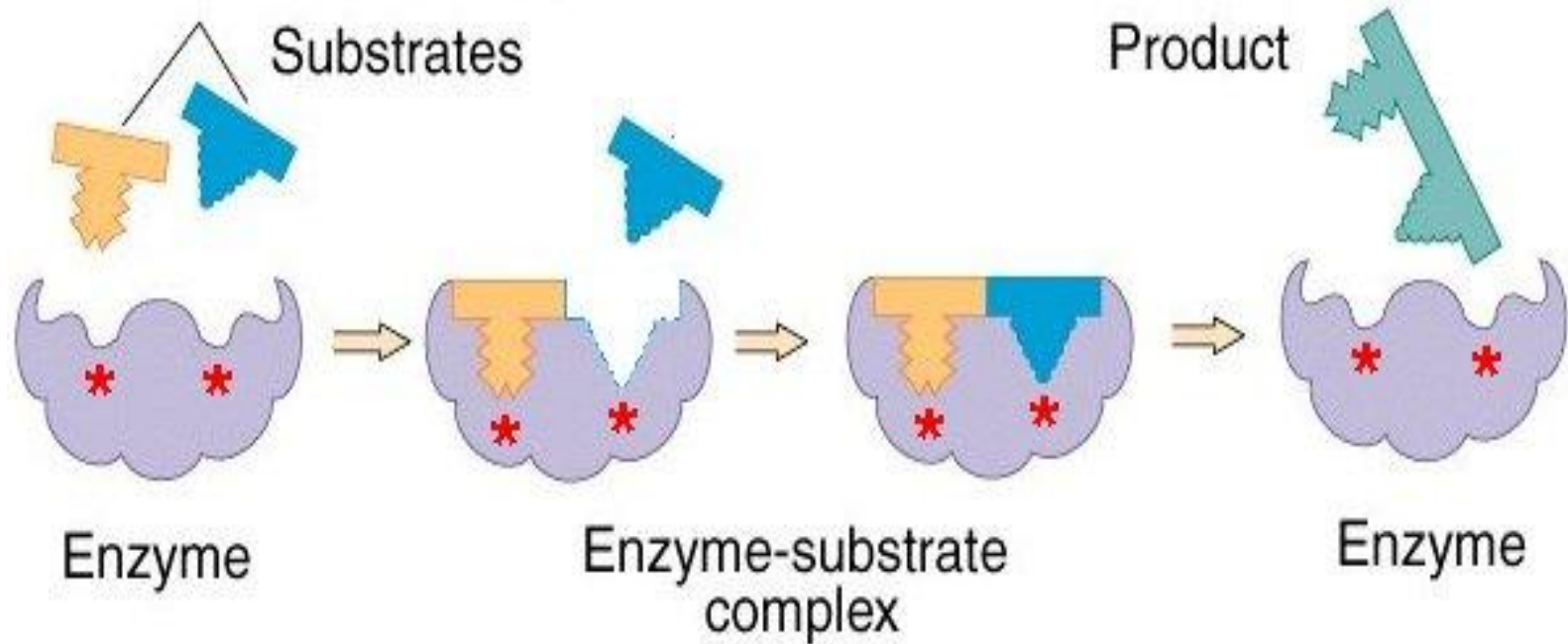


Theory of Enzyme Catalysis



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-variably 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.

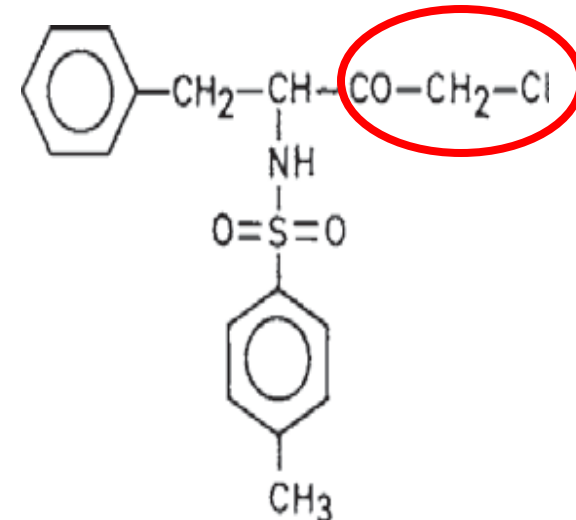
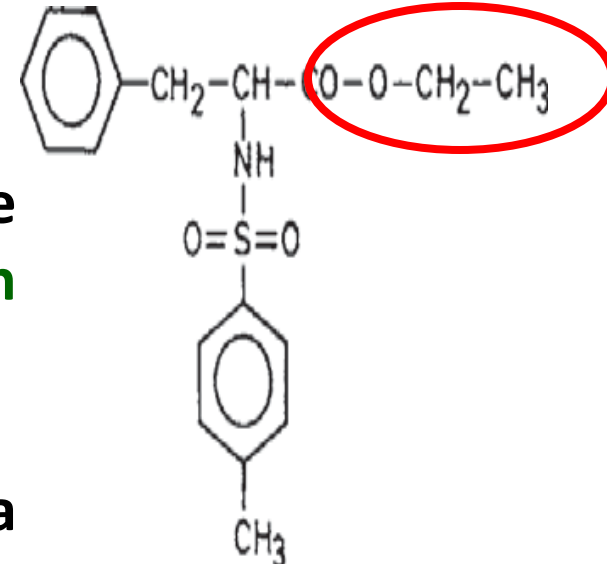
1- Active Site

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 (**N α -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**.

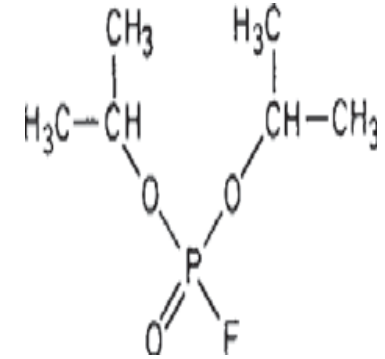
-Analysis of the enzyme inhibitor complex reveals that, of the two **histidine residues** present in **chymotrypsin**, only **His⁵⁷** is alkylated at one of its ring **nitrogens**. Hence, the modified **His** residue is part of the active site.

-TPCK (**N α -tosyl-L-phenylalanine chloromethylketone**) binds highly specifically, thus the **proteinase trypsin** is not inhibited.

1- Active Site

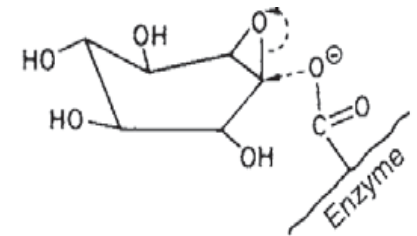
A- Active Site Localization

-Reaction of **diisopropylfluorophosphate (DIFP)** with a number of **proteinases** and **esterases** alkylates the unusually reactive **serine residue** at the active site.

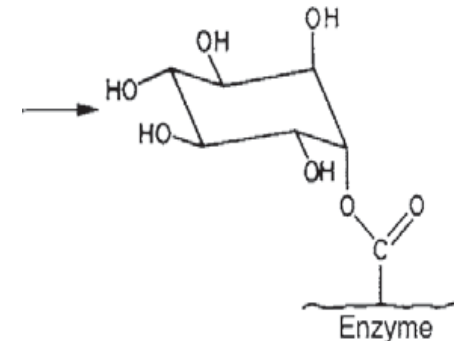


-Thus, of the **28 serine residues** present in **chymotrypsin**, only **Ser¹⁹⁵** is alkylated, while the other **27 residues** are unaffected by the reagent.

-It appears that the **reactivity** of **Ser¹⁹⁵** is enhanced by its interaction with the neighboring **His⁵⁷**.



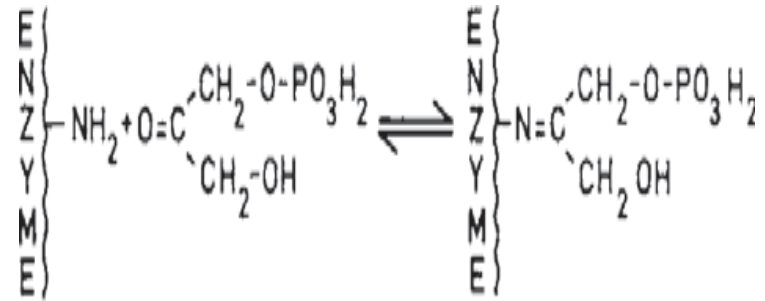
-The participation of **carboxyl group** at the active site in **β -glucosidase** catalysis has been confirmed with the help of **conduritol B-epoxide**, an inhibiting substrate analogue:



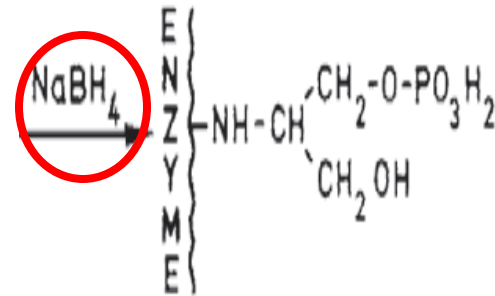
1- Active Site

A- Active Site Localization

-A **lysine residue** is involved in enzyme catalysis in a number of **lyase enzymes** and in enzymes in which **pyridoxal phosphate** is the cosubstrate.

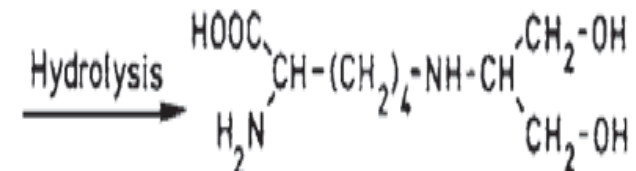


-An intermediary **Schiff base** product is formed between an **amino group** of the enzyme and the substrate or **pyridoxal phosphate**.



-The reaction site is then identified by reduction of the **Schiff base** with NaBH_4 .

-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:



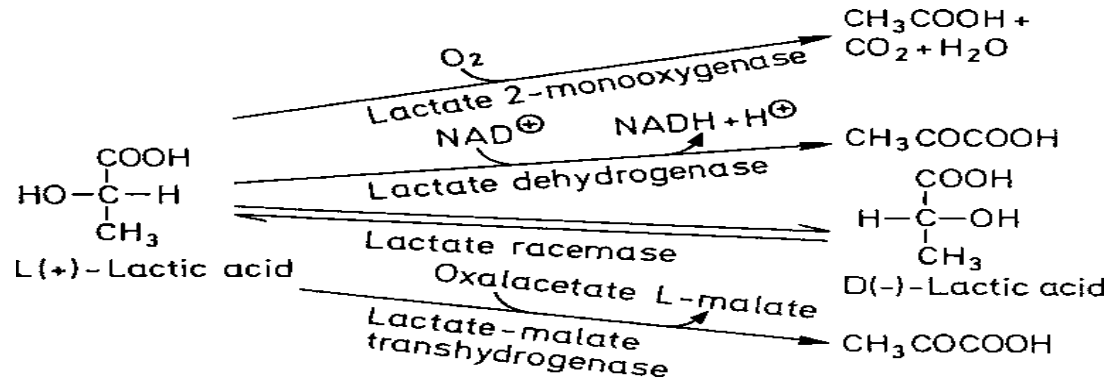
1- Active Site

B- Substrate Binding

1- Stereospecificity

-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.

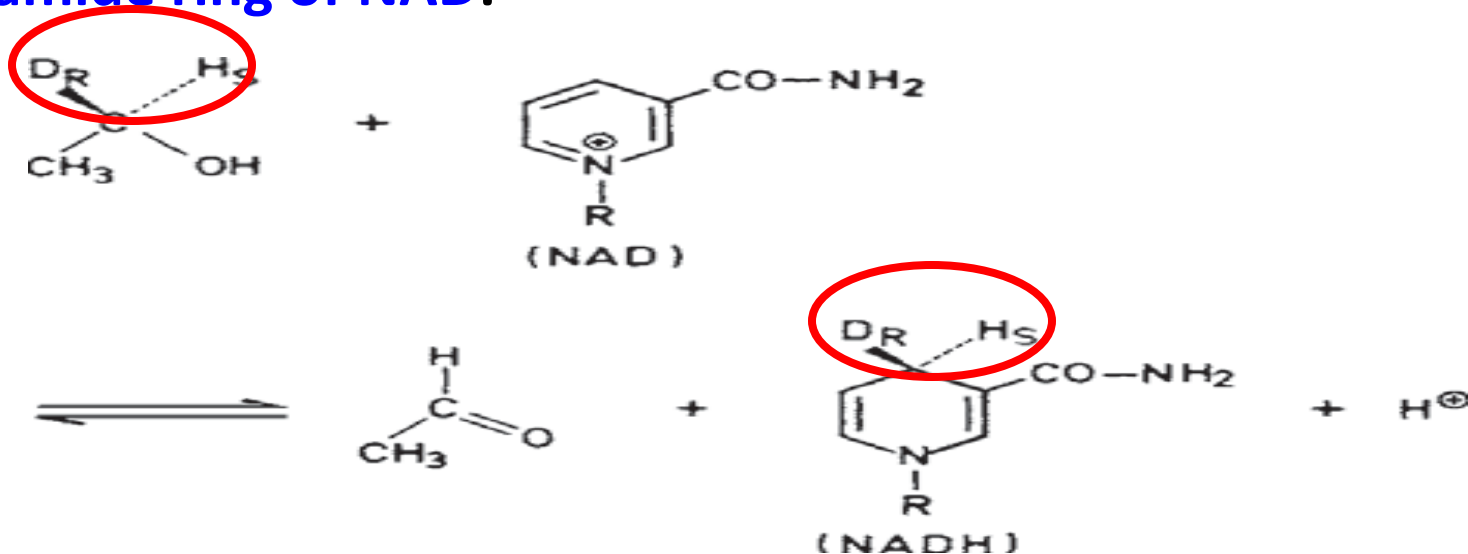
1- Active Site

B- Substrate Binding

1- Stereospecificity

-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**:

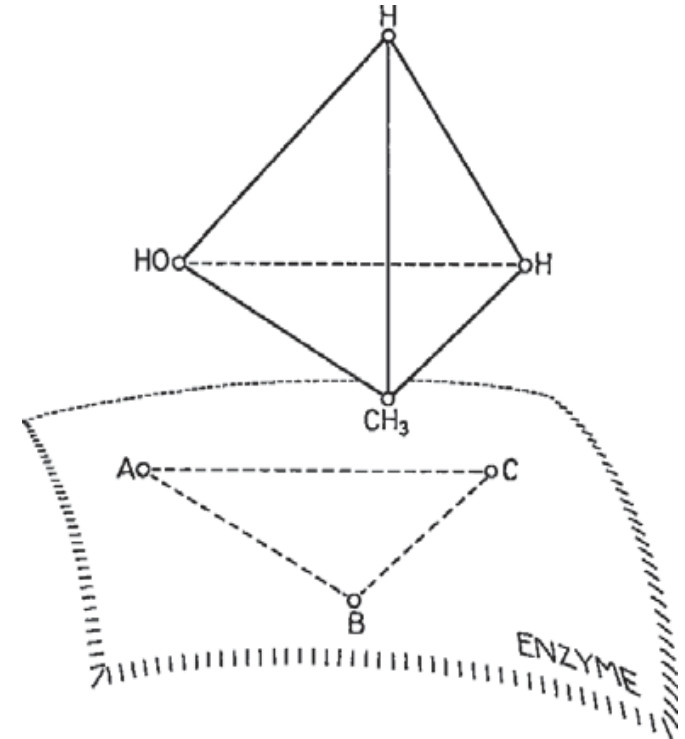


1- Active Site
B- Substrate Binding
1- Stereospecificity

-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.



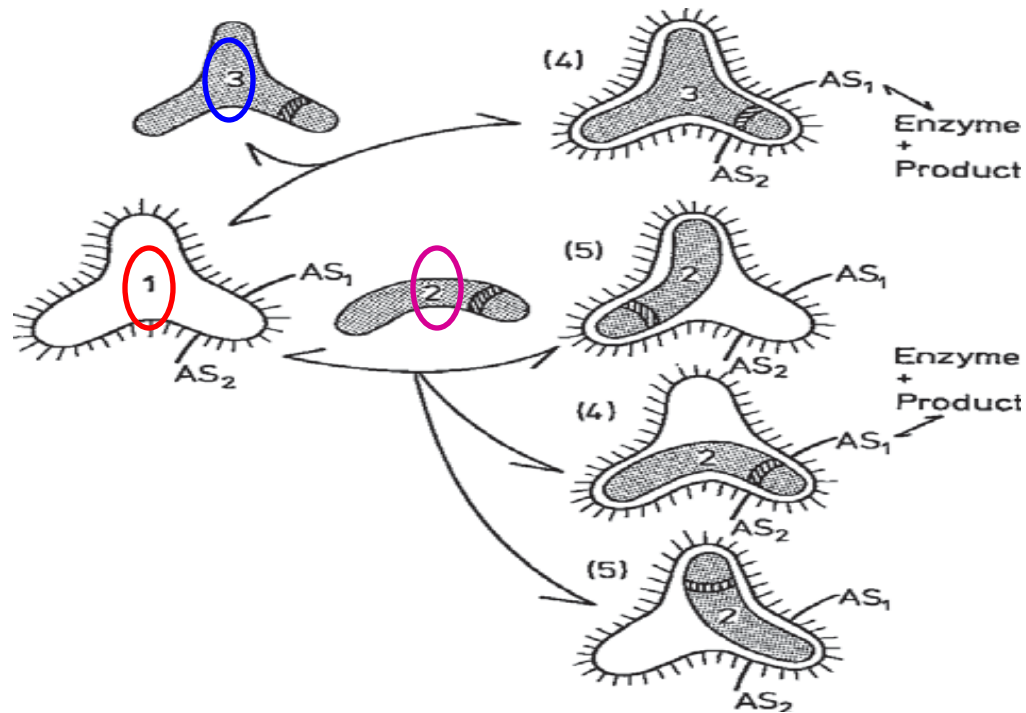
A model for binding of a prochiral substrate (ethanol) by an enzyme

1- Active Site

B- Substrate Binding

2- “Lock and Key” Hypothesis

-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

1- Active Site

B- Substrate Binding

2- “Lock and Key” Hypothesis

-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.

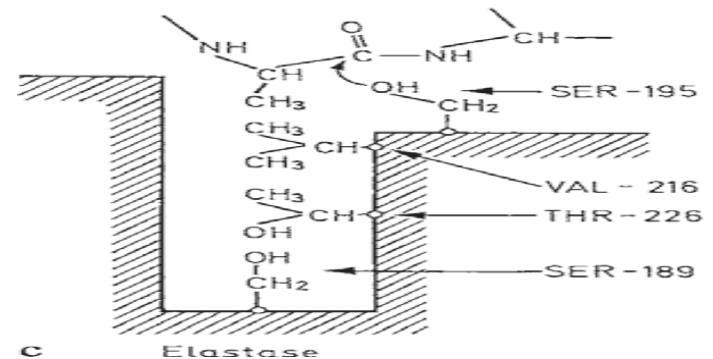
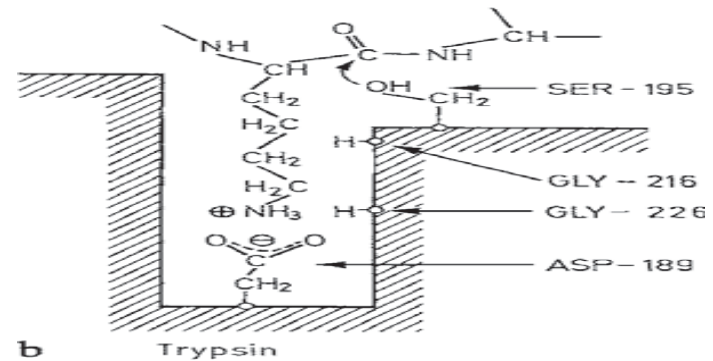
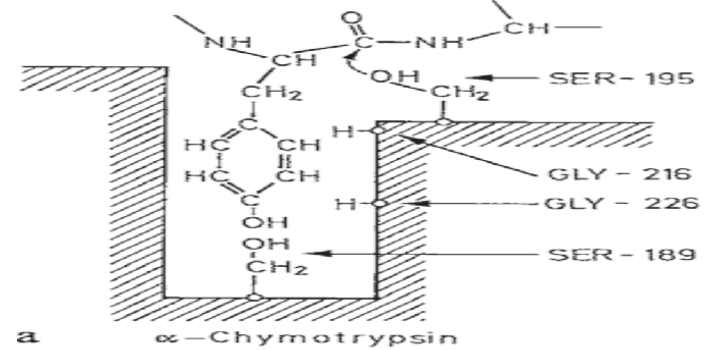
1- Active Site

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 **Val²¹⁶** and **Thr²²⁶** 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

1- Active Site

B- Substrate Binding

3- Induced-fit Model

-The conformation of a number of **enzymes** is changed by the binding of the substrate.

-An example is **carboxypeptidase A**, in which the **Try²⁴⁸** located in the active site moves approximately 12 Å towards the substrate, **glycyl-L-phenylalanine**, to establish contact.

1- Active Site

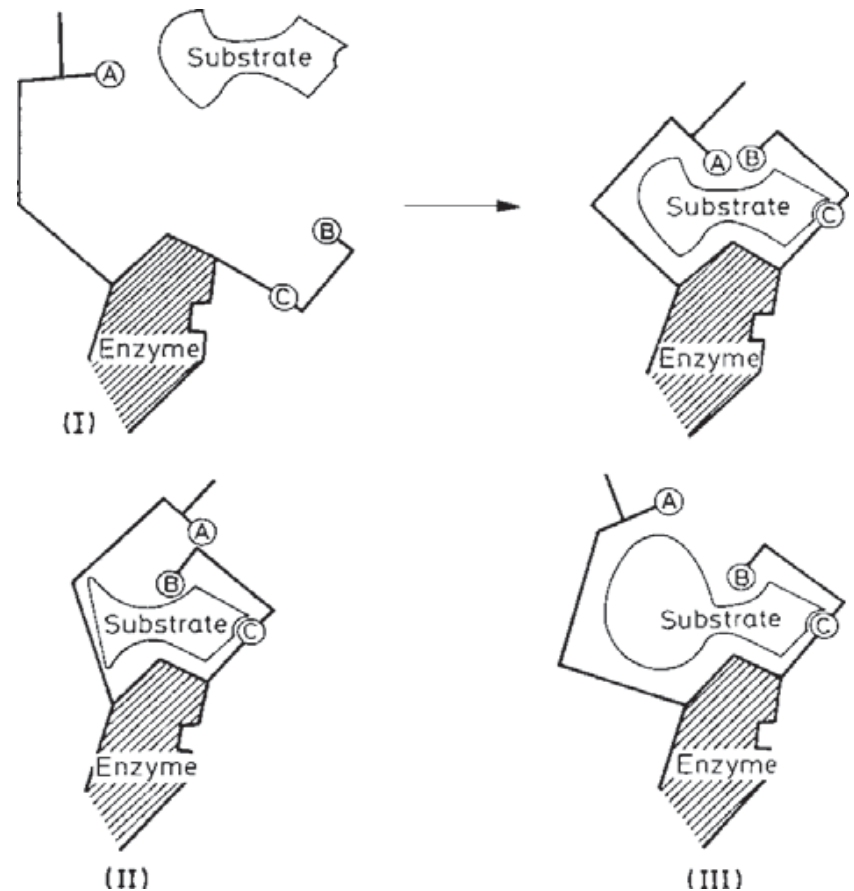
B- Substrate Binding

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

1- Active Site

B- Substrate Binding

3- Induced-fit Model

-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**.

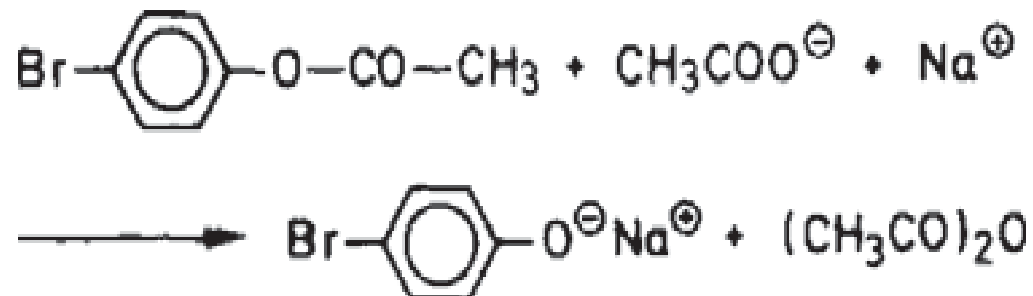
2- Reasons for Catalytic Activity

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**:



2- Reasons for Catalytic Activity

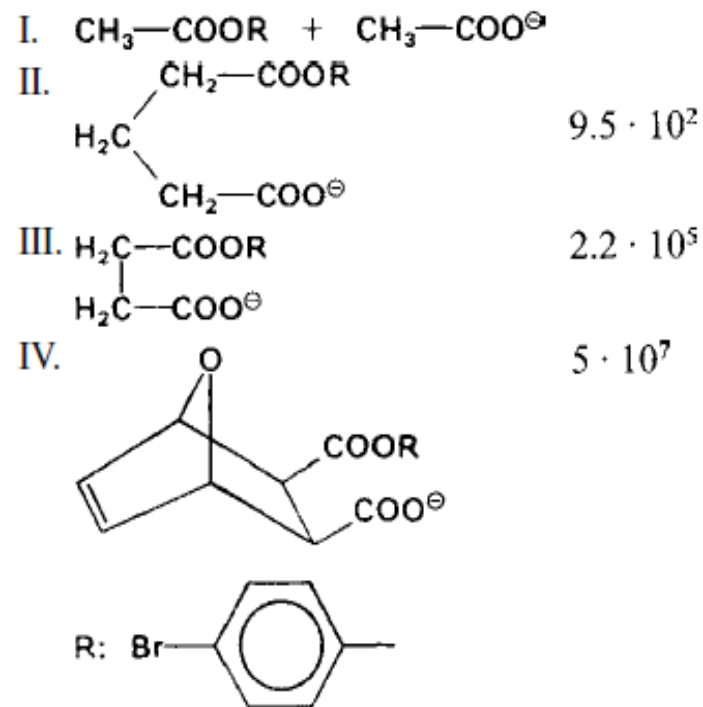
A- Steric Effects – Orientation Effects

-**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 acid
anhydrides**

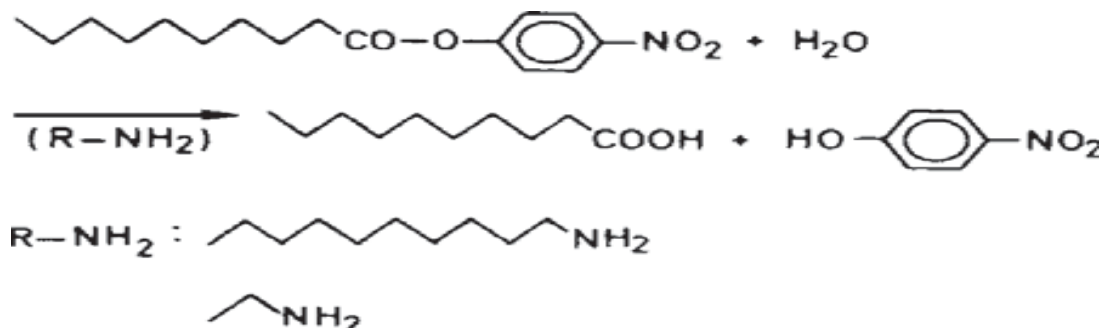
2- Reasons for Catalytic Activity

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 **p-nitrophenyldecanoic acid ester** is catalyzed by an **alkylamine**:



-The reaction rate in the presence of **decylamine** is faster than that in the presence of **ethylamine** by a factor of **700**.

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

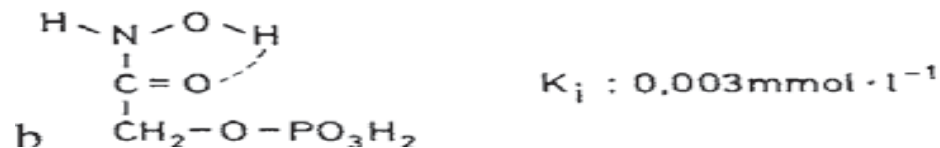
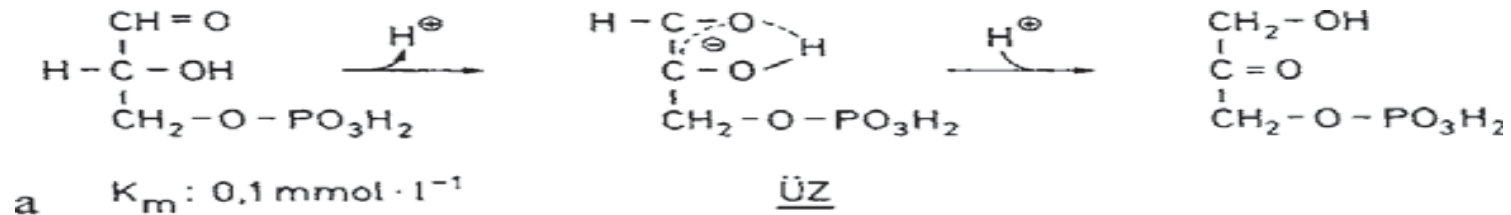
-Correspondingly, there is a decline in the reaction rate as the **alkyl amine group** is lengthened further.

2- Reasons for Catalytic Activity

B- Structural Complementarity to Transition State

-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^- 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**).

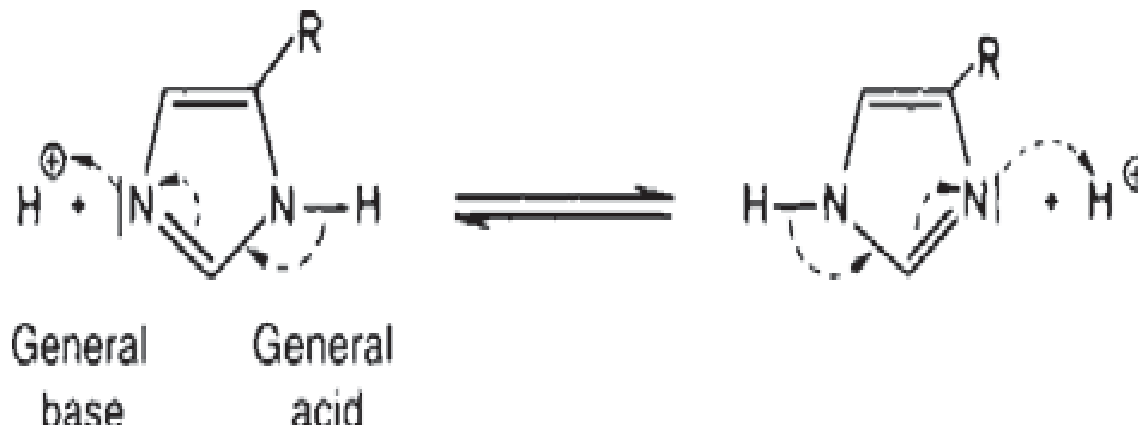
-Most of the **amino acids** located on the active site of the enzyme influence the reaction rate by **general acid-base catalysis**.

2- Reasons for Catalytic Activity

C- General Acid–Base Catalysis

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

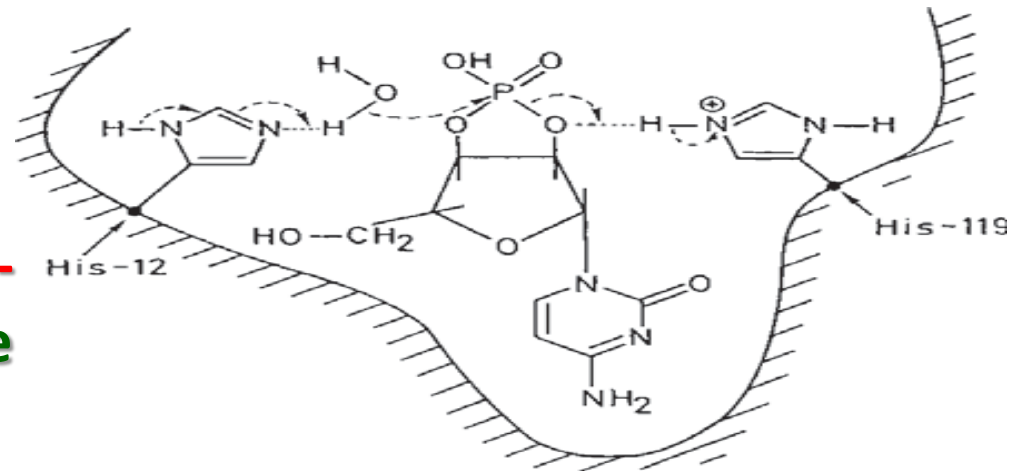
-Of these, the **imidazole ring** of **histidine** is of special interest since it can perform **both functions** simultaneously:



2- Reasons for Catalytic Activity

C- General Acid–Base Catalysis

- 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.



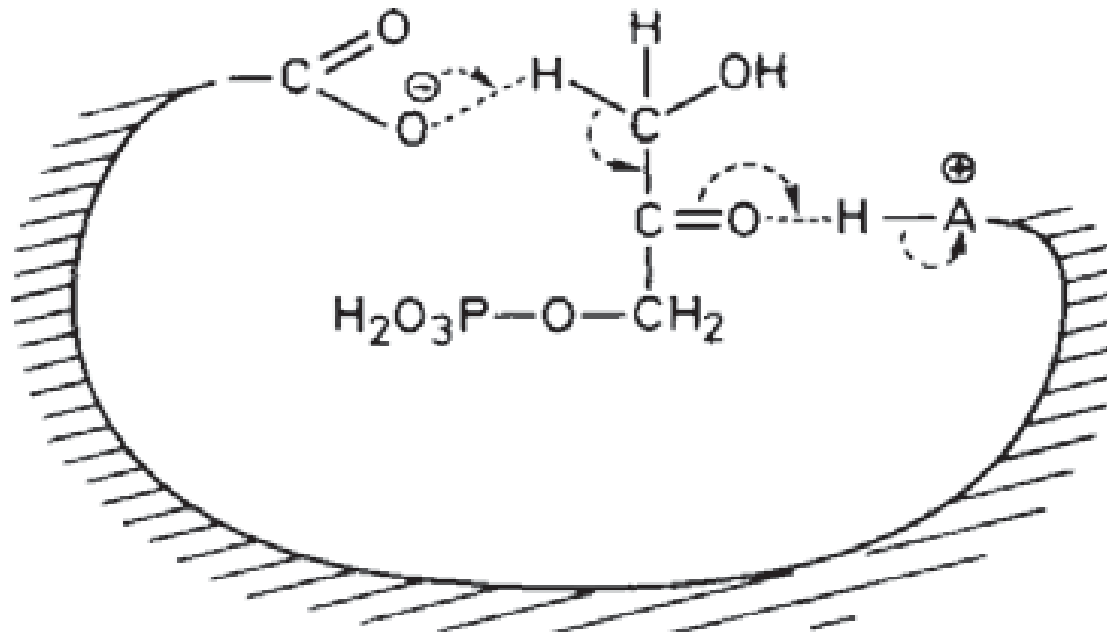
Hydrolysis of cytidine-2,3-phosphate by ribonuclease

- His¹² serves as a general **base**, removing the proton from a water molecule. This is followed by nucleophilic attack of the intermediary **OH⁻** ions on the electrophilic **phosphate group**.

2- Reasons for Catalytic Activity

C- General Acid–Base Catalysis

-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

Enzyme	Reactive functional group	Intermediate
1. Chymotrypsin	HO-(Serine)	Acylated enzyme
2. Papain	HS-(Cysteine)	Acylated enzyme
3. β -Amylase	HS-(Cysteine)	Maltosylenzyme
4. Aldolase	ϵ -H ₂ N-(Lysine)	<i>Schiff</i> base
5. Alkaline phosphatase	HO-(Serine)	Phosphoenzyme
6. Glucose-6-phosphatase	Imidazole-(Histidine)	Phosphoenzyme
7. Histidine decarboxylase	O=C < (Pyruvate)	<i>Schiff</i> base

2- Reasons for Catalytic Activity

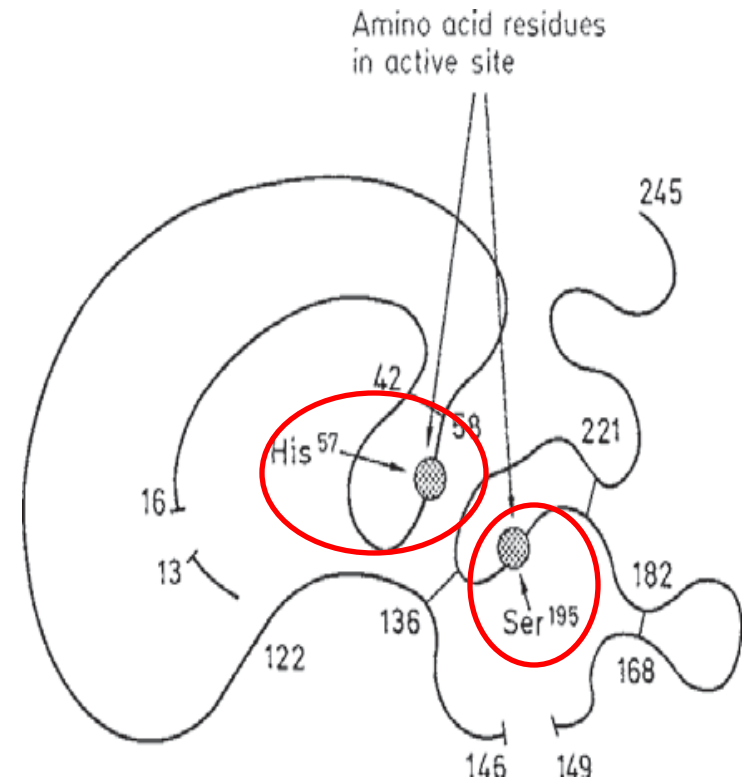
D- Covalent Catalysis

-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 **His⁵⁷** and **Ser¹⁹⁵**, which are **positioned** in close proximity within the active site of the enzyme because of folding of the peptide chain.



Polypeptide chain conformation in the chymotrypsin molecule

2- Reasons for Catalytic Activity

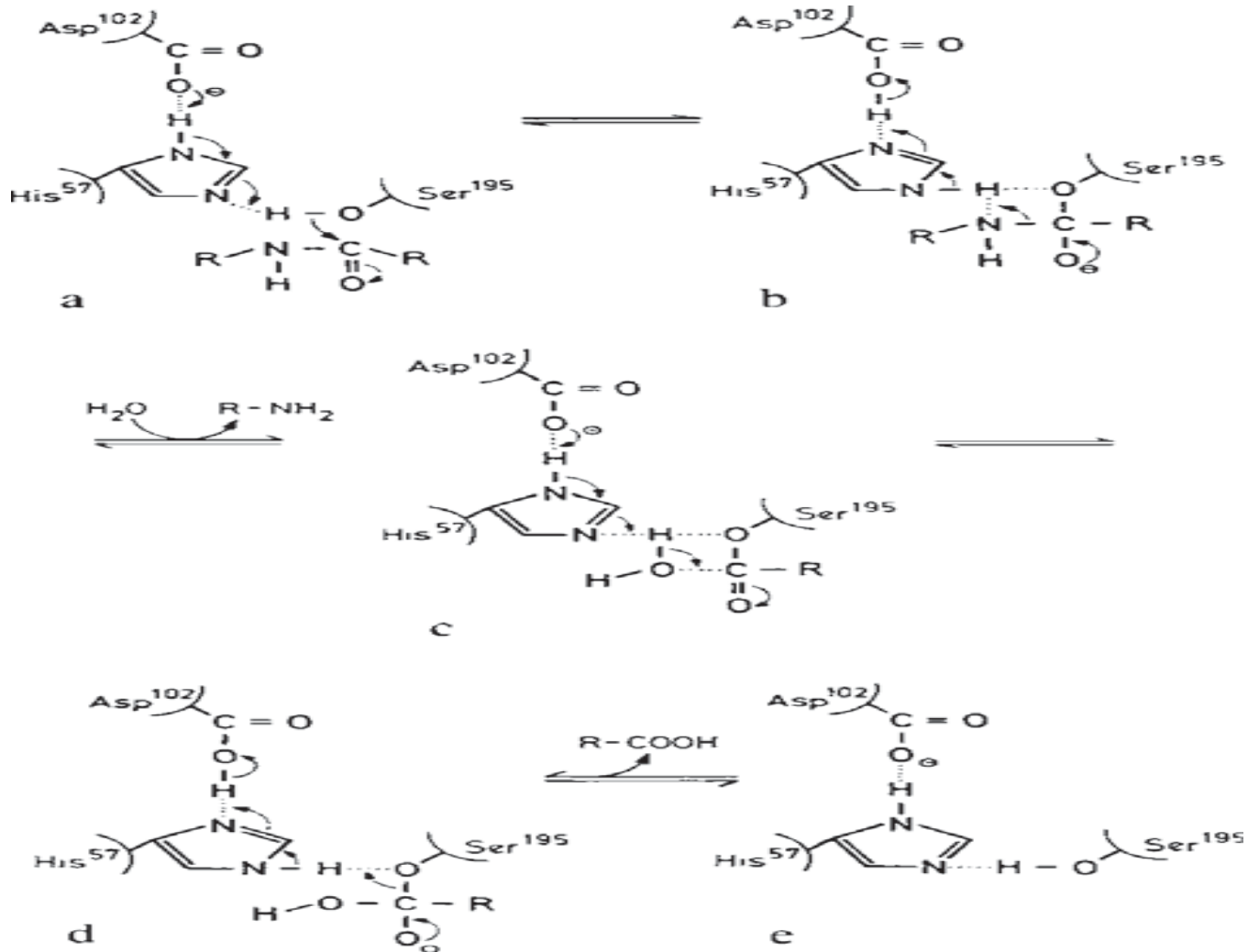
D- Covalent Catalysis

Postulated reaction mechanism for chymotrypsin activity

- Because **Asp¹⁰²** is located in hydrophobic surroundings, it can polarize the functional groups in close proximity to it. Thus, **His⁵⁷** acts as a strong general base and abstracts a proton from the **OH-group** of the neighboring **Ser¹⁹⁵** residue (**step 'a', Fig.**).
- The oxygen remaining on **Ser¹⁹⁵** 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, **His⁵⁷**, through support from **Asp¹⁰²**, 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.

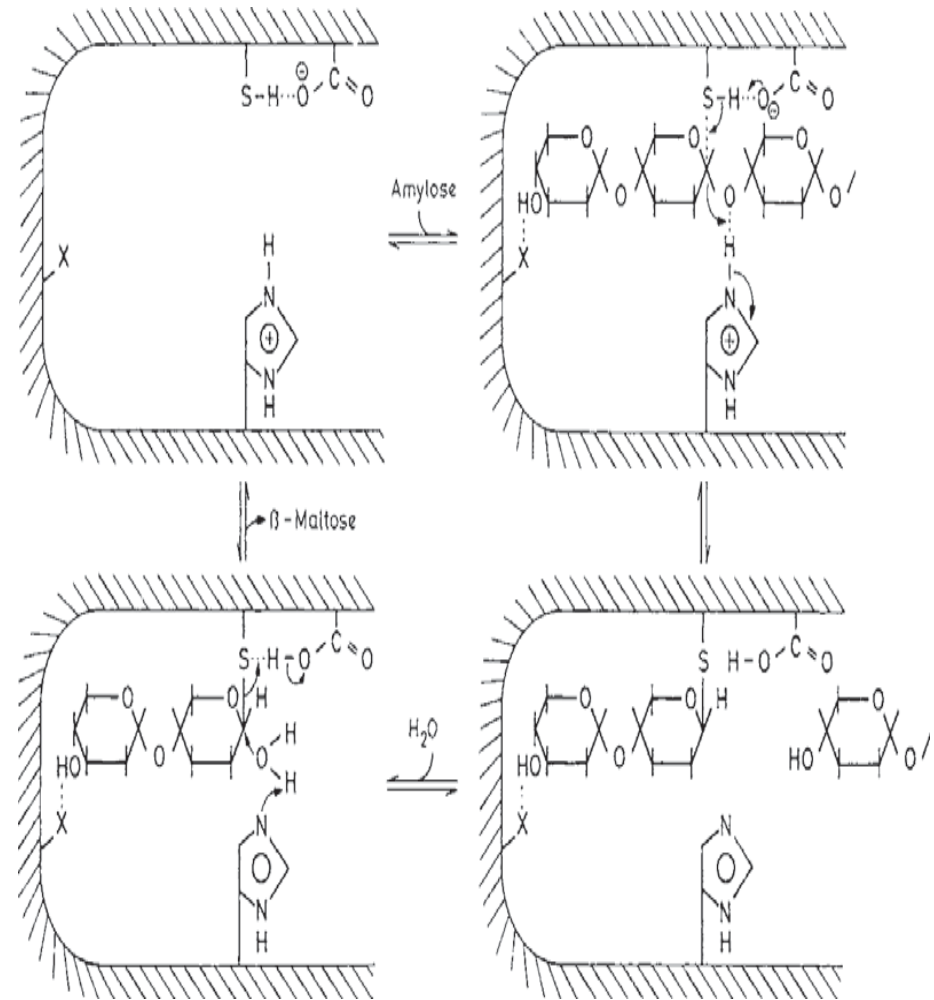
2- Reasons for Catalytic Activity

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

2- Reasons for Catalytic Activity

D- Covalent Catalysis

-**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

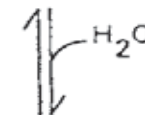
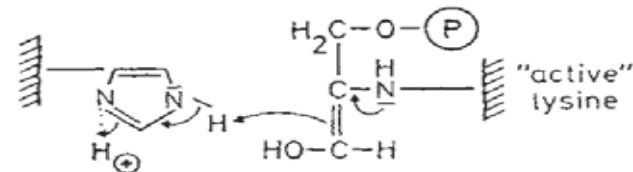
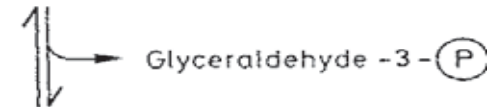
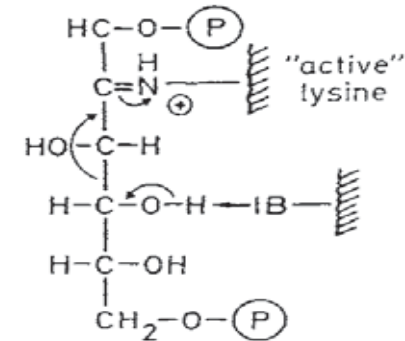
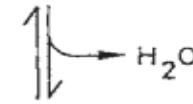
-As an example, the details of the reaction involved will be considered for **aldolase**.

-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**.

Fructose-1,6-diphosphate + Enzyme



Dihydroxyacetone-P + enzyme

Aldolase of rabbit muscle tissue.
A model for its activity; P: PO₃H₂

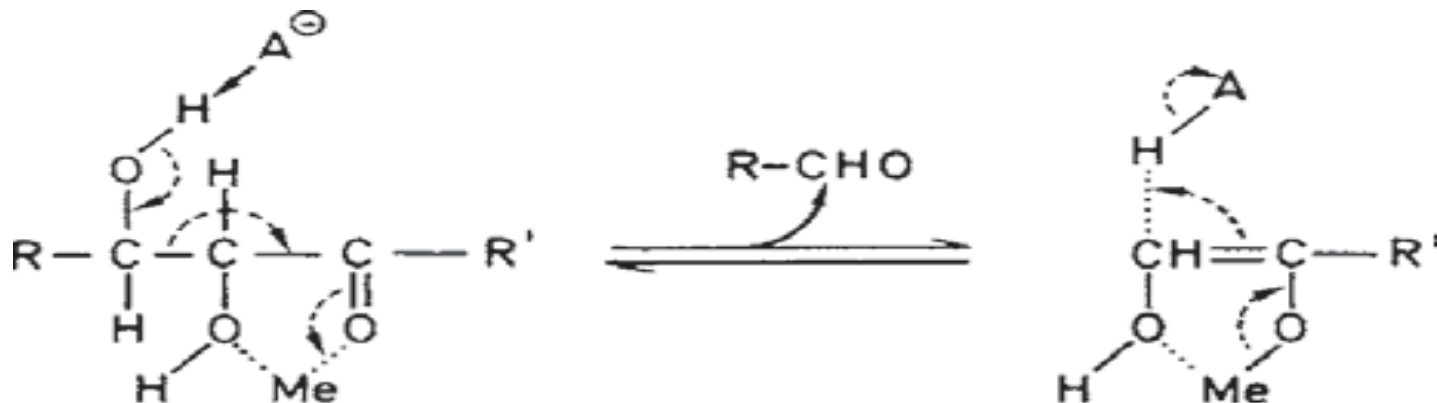
2- Reasons for Catalytic Activity

D- Covalent Catalysis

-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**:



Me : Metal ion, probably Zn^{2+}

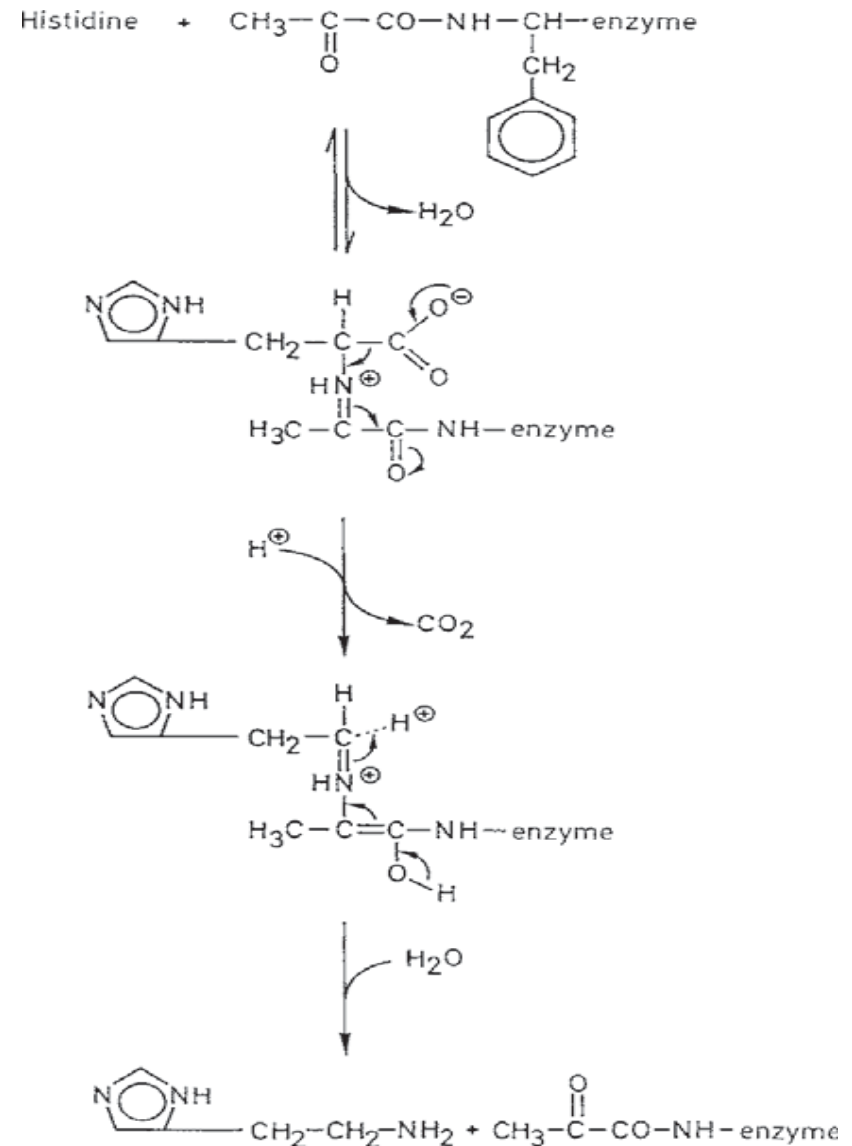
2- Reasons for Catalytic Activity

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.