BY MS. PAMELA ANGUDEYO

MECHANISM OF ENZYME ACTION

There are two theories put forward to describe the mechanism of enzyme action i.e.

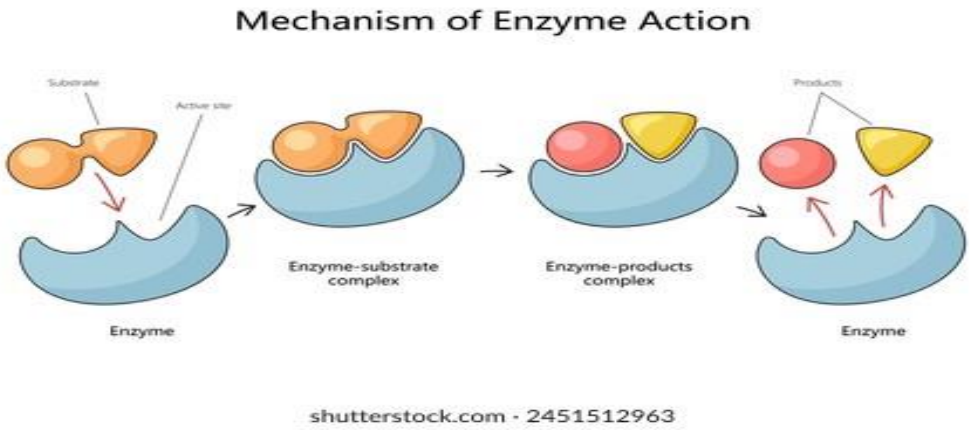
I. The Lock and key hypothesis (By Fischer in 1890)
II. The Induced fit hypothesis (By Koshland in 1959)

The lock and key hypothesis/theory

The theory suggests that the enzyme has a portion with specific rigid shape known as the active site into which the substrate fits exactly like a key fit in a lock. The substrate is like a key and the enzyme like a lock. The substrate has a complementary shape to the active site of the enzyme.

Hydrogen and ionic bonds hold the substrate in the active site to form an enzyme-substrate complex. Once formed, the substrate is either split apart by the enzyme or its pieces are linked together to form products. The products no longer fit into the active site of the enzyme, and escape into the surrounding medium, leaving the active site free to pick more substrate molecules.

Illustration



Advantages of the lock and key hypothesis

1. It explains why enzymes are specific in action i.e. only substrates with complementary shapes to the active site of the enzyme fit into the active sites and are converted to products.
2. It explains why the rate of enzyme-controlled reaction is affected by substrate concentration. If all the active sites are in use, no more substrates a can fit or occupy the active site hence the rate is constant.

3. It explains why and enzymes can be inhibited.
4. It explains why enzymes are able to lower the activation energy in a chemical reaction.

The induced fit theory

This is a modified version of the lock and theory.

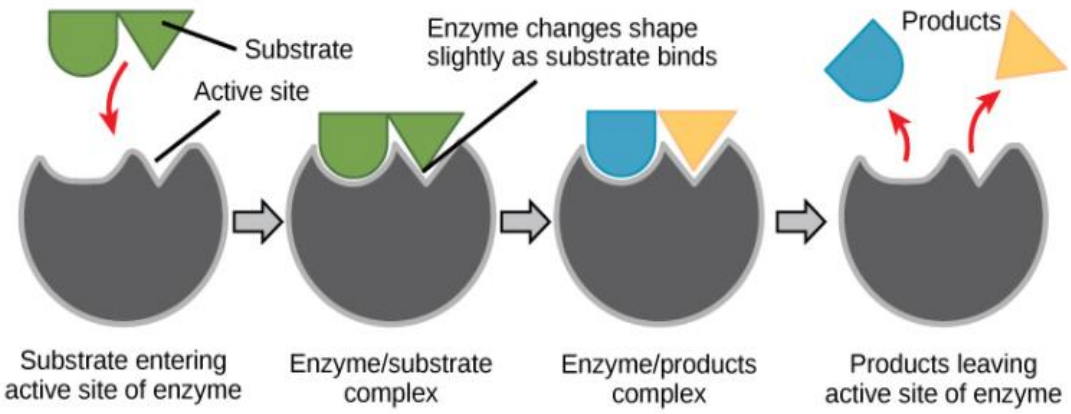
According to this theory, the active site of the enzyme is not rigid but flexible, its shape changes according to that of the substrate, enabling the enzyme to perform its catalytic function most effectively.

When the substrate molecule binds to the enzyme's active site, the bonds between the amino acids of the active site are distorted, causing the shape to be modified (moulded) to a precise shape, so that the two molecules fit together more tightly forming an enzyme-substrate complex.

The substrate molecule is distorted due to straining or twisting of its bonds by the enzyme, making it less stable with reduced potential energy. Reaction takes place and an enzyme-product complex is formed, which later breaks down to form the enzyme and products.

The products formed no longer bind to the active site and are therefore released while the flexible active site returns to its original shape to pick more substrate molecules.

Illustration



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FACTORS AFFECTING THE RATE OF ENZYME-CONTROLLED REACTIONS

The rate of an enzyme-controlled reaction is determined by either measuring the amount of substrate used or the amount of product formed. This is done by measuring the slope of the tangent to the curve on a graph, in the initial stage of the reaction. The steeper the slope, the greater is the rate.

When investigating the effect of a given factor on the rate of an enzyme-controlled reaction, all other factors should be kept constant and at optimum levels wherever possible.

Several factors affect the rate of enzyme-controlled reactions. These include:

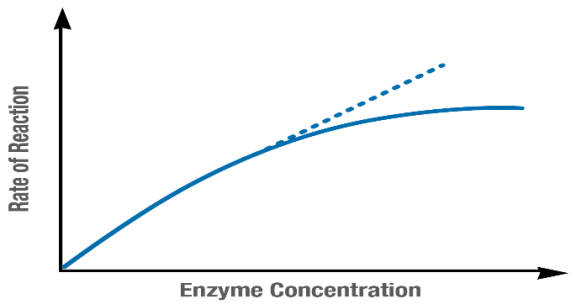
a. Enzyme concentration
b. Substrate concentration
c. Temperature
d. pH of the medium
e. Presence of inhibitors
f. Presence of cofactors

a. Enzyme concentration

As the concentration of the enzymes increases, the rate of reaction also increases, provided the substrate concentration is high and other conditions such as pH and temperature are kept constant. This is because increase in enzyme concentration increases the number of active sites available for the substrate molecules to fit in to form an enzyme-substrate complex, increasing the rate of reaction.

At very high enzyme concentration, the rate of reaction becomes constant because all the substrates might have been acted upon.

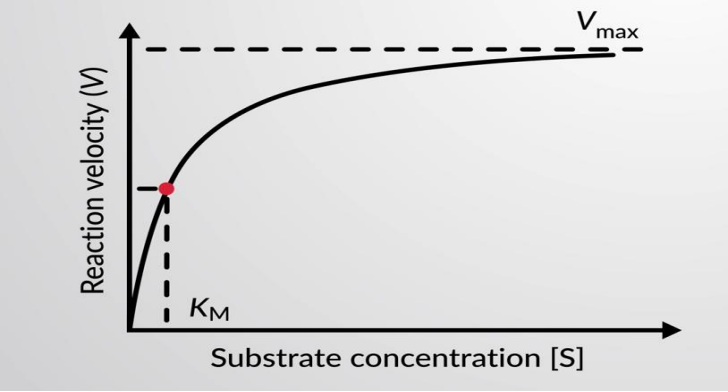
Illustration



b. Substrate concentration

The rate of enzyme-controlled reaction increases with increase in substrate concentration. However, further increase in substrate concentration will not increase the reaction rate since all the active sites of the enzyme are fully saturated with the substrate. At this point the rate of reaction can only be increased by increasing the enzyme concentration.

Illustration



Explanation

At low substrate concentration, rate of reaction is low, because there are few substrate molecules, the chances of collisions are reduced making few substrate molecules fit into the active site of the enzymes to form enzyme-substrate complexes, hence releasing few products.

As substrate concentration increases, rate of reaction also increases because more substrate molecules collide with the enzymes and fit into their active sites to form enzyme-substrate complexes, releasing more products at the end of the reaction.

At higher substrate concentration, rate of reaction remains constant. This is because all the enzyme active sites are occupied by substrates. Any more substrate molecules added will have no active sites to fit in hence constant rate of reaction.

At this point, the limiting factor is the low enzyme concentration, so if the concentration of enzymes is increased, the rate of reaction also increases.

The Michaelis-Menten constant, Km

This is used to compare the affinity of different enzymes for their substrates.

Km is the concentration of substrate required to make the reaction go at half its maximum rate. i.e. Km=[S]= ½ Vmax. The lower the value of Km, the greater the

affinity of the enzyme for its substrate, and the faster the rate of reaction and vice versa.

c. Temperature

The rate of an enzyme-catalysed reaction increases with increase in temperature up to a maximum, called the **optimum temperature,** and decreases at higher temperatures beyond the optimum.

At low temperatures near or below freezing point, rate of reaction is low because enzymes are inactivated, making it impossible for them to break down the substrates.

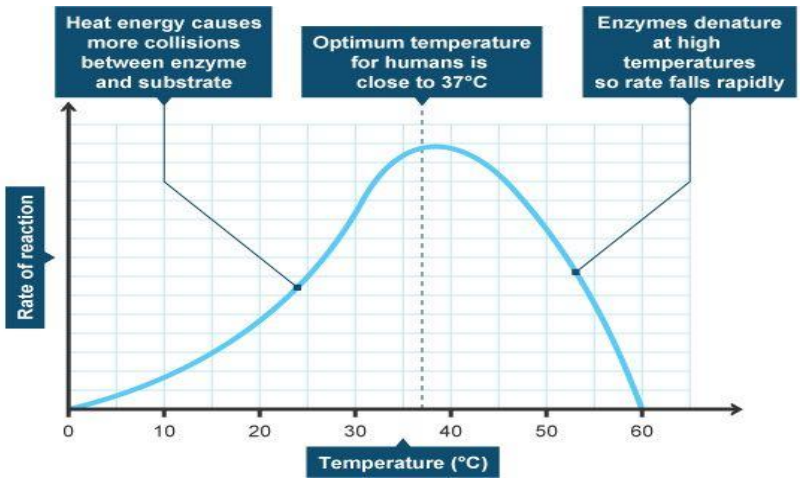
Increase in temperature increases the kinetic energy of the reactants hence, they move faster. This increases the chances of collision and interaction between the substrate molecules and with the enzymes, causing them to react.

Above the optimum temperature (40°C), the rate of the reaction decreases rapidly due to breakage of some bonds that maintain the enzyme's 3-dimensional structure; therefore, the enzyme is denatured. This changes the shape of the active site such that the substrate can no longer fit into it hence no enzyme reaction takes place.

Note: The optimum temperature for an enzyme-catalysed reaction is related to the enzyme's usual thermal environment.

In humans, many enzymes work best at core body temperature of about 37 ℃ but enzymes of other organisms that have evolved to live in much higher or lower temperatures may have much higher or lower optimum temperature.

Illustration



d. Hydrogen ion concentration (pH)

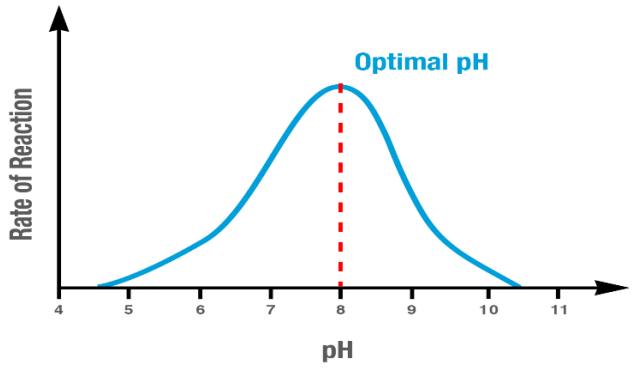
Most enzymes work efficiently within a narrow pH range. However, there is an optimum pH for each enzyme at which its activity is greatest. Any deviation from this value either below or above causes the rate of reaction to decrease.

When pH is low, acidity increases and the concentration of H^+ ions also increases. This causes the hydrogen and ionic bonds that maintain the specific shape of the enzyme to be broken and its shape including the active site is altered. The substrate no longer fits tightly into the active site to an enzyme-substrate complex, so the enzyme becomes ineffective hence decrease in the rate of reaction.

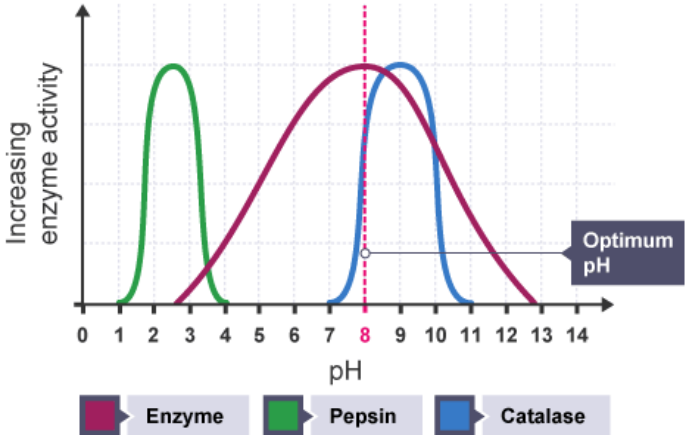
On the other hand, when pH is high, alkalinity increases, and the concentration of H^+ ions decreases (-OH ions concentration increases) leading to breakage of bonds and the enzyme becomes denatured.

Optimum pH values for some enzymes

Enzyme	Optimum pH
Pepsin	2.00
Sucrase	4.50
Enterokinase	5.50
Salivary amylase	6.80
Catalase	7.60
Chymotrypsin	7.00-8.00
Pancreatic lipase	9.00
Arginase	9.70



Effect of pH on the rate of an enzyme-controlled reaction.



The effect of pH on the activity of two enzymes; Pepsin and catalase.

e. Presence of enzyme inhibitors

These lower the rate of enzyme-controlled reactions or they may stop enzyme action altogether. i.e. Enzyme activities decrease in presence of enzyme inhibitors and increase in their absence.

f. Presence of cofactors

For some enzymes, the rate of enzyme-controlled reaction depends on cofactors. Enzyme activities increase with presence of enzyme activators and decrease with absence of enzyme activators.

Enzyme inhibitors

These are substances which reduce the rate of enzyme-controlled reactions, in a process known as inhibition. Inhibition may be competitive or non-competitive, and non-competitive inhibition maybe reversible or non-reversible.

Competitive inhibition

This occurs when a compound (an inhibitor) has a structure which is similar to that of the actual substrate of an enzyme, so that it binds to the enzyme active site and prevents the true substrate from entering it. The normal substrate and the inhibitor therefore **compete** for a position in the active site of the enzyme. Such inhibitors are known as **competitive inhibitors**. They only affect the enzyme when they are attached to it but as soon as they are detached, the enzyme can function normally again. The rate of reaction increases if the substrate concentration is increased, but reduces if the concentration of the inhibitor is increased.

Examples of competitive inhibitors include antibiotic drugs such as **sulphonamides** which have similar shape to para-aminobenzoate (PAB), a

substrate used by some harmful bacteria to synthesize folic acid. Sulphonamides compete with PAB for the active site of the enzyme which catalyses the conversion of PAB into folic acid. So, if the concentration of sulphonamide is high, the enzyme will be inhibited and the bacteria will lack folic acid and die.

Non-competitive reversible inhibition

This happens when an inhibitor has no structural similarity to the true substrate, and combines with an enzyme at a point other than its active site. Its presence does not affect the ability of the substrate to bind with an enzyme, but makes it impossible for catalysis to take place.

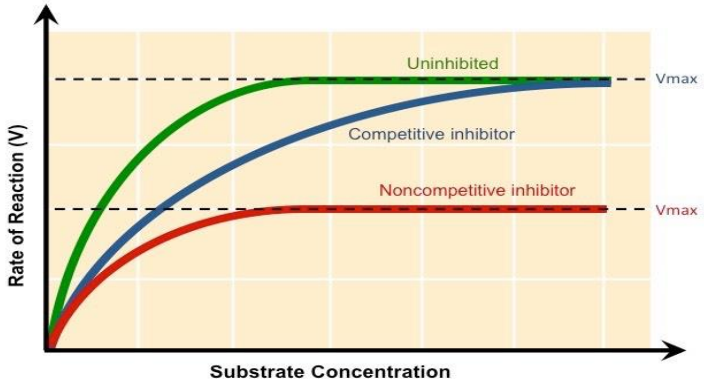
The rate of reaction decreases with increasing inhibitor concentration up to almost zero when inhibitor saturation is reached. In this case, an increase in substrate concentration does not affect the rate of reaction.

Non-competitive irreversible inhibition

This occurs when the inhibitor combines permanently with the enzyme either at the active site or elsewhere to form an inactive non-enzymatic end product. This causes a change in the structure of the enzyme making it ineffective as a catalyst.

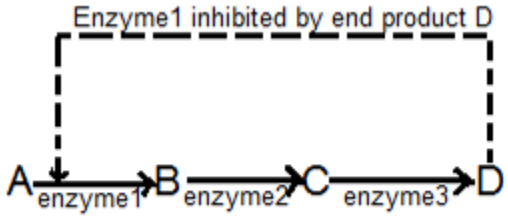
Examples of non-competitive inhibitors include; **heavy metal ions** (mercury, lead, silver and arsenic), **some iodine-containing compounds**, [which completely inhibit some enzymes by combining permanently with the sulphydryl groups of the amino acid cysteine within their structure], **cyanide** [which inhibits cytochrome c oxidase in respiration by attaching itself to the prosthetic group, preventing transfer of electrons to oxygen], **nerve gas DFP** (diisopropylfluorophosphate) [which completely inhibits the enzyme acetylcholinesterase by combining with the amino acid serine at its active site], **insecticides, herbicides**, etc.

The effect of a competitive inhibitor and a non-competitive inhibitor on the rate of an enzyme-catalysed reaction.



End product inhibition

This takes place in some metabolic pathways when the end product begins to accumulate, it combines with the first enzyme controlling the first step of the pathway for its production so that further formation of the end product is slowed or stopped. This is an example of negative feedback mechanism which is important in regulation of body processes.



Allosteric enzymes

These are enzymes which are meant to change shape (*allo*, different; *steric*, shape). They are regulated by non-competitive inhibitors which bind to specific parts of the enzyme but not the active site. i.e. allosteric enzymes have a site separate from the active site to which another substrate can bind. The inhibitors cause a reversible change in the structure of the enzyme's active site so that the substrate cannot bind to the enzyme. (unlike non-competitive reversible inhibition)

Compounds which bring about this are known as **allosteric inhibitors.** They are useful in regulating metabolic pathways. E.g. inhibition of one of enzymes of glycolysis by high ATP concentration and vice versa. This is also an example of end-product inhibition.

Commercial application of enzyme inhibition

- Applied in medicine and pharmaceuticals to manufacture antibiotics and cancer drugs.
- Applied in agriculture to produce pesticides, herbicides and fungicides.
- In industrial biotechnology to improve food and beverage quality through controlled fermentation by regulating the different stages of the metabolic pathways.
- In research, enzyme inhibitors are used to study enzyme mechanisms in the laboratory. They provide important information about the shapes and properties of enzymes.
- In analytical sensors for monitoring environmental factors by detecting specific enzyme activities.

Enzyme cofactors

Co-factors are non-protein components required by enzymes for their efficient functioning. There are three types of co-factors:

a) Activators; these are inorganic in nature. They mould either the enzyme or the substrate into a shape that allows an enzyme-substrate complex to be formed, hence increasing the chances of reaction occurring between them which in turn increases the rate of reaction catalysed by the enzyme.e.g. salivary amylase activity is increased in the presence of chloride ions. Other examples of enzyme activators include metal atoms like copper, iron and zinc.

b) Prosthetic groups; these are organic molecules tightly bound to the active site of the enzyme on a permanent basis. e.g. **Flavine adenine dinucleotide (FAD),** which contains vitamin B_2 (riboflavin). It accepts hydrogen in respiration.

Haem, an iron-containing prosthetic group found in cytochromes, where it functions as an electron carrier in E.T.C in respiration. It is found in haemoglobin and myoglobin where it functions as an oxygen carrier. Haem is also found in other enzymes such as catalases, peroxidases e.t.c.

c) Coenzymes; these are organic molecules loosely associated with the enzyme. They are usually vitamins or compounds made from vitamins. e.g. Nicotinamide adenine dinucleotide (NAD), derived from vitamin B_3 which transfers hydrogen during cellular respiration, NADP, coenzyme A and ATP. Therefore, prosthetic groups and coenzymes act as carriers of groups of atoms.