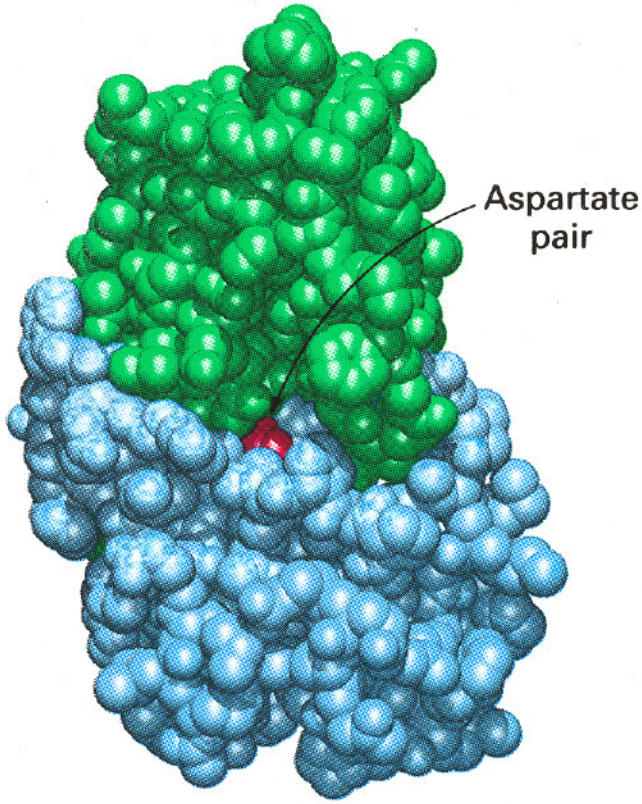
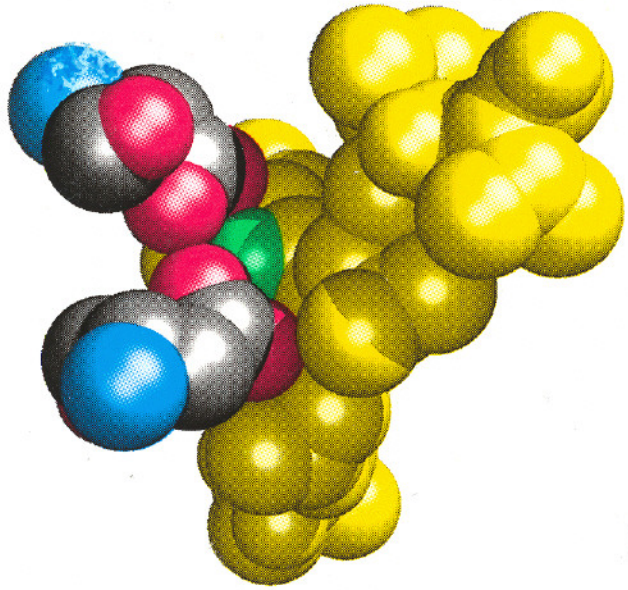




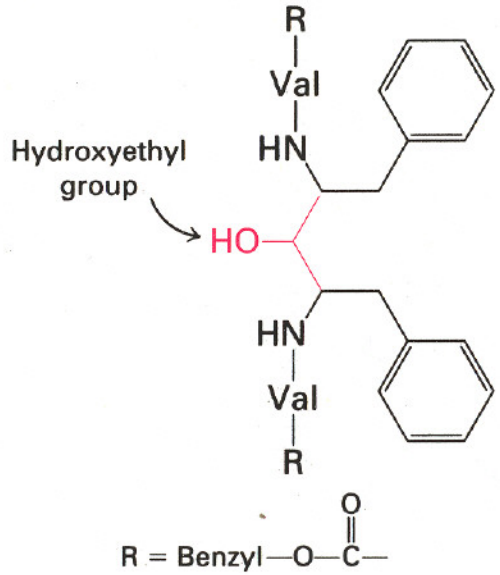
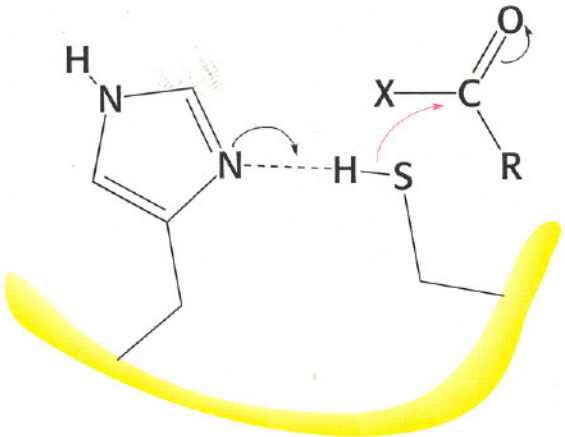


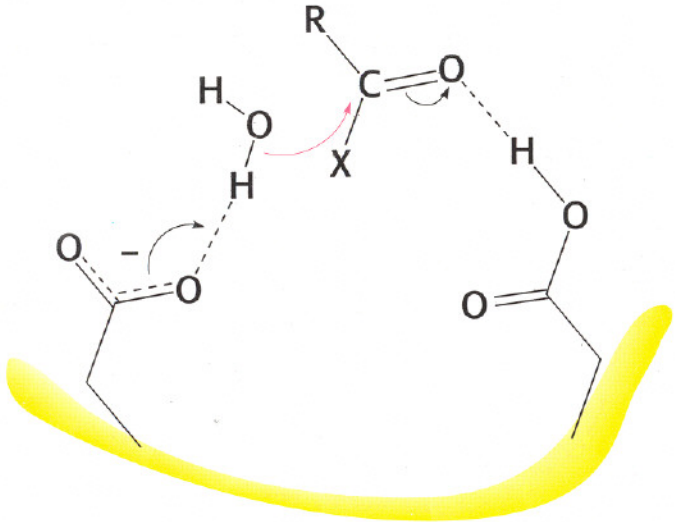
Figure 9-44, page 229; Figures 9-45 and 9-46, page 230

Stryer: *Biochemistry,* Fourth Edition

(A) CYSTEINE PROTEASES



(B) ASPARTYL PROTEASES



(C) METALLOPROTEASES

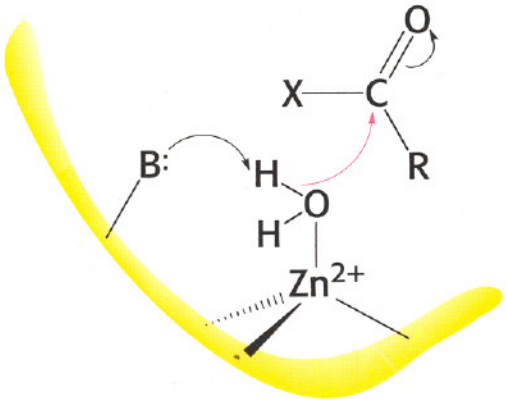


Figure 9-18
Stryer, Tymoczko & Berg, BIOCHEMISTRY, Fifth Edition.

CHAPTER 9 (Proteases)

Problems

- from textbook

  # 1, 2, 3, 4

- from companion

  # 2, 9, 10, 12