River Valley High School
2025 JC1 H2 Biology

Lecture Topic 6: Proteins - Enzymes

Name: _____ ( ) Class: 25J__ Date: _____

References

Title	Authors
Biology (9th Edition)	Campbell and Reece
Longman A-Level Course in Biology: Core Syllabus Volume 1	Hoh
Biological Science 1. Organisms, Energy and Environment (3rd Edition)	Taylor, Green, Stout and Soper
Harper's Illustrated Biochemistry (27th Edition)	Murray, Granner and Rodwell

H2 Biology Syllabus 9477 (2025)

Candidates should be able to use the knowledge gained in the following section(s) in new situations or to solve related problems.

Related Topics	Content
Proteins	Mode of action of enzymes
	Enzyme inhibitors

Learning Outcomes

1C. Proteins

p. 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 hypotheses.

q. 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 (e.g. measuring gas produced using catalase) or rate of disappearance of substrate (e.g. using amylase, starch, and iodine).

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

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

Lecture Outline

I. Importance of Enzymes

II. Nature of Enzymes
- A. Terminology
- B. Structure
- C. Property

III. Mode of Enzyme Action
- A. Activation Energy
- B. Mechanism of Enzyme Action
- C. Enzyme Specificity

IV. Measuring Rate of Enzyme-Catalysed Reaction

V. Factors Affecting Rate of Enzyme-Catalysed Reactions

A. Enzyme Concentration
B. Substrate Concentration
C. Temperature
D. pH

VI. Enzyme Inhibition

A. Competitive Inhibition
B. Non-competitive Inhibition
C. Allosteric Regulation

VII. Enzyme Cofactors

A. Inorganic ions
B. Coenzymes

Websites

URL	Description
https://www.youtube.com/watch?v=qgVFkRn8f10	Summary video on enzymes
https://www.youtube.com/watch?v=6EDBlowVST0	Summary video on enzyme reactions

I. Importance of Enzymes

- Industrial processes use catalysts to speed up the production of important chemicals, reducing the need for high temperatures and pressures.
- The reactions inside living organisms must take place at atmospheric pressure and much lower temperatures when compared with industrial reactions.

  At the human body temperature of 37°C, reactions would be too slow to sustain life without catalysts.
- **Extracellular enzymes** are secreted by some cells to **catalyse** reactions.

  Digestive enzymes (e.g. amylase, peptidase, lipase) secreted by the pancreatic acinar cells into the duodenum *catalyse* the breakdown of food into smaller molecules.
- All cells contain **intracellular enzymes** to **catalyse** biochemical reactions taking place within the cell.
- The chemical reactions of life are collectively known as **metabolism**, which comprises:
  - **Anabolic** reactions, in which complex compounds are <u>synthesised</u> from simpler ones, usually with energy absorption.

    E.g. Condensation reactions such as synthesis of starch from glucose
  - **Catabolic** reactions, in which complex compounds are <u>broken down</u> into simpler ones, usually with energy release.

    E.g. Hydrolytic reactions and oxidative reactions such as cellular respiration.

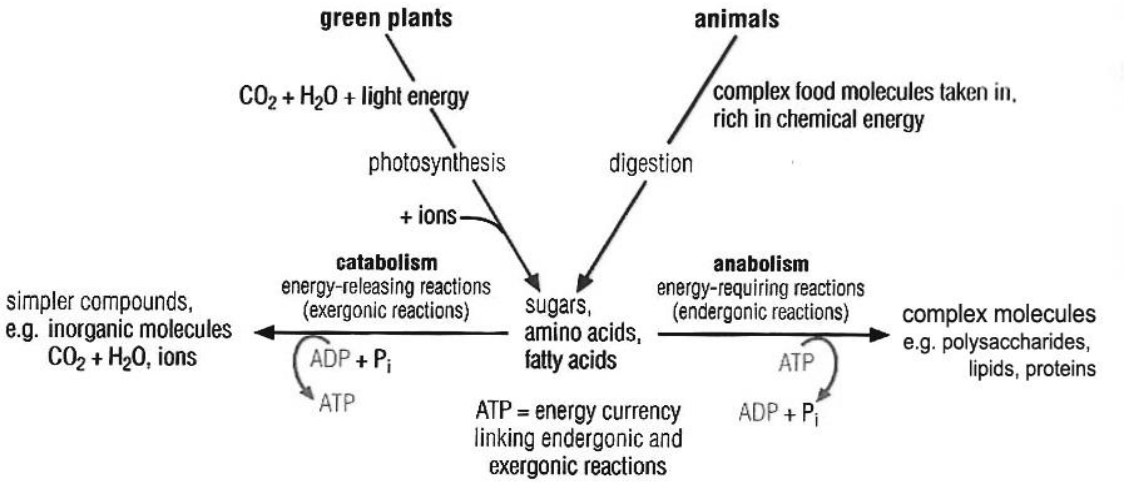
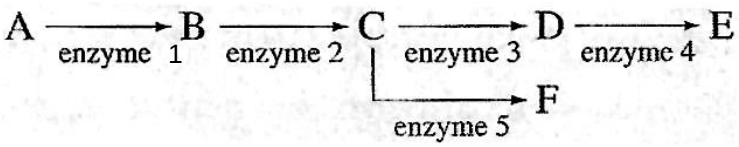


Figure showing Metabolism = anabolism + catabolism

Source: Advanced Biology Principles and Applications (2nd Edn). pp.170

- A sequence of metabolic reactions makes a **metabolic pathway**. The molecules involved in the steps in the pathway are known as **metabolites** or **intermediates**. E.g. Enzyme 1, 2, 3, 4 and 5 are enzymes involved in the metabolic pathway below. A is the precursor, B, C, D are the



In a metabolic pathway,

- a specific enzyme catalyses each step
- an intermediate can be used as a starting point for many other pathways.
  E.g. Intermediate **C** can be used to produce **D** and **F**, where both pathways use different enzymes
- enzymes control the chemical reactions that occur within cells to ensure that they proceed in an orderly manner and at a sufficient rate.

Table 8.2 Industrial and medical uses of enzymes: many of today's uses of enzymes are recent developments, but the use of enzyme preparations in industry has a very long history

Application	Enzyme	Use, and notes
1 Dairy industry	rennin from calves, lambs etc.	used in cheese manufacture to coagulate milk proteins; rennin of microbial origin is now commonly used, obtained from genetically engineered bacteria
2 Brewing industry	amylase from germinating barley, protease	breakdown of starch to glucose for fermentation by yeast in brewing, and breakdown of proteins to amino acids for benefit of yeast; enzymes of microbial origin are now used to prevent cloudiness during storage of beers (protease) and to produce 'low calorie' beers (amyloglucosidase)
3 Baking industry	protease	breakdown of proteins in flour for biscuit manufacture
	amylase	breakdown of some starch to glucose in flour for white bread, buns and rolls
4 Biological detergents	protease, lipase, amylase	removal of organic stains (e.g. food, saliva, blood) from clothes
	amylase	removal of starch residues in dishwashing machines
5 Leather tanning industry	protease in dog and pigeon faeces	treatment (called 'bating') of hides to remove hair and make the leather pliable; now replaced by enzymes obtained from slaughter houses and (most recently) of microbial origin
6 Textile industry	amylase	removal of the starch that is applied to the threads of some fabrics to protect from mechanical damage during weaving
7 Processed foods	amylase	manufacture of glucose syrup from starch
	glucose-isomerase	manufacture of fructose syrup from glucose, for 'low calorie' sweetening of manufactured foods (USA and Japan)
	trypsin	pre-digestion of some baby foods
8 Forestry and paper industry	ligninases from fungi	removal of lignin from pulverised wood, prior to use of wood cellulose in manufacturing processes
	amylase	partial breakdown of starch for sizing of paper – filling the gaps between the fibres to produce a smooth, 'quality' paper
9 Photographic industry	protease	digestion of gelatin of old film to allow recovery of silver (current 'film' and photographic 'papers' are actually plastics)
10 Medical/ pharmaceutical uses and analytical chemistry	trypsin	removal of blood clots, and in wound cleaning
	various enzymes	used in biosensors, e.g. for blood glucose etc.

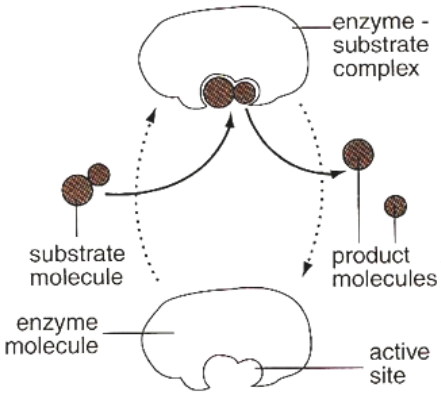
II. Nature of Enzymes

A. Terminology

- Enzymes are **biological catalysts** because they are protein / RNA molecules synthesised by living cells.

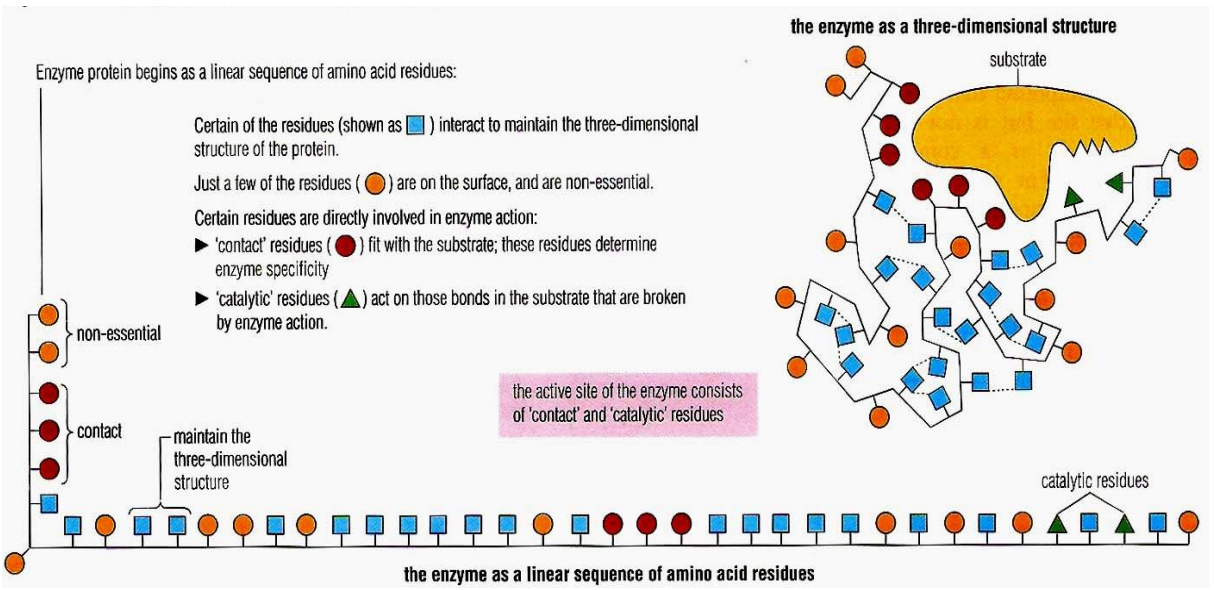
  As catalysts, enzymes speed up chemical reactions but remain unchanged at the end.
- The chemical(s) which an enzyme works on is called its **substrate(s)**.
- An enzyme combines with its substrate to form a short-lived **enzyme-substrate complex**.

  This proximity of the substrates in the complex greatly increases the probability / chances of a reaction occurring.
- Once a reaction has occurred, the complex breaks up into **product(s)** and enzyme.



- The enzyme <u>remains unchanged</u> at the end of the reaction and is free to interact again with more substrates.

B. Structure



- Only a small portion of the enzyme molecule (ranging from 3 to 12 amino acid residues) comes into direct contact with the substrate. This region is called the **active site** of the enzyme.
- The amino acid residues at the active site can be divided into two groups,
  - **contact residues**, which are responsible for the specificity of the enzyme and form a shape complementary to that of the substrate.
  - **catalytic residues**, which are responsible for the ability of the enzyme to catalyse a particular chemical reaction. They are responsible for the breakage and formation of bonds.
- The remaining amino acid residues, which make up the bulk of the enzyme, function to maintain the globular shape of the enzyme. This is essential for the optimal function of the active site.

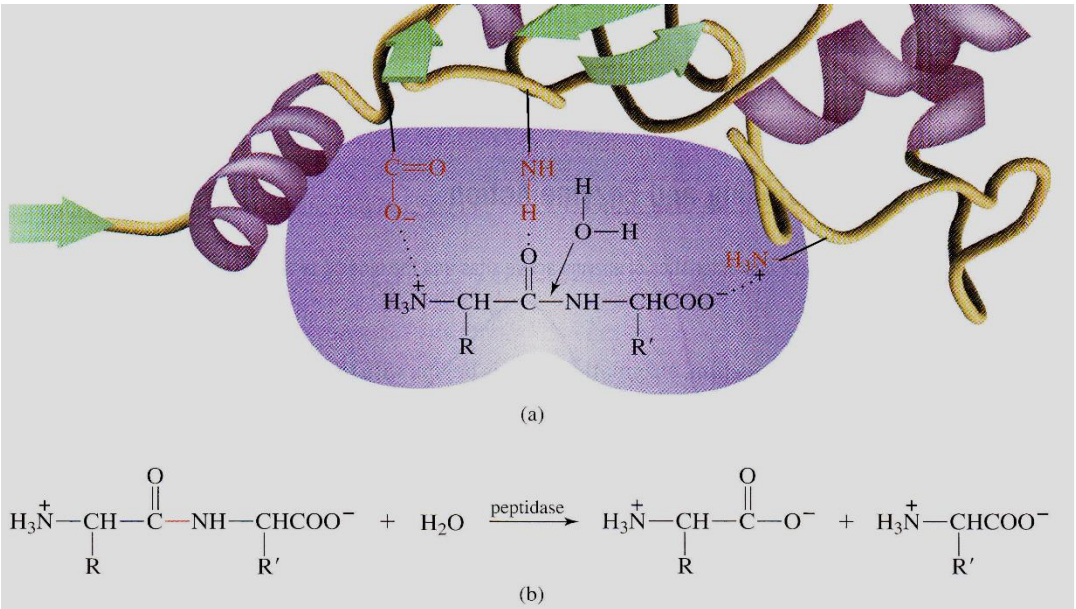


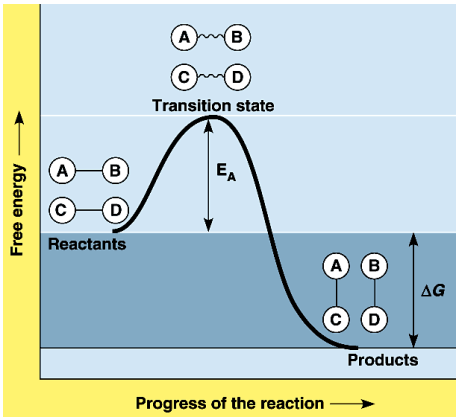
Figure 5.8 (a) The binding of a dipeptide substrate molecule to a hypothetical active site in a peptidase. Note the importance of noncovalent interactions (hydrogen bonds, ionic bonds) between substrate and enzyme molecule. The arrow identifies the scissile bond and shows where the peptide bond is attacked by water. The amino and carboxyl groups shown are side chains of amino acid residues of the peptidase. (b) A reaction showing the dipeptide in (a) being hydrolyzed to amino acid products.

C. Property

- Most enzymes are <u>globular proteins</u>. Other enzymes are RNA in nature (e.g. ribozyme).
  - Being proteins or RNA in nature, enzymes are coded for by DNA. *(KIV: Transcription & Translation)*
- Enzymes are <u>catalysts</u>.
  - They lower the **activation energy** of reactions they catalyse.
- Enzymes are <u>not changed or consumed by the reaction</u>; hence they can be used over and over again.
- The catalysed reaction is <u>reversible</u>.
- Enzymes are <u>highly efficient</u>.
  - A very small amount of catalyst brings about the change of a large amount of substrate. E.g. one molecule of the enzyme catalase can catalyse the decomposition of 600 000 molecules of H_2O_2 per second at body temperature.
- Enzymes are <u>highly specific in action</u>. E.g. catalase will only catalyse the decomposition of H_2O_2.
  - The specificity of the enzyme is attributed to a compatible fit between the shape of its active site and the shape of its substrate.
- Enzyme activity is affected by
  - enzyme concentration
  - substrate concentration
  - temperature
  - pH

III. Mode of Enzyme Action

A. Activation Energy



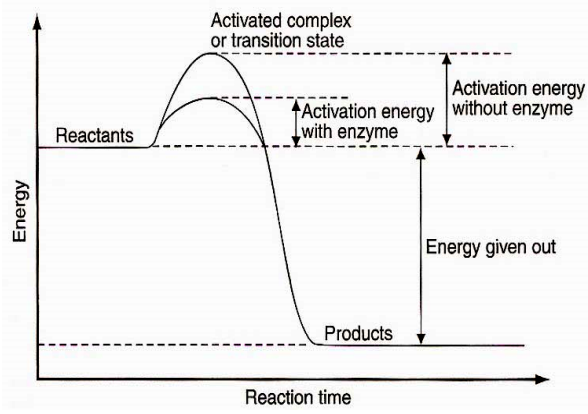
- The laws of thermodynamics predict what will and will not happen under given conditions. They do not predict the rate of these processes.

  However, consider a mixture of petrol and oxygen maintained at room temperature. Although a reaction between the two substances is thermodynamically possible, it does not occur unless energy is applied to it, such as a simple spark.

  Energy must be supplied to get the reaction started.

  This is called the **activation energy**, which is required to make substances react.
- Activation energy represents the <u>energy barrier</u> that has to be overcome before a reaction can take place to form products.
- The greater the activation energy, the slower the reaction at any particular temperature.
- If the activation energy of a reaction is decreased, the rate of reaction would be increased.

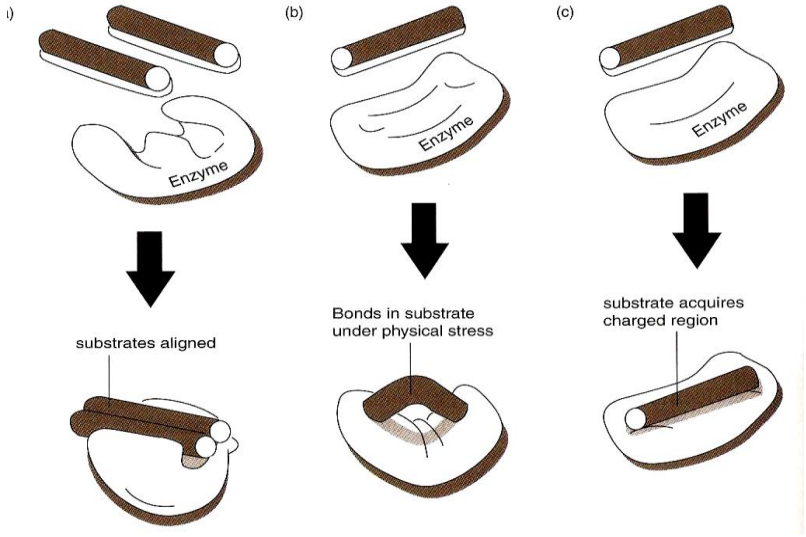
B. Mechanism of Enzyme Action



- The activation energy of a reaction can be supplied by heating the reactants and the reaction will proceed at a higher rate than without heating.

  This is not possible in living cells, which can only survive within a narrow range of relatively low temperatures (from just above freezing to almost 40 °C).
- An <u>enzyme lowers the activation energy required for a reaction</u>. It increases the rate of a reaction without altering, to any great extent, the temperature at which it occurs.

- How enzymes lower activation energy:



a) Substrate alignment
   - Collision between substrate and enzyme in the correct orientation causes the substrate molecule(s) to bind the enzyme molecule at its active site to form an enzyme-substrate complex.
   - The enzyme molecule thus holds the different substrate molecules (the reactants) in an arrangement that forces them <u>close together</u> in the <u>correct orientation</u>.
   - The <u>proximity of the substrates</u> within the enzyme-substrate complex greatly increases the probability of a reaction occurring.
   - Once the reaction has occurred, the complex breaks up to give (i) the product(s) and (ii) the unchanged enzyme molecule, which is then available to catalyse another cycle of reaction.
   - In the absence of enzymes, it is unlikely that the various substrate molecules will collide with each other in the correct orientation for the reaction to occur. The rate of reaction would thus be very low.

b) Bonds in substrate under physical stress
   - Once a substrate binds to the active site of an enzyme, certain bonds within the substrate molecule may be placed under <u>physical stress</u>.

     This increases the likelihood that the bonds will break.

c) Substrates acquire charged region
   - When the R-groups of amino acid residues at the active site are very close to part of the substrate, the R-groups can
     i. change the charge on the substrate, altering the distribution of electrons within the bonds of the substrate
     ii. cause other changes that will <u>increase the reactivity</u> of the substrate.

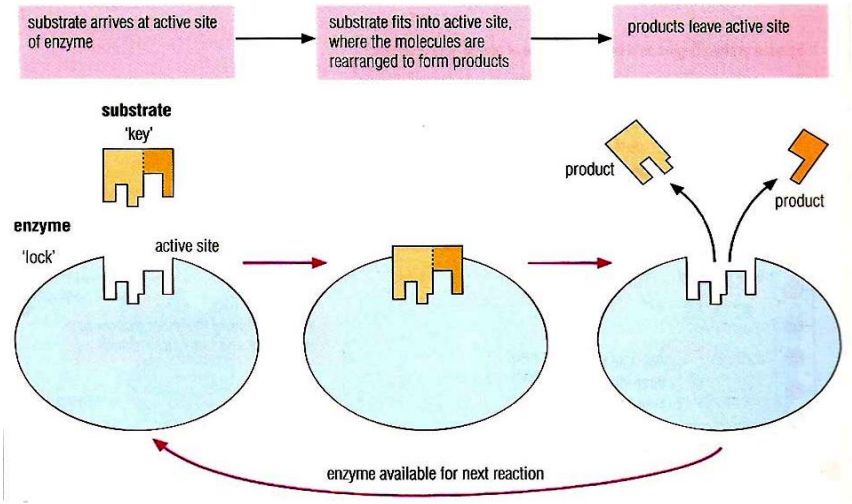
C. Enzyme Specificity

- Enzymes are highly specific in the reactions they catalyse, the specificity of which can be grouped as follows:
   - absolute specificity: the enzyme will catalyse only one particular reaction
   - group specificity: the enzyme will act on only on molecules that have specific functional groups, such as amino, methyl, phosphate groups.
   - linkage specificity: the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure
   - stereochemical specificity: the enzyme will act on a particular stereoisomer

- Enzymes are **specific** because:
  1. Only substrate of complementary shape will fit into the active site – **spatial fit**
  2. Enzyme and substrate must have charge or hydrophilic/hydrophobic complementarity – **chemical fit**

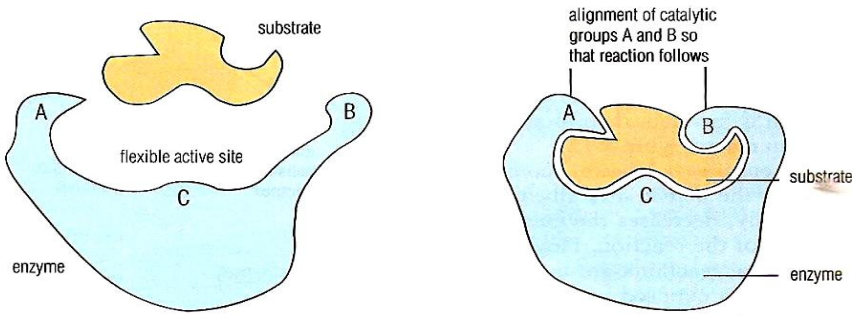
Spatial fit

- Two hypotheses have been put forward to explain enzyme specificity in terms of spatial fit:
  a) **'Lock and key' hypothesis**, proposed by Emil Fischer (1890)



  - The substrate is analogous to a 'key', whose shape is **complementary** to that of the enzyme's active site - the 'lock'.
  - When an enzyme-substrate complex is formed, chances of successful reaction increases.
  - Once the products are formed, they no longer fit into the active site. The products escape into the surrounding medium, leaving the active site free to receive further substrate molecules.

  b) **'Induced fit' hypothesis**, proposed by Daniel Koshland (1959)



  - Some enzymes and their active sites are physically more flexible than previously described.
  - The initial shape of the active site may not be complementary to the shape of the substrate(s).
  - Binding of substrate(s) to active site induces a **conformational change** in the enzyme. Through small rearrangement of chemical groups, this enables the substrate to fit more snugly into the active site. This enables the enzyme to perform its catalytic function more effectively.
  - The product formed can no longer fit into the active site and is expelled.
  - The enzyme reverts to its original conformation and is ready to receive another substrate.

Chemical fit

- Enzyme and substrate are held together by weak bonds, e.g. ionic bonds, hydrogen bonds and hydrophobic interactions, which can be made and broken readily during the transition state.
- The type of substrate to which an enzyme molecule can bind to depends on the nature of the amino acids making up its active site i.e. if the exposed R groups of the amino acids at the active site are electrically charged, then the reactive portion of the substrate must be complementarily charged.

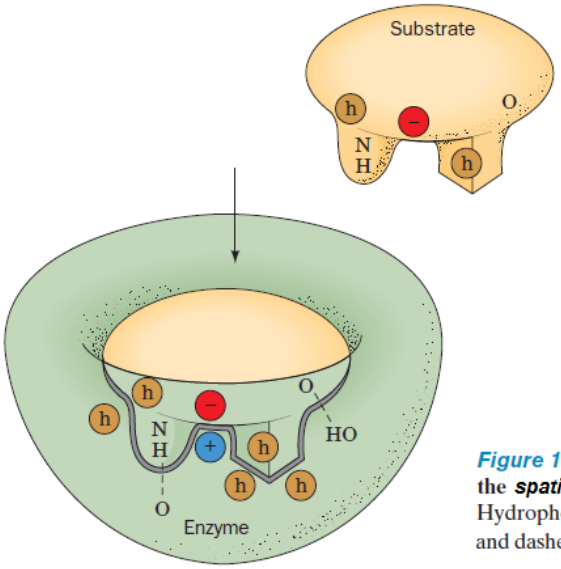
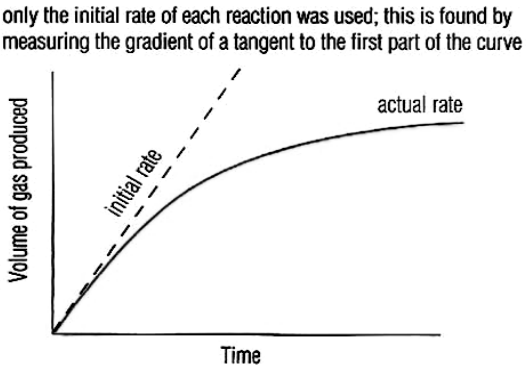


Figure 13-1 **An enzyme–substrate complex illustrating both the *spatial* and the *chemical fit* between enzymes and substrates.** Hydrophobic groups are represented by an h in a brown circle, and dashed lines represent hydrogen bonds.

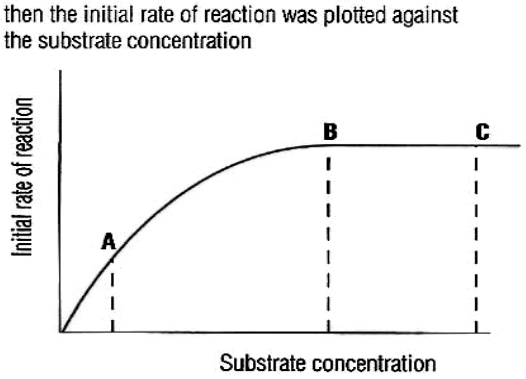
IV. Measuring Rate of Enzyme-Catalysed Reaction

- Consider the following chemical equation: **A** + **B** → **C** + **D**
  - As the reaction progresses, the amounts of **A** and **B** (the reactants) decrease, whilst the amounts of **C** and **D** (the products) increase.
- Rate of reaction can be determined by measuring:
  - rate of disappearance of reactants / substrate(s) in enzyme-catalysed reactions: **A** or **B**
  - rate of formation of products: **C** or **D**
- Hence, the time course of an enzyme-catalysed reaction is tracked by:
  - the amount of substrate that has disappeared or
  - the amount of products formed over a period of time.
- The rate is determined by measuring the slope of the tangent to the curve in the **initial stage** of the reaction. The steeper the slope, the higher is the rate of reaction.

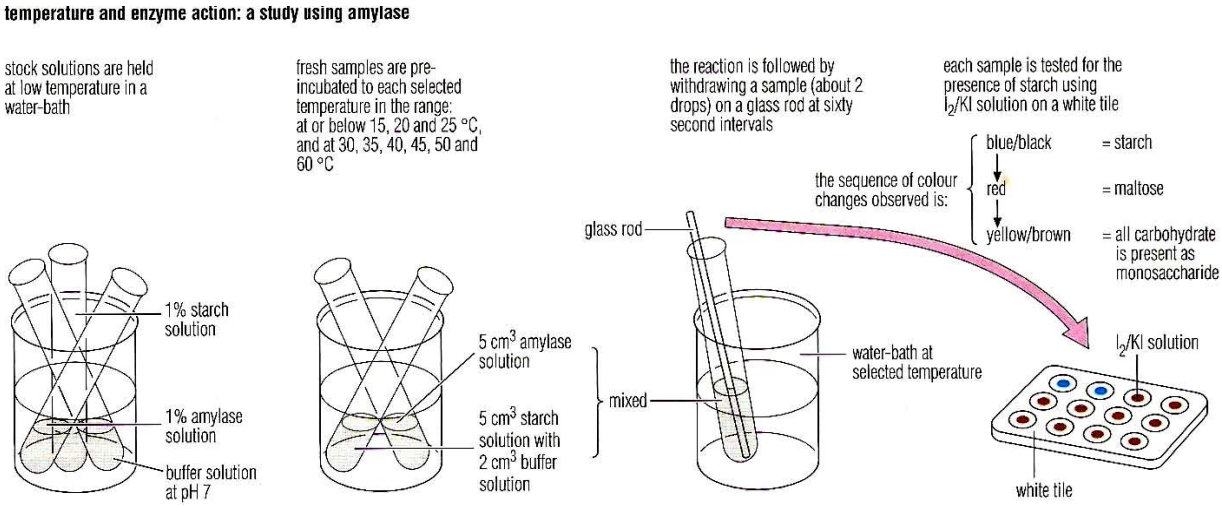


- If the activity is measured over a period of time, the rate of reaction usually falls, most commonly as a result of a fall in substrate concentration.

- When investigating the effect of a given factor (the independent variable) on the rate of an enzyme-catalysed reaction (the dependent variable), all other factors should be kept constant and at optimum levels whenever possible.
- Measure the **initial rate** for each interval of the independent variable. Plot this initial rate against the independent variable in a graph.



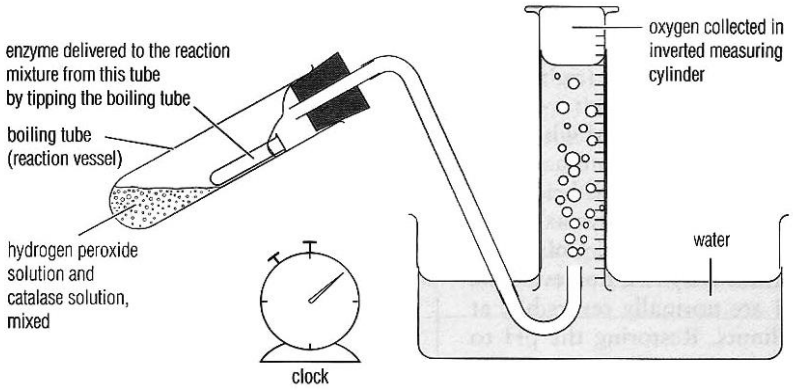
- E.g. in the reaction catalysed by amylase, starch is hydrolysed to maltose.



  - It is easier to determine the rate of reaction by measuring the rate of starch disappearance through determining the time taken to reach achromic point. Once starch is completely hydrolysed to maltose, it fails to form a complex with iodine to give a blue-black colour.

- E.g. in the reaction catalysed by catalase, hydrogen peroxide is decomposed to oxygen and water:

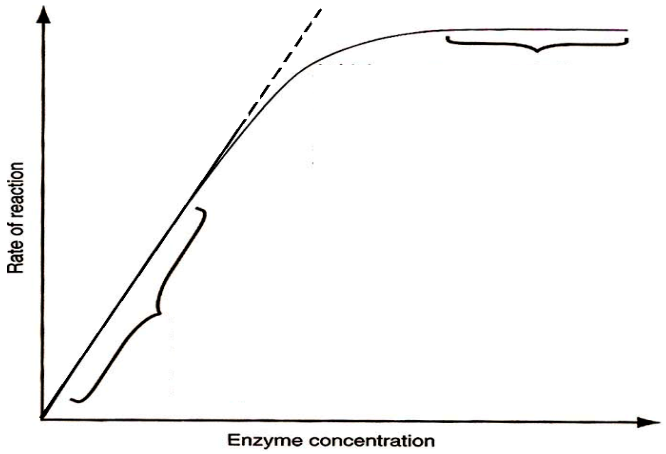
$$2\ H_2O_2 \rightarrow O_2 + 2\ H_20$$



  - It is easier to determine the rate of reaction by measuring the rate of oxygen formation, since oxygen is a gas and is easily separated from hydrogen peroxide and water.
  - The amount of oxygen formed over time can be measured through volume of gas produced or pressure exerted by gas.

V. Factors Affecting Rate of Enzyme-Catalysed Reactions

A. Enzyme concentration



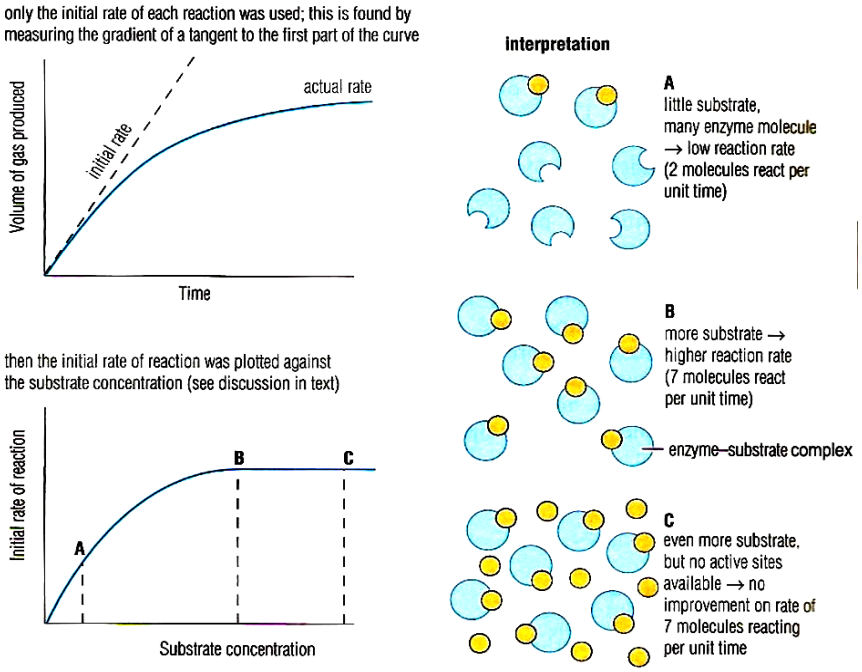
At low enzyme concentrations

- If the substrate is in excess and other factors such as temperature and pH are kept constant, the rate of reaction (velocity) increases in proportional to the enzyme concentration.
  - As enzyme concentration increases, the frequency of successful collisions between the enzyme and substrate molecules increases.
  - More enzyme-substrate complexes are formed per unit time, thus rate of reaction increases.
  - Hence, the rate of reaction is limited by enzyme concentration

At high enzyme concentrations

- If the substrate concentration is limiting, an increase in enzyme concentration would not result in further increase in the rate of reaction.

B. Substrate concentration



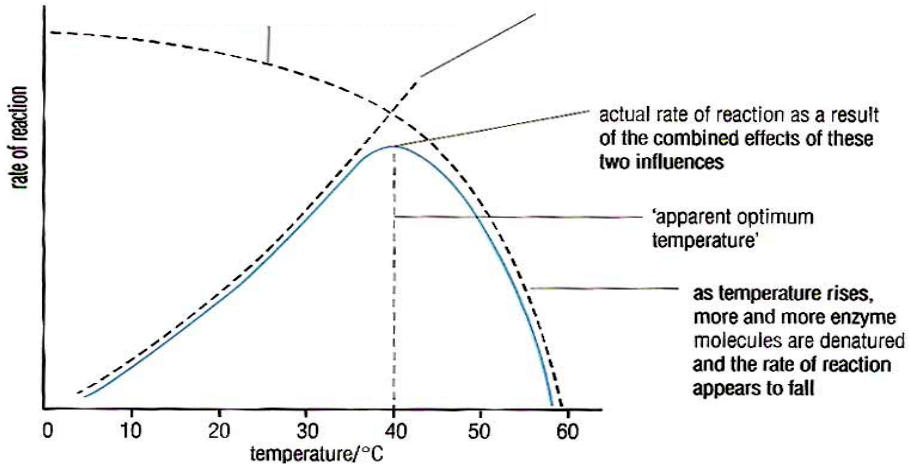
At low substrate concentrations

- For a fixed / constant enzyme concentration, the rate of reaction increases with increasing substrate concentration.
  - An increase in the number of substrate molecules will result in an increase in the frequency of successful collisions between enzyme and substrate molecules.
  - More enzyme-substrate complexes will be formed per unit time and the rate of reaction will increase.
  - Hence, the rate of reaction is limited by substrate concentration.

At higher substrate concentrations

- The rate of reaction will continue to increase with increases in substrate concentration, until a point when further increase in substrate concentration will no longer produce an increase in rate of reaction i.e. rate of reaction remains constant.
  - The active sites of the enzyme molecules are **saturated** with substrate molecules.
  - Any extra substrate molecule has to wait until the enzyme-substrate complex has released the products before it can enter the active site of the enzyme.
  - The rate of reaction is now limited by enzyme concentration.

C. Temperature



Below optimum temperature

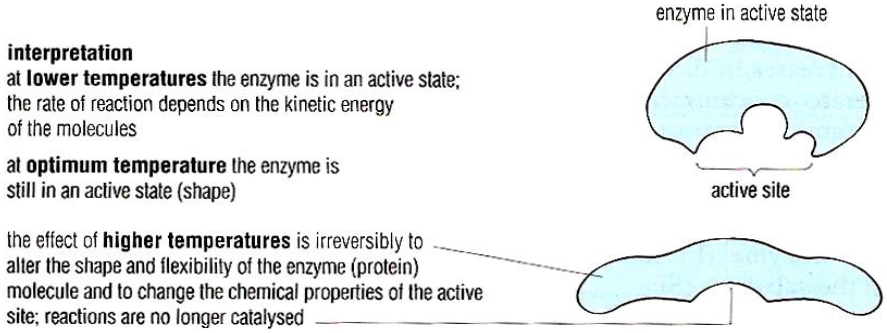
- **Optimum temperature** is the temperature at which the enzyme is functioning at its maximum rate.
- The rate of reaction increases with temperature until the optimum temperature is reached.
  - As temperature increases, there is an increase in the kinetic energy of the enzyme and substrate molecules. i.e. enzyme and substrate molecules move more quickly.
  - This results in an increase in the frequency of successful collisions between enzyme and substrate molecules.
  - More enzyme-substrate complexes are formed per unit time and thus, the rate of reaction increases
- The effects of temperature on the rate of an enzyme-catalysed reaction is expressed as the temperature coefficient, Q_{10} value, which is:

$$\frac{\text{Rate of enzyme-catalysed reaction at } (x + 10)\ ^\circ\text{C}}{\text{Rate of enzyme-catalysed reaction at } x\ ^\circ\text{C}}$$

  - For most enzymes, the Q_{10} value is approximately 2. This means that the rate of reaction is doubled for every increase of 10 °C.

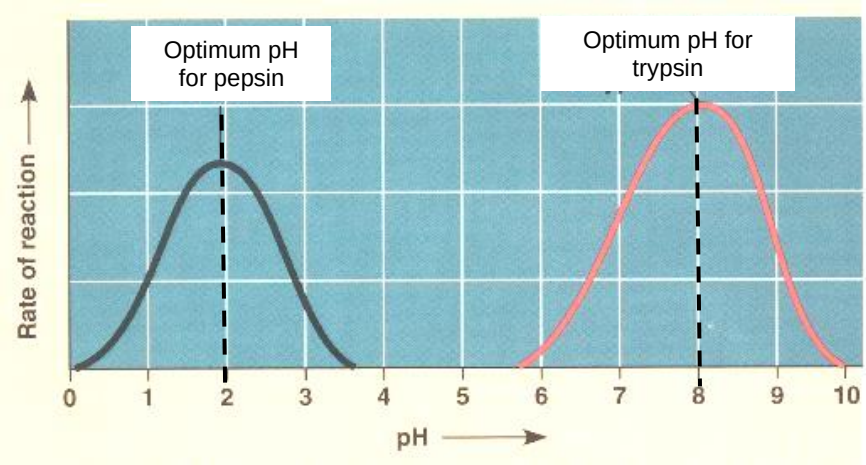
Above optimum temperature

- If the temperature is increased beyond the optimum temperature, the rate of enzyme catalysed reaction decreases rapidly.
  - Above the optimum temperature, the atoms which make up the enzyme molecule vibrate so vigorously that the hydrogen bonds and hydrophobic interactions between the R groups of amino acid residues begin to break.
  - The enzyme is said to be **denatured** and the shape of its active site is no longer complementary to that of substrate.
  - This loss of structure and function of the enzyme is often irreversible.



- Hence, the actual rate of reaction is the combined effect of
  - increased kinetic energy of enzyme and substrate molecules; and
  - denaturation of enzyme molecules.

D. pH



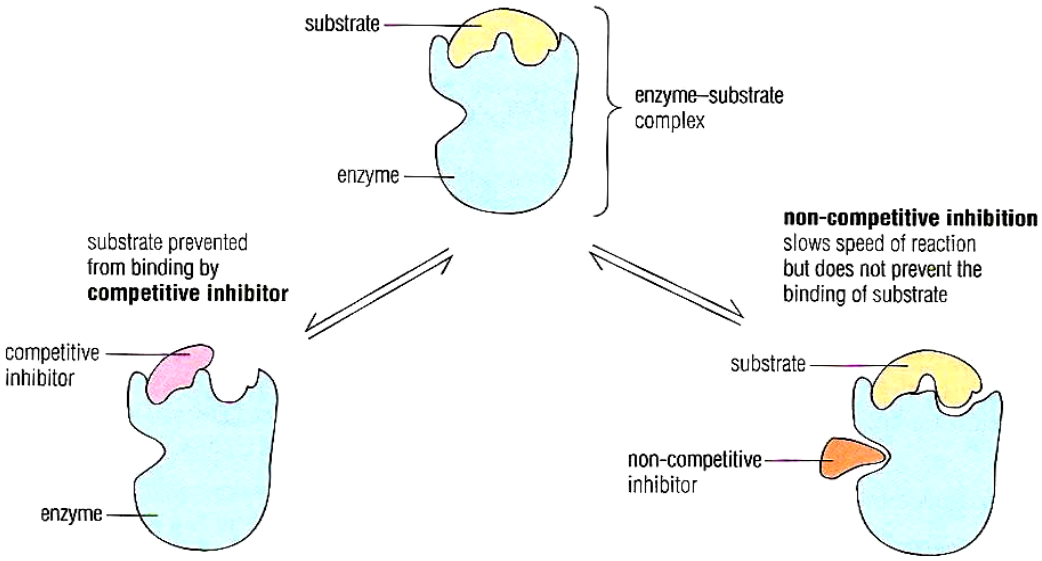
At optimum pH

- Rate of an enzyme catalysed reaction is at its maximum.
  - At this pH, the intramolecular bonds that maintain the 3-dimensional conformation of the enzyme molecule are intact.
  - Majority of enzymes have shape of active site most ideal for binding with substrate.
  - Frequency of successful collisions between enzyme and substrate molecules is the highest.
  - Hence, the number of enzyme-substrate complexes formed per unit time is at its highest and the rate of reaction is at its maximum.

At pH above or below the optimum pH

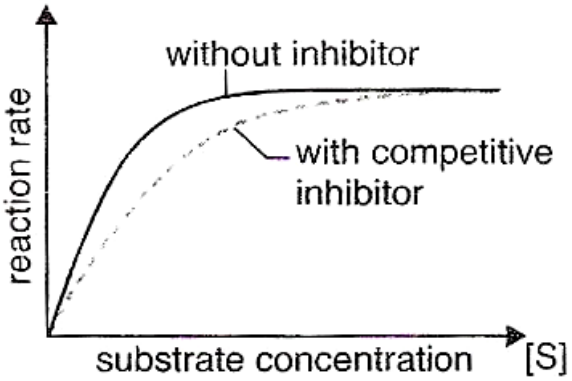
- Rate of an enzyme catalysed reaction decreases.
  - Change in concentration of H^+ (in the environment)
  - Alter the charges in the acidic and basic R groups of the amino acid residues of the enzyme molecule.
  - This disrupts ionic bonds and hydrogen bonds between R groups of amino acid residues (that helps to maintain the specific 3-dimensional conformation of the enzyme, including that of the active site).
  - The shape of active site is no longer complementary to that of the substrate. Enzymes are **denatured**.
  - Hence, less / no enzyme-substrate complexes are formed per unit time and the rate of reaction decreases.
- Most enzymes work within narrow pH range.
- If the pH is altered by a small extent from the optimum, the effects are normally reversible. If the pH is restored to the optimum, the maximum activity of the enzyme will be restored.
- If the pH is altered by a large extent, the conformation of the enzyme molecule would be severely affected and denaturation of the enzyme might be irreversible.

VI. Enzyme Inhibition



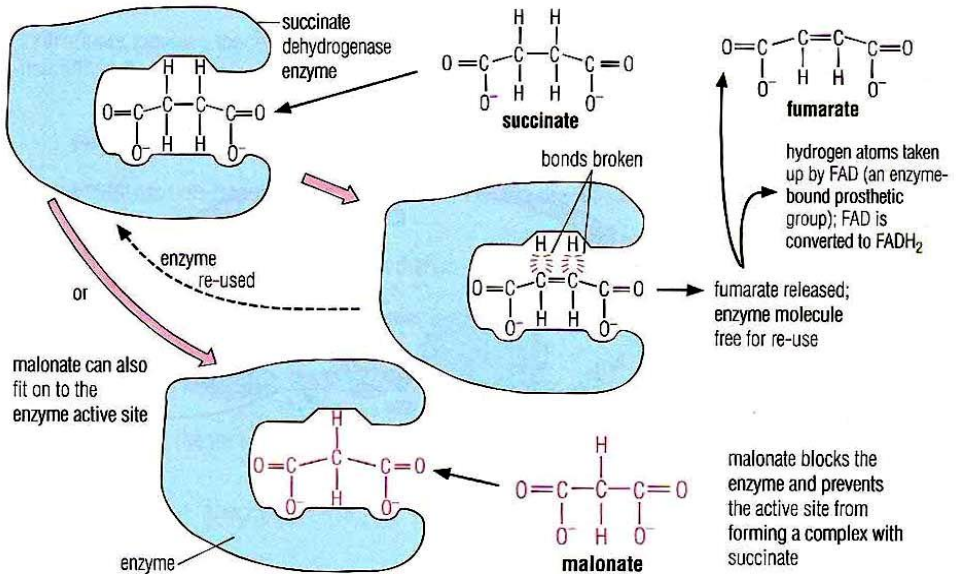
- A variety of small molecules exists which can reduce the rate of an enzyme-controlled reactions. These molecules are called **enzyme inhibitors**.
- Inhibition is a normal part of regulation of enzyme activity within cells.
- Many drugs and poisons also act as enzyme inhibitors.
- Inhibitors can interact with enzymes via covalent bonds (inhibition is usually irreversible) or weak bonds like hydrogen bonds and hydrophobic interaction (inhibition is usually reversible).
- There are generally three types of enzyme inhibition:
  - **Competitive inhibition**
  - **Non-competitive inhibition**
  - **Allosteric regulation**

A. Competitive Inhibition

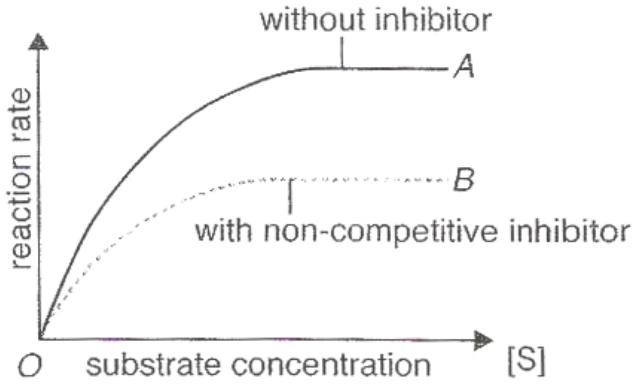


- A competitive inhibitor has a <u>similar shape</u> as the substrate. It fits <u>temporarily</u> into the <u>active site</u> of the enzyme and prevents binding of the enzyme with its substrate.
- Thus the inhibitor and substrate <u>compete</u> for the same active site.
- Competitive inhibitors <u>reduced the rate of reaction</u> by lowering the proportion of enzyme molecules bound to the substrate. i.e. It lowers the amount of enzyme-substrate complexes formed per unit time.
- The effect of competitive inhibition can be overcome by <u>increasing the substrate concentration</u>.
  - Increased substrate concentration increases the frequency of collisions between enzyme and substrate, rather than between enzyme and inhibitor.
  - At very high substrate concentrations, the rate of reaction <u>can reach the same maximum value</u> as that in the absence of inhibitors.
- An example of competitive inhibitor is malonate, which is a competitive inhibitor of succinate for the active site of succinate dehydrogenase

Figure 8.23 An example of competitive inhibition: succinate dehydrogenase catalyses the oxidation of succinate to fumarate (succinate and fumarate are intermediates of the Krebs cycle, p. 315); malonate is a competitive inhibitor of the enzyme



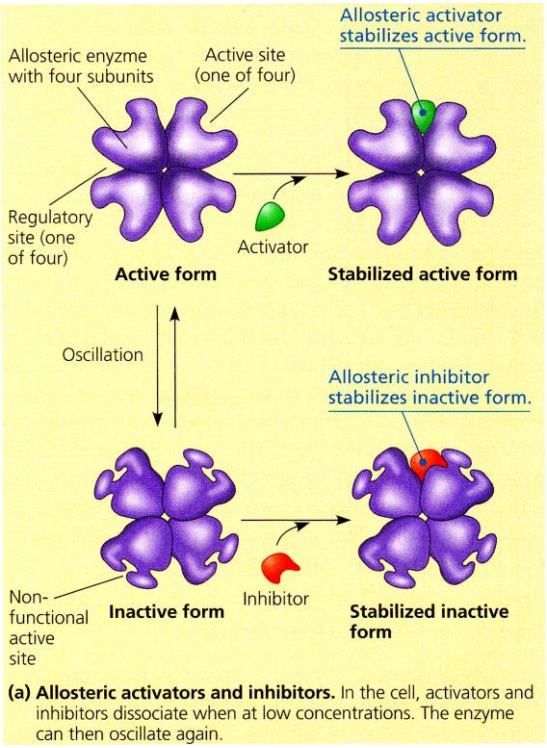
B. Non- competitive Inhibition



- A non-competitive inhibitor has no structural similarity to the substrate. It binds the enzyme at a region other than the active site.
- Interaction between the non-competitive inhibitor and the enzyme causes the enzyme molecule to change its shape, hence the shape of the active site is no longer complementary to the shape of substrates.
- Hence, a non-competitive inhibitor renders a proportion of the enzyme molecules out of action, decreasing the number of enzyme-substrate complexes formed per unit time, resulting in a drop in the effective enzyme concentration.
- The rate of reaction will not reach the maximum value as that in the absence of inhibitors.
- The effect of non-competitive inhibition cannot be overcome by increasing the substrate concentration.
- Examples of non-competitive inhibitors include metal ions such as Fe^{3+} and Al^{3+}. These ions form complexes with oxygen or sulfur atoms in amino acid residues (Ser, Thr, Asp, Cys, etc) at a site other than the active site of the enzyme.

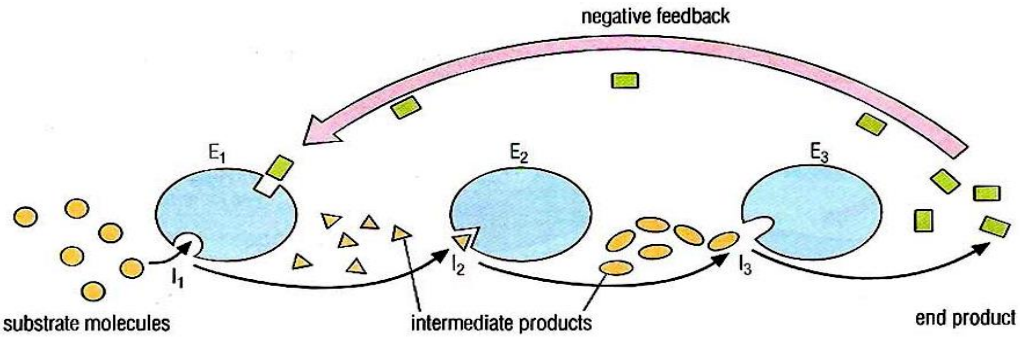
C. Allosteric Regulation

- Metabolic control often depends on **allosteric regulation**.
- In many cases, the molecules that normally regulate enzyme activity in a cell.
- These regulatory molecules change an enzyme's 3-dimensional conformation and function by binding weakly to the **allosteric site**, which is a specific site on a part of the enzyme molecule remote from the active site. The binding is weak and reversible.
- The effect of this allosteric regulation may be inhibition or stimulation of the enzyme's activity.
- Most allosterically-regulated enzymes are constructed from two or more polypeptide chains (i.e. 2 or more subunits).
    - Each subunit has its own active site, and the allosteric sites are often located where subunits are joined.
    - The entire complex oscillates between two conformational states: one catalytically active and the other inactive.
    - The binding of an **activator** to the allosteric site stabilizes the conformation that has a functional active site, whilst the binding of an **inhibitor** stabilizes the inactive form of the enzyme.
    - The areas of contact between the subunits of an allosteric enzyme fit together in such a way that a conformational change in one subunit is transmitted to all others.
    - Through this interaction of subunits, a single activator or inhibitor molecule that binds one allosteric site will affect the shape of the active sites of all subunits.



(a) Allosteric activators and inhibitors. In the cell, activators and inhibitors dissociate when at low concentrations. The enzyme can then oscillate again.

- Example of allosterically-regulated enzyme is phosphofructokinase (PFK).
  - PFK catalyses the phosphorylation of fructose phosphate by ATP to give fructose bisphosphate, an intermediate in glycolysis.
  - When ATP is at high concentration (e.g. when the mitochondria are actively producing it), it acts as an allosteric inhibitor of PFK and glycolysis is inhibited.
  - When ATP starts to be used up in the cell (e.g. when muscles are contracting), ATP concentration falls and PFK is no longer inhibited, thus glycolysis proceeds.



- This allosteric inhibition is a type of **negative feedback**, where the final molecule in the metabolic pathway acts on an enzyme catalysing the same pathway, to prevent accumulation of unnecessary intermediates.

VII. Enzyme Cofactors

- Many enzymes require non-protein components called **cofactors** for their efficient activity.
- Cofactors may vary from simple inorganic ions to complex organic molecules, and may either remain unchanged at the end of a reaction or be regenerated by a later process.
- There are two main types of cofactors:
  - Inorganic ions
  - Coenzymes

A. Inorganic ions

- These are thought to mould the enzyme into a shape that allows an enzyme-substrate complex to form more easily, thereby increasing the rate of an enzyme-catalysed reaction.
- E.g. salivary amylase activity is increased in presence of chloride ions

B. Coenzymes

- Small organic molecules which are required for the catalytic activity of an enzyme.
- They may be bound tightly to the enzyme permanently, or they may bind loosely and reversibly along with the substrate.
- E.g. nicotinamide adenine dinucleotide (NAD) which serves as a hydrogen acceptor. *(KIV: cellular respiration)*