UNIT- 1. ENZYMES

Introduction

Enzymes are nature's sustainable catalysts. They are biocompatible, biodegradable and are derived from renewable resources. Enzymes constitute a large biological globular protein molecule responsible for thousands of metabolic processes that sustain life , and function as catalysts to facilitate specific chemical reactions within the cell. These reactions are essential for the life of the organism. The living cell is the site of tremendous biochemical activity called metabolism. This is the process of chemical and physical changes which go on continually in the living organism; enzyme facilitate life processes in essentially all life-forms from viruses to man.

Enzymes have been naturally tailored to perform under different physiological conditions. Build-up of new tissues , replacement of old tissues , conversion of food into energy disposal of toxic materials , reproduction almost all the activities that can be characterized as "life." Enzymes act as life catalysts, substances that accelerate the rate of a chemical reaction. By reducing the activation energy (*E*a) necessary to initiate the reaction, thus dramatically increasing the rate of reaction. Enzymes do not initiate reactions that would not naturally occur but they accelerate any reaction that is already underway. Enzymes enable the reaction to take place more rapidly at a safer, relatively low temperature that is consistent with living systems. During an enzyme-mediated reaction, the substrate physically attaches to the enzyme at its active site, allowing the substrate(s) to be converted to new product molecule(s). Most enzyme reaction rates are millions of times faster than those of comparable uncatalyzed reactions. Enzymes are neither consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions. Enzymes are known to catalyze about 4,000 biochemical reactions.

History of evolution of enzymes

The existence of enzymes has been known for well over a century**.** Biological catalysis was first recognized and described in the early 1800s, in studies of the digestion of meat by secretions of the stomach and the conversion of starch into sugar by saliva and various plant extracts. In 1835, Swedish chemist Jon Jakob Berzelius termed their chemical action as catalytic in nature. In 1860 Louis Pasteur recognized that enzymes were essential to

fermentation but assumed that their catalytic action was inextricably linked with the structure and life of the yeast cell. Not until 1897 was it shown by German chemist Edward Buchner that cell-free extracts of yeast could ferment sugars to alcohol and carbon dioxide, Buchner denoted his preparation as *zymase.* The term enzyme comes from *zymosis,* the Greek word for fermentation, a process accomplished by yeast cells and long known to the brewing industry . In 1876, William Kuhne proposed that the name 'enzyme' be used as the new term to denote phenomena previously known as 'unorganised ferments', that is, ferments isolated from the viable organisms in which they were formed. The word itself means 'in yeast' and is derived from the Greek 'en' meaning 'in', and 'zyme' meaning 'yeast' or 'leaven'. This important achievement was the first indication that enzymes could function independently of the cell. It was not until 1926, however, that the first enzyme was obtained in pure form, a feat accomplished by American biochemist James B. Sumner of Cornell University. Sumner was able to isolate and crystallize the enzyme urease from the jack bean. His work was to earn him the 1947 Nobel Prize. John H. Northrop and Wendell M. Stanley of the Rockefeller Institute for Medical Research shared the 1947 Nobel Prize with Sumner They discovered a complex procedure for isolating pepsin. This precipitation technique devised by Northrop and Stanley has been used to crystallize several enzymes.

Proteinaceous nature of enzymes

Enzymes are proteins and are nature’s own biocatalyst and their function is determined by their complex structure. With the exception of a small group of catalytic RNA molecules, all enzymes are proteins which are made up of amino acids linked together by peptide bonds. By the early 1800s, the proteinaceous nature of enzymes had been recognized. Knowledge of the chemistry of proteins drew heavily on improving techniques and concepts of organic chemistry in the second half of the 1800s; it culminated in the peptide theory of protein structure, usually credited to Fischer and Hofmeister. However, methods that had permitted the separation and synthesis of small peptides were unequal to the task of purifying enzymes. Indeed, there was no consensus that enzymes were proteins. After isolation of a series of crystalline proteolytic enzymes beginning with pepsin by Northrop et al., in 1930, the proteinaceous nature of enzymes was established. They are high molecular weight compounds made up principally of chains of amino acids linked together by peptide bonds. Their catalytic activity depends on the integrity of their native protein conformation. If an enzyme is denatured or dissociated into its subunits, catalytic activity is usually lost. Thus the

primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity .

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1.4.Structure of enzymes

Enzymes are proteins and, are agreeable to structural analysis by the methods of protein chemistry, molecular biology, and molecular biophysics. Like all proteins, enzymes are composed mainly of the 20 naturally occurring amino acids. The structures of enzymes can be elucidating by the physical methods such as Spectroscopic methods , x-ray crystallography , and more recently, multidimensional NMR methods . On the basis of arrangement of amino acids enzyme structure can be classified into following types,

(i). Primary structure

The structure and reactivity of a protein are defined by the identity of the amino acids that make up its polypeptide chain, this amino acid sequence of the peptide chains is the primary structure of the enzyme.

(ii) Secondary structure

Secondary structure is due to the interaction of amino acids with each other in the same chain of protein. As a result the protein chain can fold up on itself in two ways, namely α-helix or β-sheet resulting secondary structures.

(iii). Tertiary structure

The arrangement of secondary structure elements and amino acid side chain interactions that define the three-dimensional structure of the folded protein. So that specific contacts are made between amino acid side chains and between backbone groups. The resulting folded structure of the protein is referred to as its tertiary structure.

(iv) Quaternary structure and domains

Many enzymes consist of more than one polypeptide chain (or subunit) that aggregate to confer catalytic activity. In some enzymes the subunits are identical, in others they differ in sequence and structure. This description of subunit arrangement in such enzymes is called the quaternary structure. A typical enzyme is not an entity completely folded as a whole, but may consist of apparently autonomous or semiautonomous folding units called domains.

Chemical nature of enzyme

Enzymes are proteins that are produced by all living organisms. They are high molecular weight compounds made up principally of chains of amino acids linked together by peptide

bonds. They have molecular weights ranging from 10,000 to 2,000,000. The important terminologies related to enzymes are,

Cofactor: A non-protein chemical component required for proteins biological activity are called co-factor.

Apoenzyme: The protein part of an active enzyme is called apoenzyme.

Holoenzyme: the active enzyme composed of Apoenzyme and a co-factor is termed as holoenzyme.

Coenzyme: coenzyme is a non –protein compound or substance that is necessary for an enzyme to initiate the function of the enzyme.

Prosthetic group: A coenzyme or metal ion that is very tightly or even covalently bound to the protein component of the enzyme is called a prosthetic group.

1.5. Specificity of enzymes

Enzymes are the most remarkable and highly specialized proteins, they have a high degree of specificity for their substrates, and they accelerate chemical reactions tremendously. In general, four types of behavior can be described:

1. Absolute specificity - Catalyze only one reaction.

2. Group specificity – catalyses a particular type of functional group, which can occur in a variety of substrate.

3. Linkage specificity - Catalyses a particular type of chemical bond regardless of the rest of the molecular structure.

4. Stereochemical specificity - the enzyme will act on a particular steric or optical isomer.

Naming and enzyme classification

In general many enzymes have been named by adding the suffix "-ase" to thebname of their substrate or to a word or phrase describing their activity. In 1961,baccording to the report of the first Enzyme Commission (EC) of International Union of Pure and Applied Chemistry (IUPAC), Enzymes are classified in to six types on the basis of reaction they catalyze . They were assigned code numbers, prefixed by E.C., which contain four elements separated by points and have the following meaning as shown in **Fig 1.1.**

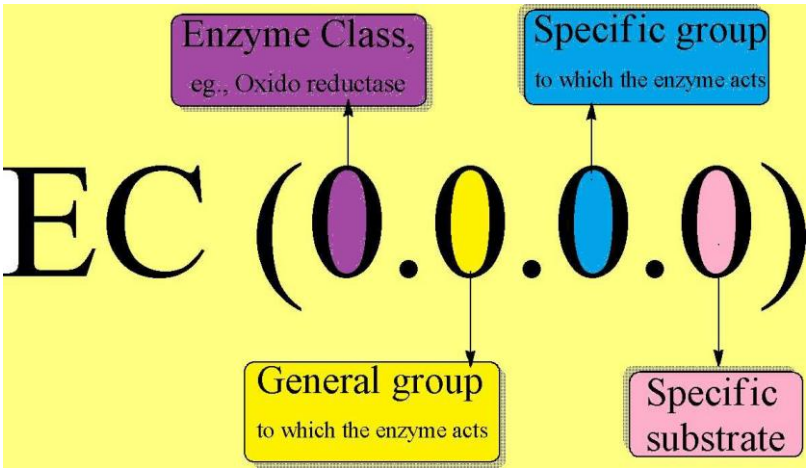

Fig 1.1. Naming of enzyme according to 'International Union of Biochemistry' formed a 'Commission on Enzyme Nomenclature.

The enzyme can be classified in to six main classes as shown in the **Fig 1.2**

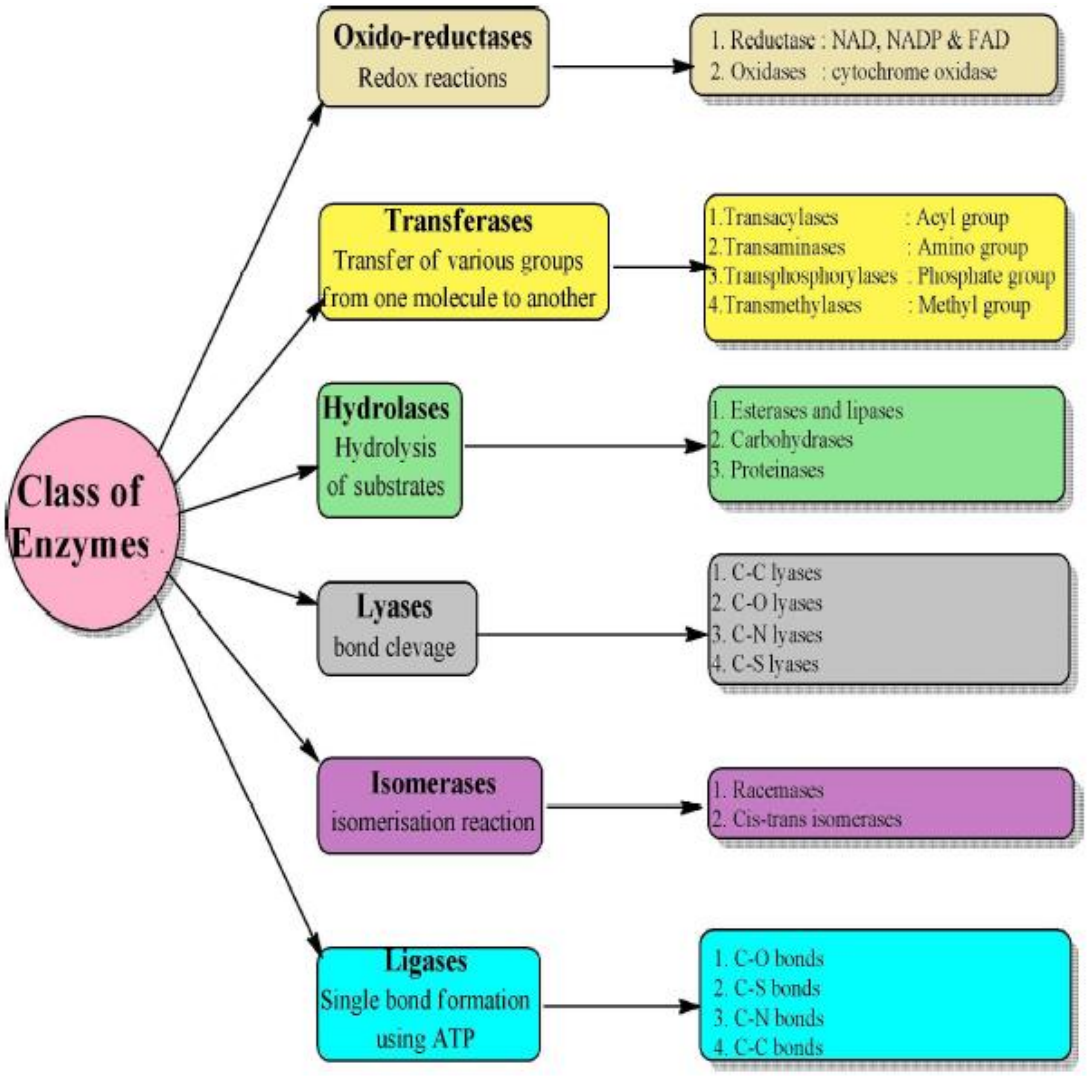

Fig 1.2: Classification of enzyme and examples of related enzyme classes

EC 1, Oxidoreductases: catalyze oxidation/reduction reactions

EC 2, Transferases: transfer a functional group (*e.g.* a methyl or phosphate group)

EC 3, Hydrolases: catalyze the hydrolysis of various bonds

EC 4, Lyases: cleave various bonds by means other than hydrolysis and oxidation

EC 5, Isomerases: catalyze isomerization changes within a single molecule

EC 6, Ligases: join two molecules with covalent bonds.

These sections are subdivided by other features such as the substrate, products, and chemical mechanism. An enzyme is fully specified by four

numerical designations. For example, hexokinase (EC 2.7.1.1) is a transferase (EC 2) that adds a phosphate group (EC 2.7) to a hexose sugar, a molecule

containing an alcohol group (EC 2.7.1).[

Lock and key model

In "lock and key" model the active site of the enzyme is complementary in shape to that of the substrate. The substrate is held in such a way that its conversion to the reaction products is more favourable. It was thought that the substrate exactly fitted into the active site of the enzyme molecule like a key fitting into a lock. In the Figure 1.3 "lock" refers to enzyme and "key" refers to its complementary substrate

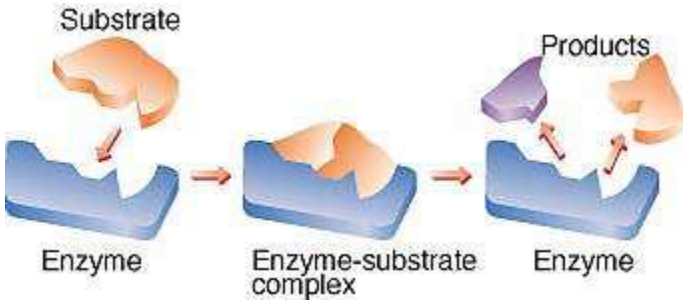

Figure. 1.3 Lock and key model for enzyme – substarte

(i) Induced fit theory

Lock and key model does not explain the stability of the transition state for it would require more energy to reach the transition state complex. To explain this concept Koshland in 1958,

first proposed the induced-fit model, this suggests that the enzyme active site is conformationally fluid. Enzyme itself usually undergoes a change in conformation when the substrate binds, induced by multiple weak interactions and hydrophobic characteristics on the enzyme surface mold into a precise formation

(ii)Transition state theory

According to this theory when an enzyme catalysis, the enzyme binds more strongly to its *"transition state complex rather than its ground state reactants."* This indicates, the transition state is more stable . A simple enzymatic reaction can be written as,

$$E + S \leftrightarrow ES \leftrightarrow EP \leftrightarrow E + P$$

Where E, S, and P represent the enzyme, substrate, and product respectively; ES and EP are transient complexes of the enzyme with the substrate and with the product respectively.

The transition state is not a chemical species with any significant stability and should not be confused with a reaction intermediate (such as ES or EP). It is simply a fleeting molecular moment in which events such as bond breakage, bond formation, and charge development have proceeded to the precise point at which decay to either substrate or product is equally likely. The difference between the energy levels of the ground state and the transition state is the activation energy; the rate of a reaction reflects this activation energy: higher activation energy corresponds to slower reaction. Reaction rates can be increased by raising the temperature, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier. Alternatively, the activation energy can be lowered by adding a catalyst.

Michaelis–Menten equation (Km)

The Michaelis-Menten equation is the elementary equation of enzyme kinetics, even though it is originally derived for the simplest case of an irreversible enzyme reaction. A relationship between the value of Michaelis – Menten constant **(**Km) for an enzyme and the physiological concentration of substrate was mentioned over two decades ago. Cleland stated a general rule that enzyme will function with reactant concentrations in the region of their apparent Michaelis constants or above, when at the pathway they are a part of is operating at full capacity; otherwise the catalytic potential of the enzyme is wasted. Apart from this Fersht explained that the tight binding of substrate implies a stable, and therefore unreactive

substrate. He argued that should be high, even as high as 10 times the concentration of substrate under physiological conditions.

The Michaelis –Menten constants **(**Km) can be evaluated by four different methods namely,

i. Plot of V_0 *versus* $[S]_0$ wherein the constants are determined from a graph of initial rate *versus* initial substrate concentration [45].

ii. Eadie - Hofstee transformation: It is the plot of initial rate (V_0) *versus* ratio of initial rate to initial substrate concentration $\frac{V_0}{[S]_0}$ which will give a straight line with an intercept of V_{max} and slope of m[46, 47].

iii. Lineweaver-Burke plot: it is also called a double reciprocal plot. It is obtained by plotting $\frac{1}{V}$ *versus* $\frac{1}{[S]}$, where V is the rate of the reaction, [S] is the concentration of the substrate.[48].

iv. Hanes-Woolf plot is constructed by plotting the ratio of product of substrate and co-substrate concentration to initial rate of reaction *versus* substrate or co-substrate concentration [49].

$$\text{i.e} \quad \left[\frac{H_0 D_0}{V_0}\right] \text{ v/s } H_0 \text{ or } D_0$$

Where, D_0 and H_0 are initial concentrations of any phenol or other aromatic co-substrates and substrate (H_2O_2).

Significances of K_m : When

$[S] = K_m$, then

$V = \frac{V_{max}}{2}$.

Hence is equal to the substrate concentration at which the reaction rate is half its maximum value. In other words, if an enzyme has small value of **(**Km), it achieves its maximum catalytic efficiency at low substrate concentrations. Hence, the smaller the value of Km the more efficient is the catalyst. The value of Km for an enzyme depends on the particular substrate. It also depends on the pH of the solution and the temperature at which the reaction is carried out. For most enzymes Km lies between 10-1 and 10-7 M.

Substrate concentration

The rate of an enzyme reaction depends on the substrate concentration. Coenzymes behave as substrates from the point of view of reaction kinetics. With increasing coenzyme concentration, the rate of the reaction becomes greater until it reaches the limiting value . If

the optimum reaction rate *V* of an enzyme reaction is to be attained, the Michaelis constant K*m* must be negligibly small in comparison with the concentration of the substrate. In ideal condition according to Michaelis – Menten equation,

$$v = \frac{V[S]}{K_m + [S]},$$

If $[S] >> K_m$ then

$v = V$, is probably rare, since practical factors often prevent it.

Enzyme Inhibitors

Enzyme reaction rates can be decreased by various types of enzyme inhibitors.

(i)Competitive inhibitor

A competitive inhibitor and substrate cannot bind to the enzyme at the same time. Often competitive inhibitors strongly resemble the real substrate of the enzyme. For example, the drug methotrexate is a competitive inhibitor of the enzyme dihydrofolate reductase, which catalyzes the reduction of dihydrofolate to tetrahydrofolate.. The similarity between the structures of dihydrofolate and this drug are shown in the accompanying figure. This type of inhibition can be overcome with high substrate concentration. In some cases, the inhibitor can bind to a site other than the binding-site of the usual substrate and exert an allosteric effect to change the shape of the usual binding-site.

(ii) Non-competitive inhibitor

A non-competitive inhibitor binds to a site other than where the substrate binds. The substrate still binds with its usual affinity and hence Km remains the same. However the inhibitor reduces the catalytic efficiency of the enzyme so that Vmax is reduced. In contrast to competitive inhibition, noncompetitive inhibition cannot be overcome with high substrate concentration.

(iii) Uncompetitive inhibitor

An uncompetitive inhibitor cannot bind to the free enzyme, only to the enzyme-substrate complex; hence, these types of inhibitors are most effective at high substrate concentration. In the presence of the inhibitor, the enzyme-substrate complex is inactiveThis type of inhibition is rare.

(iv) Mixed inhibitor

A mixed inhibitor binds to an allosteric site and the binding of the substrate and the inhibitor affect each other. The enzyme's function is reduced but not eliminated when bound to the inhibitor. This type of inhibitor does not follow the Michaelis–Menten equation.

Isoenzymes

Isozymes (also known as **isoenzymes** or more generally as **multiple forms of enzymes**) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters (e.g. different *K*M values), or different regulatory properties. The existence of isozymes permits the finetuning of metabolism to meet the particular needs of a given tissue or developmental stage. In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants) of enzymes. In many cases, they are coded for by homologous genes that have diverged over time. Although, strictly speaking, allozymes represent enzymes from different alleles of the same gene, and isozymes represent enzymes from different genes that process or catalyse the same reaction, the two words are usually used interchangeably.

Isozymes were first described by R. L. Hunter and Clement Markert (1957) who defined them as *different variants of the same enzyme having identical functions and present in the same individual*. This definition encompasses

(1) enzyme variants that are the product of different genes and thus represent different loci (described as *isozymes*) and

(2) enzymes Introduction that are the product of different alleles of the same gene (described as *allozymes*).

Isozymes are usually the result of gene duplication, but can also arise from polyploidisation or nucleic acid hybridization. Over evolutionary time, if the function of the new variant remains *identical* to the original, then it is likely that one or the other will be lost as mutations accumulate, resulting in a pseudogene. However, if the mutations do not immediately prevent the enzyme from functioning, but instead modify either its function, or its pattern of expression, then the two variants may both be favoured by natural selection and become specialised to different functions. For example, they may be expressed at different stages of development or in different tissues. Allozymes may result from point mutations or from insertion-deletion (indel) events that affect the coding sequence of the gene. As with any other new mutations, there are three things that may happen to a new allozyme: It is most likely that the new allele will be non-functional—in which case it will probably result in low fitness and be removed from the population by natural selection. Alternatively, if the amino acid residue that is changed is in a relatively unimportant part of the enzyme (e.g., a long way from the active site), then the mutation may be selectively neutral and subject to genetic drift. In rare cases, the mutation may result in an enzyme that is more efficient, or one that can catalyse a slightly different chemical reaction, in which case the mutation may cause an

increase in fitness, and be favoured by natural selection.[6] An example of an isozyme is glucokinase, a variant of hexokinase which is not inhibited by glucose 6- phosphate. Its different regulatory features and lower affinity for glucose
Examples
(compared to other hexokinases), allow it to serve different functions in cells of specific organs, such as control of insulin release by the beta cells of the pancreas, or initiation of glycogen synthesis by liver cells. Both these processes must only occur when glucose is abundant

1.) lactate dehydrogenase

The enzyme lactate dehydrogenase is a tetramer made of two different subunits, the H-form and the M-form. These combine in different combinations depending on the tissue

Type	Composition	Location	Electrophoretic Mobility	Whether destroyed by Heat (at 60° C)	Percentage of normal serum in humans
LDH_1	HHHH	Heart and Erythrocyte	Fastest	No	25%
LDH_2	HHHM	Heart and Erythrocyte	Faster	No	35%
LDH_3	HHMM	Brain and Kidney	Fast	Partially	27%
LDH_4	HMMM	Skeletal Muscle and Liver	Slow	Yes	8%
LDH_5	MMMM	Skeletal Muscle and Liver	Slowest	Yes	5%

2.) Isoenzymes of creatine phosphokinase :

Creatine kinase (CK) or creatine phosphokinase (CPK) catalyses the interconversion of phosphor creatine to creatine . CPK exists in 3 isoenzymes. Each isoenzymes is a dimer of 2 subunits M (muscle) , B (brain) or both

Isoenzyme	Subunit	Tissue of Origin
CPK_1	BB	Brain
CPK_2	MB	Heart
CPK_3	MM	Skeletal muscle

3.) Isoenzymes of alkaline phosphatase

: Six isoenzymes have been identified. The enzyme is a monomer, the isoenzymes are due to the differences in the carbohydrate content (sialic acid residues). The most important

ALP isoenzymes are α1- ALP , α2- heat labile ALP , α2- heat stable ALP , pre-β ALP and γ-ALP.Increase in α2- heat labile ALP suggests hepatitis whereas pre-β ALP indicates bone diseases.

Active Site

The **active site** is the region of an enzyme where substrate molecules bind and undergo a chemical reaction. The active site consists of amino acid residues that form temporary bonds with the substrate (binding site) and residues that catalyse a reaction of that substrate (catalytic site). Although the active site occupies only ~10–20% of the volume of an enzyme, it is the most important part as it directly catalyzes the chemical reaction. It usually consists of three to four amino acids, while other amino acids within the protein are required to maintain the tertiary structure of the enzyme. Each active site is evolved to be optimised to bind a particular substrate and catalyse a particular reaction, resulting in high specificity. This specificity is determined by the arrangement of amino acids within the active site and the structure of the substrates. Sometimes enzymes also need to bind with some cofactors to fulfil their function. The active site is usually a groove or pocket of the enzyme which can be located in a deep tunnel within the enzyme, or between the interfaces of multimeric enzymes. An active site can catalyse a reaction repeatedly as residues are not altered at the end of the reaction (they may change during the reaction, but are regenerated by the end). This process is achieved by lowering the activation energy of the reaction, so more substrates have enough energy to undergo reaction.

Binding site

Usually, an enzyme molecule has only two active sites, and the active sites fit with one specific type of substrate. An active site contains a binding site that binds the substrate and orients it for catalysis. The orientation of the substrate and the close proximity between it and the active site is so important that in some cases the enzyme can still function properly even though all other parts are mutated and lose function. Initially, the interaction between the active site and the substrate is noncovalent and transient. There are four important types of interaction that hold the substrate in a defined orientation and form an enzyme-substrate complex (ES complex): hydrogen bonds, vander Waals interactions, hydrophobic interactions and electrostatic force interactions

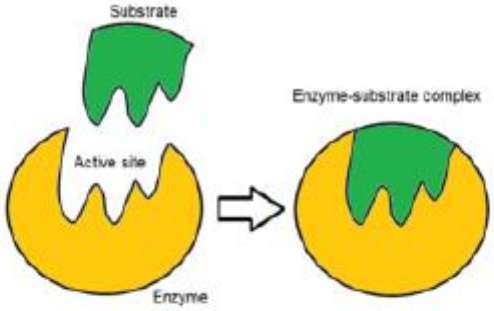

Diagram of the lock and key hypothesis

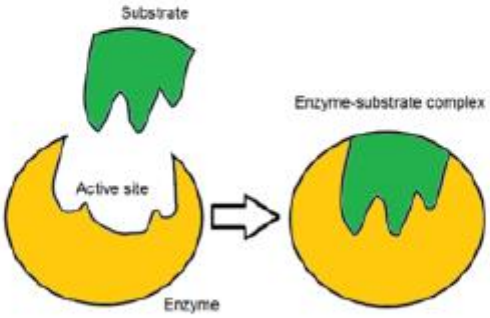

Diagram of the induced fit hypothesis

The charge distribution on the substrate and active site must be complementary, which means all positive and negative charges must be cancelled out. Otherwise, there will be a repulsive force pushing them apart. The active sit usually contains non-polar amino acids, although sometimes polar amino acids may also occur. The binding of substrate to the binding site requires at least three contact points in order to achieve stereo-, regio-, and enantioselectivity. For example, alcohol dehydrogenase which catalyses the transfer of a hydride ion from ethanol to NAD+ interacts with the substrate methyl group, hydroxyl group and the pro-*(R)* hydrogen that will be abstracted during the reaction.

In order to exert their function, enzymes need to assume their correct protein fold (*native fold*) and tertiary structure. To maintain this defined three-dimensional structure, proteins rely on various types of interactions between their amino acid residues. If these interactions are interfered with, for example by extreme pH values, high temperature or high ion concentrations, this will cause the enzyme to denature and lose its catalytic activity. A tighter fit between an active site and the substrate molecule is believed to increase the efficiency of a reaction. If the tightness between the active site of DNA polymerase and its

substrate is increased, the fidelity, which means the correct rate of DNA replication will also increase. Most enzymes have deeply buried active sites, which can be accessed by a substrate via access channels.

Reference:
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2.Shodhaganga websource
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