Lysozyme

Bacterial cell wall lysis

Hydrolysis reaction

β(1-4) glycosidic linkage of NAM-NAG and poly(NAG)

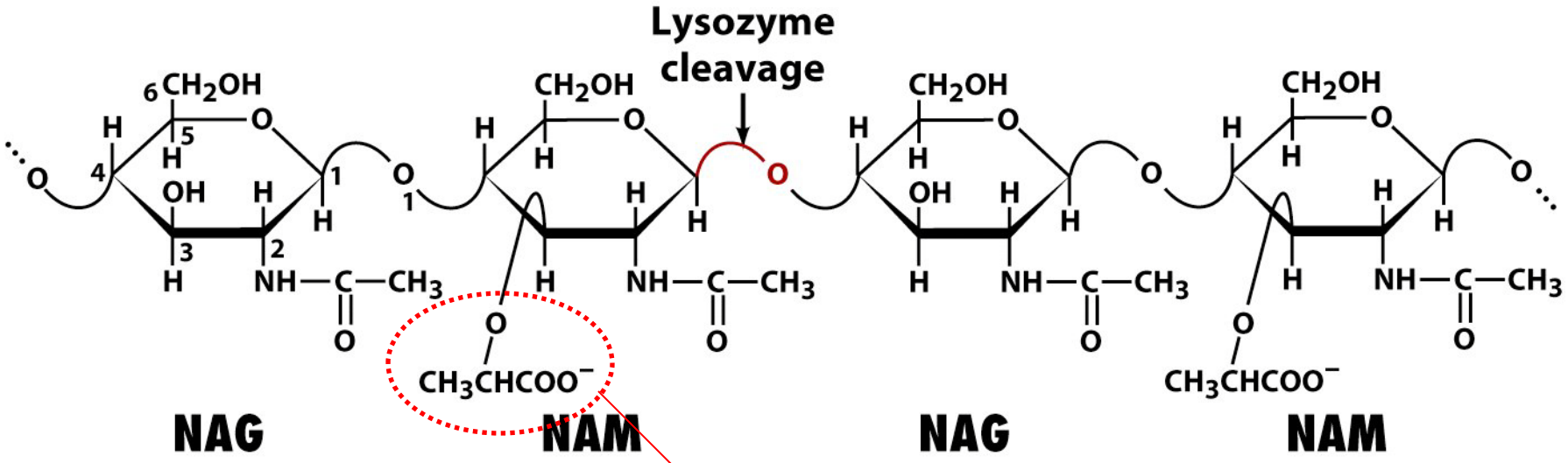


Figure 11-16 Fundamentals of Biochemistry, 2/e

Enzyme structure (Hen Egg White)
Substrate binding site traversing one face
At least 6 units of NAG to be catalyzed

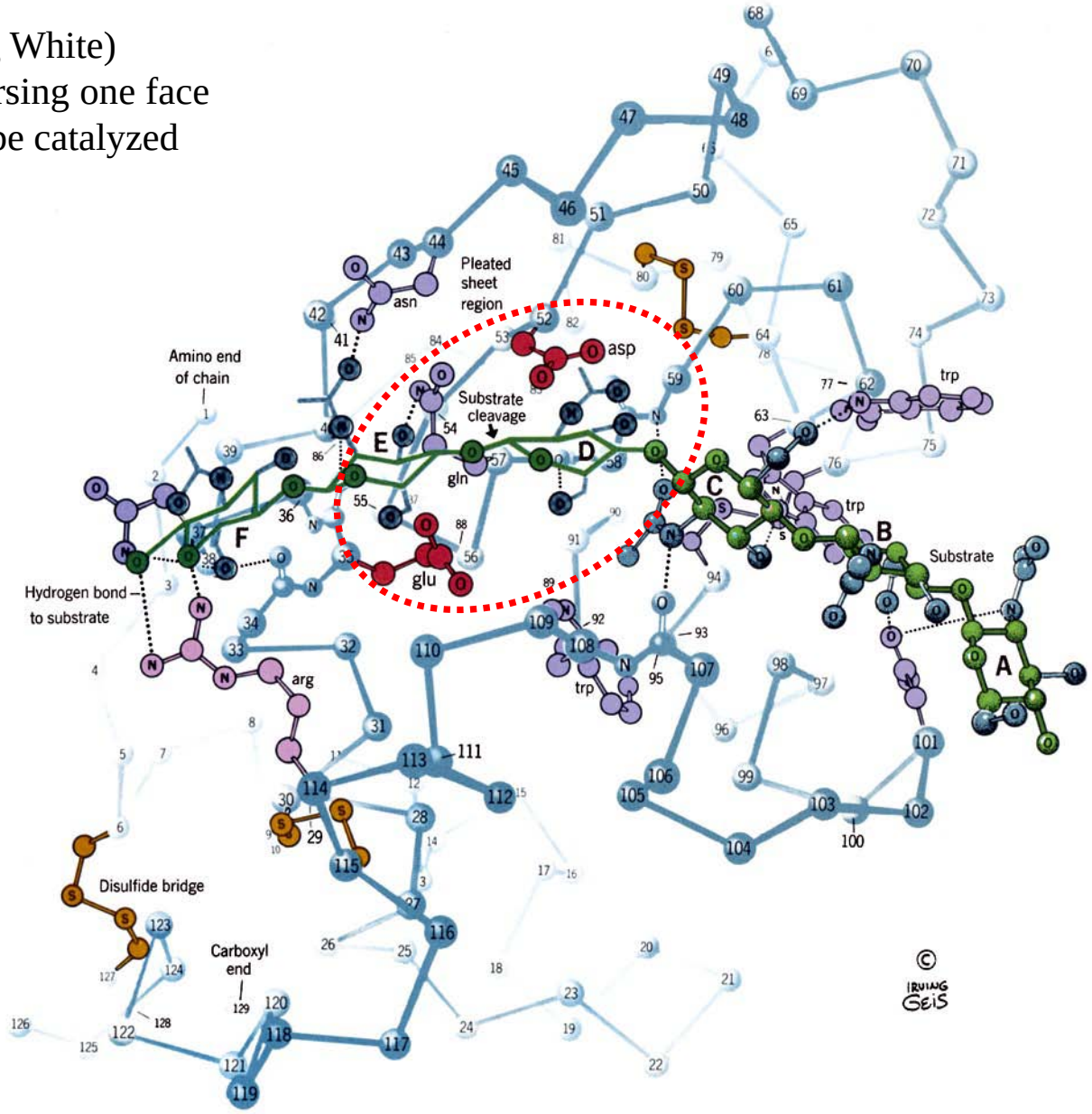


Figure 11-17a Fundamentals of Biochemistry, 2/e

Secondary structure

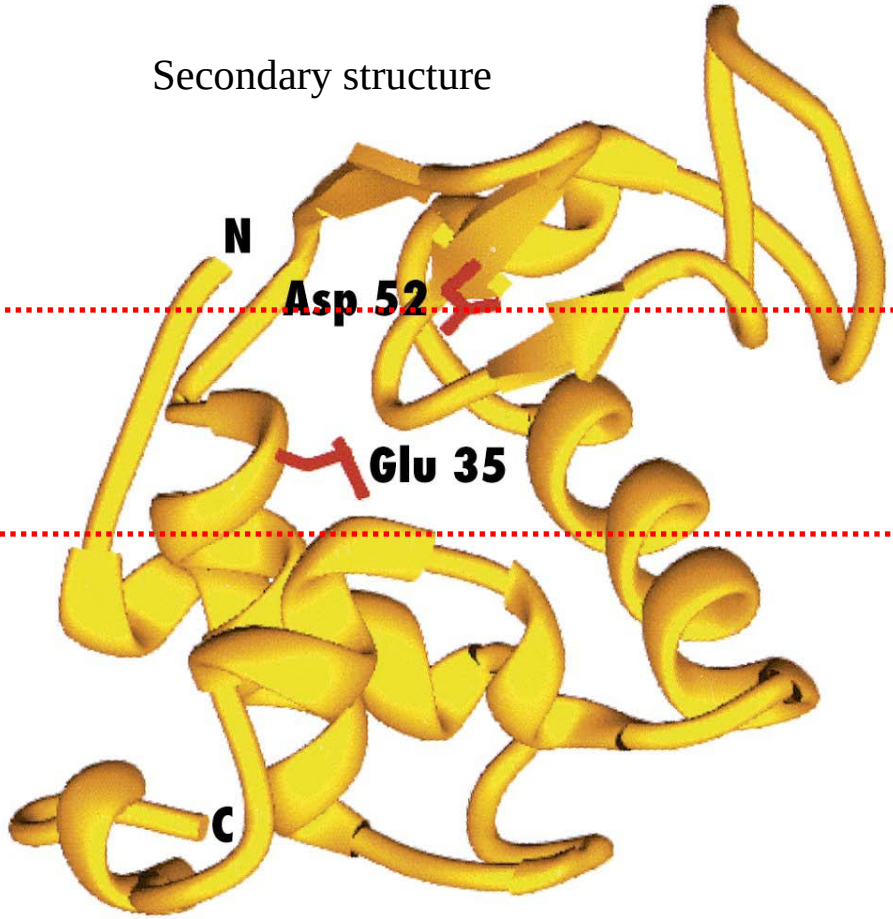


Figure 11-17b Fundamentals of Biochemistry, 2/e

Computer generated model

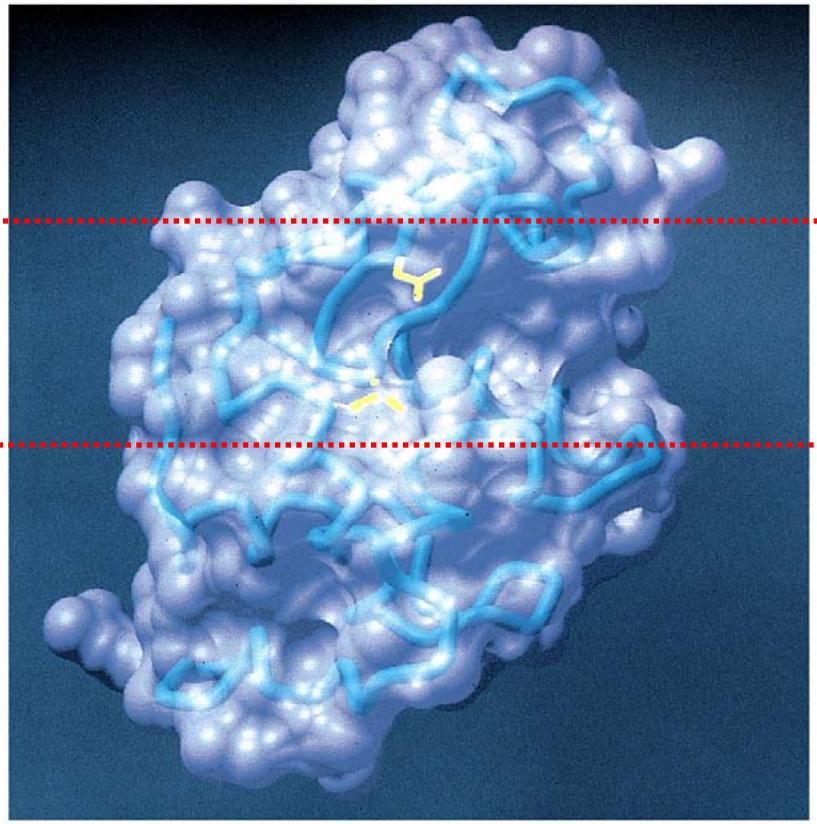


Figure 11-17c Fundamentals of Biochemistry, 2/e

Cleavage site: glycosidic bond between residue D and E
Residue D: normal conformation is not allowed to bind
distorted conformation

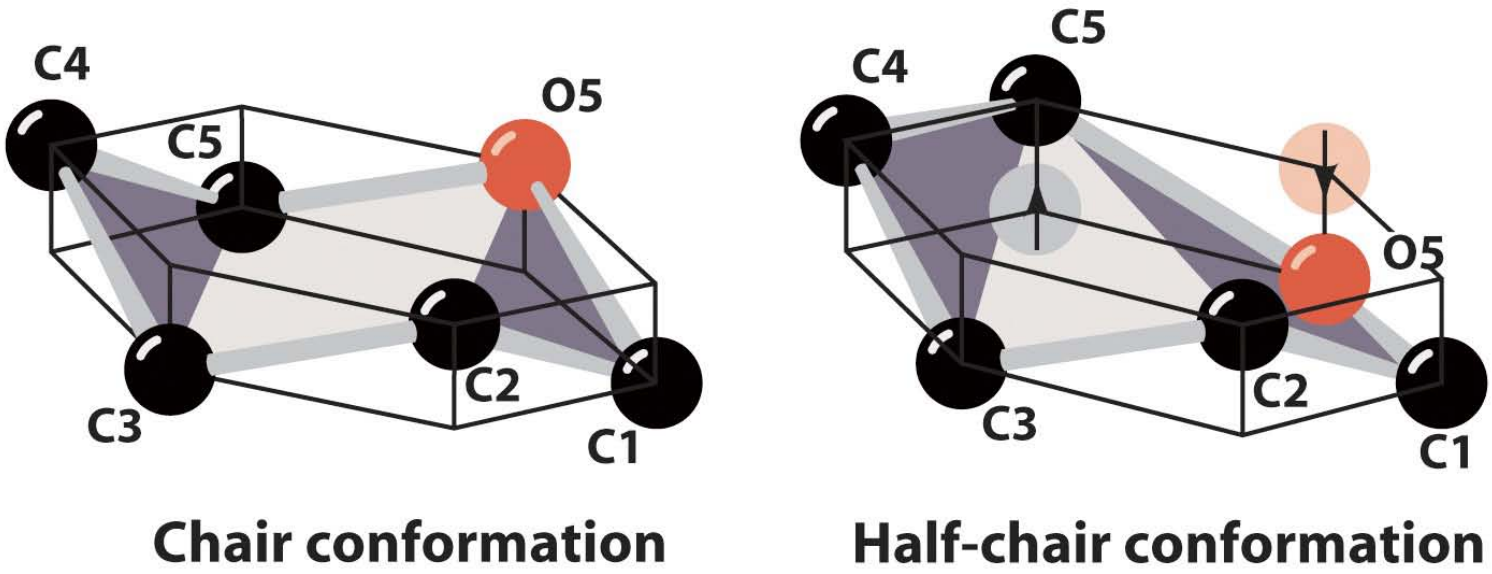
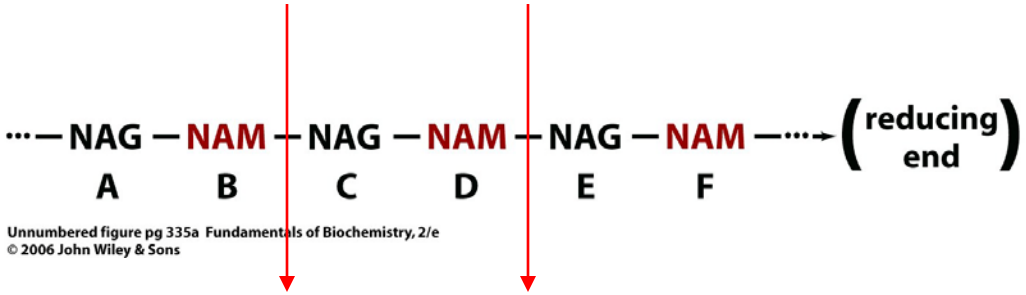


Figure 11-18 Fundamentals of Biochemistry, 2/e

The interactions of lysozyme with its substrate
Through computer model building

(NAG)3 can be stably bound
Cleaved between NAM and NAG



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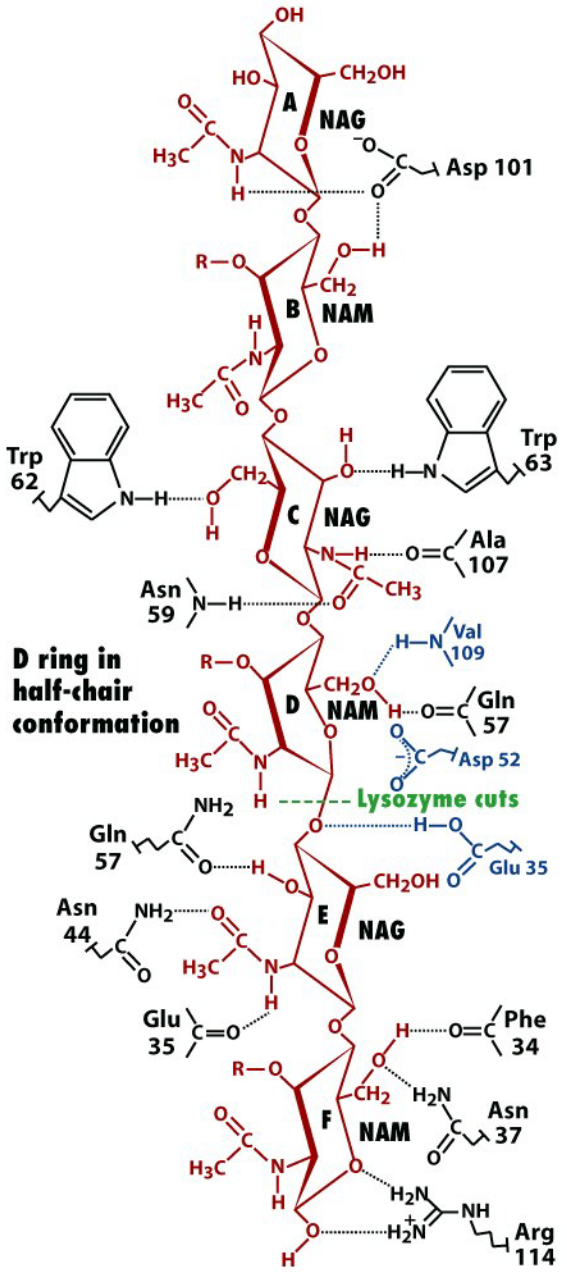

Figure 11-19 Fundamentals of Biochemistry, 2/e
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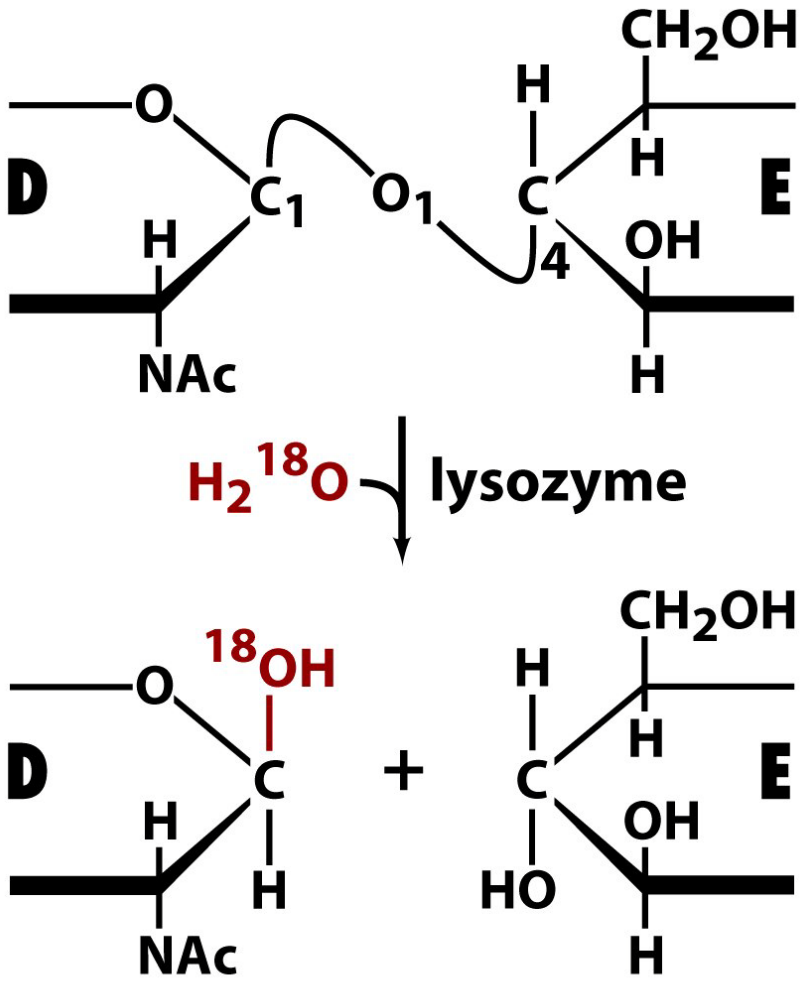
Which side is cleaved between NAM and NAG?

Using isotope labeled H_2O
Cleave between C1 and the bridge oxygen O1
Producing β anomer



Unnumbered figure pg 335b Fundamentals of Biochemistry, 2/e

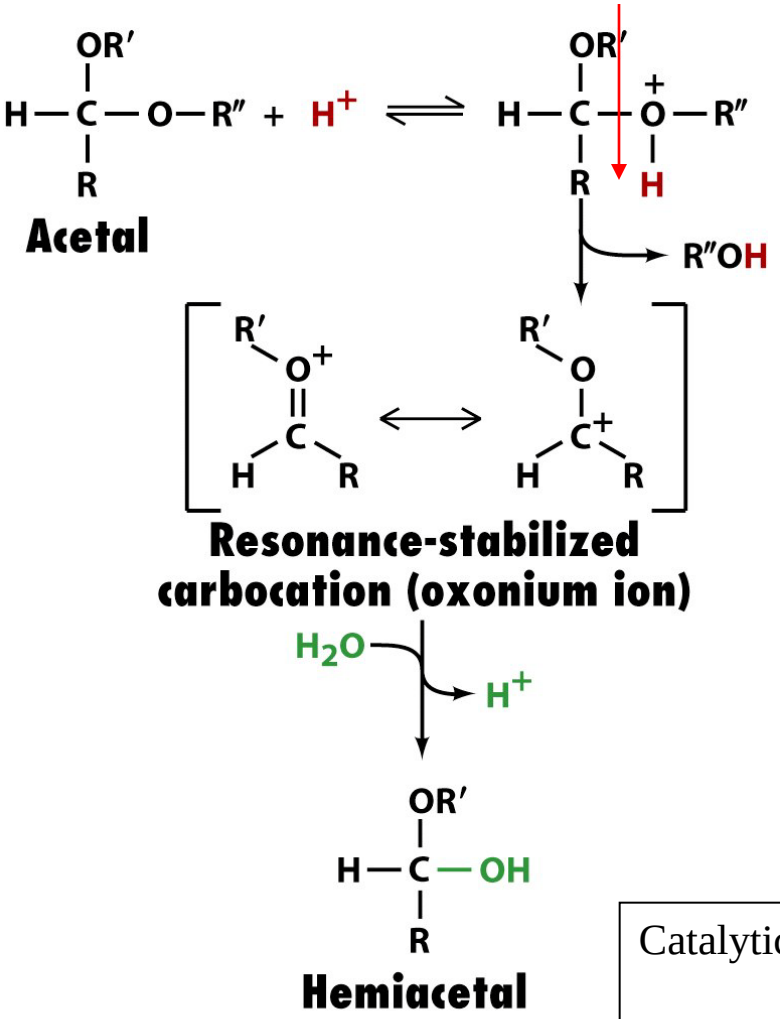


Figure 11-20 Fundamentals of Biochemistry, 2/e

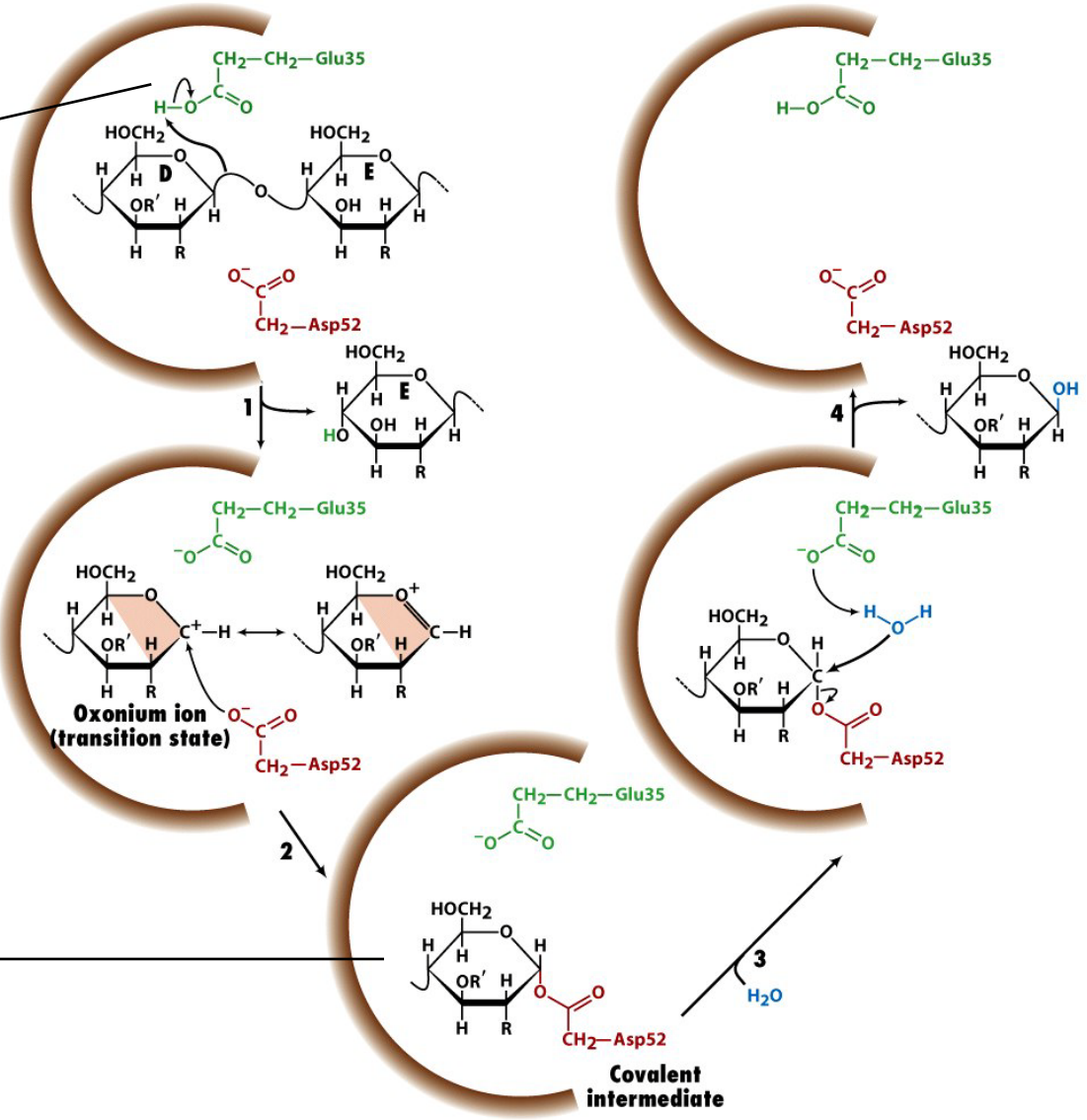
The mechanism of nonenzymatic acid-catalyzed hydrolysis of an acetal to a hemiacetal

Catalytic mechanism

Glu 35 and Asp 52
Asp 52 surrounded by polar residues: charged and stabilizing oxonium ion
Glu 35 in nonpolar pocket: uncharged & acid catalyst

The lysozyme reaction mechanism: proceeds in 4 steps

General acid catalysis



Covalent intermediate

Figure 11-21 Fundamentals of Biochemistry, 2/e

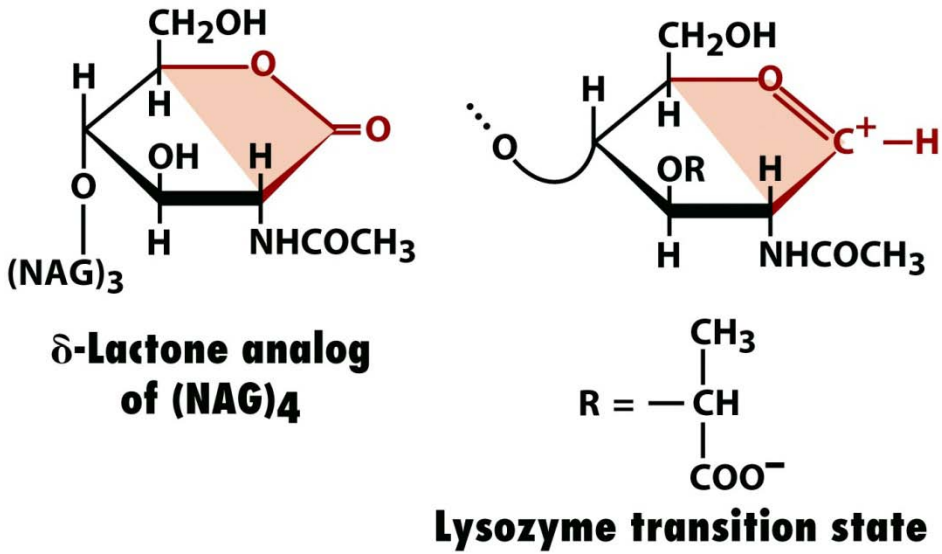
Experimental support for the lysozyme mechanism

Table 11-4 Binding Free Energies of HEW Lysozyme Subsites

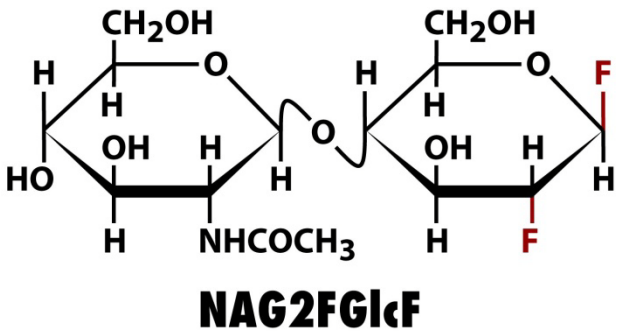
Site	Bound Saccharide	Binding Free Energy ($kJ \cdot mol^{-1}$)
A	NAG	−7.5
B	NAM	−12.3
C	NAG	−23.8
D	**NAM**	**+12.1**
E	NAG	−7.1
F	NAM	−7.1

Source: Chipman, D.M. and Sharon, N., *Science* **165,** 459 (1969).

Transition state analog inhibition
coplanar structure: shaded



Covalent bond formation



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E35Q lysozyme

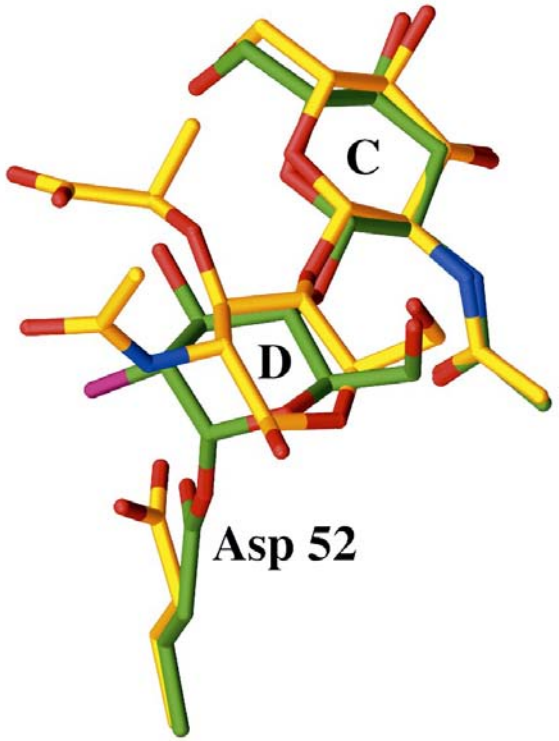

Figure 11-23 Fundamentals of Biochemistry, 2/e
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Serine proteases

- serine residue at the active site
- chymotrypsin, trypsin, elastase

Cysteine proteases: papain, plant & viral proteases
Asparatic proteases: pepsin
Metalloproteases: carboxypeptidase
Unknown mechanism

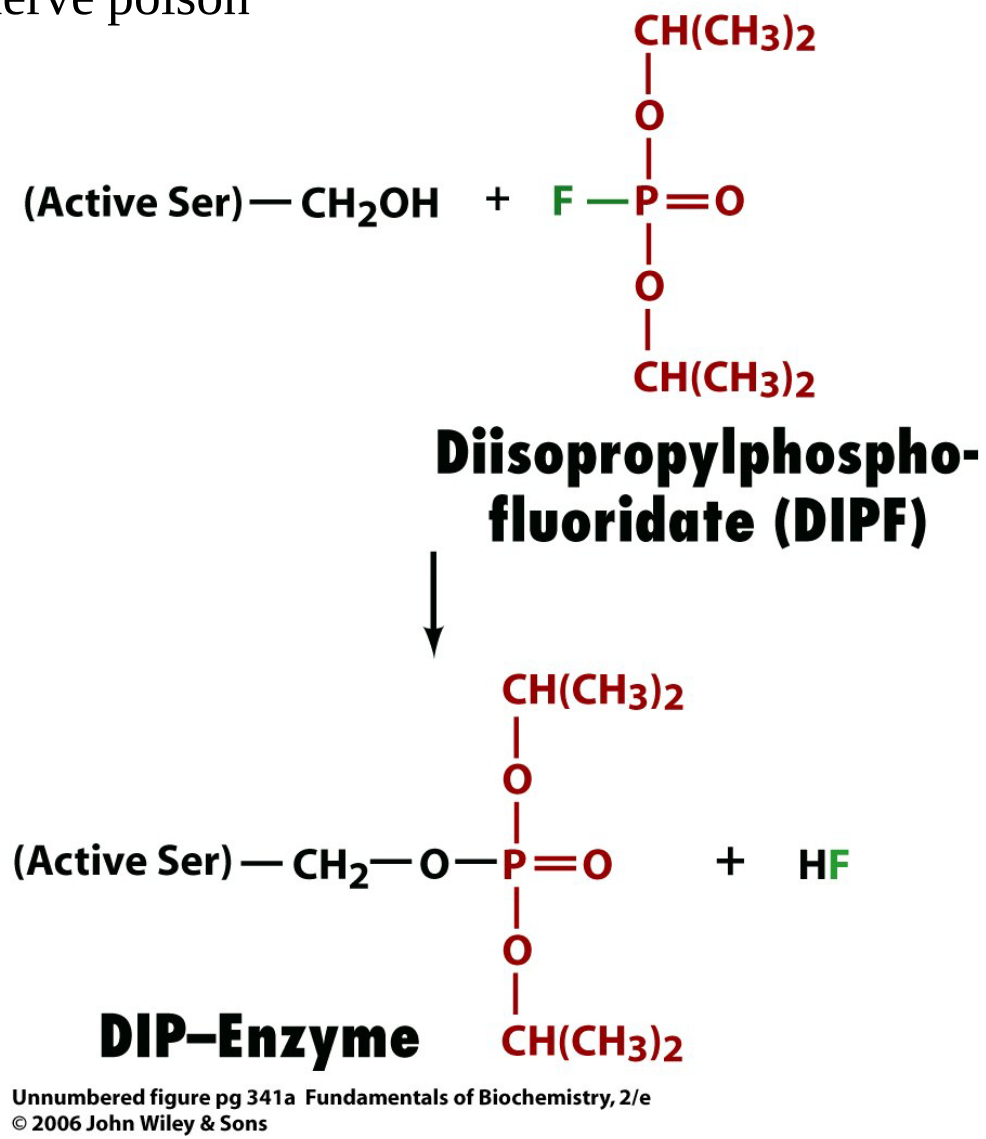
The active site

- Serine 195: DIPF (diisopropylphosphofluoridate)
- His 57: affinity labeling
  - TPCK
  - TLCK

DIPF

Irreversible inactivator: potent nerve poison

Specific binding to Ser 195



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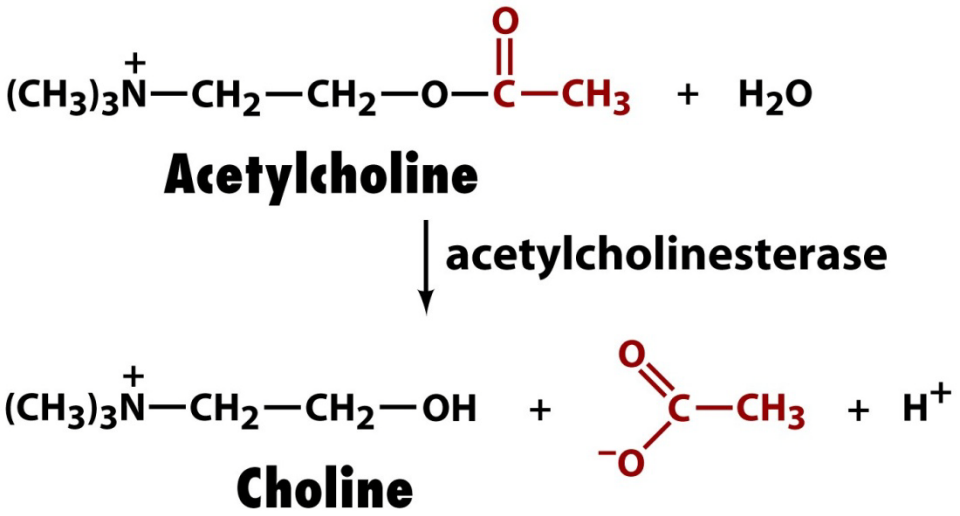
Nerve poisons

Organophosphorous groups
- nerve gas: DIPF, sarin
- insecticide: parathion, malathion

Acetylcholine esterase inhibitor



Box 11-3 figure 1 Fundamentals of Biochemistry, 2/e
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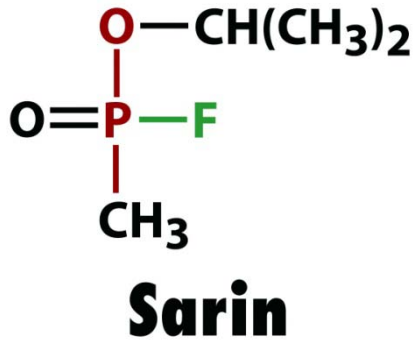

Box 11-3 figure 3 Fundamentals of Biochemistry, 2/e
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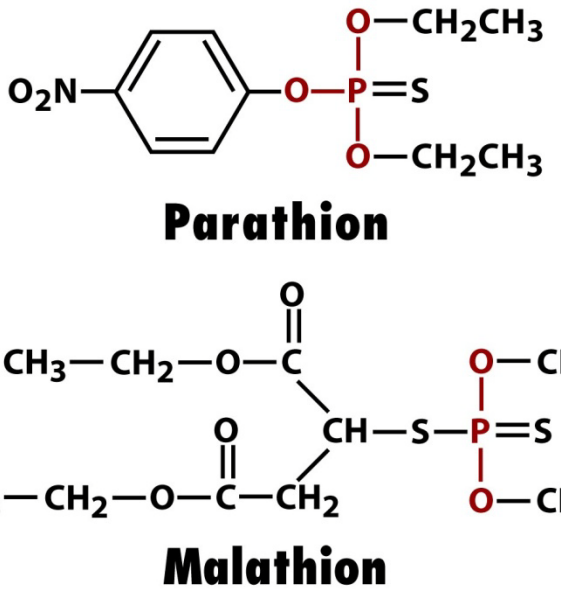

Box 11-3 figure 2 Fundamentals of Biochemistry, 2/e
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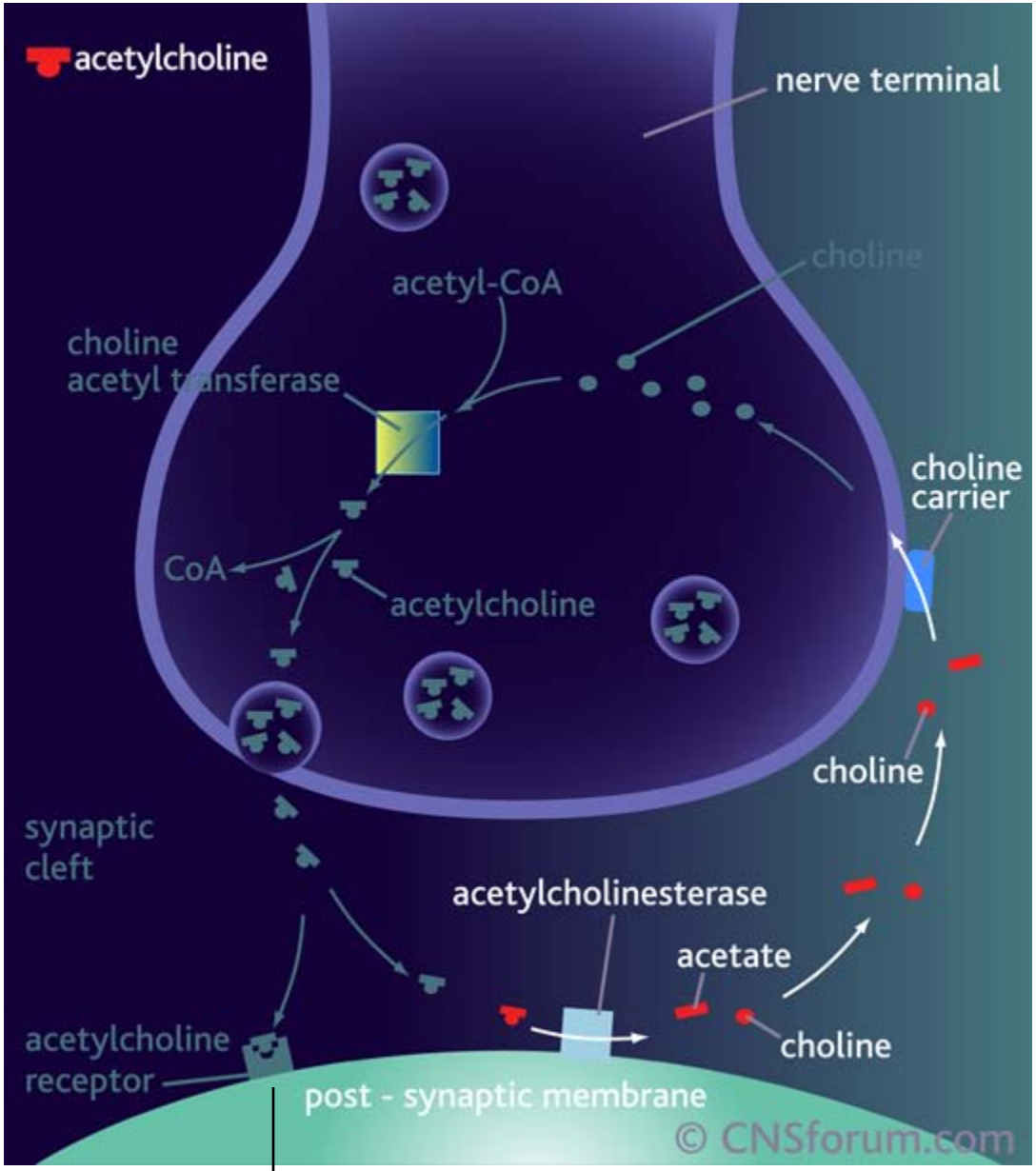

stimulation of muscle contraction

Affinity labeling of His 57

substrate analogs

covalent binding: useful for identification of the reactive group

tosyl-L-phenylalanine chloromethyl ketone

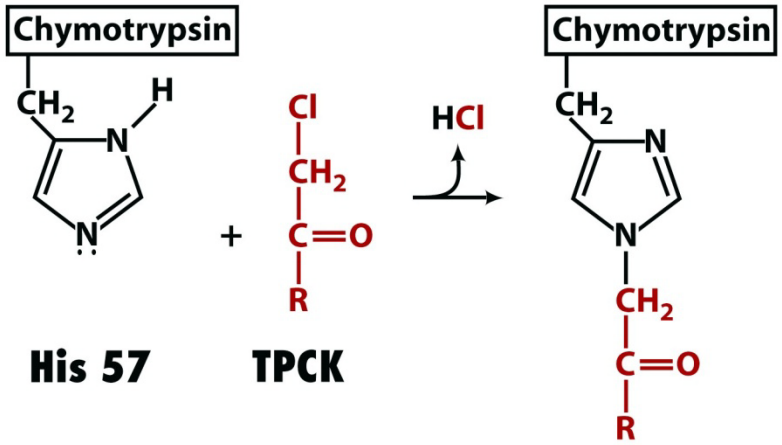

Figure 11-24 Fundamentals of Biochemistry, 2/e
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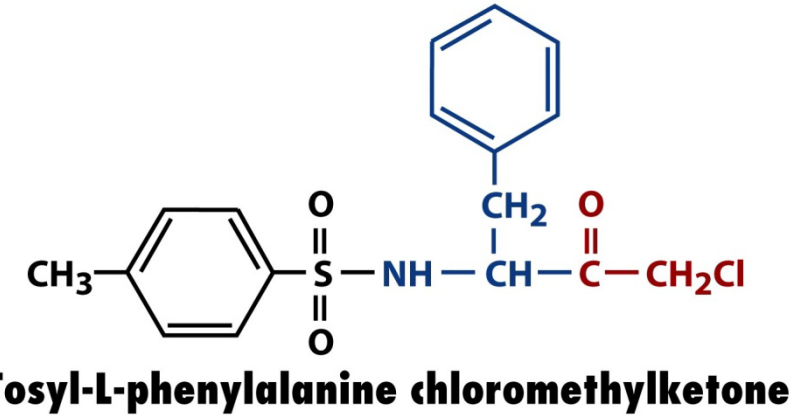
tosyl-L-lysine chloromethyl ketone



Unnumbered figure pg 341b Fundamentals of Biochemistry, 2/e
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X-ray structure of bovine trypsin

trypsin, chymotrypsin. Elastase
Similar structures
~40% sequence identities

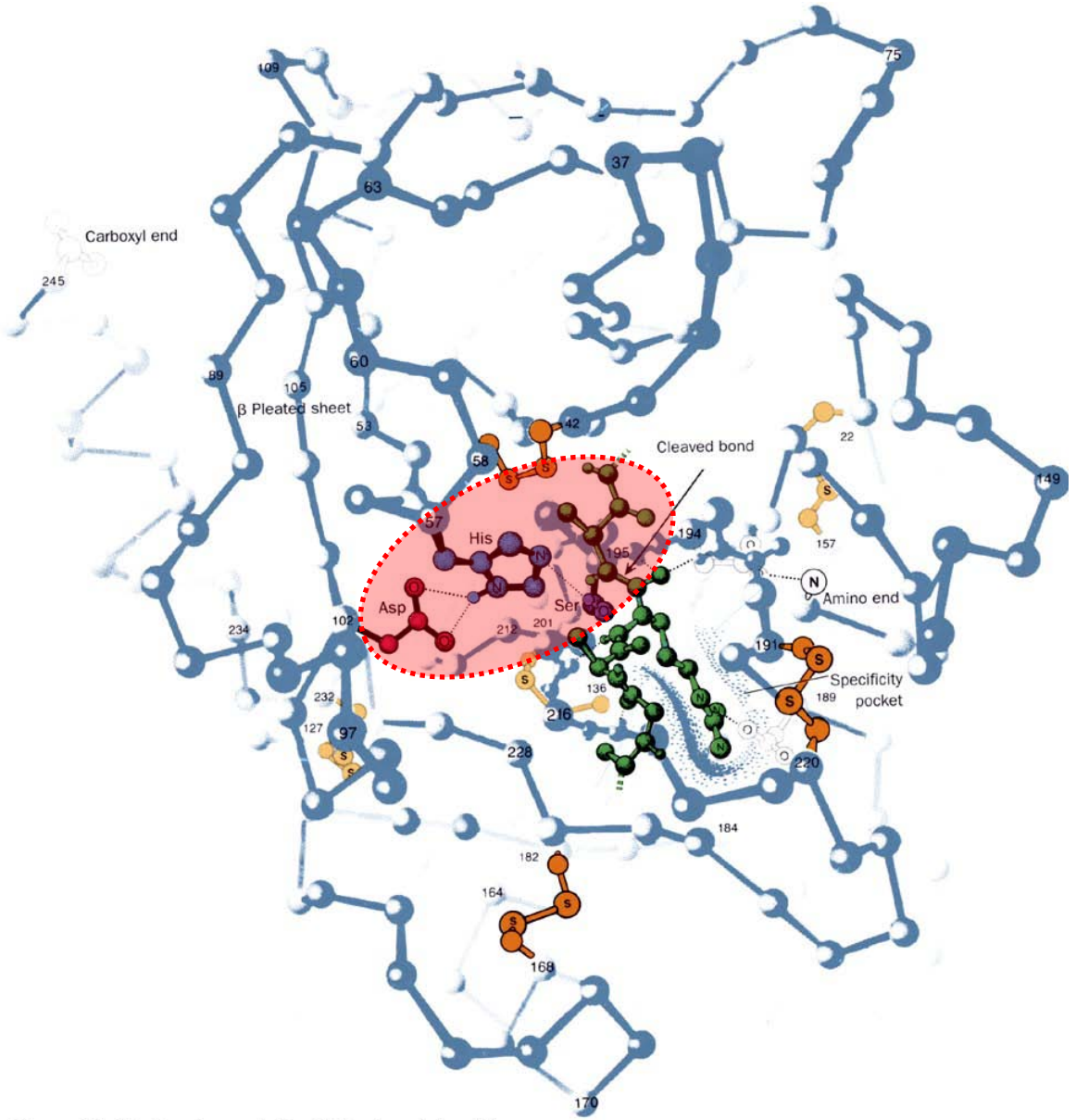

Figure 11-25 Fundamentals of Biochemistry, 2/e

The active site residues of chymotrypsin

Three invariant residues: catalytic triad

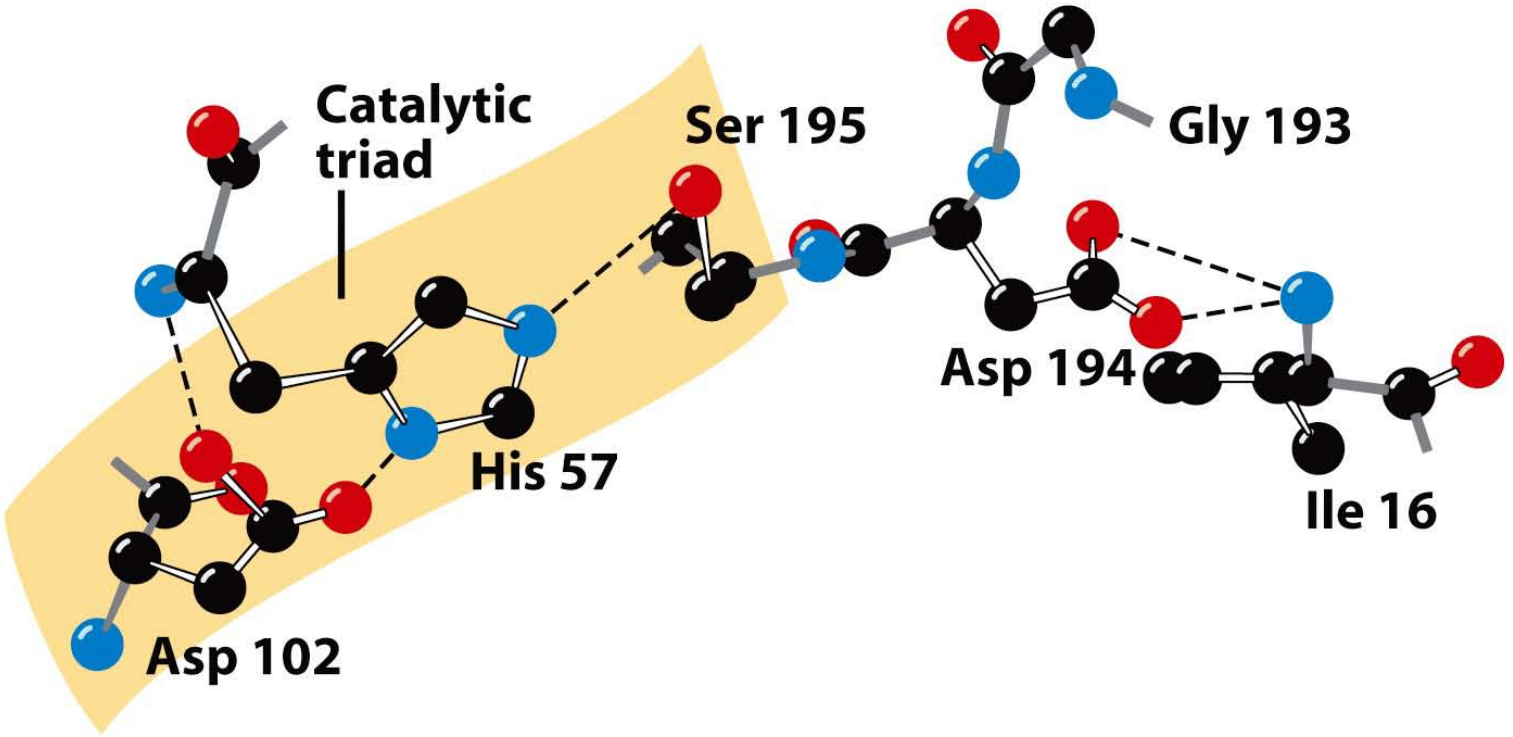

Figure 11-26 Fundamentals of Biochemistry, 2/e

Specificity pockets

Substrate specificities
Aromatic side chains
Cationic side chains
Nonpolar Small side chains

Site-directed mutagenesis
Trypsin Asp 189 to Ser
Unexpected results: poor nonspecific activity

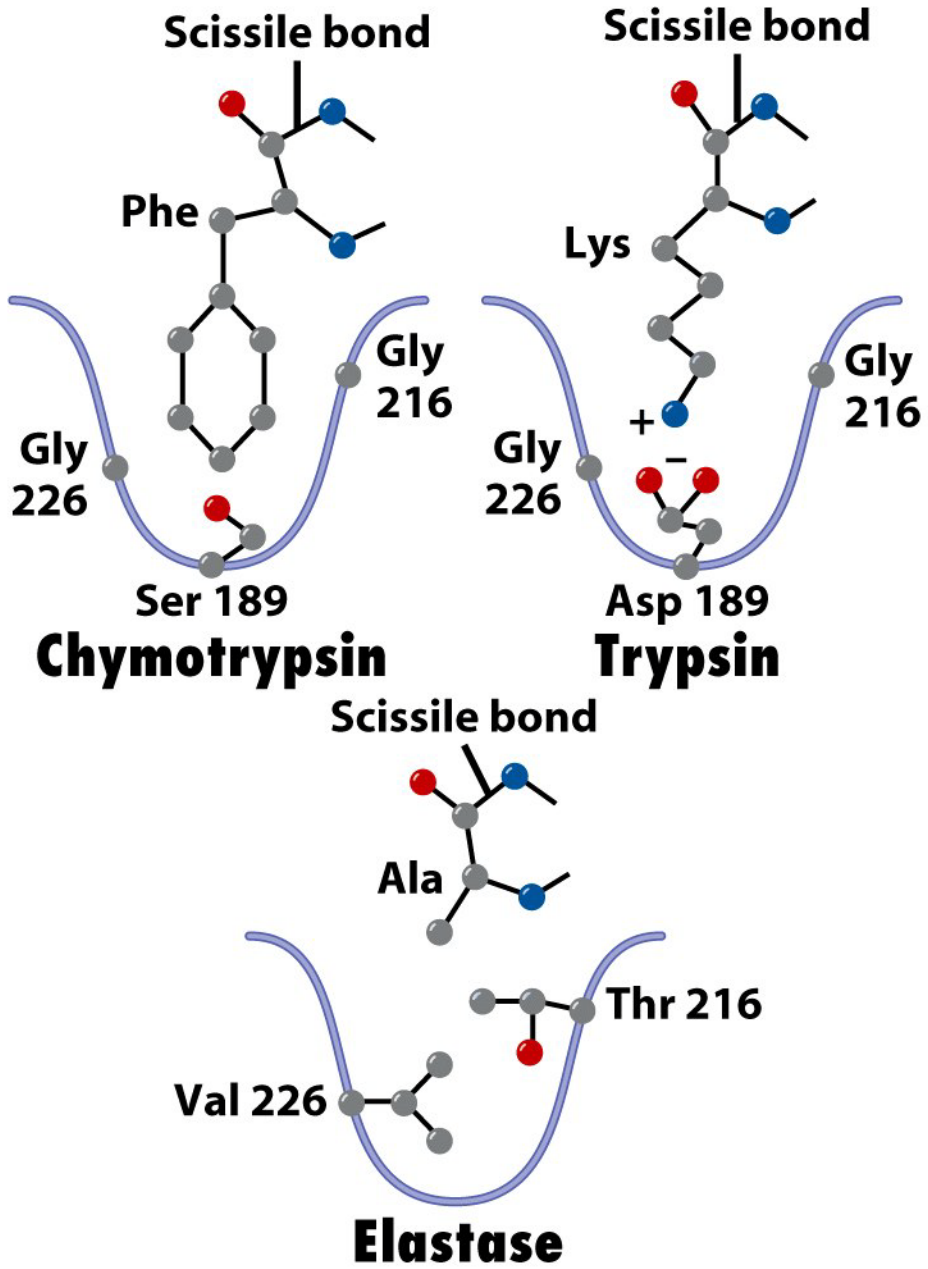

Figure 11-27 Fundamentals of Biochemistry, 2/e

Evolutionary relationships among serine proteases

Divergent evolution: trypsin, chymotrypsin
Convergent evolution: subtilisin

Conserved catalytic triad
But low homology in primary structures

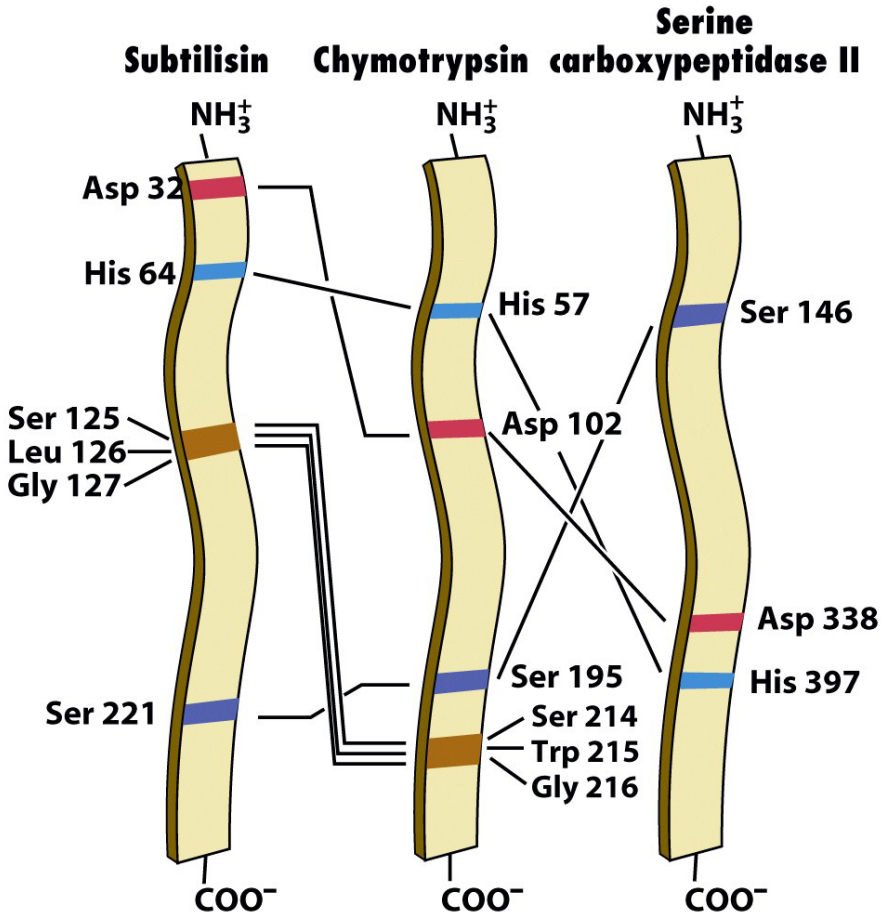

Figure 11-28 Fundamentals of Biochemistry, 2/e

Catalytic Mechanism

4 steps

Tetrahedral intermediate
Acyl-enzyme intermediate
Tetrahedral intermediate

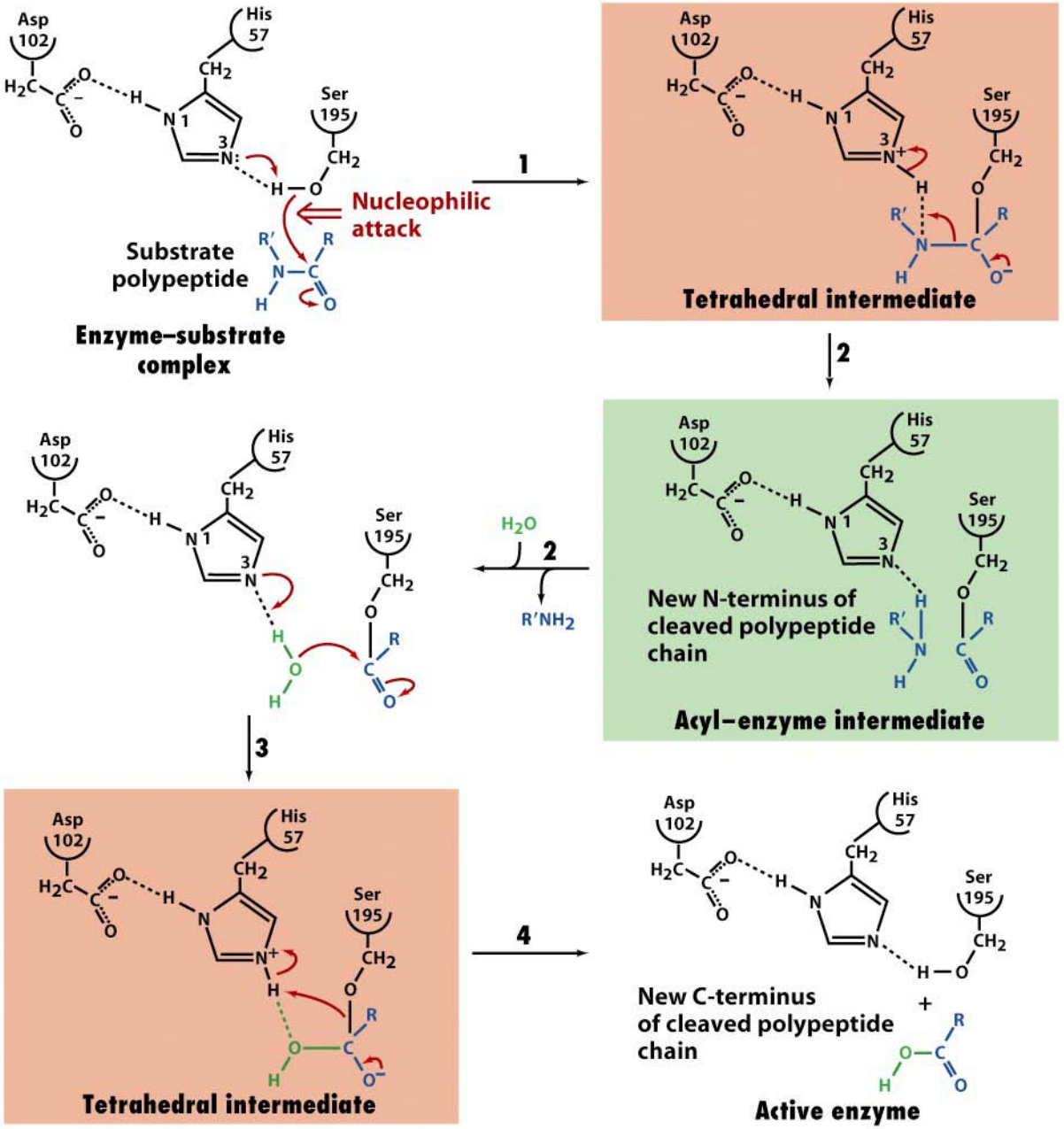

Figure 11-29 Fundamentals of Biochemistry, 2/e

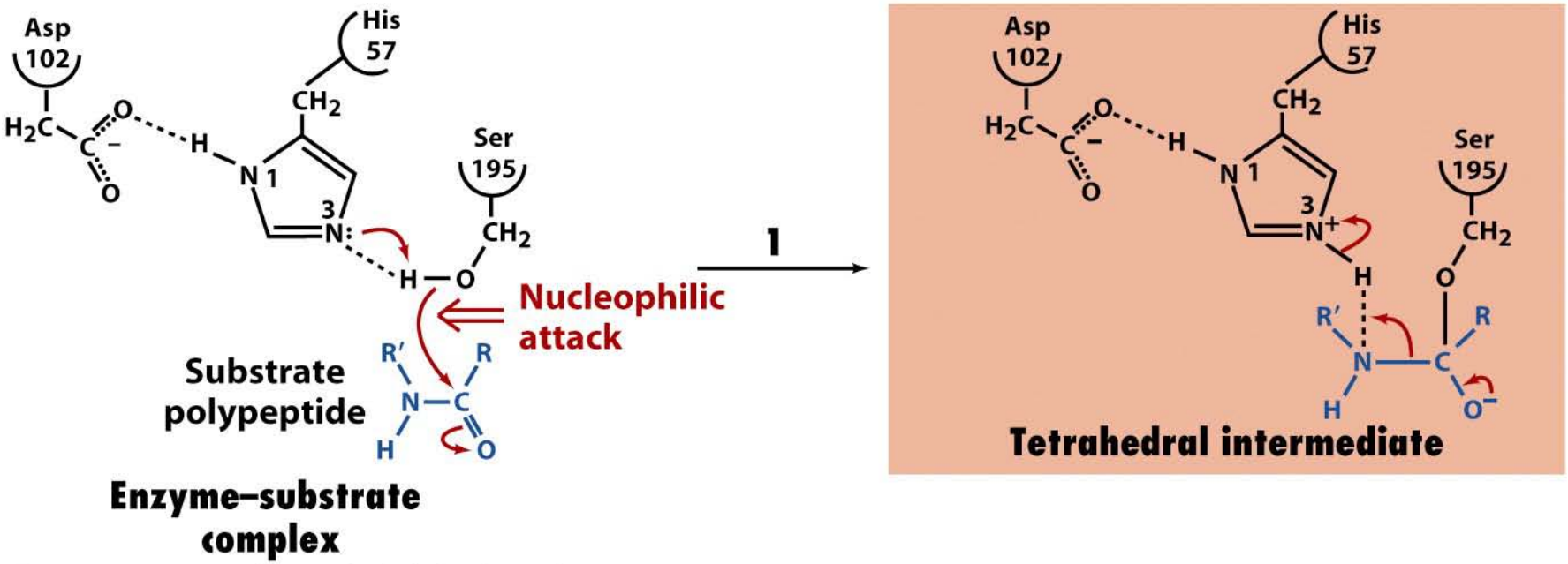

Figure 11-29 part 1 Fundamentals of Biochemistry, 2/e

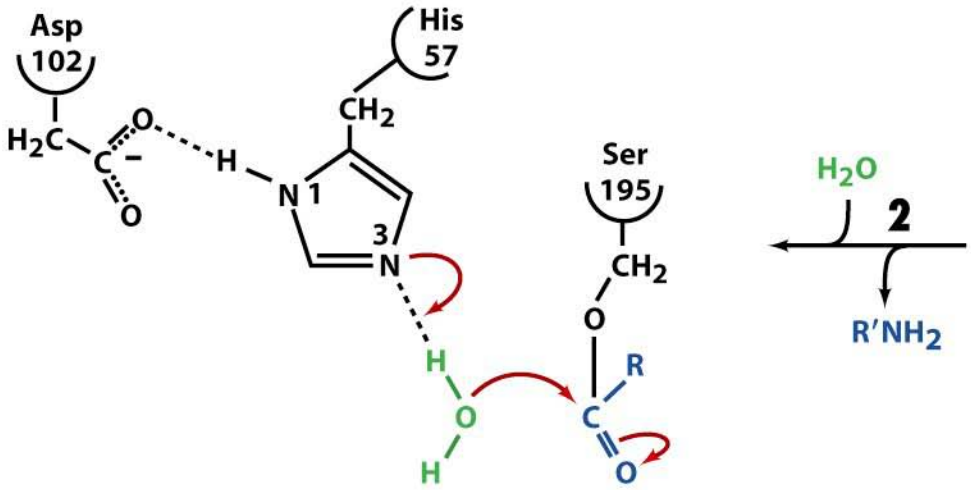

Figure 11-29 part 2 Fundamentals of Biochemistry, 2/e

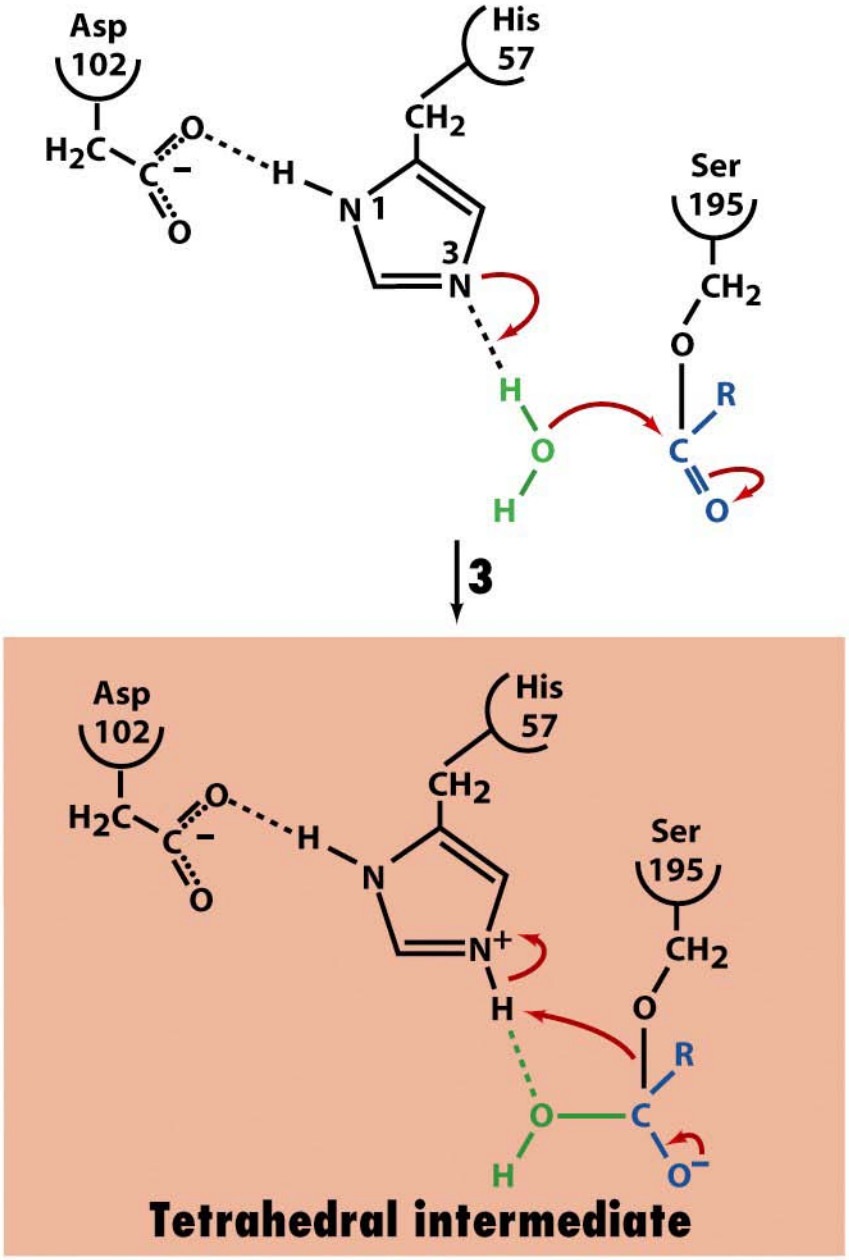

Figure 11-29 part 3 Fundamentals of Biochemistry, 2/e

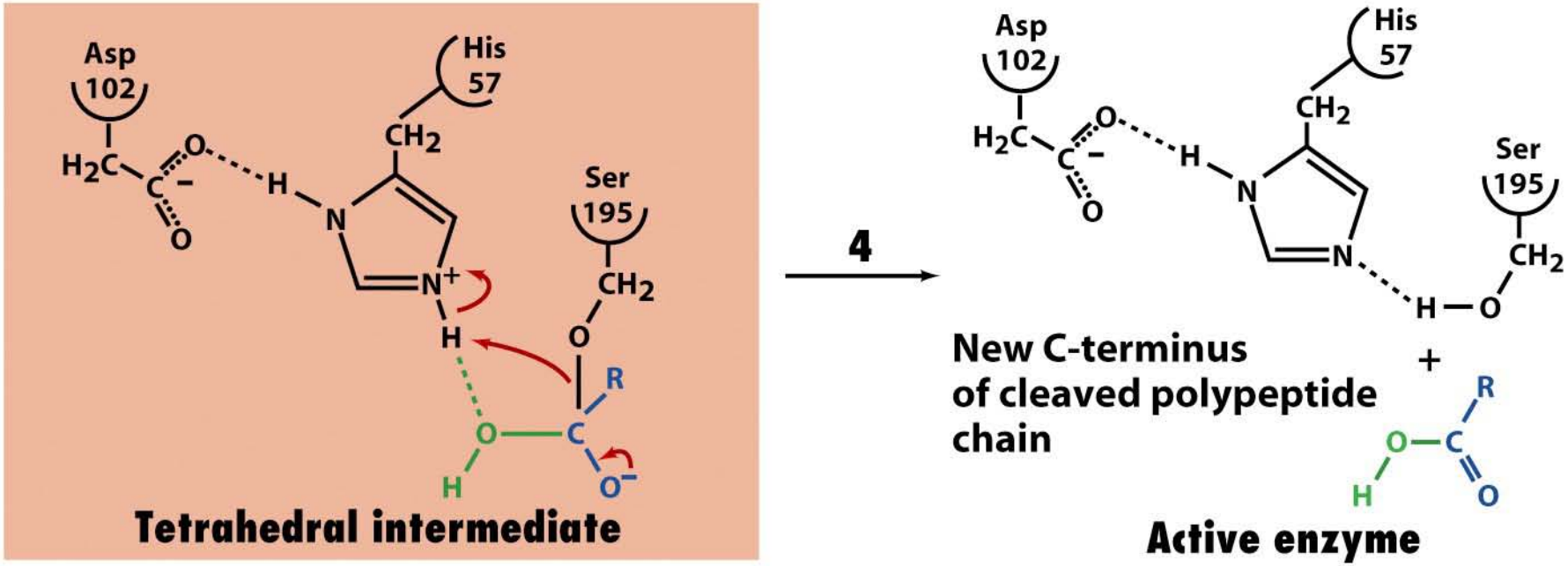

Figure 11-29 part 4 Fundamentals of Biochemistry, 2/e

Serine proteases preferentially bind the transition state

Oxyanion hole
Tetrahedral distortion allows H-bond formation
Hydrogen bonds: low-barrier hydrogen bonds (LBHBs)

Transition state stabilization in the serine proteases

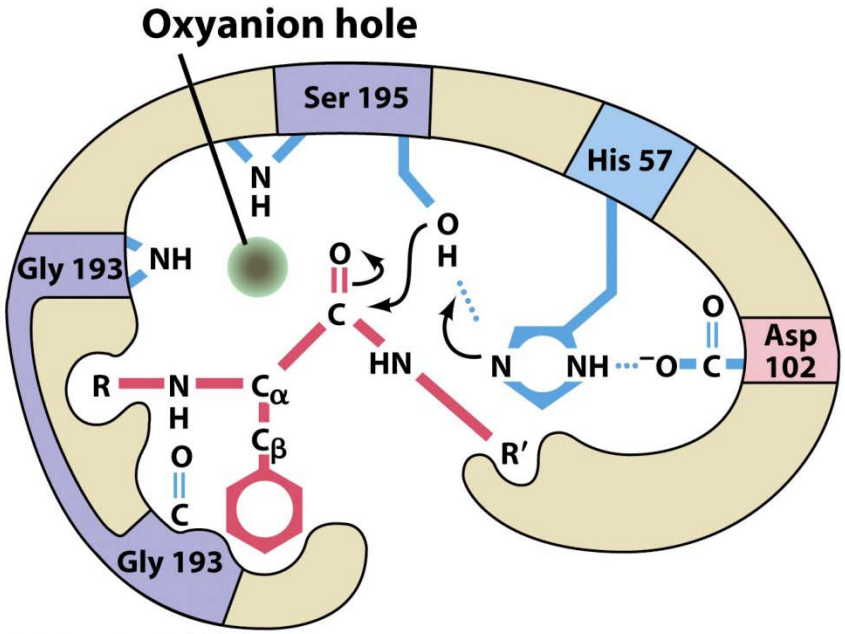

Figure 11-30a Fundamentals of Biochemistry, 2/e
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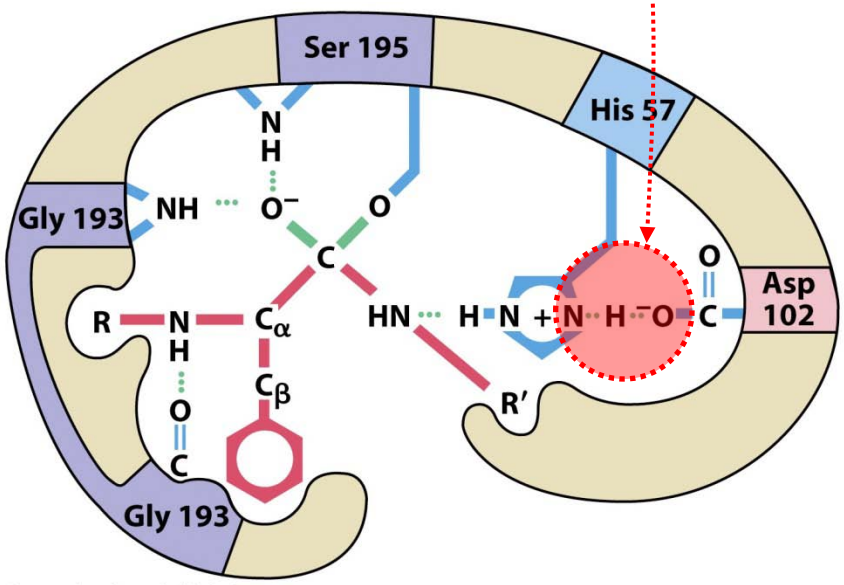

Figure 11-30b Fundamentals of Biochemistry, 2/e
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The trypsin-BPTI complex

BPTI (bovine pancreatic trypsin inhibitor) prevent premature activation
Association constant: 10^{13} M^{-1}
Resembles the tetrahedral intermediate
Reaction can not proceed: rigidity & tight sealing

***α1-proteinase inhibitor: leukocyte elastase inhibitor (pulmonary emphysema)

Computer generated drawing

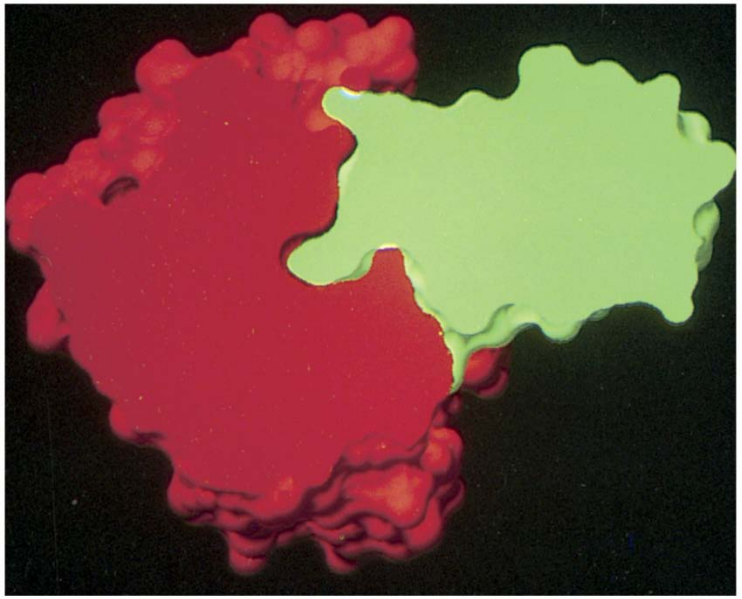


Figure 11-31a Fundamentals of Biochemistry, 2/e

Trypsin Ser 195 & BPTI's scissile peptide

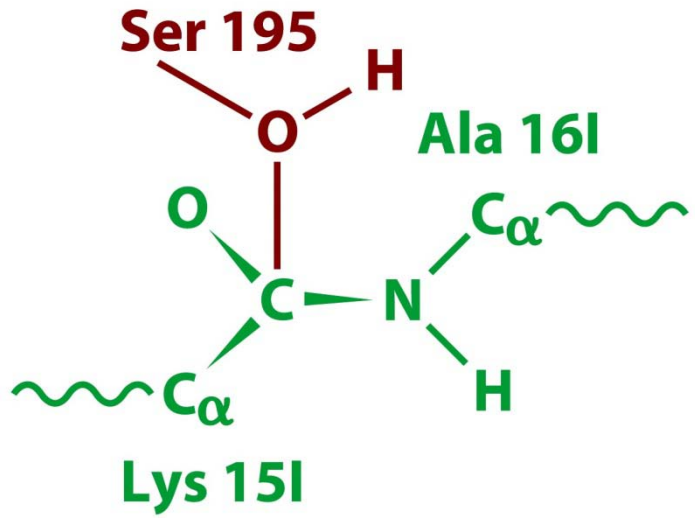
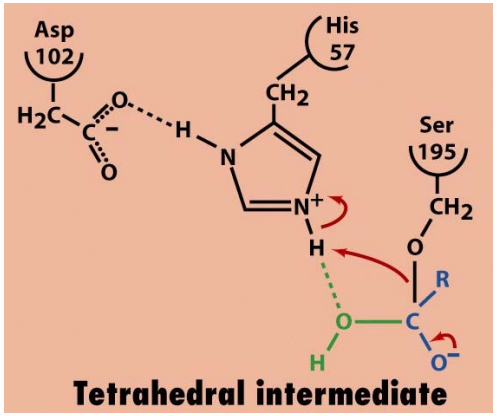


Figure 11-31b Fundamentals of Biochemistry, 2/e

Structure of the acyl-enzyme and tetrahedral intermediates in porcine pancreatic elastase

Acyl-enzyme complex trapped at pH 5

Tetrahedral intermediate trapped at pH 9



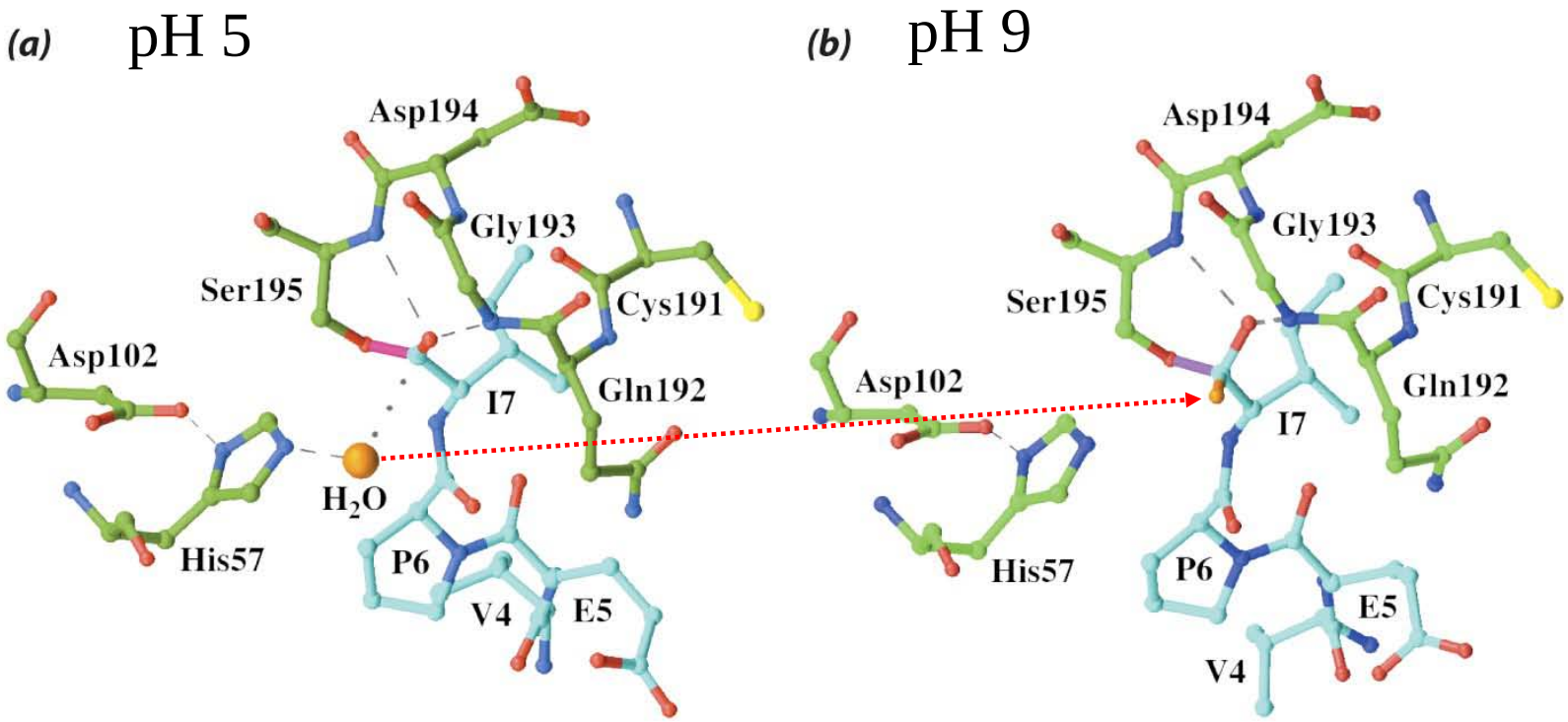


Figure 11-32 Fundamentals of Biochemistry, 2/e

Zymogens

Larger inactive precursors

Autocatalytic or catalyzed by other proteases

Distorted active sites

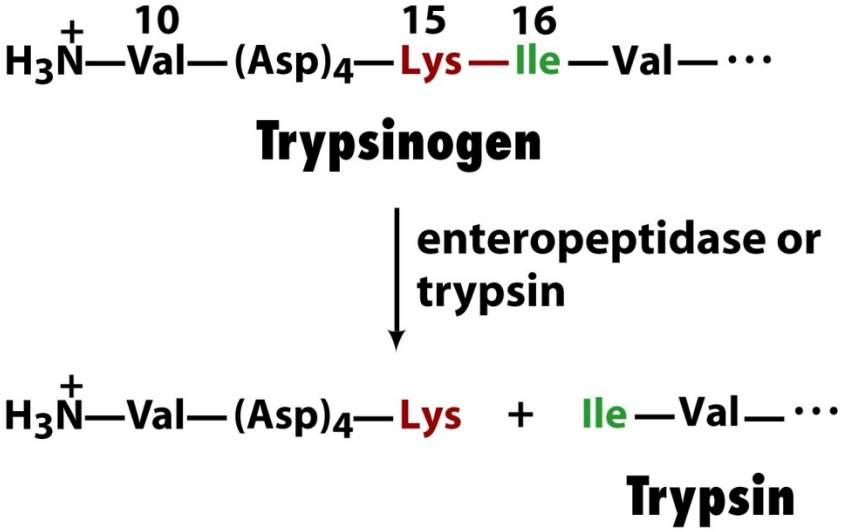


Figure 11-33 Fundamentals of Biochemistry, 2/e
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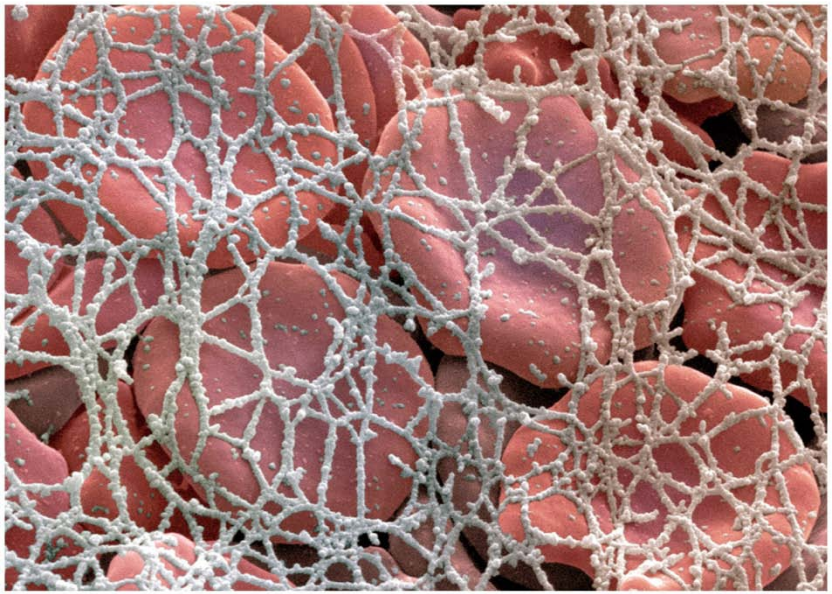
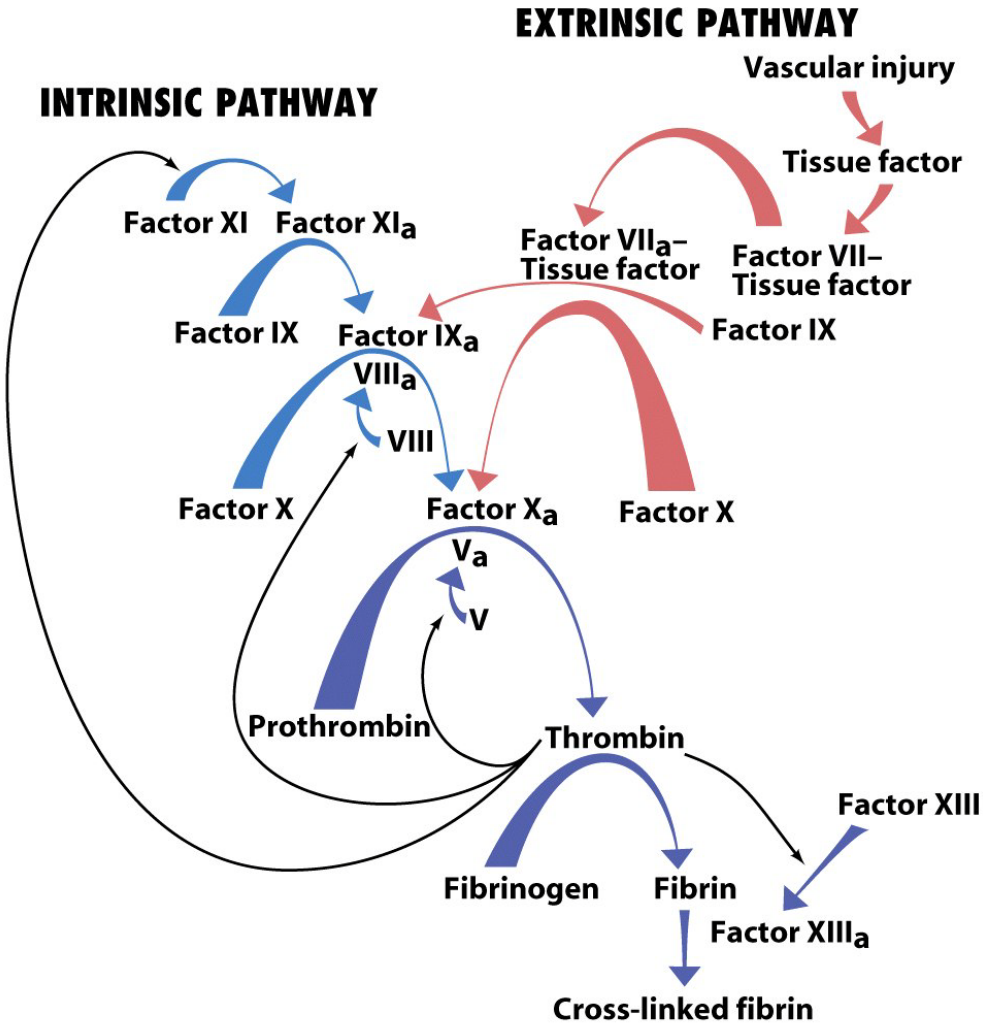
Fibrin networks



Box 11-4 figure 1 Fundamentals of Biochemistry, 2/e

Coagulation cascade



Box 11-4 figure 2 Fundamentals of Biochemistry, 2/e

Plasma Concentrations of Some Human Coagulation Factors

Factor	Concentration (μM)[a]
XI	0.06
IX	0.09
VII	0.01
X	0.18
Prothrombin	1.39
Fibrinogen	8.82

[a]Concentrations calculated from data in High, K.A. and Roberts, H.R., (Eds.), *Molecular Basis of Thrombosis and Hemostasis,* Marcel Dekker (1995).

Box 11-4 Table 1 Fundamentals of Biochemistry, 2/e