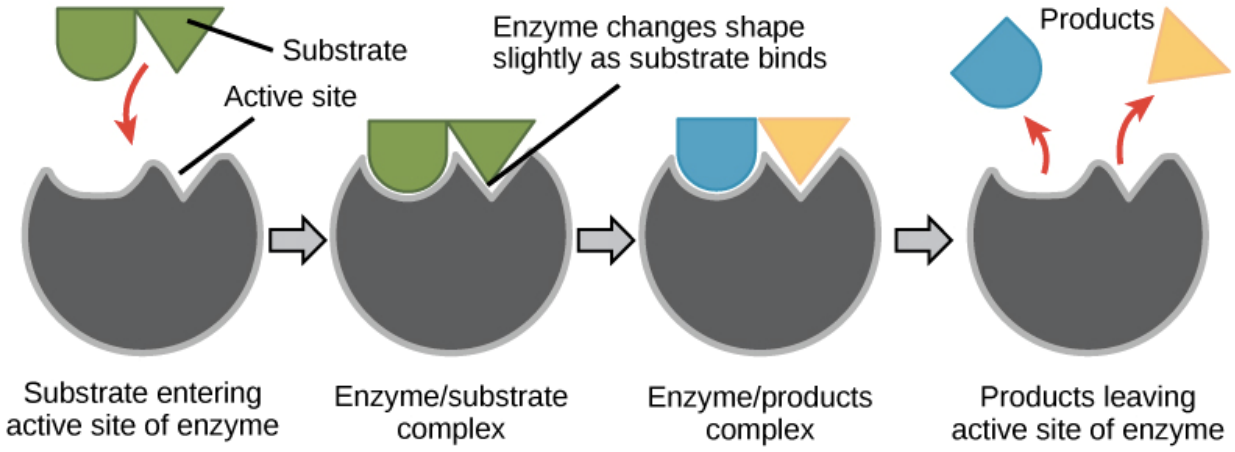
Cambridge AS level Biology

CODE: (9700)

Chapter 03



Mode of action of enzymes

Enzymes are protein molecules which can be defined as **biological catalysts.** A catalyst speeds up chemical reactions, while enzymes catalyse all metabolic reactions in living organisms, making them essential for life's existence.

Intracellular and extracellular enzymes

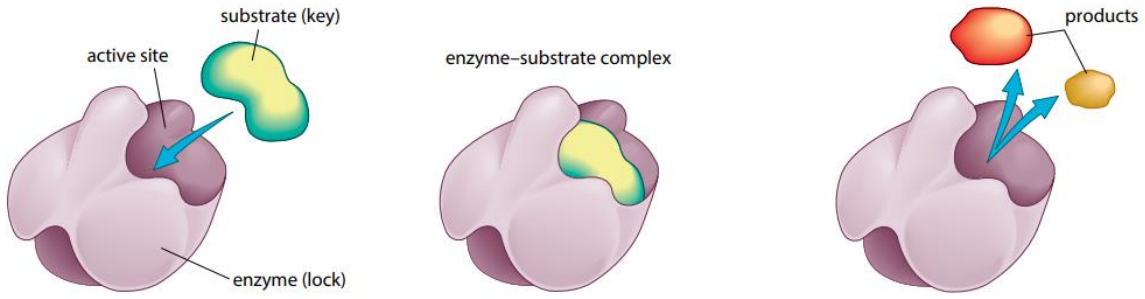
Not all enzymes operate within cells. Those that do are described as **intracellular.** Enzymes secreted by cells and catalyse reactions outside cells are defined as **extracellular.**

Lock and key and induced fit hypotheses

Enzymes are globular proteins with a precise three-dimensional shape, hydrophilic R groups, and an active site. They bind to a region in a cleft or depression. This molecule is the **substrate** of the **enzyme.**

The idea that the enzyme has a particular shape into which the substrate fits exactly is known as the **lock and key hypothesis.** The substrate is the **key** whose shape fits the **lock** of the enzyme.

The substrate is held in place by temporary bonds which form between the substrate and some of the R groups of the enzyme's amino acids. This combined structure is termed the **enzyme-substrate complex.**



a An enzyme has a cleft in its surface, called the active site. The substrate molecule has a complementary shape.

b Random movement of enzyme and substrate brings the substrate into the active site. An enzyme–substrate complex is temporarily formed. The R groups of the amino acids in the active site interact with the substrate.

c The interaction of the substrate with the active site breaks the substrate apart. An enzyme–product complex is briefly formed, before the two product molecules leave the active site, leaving the enzyme molecule unchanged and ready to bind with another substrate molecule.

Figure 3.2 How an enzyme catalyses the breakdown of a substrate molecule into two product molecules.

The enzyme is said to be **specific** for this substrate. **The induced fit hypothesis**, like the lock and key hypothesis, suggests that enzyme and substrate shape can slightly change during substrate entry, ensuring a perfect fit for efficient catalysis.

The interaction between the enzyme's R groups and the substrate's atoms can either break or encourage bond formation, resulting in the formation of one, two, or more **products.**

The enzyme lysozyme, found in tears and saliva, is a natural defence against bacteria. It breaks polysaccharide chains that form the cell walls of bacteria. The enzyme's interaction with hydrogen peroxide molecules, including the slight change in shape due to substrate binding, is demonstrated in Figure 2.20.

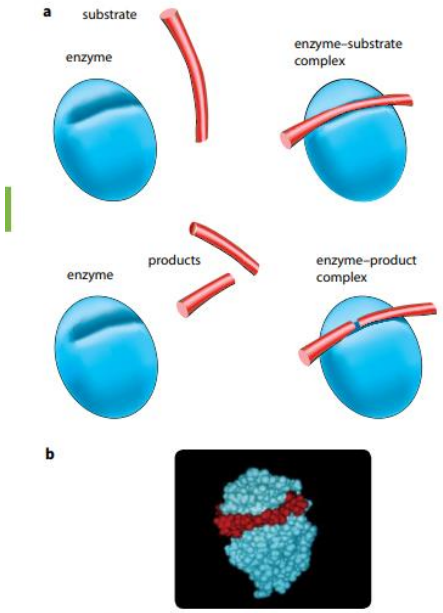


Figure 3.4 Lysozyme breaking a polysaccharide chain. This is a hydrolysis reaction. **a** Diagram showing the formation of enzyme–substrate and enzyme–product complexes before release of the products. **b** Space-filling model showing the substrate in the active site of the enzyme. The substrate is a polysaccharide chain which slides neatly into the groove (active site) and is split by the enzyme. Many such chains give the bacterial cell wall rigidity. When the chains are broken, the wall loses its rigidity and the bacterial cell explodes as a result of osmosis.

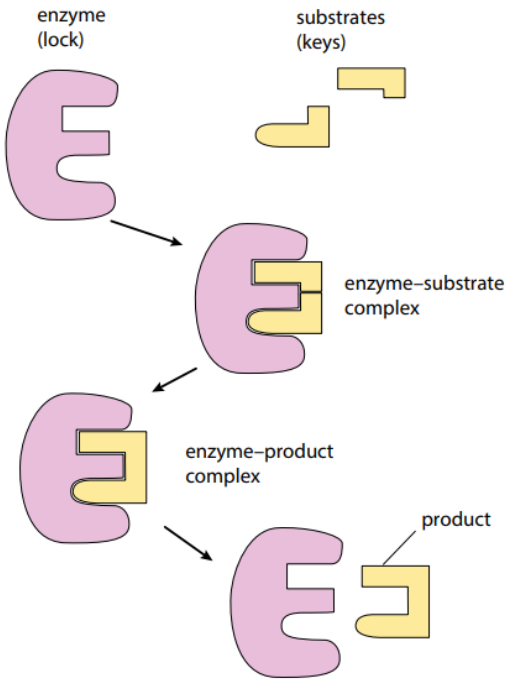


Figure 3.3 A simplified diagram of enzyme function. Note that in this example the enzyme is catalysing the joining together of two molecules.

Enzymes reduce activation energy

In many chemical reactions, the substrate will not be converted to a product unless it is temporarily given some extra energy. This energy is called **activation energy (Figure 3.5a)**

Heating reactants increase their energy, as seen in Benedict's test for reducing sugar. This process increases the rate of chemical reactions. Humans use this method to accelerate metabolic reactions, maintaining body temperature at 37°C. However, this doesn't provide enough activation energy for substrates to convert into products.

Enzymes avoid this problem because they **decrease** the activation energy of the reaction that they catalyse (Figure 3.5b).

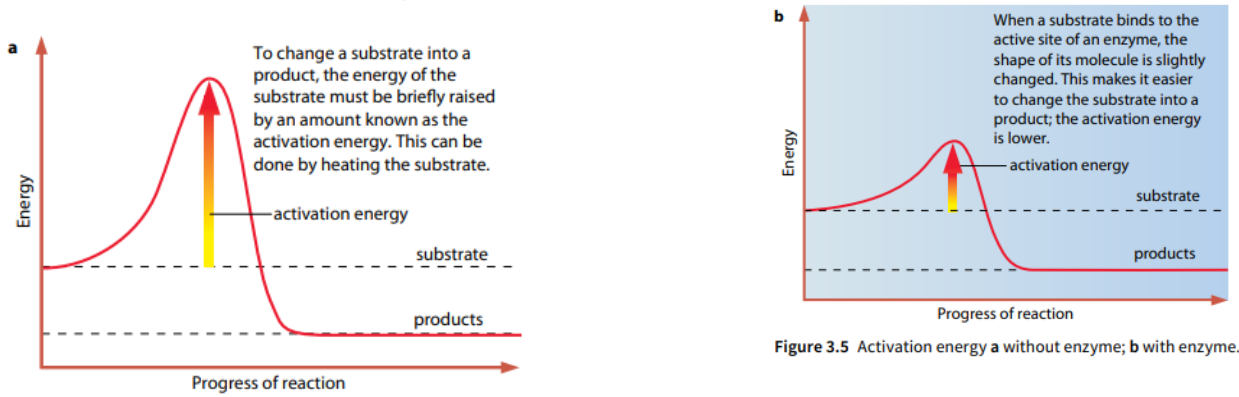


Figure 3.5 Activation energy **a** without enzyme; **b** with enzyme.

The course of a reaction

An investigation into substrate conversion during an enzyme-controlled reaction can be conducted using the enzyme catalase. This enzyme breaks down hydrogen peroxide into water and oxygen, a toxic product. The reaction begins quickly with oxygen bubbles, but as it continues, the rate of oxygen release slows down, eventually stopping completely.

The curve of a graph such as the one in Figure 3.6 is therefore steepest at the beginning of the reaction: the rate of an enzyme-controlled reaction is always fastest at the beginning. This rate is called the **initial rate of reaction.**

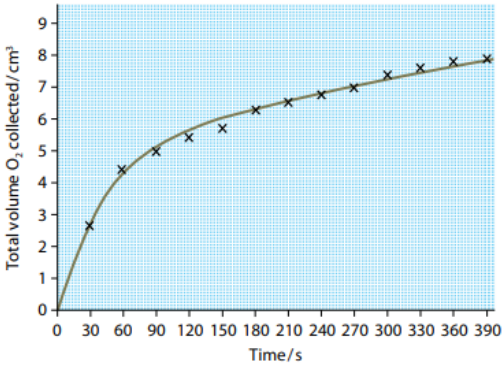


Figure 3.6 The course of an enzyme-catalysed reaction. Catalase was added to hydrogen peroxide at time 0. The gas released was collected in a gas syringe, the volume being read at 30 s intervals.

Factors that affect enzyme action

The effect of enzyme concentration

Figure 3.7a shows an investigation involving different concentrations of catalase solution from celery extract and hydrogen peroxide solution. The results show similar shapes in all five curves, with the total amount of oxygen produced being the same. If the investigation continues, all curves will meet.

To compare the rates of these five reactions, and to look at the effect of enzyme concentration on reaction rate, it is fairest to look at the rate **right at the beginning of the reaction.**

The amount of substrate in each reaction varies due to different conversion rates in the five reactions. Differences in reaction rate are caused by enzyme concentration. To calculate the initial rate, calculate the slope 30 seconds after the reaction begins, which can be plotted in Figure 3.7b.

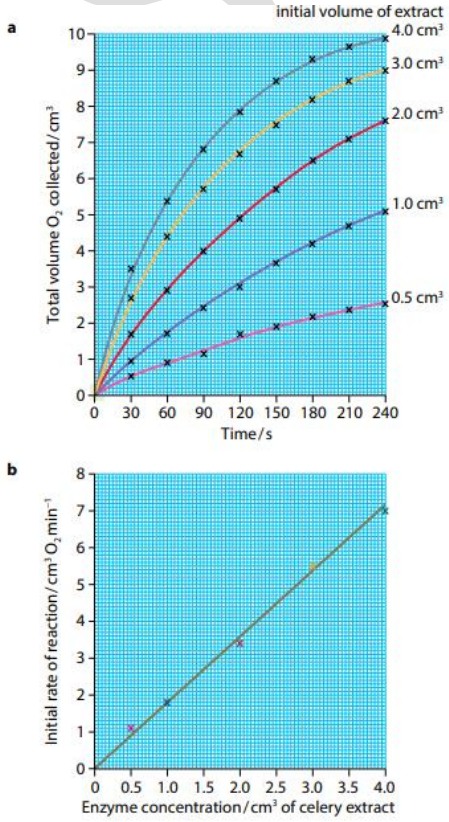


Figure 3.7 The effect of enzyme concentration on the rate of an enzyme-catalysed reaction. **a** Different volumes of celery extract, which contains catalase, were added to the same volume of hydrogen peroxide. Water was added to make the total volume of the mixture the same in each case. **b** The rate of reaction in the first 30 s was calculated for each enzyme concentration.

The effect of substrate concentration

The investigation showed that as substrate concentration increases, the initial rate of reaction also increases. However, as substrate concentration remains constant, every enzyme active site works continuously. If more substrate is added, substrate molecules queue up for an active site to become vacant, causing the enzyme to work at its maximum possible rate, known as $\mathbf{V_{max}}$.

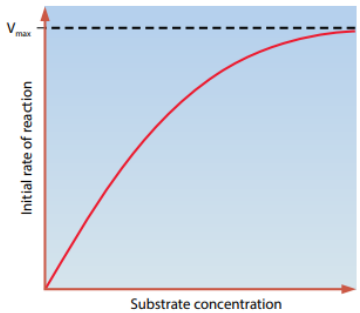


Figure 3.8 The effect of substrate concentration on the rate of an enzyme-catalysed reaction.

Temperature and enzyme activity

Figure 3.9 illustrates the rate of an enzyme-catalyzed reaction varies with temperature. At low temperatures, the reaction is slow due to the slow movement of molecules and rare substrate-enzyme collisions. As temperatures rise, collisions occur more frequently, facilitating bond formation and breaking.

As temperature rises, substrate and enzyme movement speed increases, but enzyme molecule structure vibrates energetically, breaking bonds, especially hydrogen bonds, above a certain temperature. The enzyme molecule begins to lose its shape and activity and is said to be **denatured.** This is often irreversible.

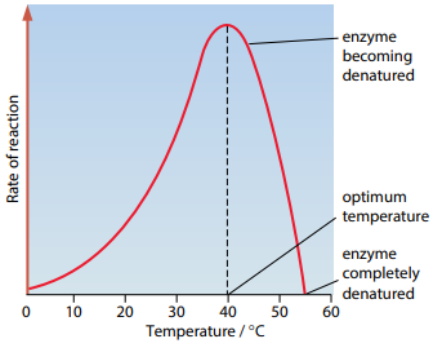


Figure 3.9 The effect of temperature on the rate of an enzyme-controlled reaction.

The temperature at which an enzyme catalyses a reaction at the maximum rate is called the **optimum temperature.**

Maintaining a body temperature of 40°C is dangerous as even a slight rise can denature enzymes. Enzymes from other organisms have varying optimum temperatures.

pH and enzyme activity

Enzymes' activity is influenced by pH, with most working at a pH of around 7, but some enzymes, like protease pepsin, have different optimum pHs. pH is a measure of hydrogen ion concentration and lower pH results in higher hydrogen ions. This affects the ionization of amino acid groups, affecting the active site's shape and substrate molecule compatibility. A pH significantly different from the optimal can cause enzyme denaturation.

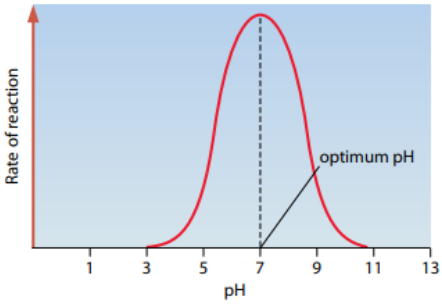


Figure 3.11 The effect of pH on the rate of an enzyme-controlled reaction.

Enzyme inhibitors

Competitive, reversible inhibition

Enzymes' active sites are designed to fit a specific substrate perfectly, but if they are similar in shape, another molecule can bind and inhibit the enzyme's function.

An **inhibitor** molecule binds briefly to a site, causing competition between it and the substrate for the site. If there is more substrate than an inhibitor, substrate molecules can easily bind, affecting enzyme function. Competitive inhibition occurs when inhibitor concentration or substrate concentration increases.

It is said to be **reversible** (not permanent) because it can be reversed by increasing the concentration of the substrate. This is therefore known as **competitive inhibition** (Figure 3.12a).

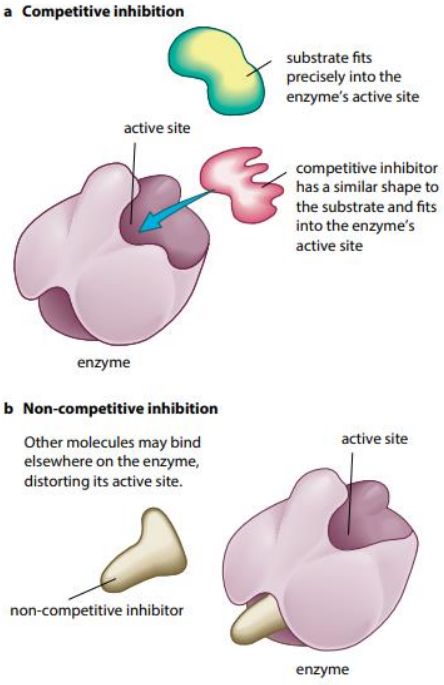


Figure 3.12 Enzyme inhibition. **a** Competitive inhibition. **b** Non-competitive inhibition.

Non-competitive, reversible inhibition

An inhibitor, when bound to an enzyme, disrupts the enzyme's three-dimensional shape, making it unsuitable for the substrate. This **non-competitive inhibition** occurs when the inhibitor blocks the enzyme's function, regardless of the amount of substrate present.

One way of controlling metabolic reactions is to use the end-product of a chain of reactions as a non-competitive, reversible inhibitor (Figure 3.13)

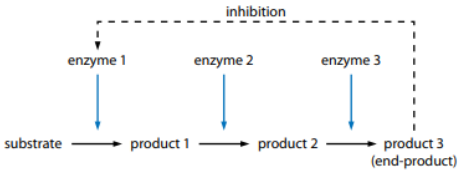


Figure 3.13 End-product inhibition. As levels of product 3 rise, there is increasing inhibition of enzyme 1. So, less product 1 is made and hence less product 2 and 3. Falling levels of product 3 allow increased function of enzyme 1 so products 1, 2 and 3 rise again and the cycle continues. This end-product inhibition finely controls levels of product 3 between narrow upper and lower limits, and is an example of a **feedback mechanism**.

However, the product can lose its attachment to the enzyme and go on to be used elsewhere, allowing the enzyme to reform into its active state. As product levels fall, the enzyme can top them up again. This is termed **end-product inhibition.**

Comparing enzyme affinities

There is enormous variation in the speed at which different enzymes work. A typical enzyme molecule can convert around one thousand substrate molecules into a product per second. This is known as the **turnover rate.**

Carbonic anhydrase is a highly efficient enzyme that can remove 600,000 carbon dioxide molecules from respiring tissue per second, 107 times faster than in the absence of the enzyme. This efficiency is due to the rapid movement of molecules within cells, with collisions occurring between enzyme and substrate molecules. Measuring enzyme activity is crucial for understanding how they control cell metabolism. The theoretical maximum rate (V_{max}) of the reaction is measured at different substrate concentrations, indicating the enzyme's efficiency.

The initial rate for substrate concentration is plotted against substrate concentration, creating an asymptotic curve. This curve never flattens out, making it impossible to accurately read Vmax from the graph. However, a double-reciprocal plot can be used, plotting 1/[S] and 1/velocity respectively. This allows for the accurate finding of Vmax, as 1/infinity is zero and the resulting graph is a straight line.

Figure 3.14a displays a double-reciprocal plot, allowing us to find V_{max} by determining $1/V_{max,}$ where the line intersects the y-axis, indicating infinite [S].

Another useful value can be obtained from the double-reciprocal plot, namely the Michaelis–Menten constant, Km. The Michaelis–Menten constant is the substrate concentration at which an enzyme works at half its maximum rate ($½V_{max}$).

The value of Km for an enzyme can vary depending on factors like substrate identity, temperature, pH, ions, ion concentration, and the presence of poisons, pollutants, or inhibitors. The point where the line intersects the x-axis is $-1/K_m$, which can be calculated from the value of $-1/K_m$. Vmax and Km are independent of each other.

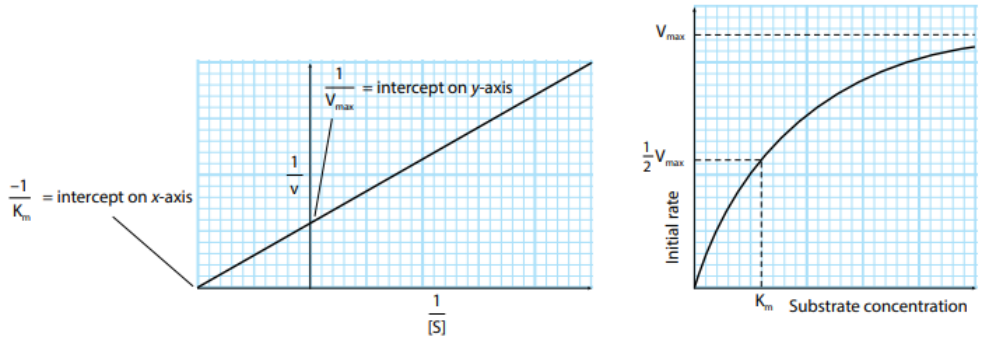


Figure 3.14 **a** A double-reciprocal plot of substrate concentration against initial rate: **b** A graph showing the effect of substrate concentration on initial rate, with V_{max}, $½V_{max}$ and K_m values shown.

Enzyme	Substrate	Maximum turnover number / per second	K_m / µmol dm^{-3}
carbonic anhydrase	carbon dioxide	600 000	8000
penicillinase	penicillin	2000	50
chymotrypsin	protein	100	5000
lysozyme	acetylglucosamine	0.5	6

Table 3.2 Turnover numbers and K_m values for four enzymes. Note that the unit for K_m is a concentration. (The turnover number per second is the number of molecules of substrate that one molecule of an enzyme converts to product per second. This is related to V_{max}.)

The significance of V_{max} and K_m values

Knowing the values of V_{max} and K_m has several applications.

■It enables scientists to make computerized models of biochemical pathways or even the behaviour of whole cells because it helps to predict how each reaction in a proposed pathway will proceed and therefore how the enzymes will interact. The consequences of changing conditions such as temperature, pH or the presence of inhibitors can be built into the models.

■An enzyme's preference for different substrates can be compared quantitatively.

■By understanding what affects enzyme efficiency, scientists may in future be able to design better catalysts, linking this to genetic engineering.

■For a commercially important enzyme, the performance of the same enzyme from different organisms can be compared.

■The calculations involved can be applied to other fields of biochemistry, such as antibody-antigen binding.

■Knowing Km means the proportion of active sites occupied by substrate molecules can be calculated for any substrate concentration.

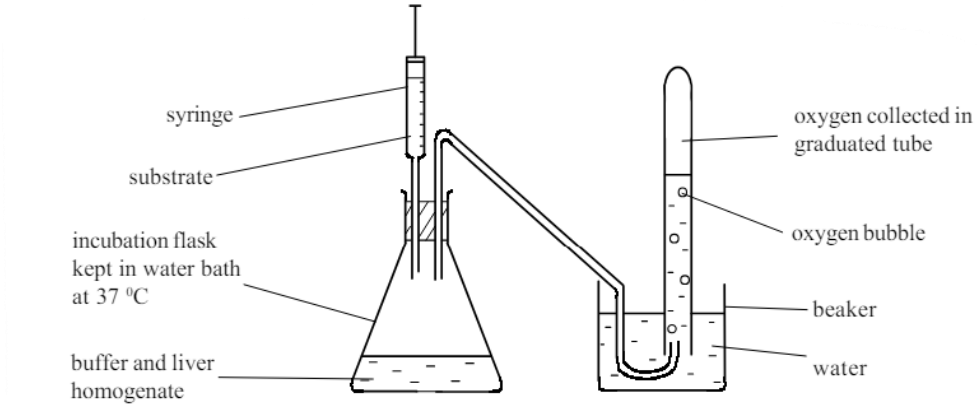
Immobilizing enzymes

Enzymes are essential in medicine, food technology, and industrial processing, but their high cost can be reduced by recycling them using **immobilized enzymes.**

Enzymes, such as lactase, can be immobilized using alginate beads, allowing milk to run through the beads. This process hydrolyzes lactose into glucose and galactose, making lactose-free dairy products. Enzyme immobilization offers advantages over mixing enzymes with substrates, as it allows for easier re-use and preservation. Additionally, immobilized enzymes are more tolerant to temperature and pH changes, as their molecules are held in shape by the alginate.

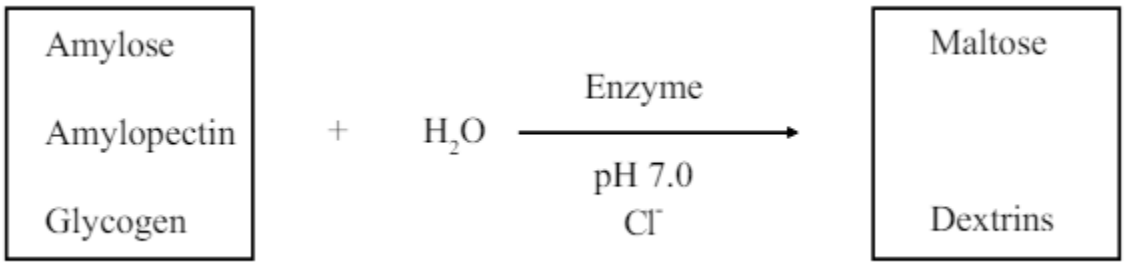
Revision Questions

1)The apparatus illustrated below can be used to investigate the activity of the enzyme catalase, which is found in the liver. The liver tissue has been ground up and mixed with a buffer solution. The substrate may be added via the syringe.



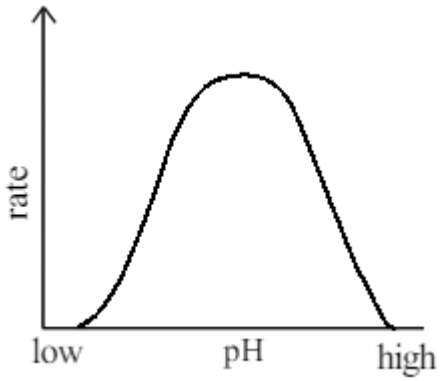
(a) Name the substrate upon which catalase acts.
(b) (i) Why is it necessary to grind up the liver tissue?
(ii) State two precautions you would take when preparing a standard homogenate of liver tissue.
(c) Describe how you would use this apparatus to investigate the effect of temperature on the activity of catalase.
(d) Suggest two possible sources of error in your investigation.

2) The equation shows the effect of an enzyme on carbohydrates in the buccal cavity.

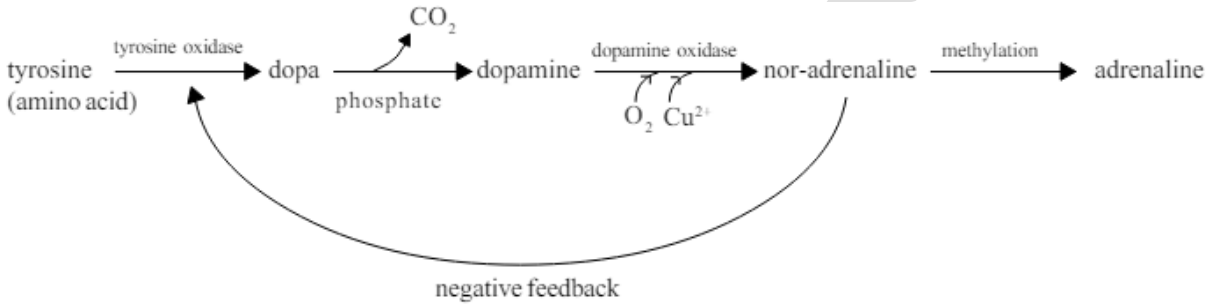


(a) Identify the enzyme involved in this reaction.
(b)Explain: (i) the role of the chloride ions in this reaction.
(ii) why this reaction does not continue in the stomach.
(c) Maltose digestion is completed elsewhere in the gut. Name the enzyme involved, the digestive juice which contains the enzyme and the product.
Enzyme
Digestive juice
Product

3) The graph shows the effect of substrate concentration on the rate of an enzyme-controlled reaction.
(a) Explain the shape of the graph between:
(i) and b.
(ii) c and d.
The figure shows the effect of pH on the rate of an enzyme-controlled reaction.
(b) Explain the shape of the curve.

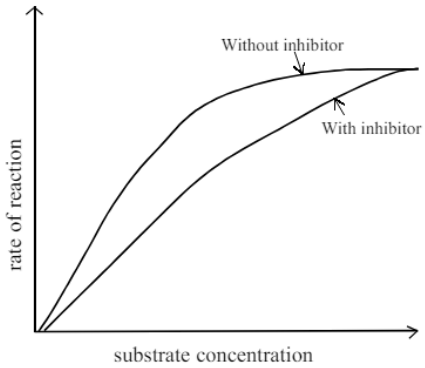


4)The figure shows some of the enzyme-controlled steps involved in the synthesis of noradrenaline and adrenaline.



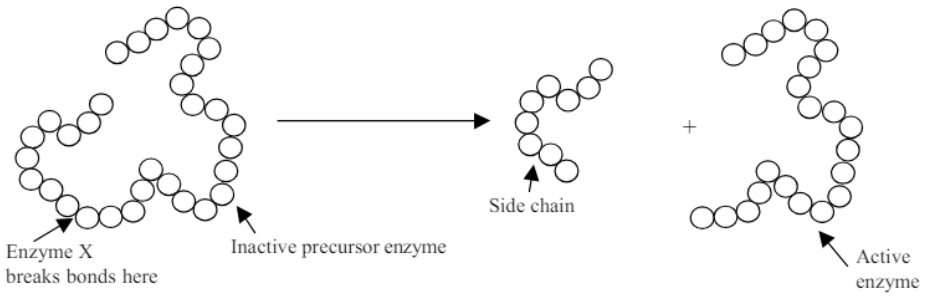
(a) Explain the term 'metabolic pathway'
(b) What is the action of the enzyme which is involved in the conversion of Dopa into Dopamine?
(c) Suggest one function of the phosphate in the reaction.
(d) Using information in the diagram explain how the body regulates the amount of adrenaline which is produced.
(e) Suggest a site in the body where the above metabolic pathway would occur.

5) The graph shows the effect of substrate concentration on the rate of an enzyme-controlled reaction with and without the addition of a competitive inhibitor.



(a) Define the term 'competitive inhibitor'.
(b) Suggest an explanation for the effect of the inhibitor on the rate of reaction when substrate concentration is high
(c) State three differences between competitive and non-competitive inhibitors.

6) The diagram shows how a hydrolytic enzyme (X) converts an inactive precursor enzyme into an active form.



(a) Explain:
(i) the effect of enzyme X.
(ii) why does enzyme X always act on the same part of the inactive precursor
(iii) why enzymes may be held in inactive forms.
(b) State an example of an enzyme that is secreted in an inactive form and name the agent that activates it.
name of enzyme:
name of activator:

7) Sucrose is a disaccharide which is broken down into glucose and fructose by the enzyme sucrase. An investigation was carried out into the effect of temperature on sucrase activity. A measured volume of sucrase solution was added to and mixed with a known volume of sucrose solution. At 60-second intervals for 5 minutes, 1cm3 samples of the mixture were removed and tested with Benedict's reagent. The investigation was repeated at 25o C, 40o C and 65o C.

(a) Describe how you would set up and maintain a mixture of sucrose and sucrase at 65o C.
(b) Explain how Benedict's reagent would indicate the activity of the enzyme.
(c) A student's recording of the first three minutes of this investigation is shown below.

Time/seconds	Temperature/°C		
	25	40	65
30	Blue solution	Pale green-yellow solution	Pale blue-green solution
60	Pale green solution	Orange-red solution	Pale blue-green solution
90	Yellow solution	Brick red precipitate	Blue solution
120	Orange solution	Brick red precipitate	Blue solution
150	Brick red precipitate	Brick red precipitate	Blue solution
180	Brick red precipitate	Brick red precipitate	Blue solution

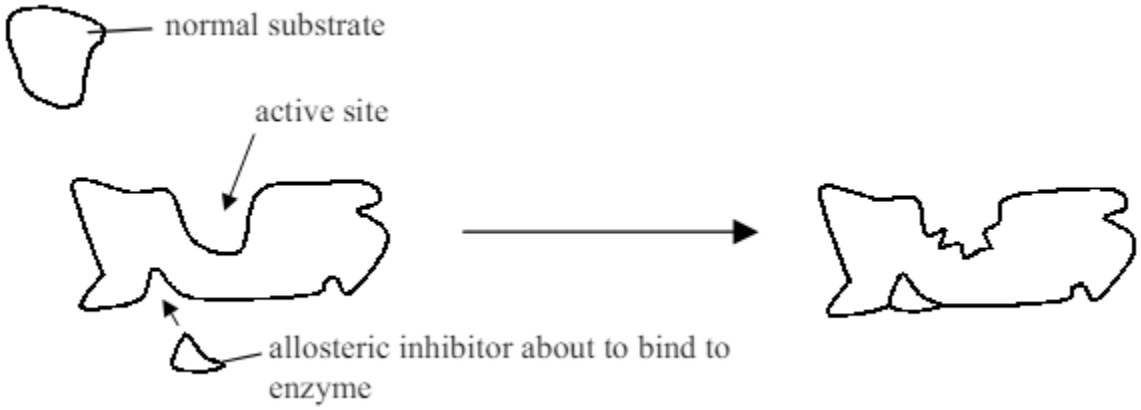
(i) Explain the difference between the results obtained at 25 C and 40 C.
(ii) Explain the results obtained at 65 C.

8) Catalase is an enzyme which breaks down hydrogen peroxide into water and oxygen. Hydrogen peroxide is a highly toxic waste product of metabolism. An investigation was carried out to determine the relative amounts of catalase in samples of potato, liver and apple. Samples of each tissue were ground up using a homogeniser. Samples of these tissues were then added to hydrogen peroxide solution in a measuring cylinder. The table below shows the height of the resulting effervescence in each cylinder.

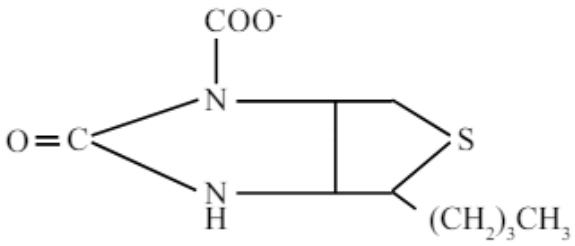
	Sample		
	Potato	Liver	Apple
Height of effervescence/cm^3	4	9	1

(a) State four precautions which should have been taken in this investigation
(b) (i) Which tissue appeared to contain the most catalase?
(ii) Suggest an explanation for this.
(c) Explain why an increase in enzyme concentration usually increases the rate of reaction.

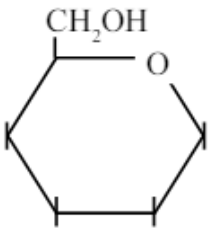
9) The diagram shows the effect of an allosteric inhibitor on an enzyme.



(a) What is the effect of the allosteric inhibitor on the enzyme?
(b) (i) What is the effect of the allosteric inhibitor on the catalytic activity of the enzyme
(ii) Explain why this occurs.
Substances X and Y, shown below, are inhibitors of glucose phosphorylase



X



Y

(c) Which substance is likely to be a competitive inhibitor of glucose phosphorylase? Explain your answer.

10) The table below refers to the enzyme's amylase and lactic dehydrogenase. If the feature is correct, place a tick in the appropriate box and if the feature is incorrect, place a cross in the appropriate box.

Feature	Amylase	Lactic dehydrogenase
Will breakdown lactose		
Found only in animals		
Requires NAD as coenzyme		
Is classed as a hydrolase		
Can be manufactured by genetic engineering		
Can be used to make yoghurt		