Biomolecules of Life (Part 2)- Enzymes

1. Overview of Topic

Many enzymes are made up of proteins. Enzymes are biological catalysts which speed up the rate of reactions. In this topic, we will examine the mode of action of enzymes in terms of their catalytic action via their activity sites. We will also study how enzyme-substrate complexes are formed through the proposed models of enzyme substrate interactions. Various factors such as temperature, pH, enzyme concentrations & substrate concentrations affect enzyme catalysed reactions. Enzyme can also be influenced by inhibitors and activators which can regulate the rates of enzymatic reactions.

2. Learning Outcomes

a. Explain the mode of action of enzymes in terms of an active site, enzyme-substrate complex, lowering of activation energy and enzyme specificity using the lock-and-key and induced-fit hypothesis.

b. Investigate and explain the effects of temperature, pH, enzyme concentration and substrate concentration of an enzyme-catalysed reaction by measuring rates of formation of products (eg measuring gas produced using catalase) or rate of disappearance of substrate (e.g. using amylase, starch and iodine).

c. Describe the structure of competitive and non-competitive inhibitors with reference to the binding sites of the inhibitor.

d. Explain the effects of competitive and non-competitive inhibitors (including allosteric inhibitors) on the rate of enzyme activity.

3. References

Campbell, N.A. and Reece, J.B. (2011), ***Biology*** (9th edition), Pearson Benjamin-Cummings, San Francisco

Brooker, R.J., Widmaier, E.P., Graham, L. and Stiling, P. (2008), ***Biology***, Mc-Graw-Hill, New York

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4. Introduction

Notes to self

In living cells, hundreds of different biochemical reactions take place rapidly and simultaneously. How is it possible for there to be such orderliness in what must be a potentially chaotic situation? How can reactions take place so rapidly at such modest temperatures? The answers to these questions come from a study of enzymes.

Enzymes act as **highly specific biological catalysts** that speed up the rate of metabolic reactions. Enzymes also provide a means by which individual reactions can be controlled. The mechanism of these regulatory processes includes allosteric control, competitive inhibition, non-competitive inhibition, covalent modification of enzyme and variation in the amount of enzymes synthesized (which we will cover later or in other topics).

Enzymes are vitally important, because in their absence, reactions in the cell would be too slow to sustain life.

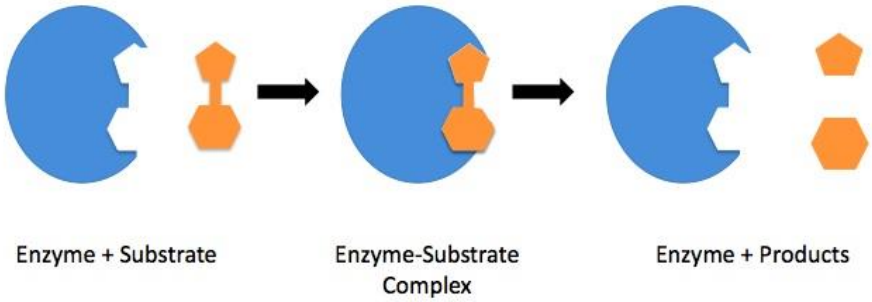


Fig.4.1: An illustration of how enzymes catalyse biological reactions

5. General Information on Enzymes

Notes to self

A. Definition

Enzymes are **biological catalysts** that increase the rate of a reaction and are **chemically unaltered** at the end of the reaction and thus can be reused. They are effective in **small amounts**.

B. General Characteristics of Enzymes

(I) Enzymes are mostly globular proteins

They consist of one or more polypeptide chains coiled and folded to form a globular unit. (ref. tertiary or quaternary level of protein organisation).

As globular proteins, they are extremely complex molecules with intricate 3-dimensional contours and distinct surface geometries.

The action of enzymes depends on their **3-dimensional structure/ conformation**.

There are exceptions because some enzymes can be composed of ribonucleic acids (RNA), e.g. ribozyme or complexes of RNA and protein, e.g telomerase.

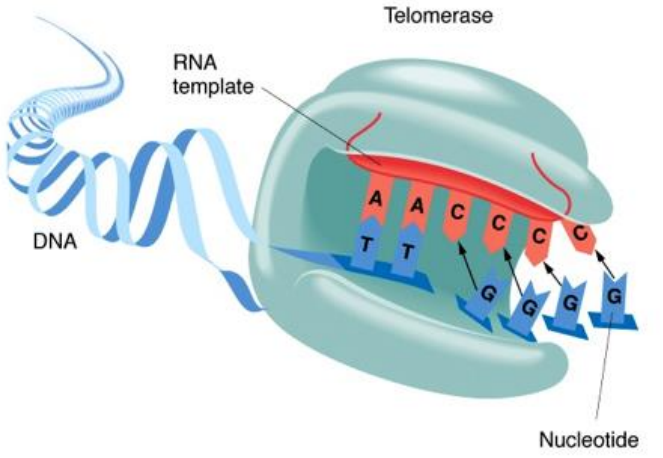


Fig.5.1: Telomerase is an example of a globular enzyme which is consists both protein and RNA (in the form a RNA template). The substrates for telomerase are DNA nucleotides and a DNA template strand.

Notes to self

(II) Enzymes increase the rates of reactions

Rate of enzymatically catalysed reactions are typically 10^6 to 10^{12} times greater than those of corresponding uncatalysed reaction.

(III) Enzymes operate at milder reaction conditions

Enzyme-catalysed reactions can occur under relatively milder conditions i.e. temperatures below 100^{o}C, atmospheric pressure and nearly neutral pH normally encountered in the organism.

In contrast, efficient chemical catalysts often requires elevated temperatures and pressures as well as extreme pH.

(IV) Enzymes exhibit substrate specificity

An enzyme or a particular type of enzyme will usually catalyze a specific chemical reaction.

- **Absolute** specificity (When an enzyme catalyzes a single specific reaction.)
  Example:

  Maltose $\xrightarrow{\textbf{\textit{Maltase}}}$ 2 Glucose

- **Group** specificity (When an enzyme acts on one type of chemical bond in a variety of substances, e.g. peptide bond.)
  Example:

  Polypeptides $\xrightarrow{\textbf{\textit{Chymotrypsin}}}$ smaller polypeptides

6. The Active Site of Enzymes

Notes to self

The Active Site is an Enzyme's Catalytic Center

The primary structure of the enzyme will determine its secondary and tertiary structure. This in turn will specify the overall 3D conformation/structure of the enzyme.

Only a small region of the enzyme binds with the substrate. This is known as the **active site**. There is a precise 3D groove on the enzyme at the active site which gives it a specific conformation/structure.

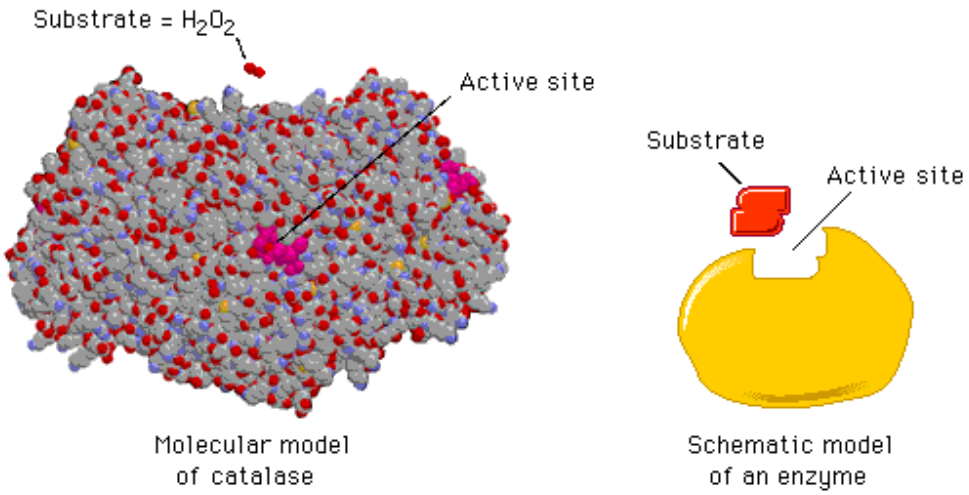
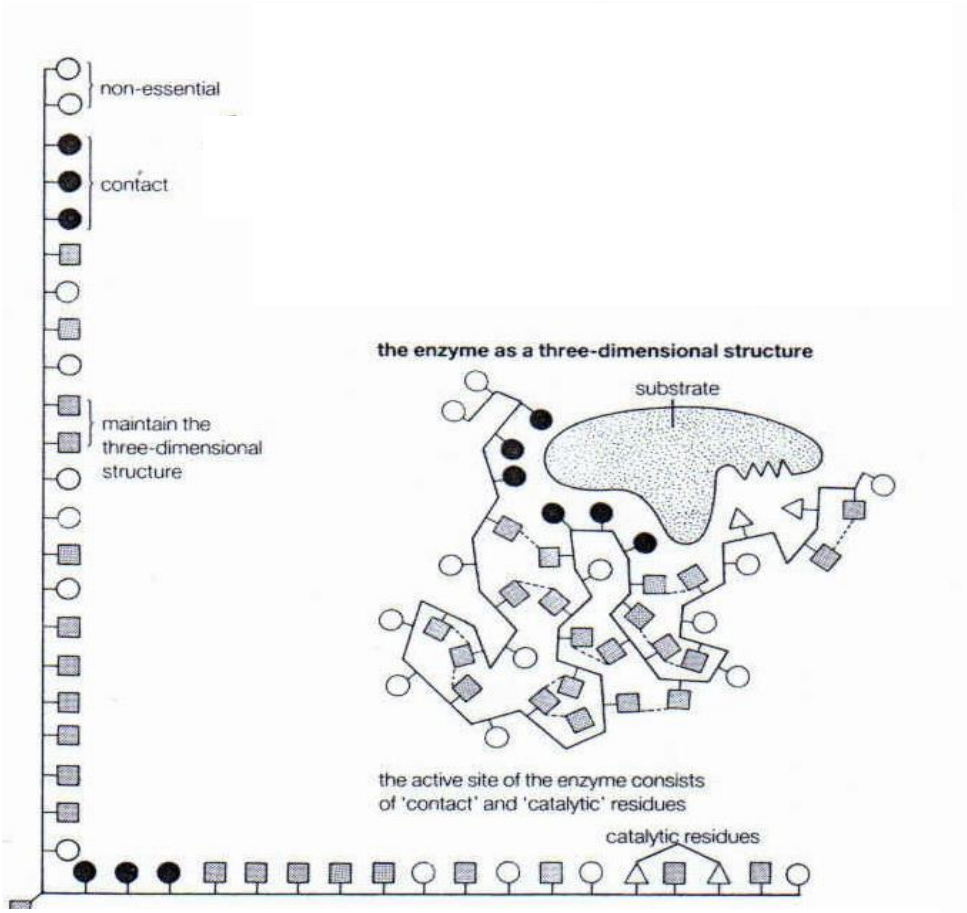


Fig.6.1: Molecular model and schematic model of catalase. Each enzyme is specific to its substrate(s).

The active site typically consists of 3-12 amino acids. Not all are involved in catalytic function. Some are contact amino acids residues which interact reversibly with the substrate, via weak hydrogen and ionic bonds, to position it in the correct orientation. The R-groups of catalytic amino acid residues, present within the active site catalyse the conversion of the substrate to its product. The rest of the polypeptide provides a framework that maintains the conformation of the active site.

The **specificity** of an enzyme is attributed to the **complementary conformation/structure and charge between substrate and active site**. The active site is not rigid. As the substrate enters, it changes its structure so that the active site fits more snugly around the substrate to form a more stable structure (ref. to induced fit model).



An enzyme consists of 4 different categories of amino acid residues:

- **Contact/binding residues** bind reversibly with the substrate while positioning it in the correct orientation. The substrate is held in active site by weak interactions like hydrogen bonds and ionic bonds. These amino acid residues determine enzyme specificity.
- **Catalytic residues** act on the bonds in the substrate molecule and the side chains/R-groups of a few of the amino acids residues catalyse the conversion of the substrate to product.
- **Structural residues** interact to maintain the overall 3D conformation of the protein for proper functioning of the protein.
- **Non-essential residues** are generally found on the surface of the protein. They have no specific functions.

Fig.6.2: Amino acids which make up the 3D structure of an enzyme

Strengthen Your Synapses

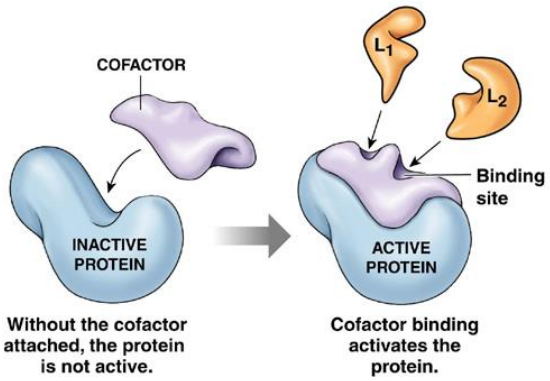
Question: **How can the two amino acids, 'A' and 'B' that are far apart along the polypeptide chain somehow end up next to each other in the active site?**

In some enzymes, which are made up of 2 or more subunits, there are sites known as allosteric sites. Molecules such as activators and inhibitors bind to these allosteric sites and influence the structure of the enzyme as well as its catalytic activity. We will look into the regulation of allosteric enzymes in the later part of this notes.

7. Enzyme co-factors

Some enzymes require additional non-protein substances for catalytic activity. These are called **cofactors**.

1. **Inorganic ions** (e.g. zinc in DNA polymerase)
   - Many enzymes require certain metal ions to change non-functioning active site to a functioning one.
   - Some of common cofactors are Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+}.
   - The attachment of the ion with the main enzyme (apoenzyme) changes the shape of the enzyme so as to allow the enzyme-substrate complex to form more easily.



2. **Coenzymes** (e.g. NAD). Cofactors are usually organic in nature.

3. **Prosthetic group** (e.g. haem group of cytochrome oxidase in electron transport chain in inner mitochondrial membrane). Prosthetic groups are permanently bound to enzyme

8. Models of Enzyme Action

Notes to self

A. The 'Lock & Key' Hypothesis

There are two well illustrated models explain why enzymes are highly specific.

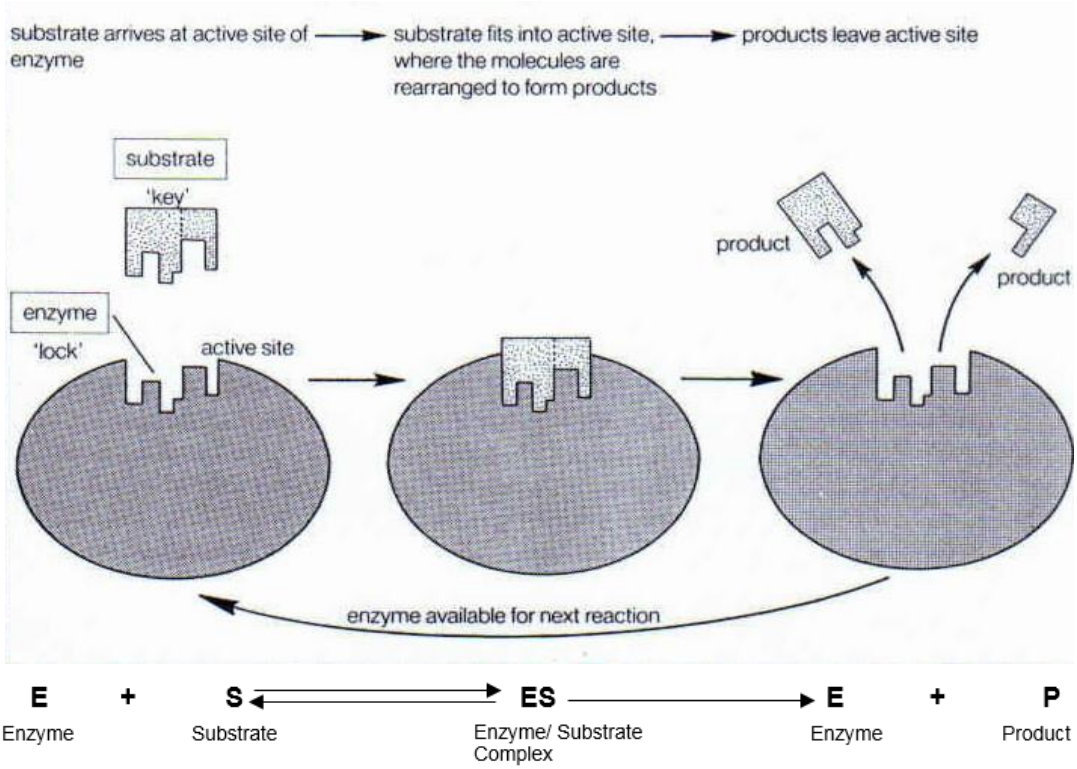


Fig. 7.1: The 'lock & key' model

The premise for the 'lock & key' model is that the enzyme's active site has a specific surface conformation/structure and charge produced by the 3-dimensional folding the polypeptide chain.

The substrate is like a "key" whose conformation/structure is complementary to the enzyme active site or "lock". When enzyme and substrate molecules collide in the correct orientation, the substrate fits into the active site of the enzyme.

A short-lived **enzyme-substrate (ES) complex** is formed. Catalysis occurs and products are formed. Once formed, the **products no longer fit** into the active site and are released into the surrounding medium, leaving the active site **free to receive further substrate molecules**.

The enzyme and its active site are not altered at the end of the reaction.

B. The 'Induced-Fit' model

Notes to self

As the substrate enters the enzyme active site, it **induces a change** in the structure of the active site of the protein. This change causes the active site to enfold the substrate and hold it in place via the formation of weak bonds.

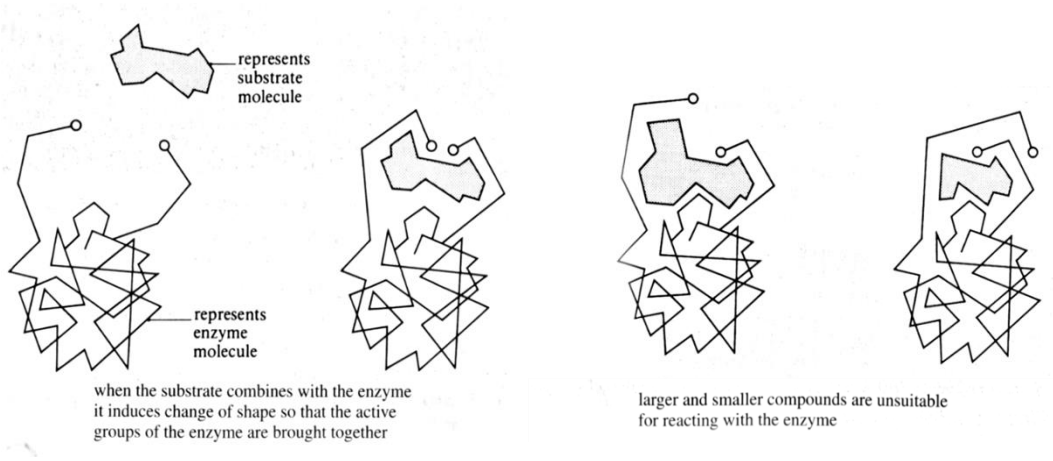


Fig 7.2: The 'Induced Fit' Hypothesis

The active site of enzymes is generally complementary in conformation/structure but not a perfect fit to the substrates it catalyzes.

However, when the substrate binds to the enzyme, it induces a change in the conformation/structure of the enzyme and its active site. The **change in conformation/structure** allows the **active site** to be moulded into a **more precise fit for the substrate**, enabling the enzyme to perform its catalytic function most effectively.

The 3D conformation/structure of the enzymes reverts to its original state upon completion of the reaction and release of the product molecules.

9. Energy Profile of Enzymatic Reactions

Notes to self

A. Lowering the Activation Energy (EA) barriers

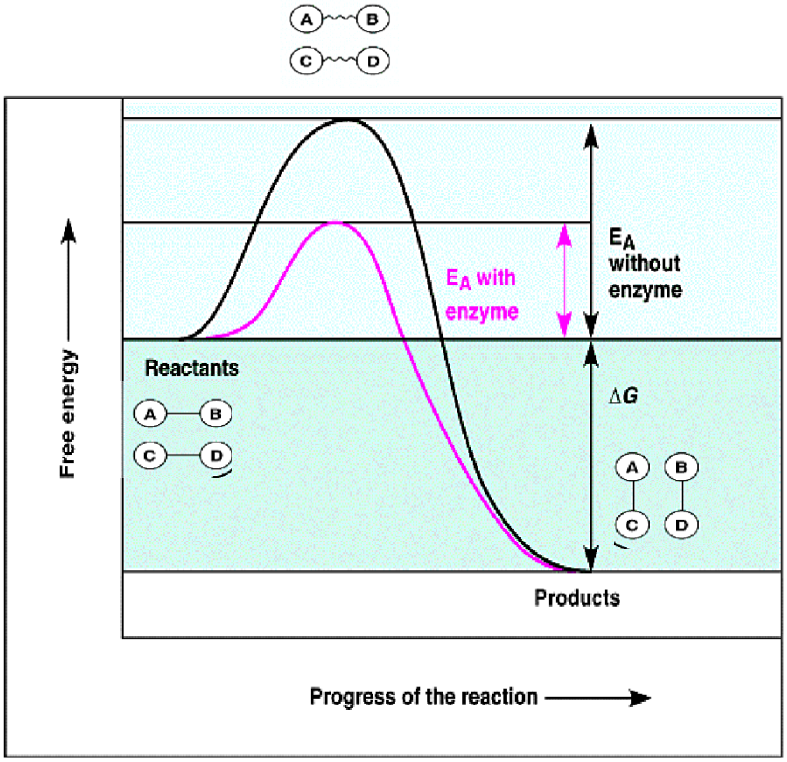


Fig. 8.1: The energy profile diagram of an enzyme catalysed reaction

The graph shows a hypothetical reaction:

$$AB + CD \xrightarrow{\text{enzyme}} AC + BD$$

Energy is needed to contort the reactant molecules so that bonds can change. Initial investment of energy = **Free energy of Activation** (or **Activation energy**) = **E_A.** Reactants AB and CD absorb energy from the surroundings. Energy comes in the form of heat from their surroundings.

The absorption of thermal energy leads to:
An increase in the kinetic energy of reactant molecules for more forceful collisions.
An increase in frequency of collision of reactants.
Thermal agitation of the atoms in molecules makes bonds more likely to break.

Without affecting the free energy (ΔG) for the reaction, an enzyme speeds up the reaction by reducing the uphill climb to the transition state at the peak of the graph. At the peak, reactant molecules have absorbed sufficient free energy to react and are unstable = Transition state. Bonds within the reactants can then break once they have absorbed enough energy to become unstable.

Notes to self

As bonds break and new bonds form, the molecules settle into their new bonding arrangements and energy is released to the surroundings = exergonic reaction. Exergonic reactions are those in which the free energy of the final state is less than the free energy of the initial state.

Enzymes **lower** the **activation energy** (E_A) barrier enabling reactant molecules to reach the transition state at moderate temperatures. An enzyme cannot change the ΔG (free energy change) for a reaction.

B. Molecular Basis of Enzyme Action

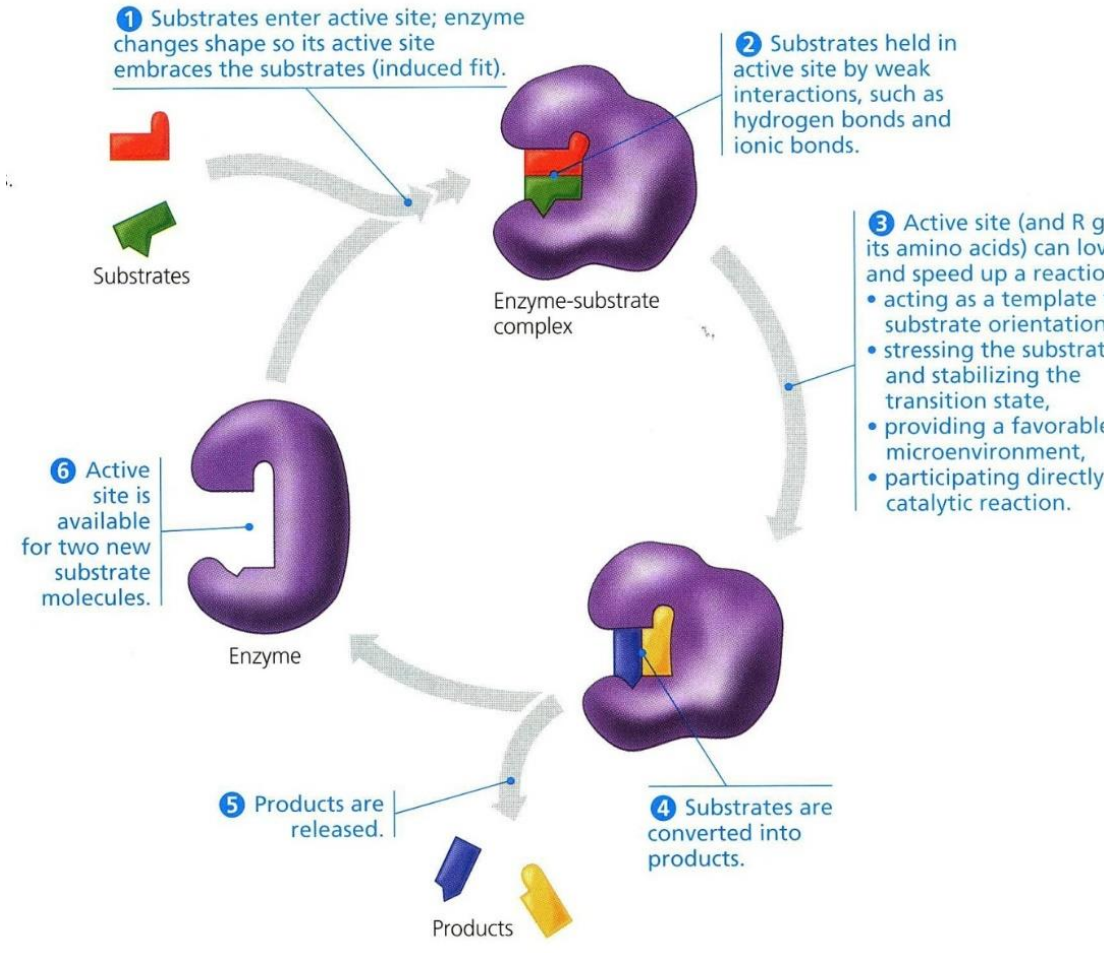
Notes to self

The following molecular mechanisms contribute to a **lowering of activation energy**. One or more of these mechanisms may work simultaneously in catalysis.

Method	Comment
1. Proximity effects	Temporary binding of reactants next to each other in the enzyme active site increases the chance of a reaction. Uncatalysed reactions depend on random collisions between reactant molecules.
2. Strain effects	Slight distortion of the reactants as they bind to the enzyme. This strains the bonds which are to be broken and increases the chance of breakage.
3. Orientation effects	Reactants are held by the enzyme in such a way that bonds are exposed to chemical reactions.
4. Microenvironment effects	Hydrophobic amino acids create a 'water-free' zone in which non-polar reactants may react more easily.
5. Acid-base catalysis	Acidic and basic amino acids in the enzyme facilitate catalysis. *(Knowledge of detailed mechanism is not needed.)*

Fig. 8.3: Mechanisms of enzymatic lowering of activation energy of reactions.

Summary:



10. Investigation of Enzyme-catalysed Reactions

Notes to self

In this section, we will learn about how to follow the time course of an enzyme-catalyzed reaction by measuring the formation of products (for example using catalase) or the disappearance of substrate (for example using amylase) over time.

A. Measuring the rate of product formed over time

Example: Investigating the conversion of hydrogen peroxide to water and oxygen by the enzyme catalase, over time.

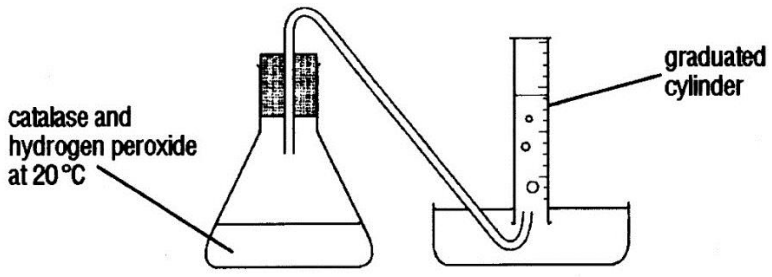
$$2H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

Independent variable: e.g. pH, temperature, concentration of substrate, concentration of enzyme (any one of these can be varied depending on the purpose of the experiment).

Dependent variable: Volume of O_2 evolved.

Constant conditions: Other than independent variable (i.e. what you are testing), all other conditions have to be kept constant.

Possible experimental set-up:



Tease your Brain

The experiment can be improved by attaching the delivery tube in the above set-up directly to a frictionless gas syringe. Why would this be a better approach?

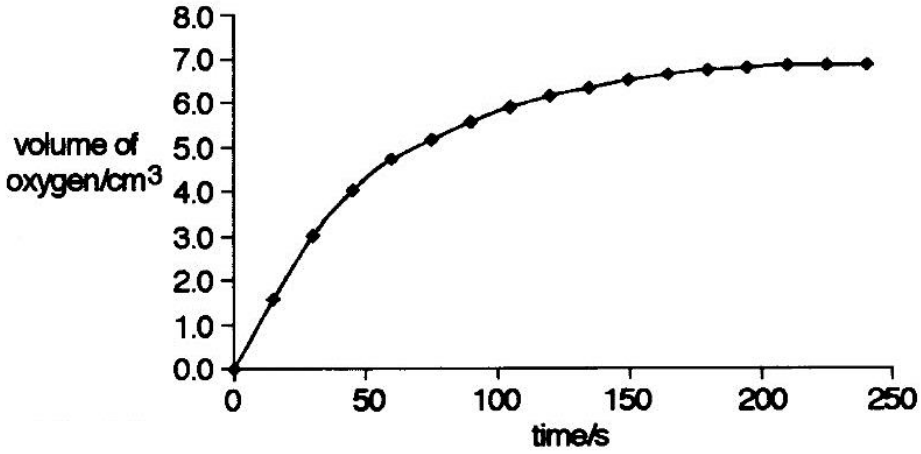
Procedure:

Notes to self

1. Add enzyme (catalase) to H_2O_2, mix and start the stopwatch.
2. O_2 evolved can be measured by the downward displacement of water in a graduated cylinder (as shown above) or using a frictionless gas syringe.
3. Record volume of O_2 evolved at fixed time intervals.

Record your data and present it in a time-course graph:

Time/s	Volume of Oxygen Collected/cm^3
15	1.5
30	3.0
45	4.0
60	4.8
75	5.3
90	5.6
.	.
.	.
.	.



Trend:

1. Volume of O_2 evolved increases with time.

2. Rate of O_2 production decreases with time.

Questions:

1. With reference to the above graph, how is the rate of O_2 production determined?

2. How is the conclusion of decrease in rate of O_2 production with time made?

Explanation:

Notes to self

Both the substrate and enzyme molecules move around freely in the solution. They collide with each other and quite often, a substrate molecule collides with an enzyme molecule in such a way that it fits into the enzyme's active site – an **effective collision**.

At time zero, the rate of reaction is at its maximum (steepest). This is because the **concentration of substrates** is the **highest** and the chances of an effective collision between an enzyme and a substrate molecule is highest i.e. **higher frequency of effective collisions** between enzyme and substrate molecules

Therefore, **rate of formation** of **enzyme-substrate (ES) complexes** is also the **highest** and the **rate of reaction** is at its **maximum**.

As time progresses, less substrate molecules remain in solution i.e. substrate concentration decreases, so there are less of them to collide with the enzyme molecules. Eventually all of the substrate molecules are changed into product molecules and the graph showing product formation becomes horizontal. The rate of reaction is now zero.

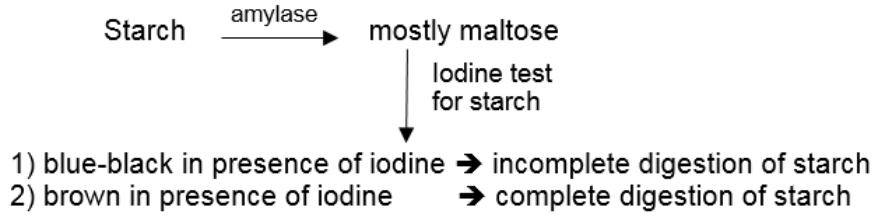
Question:

In the space below, sketch a graph of the rate of reaction against time based on the experiment above. The dependent variable for this experiment is the rate of O_2 evolved.

B. Measuring the rate of disappearance of substrate

Notes to self

Example: Conversion of starch to reducing sugars by the enzyme amylase



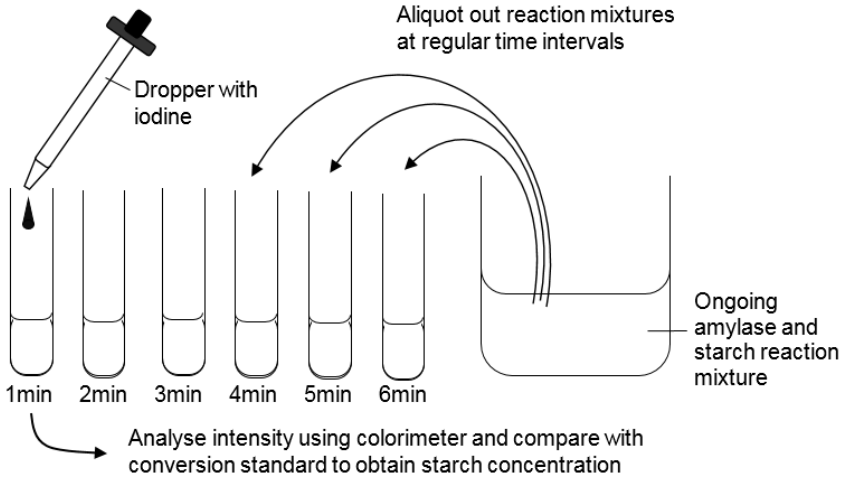
Independent variable: e.g. concentration of enzyme, pH, temperature, concentration of inhibitor

Dependent variable: Starch concentration

Measured quantity: Intensity of blue-black colouration

Constant conditions: Other than independent variable (i.e. what you are testing), all other conditions have to be kept constant

Experimental set-up:

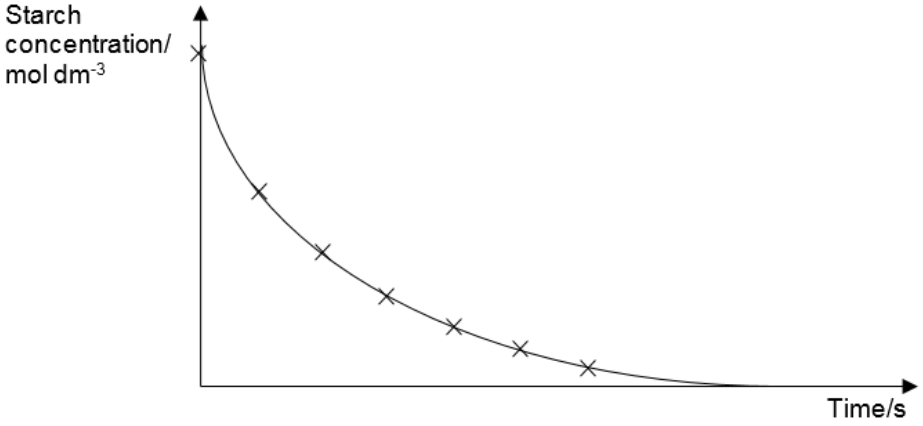


Procedure:

1. Add enzyme (amylase) to starch and start the stopwatch immediately.
2. At fixed time intervals, aliquot 1cm^3 of the reaction mixture to a test tube and add 3 drops of iodine to the reaction mixture.
3. In the presence of starch, iodine turns from yellowish brown to blue-black.
4. Intensity of the blue-black colouration can be measured using a colorimeter and this indicates the concentration of starch present.
5. Use a conversion standard to convert the colorimeter reading to starch concentration.

Record your data and present it in a time-course graph:

Notes to self



Trend:

1. Intensity of blue-black colouration / concentration of starch, decreases with time.

2. Rate of starch digestion decreases with time.

Explanation:

Notes to self

Same as for catalase reaction.

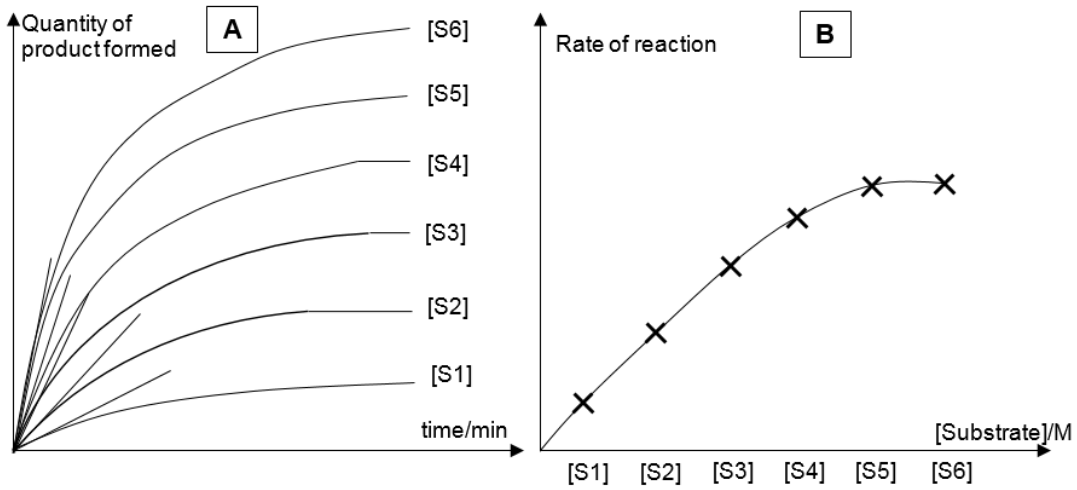
Sometimes you may be asked to investigate how the rate of enzyme reaction varies with one manipulated variable such as **substrate concentration [S].**

To do this you need to carry out several experiments at different substrate concentrations [S1], [S2], [S3], [S4], [S5] etc. To obtain a decent trend, it is recommended to have at least 5 different values for the independent variable. All other variables such as temperature and pH are kept constant and optimal.

Measure the progress of the reactions over fixed time intervals. Plot the graphs. **(Graph A)**

Find the initial rate at each concentration by finding the gradient of the tangent at time 0 min for each graph. Plot the graph of rate against [Substrate] **(Graph B).**

The graphs A and B should look like these below:



11. Factors Affecting The Rate of Enzyme Catalysed Reactions

Notes to self

A. Temperature

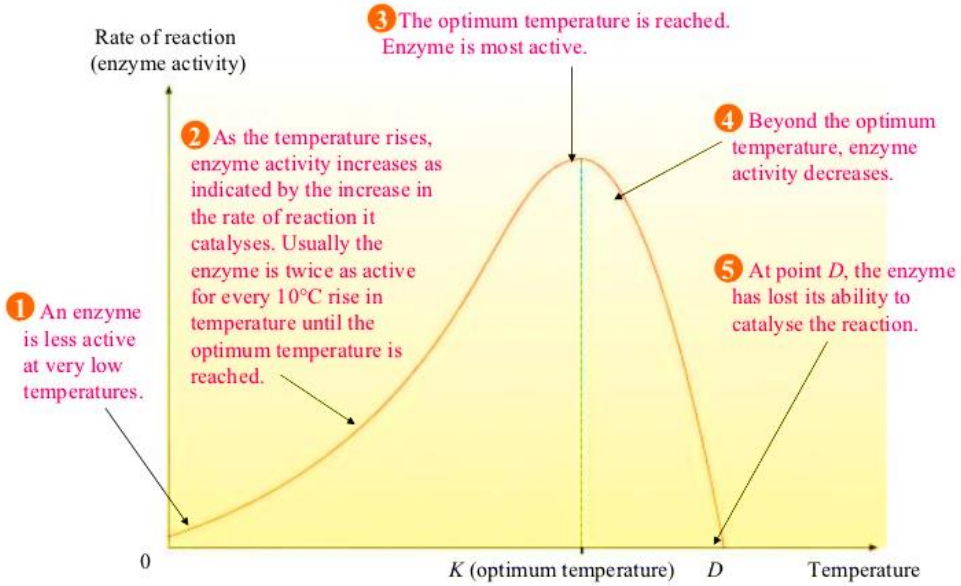


Fig 10.1: Effect of temperature on the rate of enzymatic reaction

Beginning at a low temperature, increase in temperature results in an increase in kinetic energy of the enzyme and substrate molecules, which will increase the frequency of effective collisions between substrate and enzyme active sites.

This results in an increase in rate of formation of enzyme-substrate complexes.

Increased kinetic energy also increased the number of molecules having sufficient energy to overcome the activation energy barrier to form the products of reaction.

Temperature coefficient (Q_{10}) = factor by which rate increases with each 10˚C rise in temperature

$$Q_{10} = \frac{\text{Rate of reaction at (X+ 10) ºC}}{\text{Rate of reaction at X ºC}}$$

For enzyme-catalysed reactions between 4ºC to optimum temperature i.e. physiological temperatures, the rate of chemical reaction doubles for each 10ºC rise in temperature (Q_{10}). → Q_{10} = 2 for most typical enzyme reactions.

Reaction rate increases with temperature only until the optimal temperature of the enzyme is reached. Each enzyme has an optimal temperature at which the rate of enzyme reaction proceeds at a maximum rate (humans 25 – 40^{o}C).

Notes to self

Some enzymes have a higher optimum temperature. They tend to have a higher proportion of disulfide bonds (strong covalent bonds) or numerous intramolecular interactions that hold the tertiary structure of the enzyme together.

The increase in kinetic energy at temperatures beyond the optimum temperature causes intramolecular vibrations to increase. This breaks hydrogen, ionic bonds and other weak interactions, such as hydrophobic interactions, that stabilises the conformation/structure i.e. denaturation. (At higher temperatures, covalent disulfide bonds may also break.)

The substrate is no longer complementary to the conformation/structure of active site of the enzyme. Failure of the substrate to fit into the enzyme active site results in fewer enzyme-substrate complexes being formed, resulting in the lowering of the rate of reaction.

B. pH

Notes to self

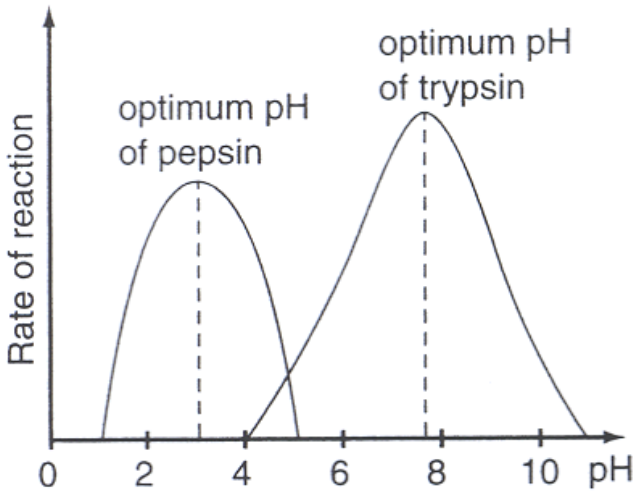


Fig. 10.2: Effect of pH on the enzymatic activity

Each enzyme has an optimal pH at which it is most active. The rate of reaction is maximum at the optimal pH.

For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 3. Other enzymes, designed to work at neutral (e.g. trypsin) or alkaline pH, are denatured by such an acidic environment.

Deviation from the optimum pH results in the lowering of the rate of reaction. Excess $[H^+]$ or $[OH^-]$ ions may affect the ionisation of the R-groups/side chains of charged amino acids e.g. where excess H^+ results in $–COO^-$ groups becoming -COOH and excess $-OH^-$ results in $-NH_3^+$ becoming $–NH_2$.

If these affected amino acids are;

1. Structural amino acid residues, it may result in the disruption of ionic and hydrogen bond formation which determine the tertiary structure of the protein. This changes the specific 3D conformation/structure of the enzyme active site. The enzyme is denatured.
2. Contact and catalytic amino acid residue in the active site, enzyme-substrate interaction may be disrupted and catalysis may not take place. E.g. catalytic activity may require that an amino group of the enzyme be in the protonated form ($-NH_3^+$). At alkaline pH, this group is deprotonated so catalysis cannot take place.

3. Part of the protein substrate (in the case where the substrate is a protein), charges on its residues will change and this will affect substrate interaction with the enzyme active site and/or catalysis as well.

Notes to self

The end result is a reduced rate of enzyme-substrate complex formation, resulting in a reduced rate of reaction.

C. Enzyme Concentration

Notes to self

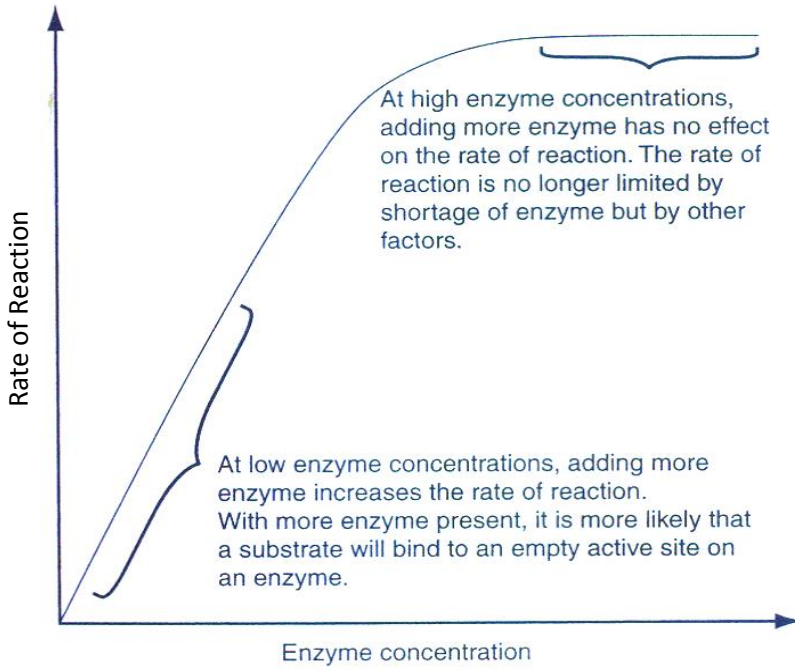


Fig 10.3: Effect of enzyme concentration on rate of reaction

The rate of an enzyme-controlled reaction is dependent on the frequency of effective collisions between molecules of enzyme and substrate.

With increased enzyme concentration, frequency of effective enzyme-substrate collisions increases resulting in increased rate of formation of enzyme-substrate complexes and reaction rate will increase.

At the linear portion of the graph, enzyme concentration is limiting. Any increase in enzyme concentration will result in a proportional increase in rate of reaction.

At the curved portion of the graph, enzyme concentration is not the only limiting factor. Some other factor is also limiting.

At the plateau, enzyme concentration is no longer the limiting factor. Other factors are limiting the rate of reaction. Increasing enzyme concentration no longer increases the rate of reaction

D. Substrate concentration

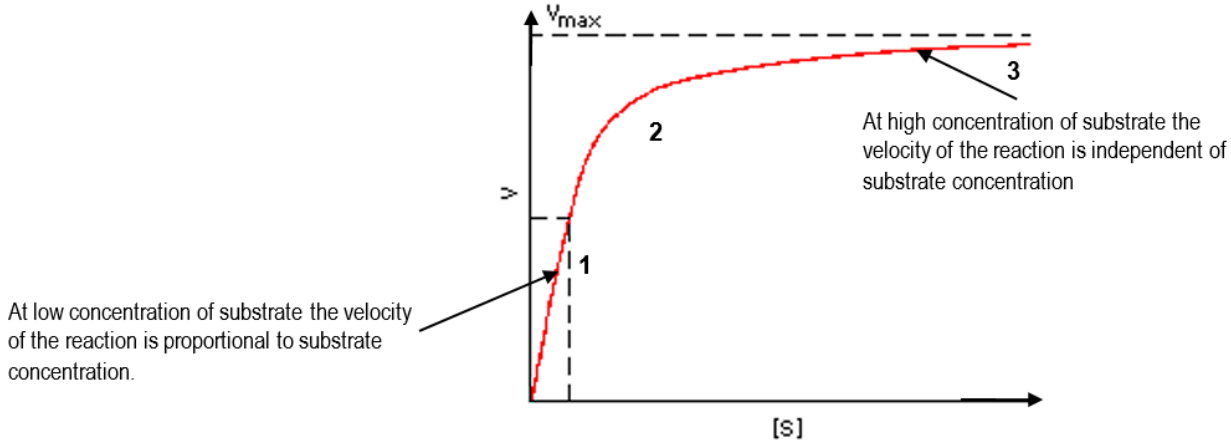


Fig. 10.4: Effect of substrate concentration on reaction velocity for an enzyme catalysed reaction

At point **1** of graph, the **rate** of reaction **increases proportionally** with an **increase in [S]**. Frequency of effective collision between enzyme and substrate molecules increases. Rate of enzyme-substrate complex formation increases which results in an increase in rate of reaction. This is because at low [S], the active sites of the enzymes are readily available to catalyze the reaction and the **substrate concentration is limiting**.

At point **2** of graph, enzyme active sites start to get **saturated** which limits the rate of reaction.

At point **3** of graph, a **plateau** is reached. **Enzyme saturation** is reached where all available active sites are occupied by substrate molecules. Substrate concentration is no longer limiting. Instead **enzyme concentration is limiting**. (Further increase in substrate concentration will not cause the rate of reaction to increase further.) Rate of reaction has reached its **maximum velocity** (V_{max})**.**

Michaelis constant (K_m) = the concentration of substrate required to make the reaction attain half its maximum rate (½ V_{max}).

Notes to self

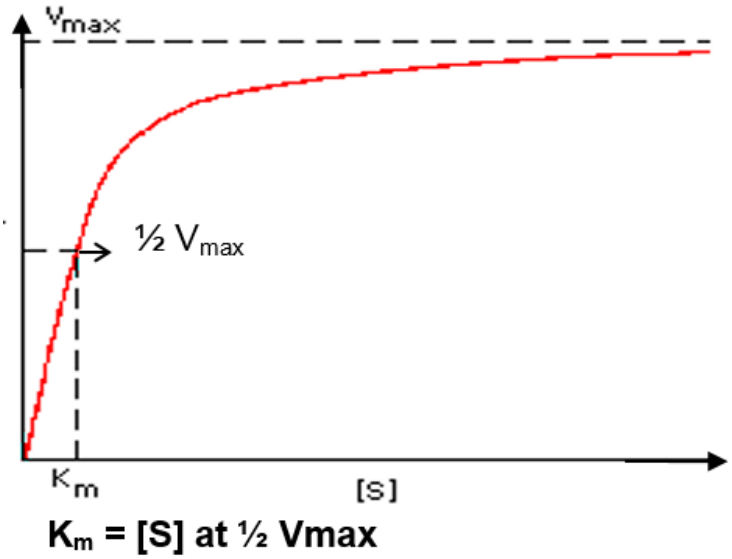


Fig. 10.5: The Km and Vmax values of an enzymatic reaction

K_m is always the same for a particular enzyme, but varies from one enzyme to the other. It is a measure of the **affinity** of the enzyme for its substrate.

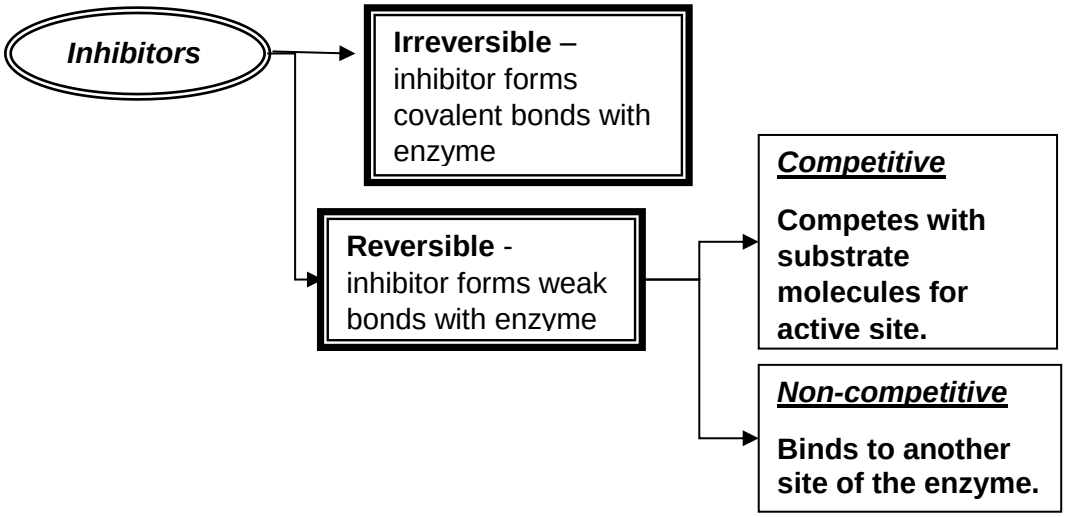
Low K_m	**High K_m**
High affinity between enzyme and substrate.	*Low affinity* between enzyme and substrate.
Low substrate concentration needed to attain half maximum velocity.	High substrate concentration needed to attain half maximum velocity.

12. Enzyme inhibition

Notes to self

Enzyme inhibitors

Some chemicals selectively inhibit the rate of enzyme-controlled reactions.



A. Competitive Inhibition

Competitive inhibitors, when bound to enzyme, prevent substrate molecules from binding to the enzyme active site. In most cases, competitive inhibitors bear **similar structure/conformation and charge to the substrate** thus **competes with substrate** for **active site**.

Most competitive inhibitors bind reversibly to the active site. The bonds involved are weak, non-covalent bonds. This reduces the availability of enzyme active sites for substrate binding and hence reduces the rate of the reaction.

Inhibition can be overcome by **increasing the substrate concentration, [S]**. This increases the chances of substrate binding to active site instead of inhibitor binding. At sufficiently high [S], reaction velocity reaches the same V_{max} observed as in the absence of inhibitor.

Notes to self

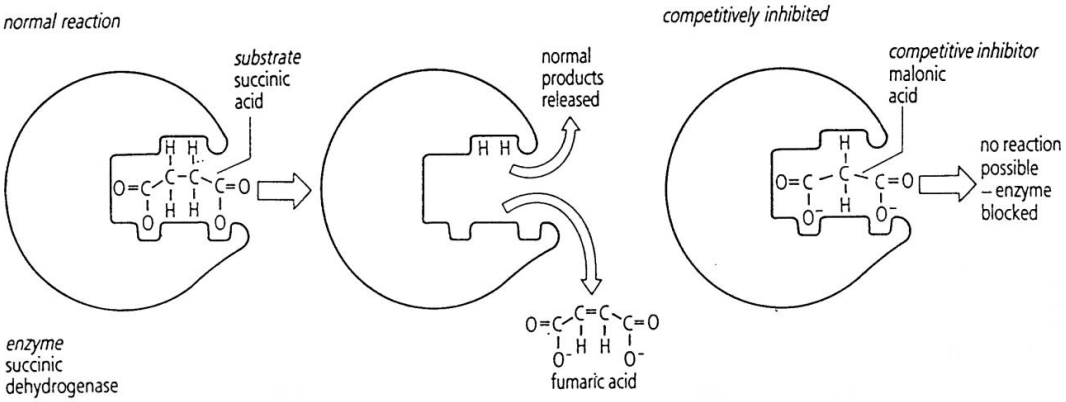


Fig. 11.1: Competitive inhibition of succinate dehydrogenase (SDH) by malonate

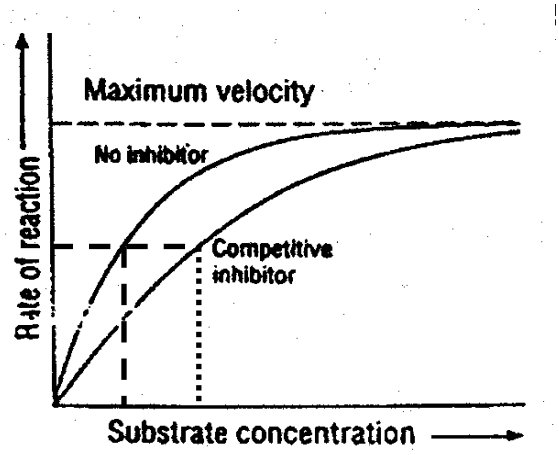
B. Non-Competitive Inhibition

The **non-competitive inhibitor** bears **no structural similarity** to the substrate molecule so it binds to a **site other than the enzyme active site.** This is sometimes also known as the allosteric site. The binding of the non-competitive inhibitor **alters the conformation/structure** of the **specific** enzyme **active site** thus the **substrate cannot bind** to active site in the correct orientation, leading to the decrease in the rate of reaction.

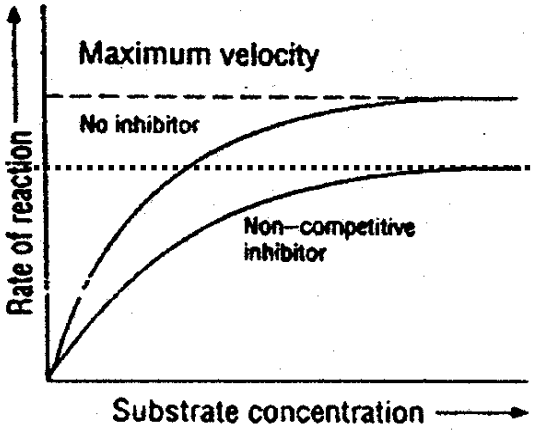
Non-competitive inhibitors effectively decrease the availability of enzymes as enzyme-inhibitor complexes are no longer able to be formed as the structure of the active site is altered.

Effects of non-competitive inhibitors **cannot be overcome by high concentration of substrate.** The rate of reaction will continue to decrease with increasing inhibitor concentration. When inhibitor saturation is reached, the rate of reaction will be almost nil.

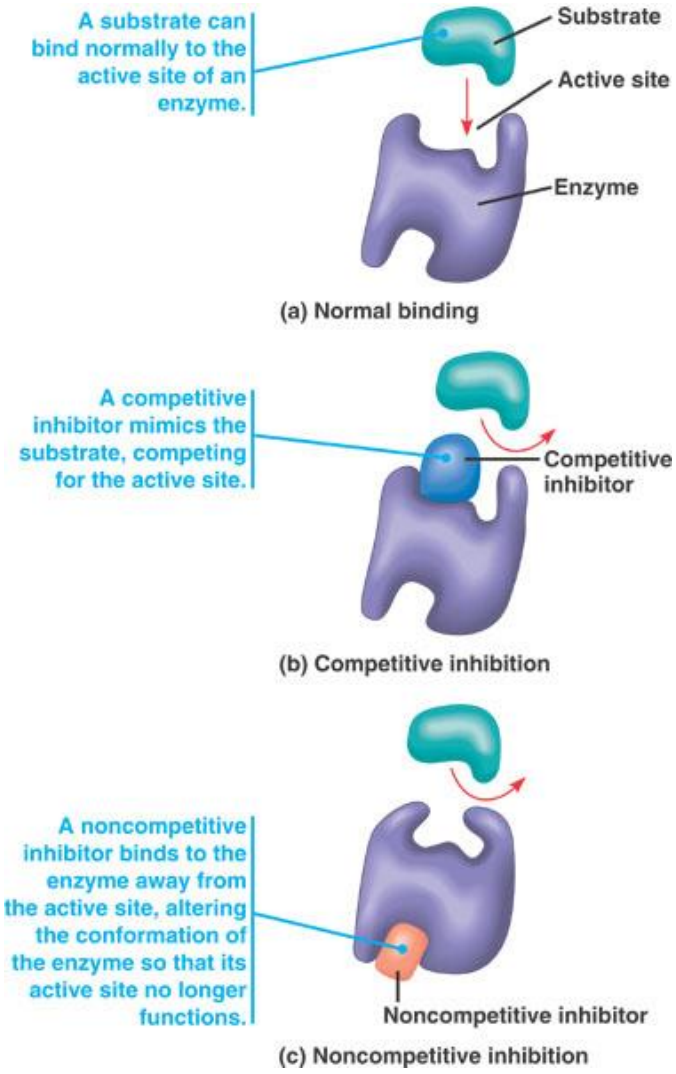
Summary – Competitive inhibition & Non-competitive inhibition



- **Competitive inhibition**



- **Non-competitive inhibition**



Effect of competitive inhibitor	Effect of non-competitive inhibitor
V_{max} the same	Decrease V_{max}

C. Allosteric Inhibition

Allosteric enzymes can be regulated by inhibitors and activators.

Allosteric enzymes usually consists of **two or more subunits** where each subunit has its own:

1. Active site that binds substrates,
2. *Allosteric site* that binds activators or inhibitors

These sites are at different locations within the same subunit. We will focus on the effects of allosteric inhibition.

Allosteric enzymes can exist in two conformational states. The binding of an allosteric inhibitor stabilizes the inactive conformation of the enzyme. A single inhibitor is sufficient to inhibit the activity of the enzyme.

The effect of an allosteric inhibitor on the rate of reaction is opposite to that of increasing substrate concentration. Hence, in the presence of an allosteric inhibitor, the same V_{max} can be reached at higher substrate concentrations.

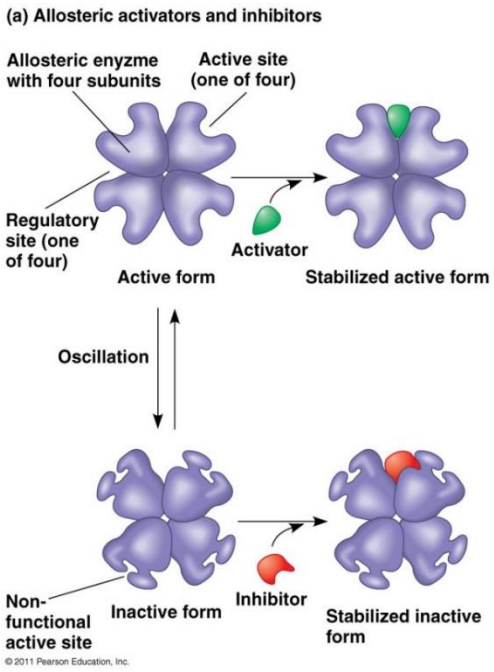


Fig 12.1: Allosteric activators & inhibitors

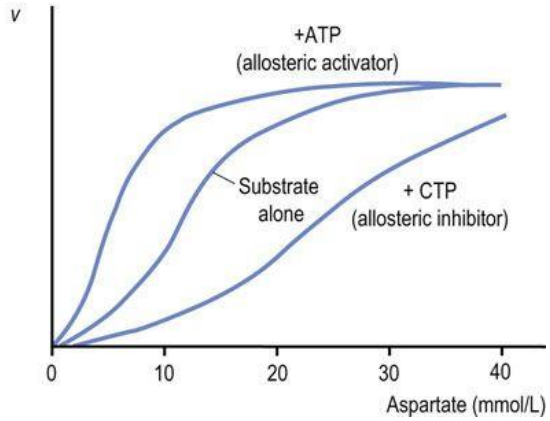


Fig. 12.2: Effect of CTP as an allosteric inhibitor of ATCase. Aspartate is the substrate of ATCase.

Question: How is allosteric inhibition different from non-competitive inhibition?

- Allosteric enzymes are multimeric while enzymes regulated by non-competitive inhibition may be made up a single polypeptide only.
- Allosteric enzymes oscillate between active and inactive conformations and are stabilised in their inactive conformation by allosteric inhibitors.
- An enzymatic reaction regulated by allosteric inhibition usually displays a sigmoidal trend (in a velocity vs substrate concentration graph) as allosteric inhibitors display cooperativity in regulation.

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Notes to self

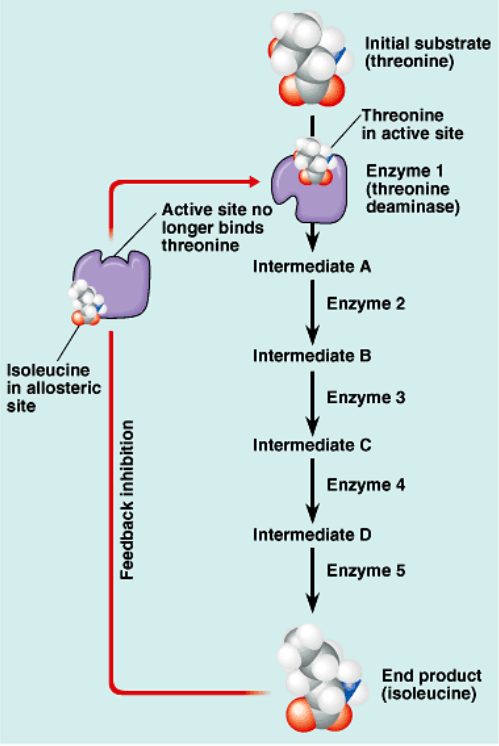
Feedback/End-product Inhibition

In end-product inhibition, a metabolic pathway is inhibited by the binding of the end product of a biochemical pathway to an enzyme that acts early in the pathway. e.g. amino acid isoleucine is produced from threonine.

As isoleucine (end product) accumulates it inhibits enzyme threonine deaminase in the first step of the reaction by binding to the allosteric site of the enzyme. Hence, the end product alters the conformation/structure of the specific enzyme active site thus substrate cannot bind to active site in the correct orientation, so rate of reaction is decreased.

This prevents the cell from wasting resources in producing excess isoleucine.

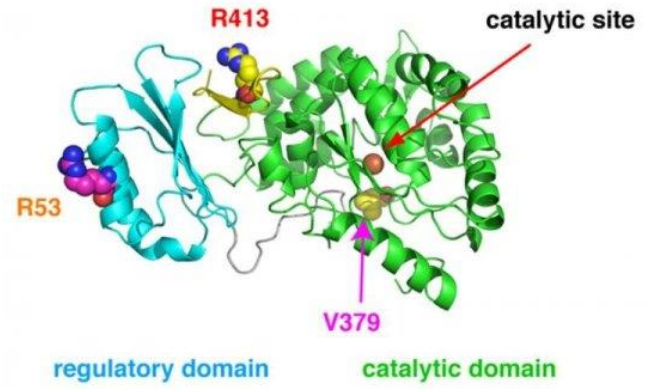
Note: Although Fig. 13.1 (below) shows threonine deaminase to be a monomeric enzyme it is actually multimeric as with most allosteric enzymes.



Notes to self

Practices of Science

New Research Finding: Genetic diversity of enzymes alters metabolic individuality



Scientists from Tohoku University's Tohoku Medical Megabank Organization (ToMMo) have published research about genetic diversity and metabolome.

In their analyses, researchers found the following results:

- The relationship between structural variants of enzymes and metabolic phenotypes in the human population was surveyed in the association study of metabolite concentrations with whole genome sequence analysis data.
- Five associations between metabolites and gene variants were identified. Four of the gene variants are known to be related to metabolic diseases. The residues substituted by these variants are located in peripheral regions of the catalytic sites or related regulatory domains of enzymes.
- Two people have larger changes of metabolite levels of phenylalanine. They had rare gene variants, which substitute residues located near the catalytic site.
- These data demonstrate that variant frequency, structural location and effect for phenotype correlate with each other in the human population.

ToMMo will study environmental and genetic influence on individual differences of proteomics and metabolomics. ToMMo aims to discover useful biomarkers for disease prevention and early diagnosis through the identification and quantification of metabolites in blood. Such studies can contribute to the advancement of personalized prevention and treatment of diseases, as well as the identification of disease mechanisms and development of new therapeutics.