VIVEKANANDA COLLEGE
THAKURPUKUR
KOLKATA-700063

NAAC ACCREDITED ‘A’ GRADE

Topic	: Mechanism of Enzyme Catalysis
Course Title	: Enzymes
Paper	: CC4
Unit	: I
Semester	: 2
Name of the Teacher	: Dr. Kakali Roy
Name of the Department	: Biochemistry

Mechanism of enzyme catalysis

Acid-Base catalysis

General acid-base catalysis involves a molecule besides water that acts as a proton donor or acceptor during the enzymatic reaction. Acid-base catalysis facilitates a reaction by stabilizing the charges in the transition state through the use of an acid or base, which donates protons or accepts them, respectively.

Mechanisms of catalysis

Acid-base catalysis

- Very often-used mechanism in enzyme reactions, e.g., hydrolysis of ester/ peptide bonds, phosphate group reactions, addition to carbonyl groups, etc.
- Enzyme *avoids unstable charged intermediates in reaction* (which would have high free energies) by:
  - **donating a proton** (act as a ***general acid***), or
  - **accepting a proton** (abstract a proton, act as a ***general base***)
- If a group *donates* a proton (acts as a **general acid**) in chemical mechanism, it has to *get a proton (a different one!) back* (act as a **general base**) by end of catalytic cycle, and vice versa
- Protein functional groups that can function as general acid/base catalysts:
  - e.g. His imidazole, α-amino group, α-carboxyl group, thiol of Cys, R group carboxyls of Glu, Asp, aromatic OH of Tyr, etc

ACID–BASE CATALYSIS

- General acid catalysis is a process in which proton transfer from an acid lowers the free energy of a reaction's transition state.
- The ionisable functional groups of amino acyl side chains and (where present) of prosthetic groups contribute to catalysis by acting as acids or bases.
- The ability of enzymes to arrange several catalytic groups around their substrates makes concerted acid-base catalysis a common enzymatic mechanism.

6

The catalytic activity of these enzymes is sensitive to pH, since the pH influences the state of protonation of side chains at the active site.

- RNase A is an Acid-Base Catalyst. This digestive enzyme is secreted by the pancreas into the small intestine, where it hydrolyses RNA to its component nucleotides.

Amino Acids in General Acid-Base catalysis

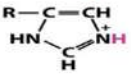
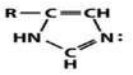
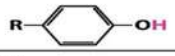
Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	R—COO⁻
Lys, Arg	R—$\overset{+}{\underset{H}{N}}$H	R—$\ddot{N}H_2$
Cys	R—SH	R—S⁻
His		
Ser	R—OH	R—O⁻
Tyr		

Figure 6-9
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W.H. Freeman and Company

Reaction Mechanism :

Ribonuclease (RNase): an example of acid-base catalysis

Step 1: the 2'OH belonging to the substrate acts as the nucleophile. His12 acts as general base to activate the nucleophile. His119 acts as a general acid, donating a proton to the leaving group 5'OH.

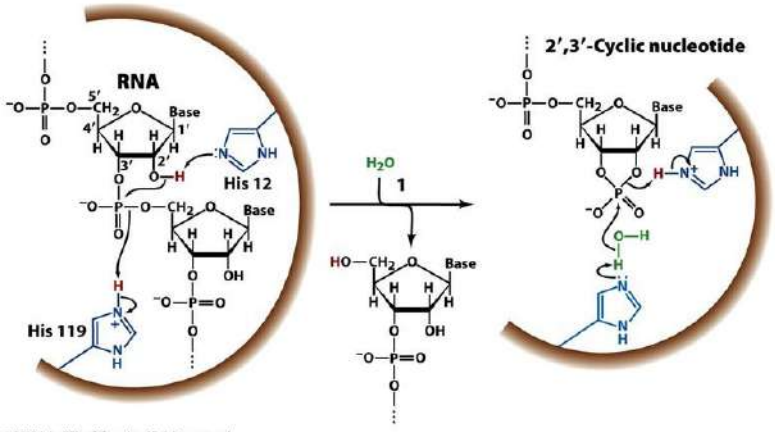


Figure 11-10 part 1

Mechanism of RNase A

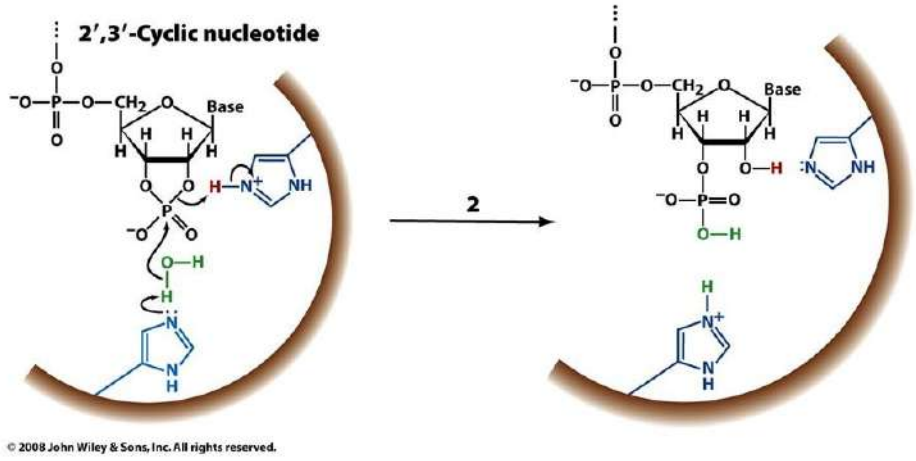


Figure 11-10 part 2

Step 2 :The 2'-3' cyclic intermediate is hydrolysed through the reverse of the 1st step in which water replaces the leaving group. Thus His 12 acts as a general acid and His 119 as a general base to yield the hydrolysed RNA and the enzyme in its original state.

Metal ion Catalysis

Two classes of metal ion dependent enzymes

1) Metalloenzymes contain tightly bound transition metal ions (eg. Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{3+}) : for example Carbonic anhydrase

2) Metal-activated enzymes loosely bind metal ions (eg. Alkali or alkaline metal including Na^{+}, K^{+} , Mg^{2+} and Ca^{2+}) : for example Hexokinase

Metal ions enhance catalysis in three major ways :

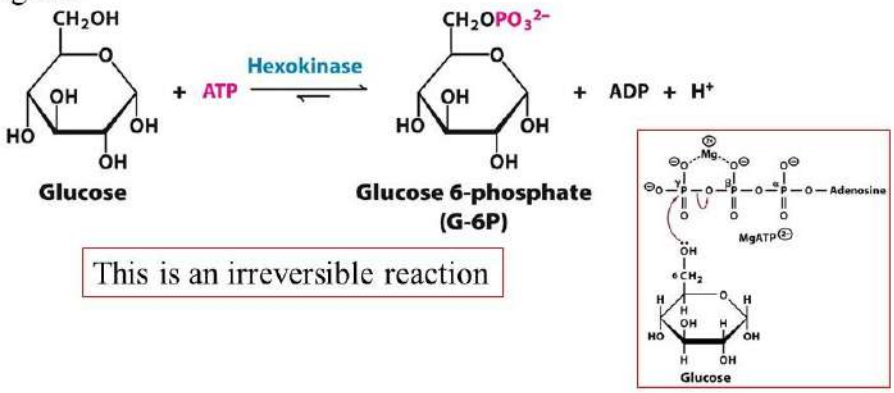
1) **Binding to and orienting substrates for reaction**
   eg. Mg^{2+} binding to ATP
2) **Mediating redox reaction through changes in oxidation state**
   eg. Reduction of O_2 to H_2O through electron transfer
3) **Electrostatic stabilization or shielding of negative charges**
   eg. Mg^{2+} binding to ATP

Metal activated enzyme : Hexokinase

Enzyme 1 Hexokinase

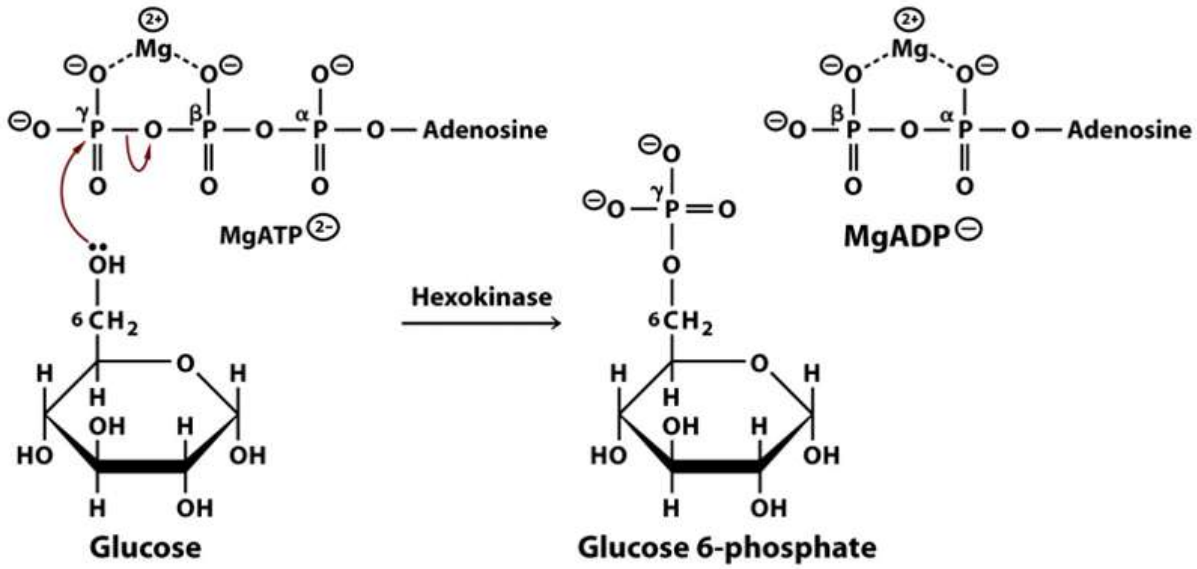
- Transfers the γ-phosphoryl of ATP to glucose C-6 oxygen to generate glucose 6-phosphate

- Mechanism: Mg^{2+} is an important cofactor, attack of C-6 hydroxyl oxygen of glucose on the γ-phosphorous of $MgATP^{2-}$ displacing $MgADP^{-}$



This is an irreversible reaction

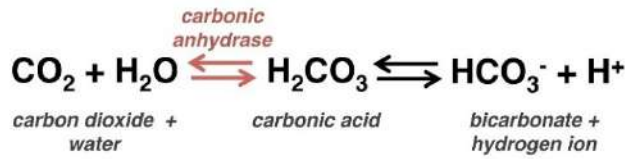
Mechanism of action :



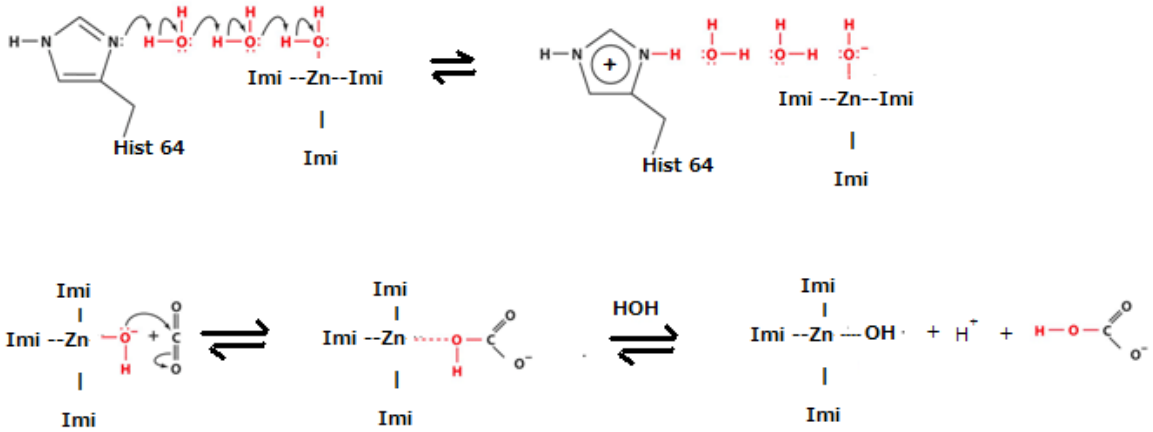
In the first reaction of glycolysis, the gamma-phosphoryl group of an ATP molecule is transferred to the oxygen at the C-6 of glucose. The magnesium ion is required as the reactive form of ATP in the complex with magnesium (II) ion. This step is a direct nucleophilic attack of the hydroxyl group on the terminal phosphoryl group of the ATP molecule. This produces glucose-6-phosphate and ADP. Hexokinase is the enzyme that catalyses this phosphoryl group transfer. Hexokinase undergoes an induced-fit conformational change when it binds to

glucose, which ultimately prevents the hydrolysis of ATP. It is also allosterically inhibited by physiological concentrations of its immediate product, glucose-6-phosphate.

Metalloenzyme :Carbonic Anhydrase

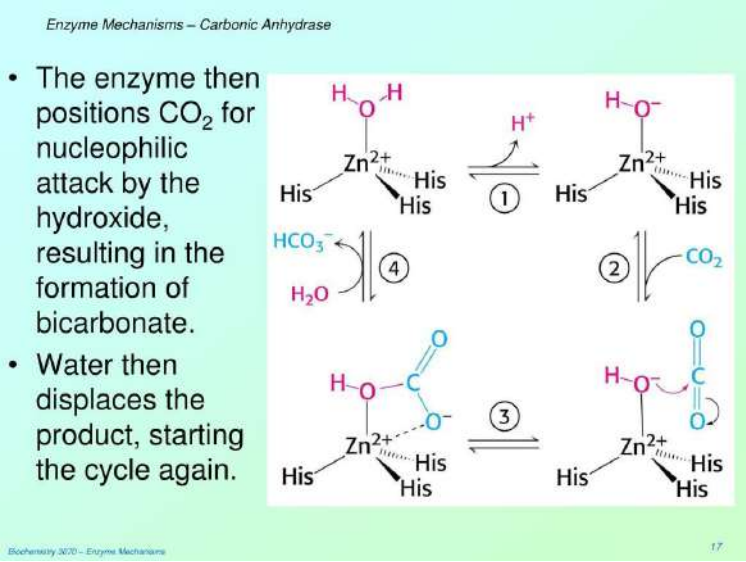


Reaction mechanism :



(1) His64 abstracts proton from Zn^{2+} bound water molecule generating Zn^{2+} bound hydroxide ion (via proton shuttle) – Electrostatic stabilization of OH^-

(2) Zn^{2+} bound hydroxide ion nucleophilically attacks bound CO_2 converting it to bicarbonate
(3) His64 releases proton to solvent to regenerate enzyme (not shown)



Covalent Catalysis

Covalent catalysis accelerates reaction rates through transient formation of enzyme-substrate covalent bond.

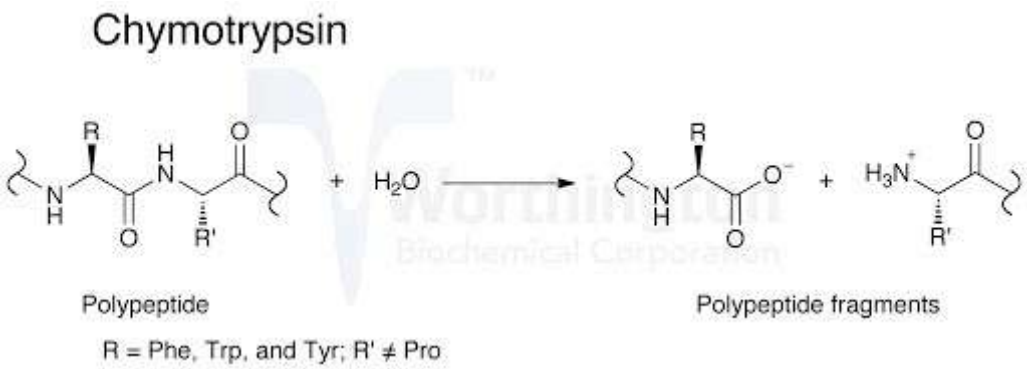
Three stages in covalent catalysis

1) nucleophilic reaction between enzyme and substrate

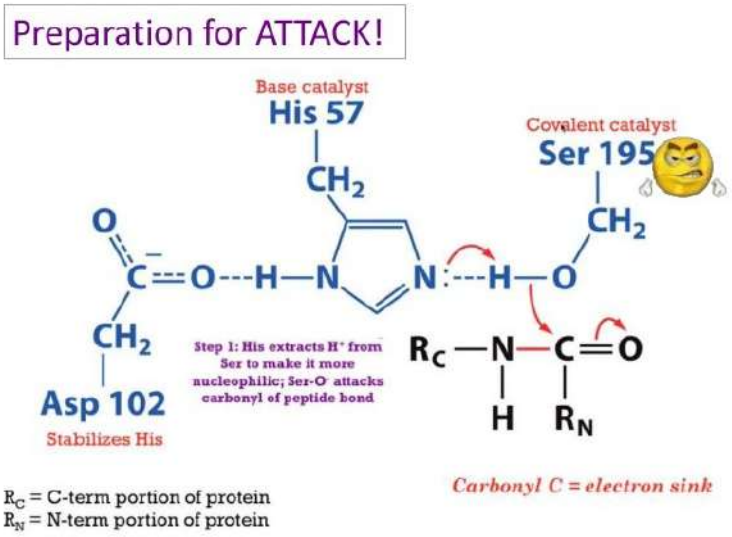
2) electrophilic withdrawl of electrons from substrate to form E-S covalent intermediate

3) elimination reaction (reverse of stage 1)

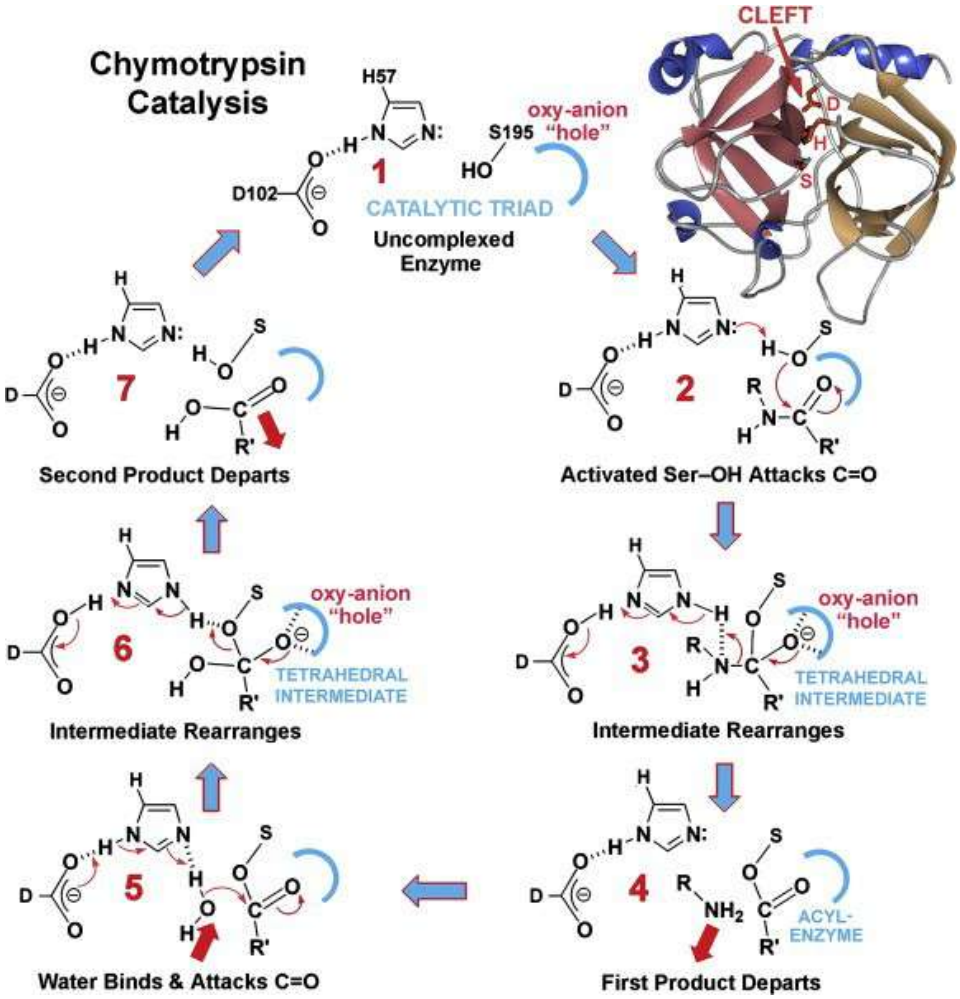
Example : Chymotrypsin and Lysozyme



Chymotrypsin is a serine protease enzyme capable of cleaving peptide bonds at the C-terminal of amino acid residues containing aromatic non-polar side chains (i.e. tryptophan, phenylalanine, tyrosine).



Reaction Mechanism

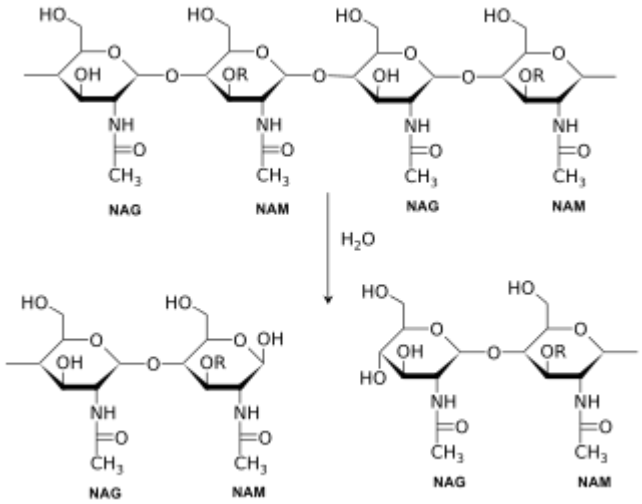


The reaction involves

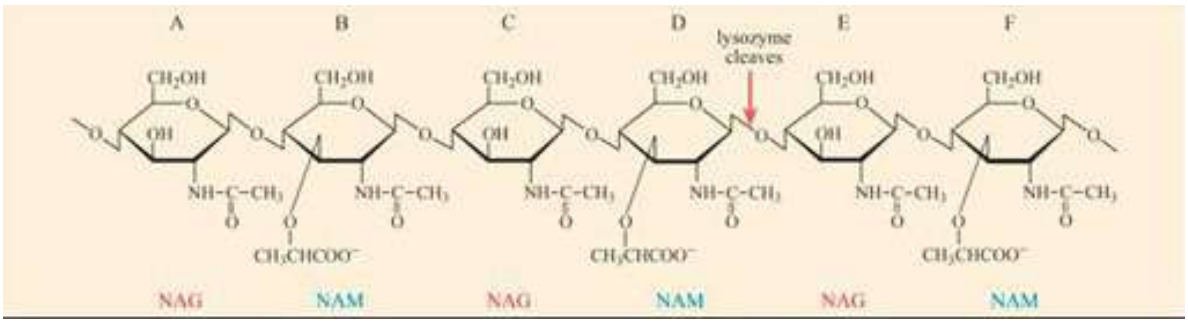
- The nucleophilic attack of the active site Serine on the carbonyl carbon atom of the scissile peptide bond to form the tetrahedral intermediate
- The decomposition of the tetrahedral intermediate to the acyl-enzyme intermediate through general acid catalysis by the active site Asp-polarized His, followed by loss of the amine product (first product) and its replacement by a water molecule
- The reversal of Step 2 to form a second tetrahedral intermediate
- The reversal of Step 1to yield the reaction's carboxyl product (second product) and the active enzyme

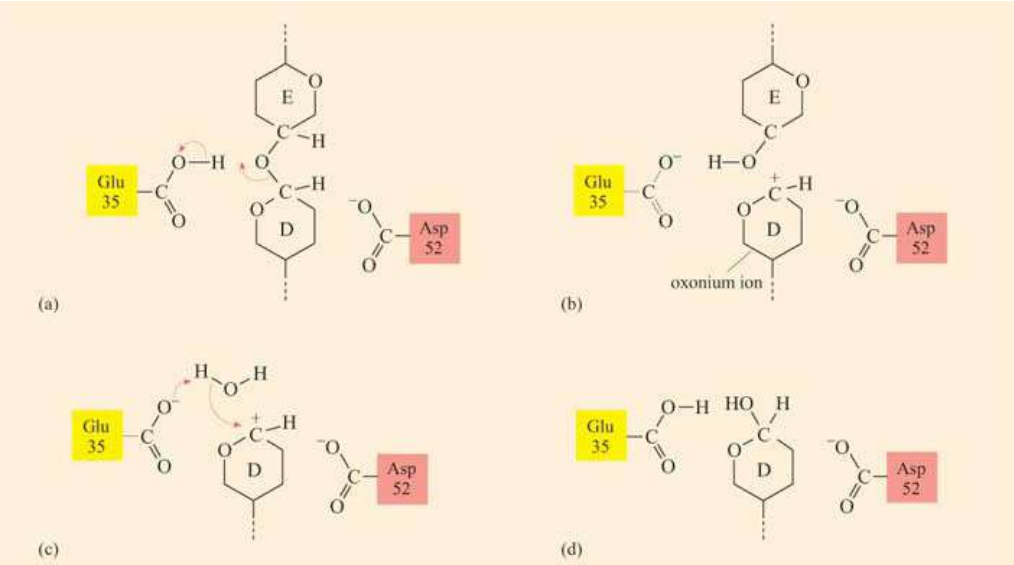
Lysozyme

Lysozyme is found widely in the cells and secretions of vertebrates, and hen egg white is particularly rich in this enzyme. Lysozyme catalyses the hydrolysis of β(1→4) glycosidic bonds that link *N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG) in polysaccharides of bacterial cell walls.



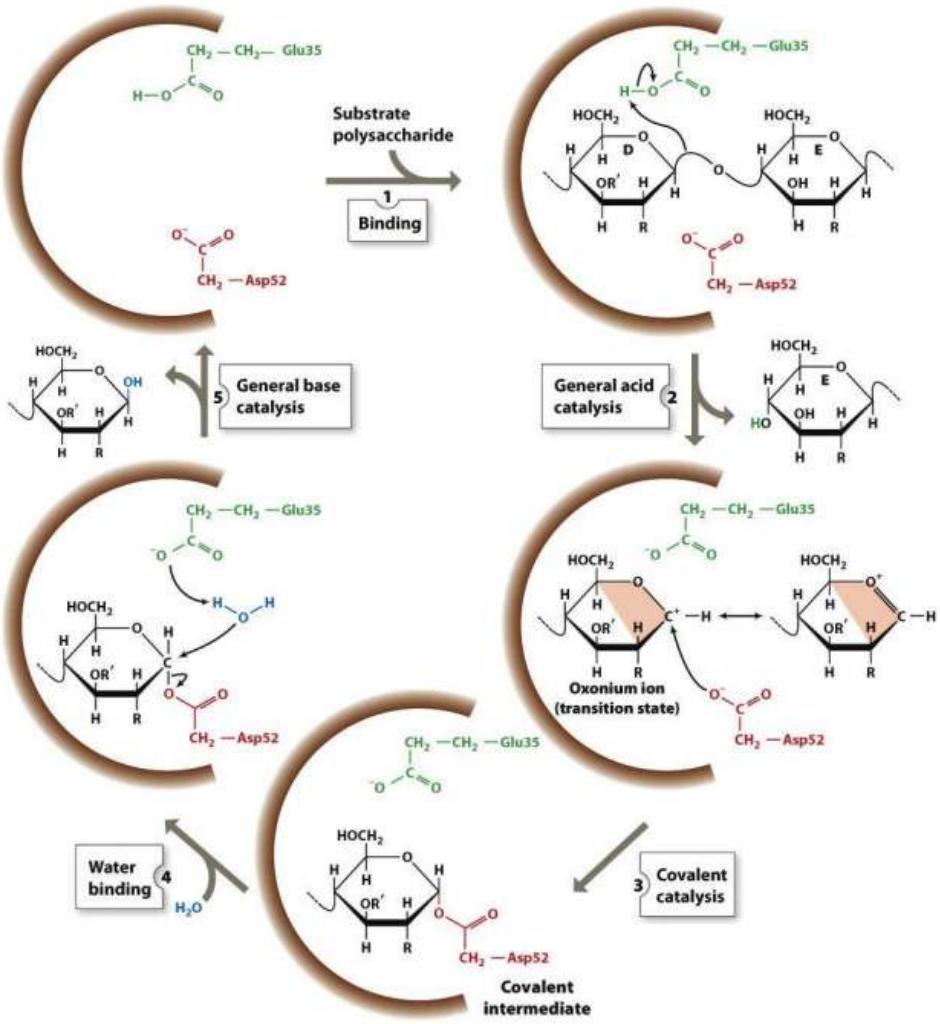
Catalytic Mechanism of Lysozyme (Based on Phillips, 1966)





Asp 52 and Glu 35, key residues in the active site, are highlighted in red and yellow respectively.

1. On binding to the enzyme, the substrate adopts a strained conformation. Residue D is distorted (not shown in the diagram) to accommodate a $–CH_2OH$ group that otherwise would make unfavourable contact with the enzyme. In this way, the enzyme forces the substrate to adopt a conformation approximating to that of the transition state.
2. Residue 35 of the enzyme is glutamic acid (Glu 35) with a proton that it readily transfers to the polar O atom of the glycosidic bond. In this way, the C–O bond in the substrate is cleaved (Figure a and b).
3. Residue D of the polysaccharide now has a net positive charge; this reaction intermediate is known as an **oxonium ion** (Figure b). The enzyme stabilises this intermediate in two ways. Firstly, a nearby aspartate residue (Asp 52), which is in the negatively charged carboxylate form, interacts with the positive charge of the oxonium ion. Secondly, the distortion of residue D enables the positive charge to be shared between its C and O atom. (Note that this sharing of charge between atoms is termed **resonance** in the same way as the sharing of electrons between the atoms of the peptide group.) *Thus the oxonium ion intermediate is the transition state*. Normally, such an intermediate would be very unstable and reactive. Asp 52 helps to stabilise the oxonium ion, but it does not react with it. This is because, at 3 Å distance, the reactive groups are too far apart.
4. The enzyme now releases residue E with its attached polysaccharide, yielding a glycosyl-enzyme intermediate. The oxonium ion reacts with a water molecule from the solvent environment, extracting a hydroxyl group and re-protonating Glu 35 (Figure c and d).
5. The enzyme then releases residue D with its attached polysaccharide and the reaction is complete.



Reference

1. Voet D, Voet JG (2011), Biochemistry (4th ed.), New York : Wiley
2. www.open.edu>biology>proteins>content-section-6
3. www.google.com