

Slide : Enzymes 3

Dr. Name : Nayef Karadsheh

Sections : 4, 5, 6

■ Slide □ Sheet



Medical Committee  
The University of Jordan

# Biochemistry

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biometrics  
biochemistry  
ecology  
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taxonomy  
biophysics  
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virology  
zoology  
biometry  
cryobiology  
enzymology  
cell  
genetics  
bionics



Mousa Suboh

# General Strategies of Enzyme Catalysis

- Proximity and Orientation
- Transition State Stabilization
- Acid-Base Catalysis
- Nucleophilic Catalysis
- Electrophilic Catalysis
- Covalent Catalysis

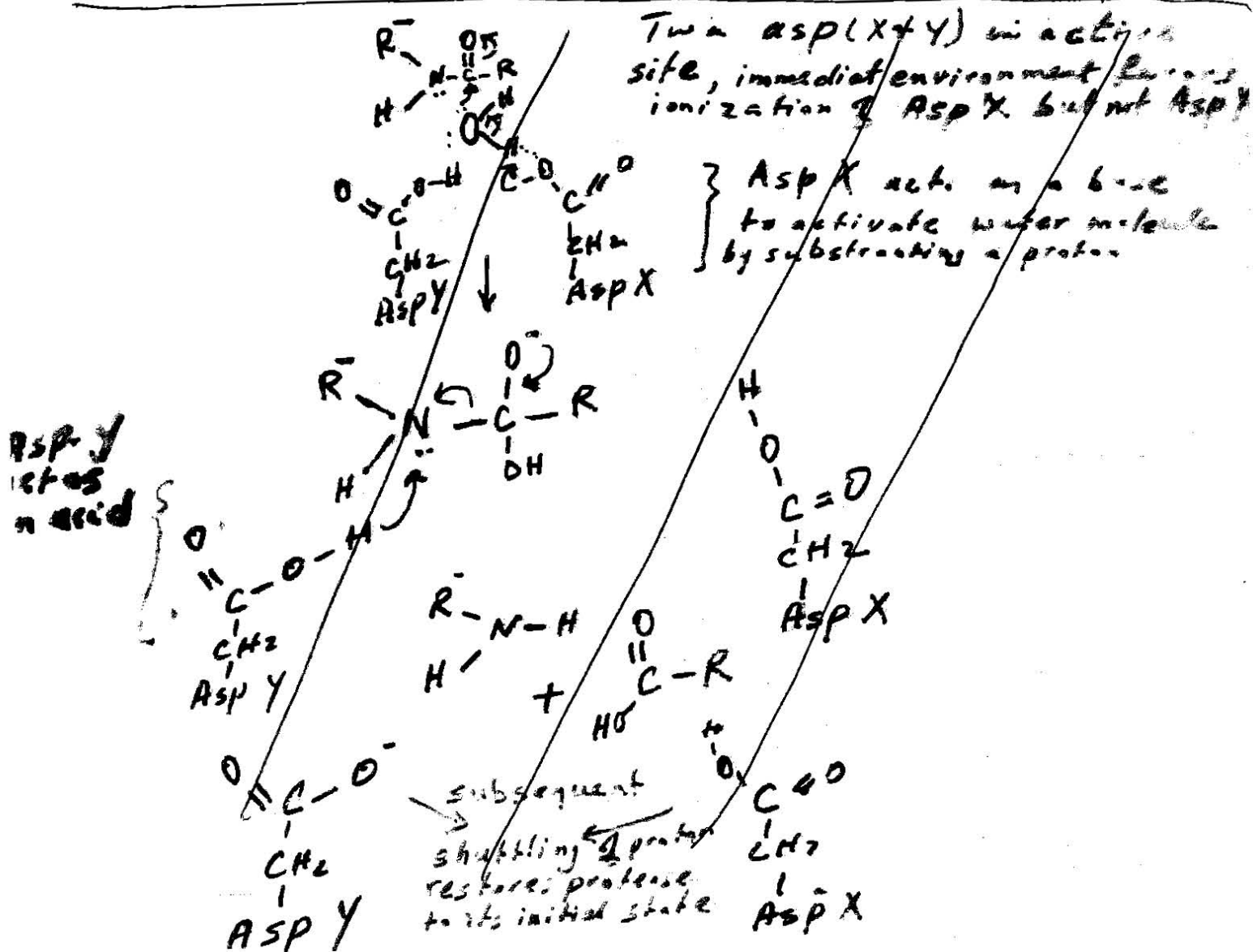
Chymotrypsin:-  
Employs

# Proteins With A Common Catalytic Mechanism:

- Proteolytic enzymes are classified by their catalytic mechanism
- Serine proteases
- Aspartate proteases
- metalloproteases
- endopeptidases
- exopeptidases

## Aspartic protease family

e.g. pepsin, lysosomal cathepsin, HIV protease



B. The active site of chymotrypsin

Chymotrypsin is a proteolytic enzyme secreted into the small intestine by the pancreas in the form of an inactive precursor or zymogen called chymotrypsinogen. Chymotrypsinogen, which has a single polypeptide chain of 245 residues and five intrachain disulfide cross-links contributed by five cystine residues, is activated by the action of trypsin, another proteolytic enzyme in the intestine. Trypsin removes two dipeptides from positions 14-15 and 147-148 of chymotrypsinogen by hydrolysis to yield active chymotrypsin, which thus has three polypeptide chains, covalently connected by two disulfide cross-links, one between chains A and B and the other between chains B and C.

as shown in Figure 1. Chymotrypsin requires for activity histidine residue 57 and aspartic acid residue 102 in chain B, as well as serine residue 195 in chain C. Although they are far apart in the sequence, and one is actually in a different chain from the others, these three residues lie very close together in the three-dimensional structure of the enzyme molecule. This is shown in the scale drawing of the backbone of the chymotrypsin molecule (Figure 2), as deduced from the x-ray diffraction pattern of crystalline chymotrypsin by David M. Blow and his colleagues of the University of Cambridge. In this drawing the R groups of only the three specific residues at the active site are shown.

Figure 1

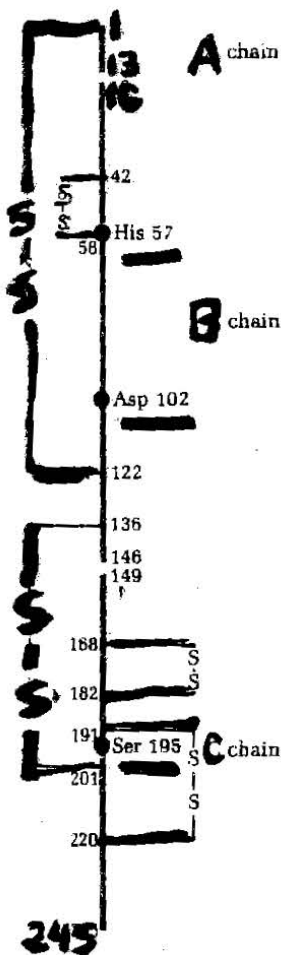
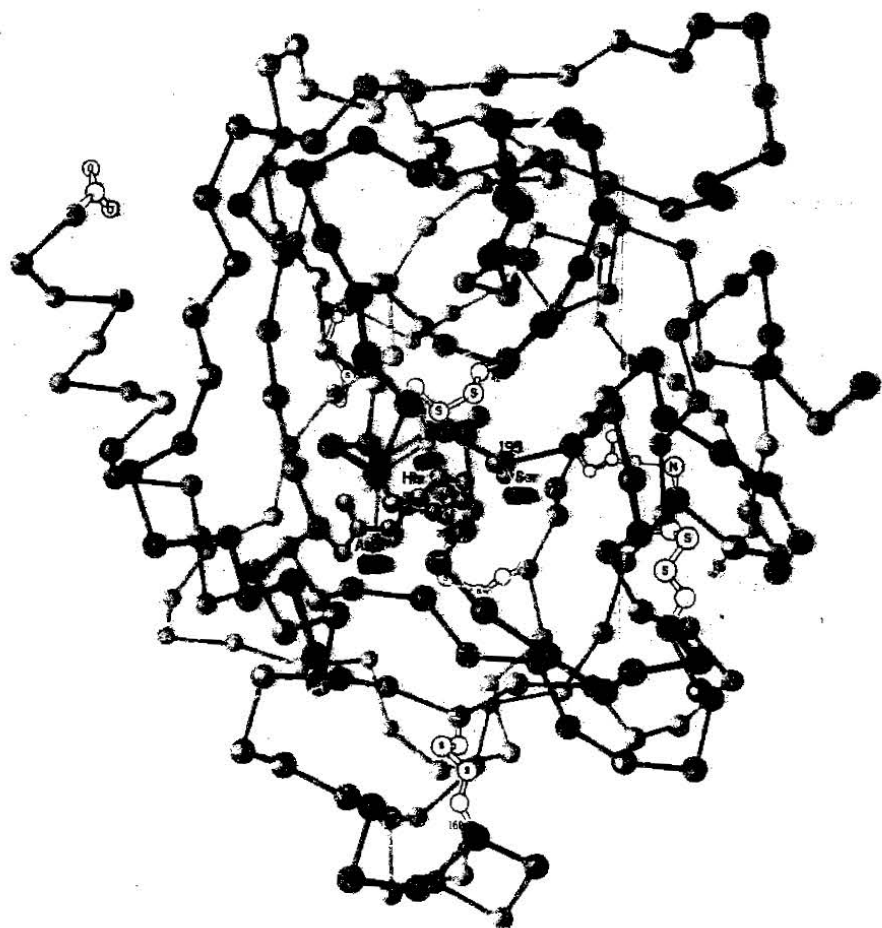
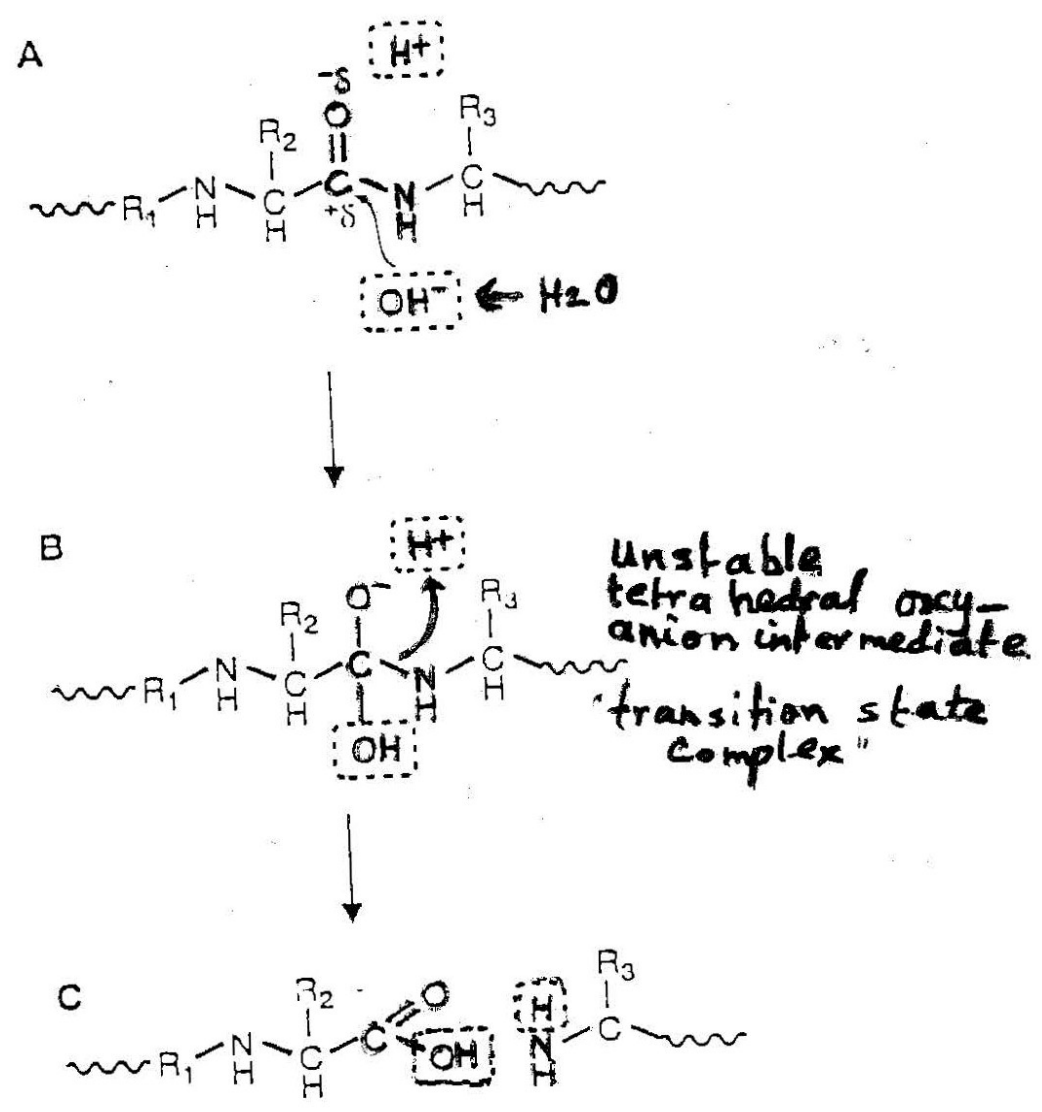


Figure 2

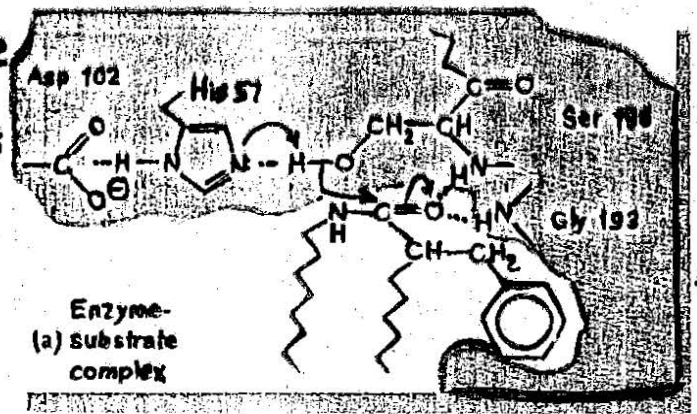


**TERTIARY  
STRUCTURE**

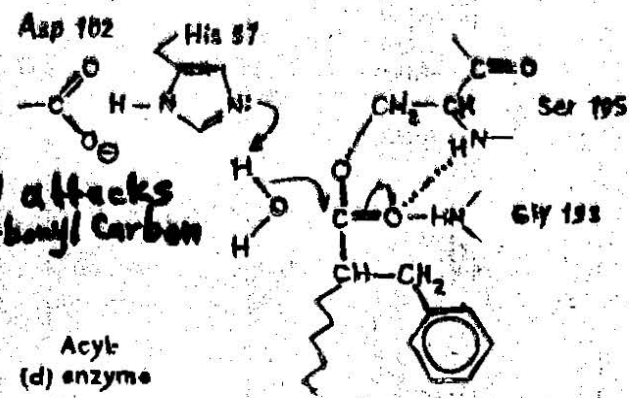
# Proteolysis in the absence of chymotrypsin:



Substrate binding  
His activates Ser for nucleophilic attack



H<sub>2</sub>O attacks the carbonyl carbon



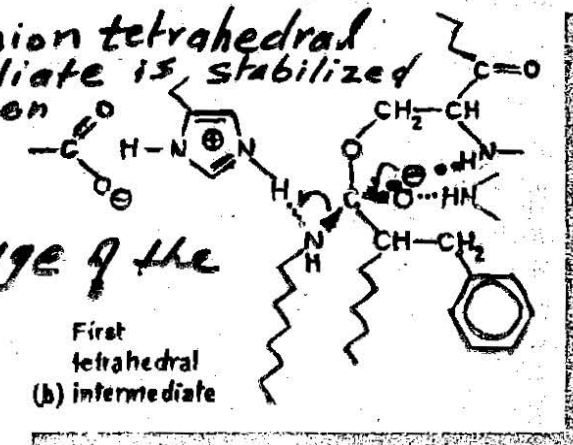
Serine Proteases:  
family of enzymes  
uses serine residue  
in catalytic site to  
hydrolyze peptide  
bonds.

e.g. trypsin,  
Chymotrypsin and  
thrombin

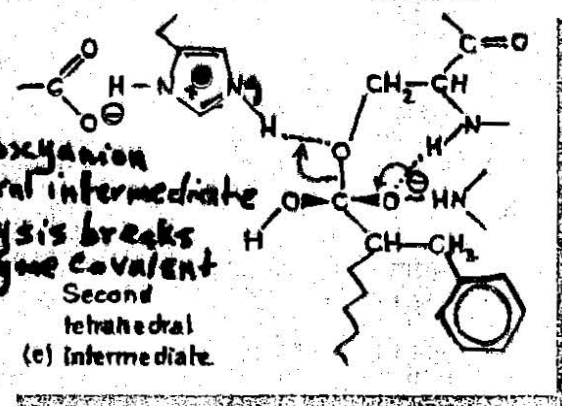
- have a catalytic triad of Ser, His and Asp.
- form covalent-acyl enzyme intermediate.

The oxyanion tetrahedral intermediate is stabilized by hydrogen bonds

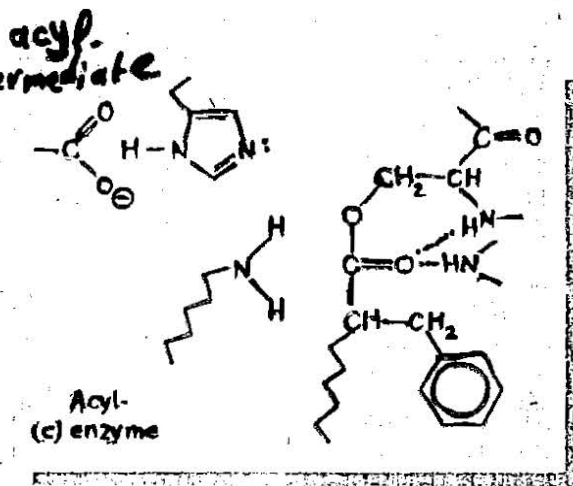
Cleavage of the Peptide bond



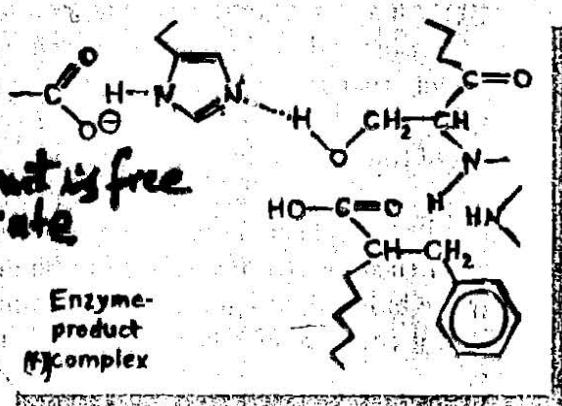
Second oxyanion tetrahedral intermediate  
Acid catalysis breaks the acyl-enzyme covalent bond



Covalently acyl-enzyme intermediate



The product is free to dissociate



# Steps in the cleavage of a peptide bond by chymotrypsin:-

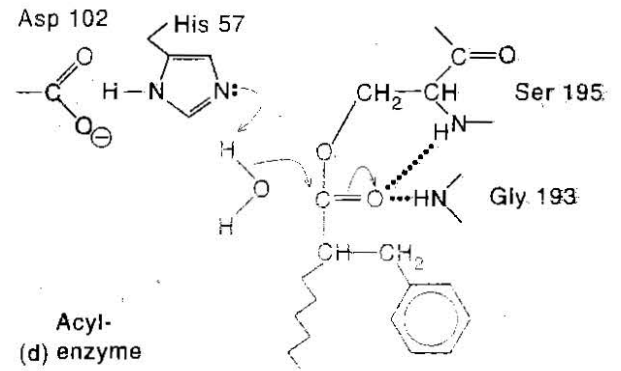
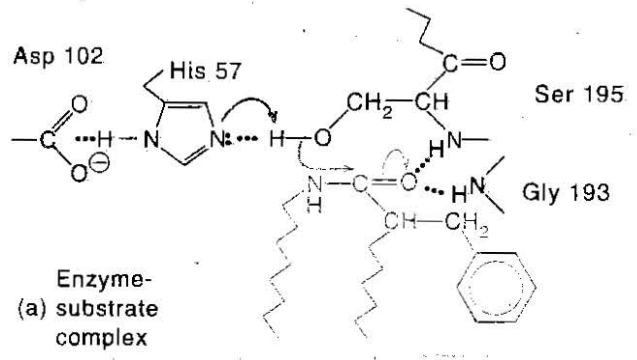
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Conformational change

- Covalent Catalysis
- General Acid-Base Catalysis

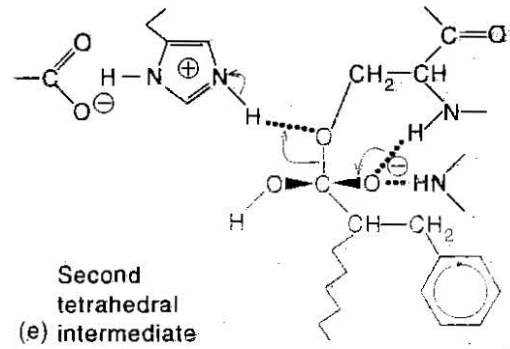
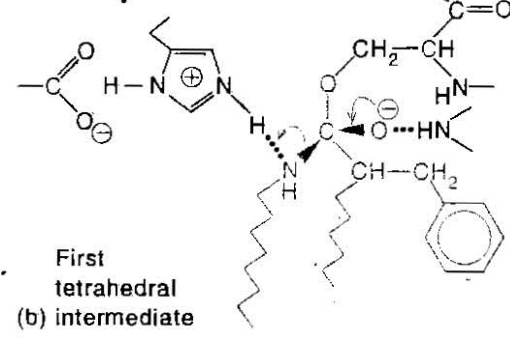
His activates ser for nucleophilic attack

Water attacks the carbonyl carbon



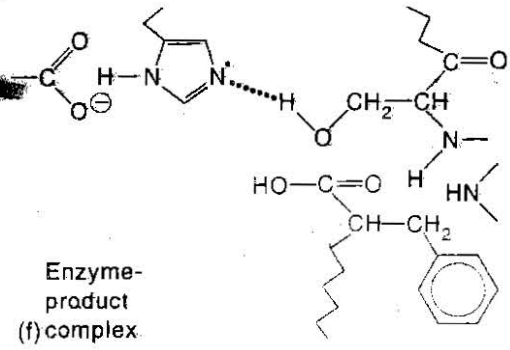
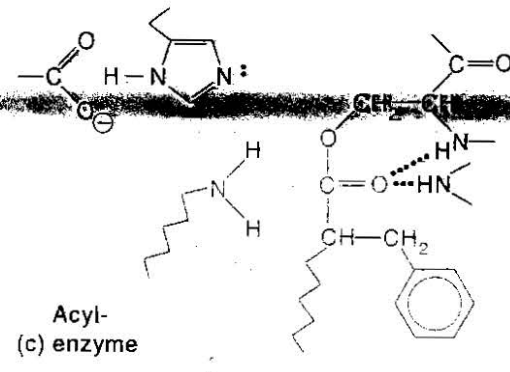
1st oxyanion tetrahedral intermediate is stabilized by hydrogen bonds

Second oxyanion tetrahedral intermediate



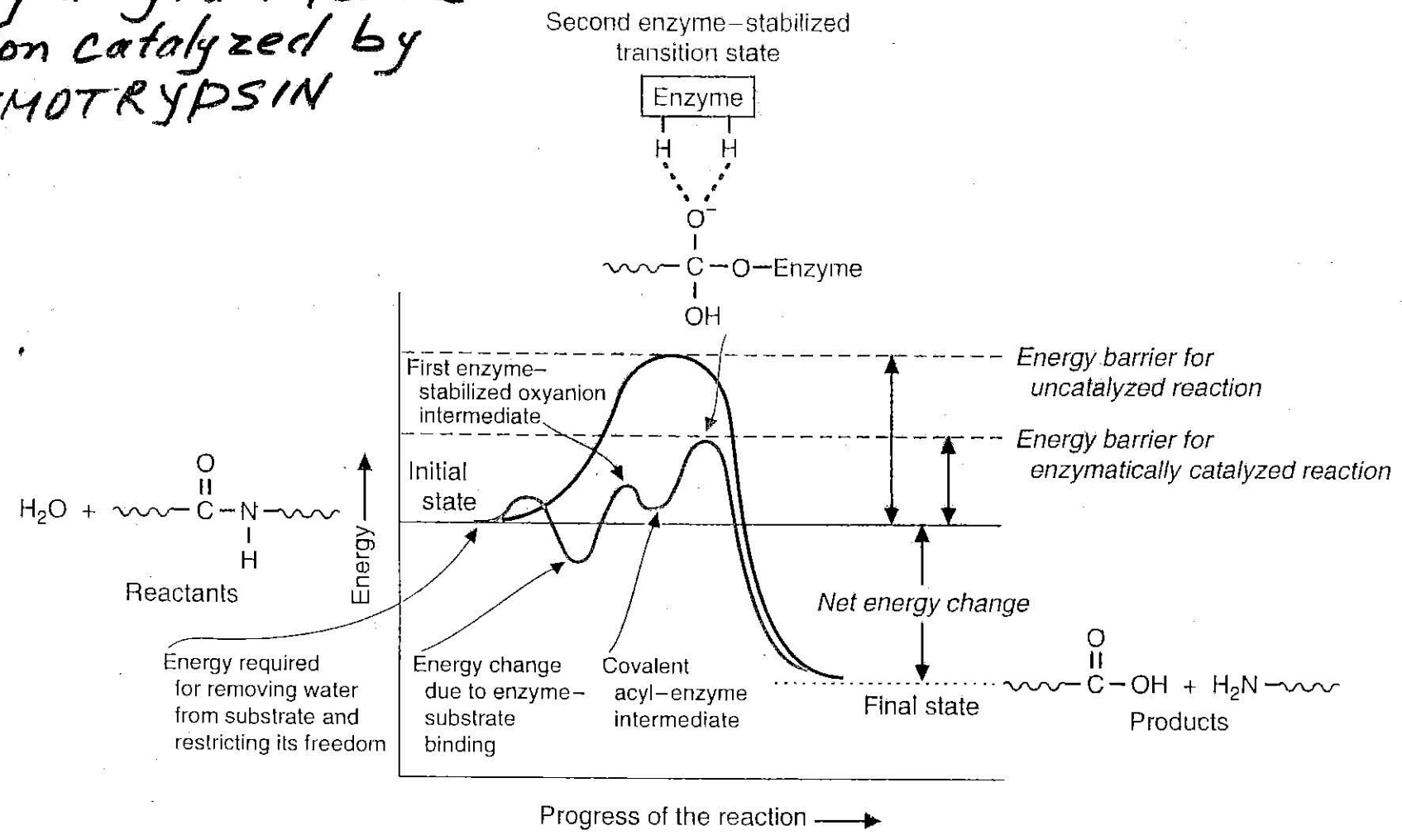
Cleavage of peptide bond

Acid catalysis breaks the acyl-enzyme covalent bond



38 Probable Mechanism of Chymotrypsin Action Figure 9.11

# Energy diagram for the reaction catalyzed by CHYMOTRYPSIN





# Mechanism of Enzyme Action

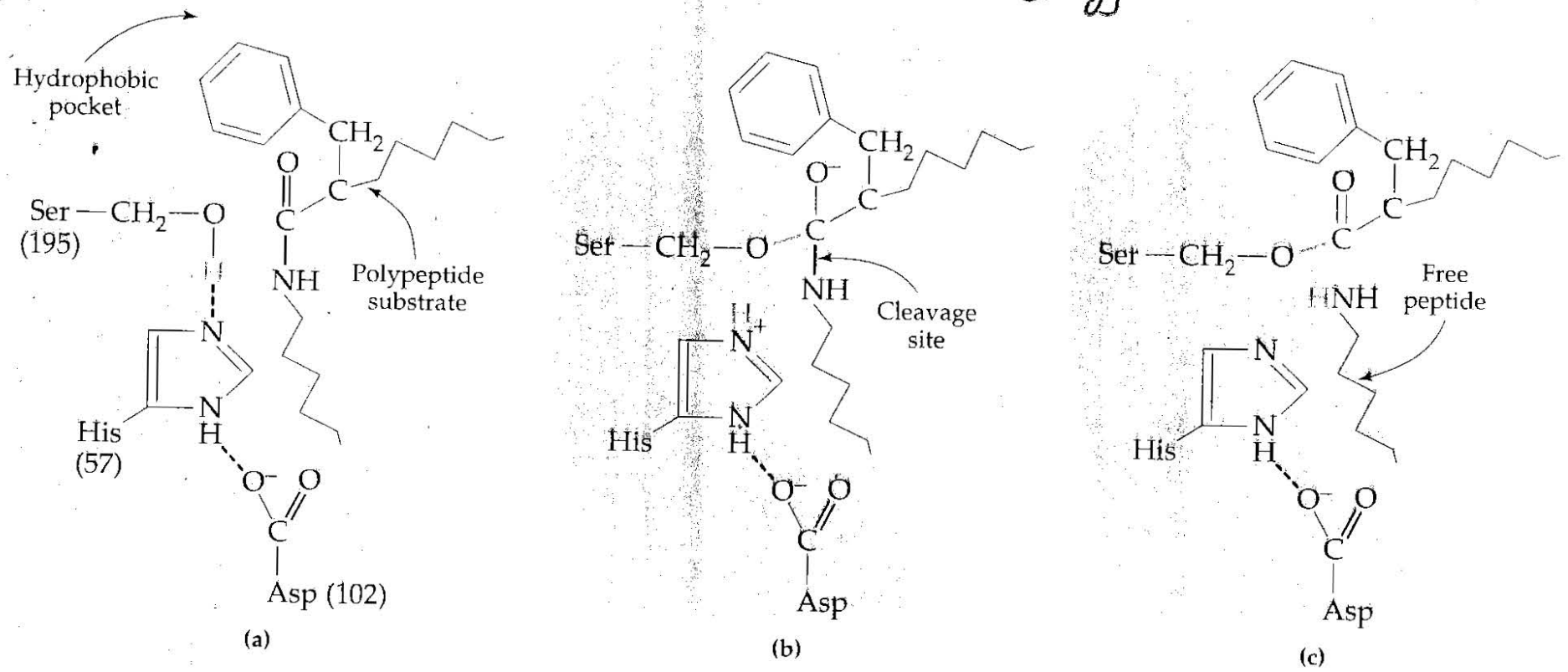
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*Summary*

Figure 19.4 Active site of chymotrypsin

- 1- Proximity effect
- 2- Orientation effect

- 3- Catalytic effect (acid-base catalysis)
- 4- Energy effect: lower the energy barrier by inducing strain in bonds



N — Tyr — phe — Trp — C