CATALYTIC MECHANISMS

Catalysis is a process that increases the rate at which a reaction approaches equilibrium.

- The rate of a reaction is a function of its free energy of activation (_G‡), a catalyst acts by lowering the height of this kinetic barrier
- Enzymes catalytic mechanisms have been classified as;

1. Acid–base catalysis.
2. Covalent catalysis.
3. Metal ion catalysis.
4. Electrostatic catalysis.
5. Proximity and orientation effects.
6. Preferential binding of the transition state complex.

References

1. Lehninger Principles Of Biochemistry Fourth Edition: David L. Nelson And Michael M. Cox
2. Biochemistry; Donald Voet And Judith G. Voet

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CATALYTIC MECHANISMS

A. *Acid–Base Catalysis*

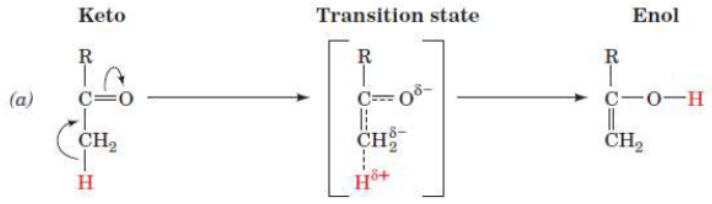
General acid catalysis *is a process in which partial proton transfer from a Brønsted acid lowers the free energy of a reaction's transition state.*

Catalysis of the type that uses only the H_ (H3O_) or OH_ ions present in water is referred to as **specific acid-base catalysis**.

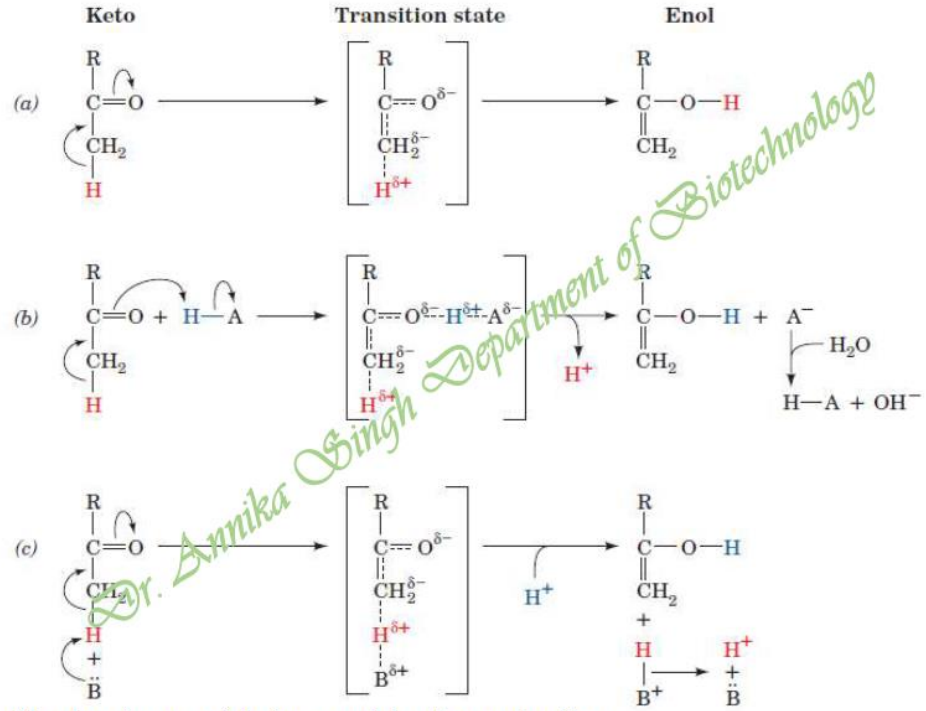
The term **general acid-base catalysis** refers to proton transfers mediated by weak acids and bases other than water.

For example,

a) an uncatalyzed keto–enol tautomerization reaction occurs quite slowly as a result of the high energy of its carbanion like transition state



CATALYTIC MECHANISMS



Mechanisms of keto–enol tautomerization.
(*a*) Uncatalyzed, (*b*) general acid catalyzed, and (*c*) general base

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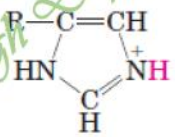
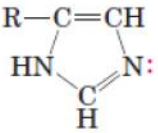
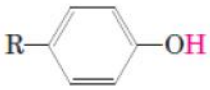
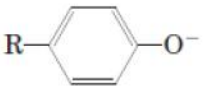
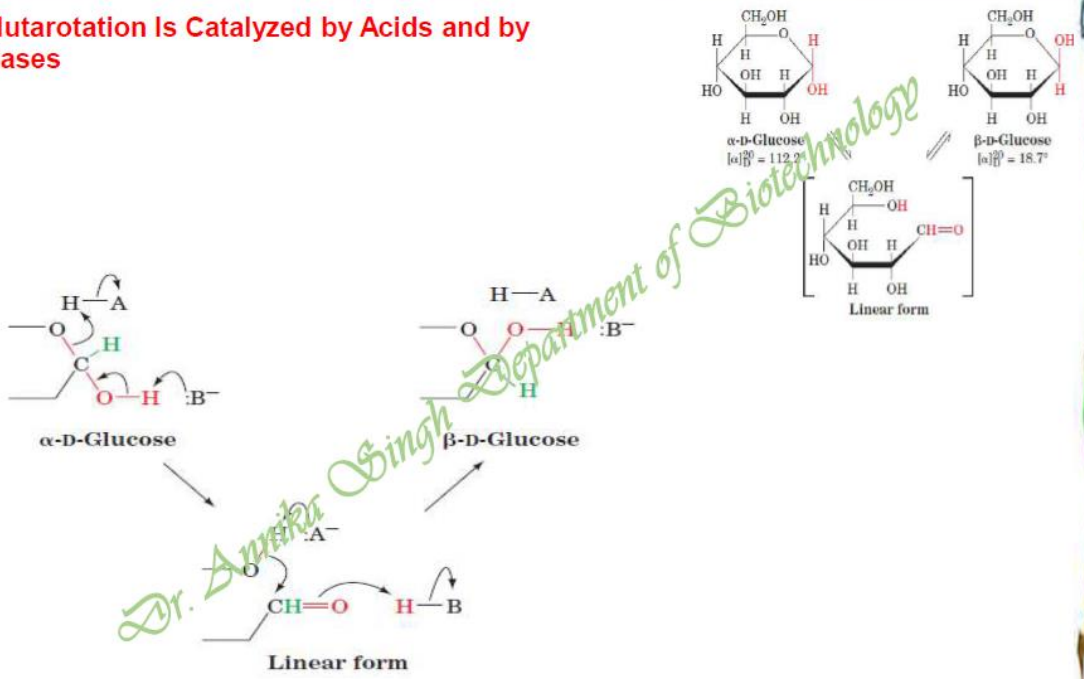
Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	R—COO⁻
Lys, Arg	R—⁺NH(H)H	R—NH₂
Cys	R—SH	R—S⁻
His		
Ser	R—OH	R—O⁻
Tyr		

Amino acids in general acid-base catalysis

CATALYTIC MECHANISMS

Mutarotation Is Catalyzed by Acids and by Bases



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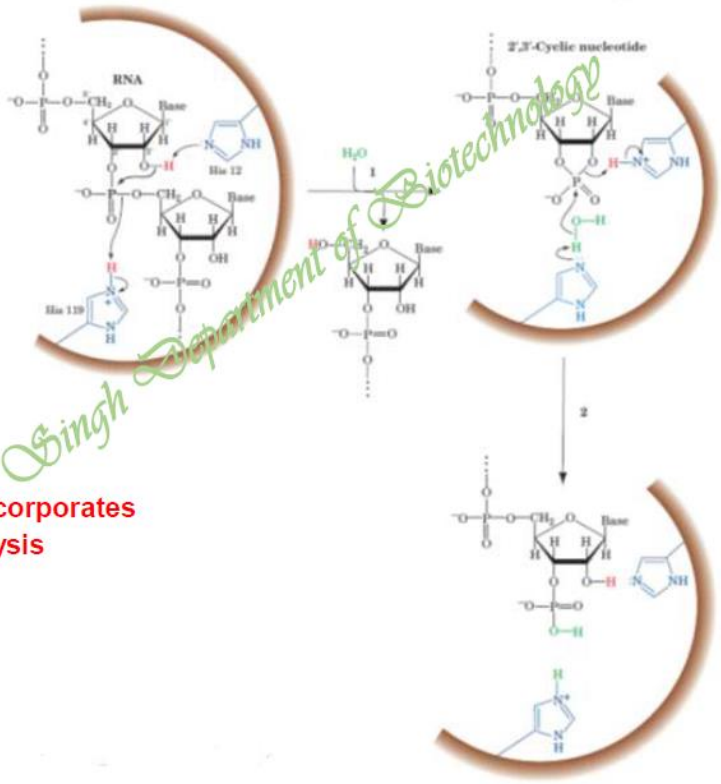
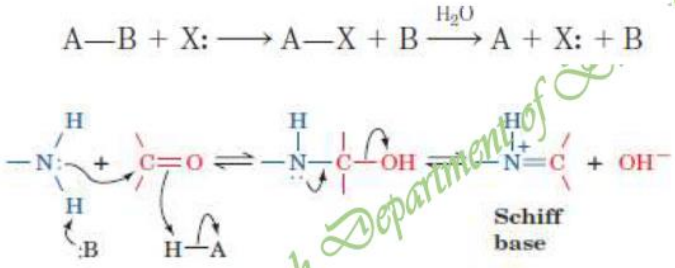


The RNase A Reaction Incorporates General Acid–Base Catalysis

B. *Covalent Catalysis*

Covalent catalysis *involves rate acceleration through the transient formation of a catalyst–substrate covalent bond.*



a. Covalent Catalysis Has Both Nucelophilic and Electrophilic Stages

As the preceding example indicates, covalent catalysis may be conceptually decomposed into three stages:

1. The nucleophilic reaction between the catalyst and the substrate to form a covalent bond.

2. The withdrawal of electrons from the reaction center by the now electrophilic catalyst.

3. The elimination of the catalyst, a reaction that is essentially the reverse of stage 1.

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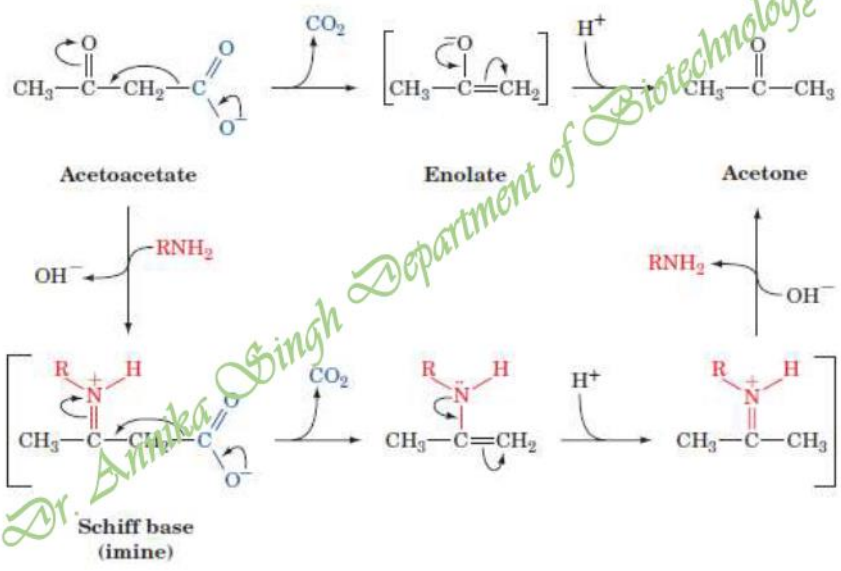
The decarboxylation of **acetoacetate,** as chemically catalyzed by primary amines, is an example of such a process.



- The amine nucleophilically attacks the carbonyl group of acetoacetate to form a **Schiff base** (imine bond).

CATALYTIC MECHANISMS

Certain Amino Acid Side Chains and Coenzymes Can Serve as Covalent Catalysts

- Covalent catalysis include the imidazole moiety of His, the thiol group of Cys, the carboxyl function of Asp, and the hydroxyl group of Ser.
- Several coenzymes, most notably **thiamine pyrophosphate** and **pyridoxal phosphate** function in association with their apoenzymes mainly as covalent catalysts.

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CATALYTIC MECHANISMS

Metal Ion Catalysis

There are two classes of metal ion–requiring enzymes that are distinguished by the strengths of their ion–protein interactions:

1. ***Metalloenzymes*** *contain tightly bound metal ions,* most commonly transition metal ions such as Fe^{2+} , Fe^{3+} , Cu^{2+} , Zn^{2+} , Mn^{2+} , or Co^{3+} .
2. ***Metal-activated enzymes*** *loosely bind metal ions from solution,* usually the alkali and alkaline earth metal ions Na^+, K^+, Mg^{2+}, or Ca^{2+} .

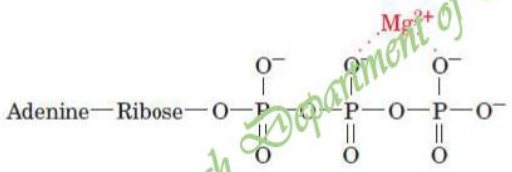
Metal ions participate in the catalytic process in three major ways:

1. By binding to substrates so as to orient them properly for reaction.
2. By mediating oxidation–reduction reactions through reversible changes in the metal ion's oxidation state.
3. By electrostatically stabilizing or shielding negative charges.

CATALYTIC MECHANISMS

Metal Ions Promote Reactions through Charge Shielding

- Another important enzymatic function of metal ions is **charge shielding.**
  For example, the actual substrates of **kinases** (phosphoryl-transfer enzymes utilizing ATP) are Mg^{2+}ATP complexes such as rather than just ATP.



- The Mg^{2+} ion's role, in addition to its orienting effect, is to shield electrostatically the negative charges of the phosphate groups.
- These charges would tend to repel the electron pairs of attacking nucleophiles, especially those with anionic character.

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Electrostatic catalysis

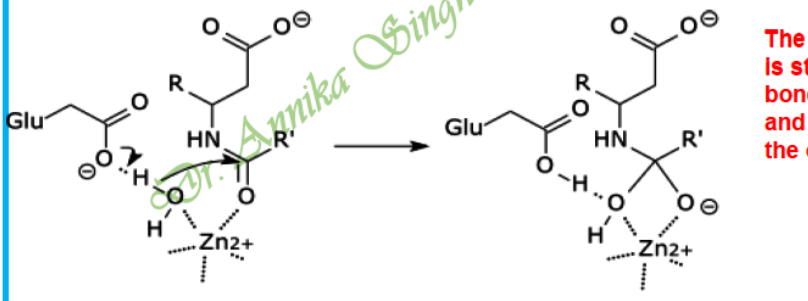
A transition-state may be stabilized by electrostatic interaction between its charged groups and charged groups on a catalyst.

Thus, the positive charge on a carbonium ion can be stabilized by interaction with a negatively charged carboxylate ion

OR the negative charge on an oxyanion can be stabilized by a positively charged metal ion.

For example:

Carboxypeptidase catalytic mechanism



Enzyme substrate complex

Tetrahedral intermediate

The tetrahedral intermediate is stabilised by a partial ionic bond between the Zn^{2+} ion and the negative charge on the oxygen.

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E. *Catalysis through Proximity and Orientation Effects*

- The catalytic efficiency of an enzyme must arise from the specific physical conditions at enzyme catalytic sites that promote the corresponding chemical reactions.
- The most obvious effects are **proximity** and **orientation:** Reactants must come together with the proper spatial relationship for a reaction to occur.
- There is a great loss of entropy when reactants leave their random existence in free solution to become bound in the transition-state, so $-T\Delta S^{*}$ will generally have a large positive value, possibly making up about half of the total free energy of activation where there is more than one reactant.
- In enzyme-catalysed reactions, there is inevitably a similar loss of entropy at some stage, but it occurs largely in the binding steps when enzyme-substrate complexes are formed, and not to the same degree in the actual conversion of substrates to products.
- The binding of substrate molecules in close proximity to each other on the enzyme surface effectively increases their concentrations and reduces the entropy loss for the subsequent formation of a transition-state.
- This has been called the **proximation (proximity) effect, (**Thomas Bruice, William Jencks).
- The enzyme may also ensure that the reacting groups of the bound substrates approach each other with their electronic orbitals correctly orientated, thus ensuring that the reaction takes place under optimal conditions this property of enzymes **orbital steering (**Daniel Koshland **) or orientation effect.**

References

1. Lehninger Principles Of Biochemistry Fourth Edition: David L. Nelson And Michael M. Cox
2. Biochemistry; Donald Voet And Judith G. Voet

Lysozyme

- **Lysozyme** is an enzyme that destroys bacterial cell walls by hydrolyzing the β(1→4) glycosidic linkages from ***N*-acetylmuramic acid (NAM)** to ***N*-acetylglucosamine (NAG)** in the alternating NAM–NAG polysaccharide component of cell wall peptidoglycans
- It also hydrolyzes β(1 → 4) linked poly (NAG) (chitin), a cell wall component of most fungi.
- Lysozyme occurs widely in the cells and secretions of vertebrates, where it may function as a bactericidal agent.
- However, the observation that few pathogenic bacteria are susceptible to lysozyme alone has prompted the suggestion that this enzyme mainly helps dispose of bacteria after they have been killed by other means.

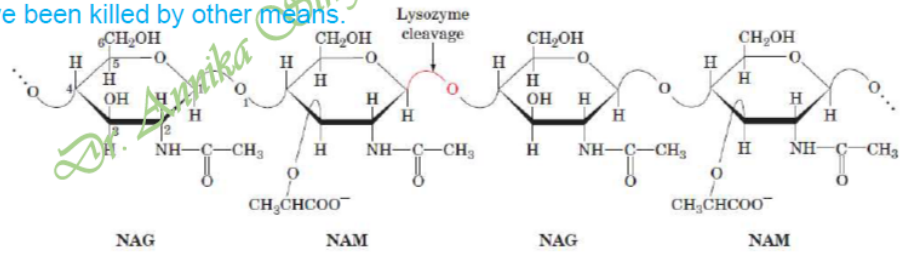

Fig. : **The alternating NAG–NAM polysaccharide component of bacterial cell walls.**

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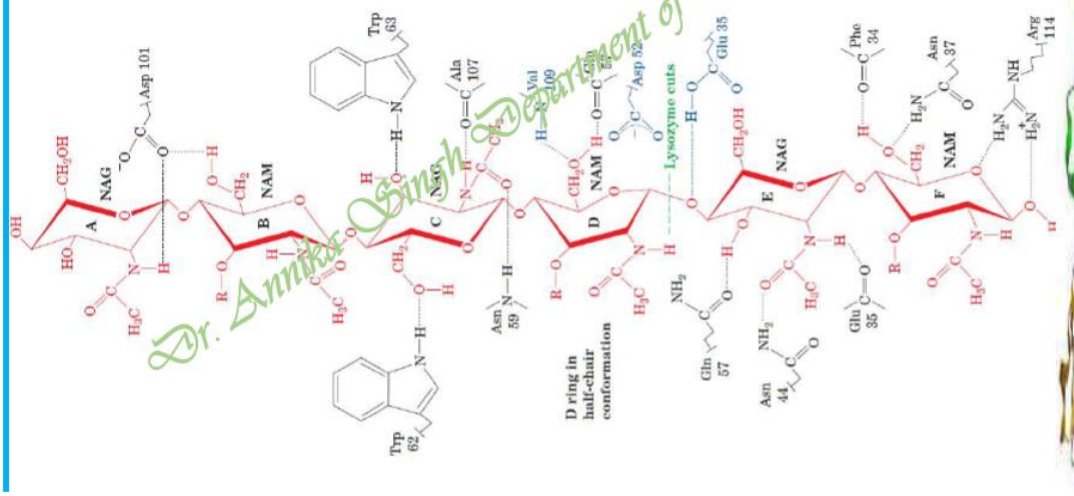

1. Lysozyme attaches to a bacterial cell wall by binding to a hexa saccharide unit. In the process, the D-ring is distorted toward the half-chair conformation in response to the unfavorable contacts that its $-C6H_2OH$ group would otherwise make with the protein.

2. Glu 35 transfers its proton to the O1 atom linking the D- and E-rings, the only polar group in its vicinity, thereby cleaving the C1¬O1 bond (general acid catalysis).This step converts the D-ring to a planar resonance-stabilized oxonium ion transition state, whose formation is facilitated by the strain distorting it to the half-chair conformation (catalysis by the preferential binding of the transition state).The positively charged oxonium ion is stabilized by the presence of the nearby negatively charged Asp 52 carboxylate group (electrostatic catalysis).The E-ring product is released.

3. The Asp 52 carboxylate group nucleophilically attacks the now electron-poor C1 of the D ring to form a covalent **glycosyl–enzyme intermediate** (covalent catalysis).

4. Water replaces the E-ring product in the active site.

5. Hydrolysis of the covalent bond with the assistance of Glu 35 (general base catalysis), which involves another oxonium ion transition state, regenerates the active site groups. The enzyme then releases the D-ring product, completing the catalytic cycle.

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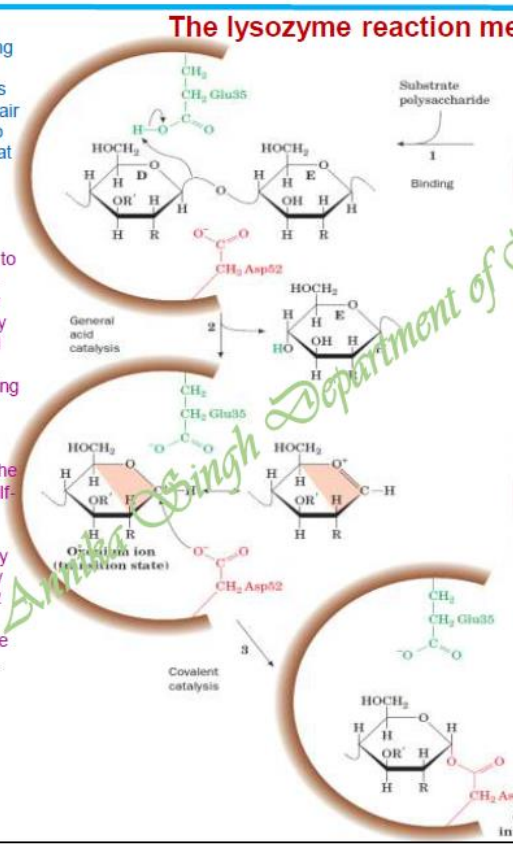

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