Enzymes

- Historical Background

I Enzyme Catalyzed Reaction

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

- Enzymes are Proteins (Exception Ribozymes)
- High Catalytic Power increase rate by 10^6 to 10^{14}-fold
- High Specificity
- Enzymes are Regulated

A. The Active site

- 3-dimensional structure
- Role of functional groups, cofactors
- Transition state

B. Substrate Binding site

1. Lock-and-key Model
2. Induced-fit Model

C. The Transition State Complex

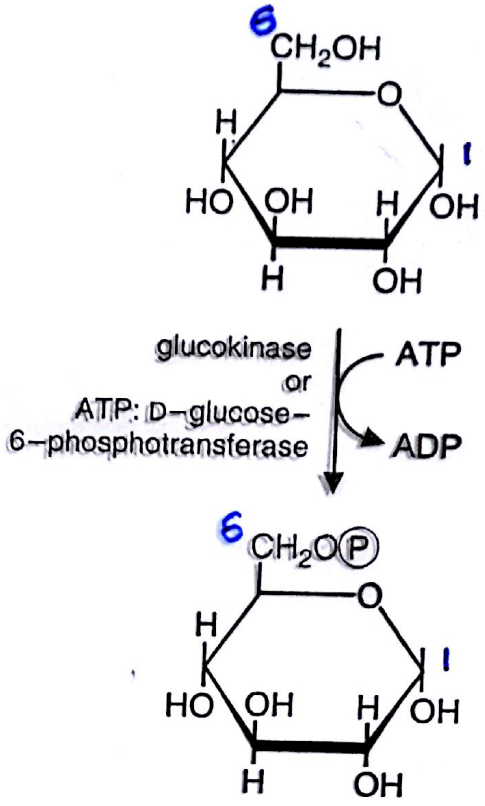
Active Sites of Enzymes Have Some Common Features :-

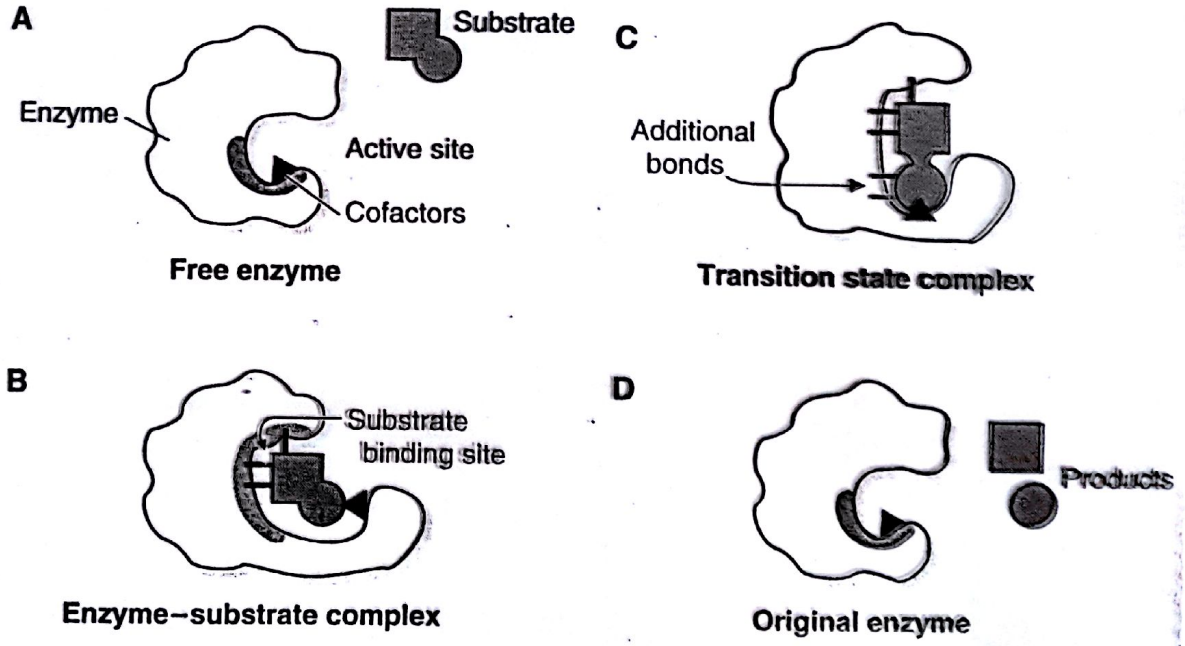
- The catalytic groups
- The active site takes up a relatively small part of the total volume of an enzyme
- The active site is a three-dimensional entity formed by groups that come from different parts of the linear amino-acid sequence
- Substrates are bound to enzymes by multiple weak interactions.
- Active sites are clefts or crevices
- The specificity of binding depends on the precisely defined arrangements of atoms in an active site.

Emil's Fisher's Lock & Key model

Koshland's Induced Fit model

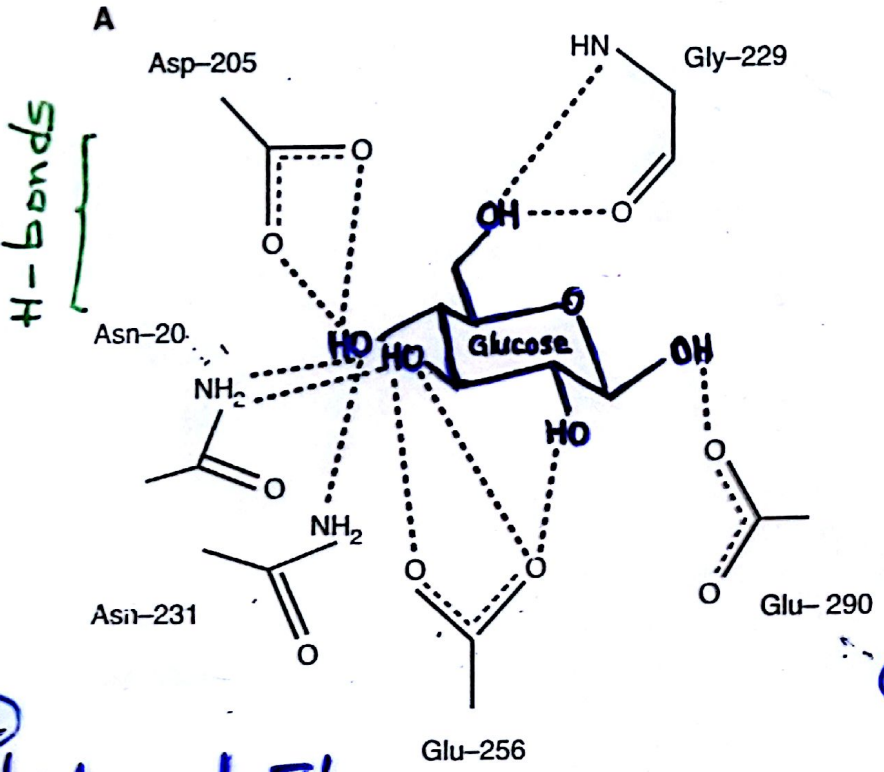
A-The Active site

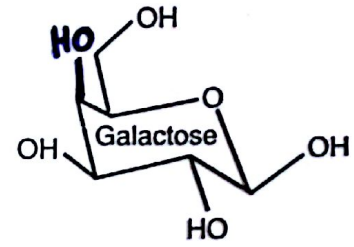




B- Substrate Binding sites

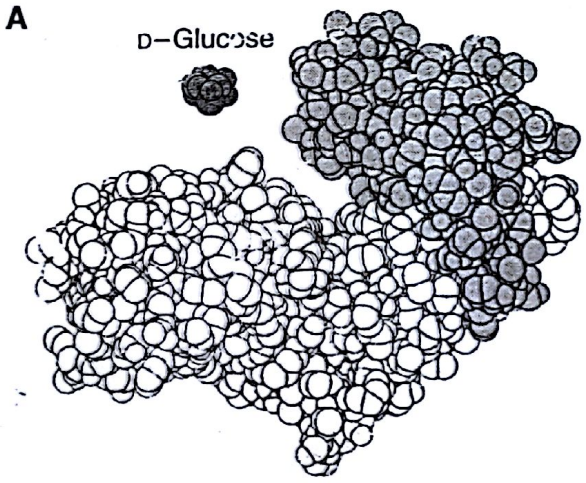
S binds E through 3
- hydrophobic
- Electrostatic
- H-bonds



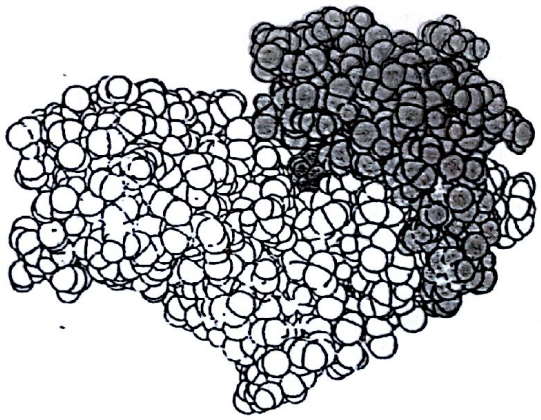


① Lock-and-key model for substrate binding

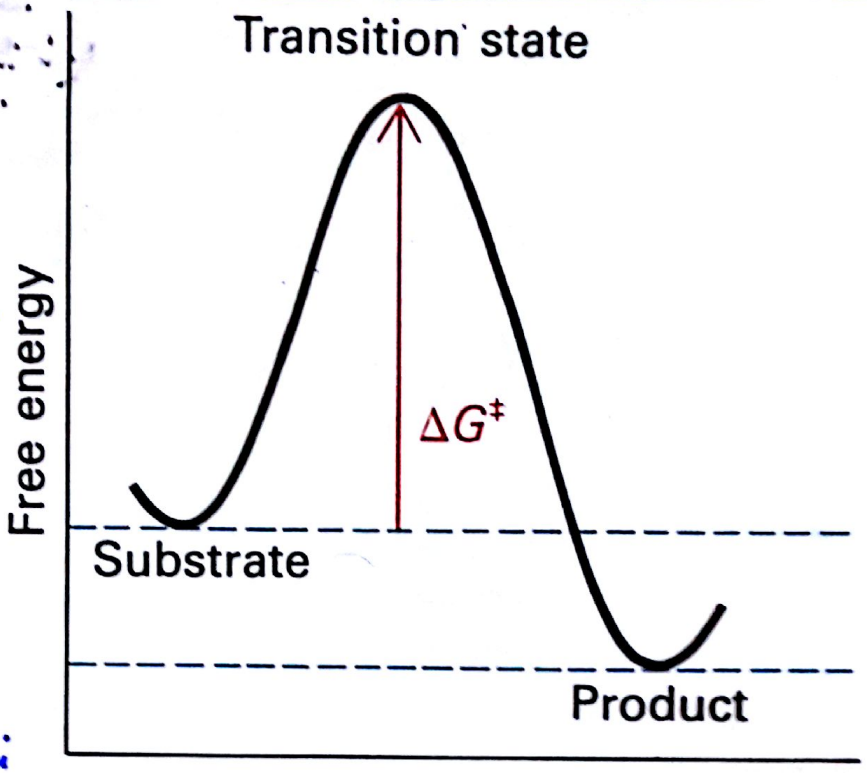
② Induced Fit Model



Yeast HK (or human Gk)



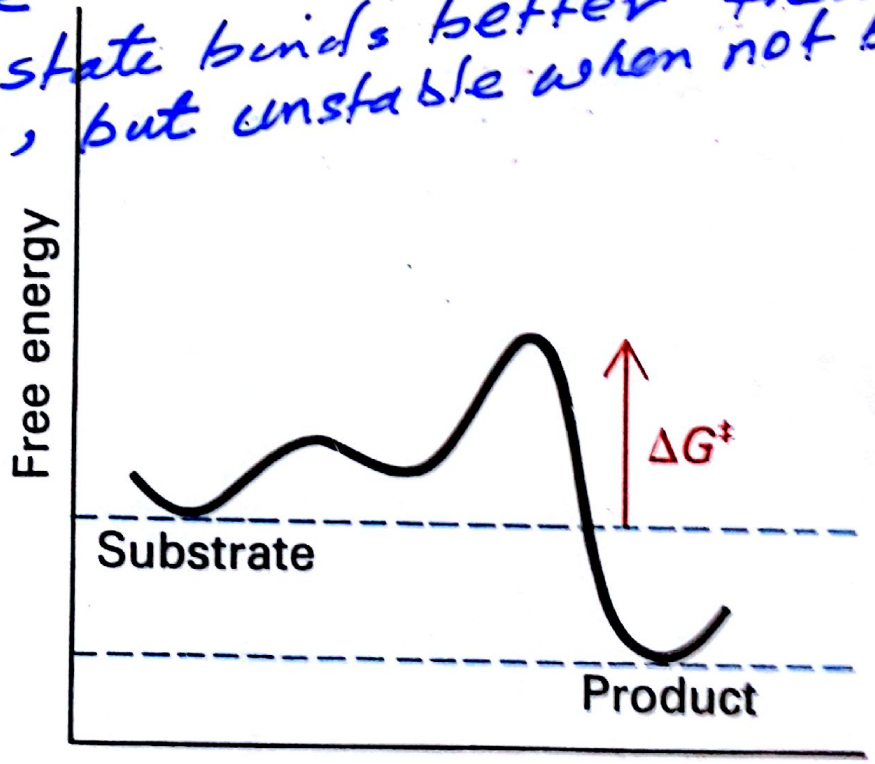
C- The Transition State Complex



A

In transition state:
- Bonds in substrate are max. strained
- In others Electronic configuration in substrate becomes very strained and unstable

Transition state binds better than the substrate, but unstable when not bound to enzyme



B

Figure 8-8

Stryer: *Biochemistry*, Third Edition

The energy diagram for the decomposition of

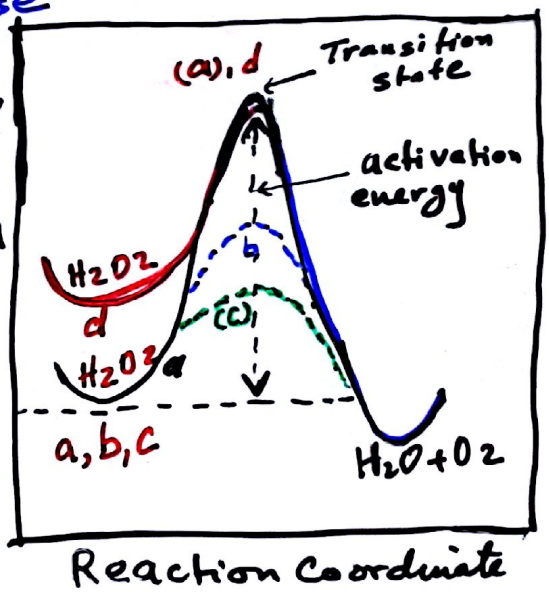
$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$

Curve a :- uncatalyzed

Curve b :- + iron catalyst ↑ 30,000

Curve c :- + Catalase ↑ 100,000,000

Curve d : uncatalyzed but at elevated temp



$2H_2O_2 \rightleftharpoons 2H_2O + O_2$

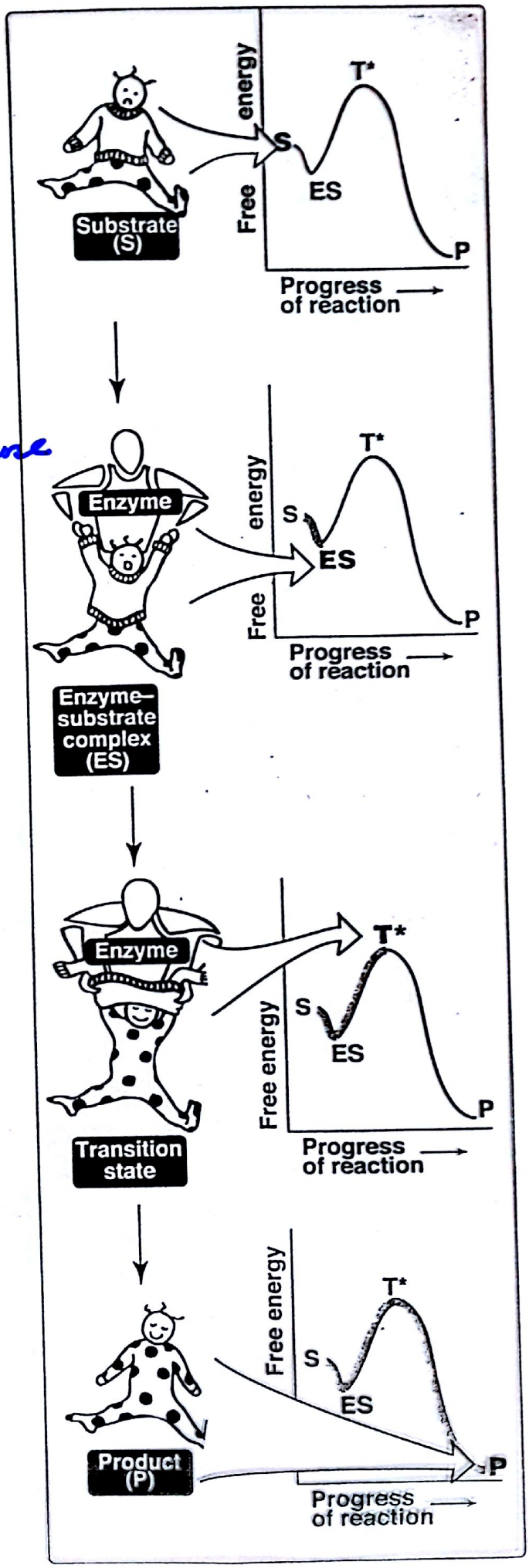
Uses of Transition State Analogs

4c

- Better inhibitors than substrate analogs
- Drugs
- Pro-drugs
- to make Catalytic Antibodies "Abzymes"

  e.g. Cocaine esterase

A dip occur because of initial multiple weak bonds between E and S



Enzymes Nomenclature

I : shortnames - recommended + convenient

- substrate + "ase"
  e.g. glucosidase, urease, sucrase
- "ase" added to description of the action
  lactate dehydrogenase, adenyl cyclase
- trivial names
  trypsin, pepsin

II Systematic name

Enzyme Commission (EC) of the IUBMB classified enzymes into six major groups according to type of reaction catalyzed. The suffic ase is added to a fairly complete description of the chemical reaction catalyzed and naming the substrates:-

e.g. D-glyceraldehyde 3-phosphate:NAD oxidoreductase

glucokinase (common name)

ATP: D-hexose 6-phosphotransferase

EC number is: (EC 2.7.1.2)

2 → EC general class (transferase)

7 → e.g subclass for transfer of phosphorus-containing group

1 → refers to (sub subclass) transfer to an alcoholic acceptor

2 → specific number of the enzyme

1. Oxidoreductases

. Very common reactions and a broad class

- e.g. dehydrogenases

accepts or donate hydride ions or hydrogen atoms

- i Hydroxylases, ii Oxidases, iii Oxygenase
  - O_2 → acceptor by [S] (both)
  - O_2 → Water
  - or O_2 → Water or H_2O_2 (Requires metal ion)

$$CH_3CH_2OH + NAD^+ \xrightarrow{AD} CH_3C(=O)H + NADH + H^+$$

NADH: hydride ion

2. Transferases

Catalyze transfer of C-, N- or P- containing groups.

e.g.
- Kinase : transfer of ~P
- glycosyl transferase : carbohydrate residue
- acyl transferase : fatty acyl group

(good leaving group)

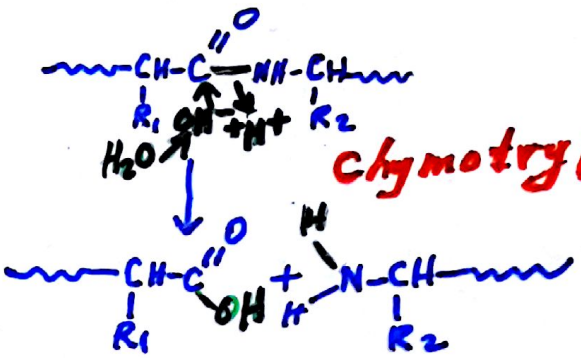
- Aminotransferase or transaminases
- Syntheses : synthesis of physiologically impt. compd. e.g. GS.

Asp + α-KG $\xrightleftharpoons[\text{Aminotransferase}]{PLP}$ O.A. + Glu

3- Hydrolases

Cleavage of C-O, C-N or C-S bonds by addition of water as OH^- and H^+

e.g. Proteases

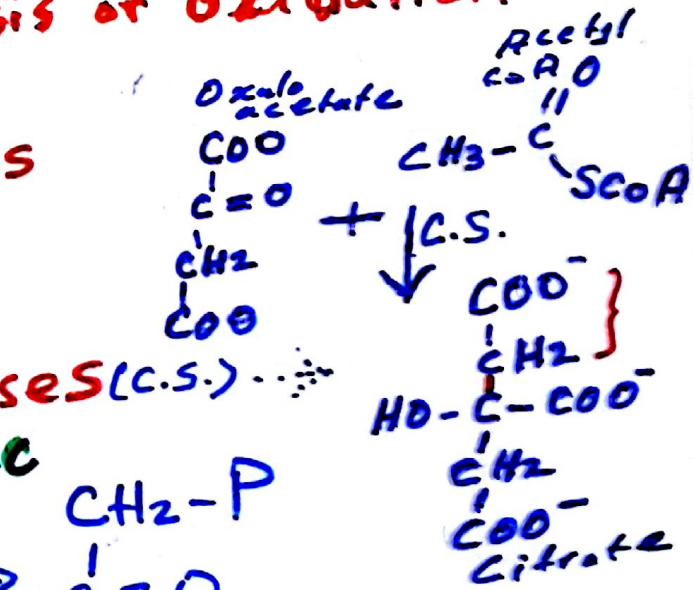


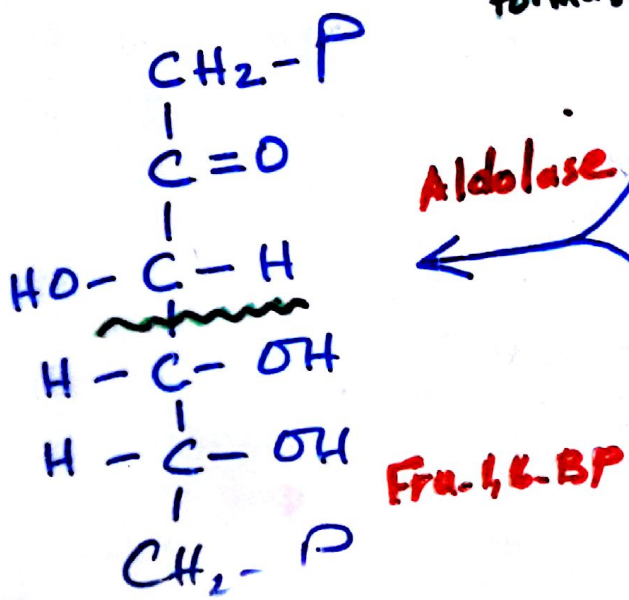
4- Lyases

Cleavage of C-C, C-O & C-N bonds by means other than hydrolysis or oxidation.

giving rise to compounds with double bond or the reverse addition to a double bond

- Aldolases
- decarboxylases
- thiolases
- Dehydratases (double bond ← H_2O)
- Some synthases (C.S.) ... formation of C-C





5- Isomerases

Isomerases — rearranging bond structures (e.g. optical, geometrical isomers)

e.g. Mutases

G6P ⟷ G-1-P

eg.: DHAP (Ketotriose) ⟷ GA3P (aldotriose) (isomerase)

6- Ligases

synthesize C-C, C-S, C-O & C-N bonds coupled to cleavage of high energy phosphate bond

e.g. Carboxylases: Add CO_2, require biotin

Synthetases — to be distinguished from syntheses.

Synthetase are different from synthase under "Lyases" and "Transferases" as they derive energy from ATP

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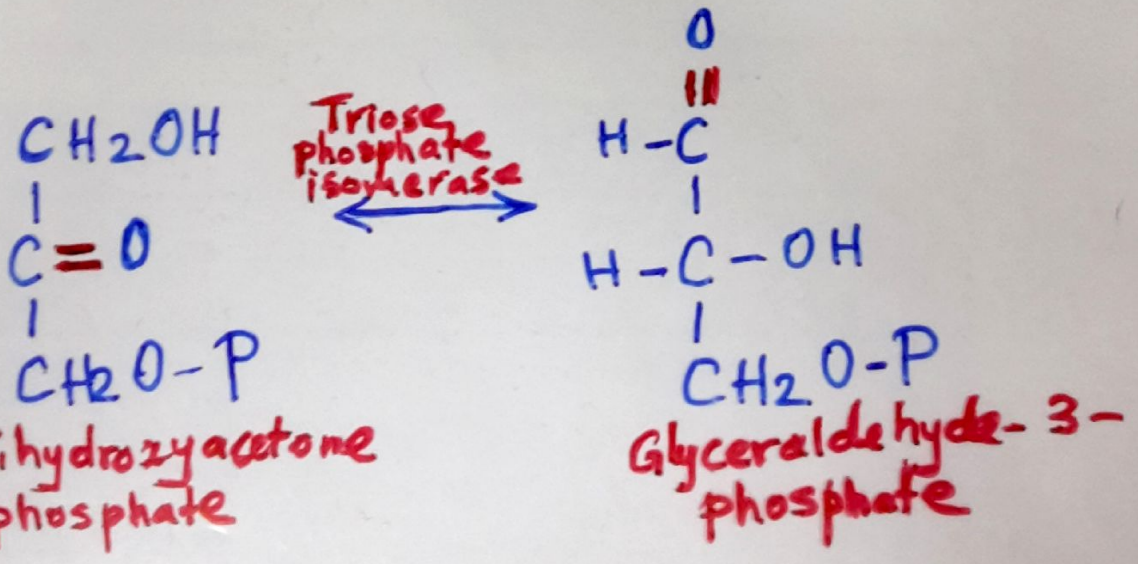
eg. for Isomerase



Dihydroxyacetone phosphate

Glyceraldehyde-3-phosphate

General Strategies of Enzyme Catalysis:-

- Proximity and Orientation
- Transition state Stabilization
- Acid-base catalysis
- Nucleophilic Catalysis
- Electrophilic Catalysis
- Covalent Catalysis

Chymotrypsin:-

Employs

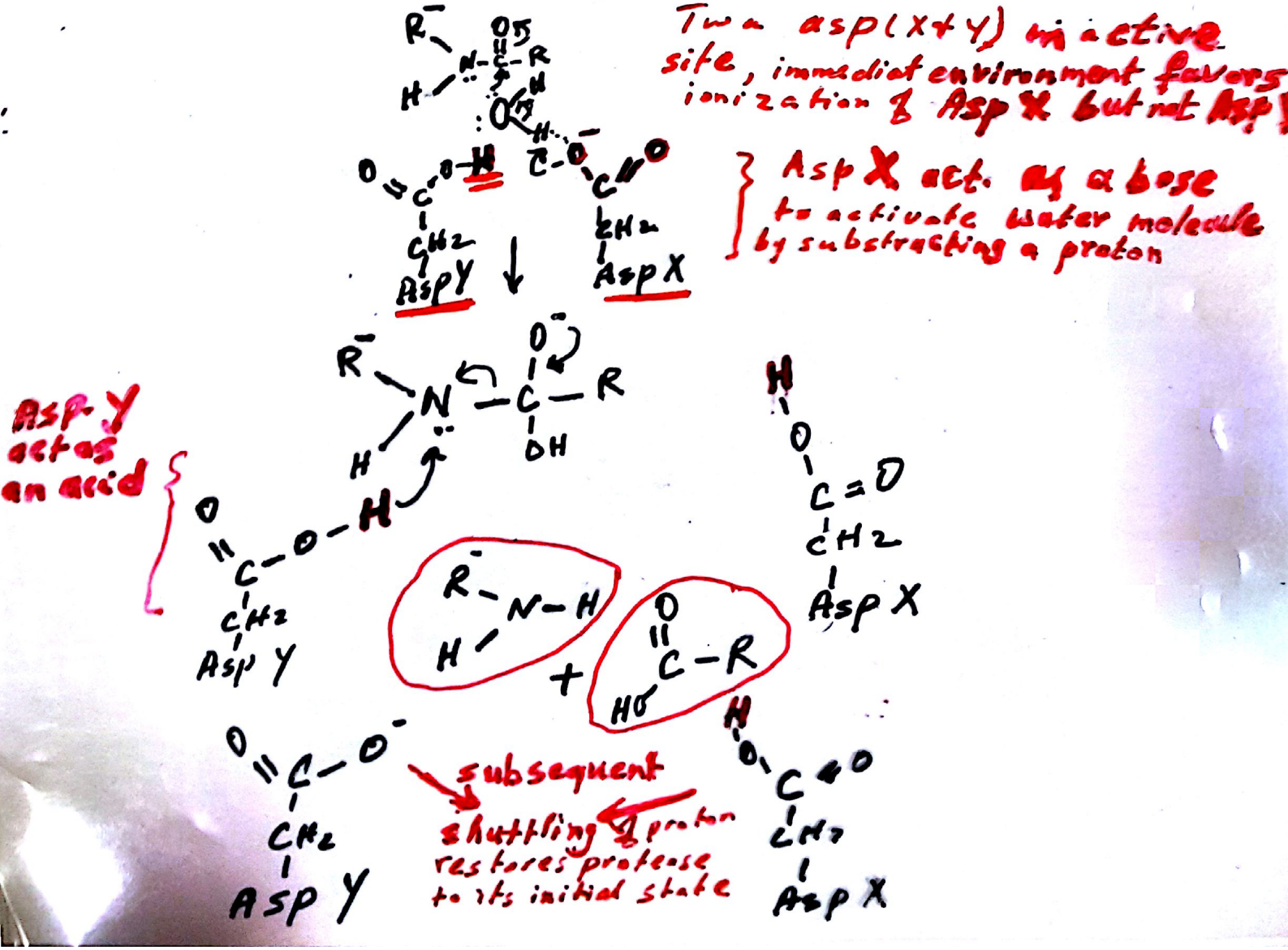
Proteins With A Common Catalytic Mechanism:

- 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

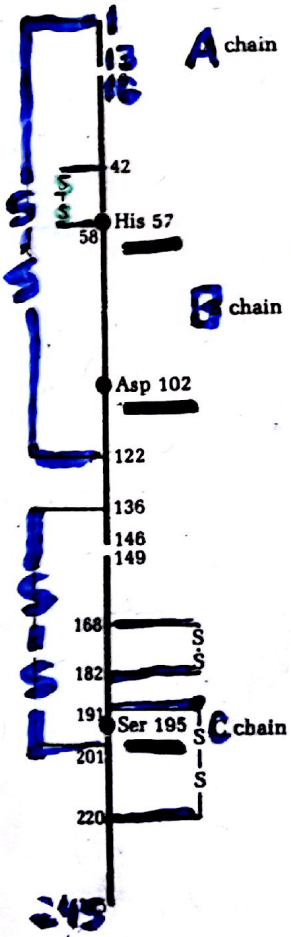
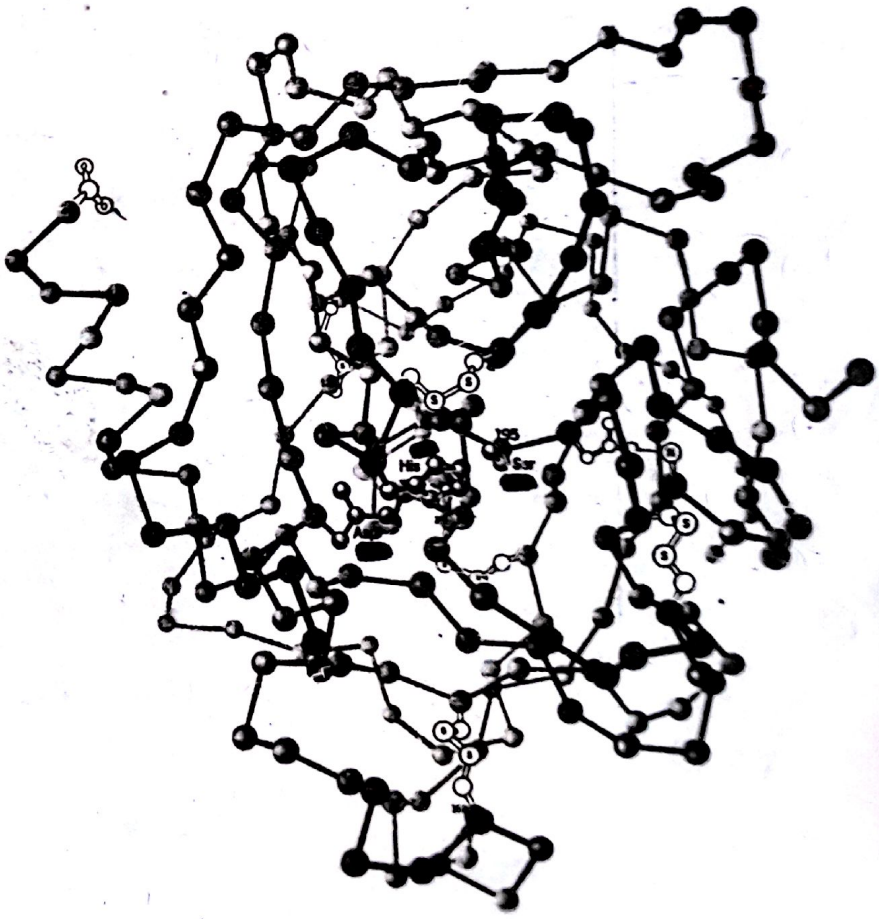
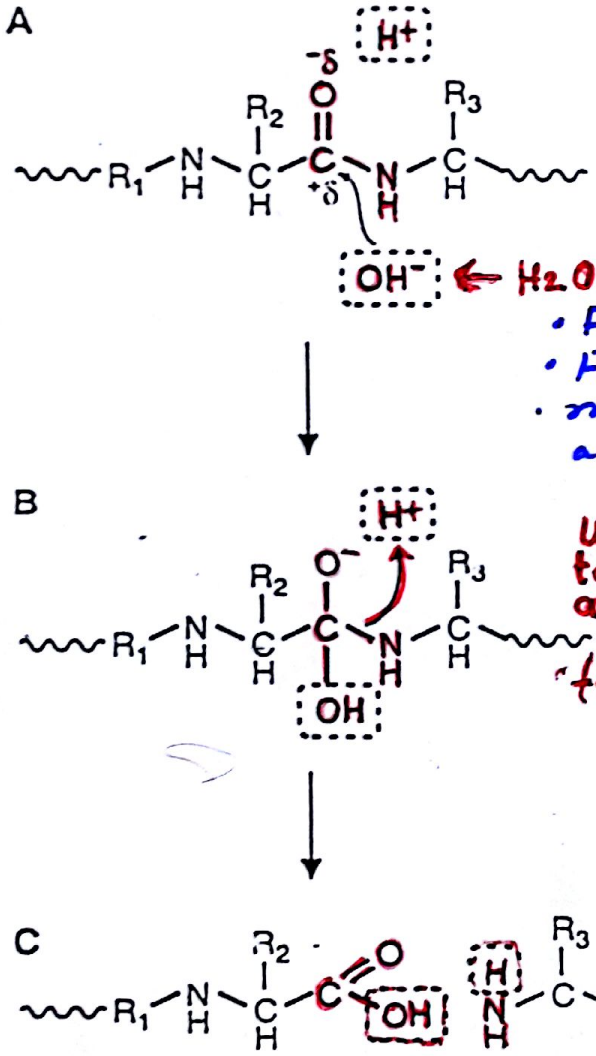


Figure 2



TERTIARY STRUCTURE

Proteolysis in the absence of chymotrypsin:

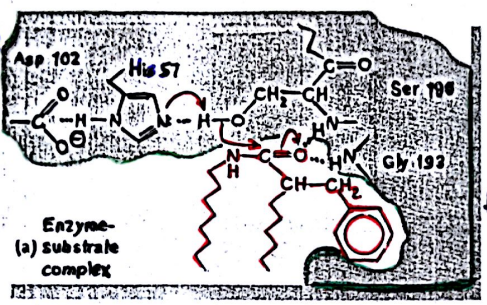


- Few OH^- are present
- Fewer are energized
- much fewer approximate and in right orientation

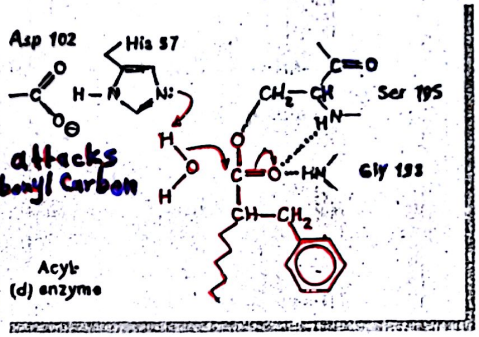
Unstable tetrahedral oxyanion intermediate "transition state complex"

Figure 9.11 Catalytic Mechanism of Chymotrypsin

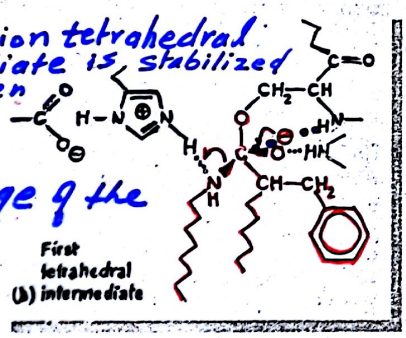
5a



· Substrate binding
· His activates Ser for nucleophilic attack

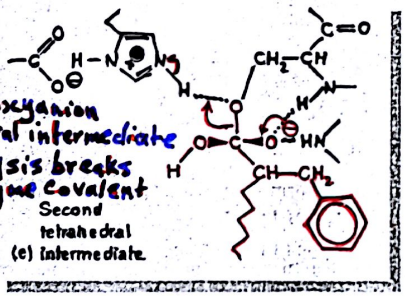


· H_2O attacks the carbonyl carbon

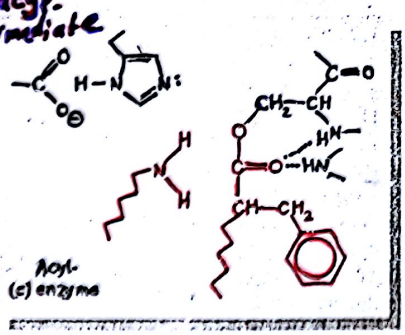


The oxyanion tetrahedral intermediate is stabilized by hydrogen bonds

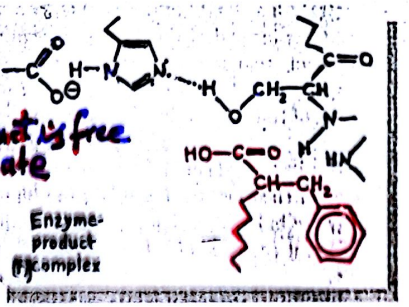
Cleavage of the Peptide bond



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



· Covalently acyl-enzyme intermediate



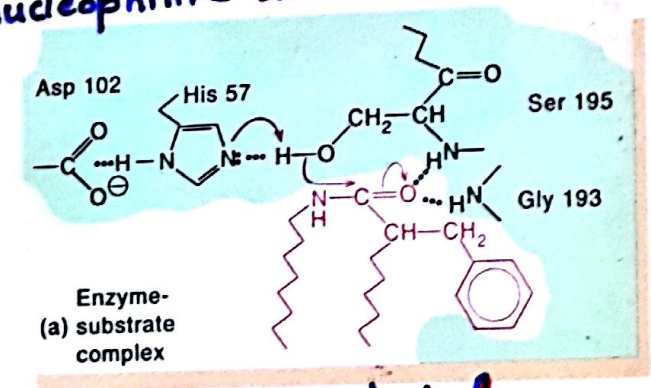
· The product is free to dissociate

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

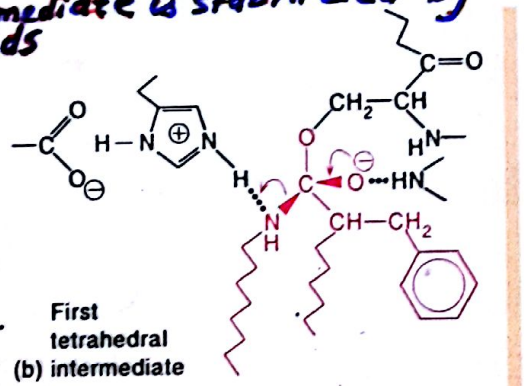
Steps in the cleavage of a peptide bond by chymotrypsin:-

- Covalent Catalysis
- General Acid-Base Catalysis

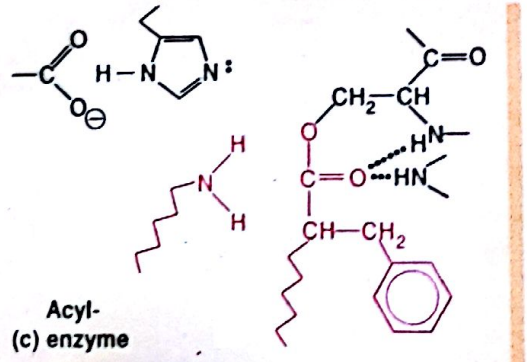
- Conformational change
- His activates ser for nucleophillic attack



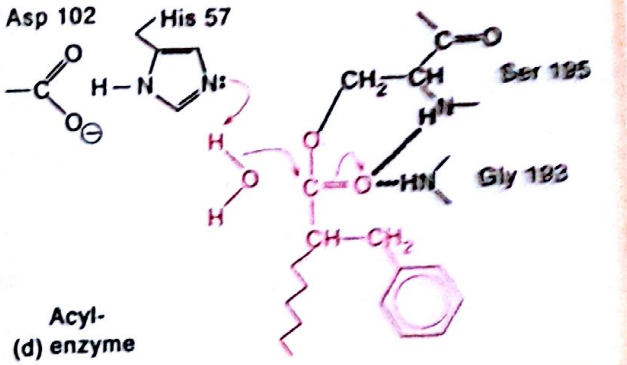
The oxyanion tetrahedral intermediate is stabilized by H-bonds



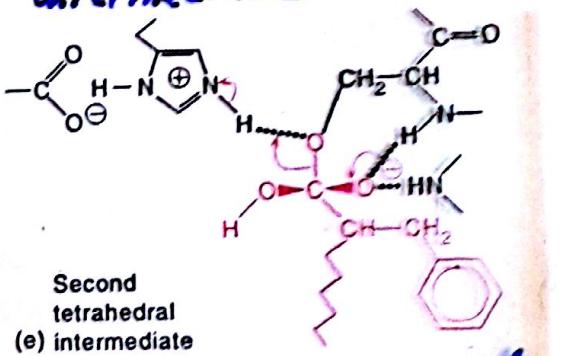
Cleavage of peptide bond



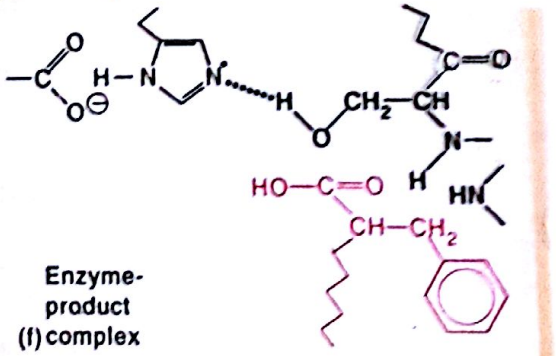
Water attacks the carbonyl carbon



Second oxyanion tetrahedral intermediate

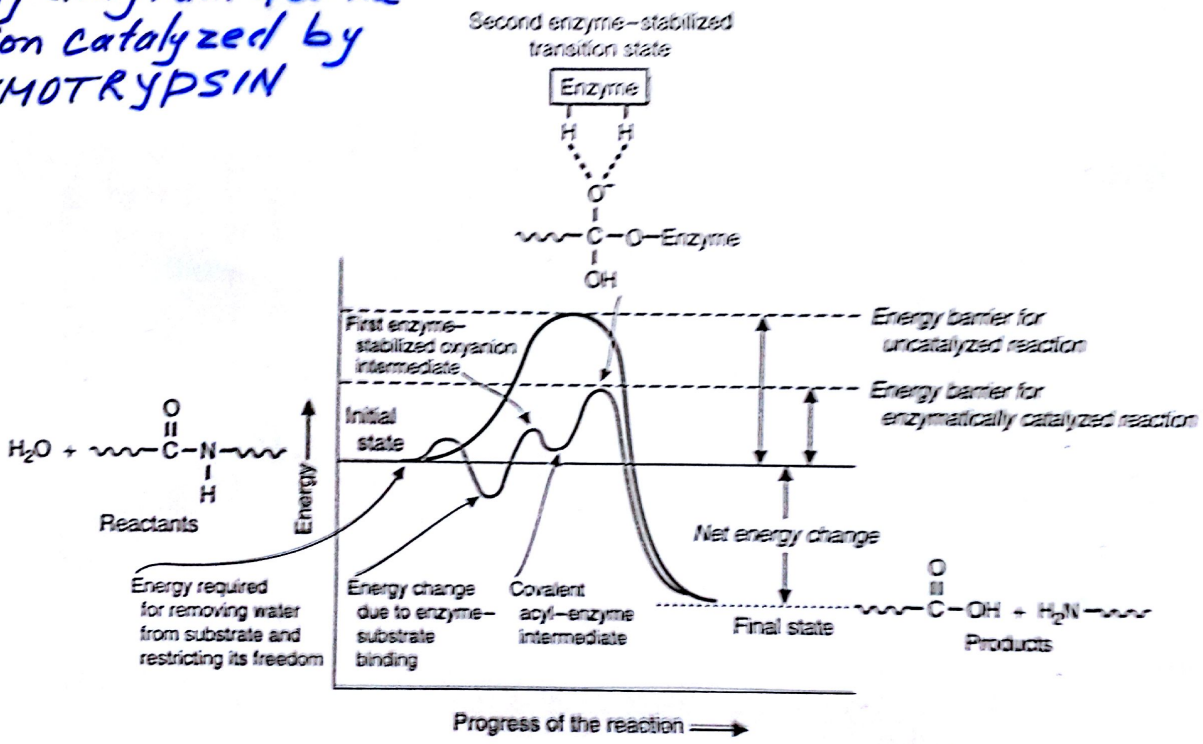


Acid catalysis breaks the acyl-enzyme covalent bond



8 Probable Mechanism of Chymotrypsin Action

Energy diagram for the reaction catalyzed by CHYMOTRYPSIN



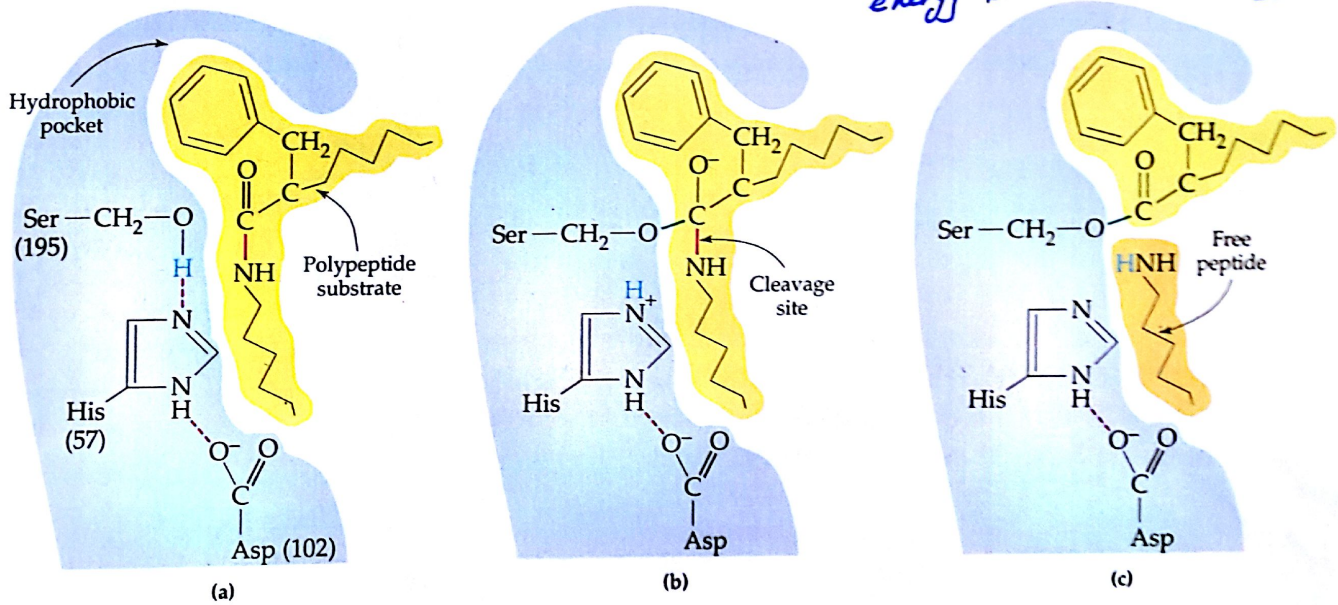
Mechanism of Enzyme Action

SUMMARY:-

T-167

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 Tryp C

Fundamentals of General, Organic, and Biological Chemistry, 6e
by John McMurry, Mary Castellion, David S. Ballantine, Carl A. Hoeger, and Virginia E. Peterson