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F.Y. B.Sc. (Sem. II) (CBCS)

MICROBIOLOGY

[201]: BASICS OF BIOCHEMISTRY AND MICROBIAL CONTROL

Unit 3
ENZYMOLOGY

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INTRODUCTION TO ENZYME

- Enzymes are biological catalysts. They increase the rate of chemical reactions taking
- place within living cells without themselves suffering any overall change. The
- reactants of enzyme-catalysed reactions are termed **substrates.** Each enzyme is
- quite specific in character, acting on a particular substrate or substrates to produce a particular product or products.
- All enzymes are proteins. However, without the presence of a non-protein
- component called a **cofactor,** many enzyme proteins lack catalytic activity. When this is the case, the inactive protein component of an enzyme is termed the **apoenzyme,** and the active enzyme, including cofactor, the **holoenzyme.**
- The cofactor may be an organic molecule, when it is known as a **coenzyme,** or it may be a metal ion. Some enzymes bind cofactors more tightly than others. When a cofactor is bound so tightly that it is difficult to remove without damaging the enzyme, it is sometimes called a **prosthetic group.**

To summarize diagrammatically:

ENZYME < INACTIVE PROTEIN+ COFACTOR < METAL ENZYME
ACTIVE PROTEIN COENZYME

As we shall see later, both the protein and cofactor components may be directly involved in the catalytic processes taking place.

A BRIEF HISTORY OF ENZYMES

- Until the nineteenth century, it was considered that processes such as the souring of milk and the fermentation of sugar to alcohol could only take place through the action of a living organism. In 1833, the active agent breaking down the sugar was partially isolated and given the name **diastase** (now known as **amylase).**
- A little later, a substance which digested dietary protein was extracted from gastric juice and called pepsin. These and other active preparations were given the general name ferments. Justus von Liebig recognized that these ferments could be non-living materials obtained from living cells, but Louis Pasteur an others still maintained that ferments must contain living material.
- While this dispute continued, the term ferment was gradually replaced by the name enzyme.
- This was first proposed by Wilhelm Kuhne in 1878, and comes from the Greek, *enzume ,* meaning 'in yeast'. Appropriately, it was in yeast that a
- factor was discovered which settled the argument in favour of the inanimate theory of catalysis: brothers Eduard and Hans Buchner showed,

in 1897, that sugar fermentation could take place when a yeast cell extract was added even though no living cells were present.

- In 1926, James Sumner crystallized urease from jack-bean extracts and, in the next few years, many other enzymes were purified and crystallized. Once pure enzymes were available, their structure and properties could be determined, and the findings form the material for most of this book.
- Today, enzymes still form a major subject for academic research.
- They are investigated in hospitals as an aid to diagnosis and, because of their specificity of action, are of great value as analytical reagents.
- Enzymes are still widely used in industry, continuing and extending many processes which have been used since the dawn of history.

Sr.no.	Question	Answer
1	The reactants of enzymes catalyzed reaction are termed.....	substrate
2	Active enzymes and co factor together called.....	holoenzymes
3	The name enzymes are proposed by.....	Wilhelm kuhne
4	The first enzyme discovered was.....	diastase
5	Urease was crystallized from which extract?	Jack-bean

CHARACTERISTICS OF ENZYME AND PROPERTIES OF ENZYMES NOMENCLATURE AND CLASSIFICATION OF ENZYMES

Physical properties of enzymes

Enzyme are in general globular proteins and range from just 62 amino acid residues in size, for the monomer of 4 oxalocrotonate tautomerase, to over 2,500 residues in the animal fatty acid synthase.

a) **Solubility:** enzymes are proteins are soluble in water or dilute salt solution.
b) **Molecular weight:** enzyme have high Mw (ranging from 10000- several thousands)
c) **Enzymes are charged molecules:** due to presence of amino acid each enzyme has a charge. The charge depends on the pH of the solution. At very low pH the amino acids are fully protonated and there is a positive charge on the proteins; as pH is increased. The protein losses a proton to neutralize the OH group and becomes a zwitter ion (a charged molecule with equal number of +ve and -ve charges. As more alkali is added, the ammonium group gives its proton and protein becomes positively charged.
d) **Enzymes have buffering capacity(acid-base):** they are amphoteric

molecule behave both as acid and base. They act as buffer. At pKa they make the most efficient buffer.

e) **Each enzyme has a specific isoelectric pH(PI):** it is the pH at which the net charge on protein equal to zero- so they do not move in an electric field . above PI negatively charged can move under an electric field.

f) **Denaturation:** when protein are heated, or subjected to extremes temperature, high salt . organic solvents etc, the non covalent bonds break changing the Native structure to random coil. This unfolding of protein is due to loss of secondary, tertiary and quarternary structure. It does not affect primary sturctue.

Effect of denaturation:

Loss of activity due to loss of shape and active site,
Heat
Changing in pH
Radiation
Heavy metals
Detergents
Digestive enzymes
Urea
Repeated freezing and thawing. Change of shape cause loss of activity . if the active site is altered E will lose activity

g) **Absorption spectra:** enzyme are proteins give maximum absorption at 280nm.

h) **Specificity:** enzymes are highly specific for the reactions they catalyze. A given enzyme is very selective, both in the substance with which it interact and in the reaction that is catalyzes. The substances upon which an enzyme acts are traditionally called substrates. In an enzyme catalyzed reaction none of the substrate is diverted into nonproductive side reactions, so no wasteful by products are produced. It follows then that the products formed by a given enzyme are also very specific.

i) **Group specificity:** broad specificity. Enzymes acts on a group of related substrates. The substrates have a common group on which the enzyme acts: eg esterase can act on different esters- protease can act on different proteins.

j) **The specificity of enzymes is determined by:**

1) **Functional groups of enzymes:** specific amino acid side chains, metal ions, and coenzymes

2) **Functional groups of substrate:** during enzyme action, there is a temporary combination between enzyme and its substrate forming enzyme substrate complex by relatively week forces. This occur at the active site of the enzyme relatively week forces.

Sr.no.	Question	Answer

1	What is the range of molecular weight of enzymes?	1000 to several thousends
2	What are zwitter ions?	a charged molecule with equal number of +ve and -ve charges
3	Enzymes give maximum absorption spectrum at.....	280nm
4	The specificity of enzymes is determined by what?	Functional groups of enzymes and Functional groups of substrate
5	Give to reason for denaturation of enzymes	Changing in pH and radiation

- **Why classify enzymes?**
- There is a long tradition of giving enzymes names ending in '-ase'. The only major exceptions to this are the proteolytic enzymes, i.e. ones involved in the breakdown of proteins, whose names usually end with '-in', e.g. trypsin.
- The names of enzymes usually indicate the substrate involved. Thus, lactase catalyses the hydrolysis of the disaccharide lactose to its component monosaccharides, glucose and galactose:

- **Lactose→ glucose + galactose**

- The name lactase is a contraction of the clumsy, but more precise, lactosase. The former is used because it sounds better but it introduces a possible trap for the unwary because it could easily suggest an enzyme acting on the substrate lactate.
- There is nothing in the name of this enzyme or many others to indicate the type of reaction being catalysed. Fumarase, for example, by analogy with lactase might be supposed to catalyse a hydrolytic reaction, but, in fact, it *hydrates* fumarate to form malate:
- -02C.CH=CH.co2 + H20→02C.CHOH.CH2C02
- **Fumarate malate**

- The names of other enzymes, e.g. **transcarboxylase,** indicate the nature of the reaction without specifying the substrates (which in the case of

transcarboxylase are methylmalonyl-CoA and pyruvate). Some names, such as **catalase,** indicate neither the substrate nor the reaction (catalase mediates the decomposition of hydrogen peroxide).

- Needless to say, whenever a new enzyme has been characterized, great care has usually been taken not to give it exactly the same name as an enzyme catalysing different reaction.
- Also, the names of many enzymes make clear the substrate and
- the nature of the reaction being catalysed. For example, there is little ambiguity about the reaction catalysed by **malate dehydrogenase.** This enzyme mediates the removal of hydrogen from malate to produce oxaloacetate.
- However, malate dehydrogenase, like many other enzymes, has been known by more than one name.
- So, because of the lack of consistency in the nomenclature, it became apparent as the list of known enzymes rapidly grew that there was a need for a systematic way of naming and classifying enzymes.
- A commission was appointed by the International Union of Biochemistry (later re-named the International Union of Biochemistry and Molecular Biology, IUBMB), and its report, published in 1964, forms the basis of the currently accepted system. Revised editions of the report were published in 1972, 1978, 1984 and 1992. An electronic version is now maintained
- by the IUBMB on an accessible web-site, and this is updated on a regular basis.

The Enzyme Commission's system of classification

The Enzyme Commission divided enzymes into six main classes, on the basis of the total reaction catalysed. Each enzyme was assigned a code number, consisting of four elements, separated by dots. The first digit shows to which of the main classes the enzyme belongs, as follows:

First digit Enzyme class

1Oxidoreductases
2 Transferases
3 Hydro lases
4 Lyases
5 Isomerases
6 Ligases

Type of reaction catalysed

Oxidation/Reduction reactions : Transfer of an atom or group between two molecules (excluding reactions in other classes)

Hydrolysis reaction : Removal of a group from substrate (not by hydrolysis)

Isomerization reactions: The synthetic joining of two molecules, coupled with

the breakdown of the pyrophosphate bond in a nucleoside triphosphate

The naming and classification of enzymes

- The second and third digits in the code further describe the kind of reaction being catalysed. There is no general rule, because the meanings of these digits are defined separately for each of the main classes. Some examples are given later in this chapter
- Note that, for convenience, and in line with normal practice, some structures are written in a slightly simplified form in the lists provided. So, for example, in the case of the acyl group, which is transferred in reactions catalysed by E.C. 2.3 enzymes, it should be understood that the structure written -COR
- represents:

--C--R
II
O

- Enzymes catalysing very similar but non-identical reactions, e.g. the hydrolysis of different carboxylic acid esters, will have the same first three digits in their code.
- The fourth digit distinguishes between them by defining the actual substrate, e.g. the actual carboxylic acid ester being hydrolysed.
- However, it should be noted that **isoenzymes,** that is to say, different enzymes catalysing identical reactions, will have the same four figure classification.
- There are, for example, five different isoenzymes of **lactate dehydrogenase** within the human body and these will have an identical code.
- The classification, therefore, provides only the basis for a unique identification of an enzyme.
- The particular isoenzyme and its source still have to be specified.
- It should also be noted that all reactions catalysed by enzymes are reversible to some degree and the classification which would be given to the enzyme for the catalysis of the forward reaction would not be the same as that for the reverse reaction.
- The classification used is that of the most important direction from the biochemical point of view, or according to some convention defined by the Commission.
- For example, for oxidation/reduction involving the interconversion of
- NADH and NAD+ (see section 11.5.2) the classification is usually based on the direction where NAD+ is the electron acceptor rather than that where NADH is the electron donor.
- Some problems are given at the end of this chapter to help the student become familiar with this system of classification.

Sr.no.	Question	Answer

1	In traditional method enzymes involved in the breakdown of proteins whose names usally end with.....	in
2	Lactose is hydrolysed into ..... monosaccharides	Glucose & Galactose
3	Give full form of IUBMB	International union of biochemistry and molecular biology
4	Enzymes commission divided enzymes into how many classes?	6
5	What is hydrolysis reaction?	Removal of a group from substrate

The Enzyme Commission's recommendations on nomenclature

- The Commission assigned to each enzyme a systematic name in addition to its existing trivial name. This systematic name includes the name of the substrate or substrates in full and a word ending in '-ase' indicating the nature of the process catalysed.
- This word is either one of the six main classes of enzymes or a subdivision of one of them. When a reaction involves two types of overall change,
- e.g. oxidation and decarboxylation, the second function is indicated in brackets, e.g. oxidoreductase (decarboxylating). Examples are given below.
- The systematic name and the Enzyme Commission (E.C.) classification number unambiguously describe the reaction catalysed by an enzyme and should always be included in a report of an investigation of an enzyme, together with the source of enzyme, e.g. rat liver mitochondria.
- However, these names are likely to be long and unwieldy. Trivial names may, therefore, be used in a communication, once they have been introduced and defined in terms of the systematic name and E.C. number. Trivial names are also inevitably used in everyday situations in the laboratory.
- The Enzyme Commission made recommendations as to which trivial names were acceptable, altering those which were considered vague or misleading. Thus, 'fumarase', mentioned above, was considered unsatisfactory and was replaced by 'fumarate hydratase'.

The six main classes of enzymes

Main Class 1: Oxidoreductases

These enzymes catalyse the transfer of H atoms, 0 atoms or electrons from one substrate to another. The second digit in the code number of oxidoreductases indicates the donor of the reducing equivalents (hydrogen or electrons) involved in the reaction. For example:

Second digit

Hydrogen or electron donor

1. alcohol (>CHOH)
2. aldehyde or ketone (>C=O)
3. --CH.CHprimary
4. amine (>CHNH2 or >CHNH3 "')
5. secondary amine (>CHNH-)
6. NADH or NADPH (only when some other redox catalyst

is the acceptor)

The third digit refers to the hydrogen or electron acceptor, as follows:

Third digit

Hydrogen or electron acceptor

1. NAD+ or NADP+
2. Fe3+ (e.g. cytochrome)
3. O2

99. An otherwise unclassified acceptor

Trivial names of oxidoreductases include oxidases (transfer of H to 0 2) and dehydrogenases (transfer ofH to an acceptor other than 0 2). These often indicate the identity of the donor and/or acceptor. Here are some examples:

(S)-lactate: NAD+ oxidoreductase (E.C. 1.1.1.27), trivial name lactate dehydrogenase, catalyses the reaction:

CH3.CH.co2 +NAD+--→ CH3 c.co-2 + NADH + H+ I

OH

(S)-lactate pyruvate

Note that it is the alcohol group of lactate, rather than the carboxyl group, which is involved in the reaction and this is indicated in the classification. The naming and classification of enzymes

Isocitrate: NAD+ oxidoreductase (decarboxylating) (E.C. 1.1.1.41), trivial name isocitrate dehydrogenase, catalyses:

isocitrate

-02C.CH2.CH.c.co2 + NADH + H+ + C02

II

0 (1.5)

2-oxoglutarate

D-amino acid: oxygen oxidoreductase (deaminating) (E.C. 1.4.3.3), trivial name D-amino acid oxidase, catalyses:

D-amino acid oxoacid

Note that this enzyme is less specific than most and will act on any D-amino

acid.

Main Class 2: Trans/erases

These catalyse reactions of the type:

AX+ B BX + A

but specifically exclude oxidoreductase and hydrolase reactions. In general, the Enzyme Commission recommends that the names of transferases should end 'Xtransferase',

where Xis the group transferred, although a name ending 'trans-X-ase'

is an acceptable alternative. The second digit in the classification describes the type

of group transferred. For example:

Second digit

Group transferred

1. I -carbon group
2. aldehyde or ketone group (>C=O)
3. acyl group (--COR)
4. glycosyl (carbohydrate group)

7. phosphate group

In general, the third digit further describes the group transferred. Thus:

E.C. 2.1.1 enzyme are methyltransferases (transfer --CH3), whereas

E.C. 2.1.2 enzymes are hydroxymethyltransferases (transfer --CH20H) and

E.C. 2.1.3 enzymes are carboxyl transferases (transfer --COOR)

or carbamoyl transferases (transfer --CONH2).

Similarly,

E.C. 2.4.1 enzymes are hexosyltransferases (transfer hexose units), and

E.C. 2.4.2 enzymes are pentosyltransferases (transfer pentose units).

The exception to this general rule for transferases is where there is transfer of phosphate groups: these cannot be described further, so there is opportunity to indicate the acceptor.

E.C. 2.7.l enzymes are phosphotransferases with an alcohol group as acceptor,

E.C. 2.7.2 enzymes are phosphotransferases with a carboxyl group as acceptor,

E.C. 2.7.3 enzymes are phosphotransferases with a nitrogenous group as acceptor.

Phosphotransferases usually have a trivial name ending in '-kinase'. Some examples oftransferases are:

(S)-2-methyl-3-oxopropanoyl-CoA: pyruvate carboxyltransferase (E.C. 2.1.3.1)

(trivial name: methylmalonyl-CoA carboxyltransferase, formerly transcarboxylase)

which catalyses the transfer of a carboxyl group from methylmalonyl-CoA to pyruvate:

CH3.CH.COSC0A + CH3CO.co2 ~ CH3_CH2.COSC0A + H2.CO.co2

co2
methylmalonyl-CoA pyruvate propionyl-CoA oxaloacetate
ATP: D-hexose-6-phosphotransferase (E.C. 2.7.l.1) (trivial name: hexokinase) which catalyses:
C5H90 5.CH20H + ATP ~ C5H90 5.CH20Poi- + ADP (1.8)
D-hexose D-hexose-6-phosphate
This enzyme will transfer phosphate to a variety of D-hexoses.

Main Class 3: Hydrolases

These enzymes catalyse hydrolytic reactions of the form:
A-X + H20 ~ X-OH + HA
They are classified according to the type of bond hydrolysed. For example:
Second digit
Bond hydrolysed
1. ester
2. glycosidic (linking carbohydrate units)
4. peptide (-CONH-)
5. C-N bonds other than peptides

The third digit further describes the type of bond hydrolysed. Thus:
E.C. 3.1.1 enzymes are carboxylic ester (-COO-) hydrolases,
E.C. 3.1.2 enzymes are thiol ester (-COS-) hydrolases,
E.C. 3.1.3 enzymes are phosphoric monoester (-0 - Poi-) hydrolases,
E.C. 3.1.4 enzymes are phosphoric diester (-O-P02 -0-) hydrolases.
Sec. 1.3 The naming and classification of enzymes 9
For example, orthophosphoric monoester phosphohydrolase (E.C. 3.1.3.1) (alkaline phosphatase) catalyses:
R-0-POj- + H20
organic phosphate
R-OH + HO-Pojinorganic
Phosphate Alkaline phosphatases are relatively non-specific, and act on a variety of
substrates at alkaline pH.
The trivial names of hydrolases are recommended to be the only ones to consist simply of the name of the substrate plus '-ase'.

Main Class 4: Lyases

These enzymes catalyse the non-hydrolytic removal of groups from substrates, often
leaving double bonds.
The second digit in the classification indicates the bond broken, for example:
Second digit

Bond broken

1. C-C
2. C-0
3. C-N
4. C-S

The third digit refers to the type of group removed. Thus, for the C-C lyases:

Third digit

Group removed

1. carboxyl group (i.e. C02)
2. aldehyde group (-CH=O)
3. ketoacid group (-co.coz-)

For example, L-histidine carboxy-lyase (E.C. 4.1.1.22) (trivial name: histidine decarboxylase, catalyses:

C3N2H3.CH2CH.NH; ~ C3N2H3.CH2.CH2.NH; + C02

I

co2

histidine histamine

- (Note the importance of the hyphen and the extra 'y' in the systematic name,
- because carboxy-lyase and carboxylase do not mean the same thing: carboxylase

simply refers to the involvement of C02 in a reaction without being specific.)

- Also classified as lyases are enzymes catalysing reactions whose biochemically
- important direction is the reverse of the above, i.e. addition across double bonds.
- These may have the trivial name synthase or, if water is added across the double
- bond, hydratase, as discussed earlier in the example of fumarate hydratase (fumarase), the systematic name of this particular enzyme being (S)-malate hydrolyase (E.C. 4.2.1.2).

Main Class 5: Isomerases

Enzymes catalysing isomerization processes are classified according to the type of

reaction involved. For example:

Second digit

Type of reaction

1. Racemization or epimerization (inversion at an

asymmetric carbon atom)

2.cis-trans isomerization

3.intramolecular oxidoreductases
4.intramolecular transfer reaction
The third digit describes the type of molecule undergoing isomerization. Thus, for racemases and epimerases:
Third digit
Substrate
2. amino acids
3. hydroxy acids
4. carbohydrates
An example is alanine racemase (E.C. 5.1.1.1) which catalyses:
L-alanine→ D-alanine

Main Class 6: Ligases
These enzymes catalyse the synthesis of new bonds, coupled to the breakdown of
ATP or other nucleotide triphosphates. The reactions are of the form:
X + Y + ATP """" X-Y + ADP + Pi
or X + Y + ATP """" X-Y + AMP + (PP)i
The second digit in the code indicates the type of bond synthesized. For example:
Second digit
Bond synthesized
1. C-0
2. C-S
3. C-N
4. C-C
The third digit further describes the bond being formed. Thus
E.C. 6.3.l enzymes are acid-ammonia ligases (amide, -CONH2 , synthases) and E.C. 6.3.2 enzymes are acid-amino acid ligases (peptide, -CONH-, synthases). Prior to 1984, such enzymes could also be known as synthetases.
An example is L-glutamate: ammonia ligase (E.C. 6.3.1.2), trivial name: glutamate-ammonia ligase, formerly glutamate synthetase, which catalyses:
O=C.CH2CH2.CH.C02 +ATP+ NH3 -oI I +NH3→ ~ O=C.CH2CH2 .CH.C02 +ADP +Pi
I I
NH2 +NH3
L-glutamate

Sr.no.	Question	Answer
1	What is the name of class 1 enzyme?	oxidoreductase
2	What is the X in trans/erases enzyme?	Group transferred
3	Hydrolyses enzymes catalysed which type of	Hydrolytic reaction

	reaction?	
4	What dose second digit indicates in class 4 enzyme?	Broken bond
5	Give example of isomerases	Alanine racemase

ENZYME ACTIVITY

- A characteristic feature of enzymes is that they are specific in action. Some enzymes exhibit **group specificity,** i.e. they may act on several different, though closely related, substrates to catalyse a reaction involving a particular chemical group.
- An example of this kind of enzyme is alcohol dehydrogenase, which will catalyse the oxidation of a variety of alcohols. Another is hexokinase, which will assist the transfer of phosphate from ATP to several different hexose sugars. Other enzymes

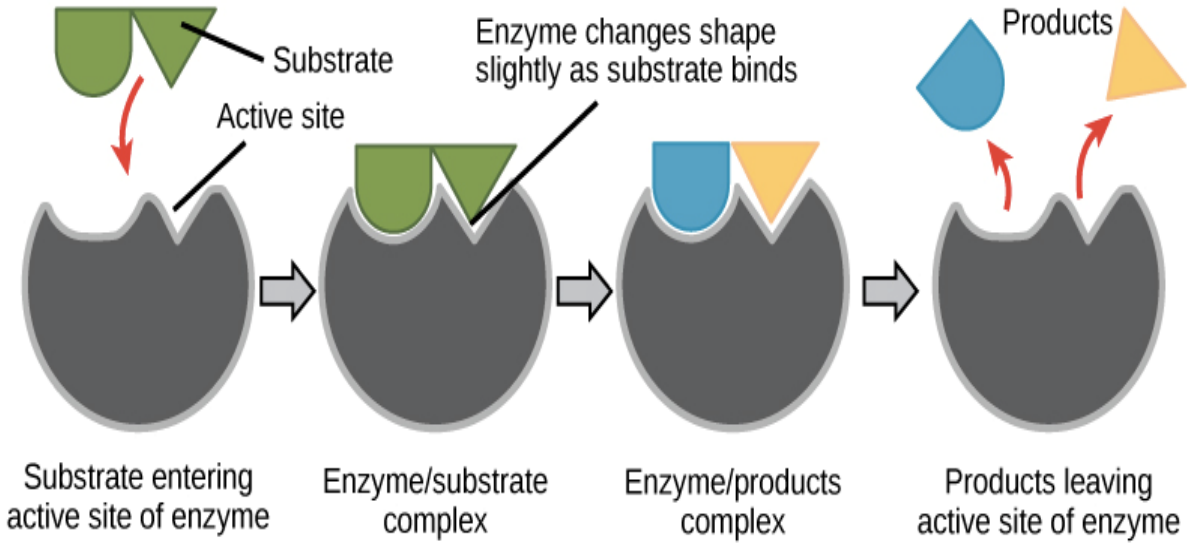
will act only on one particular substrate, when they are said to exhibit **absolute specificity.**

- For example, glucokinase catalyses the transfer of phosphate from ATP to glucose and to no other sugar (although the specificity of the so-called 'glucokinase' found in liver is less clear-cut, in contrast to the true glucokinases of bacteria and invertebrates).
- Uncatalysed reactions often give rise to a wide range of products, but enzyme catalysed reactions are **product-specific** as well as being **substrate-specific.** Also, in addition to showing chemical specificity, enzymes exhibit **stereochemical specificity:** if a substrate can exist in two stereochemical forms, chemically identical but with a different arrangement of atoms in three-dimensional space (section
- then only one of the isomers will undergo reaction as a result of catalysis by a particular enzyme. For example, L-amino acid oxidase mediates the oxidation of Lamino acids to oxo acids.
- A separate enzyme, D-amino acid oxidase, is required for the corresponding oxidation ofD-amino acids.
- Even greater specificity is shown by the fungal enzyme glucose oxidase, which
- catalyses the reaction: 68 Specificity of Enzyme Action
- No other naturally-occurring sugar, including a-D-glucose and P-D-galactose, can be acted upon to any appreciable extent. CH20H
- The only enzymes which act on both stereoisomeric forms of a substrate are those whose function is to interconvert L- and D-isomers. An example is alanine racemase, which catalyses the reaction:
- L-alanine ~ D-alanine Enzyme-catalysed reactions may yield stereospecific products even when the substrate possesses no asymmetric carbon atom.

- For example, the action of glycerol kinase on glycerol always results in the production of L-glycerol-3-phosphate (snglycerol-
- 3- phosphate).

Sr.no.	Question	Answer
1	What type of reaction is glucokinase dose?	It transfer phosphate from ATP to glucose
2	Enzymes catalyses reactions are.....specific	Product -specific
3	..... enzyme is required for oxidation of d amino acid	d- amino acid oxidase
4	..... enzymes act on both steroisomeric form	Alanine racemase
5	The action of glycerol kinase on glycerol always results in the production of.....	L-glycerol-3-phosphate

THE ACTIVE SITE



- In order to explain the stereochemical specificity of enzymes, Alexander Ogston (1948) pointed out that there must be *at least three different points of interaction* between enzyme and substrate.
- These interactions can have either a binding or a catalytic function: binding sites link to specific groups in the substrate, ensuring that the enzyme and substrate molecules are held in a fixed orientation with respect to each other, with the reacting group or groups in the vicinity of catalytic sites.
- For example, sites A" and A"' might represent binding sites for R" and R"' respectively, and A' a catalytic site for a reaction involving R'. Thus, even if R' and R" are chemically identical (as with glycerol in the glycerol , the asymmetry of the enzyme-substrate complex means that only R' can react, providing binding site A"' is specific for R"'. R" can never undergo reaction under these conditions, since it is not brought into the vicinity Of site A' even when R' binds to site A".
- Generally similar considerations apply to enzymes catalysing reactions involving more than one substrate.
- In this case, the reacting groups of each substrate are brought together in the vicinity of one or more catalytic sites.
- The region which contains the binding and catalytic sites is termed the active site, or active centre, of the enzyme.
- This comprises only a small proportion of the total volume of the enzyme and is usually at or near the surface, since it must be accessible to substrate molecules.
- In some cases, X-ray diffraction studies have revealed a clearly-defined pocket or cleft in the enzyme molecule into which the whole or part of each substrate can fit.
- Although the active site is given a planar representation in it should be realised that it has, in fact, a three-dimensional structure since it consists of portions of a polypeptide chain.

- The amino acid residues involved may be widely separated in the primary structure, being brought together in space because of the twists and turns within the molecule. The binding and catalytic sites must be either amino acid residues or cofactors, the latter being themselves bound to amino acid side chains.
- Substrate binding may involve a variety of linkages, but the bonds formed are usually relatively weak.
- Those amino acid residues in the active site which do not have a binding or catalytic function may nevertheless contribute to the specificity of the enzyme.
- Their side chains must be of suitable size, shape and character not to interfere with the binding of the substrate, but they might interfere with the binding of other, chemically similar, substances.
- The active site often includes both polar and non-polar amino acid residues, creating an arrangement of hydrophilic and hydrophobic microenvironments not found elsewhere on an enzyme molecule.
- Hence, the function of an enzyme may depend not only on the spatial arrangement of binding and catalytic sites, but also on the environment in which these sites occurs Thus it can be seen that the three-point interaction theory provides only a limited explanation of enzyme specificity, a more complete view coming from consideration of a whole range of interactions in three-dimensional space.

Sr.no.	Question	Answer
1	What is active site?	The region which contains the binding and catalytic sites
2	The binding or catalytic site must be.....	Amino acid residue or co- factor
3	Who gave three point interaction theories?	Alexander Ogston
4	Bond form between substrate and enzymes are.....	Relatively weak bond
5	Where active site is seen in enzyme?	Near the surface

THE FISCHER 'LOCK-AND-KEY' HYPOTHESIS

- As early as 1890, Emil Fischer suggested that enzyme specificity implied the presence of complementary structural features between enzyme and

substrate: a substrate might fit into its complementary site on the enzyme as a key fits into a lock.

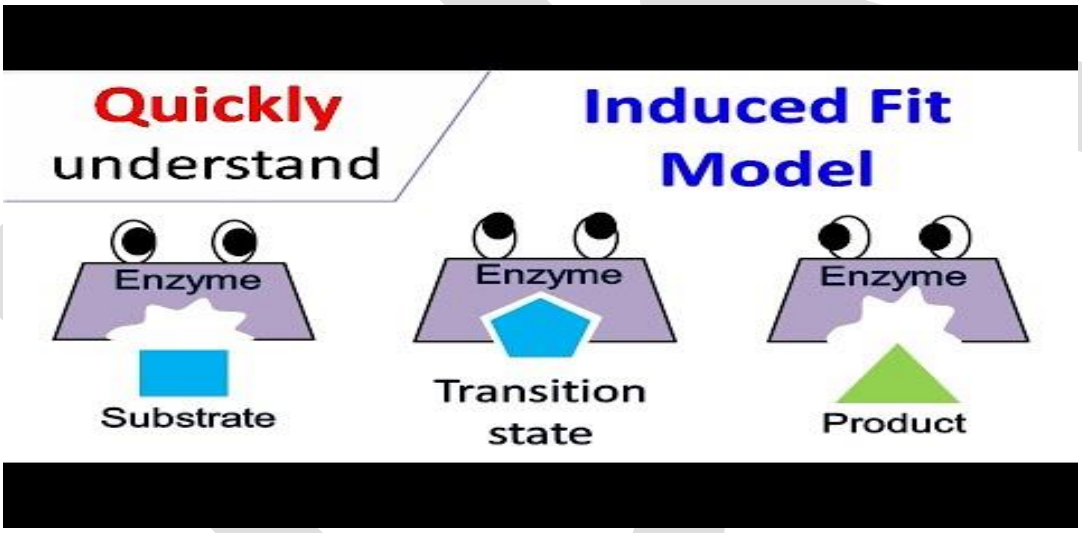
- This is entirely consistent with the more detailed aspects of active site structure.
- According to the **lock-and-key** model, all structures remain fixed throughout the binding process.

THE KOSHLAND 'INDUCED-FIT' HYPOTHESIS

- The lock-and-key hypothesis explains many features of enzyme specificity, but takes no account of the known flexibility of proteins.
- X-ray diffraction analysis and data from several forms of spectrometry, including nuclear magnetic resonance (NMR), have revealed differences in structure between free and substrate-bound enzymes. Thus, the binding of a substrate to an enzyme may bring about a **conformational change,** i.e. a change in three-dimensional structure but not in primary structure.
- This is not necessarily surprising, for the bonds formed between a substrate and its binding sites may have replaced previously existing linkages between each binding site and neighbouring groups on the enzyme.
- Also, the presence of a substrate at the active site may exclude water molecules and thus make the region more non-polar.
- Both of these factors could be responsible for some degree of change in tertiary structure taking place.
- Daniel Koshland, in his **induced-fit** hypothesis of 1958, suggested that the structure of a substrate may be complementary to that of the active site in the enzyme-substrate complex, but not in the free enzyme: a conformational change takes place in the enzyme during the binding of substrate which results in the required matching of structures .
- The induced-fit hypothesis essentially requires the active site to be floppy and the substrate to be rigid, allowing the enzyme to wrap itself around the substrate, in this way bringing together the corresponding catalytic sites and reacting groups.
- In some respects, the relationship between a substrate and an active site is similar to that between a hand and a woollen glove: in each interaction the

structure of one component (substrate or hand) remains fixed and the shape of the second component (active site or glove) changes to become complementary to that of the first.

- Such a mechanism could help to achieve a high degree of specificity for the enzyme. In the lock-and-key mechanism, the active site is always structurally intact, with the catalytic sites aligned and freely accessible. Thus a suitable reacting group whether part of an appropriately-bound substrate or not, can come into contact with the region of catalytic activity and some degree of reaction take place.
- In the induced-fit mechanism, on the other hand, different catalytic components might be separated by a considerable margin in the free enzyme, minimising the risk of chance collision of a reactive group with both of them.
- It is also possible that access to the catalytic groups of the free enzyme might be blocked. Only when a binding group of the substrate is recognized by the corresponding site of the enzyme and the binding process proceeds does the conformational change take place, which results in all the relevant groups in substrate and enzyme comin together.

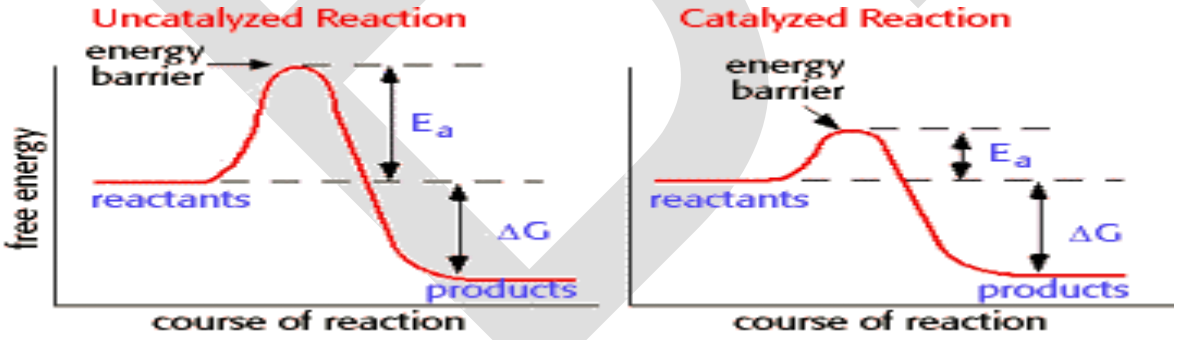


- Of course, a similar binding group in a substance other than the substrate might trigger off a conformational change but, in general, this would not result in catalytic groups being brought together in the vicinity of an appropriate reacting group, so no reaction would take place .
- This would be termed **nonproductive binding.** An example of a reaction which appears to proceed via an induced-fit mechanism is that catalysed by yeast hexokinase:
- D-hexose + ATP ~ D-hexose-6-P + ADP
- In the absence of hexose, bound ATP is hydrolysed extremely slowly, even though, in chemical terms, this hydrolysis could be brought about by the action of a water molecule in the solvent just as well as by an -OH group in the hexose. This, together with X-ray diffraction evidence, suggests that the binding of the hexose causes a conformational change in the enzyme which activates the ATP.

- Conformational changes of the type discussed in this section have been shown to play a part in the mechanism of action of several other enzymes, e.g. carboxypeptidase A, They have also been useful in explaining the behaviour of allosteric enzymes.
-

Sr.no.	Question	Answer
1	Who gave first lock & key hypothesis?	Emil fischer
2	What dose fischer lock & key hypothesis suggest?	All structure remains fixed throughout the binding site
3	Give full form of NMR	Nuclear magnetic resonance
4	Who gave induced fit hypothesis?	Denial koshland
5	In induced fit hypothesis active site remains.....	Floppy

ENZYME CAN ACT IN SEVERAL WAYS, ALL OF WHICH ▲G:(Gibbs energy)



Enzymes are biological catalysts. Catalysts lower the **activation energy** for reactions. The lower the **activation energy** for a reaction, the faster the rate. Thus **enzymes** speed up reactions by lowering **activation energy**.

activation energy is the minimum amount of energy that must be provided to compounds to result in a chemical reaction.[1] The activation energy (E_a) of a reaction is measured in joules per mole (J/mol), kilojoules per mole (kJ/mol) or kilocalories per mole (kcal/mol).[2] Activation energy can be thought of as the magnitude of the potential barrier (sometimes called the energy barrier)

separating minima of the potential energy surface pertaining to the initial and final thermodynamic state. For a chemical reaction to proceed at a reasonable rate, the temperature of the system should be high enough such that there exists an appreciable number of molecules with translational energy equal to or greater than the activation energy

FACTORS AFFECTING ENZYME ACTIVITY

Enzyme activity can be affected by a variety of factors, such as temperature, pH, and concentration.

Enzymes work best within specific temperature and pH ranges, and sub-optimal conditions can cause an enzyme to lose its ability to bind to a substrate.

- **Temperature:** Raising temperature generally speeds up a reaction, and lowering temperature slows down a reaction. However, extreme high temperatures can cause an enzyme to lose its shape (denature) and stop working.
- **pH:** Each enzyme has an optimum pH range. Changing the pH outside of this range will slow enzyme activity. Extreme pH values can cause enzymes to denature.
- **Enzyme concentration**: Increasing enzyme concentration will speed up the reaction, as long as there is substrate available to bind to. Once all of the substrate is bound, the reaction will no longer speed up, since there will be nothing for additional enzymes to bind to.
- **Substrate concentration:** Increasing substrate concentration also increases the rate of reaction to a certain point. Once all of the enzymes have bound, any substrate increase will have no effect on the rate of reaction, as the available enzymes will be saturated and working at their maximum rate.

Sr.no.	Question	Answer
1	How enzymes speed up reaction?	By lower the activation energy
2	Activation energy is measured in	Joules per mole or kj/mol or kcl/mol
3	Enzymes activity affected by ..... factors	Temperature, pH and concentration
4	Extreme pH value can cause enzymes to.....	Denature
5	Extreme temperature can cause to enzyme.....	Loose its shape and stop working

- **ENZYME INHIBITION**

Inhibitors are substances which tend to decrease the rate of an enzyme-catalysed reaction. Although some act on a substrate or cofactor, we will restrict our discussion here to those which combine directly with an enzyme. **Reversible inhibitors** bind to an enzyme in a reversible fashion and can be removed by dialysis (or simply dilution) to restore full enzymic activity, whereas **irreversible inhibitors** cannot be removed from an enzyme by dialysis. Sometimes it may be possible to remove an irreversible inhibitor from an enzyme by introducing another component to the reaction mixture, but this would not affect the classification of the original interaction.

Reversible inhibitors usually rapidly form an equilibrium system with an enzyme to show a definite degree of inhibition (depending on the concentration of enzyme, inhibitor and substrate) which remains constant over the period when initial velocity studies are normally carried out. In contrast, the degree of inhibition by irreversible inhibitors may increase over this period of time.

In this chapter we shall be concerned mainly with the inhibition of simple singlesubstrate enzyme-catalysed reactions. This group includes most single-substrate reactions obeying Michaelis-Menten kinetics (section 7.1.3). The inhibition oftwosubstrate enzyme-catalysed reactions will be discussed.

REVERSIBLE INIDBITION

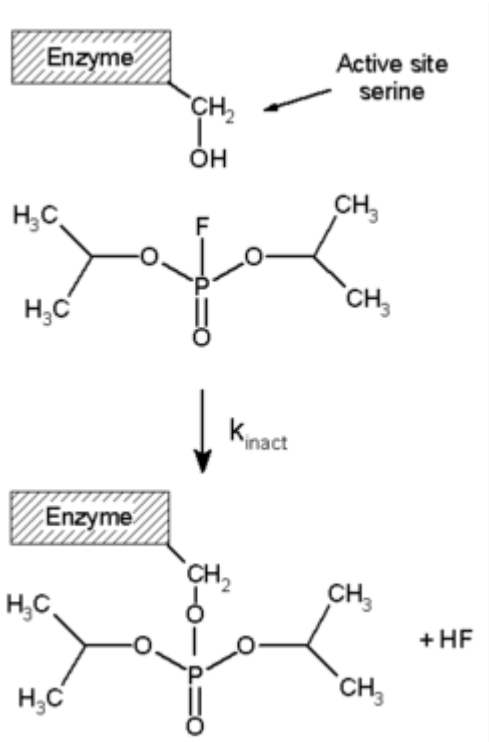
Reversible inhibitors attach to enzymes with non-covalent interactions such as hydrogen bonds, hydrophobic interactions and ionic bonds. Multiple weak bonds between the inhibitor and the active site combine to produce strong and specific binding. In contrast to substrates and irreversible inhibitors, reversible inhibitors generally do not undergo chemical reactions when bound to the enzyme and can be easily removed by dilution or dialysis.

There are four kinds of reversible enzyme inhibitors. They are classified according to the effect of varying the concentration of the enzyme's substrate on the inhibitor.

- In **competitive inhibition**, the substrate and inhibitor cannot bind to the enzyme at the same time, as shown in the figure on the right. This usually results from the inhibitor having an affinity for the active site of an enzyme where the substrate also binds; the substrate and inhibitor *compete* for access to the enzyme's active site. This type of inhibition can be overcome by sufficiently high concentrations of substrate (V_{max} remains constant), i.e., by out-competing the inhibitor. However, the apparent K_m will increase as it takes a higher concentration of the substrate to reach the K_m point, or half the V_{max}. Competitive inhibitors are often similar in structure to the real substrate (see examples below).
- In **uncompetitive inhibition**, the inhibitor binds only to the substrate-enzyme complex. This type of inhibition causes V_{max} to decrease (maximum velocity decreases as a result of removing activated complex) and K_m to decrease (due to better binding efficiency as a result of Le Chatelier's principle and the effective elimination of the ES complex thus decreasing the K_m which indicates a higher binding affinity).
- In **non-competitive inhibition**, the binding of the inhibitor to the enzyme reduces its activity but does not affect the binding of substrate. As a result, the extent of inhibition depends only on the concentration of the inhibitor. V_{max} will decrease due to the inability for the reaction to proceed as efficiently, but K_m will remain the same as the actual binding of the substrate, by definition, will still function properly.
- In **mixed inhibition**, the inhibitor can bind to the enzyme at the same time as the enzyme's substrate. However, the binding of the inhibitor affects the binding of the substrate, and vice versa. This type of inhibition can be reduced, but not overcome by increasing concentrations of substrate. Although it is possible for mixed-type inhibitors to bind in the active site, this type of inhibition generally results from an allosteric effect where the inhibitor binds to a different site on an enzyme. Inhibitor binding to this allosteric site changes the conformation (i.e., tertiary structure or three-dimensional shape) of the enzyme so that the affinity of the substrate for the active site is reduced.

These types can also be distinguished by the effect of increasing the substrate concentration [S] on the degree of inhibition caused by a given amount of inhibitor. For competitive inhibition the degree of inhibition is reduced by increasing [S], for noncompetitive inhibition the degree of inhibition is unchanged, and for uncompetitive (also called anticompetitive) inhibition the degree of inhibition increases with [S]

ypes of irreversible inhibition (covalent inactivation) **[edit]**



Reaction of the irreversible inhibitor diisopropylfluorophosphate (DFP) with a serine protease

Irreversible inhibitors usually covalently modify an enzyme, and inhibition can therefore not be reversed. Irreversible inhibitors often contain reactive functional groups such as nitrogen mustards, aldehydes, haloalkanes, alkenes, Michael acceptors, phenyl sulfonates, or fluorophosphonates. These nucleophilic groups react with amino acid side chains to form covalent adducts. The residues modified are those with side chains containing nucleophiles such as hydroxyl or sulfhydryl groups; these include the amino acids serine (as in DFP, right), cysteine, threonine, or tyrosine.[28]

Irreversible inhibition is different from irreversible enzyme inactivation. Irreversible inhibitors are generally specific for one class of enzyme and do not inactivate all proteins; they do not function by destroying protein structure but by specifically altering the active site of their target. For example, extremes of pH or temperature usually cause denaturation of all protein structure, but this is a non-specific effect. Similarly, some non-specific chemical treatments destroy protein structure: for example, heating in concentrated hydrochloric acid will hydrolyse the peptide bonds holding proteins together, releasing free amino acids.[29]

Irreversible inhibitors display time-dependent inhibition and their potency therefore cannot be characterised by an IC_{50} value.[30][31] This is because the amount of active enzyme at a given concentration of irreversible inhibitor will be different depending on how long the inhibitor is pre-incubated with the enzyme. Instead, $k_{obs}/[I]$ values are used,[32] where k_{obs} is the observed pseudo-

first order rate of inactivation (obtained by plotting the log of % activity vs. time) and [*I*] is the concentration of inhibitor. The k_{obs}/[*I*] parameter is valid as long as the inhibitor does not saturate binding with the enzyme (in which case $k_{obs} = k_{inact}$).

Sr.no.	Question	Answer
1	What are inhibitors?	Inhibitors are substances which tend to decrease the rate of an enzyme-catalysed Reaction.
2	How reversible inhibitos attach to substrate?	By non- covelent interactions
3	In competitive inhibitors what is the value of Vmax?	Vmax remains constant throughout reaction
4	In non- competitive inhibitors Vmax is.....	decreased
5	What is the full form of DFP	Diisopropylflurophosphate

ENZYME PREPARATION

The source of enzyme for any microbiological experiment may be one of the following:.

1) Growing culture techinique

a) Inoculation of bacteria into medium containing substrate.

b) Incubation of bacteria at optimum temperature

c) Examines for change or disappearance of substrate and presence of end products.

2) Resting cell technique

a) Cells removed from growing culture and resuspended in a non nutrient solution.

b) Growth of bacteria in a suitable medium

c) Preparation of a resting cell suspension. The cells are separated from the medium by centrifugation and resuspended in a non nutrient solution. The process in repeated to free the cells of all material from the medium and is known as washing cells. The final suspension is referred to as resting cell suspension.

d) Addition of resting cells and substrate to a special testing unit

e) Incubation of the apparatus at optimum temperature.

f) Examination for disappearance of substance and appearance of products.

3) Cell free enzyme technique

a) Enzyme extracted from cell. Cell free preparation may be of different degrees of purity, from very crude extrates to very pure. Sometimes the cellular organelles are separated to study enzyme system housed in it.

b) Preparation of concentrated resting cells suspension by centrifugation of actively growing cultures.

c) Disintegrate of centrifuged cells by special technique to release enzymes, eg. Grinding sonic treatment.

d) Addition of cell free enzymes and substrates to special testing unit.

e) Incubation of the unit at optimum temperature.

f) Examining for disappearance of substrate and appearance of products.

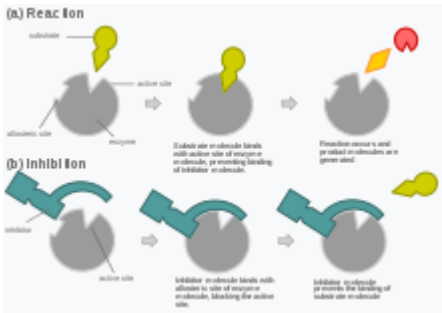
Sr.no.	Question	Answer
1	In which technique bacteria are incubated in a medium containing substance?	Growing culture technique
2	In..... technique culture are suspended in to nutrient solution	Resting cell technique
3	In which technique grinding sonic treatment is used?	Cell free enzyme technique
4	In resting cell technique cells are separated from medium by	centrifugation
5	Which specific technique is used to disintegrate centrifuged cells?	Grinding sonic treatment

MECHANISMS AND REGULATION OF ENZYME ACTIVITY

A **regulatory enzyme** is an enzyme in a biochemical pathway which, through its responses to the presence of certain other biomolecules, regulates the pathway activity. This is usually done for pathways whose products may be needed in different amounts at different times, such as hormone production. Regulatory enzymes exist at high concentrations (low Vmax) so their activity can be increased or decreased with changes in substrate concentrations.

The enzymes which catalyse chemical reactions again and again are called regulatory enzymes.

1) Allosteric enzymes[edit]



In a) the allosteric enzyme functions normally. In b), it is inhibited

This type of enzymes presents two binding sites: the substrate of the enzyme and the effectors. Effectors are small molecules which modulate the enzyme activity; they function through reversible, non-covalent binding of a regulatory metabolite in the allosteric site (which is not the active site). When bound, these metabolites do not participate in catalysis directly, but they are still essential: they lead to conformational changes in a concrete part of the enzyme. These changes affect the overall conformation of the active site, causing modifications on the activity of the reaction.[1]

Properties

Allosteric enzymes are generally larger in mass than other enzymes. Different from having a single subunit enzyme, in this case they are composed of multiple subunits, which contain active sites and regulatory molecule binding sites.

They present a special kinetics: the cooperation. In here, configuration changes in each chain of the protein strengthen changes in the other chains. These changes occur at the tertiary and quaternary levels of organisation.

Based on modulation, they can be classified in two different groups:

- *Homotropic allosteric enzymes*: substrate and effector play a part in the modulation of the enzyme, which affects the enzyme catalytic activity.
- *Heterotropic allosteric enzymes*: only the effector performs the role of modulation.

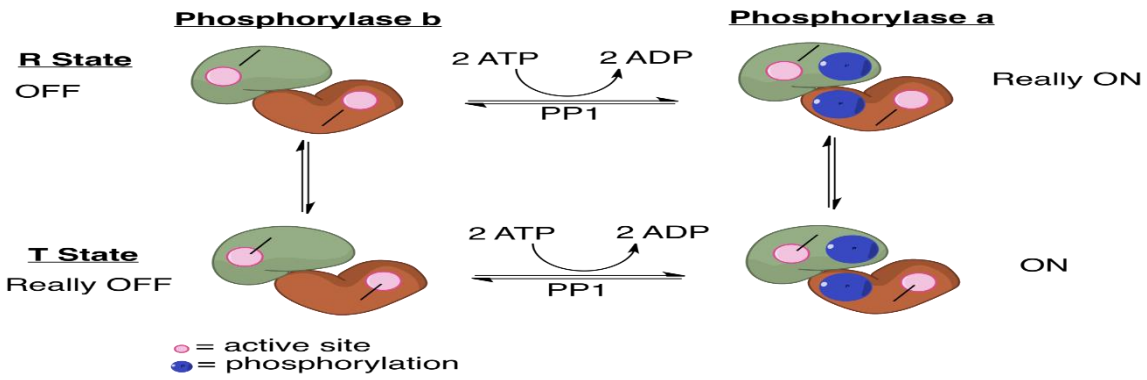
2) FEEDBACK INHIBITION

- In some multienzyme systems, the enzyme is inhibited by the end product whenever its concentration is above the requirements of the cell. So, the velocity of the reaction can be controlled by the amount of product that is needed by the cell (the lower the requirement is, the slower the reaction goes).
- Feedback inhibition is one of the most important function of proteins. Due to feedback inhibition, a cell is able to know whether the amount of a product is enough for its subsistence or there is a lack of the product (or there is too much product).

- The cell is able to react to this kind of situation in a mechanical way and solve the problem of the amount of a product. An example of feedback inhibition in human cells is the protein aconitase (an enzyme that catalyses the isomeration of citrate to isocitrate).
- When the cell needs iron, this enzyme loses the iron molecule and its form changes. When this happens, the aconitase is converted to IRPF1, a translation repressor or mRNA stabilizer that represses the formation of iron-binding proteins and favours formation of proteins that can get iron from the cell's reservations.

3) **COVALENT MODIFICATION**

- Here, the active and inactive form of the enzymes are altered due to covalent modification of their structures which is catalysed by other enzymes.
- This type of regulation consists of the addition or elimination of some molecules which can be attached to the enzyme protein. The most important groups that work as modifiers are phosphate, methyl, uridine, adenine and adenosine diphosphate ribosyl.
- These groups are joined to or eliminated from the protein by other enzymes. The most remarkable covalent modification is phosphorylation. Serine, Threonine and Tyrosine are common amino acids that participate in covalent modifications and are used to control enzyme's catalytic activities. Kinase and phosphatases are commonly known enzymes that affect these modifications, which result in shifting of conformational states of the binding affinity to substrate.
- Phosphorylation is the addition of phosphate groups to proteins, which is the most frequent regulatory modification mechanism in our cells. This process takes place in prokaryotic and eukaryotic cells (in this type of cells, a third or a half of the proteins experience phosphorylation). Because of its frequency, phosphorylation has a lot of importance in regulatory pathways in cells.
- The addition of a phosphoryl group to an enzyme is catalysed by kinase enzymes, while the elimination of this group is catalysed by phosphatase enzymes. The frequency of phosphorylation as a regulatory mechanism is due to the ease of changing from phosphorylated form to dephosphorylated form.
- Phosphorylation or dephosphorylation make the enzyme be functional at the time when the cell needs the reaction to happen. The effects produced by the addition of phosphoryl groups that regulate the kinetics of a reaction can be divided in two groups:



- Phosphorylation changes the conformation of an enzyme to a more active or inactive way (e.g. regulation of glycogen phosphorylase). Each phosphate group contains two negative charges, so the addition of this group can cause an important change in the conformation of the enzyme. The phosphate can attract positively charged amino acids or create repulsive interactions with negatively charged amino acids. These interactions can change the conformation and the function of the enzyme. When a phosphatase enzyme removes the phosphate groups, this enzyme returns to its initial conformation.
- Phosphorylation modifies the affinity of the enzyme to the substrate (e.g. phosphorylation of isocitrate dehydrogenase creates electrostatic repulsion which inhibits the union of the substrate to the active center). Phosphorylation can take place in the active center of the enzyme. It can change the conformation of this active center, so it can recognize the substrate or not. Also, the ionized phosphate can attract some parts of the substrate, which can join to the enzyme.
- Phosphorylation and dephosphorylation may take place as a result of the response to signals that warn about a change in the cell state. This means that some pathways where regulatory enzymes participate are regulated by phosphorylation after a specific signal: a change in the cell.
- Some enzymes can be phosphorylated in multiple sites. The presence of a phosphoryl group in a part of a protein may depend on the folding of the enzyme (which can make the protein more or less accessible to kinase proteins) and the proximity of other phosphoryl groups.

4) PRECURSOR ACTIVATION

- Many times, the precursor or the first metabolite of the pathway function as the effector molecule and regulates the enzymatic pathway. The concentration of the precursor or the first metabolite activates or stimulates the last or the later enzyme of the pathway, thus increasing the reaction rate. This is called precursor activation.
- To study such mechanisms either the cell free enzymes or mutant bacteria with specific enzyme defect can be used. Apart from these

mechanisms there are certain general mechanisms common to almost all the living cell for the regulation of enzyme activity. These mechanisms do not need the participation of any effector molecule.

5) **The cell membrane**

- The cell membrane controls the movement of many hydrophilic and hydrophobic molecules. It has specific mechanisms for the transport of specific molecules which generally requires energy, such systems can therefore be controlled by the availability of ATP.

6) **Substrate concentration**

- The concentration of substrate positively affects the rate of enzymatic reaction to a certain concentration of the substrate. After that increase in substance concentration does not have any stimulation effect on the activity of enzymes as all the available enzyme molecules would be occupies by the substrate molecules. Secondly the accumulated product would also exert feedback inhibition on enzyme activity, decreasing the reaction rate.

7) **Coenzyme and cofactors**

- Concentration of coenzymes and cofectors also exerts regulation on the activity of enzymes. Their absence or less quantity stops or slows the enzymatic reaction rate.

8) **Action of proteolytic enzymes**

- . in microbial cells, highley specific protein degrading enzymes are present which breackdown enzymes which are no longer required by the cell for any metabolic reactions, thus eliminating unwanted use of substrate and production of product

9) **Compartmentalization**

- Compartmentalization within cell is also a mechanisms of regulating enzyme activity. In a cell. Enzyme may be bound to many internal structure like membranes and molecules. This prevents the enzyme and its substrate to come in direct cobntact. This limites access of substrate to its enzyme is more common in eukaryotic cells. Substrate are generally present at a separate location within a cell in membrane bound organelles.

Sr.no.	Question	Answer
1	What are regulatory enzymes?	The enzymes which catalyse chemical reactions again and again are called regulatory

		enzymes.
2	Allostric enzymes have ..... binding site	Two
3	Give name of two group which act as a modifier	Uridine and adenine
4	The concentration of substrate ..... affect on the rate of reaction	Positively
5	..... absence or less quantity affect the enzymatic reaction	Coenzymes and cofectors

MECHANISMS AND REGULATION OF ENZYME SYNTHESIS

When the product of an enzymatic reaction is no longer required by the cell, the cell will exercise a mechanism by which it will stop the synthesis of that particular enzyme for the time being. This is referred to as regulation of enzyme ynthesis. The regulation operates at gene expression level.

[1] Induction and Repression of Enzyme Synthesis:

As mentioned earlier, enzymes can be divided into two groups namely,

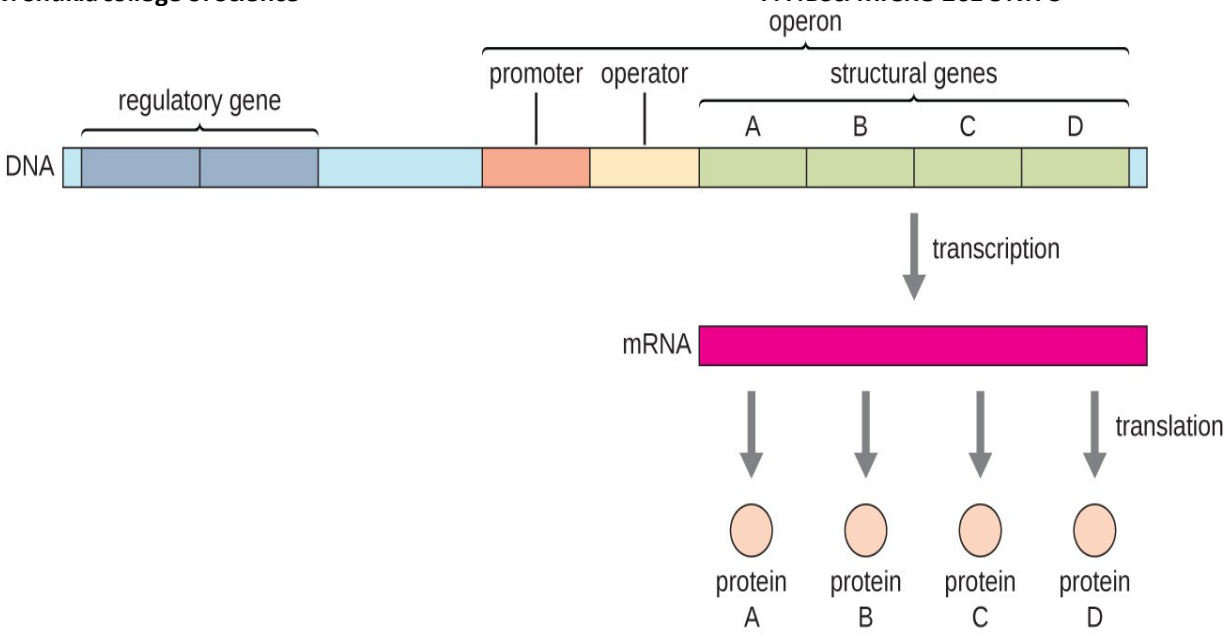
(a) **Constitutive enzymes**: Enzymes which are always present in the cell. irrespective of the presence or absence of their substrate. However, their concentration increases in presence of their substrate. Ex: enzymes of Glycolysis

(b) **Inducible enzyme**: Enzymes which are produced only in the presence of their substrate, i.e., only when required. This process is called enzyme induction and the substrate or the compounds similar in structure to the substance responsible for induction are called inducers. Ex: enzymes B-galactosidase. Its inducer is sugar Lactose.

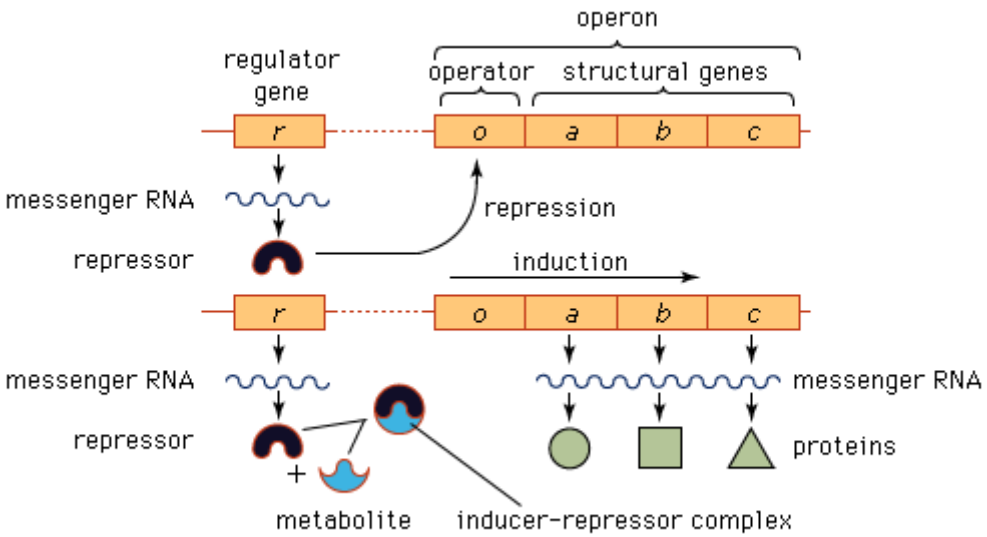
The process of induction occurs when an inducer or the effector molecule , which is either the substrate or its analogue structurally similar compound) . required for the synthesis of enzyme . When the production of enzyme is to be ceased or stopped , an effector molecule called repressor (regulatory protein comes in to nction and the process is called Repression . Effector molecules like the product of the reaction or related compounds function synthesis . as Co - repressor , which binds to the represpor and help in prevention of enzyme.

Mechanism of Regulation of Enzyme Synthesis : -

- Enzymes , being proteins , are under the direct control of the DNA or the specific genes .
- The genes responsible for the synthesis of a particular protein or enzyme are first transcribed into messenger RNA (TRNA) which the gets translated in the sequence of amino acids which forms the protein . There are set of genes responsible for the synthesis of a particular protein or enzyme .
- These genes can be broadly grouped as the operator and the structural genes .
- Operator genes (a type of Regulator genes) regulate the activity of the structural genes which are mainly responsible for the synthesis of mRNA by the process of transcription and determining the sequence amino acid in a protein - The repressor or the regulatory protein combines with the co - repressor the product or the related compound) to form as active compound which binds to the operator and block it (negative control) .
- This prevents the operator to give signal to the structural genes to produce required mRNA and hence no protein would be synthesized . When the amount of product or the co - repress is less in the cell , indicating the need for more production of that product , the repressor and co - repressor separates , and the repressor binds to the substrate or its analogue (inducer molecules) .
- This causes a conformational change is the repressor , making it unable to bind to the operator . (inactive repressor) Inactivated repressor cannot bind to the operator , hence causing de repression . This sets the operator free and capable of signaling the structural genes to transcribe mRNA .
- This mRNA will be translated into proteins thus producing the required enzyme . (positive regulation) . Francois Jacob and Jecques Monod named this group of consecutive genes which forms as operational unit as Operon Operon Promoter Operator Structural genes Regulatory gene 3 Template 3 General structure of an Operon
- .
- **STRUCTURE OF OPERON**



- An operon is made up of 4 basic DNA components : Promoter : A nucleotide sequence that enables a gene to be transcribed . Th promoter is recognized by RNA polymerase , which then initiate transcription .
- In RNA synthesis , promoters indicate which genes should used for messenger RNA creation - and , by extension , control which protein the cell produces.
- **Promotor:** These genes control the operator gene in cooperation with certain compounds called inducers and co - repressors present in the cytoplasm . A regulator gene is not necessarily adjacent to the operator gene it controls . The regulator gene codes for and produces a protein substance called repressor . The repressor substance combines with the operator gene to repress its action . A regulator gene controls an operon , but is not the part of the operon .
- **Operator** : A segment of DNA that a repressor binds to . It is classically defined in the lac operon as a segment between the promoter and the genes of the operon . In the case of a repressor , the repressor protein physically obstructs the RNA polymerase from transcribing the genes .
- **Structural genes** : The genes that are co - regulated by the operon . Not always included within the operon , but important in its function is a regulatory gene , a constantly expressed gene which codes for repressor proteins . The regulatory gene does not need to be in , adjacent to , or even near the operon . Genetic control of the rate of enzyme synthnie is directed by the regulator genes . Originally , operons were thought to exist solely in prokaryotes , but since the discovery of the first operons in eukaryotes in the early 1990s , more evidence has arisen to suggest they are more common than previously assumed . Operons are also found in viruses such as bacteriophages T7 .



- As can be seen from the above figure , regulator gener transcribes messenger RNA and synthesis a repressor protein which binds to the operator . This (3) C : prevents the attachment of RNA polymerase to promoter thus preventing the Re synthesis of mRNA from structural genes . But when inducer is present (substrate or its analogue) , the repressor binds to it and undergo a AE conformational change . This inducer - repressor complex cannot bind to the operator and hence RNA polymerase can attach to promoter and can transcribe structural genes and т protein synthesis can take place . A classic example of Enzyme regulation at synthesis level is lae operon which is responsible for the synthesis of the enzyme B - galactosidase , responsible for the metabolism of the sugar lactose E by the cell.
- **End - Product Repression** : In a biosynthetic pathway , the overall regulation is modulated by the concentration of the end product of the pathway . It has been observed many bacteria that addition of a particular metabolite (end product la amino acid) in the culture media inhibits the synthesis of enzyme responsib for the biosynthetic pathway of that particular amino acid . This process called end - product repression or feedback repression . When intracellular levels of the end product falls , derepression occurs and enzyme is again synthesized . The feedback inhibition of the enzyme active and the feedback repression of the enzyme synthesis are the coordinated a complementary and brings about a very efficient regulation of biosynthet pathways

- **Catabolite Repression** :
- Regulation of enzyme synthesis by induction (operon) and end product repression are dependent on the presence or absence of a particular metabolite or a closely related group of metaboliten called effector molecules .

- Apart from these two mechanisms , there are certain more general control mechanism .
- mechanisms which affect many operons . Catabolite Repression in one such These controls allow the cells to grow utilizing the most preferred substrate (the one which supports the most rapid growth) in presence of several substrates .
- Glucose Effect is an example of this mechanism . When cells of E.coli are grown in a medium containing both glucose and lactose , the organisms preferentially use glucose .
- Lactose is not metabolized until all the glucose is used up . The enzyme required for the catabolism of lactose (B - galactosidase) is not synthesized although the inducer or the substrate is present in the medium .
- This type of regulation is called Catabolite repression as the catabolism of one substrate inhibits the catabolism or utilization of the other substrate . This regulation mechanism is not restricted to the enzymes for utilization of carbon source only , it is equally applicable to the regulation of enzymes for utilization of nitrogen containing metabolites .
- This brings forth a fact that cells do not spend energy on the synthesis of enzymes of less efficient pathways if substrates which are easily utilizable and are used for more efficient pathways exists.

Sr.no.	Question	Answer
1	The regulation operates at.....	Gene expression level
2	What are constitutive enzymes?	Enzymes which are always present in cell
3	Give example of constitutive enzymes	Enzymes of glycolysis
4	..... induced by sugar lactose	B- galactosidase
5	What is operator?	A segment of DNA that repressor binds to

DIFFERENCES BETWEEN PROKARYOTIC AND EUKARYOTIC ENZYME REGULATION There are some basic differences in the enzyme regulation in Prokaryotes and The eukaryotes :

- The difference in the basal levels of enzymes to its induced levels (concentration of enzyme before the induction and after the induction) in Eukaryotes is less than that in prokaryotes . In Prokaryotes , the level rises to about 100 fold more while in eukaryotes , it rises to about 10 folds .
- In eukaryotic cells , the significant clustering of the genes into an operon is not observed, the structural genes for aa biosynthetic pathway are observed to be scattered over many chromosome and are not linked to each other.

Sr.no.	Question	Answer
1	Induced level in prokaryotes is about.....	100 folds
2	Induced level of enzymes rises about..... level in eukaryotes	10 folds
3	What are structure genes?	The genes that are co-regulated by operon
4	Regulator genes synthesis ..... protein	Repressor
5	..... responsible for inhibiting enzymes synthesis in biochemical pathway	End product