VIVEKANANDA COLLEGE
THAKURPUKUR
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NAAC ACCREDITED ‘A’ GRADE

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

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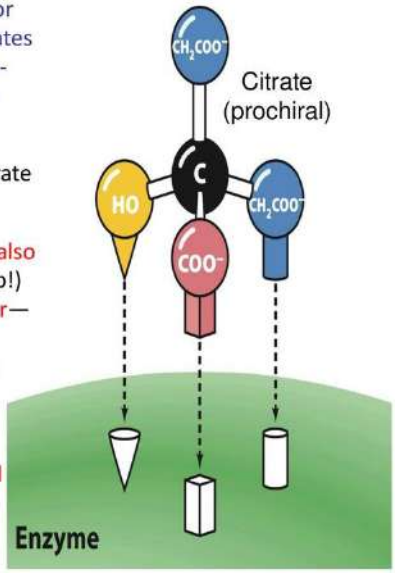
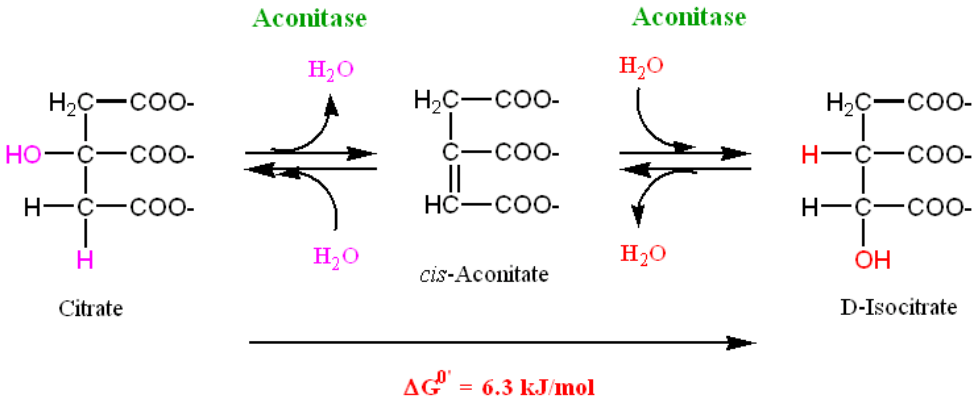
Enzyme Specificity: Stereospecificity

Stereospecificity is concerned with chirality or asymmetry (eg spatial orientation) of substrates due to the fact that enzymes (composed of L-amino acids) themselves are chiral or harbor asymmetric active sites:

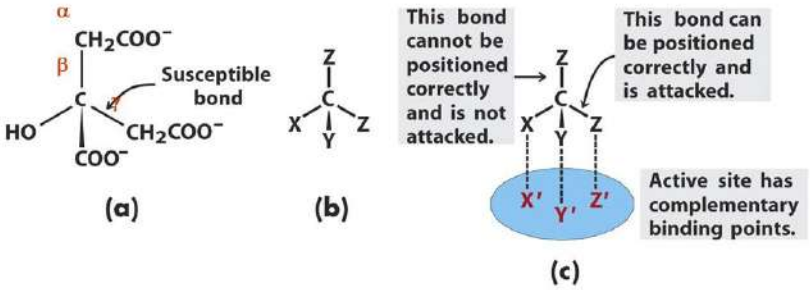
- Enzymes can only accommodate the substrate in an asymmetric manner
- Thus, enzymes catalyze not only chiral but also prochiral (can become chiral in a single step!) substrates in a highly stereospecific manner—eg citrate binds to the enzyme active site asymmetrically via three-point attachment
- This is due to the fact that while the two CH_2COO^- groups on citrate are chemically-equivalent, they occupy two distinct spatial positions relative to OH and COO^- groups—only one of these two CH_2COO^- groups can therefore undergo catalysis!





BOX 16-2

Enzymatic reaction of prochiral molecules



- the active site of aconitase may have three points to which the citrate must be bound and that the citrate must undergo a specific three-point attachment to these binding points. The binding of citrate to three such points could happen in only one way, and this would account for the formation of only one type of labeled α-ketoglutarate.
- **prochiral molecules:** Organic molecules, such as citrate, that have no chiral center but are potentially capable of reacting asymmetrically with an asymmetric active site

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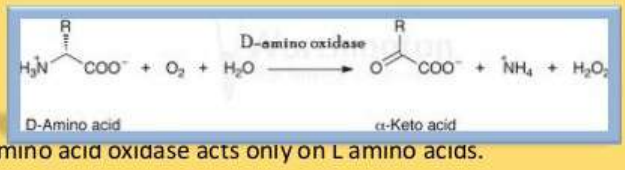
OPTICAL / STEREO-SPECIFICITY

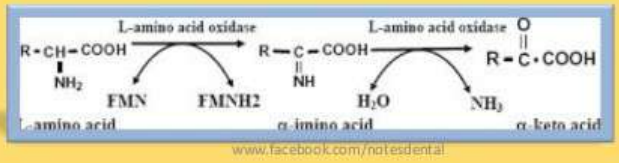
- In this type of specificity , the enzyme is not specific to substrate but also to its optical configuration

Example:

➢ D amino acid oxidase acts only on D amino acids.



➢ L amino acid oxidase acts only on L amino acids.



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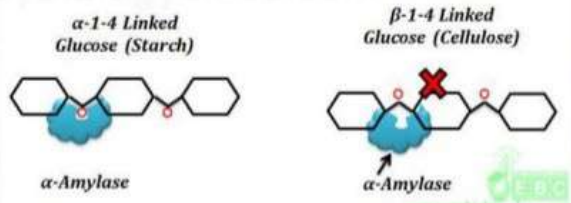
SPECIFICITY OF ENZYMES

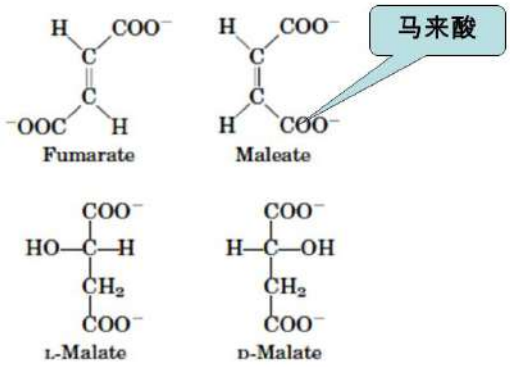
(4). Optical specificity

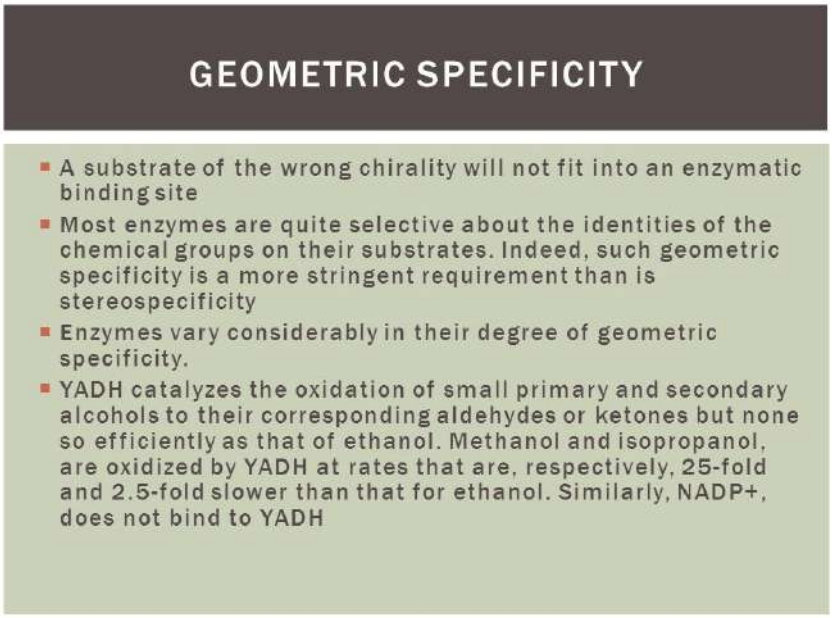
- D-amino acid oxidase acts only on D-amino acids
- α-glycosidic bonds of starch and glycogen are hydrolyzed only by α-glycosidase (α-amylase)
- β-glycosidic bonds of cellulose are hydrolyzed only by β-glycosidase (β-amylase)

Stereo specificity of Enzymes



This enzyme is highly stereospecific; it catalyzes hydration of the trans double bond of fumarate but not the cis double bond of maleate (the cis isomer of fumarate).
In the reverse direction (from L-malate to fumarate), fumarase is equally stereospecific: D-malate is not a substrate.





In **geometrical specificity**, an enzyme can bind to different substrate that have similar molecular **geometry.**

Proximity and Orientation effect

Substrate binding has additional effects that enhance reaction rates - most obvious is proximity & orientation. Reactants must come together with the proper spatial relationship for a reaction to occur. Proximity effects (minor) are most readily observed by comparing equivalent inter- and intramolecular reactions. Intramolecular reactions are typically 10-100 fold more rapid. Orientation effects are more significant though difficult to quantify. Theory suggest that molecules are maximally reactive when their orbitals are aligned so the activation energy of the transition state is minimized.

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Catalysis can occur through proximity and orientation effects

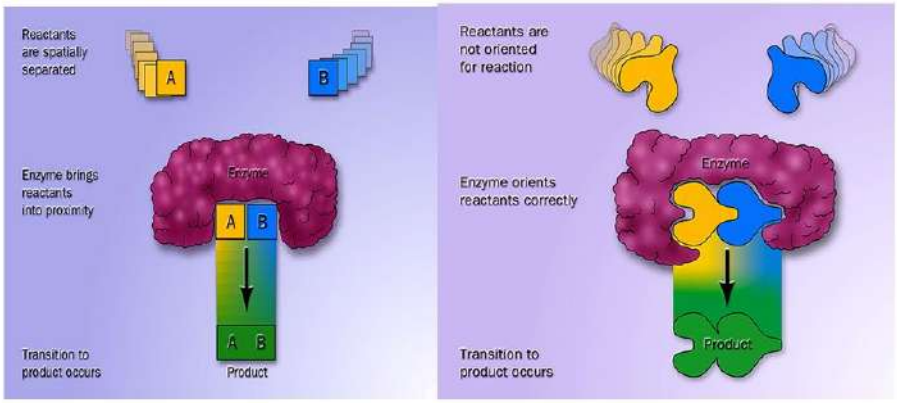
Enzymes are usually much bigger than their substrates

By oriented binding and immobilization of the substrate, enzymes facilitate catalysis by four ways:

1. bring substrates close to catalytic residues
2. Binding of substrate in proper orientation (up to 102-fold)
3. Stabilization of transition state by electrostatic interactions
4. freezing out of translational and rotational mobility of the substrate (up to 107-fold)

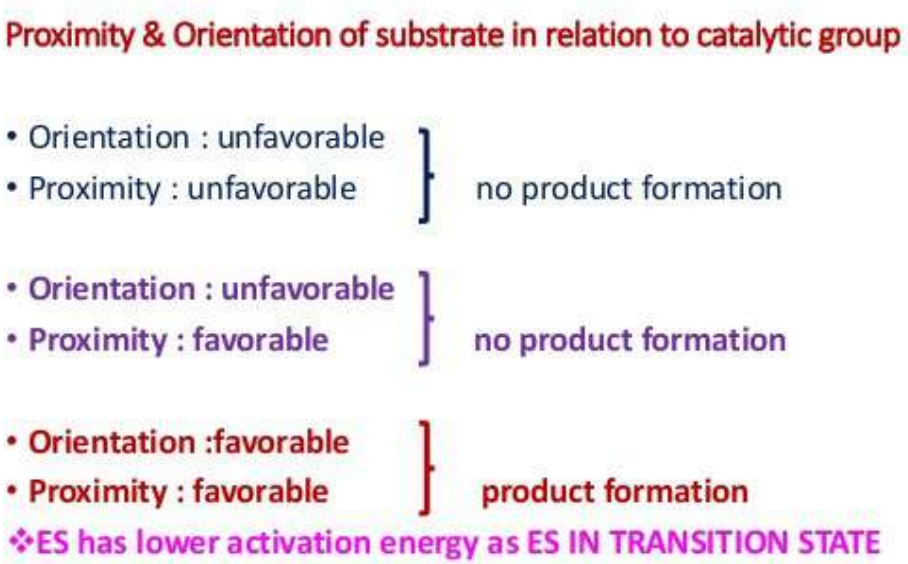
Catalytic mechanisms of enzymes

(1) Proximity and orientation effects



Orbital Steering Hypothesis

A concept related to proximity effects is that of orbital steering. The orbital steering hypothesis suggests that the juxtaposition of reactive groups among the substrates and active site residues is not sufficient for catalysis. In addition to this positioning, the enzyme needs to precisely steer the molecular orbitals of the substrate into a suitable orientation. According to this hypothesis, enzyme active site groups have evolved to optimize this steering upon substrate binding for proper alignment so that relevant orbitals overlap and the transition state can be attained with a high probability.



Strain and distortion theory

- When substrate bind the enzyme, it may induce a conformational change in the active site to fit to a transition state.
- Frequently, in the transition state, the substrate and the enzyme have slightly different structure (strain or distortion) and increase the reactivity of the substrate.

The native 3-dimensional conformation of the enzyme molecule is required for its catalytic activity, for example Ribonuclease, chymotrypsin

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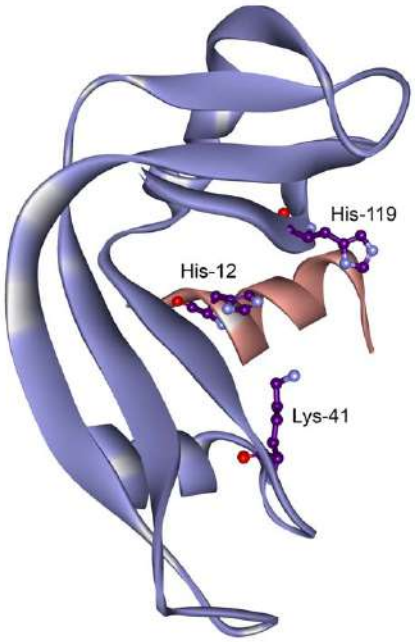
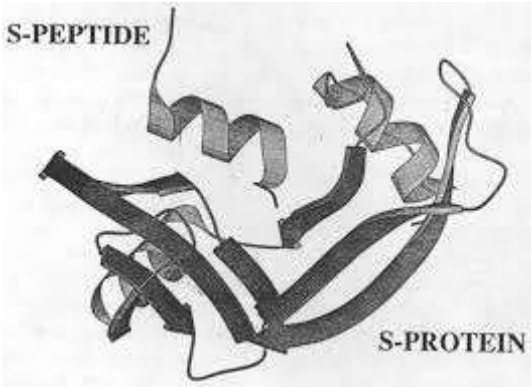
Ribonuclease

Intact Ribonuclease



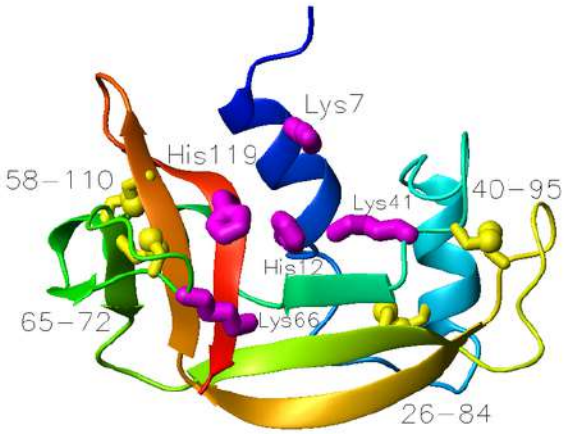
↓ Subtilisin

After Cleavage



Histidine residues 12 and 119 are required for catalytic activity of ribonuclease. The bacterial protease Subtilisin cleaves the 124 residue chain of ribonuclease between residues 20 and 21. The long fragment, S-protein contains Histidine residue 119 and the short fragment S-peptide contains Histidine residue 12. S-protein and S-peptide are catalytically inactive singly but when they are mixed at pH 7, enzymatic activity is restored, though no covalent linkage is formed between them. S-peptide binds to S-protein through weak forces like H-bonding and

hydrophobic interaction in such a way as to bring the 2 essential Histidine residues together at the active site.



X-ray analysis studies suggesting that the polypeptide chain is folded in such a way that these two Histidines (12 and 119), which are separated by 107 residues in the backbone, are brought close together at the active site.

Chymotrypsin

- Chymotrypsin is a digestive enzyme that hydrolyses proteins in the small intestine.
- Its inactive precursor, chymotrypsinogen, is synthesised in the pancreas.
- Chymotrypsinogen, a single polypeptide chain consisting of 245 amino acid residues, is devoid of enzymatic activity. It is converted into an active enzyme when the peptide bond joining Arg15 and Ile16 is cleaved by trypsin to form π – Chymotrypsin.
- π - Chymotrypsin subsequently undergoes autolysis to specifically excise two dipeptides, Ser14- Arg15 and Thr147 – Asn148, thereby yielding the active enzyme α-Chymotrypsin.
- It has a single polypeptide chain of 245 amino acid residues held together by 5 intrachain disulphide bridges.
- The active α-chymotrypsin consists of three polypeptide chains (A, B & C) held together by 2 disulphide bonds (1-122 and 136-201). Thus the two specific residues essential for catalytic activity, His-57 and Ser-195 are present in two different chains.

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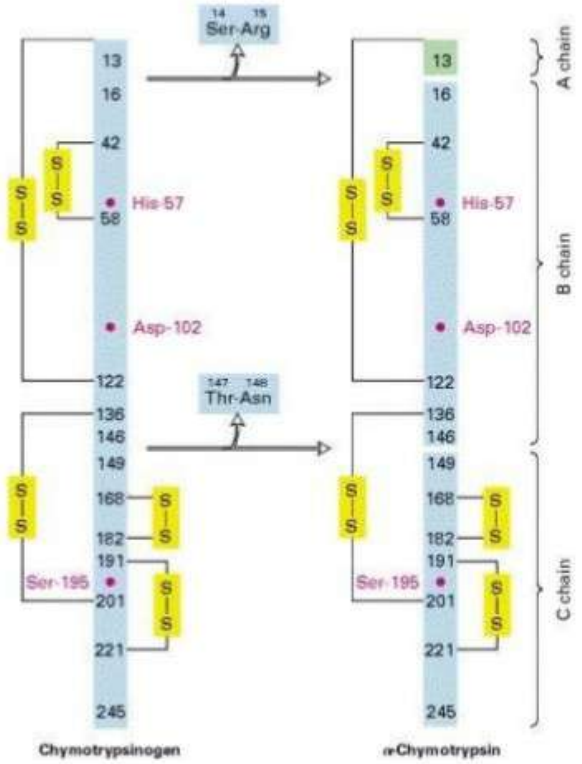

Proteolytic activation

A linear representation of the conversion of chymotrypsinogen into chymotrypsin by the excision of two dipeptides

These reactions yield three separate chains (A, B, and C), which are covalently linked by disulfide bonds (yellow) in the active enzyme. In the folded, native conformation of chymotrypsin, histidine 57, aspartate 102, and serine 195 are located in the active site.

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Chymotrypsin (Cont'd)

- Because Ser-195 and His-57 are required for activity, they must be close to each other in the active site
- Results of x-ray crystallography show the definite arrangement of amino acids at the active site
- In addition to His-57 and Ser-195, Asp-102 is also involved in catalysis at the active site
- The folding of the chymotrypsin backbone, mostly in antiparallel pleated sheet array, positions the essential amino acids around the active-site pocket

2- Reasons for Catalytic Activity

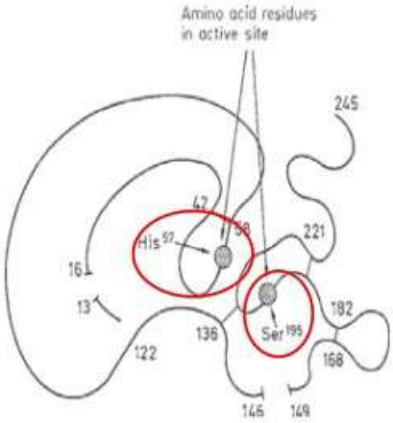
D- Covalent Catalysis

-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.



Polypeptide chain conformation in the chymotrypsin molecule

- By X-ray analysis of the tertiary structure of chymotrypsin shows that these two residues are very close to each other in the conformation of the native enzyme.
- Folded structure of chymotrypsin shows His-57 and Ser-195, active site in two separate chain close together.

- **Therefore conformational change in the tightly folded loops of the polypeptide chain contribute to the catalytic process and many enzymes undergo a change in conformation during their catalytic cycle.**

Reference :

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2. Biochemistry (2nd Edition) Albert L. Lehninger, Kalyani Publishers, New Delhi
3. www.google.com