Mechanism-Based Inhibitors

- Irreversible inhibitors
  - e.g. **DIFP**
  - **Aspirin** (Acetylsalicylic acid)

- Suicide inhibitors
  - Allopurinol
  - Pencilline

- Transition state analogues
  - **Abzymes**

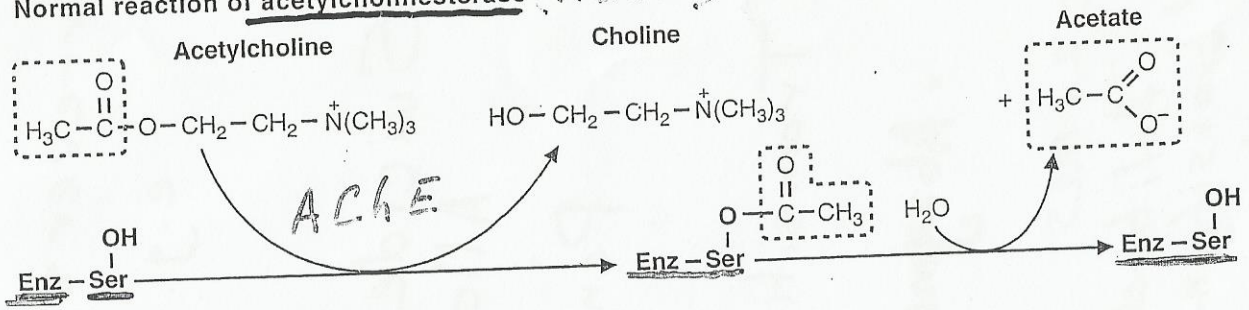
Abzymes
Antibodies raised against Analogs of transition-state complex
- Have an arrangement of amino acids in the variable region similar to the active site of the enzyme in the transition state

e.g. Abzyme for cocaine esterase transition state analogue ⟶ therapeutic in addicts

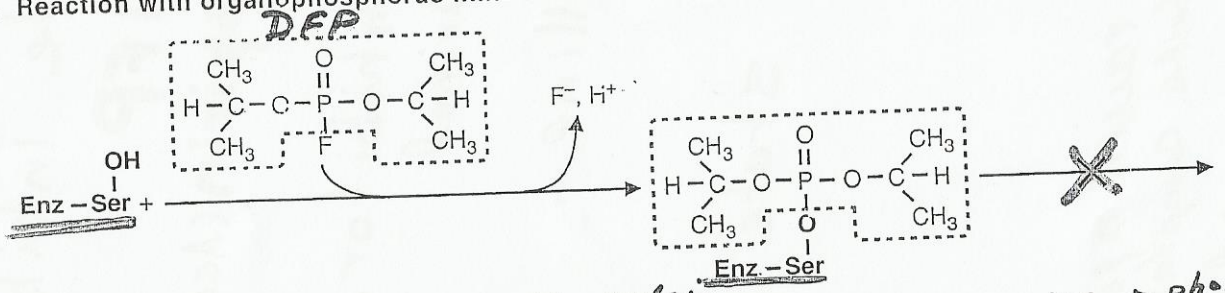
Mechanism-Based Inhibitors

A- React Irreversibly with functional groups - COVALEINT INHIBITORS

A. Normal reaction of acetylcholinesterase (AChE)



B. Reaction with organophosphorus inhibitors



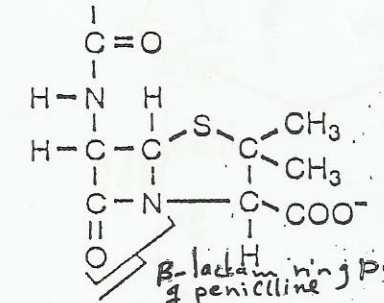
Irreversible Inhibition

of all the serines in the protein, DFP reacts only with the catalytically active serine → phosphate esters as the functional gr. is activated by other residues in the active site.

- Aspirin inhibit the prostaglandin endoperoxide synthase (Cyclo-oxygenase

B. Transition State Analogues and substrate analogues

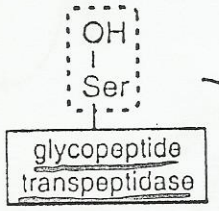
Bacterial Enzyme Glycopeptide transpeptidase



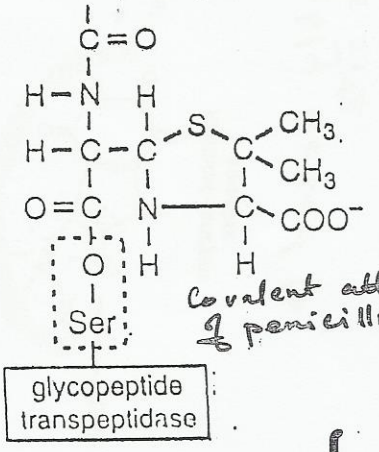
Penicilline

resemble the transition state of the substrate

bacterial serine proteases :- cleaves peptide bond between 2 D-alanine residues. (for cross-linking bacterial cell walls)



(Partial reaction to form permanent irreversible inhibitor in the active site)

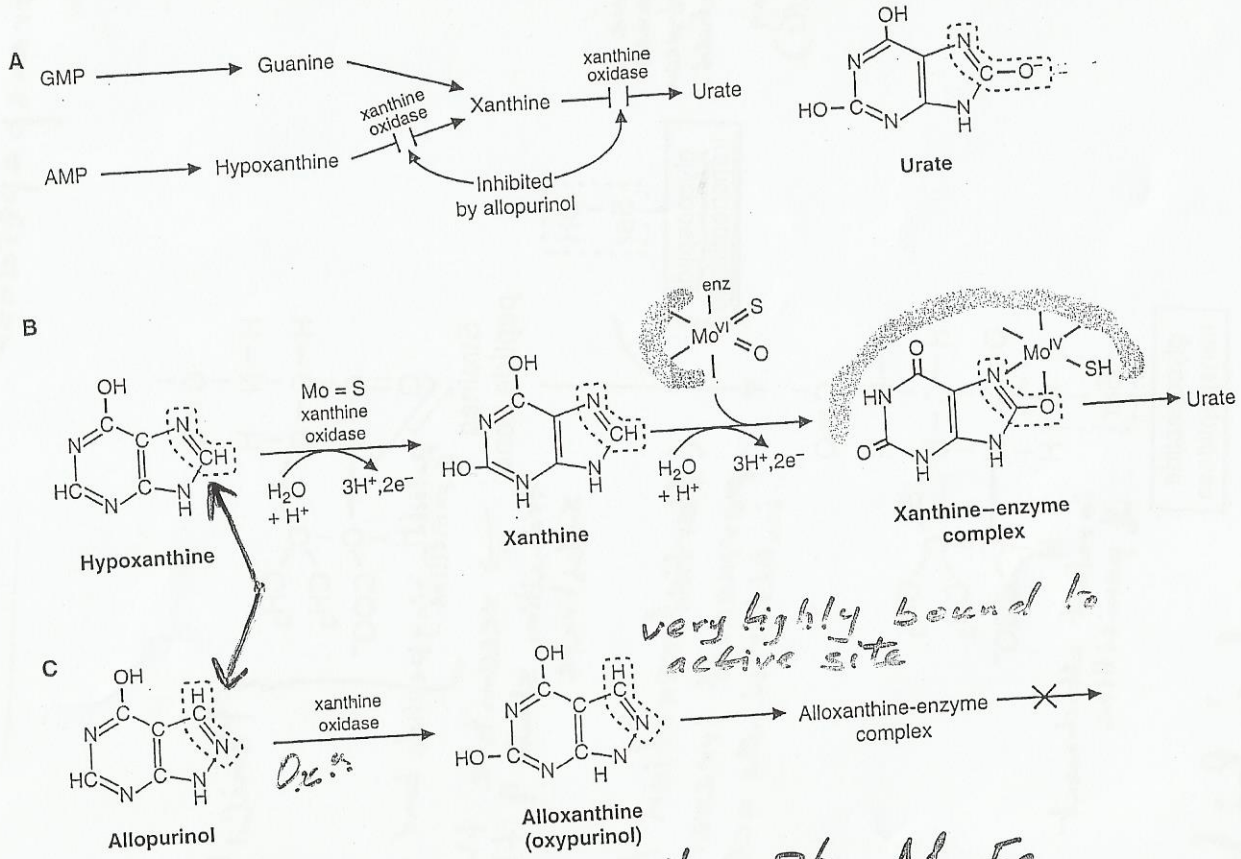


covalent attachment of penicilline

suicide inhibition

Allopurinol is a suicide inhibitor of Xanthine Oxidase

2)



C – Heavy Metals Inhibition :- Hg, Pb, Al, Fe
Bind functional groups, non-specific for enzymes
e.g. Hg binds many enzymes particularly at – SH gr.
[illegible] T.P.L. bind replacing normal functioning metal [illegible] calmodulin + Protein Kinase C

General Strategies of Enzyme Catalysis:-

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

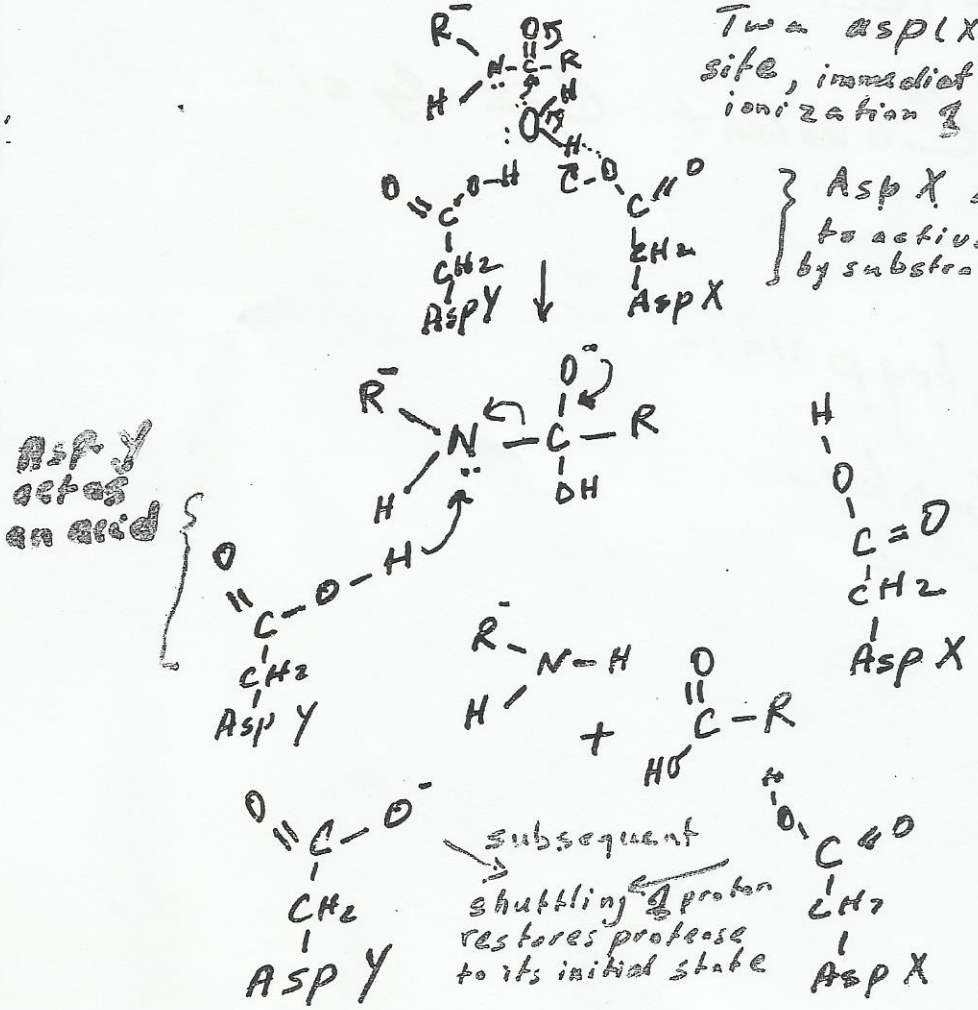
Chymotrypsin:-
Employs

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

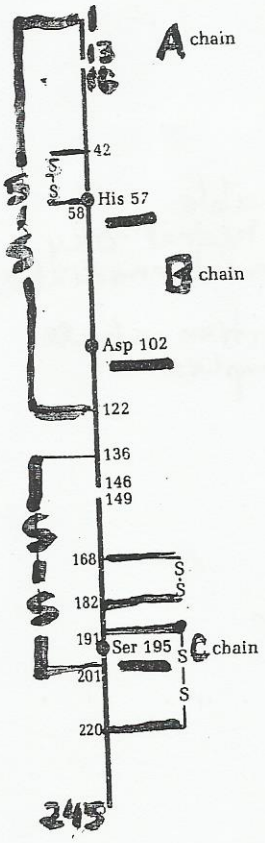
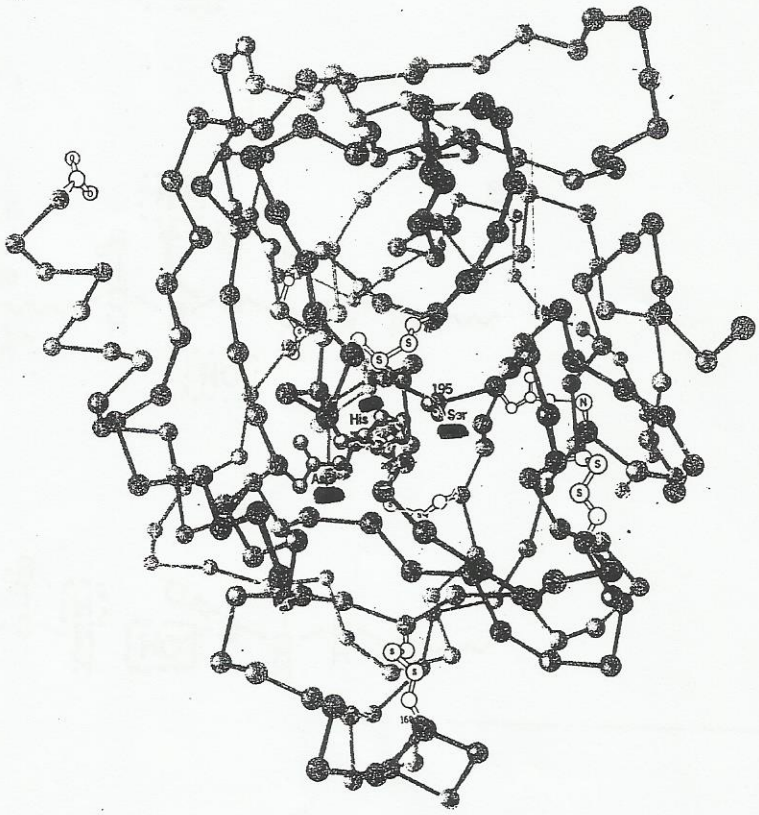


Figure 2



TERTIARY STRUCTURE

Proteolysis in the absence of chymotrypsin:

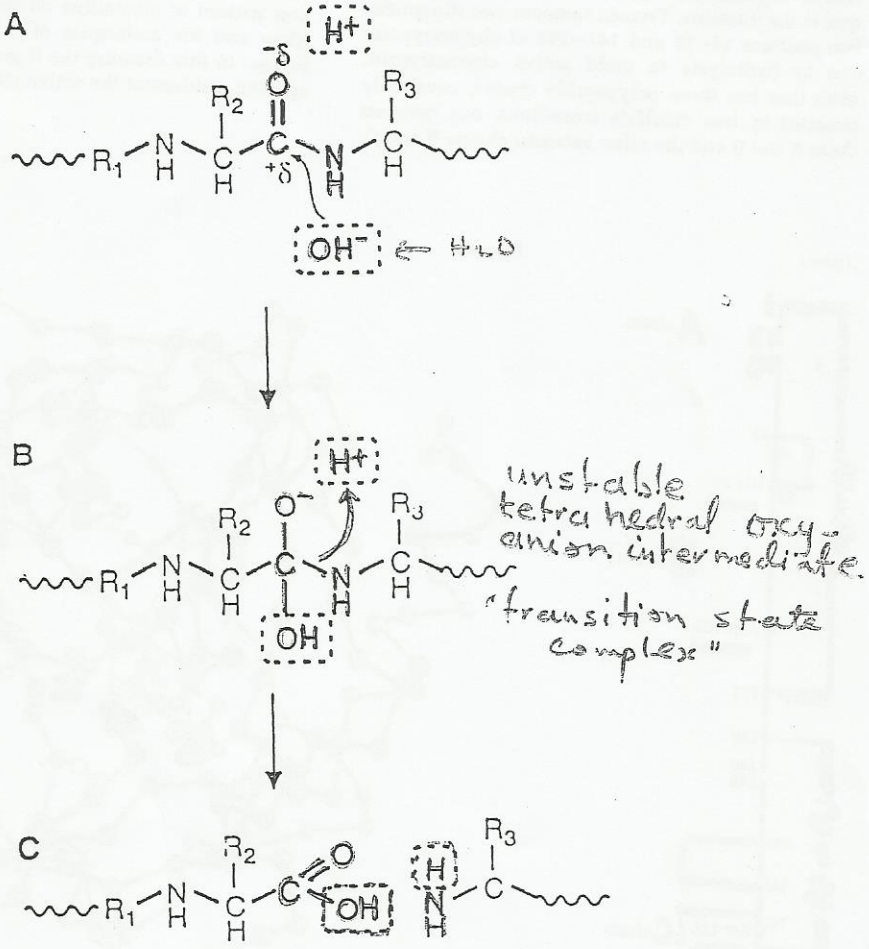
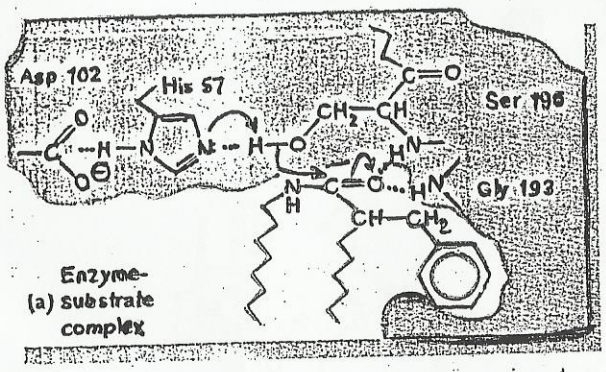


Figure 9.11 Catalytic Mechanism of Chymotrypsin

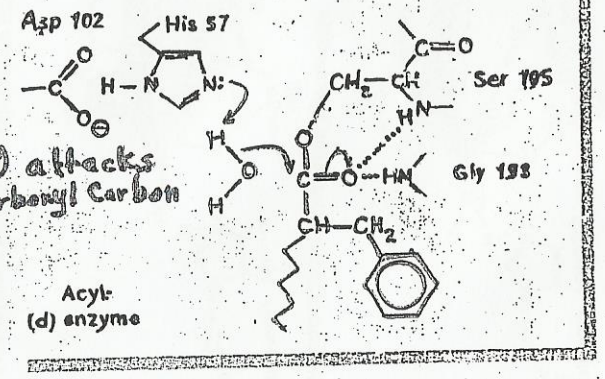
Substrate binding

His activates Ser for nucleophilic attack



Enzyme-(a) substrate complex

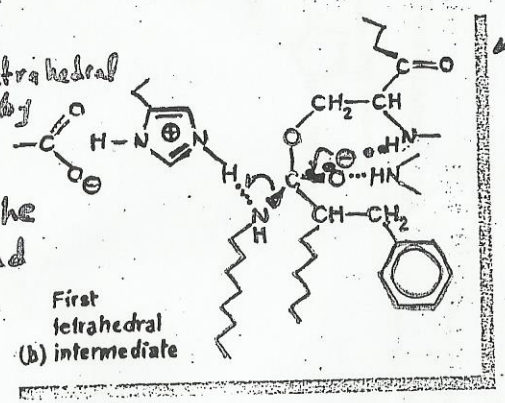
- H_2O attacks the carbonyl carbon



Acyl-(d) enzyme

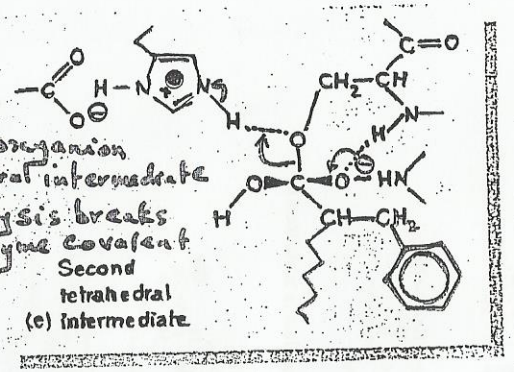
Oxyanion tetrahedral is stabilized by H-bonds

Cleavage of the peptide bond



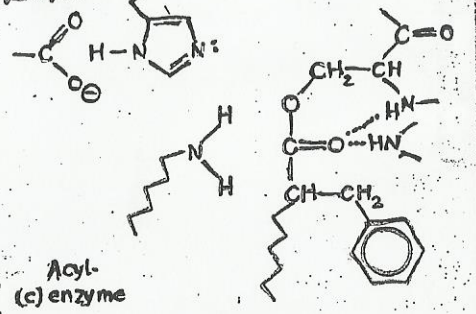
First tetrahedral (b) intermediate

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



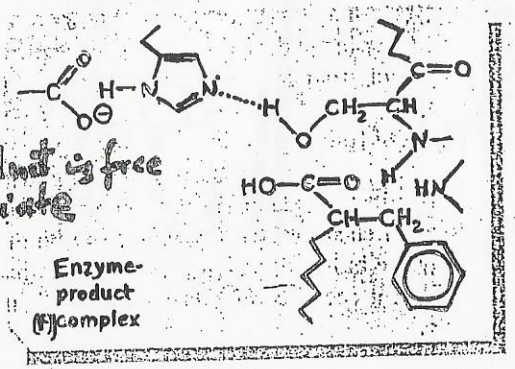
Second tetrahedral (e) intermediate

- Covalently acyl-enzyme intermediate



Acyl-(c) enzyme

- The product is free to dissociate

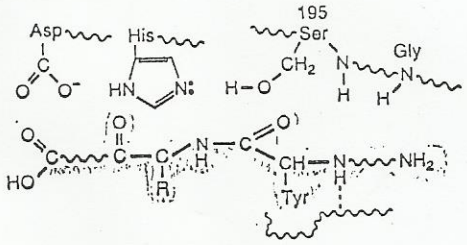


Enzyme-product (f) complex

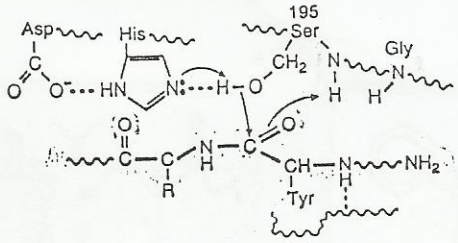
Serine Proteases: family of enz uses serine residue in catalytic site to hydrolyze peptide bonds e.g. trypsin, chymotrypsin, thrombin

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

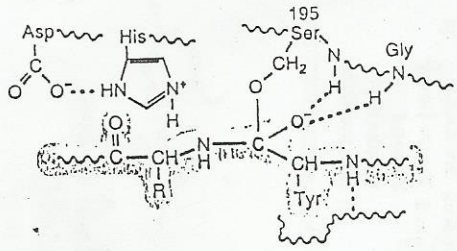
1. Substrate binding



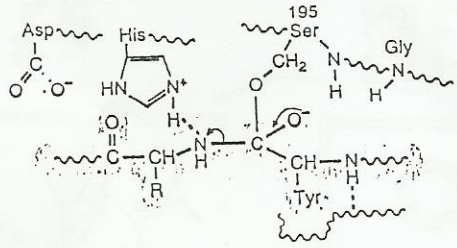
2. Histidine activates serine for nucleophilic attack



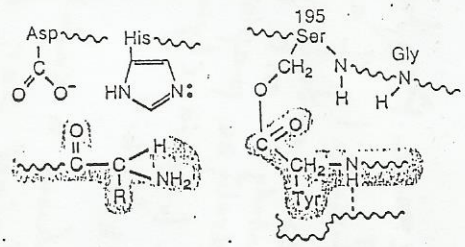
3. The oxyanion tetrahedral intermediate is stabilized by hydrogen bonds



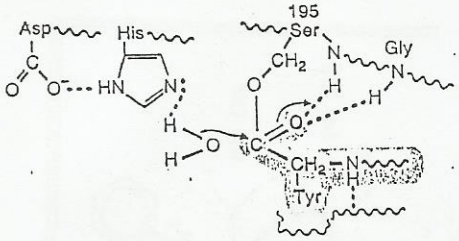
4. Cleavage of the peptide bond



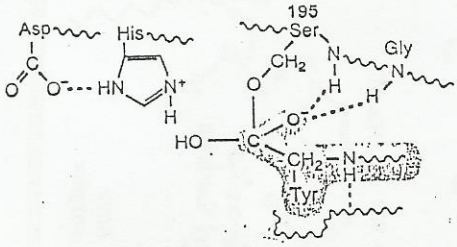
5. The covalent acyl–enzyme intermediate



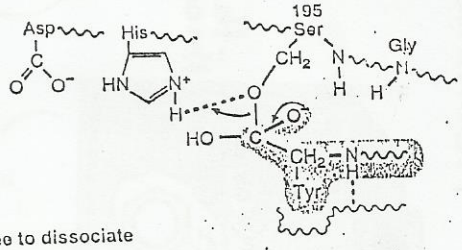
6. Water attacks the carbonyl carbon



7. Second oxyanion tetrahedral intermediate



8. Acid catalysis breaks the acyl–enzyme covalent bond



9. The product is free to dissociate

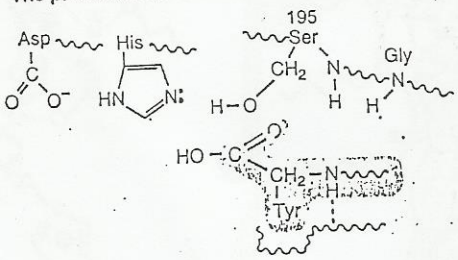


Fig. 9.10.