5TH SEM MAJOR

ENZYME,NATURE AND CLASSIFICATION,MECHANISM OF ENZYME ACTION

BY: DR. LUNA PHUKAN

Enzymes are the biological macromolecules which speed up the rate of biochemical reactions without undergoing any change. They are also called as biological catalysts.

An enzyme is a highly selective catalyst that greatly accelerates both the rate and specificity of metabolic reactions.

Properties of Enzymes

Nearly all enzymes are proteins, although a few catalytically active RNA molecules have been identified.

Enzyme catalyzed reactions usually take place under relatively mild conditions (temperatures well below 100oC, atmospheric pressure and neutral pH) as compared with the corresponding chemical reactions.

Enzymes are catalysts that increase the rate of a chemical reaction without being changed themselves in the process.

Enzymes are highly specific with respect to the substrates on which they act and the products that they form.

Enzyme activity can be regulated, varying in response to the concentration of substrates or other molecules.

They function under strict conditions of temperature and pH in the body.

Enzyme Structure

Enzymes are a linear chain of amino acids, which give rise to a three-dimensional structure. The sequence of amino acids specifies the structure, which in turn identifies the catalytic activity of the enzyme. Upon heating, enzyme's structure denatures, resulting in a loss of enzyme activity, that typically is associated with temperature.

Compared to its substrates, enzymes are typically large with varying sizes, ranging from 62 amino acid residues to an average of 2500 residues found in fatty acid synthase. Only a small section of the structure is involved in catalysis and are situated next to the binding sites. The catalytic site and binding site together constitute the enzyme's active site. A small number of ribozymes exist which serves as an RNA-based biological catalyst. It reacts in complex with proteins.

Classification of Enzymes

Oxidoreductases

Catalyze oxidation-reduction reactions where electrons are transferred.

These electrons are usually in the form of hydride ions or hydrogen atoms.

The most common name used is a dehydrogenase and sometimes reductase is used.

An oxidase is referred to when the oxygen atom is the acceptor.

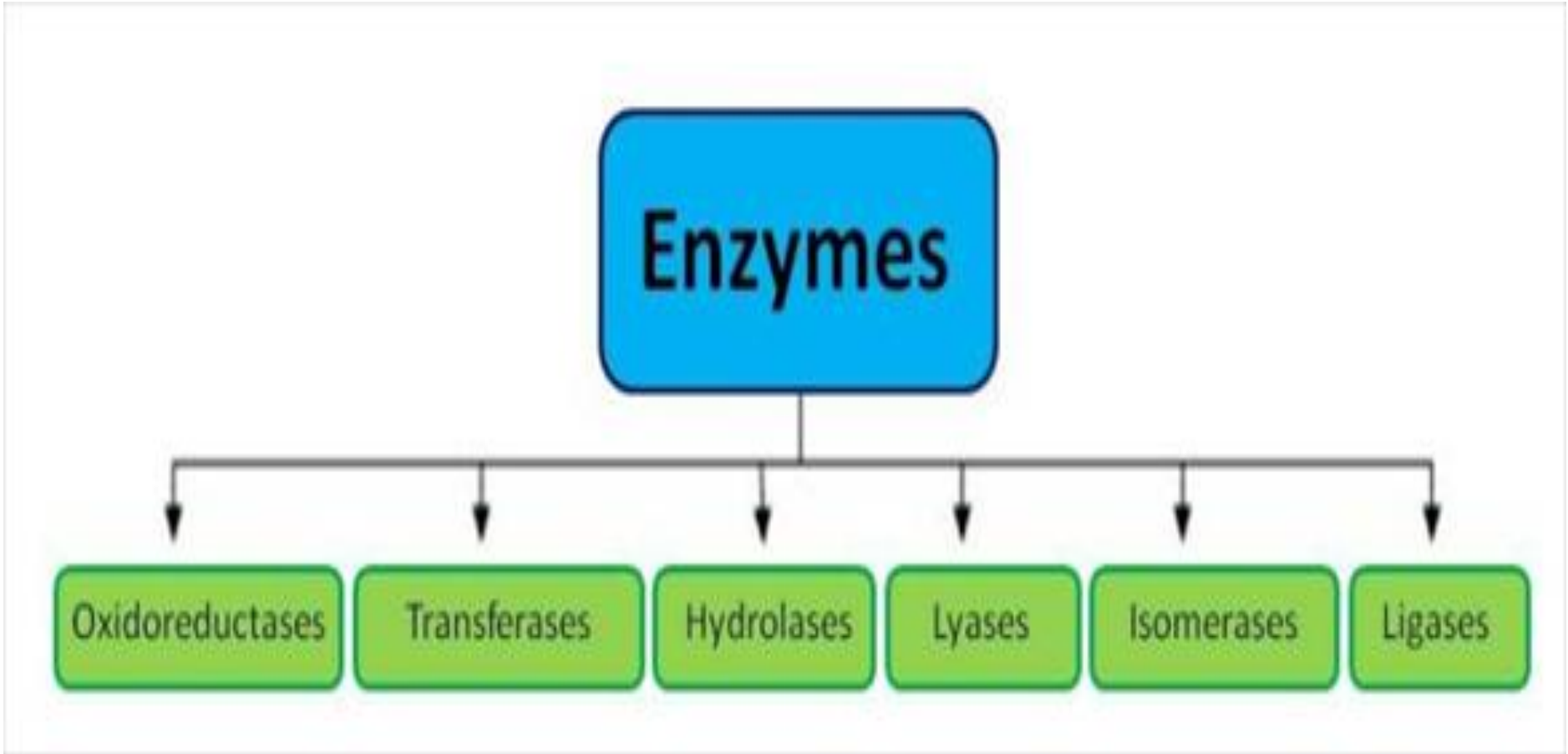
Transferases

Catalyze group transfer reactions.

The transfer occurs from one molecule that will be the donor to another molecule that will be the acceptor.

Most of the time, the donor is a cofactor that is charged with the group about to be transferred.

Example: Hexokinase used in glycolysis.


Enzymes
Oxidoreductases
Transferases
Hydrolases
Lyases
Isomerases
Ligases

According to the International Union of Biochemists (I U B), enzymes are divided into six functional classes and are classified based on the type of reaction in which they are used to catalyze. The six kinds of enzymes are hydrolases, oxidoreductases, lyases, transferases, ligases, and isomerases.

Listed below is the classification of enzymes discussed in detail:

Types Biochemical Property

Oxidoreductases: The enzyme Oxidoreductase catalyzes the oxidation reaction where the electrons tend to travel from one form of a molecule to the other.

Transferases: The Transferases enzymes help in the transportation of the functional group among acceptors and donor molecules.

Hydrolases: Hydrolases are hydrolytic enzymes, which catalyze the hydrolysis reaction by adding water to cleave the bond and hydrolyze it.

Lyases : Adds water, carbon dioxide or ammonia across double bonds or eliminate these to create double bonds.

Isomerases: The Isomerases enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule.

Ligases: The Ligases enzymes are known to charge the catalysis of a ligation process.

Hydrolases

Catalyze reactions that involve hydrolysis.

It usually involves the transfer of functional groups to water.

When the hydrolase acts on amide, glycosyl, peptide, ester, or other bonds, they not only catalyze the hydrolytic removal of a group from the substrate but also a transfer of the group to an acceptor compound

For example: Chymotrypsin.

Lyases

Catalyze reactions where functional groups are added to break double bonds in molecules or the reverse where double bonds are formed by the removal of functional groups.

For example: Fructose bisphosphate aldolase used in converting fructose 1,6-bisphospate to G3P and DHAP by cutting C-C bond.

Isomerases

They catalyze the formation of an isomer of a compound. Example: phosphoglucomutase catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate (phosphate group is transferred from one to another position in the same compound) in glycogenolysis (glycogen is converted to glucose for energy to be released quickly).

Ligases

Ligases catalyzes the association of two molecules. For example, DNA ligase catalyzes the joining of two fragments of DNA by forming a phosphodiester bond.

Cofactors

Cofactors are non-proteinous substances that associate with enzymes. A cofactor is essential for the functioning of an enzyme. An enzyme without a cofactor is called an apoenzyme. An enzyme and its cofactor together constitute the holoenzyme.

There are three kinds of cofactors present in enzymes:

Prosthetic groups: These are cofactors tightly bound to an enzyme at all times. A fad is a prosthetic group present in many enzymes.

Coenzyme: A coenzyme binds to an enzyme only during catalysis. At all other times, it is detached from the enzyme. NAD+ is a common coenzyme.

Metal ions: For the catalysis of certain enzymes, a metal ion is required at the active site to form coordinate bonds. Zn2+ is a metal ion cofactor used by a number of enzymes.

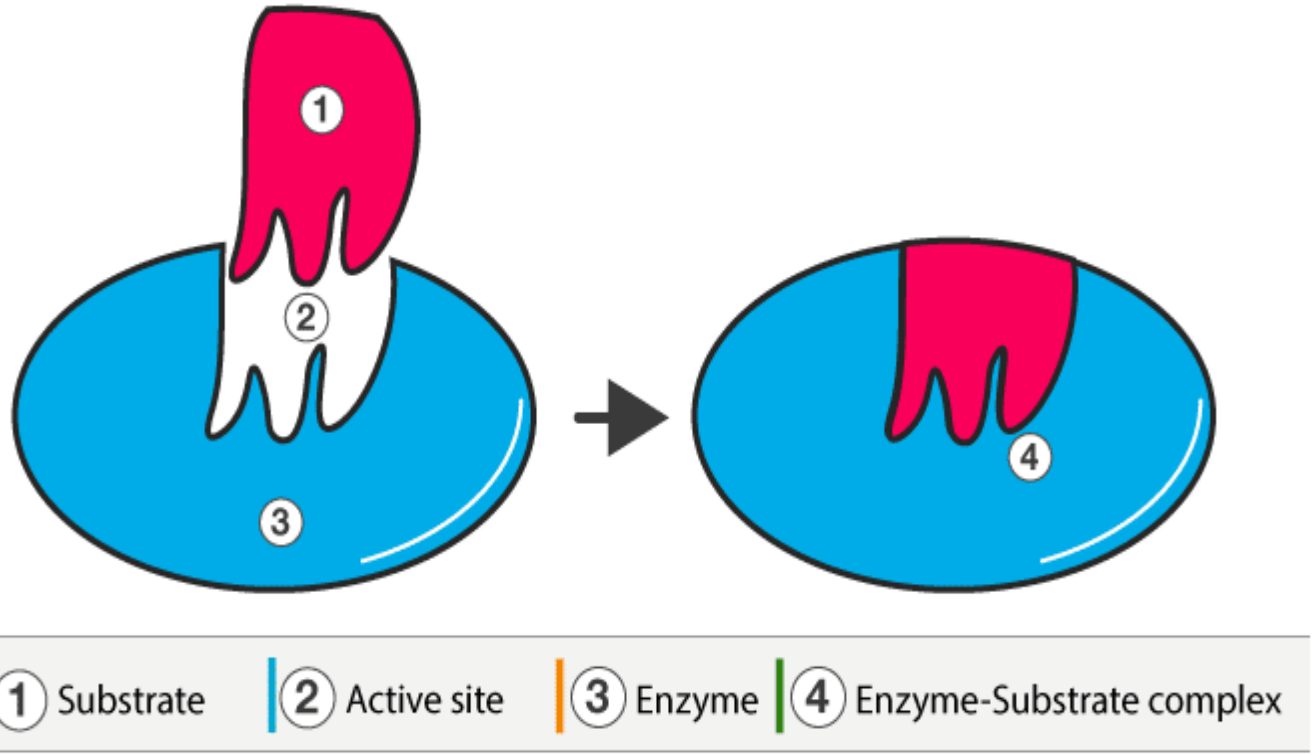
Mechanism of Enzyme Reaction

Any two molecules have to collide for the reaction to occur along with the right orientation and a sufficient amount of energy. The energy between these molecules needs to overcome the barrier in the reaction. This energy is called activation energy.

Enzymes are said to possess an active site. The active site is a part of the molecule that has a definite shape and the functional group for the binding of reactant molecules. The molecule that binds to the enzyme is referred to as the substrate group. The substrate and the enzyme form an intermediate reaction with low activation energy without any catalysts.

reactant(1)+reactant(2)→productr e a c t a n t (1) + e n zYme→intermedi a t eintermediate+reactant(2)→product+enzYme

MECHANISM OF ENZYME REACTION



The basic mechanism of enzyme action is to catalyze the chemical reactions, which begins with the binding of the substrate with the active site of the enzyme. This active site is a specific area that combines with the substrate.

Enzyme-Substrate Interactions

Enzymes are the biocatalysts with high molecular weight proteinous compound. It enhances the reactions which occur in the body during various life processes. It helps the substrate by providing the surface for the reaction to occur. The enzyme comprises hollow spaces occupying groups such as -SH, -COOH, and others on the outer surface. The substrate which has an opposite charge of the enzyme fits into these spaces, just like a key fits into a lock. This substrate binding site is called the active site of an enzyme (E).

The favourable model of enzyme-substrate interaction is called the induced-fit model. This model states that the interaction between substrate and enzyme is weak, and these weak interactions induce conformational changes rapidly and strengthen binding and bring catalytic sites close enough to substrate bonds.

There are four possible major mechanisms of catalysis:

Catalysis by Bond Strain

The induced structural rearrangements in this type of catalysis produce strained substrate bonds that attain transition state more easily. The new conformation forces substrate atoms and catalytic groups like aspartate into conformations that strain substrate bonds.

Covalent Catalysis

The substrate is oriented to active place on the enzymes in such a manner that a covalent intermediate develops between the enzyme and the substrate, in catalysis that occurs by covalent mechanisms. The best example of this involves proteolysis by serine proteases that have both digestive enzymes and various enzymes of the blood clotting cascade. These proteases possess an active site serine whose R group hydroxyl generate a covalent bond with a carbonyl carbon of a peptide bond and results in the hydrolysis of the peptide bond.

Catalysis Involving Acids and Bases

Other mechanisms add to the completion of catalytic events which are launched by strain mechanism such as the usage of glutamate as a general acid catalyst.

Catalysis by Orientation and Proximity

Enzyme-substrate interactions induce reactive groups into proximity with one another. Also, groups like aspartate are chemically reactive, and their proximity towards the substrate favours their involvement in catalysis.

Action and Nature of Enzymes

Once substrate (S) binds to this active site, they form a complex (intermediate-ES) which then produces the product (P) and the enzyme (E). The substrate which gets attached to the enzyme has a specific structure, and that can only fit in a particular enzyme. Hence, by providing a surface for the substrate, an enzyme slows down the activation energy of the reaction. The intermediate state where the substrate binds to the enzyme is called the transition state. By breaking and making the bonds, the substrate binds to the enzyme (remains unchanged), which converts into the product and later splits into product and enzyme. The free enzymes then bind to other substrates, and the catalytic cycle continues until the reaction completes.

The enzyme action basically happens in two steps:

Step1: Combining of enzyme and the reactant/substrate.

E + S → [ES]

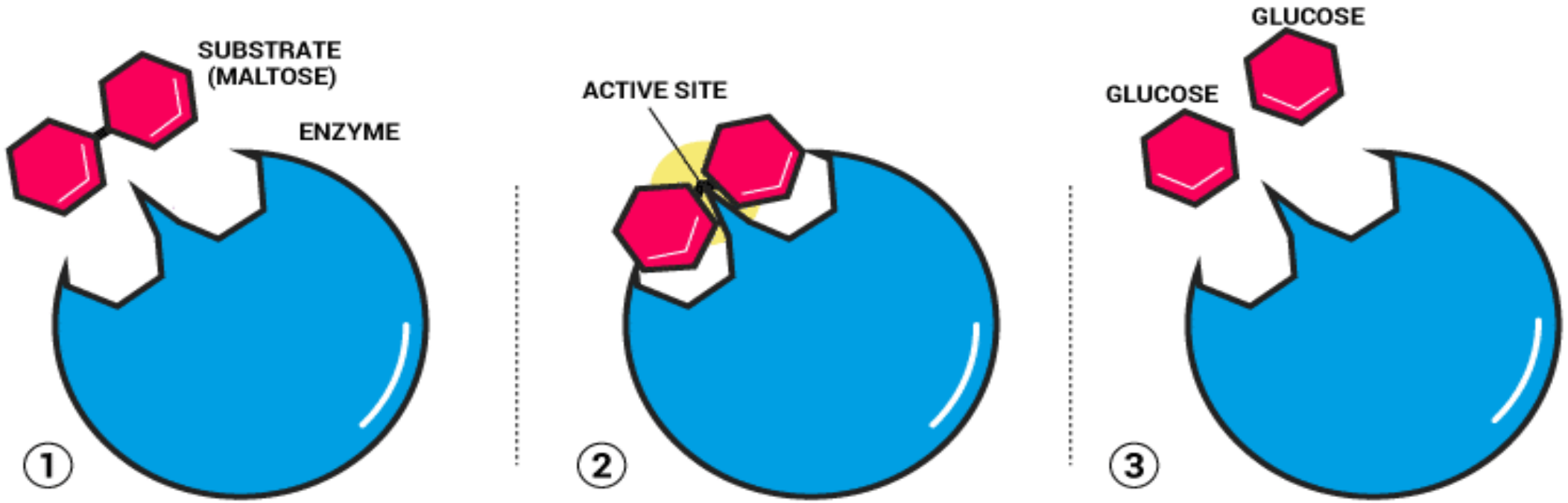
Step 2: Disintegration of the complex molecule to give the product.

[ES] → E + P

Thus, the whole catalyst action of enzymes is summarized as:

E + S → [ES] → [EP] → E + P

ENZYME ACTION



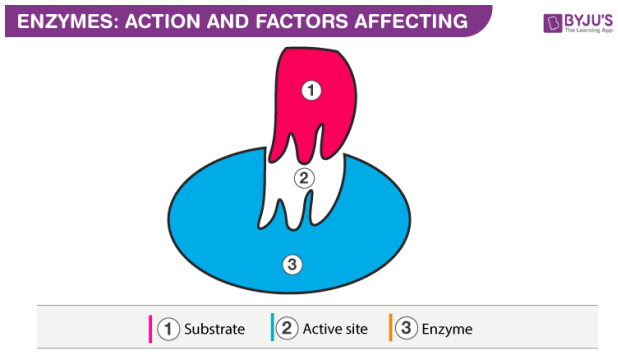
Biological Catalysts

Biological catalysts, enzymes, are extremely specific that catalyze a single chemical reaction or some closely associated reactions. An enzyme's exact structure and its active site decide an enzyme's specificity. Substrate molecules attach themselves at the active site of an enzyme. Initially, substrates associate themselves by noncovalent interactions to the enzymes which include ionic, hydrogen bonds and hydrophobic interactions. Enzymes reduce the reactions and activation energy to progress towards equilibrium quicker than the reactions that are not catalyzed. Both eukaryotic and prokaryotic cells usually make use of allosteric regulation to respond to fluctuations in the state inside the cells.

The nature of enzyme action and factors affecting the enzyme activity are discussed below.

Factors Affecting Enzyme Activity

The conditions of the reaction have a great impact on the activity of the enzymes. Enzymes are particular about the optimum conditions provided for the reactions such as temperature, pH, alteration in substrate concentration, etc.



Typically, enzyme activities are accelerated with increasing temperatures. As enzymes are functional in cells, the feasible conditions for nearly all enzymes are temperatures that are moderate. At higher temperatures, given a specific point, there is a drastic decrease in the activity with the denaturation of enzymes. In diluted solutions, purified enzymes denature quickly compared to enzymes in crude extracts. Denaturation of enzymes can also take place when enzymes are incubated for long durations. More appropriate is to utilize a shorter time duration when it comes to incubation time to gauge the starting velocities of such enzyme reactions.

The International Union of Biochemistry suggests the standard assay temperature to be 30 °C. Almost all enzymes are extremely sensitive to pH change. Just some enzymes feasibly operate with pH above 9 and below 5. Most enzymes have their pH – optimum near to neutrality. Any alteration of pH causes the ionic state of amino acid residues to change in the whole protein and in the active site. The modifications in the ionic state can modify catalysis and substrate binding. The preference of substrate concentration is critical as at lower concentrations, the rate is driven by concentration, however, at high concentrations, the rate does not depend on any increase in the concentration of the substrate.

Active site

Enzymatic catalysis depends upon the activity of amino acid side chains assembled in the active centre.

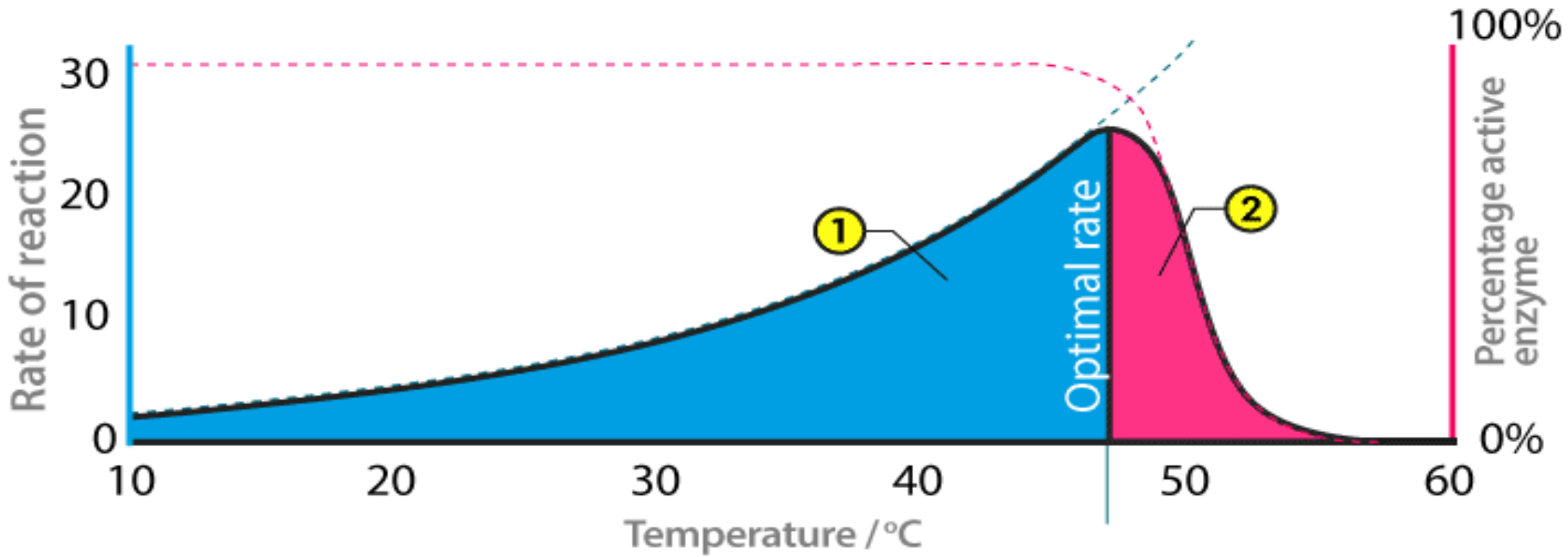
Enzymes bind the substrate into a region of the active site in an intermediate conformation.

Often, the active site is a cleft or a pocket produced by the amino acids which take part in catalysis and substrate binding. Amino acids forming an enzyme's active site is not contiguous to the other along the sequence of primary amino acid. The active site amino acids are assembled to the cluster in the right conformation by the 3-dimensional folding of the primary amino acid sequence. The most frequent active site amino acid residues out of the 20 amino acids forming the protein are polar amino acids, aspartate, cysteine, glutamate, histidine, Serine, and lysine. Typically, only 2-3 essential amino acid residues are involved directly in the bond causing the formation of the product. Glutamate, Aspartate, and histidine are the amino acid residues which also serve as a proton acceptor or donor.

Temperature and pH

Enzymes require an optimum temperature and pH for their action. The temperature or pH at which a compound shows its maximum activity is called optimum temperature or optimum pH, respectively. As mentioned earlier, enzymes are protein compounds. A temperature or pH more than optimum may alter the molecular structure of the enzymes. Generally, an optimum pH for enzymes is considered to be ranging between 5 and 7.

TEMPERATURE AND pH



1 | Temperature increases activity

2 | Denaturation decreases activity

Concentration and Type of Substrate

Enzymes have a saturation point, i.e., once all the enzymes added are occupied by the substrate molecules, its activity will be ceased. When the reaction begins, the velocity of enzyme action keeps on increasing on further addition of substrate. However, at a saturation point where substrate molecules are more in number than the free enzyme, the velocity remains the same.

The type of substrate is another factor that affects the enzyme action. The chemicals that bind to the active site of the enzyme can inhibit the activity of the enzyme and such substrate is called an inhibitor. Competitive inhibitors are chemicals that compete with the specific substrate of the enzyme for the active site. They structurally resemble the specific substrate of the enzyme and bind to the enzyme and inhibit the enzymatic activity. This concept is used for treating bacterial infectious diseases.

Functions of Enzymes

The enzymes perform a number of functions in our body. These include:

Enzymes help in signal transduction. The most common enzyme used in the process includes protein kinase that catalyzes the phosphorylation of proteins.

They breakdown large molecules into smaller substances that can be easily absorbed by the body.

They help in generating energy in the body. ATP synthase is the enzymes involved in the synthesis of energy.

Enzymes are responsible for the movement of ions across the plasma membrane.

Enzymes perform a number of biochemical reactions, including oxidation, reduction, hydrolysis, etc. to eliminate the non-nutritive substances from the body.

They function to reorganize the internal structure of the cell to regulate cellular activities.

ENZYME KINETICS

Introduction. In their 1913 paper Leonor Michaelis and Maud Menten sought to achieve “the final aim of kinetic research, namely to obtain knowledge of the nature of the reaction from a study of its progress”

Enzyme kinetics is the study of the chemical reactions that are catalysed by enzymes. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

Enzymes are usually protein molecules that manipulate other molecules—the enzymes' substrates. These target molecules bind to an enzyme's active site and are transformed into products through a series of steps known as the enzymatic mechanism

E + S ⇄ ⇄ ES ES * EP ⇄ ⇄ E + P

These mechanisms can be divided into single-substrate and multiple-substrate mechanisms. Kinetic studies on enzymes that only bind one substrate, such as triosephosphate isomerase, aim to measure the affinity with which the enzyme binds this substrate and the turnover rate. Some other examples of enzymes are phosphofructokinase and hexokinase, both of which are important for cellular respiration (glycolysis).

When enzymes bind multiple substrates, such as dihydrofolate reductase (shown right), enzyme kinetics can also show the sequence in which these substrates bind and the sequence in which products are released. An example of enzymes that bind a single substrate and release multiple products are proteases, which cleave one protein substrate into two polypeptide products. Others join two substrates together, such as DNA polymerase linking a nucleotide to DNA. Although these mechanisms are often a complex series of steps, there is typically one rate-determining step that determines the overall kinetics. This rate-determining step may be a chemical reaction or a conformational change of the enzyme or substrates, such as those involved in the release of product(s) from the enzyme.

Knowledge of the enzyme's structure is helpful in interpreting kinetic data. For example, the structure can suggest how substrates and products bind during catalysis; what changes occur during the reaction; and even the role of particular amino acid residues in the mechanism. Some enzymes change shape significantly during the mechanism; in such cases, it is helpful to determine the enzyme structure with and without bound substrate analogues that do not undergo the enzymatic reaction

Single-substrate reactions

Enzymes with single-substrate mechanisms include isomerases such as triosephosphateisomerase or bisphosphoglycerate mutase, intramolecular lyases such as adenylate cyclase and the hammerhead ribozyme, an RNA lyase.[10] However, some enzymes that only have a single substrate do not fall into this category of mechanisms. Catalase is an example of this, as the enzyme reacts with a first molecule of hydrogen peroxide substrate, becomes oxidised and is then reduced by a second molecule of substrate. Although a single substrate is involved, the existence of a modified enzyme intermediate means that the mechanism of catalase is actually a ping–pong mechanism, a type of mechanism that is discussed in the Multi-substrate reactions section below.

Michaelis–Menten kinetics

As enzyme-catalysed reactions are saturable, their rate of catalysis does not show a linear response to increasing substrate. If the initial rate of the reaction is measured over a range of substrate concentrations (denoted as [S]), the initial reaction rate ({\displaystyle v_{0}}v_ {0}) increases as [S] increases, as shown on the right. However, as [S] gets higher, the enzyme becomes saturated with substrate and the initial rate reaches Vmax, the enzyme's maximum rate

The Michaelis–Menten kinetic model of a single-substrate reaction is shown on the right. There is an initial bimolecular reaction between the enzyme E and substrate S to form the enzyme–substrate complex ES. The rate of enzymatic reaction increases with the increase of the substrate concentration up to a certain level called Vmax; at Vmax, increase in substrate concentration does not cause any increase in reaction rate as there is no more enzyme (E) available for reacting with substrate (S). Here, the rate of reaction becomes dependent on the ES complex and the reaction becomes a unimolecular reaction with an order of zero. Though the enzymatic mechanism for the unimolecular reaction

$\mathrm{ES} \xrightarrow{k_{cat}} \mathrm{E} + \mathrm{P}$ **can be quite complex, there is typically one rate-determining enzymatic step that allows this reaction to be modelled as a single catalytic step with an apparent unimolecular rate constant kcat. If the reaction path proceeds over one or several intermediates, kcat will be a function of several elementary rate constants, whereas in the simplest case of a single elementary reaction (e.g. no intermediates) it will be identical to the elementary unimolecular rate constant k2. The apparent unimolecular rate constant kcat is also called turnover number and denotes the maximum number of enzymatic reactions catalysed per second.**

The Michaelis–Menten equation[11] describes how the (initial) reaction rate v0 depends on the position of the substrate-binding equilibrium and the rate constant

$$v_0 = \frac{V_{\max}[\mathrm{S}]}{K_M + [\mathrm{S}]}$$ *(Michaelis–Menten equation)*

with the constants

$$K_M \stackrel{\text{def}}{=} \frac{k_2 + k_{-1}}{k_1} \approx K_D$$

$$V_{\max} \stackrel{\text{def}}{=} k_{cat}[\mathrm{E}]_{tot}$$

This Michaelis–Menten equation is the basis for most single-substrate enzyme kinetics. Two crucial assumptions underlie this equation (apart from the general assumption about the mechanism only involving no intermediate or product inhibition, and there is no allostericity or cooperativity). The first assumption is the so-called quasi-steady-state assumption (or pseudo-steady-state hypothesis), namely that the concentration of the substrate-bound enzyme (and hence also the unbound enzyme) changes much more slowly than those of the product and substrate and thus the change over time of the

complex can be set to zero $d[\mathrm{ES}]/dt \stackrel{!}{=} 0$. The second assumption is that the total enzyme concentration does not change over time, thus $[\mathrm{E}]_{\mathrm{tot}} = [\mathrm{E}] + [\mathrm{ES}] \stackrel{!}{=} \mathrm{const}$. A complete derivation can be found here.

The Michaelis constant K_M is experimentally defined as the concentration at which the rate of the enzyme reaction is half V_{max}, which can be verified by substituting [S] = K_M into the Michaelis–Menten equation and can also be seen graphically. If the rate-determining enzymatic step is slow compared to substrate dissociation ($k_2 \ll k_{-1}$), the Michaelis constant K_M is roughly the dissociation constant K_D of the ES complex.

If $[\mathrm{S}]$ is small compared to K_M then the term $[\mathrm{S}]/(K_M + [\mathrm{S}]) \approx [\mathrm{S}]/K_M$ and also very little ES complex is formed, thus $[\mathrm{E}]_{\mathrm{tot}} \approx [\mathrm{E}]$. Therefore, the rate of product formation is

$$v_0 \approx \frac{k_{cat}}{K_M}[\mathrm{E}][\mathrm{S}] \qquad \text{if } [\mathrm{S}] \ll K_M$$

Thus the product formation rate depends on the enzyme concentration as well as on the substrate concentration, the equation resembles a bimolecular reaction with a corresponding pseudo-second order rate constant k_2/K_M. This constant is a measure of catalytic efficiency. The most efficient enzymes reach a k_2/K_M in the range of

$10^8 - 10^{10}\ \mathrm{M^{-1}\ s^{-1}}$. These enzymes are so efficient they effectively catalyse a reaction each time they encounter a substrate molecule and have thus reached an upper theoretical limit for efficiency (diffusion limit); and are sometimes referred to as kinetically perfect enzymes.[12] But most enzymes are far from perfect: the average values of k_2/K_M and k_2 are about $10^5 \mathrm{s}^{-1}\mathrm{M}^{-1}$ and $10 \mathrm{s}^{-1}$, respectively.[13]

Michaelis–Menten kinetics with intermediate [edit]

One could also consider the less simple case

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \overset{k_2}{\longrightarrow} \mathrm{EI} \overset{k_3}{\longrightarrow} \mathrm{E} + \mathrm{P}$$

where a complex with the enzyme and an intermediate exists and the intermediate is converted into product in a second step. In this case we have a very similar equation[25]

$$v_0 = k_{cat} \frac{[\mathrm{S}][\mathrm{E}]_0}{K'_M + [\mathrm{S}]}$$

but the constants are different

$$K'_M \stackrel{\text{def}}{=} \frac{k_3}{k_2 + k_3} K_M = \frac{k_3}{k_2 + k_3} \cdot \frac{k_2 + k_{-1}}{k_1}$$

$$k_{cat} \stackrel{\text{def}}{=} \frac{k_3 k_2}{k_2 + k_3}$$

We see that for the limiting case $k_3 \gg k_2$, thus when the last step from $\mathrm{EI} \longrightarrow \mathrm{E} + \mathrm{P}$ is much faster than the previous step, we get again the original equation. Mathematically we have then $K'_M \approx K_M$ and $k_{cat} \approx k_2$.

Multi-substrate reactions

Multi-substrate reactions follow complex rate equations that describe how the substrates bind and in what sequence. The analysis of these reactions is much simpler if the concentration of substrate A is kept constant and substrate B varied. Under these conditions, the enzyme behaves just like a single-substrate enzyme and a plot of v by [S] gives apparent KM and Vmax constants for substrate B. If a set of these measurements is performed at different fixed concentrations of A, these data can be used to work out what the mechanism of the reaction is. For an enzyme that takes two substrates A and B and turns them into two products P and Q, there are two types of mechanism: ternary complex and ping–pong.

THANK YOU