Theory of Enzyme Catalysis
1- Active Site
   A- Active Site Localization
   B- Substrate Binding

1- Stereospecificity
2- “Lock and Key” Hypothesis
3- Induced-fit Model

2- Reasons for Catalytic Activity
   A- Steric Effects—Orientation Effects
   B- Structural Complementarity to Transition State
   C- General Acid–Base Catalysis
   D- Covalent Catalysis
-It has been illustrated with several examples that enzymes are substantially better catalysts than are protons or other ionic species used in non-enzymatic reactions.

-Enzymes in-varily surpass all chemical catalysts in relation to substrate and reaction specificities.

-Theories have been developed to explain the exceptional efficiency of enzyme activity. They are based on findings which provide only indirect insight into enzyme catalysis.

-Examples are
- the identification of an enzyme’s functional groups involved in catalysis,
- elucidation of their arrangement within the tertiary structure of the enzyme, and
- the detection of conformational changes induced by substrate binding.

-Complementary studies involve low molecular weight model substrates, the reactions of which shed light on the active sites or groups of the enzyme and their coordinated interaction with other factors affecting enzymatic catalysis.
1- Active Site

- An enzyme molecule is, when compared to its substrate, often larger in size by a factor of several orders of magnitude.

- For example, glucose oxidase \((M_r = 1.5 \cdot 10^5)\) and glucose \((M_r = 180)\).

- This strongly suggests that in catalysis only a small locus of an active site has direct contact with the substrate.

- Specific parts of the protein structure participate in the catalytic process from the substrate binding to the product release from the so-called active site.

- These parts are amino acid residues which bind substrate and, if required, cofactors and assist in conversion of substrate to product.

- Investigations of the structure and function of the active site are conducted to identify the amino acid residues participating in catalysis, their steric arrangement and mobility, the surrounding micro-environment and the catalysis mechanism.
1- Active Site  
A- Active Site Localization

-Several methods are generally used for the identification of amino acid residues present at the active site since data are often equivocal. Once obtained, the data must still be interpreted with a great deal of caution and insight.

-The influence of pH on the activity assay provides the first direct answer as to whether dissociable amino acid side chains, in charged or uncharged form, assist in catalysis.

-The data readily obtained from this assay must again be interpreted since neighboring charged groups, hydrogen bonds or the hydrophobic environment of the active site can affect the extent of dissociation of the amino acid residues.
1- Active Site
A- Active Site Localization

Selective labeling of side chains which form the active site is also possible by chemical modification.

When an enzyme is incubated with reagents such as iodoacetic acid or dinitrofluorobenzene, resulting in a decrease of activity, and subsequent analysis of the modified enzyme shows that only one of the several available functional groups is bound to reagent (e.g. one of several −SH groups), then this group is most probably part of the active site.

Selective labeling data when an inhibiting substrate analogue is used are more convincing.

Because of its similarity to the chemical structure of the substrate, the analogue will be bound covalently to the enzyme but not converted into product.
1- Active Site
A- Active Site Localization

We can consider the following examples:

N-tosyl-L-phenylalanine ethyl ester is a suitable substrate for the proteinase chymotrypsin which hydrolyzes ester bonds.

-When the ethoxy group is replaced by a chloromethyl group, an inhibitor whose structure is similar to the substrate is formed (Nα-tosyl-L-phenylalanine chloromethylketone, TPCK).
1- Active Site
A- Active Site Localization

-Thus, the substrate analogue binds specifically and irreversibly to the active site of chymotrypsin.

-Analysis of the enzyme inhibitor complex reveals that, of the two histidine residues present in chymotrypsin, only His$^{57}$ is alkylated at one of its ring nitrogens. Hence, the modified His residue is part of the active site.

-TPCK (Nα-tosyl-L-phenylalanine chloromethylketone) binds highly specifically, thus the proteinase trypsin is not inhibited.
Reaction of diisopropylfluorophosphate (DIFP) with a number of proteinases and esterases alkylates the unusually reactive serine residue at the active site.

Thus, of the 28 serine residues present in chymotrypsin, only Ser\textsuperscript{195} is alkylated, while the other 27 residues are unaffected by the reagent.

It appears that the reactivity of Ser\textsuperscript{195} is enhanced by its interaction with the neighboring His\textsuperscript{57}.

The participation of carboxyl group at the active site in β-glucosidase catalysis has been confirmed with the help of conduritol B-epoxide, an inhibiting substrate analogue:
-A lysine residue is involved in enzyme catalysis in a number of lyase enzymes and in enzymes in which pyridoxal phosphate is the cosubstrate.

-An intermediary Schiff base product is formed between an amino group of the enzyme and the substrate or pyridoxal phosphate.

-The reaction site is then identified by reduction of the Schiff base with NaBH₄.

-An example of a “lysine” lyase is the aldolase enzyme isolated from rabbit muscle. The intermediary product formed with dihydroxyacetone phosphate is detected as follows:
Enzymes react stereo-specifically.

Before being bound to the binding locus, the substrates are distinguished by their cis, trans-isomerism and also by their optical antipodes. The latter property was illustrated by the reactions of L(+)-lactic acid.

There are distinct recognition areas on the binding locus.

Alcohol dehydrogenase will be used to demonstrate this. This enzyme removes two hydrogen atoms, one from the methylene group and the other from the hydroxyl group, to produce acetaldehyde.
- However, the enzyme recognizes the difference between the two methylene hydrogens since it always stereospecifically removes the same hydrogen atom.

- For example, yeast alcohol dehydrogenase always removes the pro-R-hydrogen from the C-1 position of a stereospecifically deuterated substrate and transfers it to the C-4 position of the nicotinamide ring of NAD:
To explain the stereospecificity, it has been assumed that the enzyme must bind simultaneously to more than one point of the molecule.

Thus, when two substituents (e.g. methyl and hydroxyl groups of ethanol) of the prochiral site are attached to the enzyme surface at positions A and B, the position of the third substituent is fixed.

Therefore, the same substituent will always be bound to reactive position C, e.g. one of the two methylene hydrogens in ethanol. In other words, the two equal substituents in a symmetrical molecule are differentiated by asymmetric binding to the enzyme.
- To explain substrate specificity, *Fischer* proposed hypothesis a century ago in which he depicted the substrate as being analogous to a key and the enzyme as its lock.

**Binding of a good (3) and of a bad substrate (2) by the active site (1) of the enzyme. (4) A productive enzyme-substrate complex; (5) a nonproductive enzyme substrate complex.**

*As₁* and *As₂*: reactive amino acid residues of the enzyme involved in conversion of substrate to product.
In contrast, there are many possibilities for a “bad” substrate to be bound to the enzyme, but only one provides the properly positioned enzyme-substrate complex, which is converted to the product.

The proteinases chymotrypsin and trypsin are two enzymes for which secondary and tertiary structures have been elucidated by x-ray analysis and which have structures supporting the lock and key hypothesis to a certain extent.
1- Active Site
B- Substrate Binding
2- “Lock and Key” Hypothesis

- The binding site in chymotrypsin and trypsin is three-dimensional hydrophobic pocket (Fig.). Bulky amino acid residues such as aromatic amino acids fit neatly into the pocket (chymotrypsin, Fig.a), as do substrates with lysyl or arginyl residues (trypsin, Fig.b).

- The peptide substrate is hydrolyzed by the enzyme elastase by the same mechanism as for chymotrypsin. However, here the pocket is closed to such an extent by the side chains of Val^{216} and Thr^{226} that only the methyl group of alanine can enter the cleft (Fig.c). Therefore, elastase has specificity for alanyl peptide bonds or alanyl ester bonds.

A hypothesis for substrate binding by α-chymotrypsin, trypsin and elastase enzymes
-The conformation of a number of enzymes is changed by the binding of the substrate.

-An example is carboxypeptidase A, in which the $\text{Try}^{248}$ located in the active site moves approximately 12 Å towards the substrate, glycyl-L-phenylalanine, to establish contact.
1- Active Site
B- Substrate Binding
3- Induced-fit Model

- This and other observations support the dynamic induced-fit model.

- Here, only the substrate has the power to induce a change in the tertiary structure to the active form of the enzyme. Thus, as the substrate molecule approaches the enzyme surface, the amino acid residues A and B change their positions to conform closely to the shape of the substrate (I, in Fig.). Groups A and B are then in the necessary position for reaction with the substrate.

- Diagrams II and III illustrate the case when the added compound is not suitable as substrate.

A schematic presentation of “induced-fit model” for an active site of an enzyme.

— Polypeptide chain of the enzyme with catalytically active residues of amino acids, A and B; the residue C binds the substrate.
Although group C positioned the substrate correctly at its binding site, the shape of the compound prevents groups A and B from being aligned properly in their active positions and, thus, from generating the product.

In accordance with the mechanisms outlined above, one theory suitable for enzymes following the lock and key mechanism and the other theory for enzymes operating with the dynamic induced-fit model, the substrate specificity of any enzyme-catalyzed reaction can be explained satisfactorily.

In addition, the relationship between enzyme conformation and its catalytic activity thus outlined also accounts for the extreme sensitivity of the enzyme as catalyst. Even slight interferences imposed on their tertiary structure which affect the positioning of the functional groups result in loss of catalytic activity.
2- Reasons for Catalytic Activity

- Even though the rates of enzymatically catalyzed reactions vary, they are very high compared to the effectiveness of chemical catalysts.

- The factors responsible for the high increase in reaction rate will be outlined below. They are of different importance for the individual enzymes.
2- Reasons for Catalytic Activity

A- Steric Effects—Orientation Effects

-The specificity of substrate binding contributes substantially to the rate of an enzyme-catalyzed reaction.

-Binding to the active site of the enzyme concentrates the reaction partners in comparison with a dilute substrate solution.

-In addition, the reaction is now the favored one since binding places the substrate’s susceptible reactive group in the proximity of the catalytically active group of the enzyme.

-Therefore, the contribution of substrate binding to the reaction rate is partially due to a change in the molecularity of the reaction.

-The intermolecular reaction of the two substrates is replaced by an intra-molecular reaction of an enzyme-substrate complex.
2- Reasons for Catalytic Activity

A- Steric Effects—Orientation Effects

-The consequences can be clarified by using model compounds which have all the reactive groups within their molecules and, thus, are subjected to an intra-molecular reaction.

-Their reactivity can then be compared with that of the corresponding bimolecular system and the results expressed as a ratio of the reaction rates of the intra-molecular to the intermolecular reactions. Based on their dimensions, they are denoted as “effective molarity”.

-As an example, let us consider the cleavage of p-bromophenylacetate in the presence of acetate ions, yielding acetic acid anhydride:

\[
\text{Br-O-CO-CH}_3 + \text{CH}_3\text{COO}^- + \text{Na}^+ \rightarrow \text{Br-O-Na}^+ + (\text{CH}_3\text{CO})_2\text{O}
\]
2- Reasons for Catalytic Activity
A- Steric Effects – Orientation Effects

-Intra-molecular hydrolysis is faster than the inter-molecular reaction.

-The effective molarity sharply increases when the reactive carboxylate anion is in close to the ester carbonyl group and, by its presence, retards the mobility of the carbonyl group. Thus, the effective molarity increases (Table) as the C–C bond mobility decreases.

-Two bonds can rotate in a glutaric acid ester, whereas only one can rotate in a succinic acid ester.

-The free rotation is effectively blocked in a bicyclic system. Hence, the reaction rate is sharply increased. Here, the rigid steric arrangement of the acetate ion and of the ester group provides a configuration that imitates that of a transition state.

Relative reaction rate for the formation of cid anhydrides
2. Reasons for Catalytic Activity

A. Steric Effects – Orientation Effects

-In contrast to the last examples, examples should be mentioned in which substrates are not bound covalently by their enzymes.

-The following model will demonstrate that other interactions can also promote close positioning of the two reactants.

-Hydrolysis of \textit{p-nitrophenyldecanoic acid ester} is catalyzed by an \textit{alkylamine}:

\begin{align*}
&\text{\textit{p-nitrophenyldecanoic acid ester}} \rightarrow \text{\textit{decylamine}} \rightarrow \\
&\text{\textit{p-nitrophenyldecanoic acid ester}} + \text{\textit{water}} \rightarrow \text{\textit{decylamine}} + \text{\textit{water}}
\end{align*}

-The reaction rate in the presence of \textit{decylamine} is faster than that in the presence of \textit{ethylamine} by a factor of 700.

-This implies that the \textbf{reactive amino group} has been oriented very close to the susceptible \textbf{carbonyl group of the ester} by the establishment of a maximal number of \textbf{hydrophobic contacts}.

-Correspondingly, there is a decline in the reaction rate as the \textbf{alkyl amine group} is lengthened further.
- It is assumed that the active conformation of the enzyme matches the transition state of the reaction. This is supported by affinity studies which show that a compound with a structure analogous to the transition state of the reaction ("transition state analogs") is bound better than the substrate.

- Hydroxamic acid, for example, is such a transition state analog which inhibits the reaction of triosephosphate isomerase.

**Example of a transition state analog inhibitor a reaction of triosephosphate isomerase, TT: postulated transition state; b inhibitor**
2- Reasons for Catalytic Activity
C- General Acid–Base Catalysis

-When the reaction rate is affected by the concentration of hydronium \((H_3O^+)\) or \(OH^8\) ions from water, the reaction is considered to be specifically acid or base catalyzed.

-In the so-called general acid or base catalysis the reaction rate is affected by prototropic groups located on the side chains of the amino acid residues. These groups involve proton donors (denoted as general acids) and proton acceptors (general bases).

-Most of the amino acids located on the active site of the enzyme influence the reaction rate by general acid-base catalysis.
2- Reasons for Catalytic Activity

C- General Acid–Base Catalysis

-As already mentioned, the amino acid residues in enzymes have prototrophic groups which have the potential to act as a general acid or as a general base.

-Of these, the imidazole ring of histidine is of special interest since it can perform both functions simultaneously:
-The **imidazole ring** can cover the range of the **pH optima** of many enzymes.

-Thus, two **histidine residues** are involved in the catalytic activity of ribonuclease, a **phosphodiesterase**. The enzyme hydrolyzes **pyrimidine-2,3-cyclic phosphoric acids**. As shown in Figure, cytidine-2,3-cyclic phosphoric acid is positioned between two **imidazole groups** at the binding locus of the active site.

**Hydrolysis of cytidine-2,3-phosphate by ribonuclease**

-**His**$^{12}$ serves as a general **base**, removing the proton from a water molecule. This is followed by nucleophilic attack of the intermediary **OH$^e$** ions on the electrophilic **phosphate group**.
Another general acid-base catalysis is illustrated by triose phosphate isomerase, an enzyme involved in glycolysis. Here, the concerted action involves the carboxylate anion of a glutamic acid residue as a general base with a general acid which has not yet been identified:
2- Reasons for Catalytic Activity
C- General Acid–Base Catalysis

- These two examples show clearly the significant differences to chemical reactions in solutions.
- The enzyme driven acid-base catalysis takes place selectively at a certain locus of the active site.
- The local concentration of the amino acid residue acting as acid or base is fairly high due to the perfect position relative to the substrate.
- On the other hand, in chemical reactions in solutions all reactive groups of the substrate are non-specifically attacked by the acid or base.
2- Reasons for Catalytic Activity

D- Covalent Catalysis

-Studies aimed at identifying the active site of an enzyme have shown that, during catalysis, a number of enzymes bind the substrate by covalent linkages.

-Such covalent linked enzyme-substrate complexes form the corresponding products much faster than compared to the reaction rate in a non-catalyzed reaction.

-Examples of enzyme functional groups which are involved in covalent bonding and are responsible for the transient intermediates of an enzyme-substrate complex are presented in the Table.
**2- Reasons for Catalytic Activity**

**D- Covalent Catalysis**

**Examples of covalently linked enzyme-substrate intermediates**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Reactive functional group</th>
<th>Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chymotrypsin</td>
<td>HO-(Serine)</td>
<td>Acylated enzyme</td>
</tr>
<tr>
<td>2. Papain</td>
<td>HS-(Cysteine)</td>
<td>Acylated enzyme</td>
</tr>
<tr>
<td>3. β-Amylase</td>
<td>HS-(Cysteine)</td>
<td>Maltosylenzyme</td>
</tr>
<tr>
<td>4. Aldolase</td>
<td>ε-H$_2$N-(Lysine)</td>
<td><em>Schiff</em> base</td>
</tr>
<tr>
<td>5. Alkaline phosphatase</td>
<td>HO-(Serine)</td>
<td>Phosphoenzyme</td>
</tr>
<tr>
<td>6. Glucose-6-phosphatase</td>
<td>Imidazole- (Histidine)</td>
<td>Phosphoenzyme</td>
</tr>
<tr>
<td>7. Histidine decarboxylase</td>
<td>O=C &lt; (Pyruvate)</td>
<td><em>Schiff</em> base</td>
</tr>
</tbody>
</table>
2- Reasons for Catalytic Activity

D- Covalent Catalysis

-A number of peptidase and esterase enzymes react covalently in substitution reactions by a two-step nucleophilic mechanism.

-In the first step, the enzyme is acylated; in the second step, it is deacylated.

-Chymotrypsin is discussed as an example of this reaction mechanism.

-Its activity is dependent on His$^{57}$ and Ser$^{195}$, which are positioned in close proximity within the active site of the enzyme because of folding of the peptide chain.
Postulated reaction mechanism for chymotrypsin activity

- Because Asp$^{102}$ is located in hydrophobic surroundings, it can polarize the functional groups in close proximity to it. Thus, His$^{57}$ acts as a strong general base and abstracts a proton from the OH-group of the neighboring Ser$^{195}$ residue (step ‘a’, Fig.).

- The oxygen remaining on Ser$^{195}$ thus becomes a strong nucleophile and attacks the carbon of the carbonyl group of the substrate peptide bond.

- At this stage an amine (the first product) is released (step ‘b’, Fig.) and the transient covalently-bound acyl enzyme is formed. A deacylation step follows.

- The previous position of the amine is occupied by a water molecule. Again, His$^{57}$, through support from Asp$^{102}$, serves as a general base, abstracting the proton from water (step ‘c’, Fig.). This is followed by nucleophilic attack of the resultant OH$^-$ ion on the carbon of the carbonyl group of the acyl enzyme (step ‘d’, Fig.), resulting in free enzyme and the second product of the enzymic conversion.
2- Reasons for Catalytic Activity

D- Covalent Catalysis

Postulated reaction mechanism for chymotrypsin activity
2- Reasons for Catalytic Activity

D- Covalent Catalysis

-An exceptionally reactive serine residue has been identified in a great number of hydrolase enzymes, e.g., trypsin, elastase, acetylcholine esterase and some lipases.

-These enzymes appear to hydrolyze their substrates by a mechanism analogous to that of chymotrypsin.

-Hydrolases such as papain, ficin and bromelain, which are distributed in plants, have a cysteine residue instead of an “active” serine residue in their active sites. Thus, the transient intermediates are thioesters.

-Enzymes involved in the cleavage of carbohydrates can also function by the above mechanism.
2- Reasons for Catalytic Activity
D- Covalent Catalysis

- This Figure shows that amylose hydrolysis by β-amylase occurs with the help of four functional groups in the active site.

- The enzyme-substrate complex is subjected to a nucleophilic attack by an SH-group on the carbon involved in the α-glycosidic bond. This transition step is facilitated by carboxylate anion in the role of a general base and by the imidazole ring as an acid which donates a proton to glycosidic oxygen.

- In the second transition state the imidazole ring, as a general base in the presence of a water molecule, helps to release maltose from the maltosylenzyme intermediate.

Postulated mechanism for hydrolysis of amylose by β-amylase
- Lysine is another amino acid residue actively involved in covalent enzyme catalysis.

- Many lyases react covalently with a substrate containing a carbonyl group.

- They catalyze, for example, aldol condensations important for the conversion and cleavage of monosaccharides or for decarboxylation reactions of β-keto acids.
2- Reasons for Catalytic Activity
D- Covalent Catalysis

- As an example, the details of the reaction involved will be considered for aldolase.

- The enzyme-substrate complex is first stabilized by electrostatic interaction between the phosphate residues of the substrate and the charged groups present on the enzyme.

- A covalent intermediate, a Schiff base, is then formed by nucleophilic attack of the amino group of the “active” lysine on a carbonyl group of the substrate. The Schiff base cation facilitates the retroaldol cleavage of the substrate, whereas negatively charged group on the enzyme (e.g. thiolate or carboxylate anion) acts as a general base, i.e. binds the free proton. Thus, the first product, glyceraldehyde-3-phosphate, is released.

- An enamine rearrangement into a ketimine structure is followed by release of dihydroxyacetone phosphate.

Aldolase of rabbit muscle tissue. A model for its activity; P: PO₃H₂
- This is the mechanism of catalysis by aldolases which occur in plant and animal tissues (lysine aldolases).

- A second group of these enzymes often produced by microorganisms contains a metal ion (metallo-aldolases).

- This group is involved in accelerating retroaldol condensations through electrophilic reactions with carbonyl groups:

\[
\begin{align*}
\text{R-C-C-C-C-R'} & \quad \text{R-CHO} \\
\text{H-O-H} & \quad \text{H-O-H} \\
\text{Me} & \quad \text{Me} \\
\text{Me} & : \text{Metal ion, probably } \text{Zn}^{2+}
\end{align*}
\]
2- Reasons for Catalytic Activity

D- Covalent Catalysis

-Electrophilic reactions are also carried out by enzymes which have an α-keto acid (pyruvic acid or α-keto butyric acid) at the transforming locus of the active site.

-One example of such an enzyme is histidine decarboxylase in which the N-terminal amino acid residue is bound to pyruvate.

-Histidine decarboxylation is initiated by the formation of a Schiff base by the reaction mechanism shown in the figure.

A proposed mechanism for the reaction of histidine decarboxylase
The hypotheses discussed allow some understanding of the fundamentals involved in the action of enzymes.

However, the knowledge is far from the point where the individual or combined effects which regulate the rates of enzyme-catalyzed reactions can be calculated.