3 Mechanism of Enzyme Action

INTRODUCTION

Enzyme catalysis differs from chemical catalysis in following ways:

(i) Exhibits a high turnover number;

(ii) Promotes reactions in milder conditions;

(iii) Catalyses group specific as well as sterochemically specific reactions.

It has been found that a number of enzymes act as **group transferase**, *i.e.*, group (G) from donor (DG) is transfered to acceptor (A). Even hydrolytic enzymes such as phosphatases and esterases fit in this definition if water is considered as acceptor. A general reaction is illustrated below:

$$DG + Enzyme \rightleftharpoons D + Enzyme\text{-}G$$

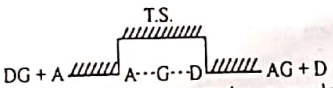
$$Enzyme - G + A \rightleftharpoons Enzyme + AG$$

or

$$DG + A \overset{Enzyme}{\rightleftharpoons} D + AG$$

For the action of hydrolytic & transfer enzymes Koshland proposed following three mechanisms:

(i) Single Displacement: Acceptor [A] makes a direct nucleophilic attack on donor substrate (DG), thus a transition state like SN^2 reactions might have existed in the reaction catalysis.



(ii) Double displacement: Enzymes make a nucleophilic attack on donor, thus an enzyme–G bond is formed followed by liberation of D. Acceptor (A), then displaces G from active site & consequently GA is formed.

$$Enzyme + DG \rightleftharpoons Enzyme\text{-}G + D$$

$$Enzyme\text{-}G + A \rightleftharpoons Enzyme + GA$$

(iii) Front-side displacement: According to this mechanism displacement is at acute angle for D—G—A rather than 180° in single displacement.

Front-side displacement

TRANSITION-STATE THEORY

The transition-state theory advocates that enzyme catalyses biochemical reaction through low energy transition state as compared to absence of enzyme. For further details see transition state theory in Unit II.

ACID BASE CATALYSIS

Catalysis of reactions in aqueous solutions containing acids & bases is known as **acid-base catalysis**. Reaction which is catalysed by $H^{\oplus}$ ion or hydroxonium ions $[H_3O^{\oplus}]$ is known as specifically proton-catalysed reaction. Few examples are inversion of sugars, solvolysis of esters and keto-enol transformation. Conversely, reactions catalysed by $\overset{\ominus}{O}H$ ions are called **specifically base-catalysed reactions**. On the other hand, catalysis by Bronsted acid & Bronstead bases are called **general acid catalysis** and **general base-catalysis**, respectively. Water acts as Bronstead acid as well as Bronstead base. Some reactions require proton acceptor as well as proton donor, *e.g.*, mutarotation of glucose. This is an example of **acid-base catalysis.**

Kinetics of Acid-Base catalysis: For a 1st order reaction involving conversion of substrate (S) to product (P) rate of reaction over a small time interval is given by the expression

$$S \longrightarrow P$$

$$\frac{-d[S]}{dt} = K[S]$$

If this reaction is taking place in a buffer solution rate constant, K, depends linearly upon $[H^{\oplus}]$, $[\overset{\ominus}{O}H]$, [HA] and $[A^{\ominus}]$. Here, HA is weak acid present in buffer & $[A^{\ominus}]$ is its conjugate base. Therefore

$$K = K_0 + K_{H^{\oplus}}[H^{\oplus}] + K_{\overset{\ominus}{O}H}[\overset{\ominus}{O}H] + K_{HA}[HA] + K_{A^{\ominus}}[\overset{\ominus}{A}] \quad \ldots (1)$$

In equation (1) K_0 is first order rate constant if reaction is carried out in absence of catalyst. $K_{H^{\oplus}}$, $K_{OH^{\ominus}}$, K_{HA} & $K_{A^{\ominus}}$ are catalytic coefficients, which may be evaluated experimentally by varying concentration of each of these species. Reaction is said to be **specific hydrogen ion catalysed** if only $K_{H^{\oplus}}[H^{\oplus}]$ is important. Similarly, it is **specific hydroxyl ion catalysed**, if $K_{\overset{\ominus}{O}H}[\overset{\ominus}{O}H]$ term is important. The reaction is **general acid catalysed** if $K_{HA}[HA]$ is important. Likewise, reaction is **general base catalysed** if term $K_{A^{\ominus}}[A^{\ominus}]$ is important. To arrive at equation (1) we have to consider following two mechanisms.

(A) First Mechanism (Acid-catalysed Mechanism): Let us consider that proton ($H^{\oplus}$) is transferred from $AH^{\oplus}$ to substrate S and protonated substrate reacts with water to give the product P.

$$S + HA^{\oplus} \underset{K_1}{\overset{K_1}{\rightleftharpoons}} \overset{\oplus}{S}H + A$$

$$\overset{\oplus}{S}H + H_2O \xrightarrow{K_2} P + H_3O^{\oplus}$$

Upon applying steady state approximation for protonated substrate $[\overset{\ominus}{S}H]$,

$$\frac{d[SH^{\oplus}]}{dt} = O = K_1[S][\overset{\oplus}{A}H] - K_{-1}[A][\overset{\oplus}{S}H] - K_2[\overset{\oplus}{S}H] \quad \ldots (2)$$

Because, water is taken in excess, $[H_2O]$ concentration is neglected in the last term of equation (2). Upon solving for $[\overset{\oplus}{S}H]$, we have

$$[\overset{\oplus}{S}H] = \frac{K_1[S][\overset{\oplus}{A}H]}{K_{-1}[A^{\ominus}] + K_2} \quad \ldots (3)$$

Therefore, rate of formation of product

$$\frac{d[P]}{dt} = K_2[\overset{\oplus}{S}H] = \frac{K_1K_2[S][\overset{\oplus}{A}H]}{K_{-1}[A] + K_2} \quad \ldots (4)$$

(a) If $K_2 >> K_{-1}$ [A]; K_{-1} [A] may be omitted from equation (4).
Then

$$\frac{d[P]}{dt} = K_1[S][\overset{\oplus}{A}H] \quad \ldots (5)$$

Equation (5) is expression for **general acid catalysis.**
(b) If $K_2 << K_{-1}$ [A]; K_2 may be omitted from the denominator of equation (4).
Then

$$\frac{d[P]}{dt} = \frac{K_1K_2[S][AH^{\oplus}]}{K_{-1}[A]} = \left(\frac{K_1K_2}{K_{-1}K}\right)[S][H^{\oplus}] \quad \ldots (6)$$

In equation (6) general acid $[\overset{\oplus}{A}H]$ of expression (4) has been replaced by hydrogen ions $[\overset{\oplus}{H}]$. This equation is expression for **specific hydrogen ion catalysis.**

(B) Second Mechanism (Base-catalysed Mechanism): Let us consider in step second protonated substrate $[\overset{\oplus}{S}H]$ reacts with base instead of water molecule:

$$S + \overset{\oplus}{A}H \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} \overset{\oplus}{S}H + A$$

$$\overset{\oplus}{S}H + A \xrightarrow{K_2} P + AH^{\oplus}$$

Upon applying steady state approximation for protonate substrate $[\overset{\oplus}{S}H]$:

$$\frac{d[\overset{\oplus}{S}H]}{dt} = O = K_1[S][\overset{\oplus}{A}H] - K_{-1}[\overset{\oplus}{S}H][A] - K_2[\overset{\oplus}{S}H][A] \quad \ldots (7)$$

Upon solving for $[\overset{\oplus}{S}H]$,

$$[\overset{\oplus}{S}H] = \frac{K_1[S][\overset{\oplus}{A}H]}{[K_{-1} + K_2][A]} \quad \ldots (8)$$

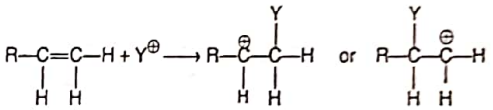
From equation (8) rate of formation of product can be given:

$$\frac{d[P]}{dt} = K_2[\overset{\oplus}{S}H][A] = \frac{K_1K_2[S][AH]^{\oplus}}{K_{-1} + K_2} \quad \ldots (9)$$

This equation (9) is expression for **general base catalysis.** If here [A] is replaced by $[\overset{\ominus}{O}H]$ it becomes case of **specific hydroxide ion catalysis.**

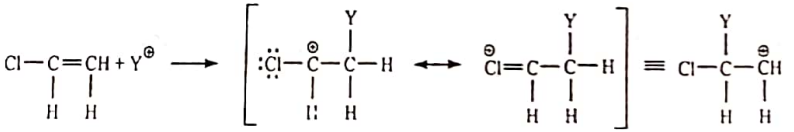
ORIENTATION AND STERIC EFFECT

When an unsymmetrical reagent is added to an unsymmetrical substrate, the question arises, which side of reagent goes to which side of double or triple bond ? For electrophilic attack the answer is given by **Markovinkov' rule** according to which negative part of reagent goes to the carbon that contains less number of hydrogen atoms. Most probable reason for this ***regioselectivity** is that electrophile $Y^{\oplus}$ attacks on the side which gives more stable carbocation.



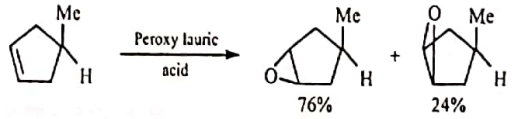
more stable

Now question is how $Y^{\oplus}$ knows which side will give more stable carbocation ? As in the similar case of **electrophilic aromatic substitution,** we invoke the fact that lower energy carbocation is preceded by lower energy transition state.† Halogeno substituted olefins & acetylenes also follow Markonikov's rule:



The fact that attack takes place from less hindered side & mroe stable product is formed through more stable intermediate or transition state also applies to enzymes. Stereochemical orientations are discussed below:

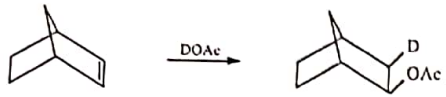
Some additions are **syn**, that is, both the group approach from the same side of double or triple bond. On the other hand, other additions are **anti**, in which two groups attack from the opposite side. In **syn** addition of unsymmetric cyclic olefins, two groups may come from more hindered or less hindered face of double bond. But, the general rule is that groups approach form less hindered side. For instance, in cycloaddition of 4-methylcylopentene 76% addition is from less hindered face & 25% is form more hindered side:



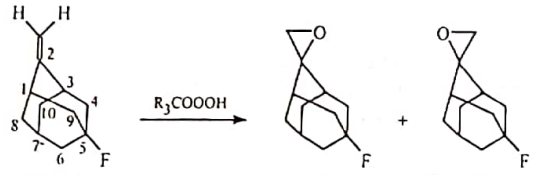
*When reaction can give rise to two or more structural isomers but produces only one, the reaction is said to be regioselective.

†Also that carbocation is stable to the greater extent in which charge delocalization is to the greater extent due to resonance or hyperconjugation.

Anti-addition also involves initial attack of the less hindered side of cyclic alkenes. But, generally additiion to norborane & some other strained molecules is **syn**. Electrophilic additions in these cases are from exo-side until & unless this side is blocked:



For example in 7, 7-dimethylnorborane **syn–endo**-epoxidation takes place. Furthermore, electronic effects also play determining role in deciding the course of attack. Admantanes behave in the same way as other olefins. Even then, hydroboration, dibromocarbene attack and epoxidation are from the side **syn** to electron withdrawing fluorine:

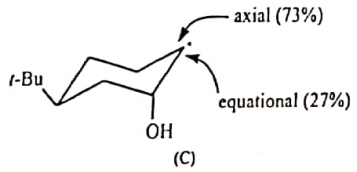


Admantane derivative (A) (B)

ratio = 66 : 34

In admantane derivatives the ratio of **syn**– and **anti**–attack is about 2 : 1. Similar results have been seen in other substrates. Electron directing field–effects, (*e.g.*, *–I* effect) direct the incoming group for **syn**–attack; + I–effect cause **anti**–attack for both nucleophilic as well as electrophilic reactions. The probable reason for this is **hypercojugation**.

Some examples of **anti**–attack are addition of HOBr & bromine through the formation of bromonium ions and free-radical addition of HBr. In case of cyclohexene, addition is not only **anti** but also confomationally specific. Unsymmetrical radicals also attack at sterically guided positions. For instance, radical (C) adds to double bond preferentially on side anti to —OH group, resulting in the formation of **trans**–product.



(C)

Electronic effects (for instance, resonance and field effects) may make faster or also slow down the rate of reactions & same is the result of steric effect. In the SN^2-reactions of alkyl halides rate of reaction depends upon the nature of alkyl groups, as shown in following Table–3.1.

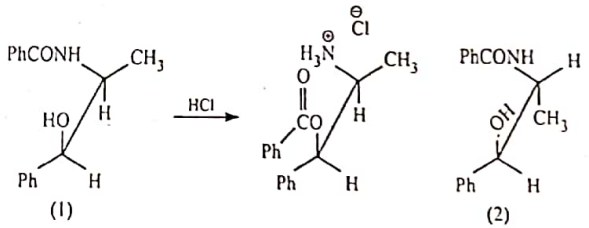
Table–3.1: Relative rates of reaction of RBr with ethanol.

R	Relative rate
CH_3	17.6
CH_3CH_2	1.0
$CH_3CH_2CH_2$	.28
$(CH_3)_2CHCH_2$	.030
$(CH_3)_3CCH_2$	4.2×10^{-6}

Although all alkyl groups are primary; but branching on second carbon atom causes steric hinderance, thus, decreases the rate of reaction.

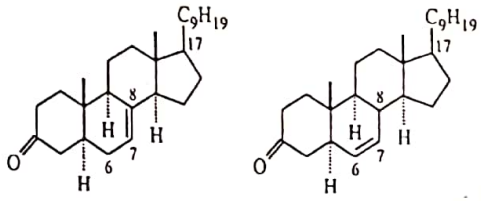
Conformational disposition also effects the reactivity and orientation on account of steric effect. A number of reactions fail because molecules are not in proper conformation, for example, rearrangement of N–benzoylnorephedrine. Two diastereomers of this compound behave entirely differently upon treatment with alcohol and hydrochloric acid. In one isomer N– to O–migration takes place; but in the other not at all. For migration to occur nitrogen must be near oxygen (gauche to it). When (1) assumes this conformation, the methyl and phenyl groups are **anti** to each other, which is a favourable position; but when (2) contains nitrogen **gauche** to oxygen, methyl and phenyl are also **gauche** which is an unfavourable situation for reaction to occur. Besides, E–Z–eliminations as well as electrophilic additions are sterically effected. On a number of occasions **axial** & **equatorial** groups behave differently.

In steroids & other such rigid systems; functional group in one part of the molecule behaves in entirely different way from the other part of molecule leading to its impact on rate of reaction by altering the conformation of whole skeleton. This effect is known as **conformational transmission**. An example of this is in ergost–7–ene–3–one (3) and cholest–6–ene–3–one (4). (4) Condenses with



(1) (2)

benzaldehyde 15 times faster than (3). The reaction site in both cases is carbonyl group and the increase in reaction rate is because moving the double bond from 7 to 6–position changes the conformation at CO group. However, difference in side chain at C–17 does not effect the rate of reaction.



(3) (4)

Strain or Distortion

A molecule comes under strain if there is deviation from normal bond angles. For an organic molecule strainless bond angle is 109°28'. Any increase or decrease from it makes molecule under strain and molecule gets distorted. Angular strain or distortion increases energy of the species making it unstable or more reactive. Strain exists generally in either very small cyclic compound or medium sized cyclic compounds. In former case bond angle is smaller than normal and is called **small ring strain**. Strain also arises due to **non-bonded interaction** between the atoms present in close proximity in a molecule. Strained molecules have strain energy associated with them, this is, why their energy is higher than unstrained molecules. Exact value of strain energy can not be calculated.

Strain in Small Rings: Three membered cyclic compounds are highly reactive because they have very large deviation form normal tetrahedral 'bond angle (109°28'). For example, ethylene oxide is more reactive than aliphatic ethers; cyclopropane is more reactive than open chain saturated hydrocarbons. In saturated hydrocarbons, each of the carbons is in sp^3 hybridized state and each of its four sp^3 hybrid orbitals contains 25% s–character and 75% p-character. But in cyclopropane each carbon atom has four hybrid orbitals which are not equivalent. Two orbitals directed outside have more s–character than sp^3 orbitals & two orbitals involved in ring formation contain more p–character than normal bond angle of 90° from the plane of molecule. The strain in **cyclopropane** molecule is the difference between preferred angle & actual angle of 60°. The additional p–character of orbital in ring relieves the strain to some extent. In reality, external orbitals have 33% s–character which is near to sp^2–hybrid orbitals. But inner orbitals have 17% *s*-character which is near to sp^5–hybrid orbitals. Therefore, each of C—C bonds of cyclopropane is formed by the overlap of approximately sp^5–orbitals. The molecular orbital calculations also reveal that these bonds are not purely σ. In normal σ–bond electron density is directed in the plane joining two sp^3 Orbitals. But in cyclopropane more electron density is directed outward from the ring & bond takes the shape of banana as shown below:

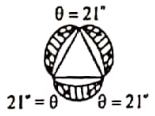
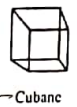


Fig. 3.1 Orbital overlap in cyclopropane. Arrows point towards the centre of electron-density

Angle θ in cyclopropane is 21° & in cyclobutane it is just 7°. Calculations also reveal that maximum electron density of C—C σ bond is bent away from the ring with θ = 9.4° in cyclopropane and 3.4° in cyclobutane. Thus σ–bonds of C—C behave in partly π–bond manner in small ring compounds; this is also a reason for their higher reactivity.

Some highly strained molecules have been prepared which have higher reactivities


Cubane


Prismane

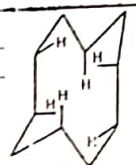
1, 3-Dehydro-adamantane

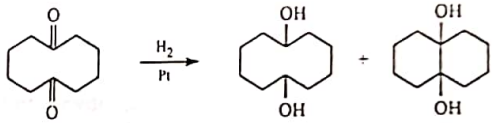
than cyclopropanes & cyclobutanes. For example: Cubane, prismane & substituted, tetrahedrane, 1, 3-Dehydroadmantane also belong to the same class.

Since strain affects chemical reactivity, measurement of latter indicates the presence of former. Chemical reactivity can be measured in terms of heat of formation, heat of combustion, dipole moment, absorption spectra etc. The **total strain** can be best calculated in terms of **heat of combustion** by the following formula:

Total strain = Number of carbon atoms in the ring × observed heat of combustion/CH_2 – observed heat of combustion/CH_2 for n–alkanes.

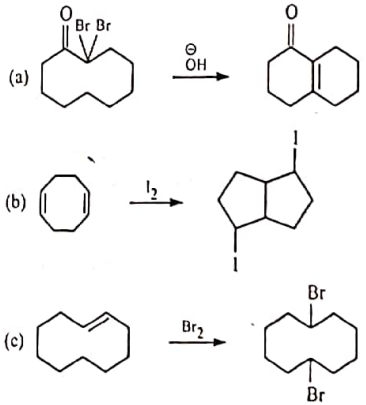
Strain in Medium Rings: Medium rings generally have larger strains in comparison to small rings. Important factors contributing to ring strain are steric repulsion, angle deformation and bond opposition forces. For instance, in cyclodecane X–ray analysis has shown its conformation to be (I), in which two cyclohexane rings are joined by 1, 3-axial bonds. Groups at 1, 2-positions are eclipsed and cause steric strain. In (I), there is steric repulsion between atoms present on the opposite side of the ring; which is also known as **transannular interaction** or **transannular strain.** Another important example of **transannular strain** is catalytic reduction of cyclodecane –1, 6–dione (II) which gives a mixture of 1, 6–dihydroxycyclodecane (III) & 9, 10–dihydroxy decalin (IV):


(I)



(II) (III) (IV)

Some other examples of transannular interaction are:



Thus transannular interaction exists mainly in 8–11 membered rings & in some other larger rings too. That conformation of cyclic compound is most stable in which

strain is minimum. For example, 'Tub-shaped'-conformation of cyclooctatetraene is most stable on account of minimum strain:

Tub-Conformation

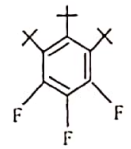
Stability of ring-compounds increases upto six-membered ring, then decreases from seven to eleven-membered rings & from tweleve-membered onwards attains the stability of six-membered ring. These conculsions are based upon heat of combustion and are summarized in the following Table:

Number of C-atoms in ring	Angle between valency bonds	Distortion	Heat of combustion in KJ/ CH_2	Total strain (KJ)
2	0°	54°44′	711	108
3	60°	24°44′	697	120
4	90°	9°44′	685	112
5	108°	0°44′	664	35
6	120°	- 5°16′	659	12
7	128°34′	- 9°33′	662	35
8-11	137-145°16′	- 12°45" to 18°54′	661-665	32-88
12-	150°	- 20°16′	657-661	0-48
n-alkanes	109°28′	0°	657	0

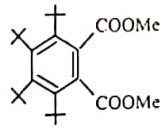
Strain in Large rings (12-membered onwards) generally have very little or no-strain.

Strain in Unsaturated rings: Unsaturated rings have more strain than saturated rings. For example, cyclopropene is about 10° more strained than cyclopropane. But, this additional strain in cyclopropene is balanced by lack of eclipsing strain of two hydrogen atoms, which are absent in it.

Besides, unavoidable crowding also increases strain. Some such molecules are:

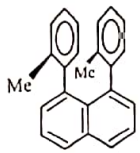


Tri-*t*-butyl-trifluorobenzene

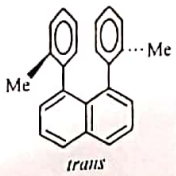


Tetra-*t*-butyl-dimethyl phthalate

Few *cis-trans* isomers are possible for of the reason that free rotation of group is not possible because of crowding. For example, *cis* & *trans* -1, 8-di-o-tolylnapthalene:



cis

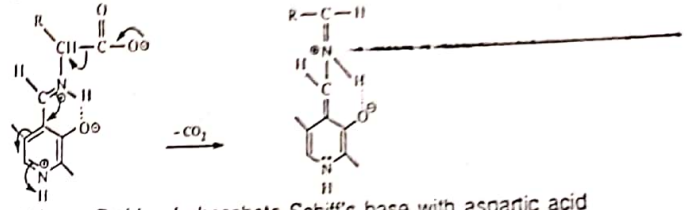


trans

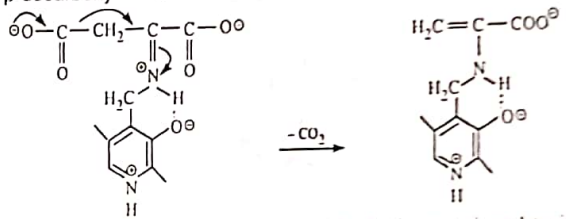
As strains also arise by non-bonded interactions between groups in close

proximity to each other, therefore, they make some enzymes & co-enzymes very reactive & they readily undergo various elimination reactions, remove like α-decarboxylations, β-decarboxylation and removal of α-hydrogen. For example, non-covalently bonded but H-bonded, pyridoxal phosphate-amino acid Schiffs base acts as electronic sink & effectively neutralizes negative charge & undergoes elimination as given below:

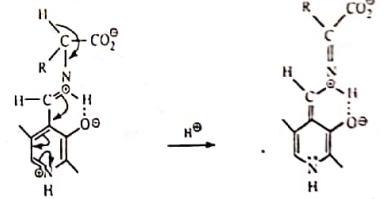
(a) **α-Decarboxylation:** Pyridoxal phosphate Schiff's base undergoes α-decarboxylation as per following scheme.



(b) **β-Decarboxylation:** Pyridoxal phosphate Schiff's base with aspartic acid undergoes β-decarboxylation:



(c) **Removal of α-hydrogen:** Removal of α-hydrogen gives key-intermediate which may react in different ways.

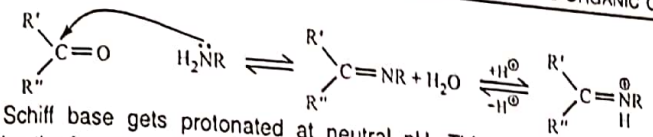


Moreover, enzymes assist in catalysis by distorting bond length or bond angles away from the normal values. If distorted structures are closer to transition state geometry than undistorted one catalysis is assisted. Activation energy for formation of enzyme substrate-complex may come from translation energy of solvent or solute molecules. Kinetic energy may channel from surface of enzyme to active-site.

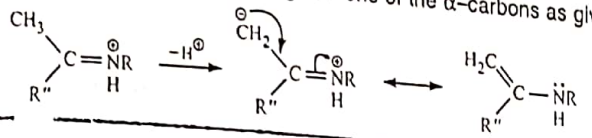
COVALENT CATALYSIS

A catalyst that adds to substrate through covalent bond is known as covalent catalyst & the phenonmenon is known as covalent catalysis. Some examples are discussed below:

1. Electrophilic catalysis by Schiff's base formation: Transient modification of a substrate can activate it for a chemical reaction. For example, Schiff base formation from the condensation of an amine with a carbonyl compound:

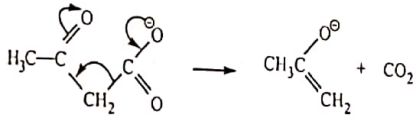


Schiff base gets protonated at neutral pH. This acts as **electron-sink** to stabilize the formation of a negative charge on one of the α-carbons as given below:

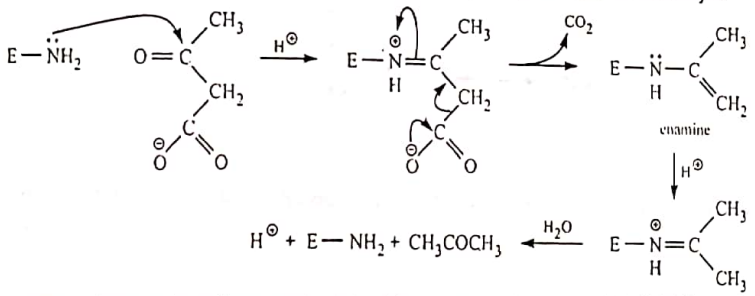


After tautomerization to form enamine, the methylene carbon is activated as neuclophile. Advantage of Schiff base formation is that the carbonyl group gets activated towards nucleophilic attack because of strong electron withdrawal by protonated nitrogen. An example is given below:

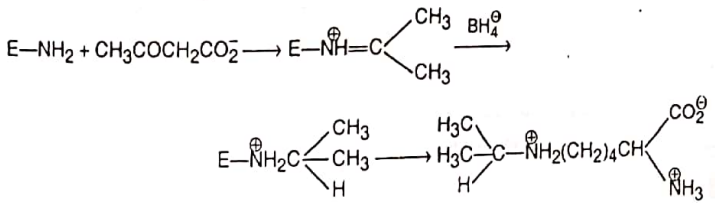
Acetoacetate decarboxylase: This enzyme catalyses decarboxylation of acetoacetate. The nonenzymatic reaction involves the explusion of highly basic enolate ion at neutral pH, but the enzymatic reaction circumvents this by the formation of a Schiff base with lysine residue. The protonated imine is then readily expelled.

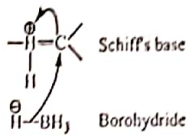


This process may be mimicked in solution by using aniline as a catalyst.



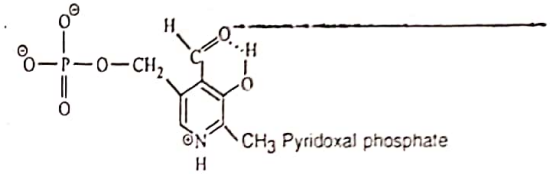
The evidence for intermediate is that the enzyme is irreversibly inhibited when sodium borohydride is added to complex with the substrate. Borohydride is known to reduce Schiff bases, and the hydrolysate of the inhibited protein is found to contain isopropyl-lysine. The carbon in the Schiff base is activated to the attack of an $H^{\ominus}$ ion from the borohydride:



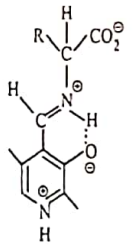


2. Pyridoxal phosphate–Electrophilic catalysis

The principles discussed above form the basis of a series of important metabolic interconversions involving the co-enzyme pyridoxal phosphate.

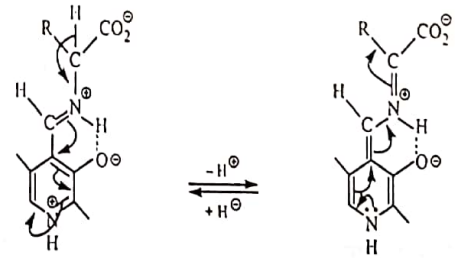


This condenses with amino acids to form a Schiff base. The pyridine ring in the Schiff base acts as an **"electron sink"** which very effectively stabilizes a negative charge:



Each of the groups around the chiral carbon of amino acid may be cleaved, forming an anion that is stabilized by the Schiff base with the pyridine ring. Different processes involved are discussed below:

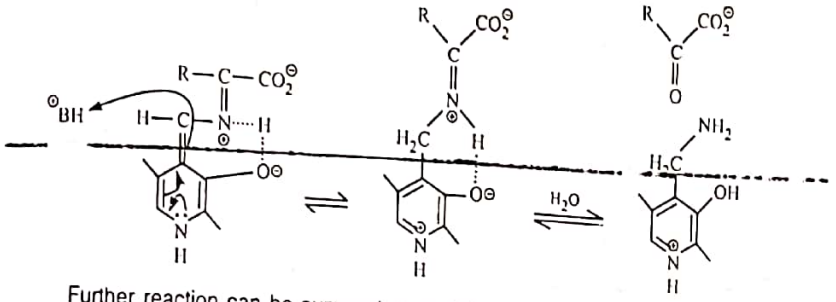
(a) Removal of α-hydrogen: The removal of the α-hydrogen gives a key intermediate.



This intermediate may react in different ways:

1. Racemization: Addition of proton back to amino acid will lead to racemization unless it is done stereospecifically.

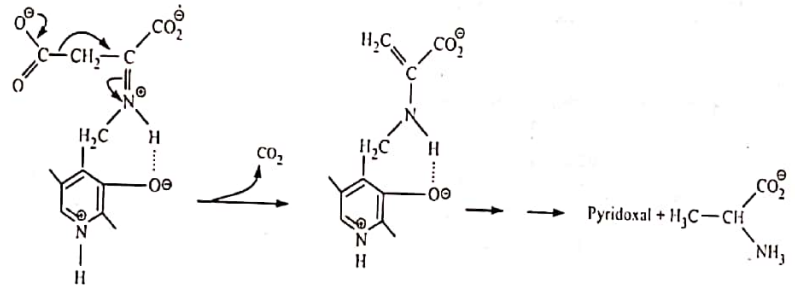
2. Transamination: Addition of a proton to carbonyl carbon of the pyridoxal leads to a compound which is Schiff base of α-keto acid & pyridoxamine. Hydrolysis of the Schiff base gives the α-keto acid & pyridoxamine, which may react with a different α-keto acid to reverse the sequence:



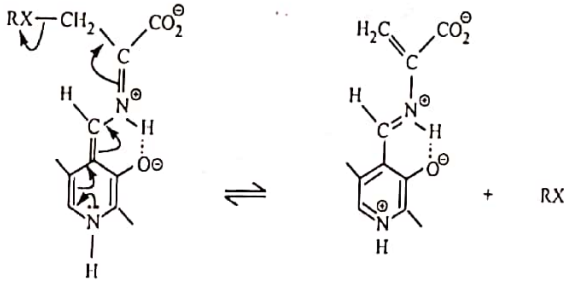
Further reaction can be summed up as follows:

$$R'CH(\overset{\oplus}{N}H_3)CO_2^{\ominus} + R''COCO_2^{\ominus} \rightleftharpoons R'COCO_2^{\ominus} + R''CH(\overset{\oplus}{N}H_3)CO_2^{\ominus}$$

3. β-Decarboxylation: When amino acid is aspartic acid β-decarboxylation is possible:

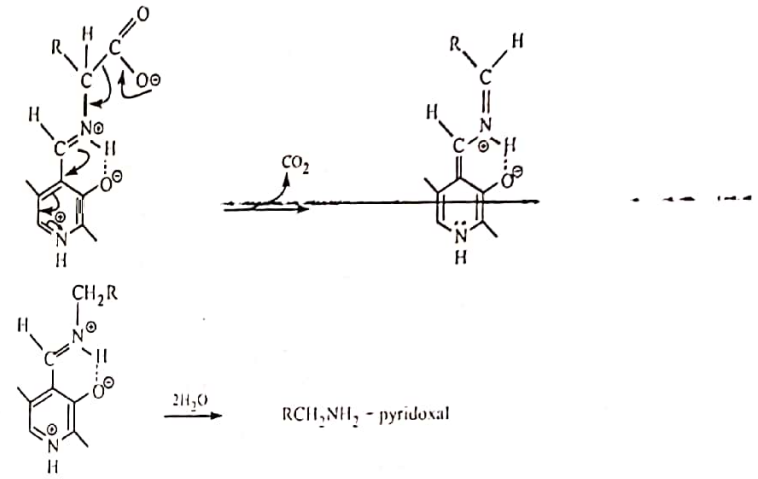


4. Elimination from side chains: When RX— is a good leaving group, it may be expelled as given below:

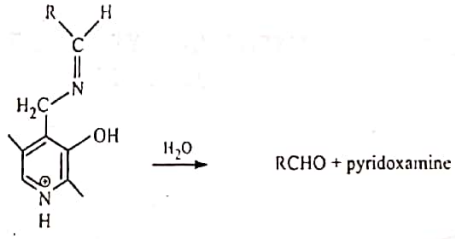


RX— may be a thiol, a hydroxyl, or an indol group. Therefore, serine, threonine, cystein, tryptophan, cystathionine, and serine as well as threonine phosphates may be degraded.

(b) α-Decarboxylation: The electronic sink allows facile decarboxylation. The decarboxylated adduct adds up a proton to amino acid carbonyl carbon & then hydrolyses to give amino acid & pyridoxal.

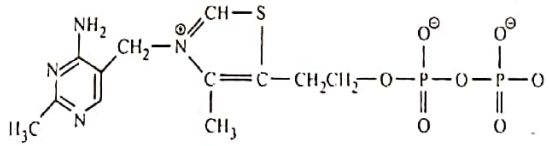


Decarboxylated adduct may also add proton to pyridoxal carbon & then hydrolyse to give the aldehyde and pyridoxamine:



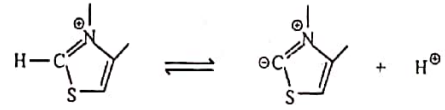
3. Thiamine pyrophosphate (Electrophilic catalyst)

It is also an coenzyme that **covalently** bonds to substrate & stabilizes a negative charge.



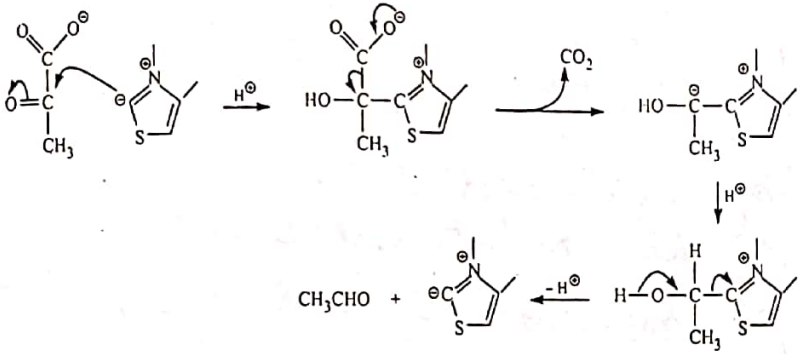
Thiamine pyrophosphate

The positive charge on nitrogen promotes the ionization of the C–2 carbon by **electrostatic stabilization**. The ionized carbon is a potent nucleophile:

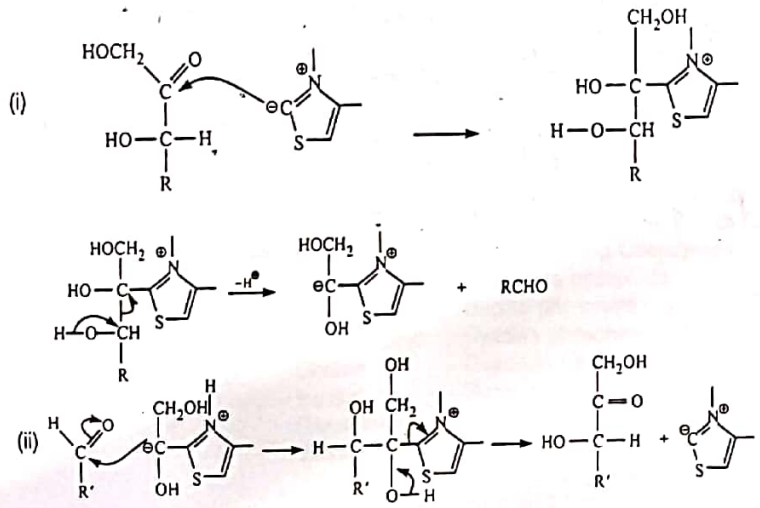


The nitrogen atom can also stabilize by delocalization the negative charge of the adduct of **thiamine** within many compounds, as for example, in **hydroxyethylamine pyrophosphate**, a form in which much of the coenzyme is found in vivo.

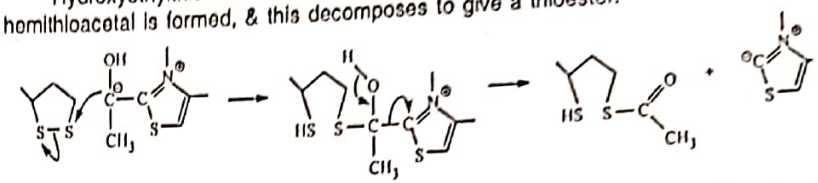
The combination of these reactions allows the decarboxylation of pyruvate as given below:



The hydroxyethylthioamine pyrophosphates are potent nucleophiles & may add to carbonyl compounds to form C—C bonds. A good illustration of the C—C bond making & breaking occurs in the reactions of **transketolase.** The enzyme contains tightly bound thiamine pyrophosphate & shuttles a dihydroxyethyl group between D–xylulose–5–phosphate & D–ribase–5–phosphate to form D–sedoheptulose 7–phoshate & D–glyceraldehyde–3–phosphate as illustrated below:



Hydroxyethylthiamine is nucleophilic towards a thiol of oxidised lipoic acid. A hemithioacetal is formed, & this decomposes to give a thioester:



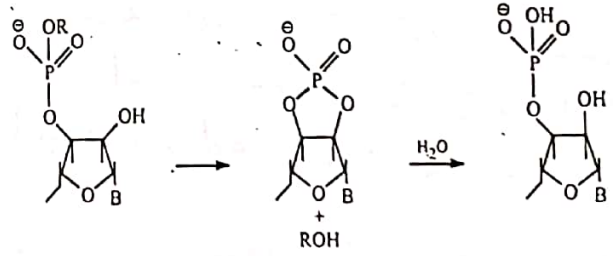
4. Nucleophilic catalysis: In enzymes, the most common nucleophilic groups that are functional in catalysis are the serine hydroxyl–which occur in the serine protease, cholinesterase, esterase, lipase, the alkaline phosphatase– & the cysteine thiol–which occurs in the thiol proteases (papain, ficin & bromelain) etc. The imidazole of histidine usually functions as an acid–base catalyst and enhances the nucleophilicity of hydroxyl and thiol groups, but it sometimes acts as a nucleophile with the phosphoryl group in phosphate transfer.

The hydrolysis of peptides by these proteases represents classic nucleophilic catalysis. The relatively inert peptide is converted to the far more reactive ester or thioester acylenzyme, which is rapidly hydrolysed. The use of serine hydroxyl rather than the direct attack of a water molecule on the substrate is favoured in several ways: alcohols are often better nucleophiles than the water molecules in both general-base-catalysed & direct nucleophilic attack; the serine reaction is intramolecular & hence favoured entropically and the arrangement of groups is more rigid & defined for the serine hydroxyl as compared with a bound water molecule.

EXAMPLES OF SOME TYPICAL ENZYME MECHANISMS

'RIBONUCLEASE'

Bovine (cattle) pancreatic nuclease catalyses the hydrolysis of RNA by a two step process in which a cyclic phosphate intermediate is formed. The cyclization is usually much faster than the subsequent hydrolysis, therefore, intermediate may be isolated. DNA is not hydrolysed, as it lacks the 2′–hydroxyl group that is essential for this reaction. There is a strong specificity for the base B on the 3′–side of the substrate which is either uracil or cytosine.



The enzyme consists of single peptide chain of 124 amino acid residues & relative molecular mass of 13680. The bond between Ala–20 & ser–21 may be cleaved by **subtilisin.** The peptide remains attached to the rest of protein by non-