2.2 3 Carbohydrates 1: Sugar

By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:

- **the ring structure and properties of glucose as an example of a hexose monosaccharide and the structure of ribose as an example of a pentose monosaccharide**
- **the chemical elements that make up biological molecules**
- **the synthesis and breakdown of a disaccharide and polysaccharide by the formation and breakage of glycosidic bonds**

KEY DEFINITIONS

carbohydrates: a group of molecules containing C, H and O

glycosidic bond: a bond formed between two monosaccharides by a hydrolysis reaction.

What are carbohydrates for?

Carbohydrates contain carbon, hydrogen and oxygen. Carbohydrates are 'hydrated carbon', which means that for every carbon there are two hydrogen atoms and one oxygen atom. The functions of carbohydrates are three-fold. They act as a source of energy (e.g. glucose), as a store of energy (e.g. starch and glycogen) and as structural units (e.g. cellulose in plants and chitin in insects). Some carbohydrates are also part of other molecules, such as nucleic acids and glycolipids. There are three main groups of carbohydrates: monosaccharides, disaccharides and polysaccharides. The common monosaccharides and disaccharides all have names ending in -ose.

Monosaccharides

Monosaccharides are the simplest carbohydrates. They are particularly important in living things as a source of energy. They are well suited to this role because of the large number of carbon–hydrogen bonds. They are sugars, which taste sweet, are soluble in water and are insoluble in non-polar solvents. Monosaccharides can exist as straight chains or in ring or cyclic forms. They have a backbone of single-bonded carbon atoms, with one double-bonded to an oxygen atom to form a carbonyl group. Different sugars can have different numbers of carbon atoms (see Table 1): hexose sugars have six carbon atoms, pentose sugars have five carbon atoms and triose sugars have three carbon atoms. Monosaccharide hexose sugars, like glucose, are the **monomers** of more complex carbohydrates, and they bond together to form **disaccharides** or **polysaccharides**.

In solution, triose and tetrose sugars exist as straight chains. However, pentoses and hexoses are more likely to be found in a ring or cyclic form (shown in Table 1).

In both forms (straight-chain and cyclic forms), glucose can exist as a number of different isomers (molecules with the same formula, but whose atoms are arranged differently in space). In the straight-chain form, the –H and –OH can be reversed. In a ring shape, isomers can also form. The ring is formed when the oxygen attached to carbon 5 bonds to carbon 1 (see Table 1). Because the –OH and –H on carbon 1 can be above or below the plane of the ring when the ring forms, there are two isomers: α- and β-glucose. This small difference appears insignificant, but it becomes very important when glucose molecules polymerise into starch or cellulose.

DID YOU KNOW?

The word saccharide derives from the Greek word sakkharon, which means sugar.

Disaccharides

Like monosaccharides, disaccharides are sweet and soluble. The most common disaccharides are maltose (malt sugar), sucrose and lactose (milk sugar). Maltose and lactose are **reducing sugars**, whereas sucrose is a **non-reducing sugar**.

Disaccharides are made when two monosaccharides join together. There are lots of different combinations, which determine the disaccharide made:

- α-glucose + α-glucose → maltose
- α-glucose + fructose → sucrose
- β-galactose + α-glucose → lactose
- β-glucose + β-glucose → cellobiose

When they join, a condensation reaction occurs to form a **glycosidic bond**. Two hydroxyl groups line up next to each other, from which a water molecule is removed (see topic 2.2.1). This leaves an oxygen atom acting as a link between the two monosaccharide units.

Disaccharides are broken into monosaccharides by a hydrolysis reaction, which requires addition of water. The water provides a hydroxyl group (–OH) and a hydrogen (–H), which help the glycosidic bond to break. For example, cellobiose is obtained by the hydrolysis of the polysaccharide cellulose.

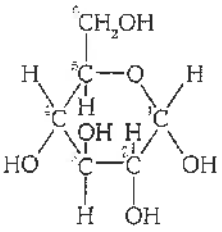
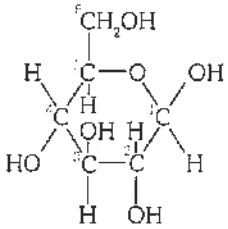
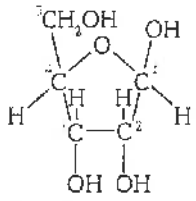
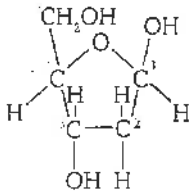
Name of sugar	Displayed formula	Molecular formula	Role in the body	Type of sugar
α-Glucose		$C_6H_{12}O_6$	Energy source. Component of starch and glycogen, which act as energy stores.	Hexose
β-Glucose		$C_6H_{12}O_6$	Energy source. Component of cellulose, which provides structural support in plant cell walls.	Hexose
Ribose		$C_5H_{10}O_5$	Component of ribonucleic acid (RNA), ATP and NAD.	Pentose
Deoxyribose		$C_5H_{10}O_4$	Component of deoxyribonucleic acid (DNA).	Pentose

Table 1 Some monosaccharides.

Like most reactions, when glycosidic bonds are formed and hydrolysed in living things, such reactions are catalysed by enzymes.

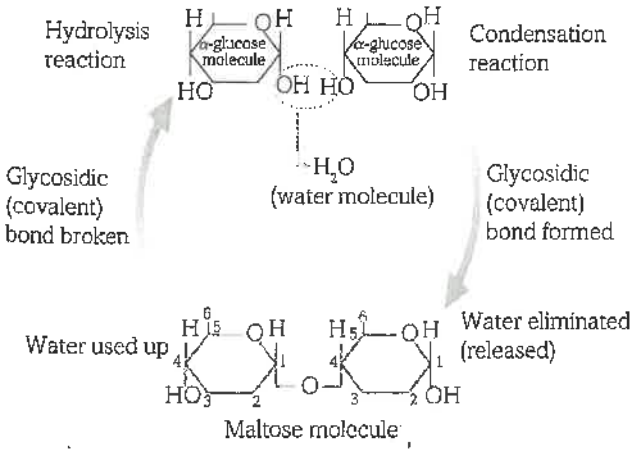
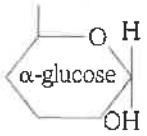


Figure 1 Formation and splitting of maltose from α-glucose. The glycosidic bond is formed between carbon 1 of one α-glucose molecule and carbon 4 of the other α-glucose molecule. It is known as a 1–4 glycosidic bond.

Questions

1. Write down the molecular formula for a triose sugar (write your answer in terms of C, H, O and *x*).
2. Write down the molecular formula for maltose.
3. To reduce complexity, many writers draw α-glucose like the diagram below. Draw β-glucose in the same way.



4. (a) Draw the displayed formula showing two β-glucose molecules joined together by a condensation reaction.
   (b) Draw the formulae to show what happens when this disaccharide is hydrolysed.
5. Study the formulae of ribose and deoxyribose. State the difference between the two.
6. Draw a trisaccharide of α-glucose.

2.2 4 Carbohydrates 2: Polysaccharides as energy stores

By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:

- the synthesis and breakdown of a disaccharide and polysaccharide by the formation and breakage of glycosidic bonds
- the structure of starch (amylose and amylopectin), glycogen and cellulose molecules
- how the structures and properties of glucose, starch, glycogen and cellulose molecules relate to their functions in living organisms

Polysaccharides are **polymers** of monosaccharides. They are made of hundreds or thousands of monosaccharide monomers bonded together. Polysaccharides made solely of one kind of monosaccharide are called homopolysaccharides. Those made of more than one monomer are called heteropolysaccharides. Starch is an example of a homopolysaccharide. Hyaluronic acid (in connective tissue) is an example of a heteropolysaccharide.

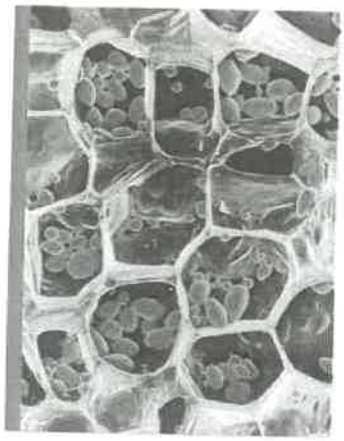


Figure 1 Starch grains in potato cells (×80).

Energy sources and energy stores

Glucose is a source of energy, as it is a reactant in **respiration**. The energy released is used to make ATP, which is the energy currency of the cell:

$$\text{Glucose} + \text{Oxygen} \xrightarrow{\text{releasing energy which is used to form ATP}} \text{Carbon dioxide} + \text{Water}$$

If you join lots of glucose molecules together into polysaccharides, you can create a *store* of energy, and this is exactly what living things do. Plants store energy as starch in chloroplasts and in membrane-bound starch grains, and humans store energy as glycogen in cells of the muscles and liver.

Why are polysaccharides good energy stores?

The structure of some polysaccharides lends itself to energy storage. **Glycogen** in *animals* and starch in *plants* (comprising **amylose** and **amylopectin**) occur within cells in the form of large granules. They form good stores of monosaccharides for the following reasons:

- Glycogen and starch are compact, which means they do not occupy a large amount of space. They both occur in dense granules within the cell.
- Polysaccharides hold glucose molecules in chains, so they can be easily 'snipped off' from the end of the chain by hydrolysis when required for respiration. Hydrolysis reactions are always catalysed by enzymes.
- Some chains are unbranched (amylose) and some are branched (amylopectin and glycogen). Branched chains tend to be more compact, but also offer the chance for lots of glucose molecules to be snipped off by hydrolysis at the same time, when lots of energy is required quickly. The enzyme amylase is responsible for hydrolysing 1–4 glycosidic linkages, and glucosidase is responsible for hydrolysing 1–6 glycosidic linkages. A 1–4 glycosidic linkage is one between carbon 1 of one glucose and carbon 4 of the other.

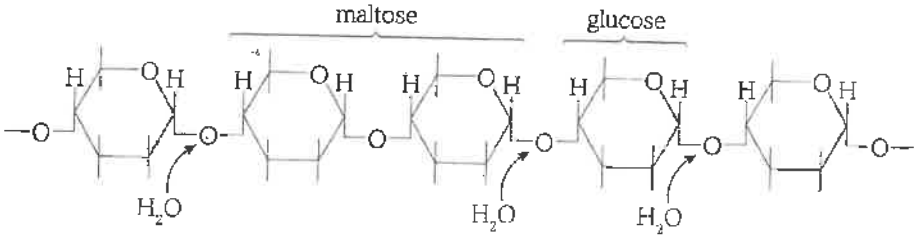


Figure 2 Starch is hydrolysed at 1–4 linkages to produce a mixture of glucose and maltose.

- Polysaccharides are less soluble in water than monosaccharides. If many glucose molecules did dissolve in the cytoplasm, the **water potential** (see topic 2.5.3) would reduce, and excess water would diffuse in, disrupting the normal workings of the cell. Polysaccharides are less soluble because of their size, but also because regions which could hydrogen-bond with water are hidden away inside the molecule. Sometimes the amylose molecule may form a double helix, which presents a hydrophobic external surface in contact with the surrounding solution.

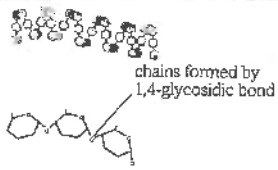
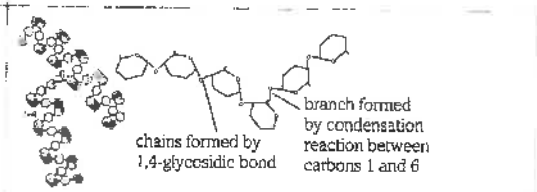
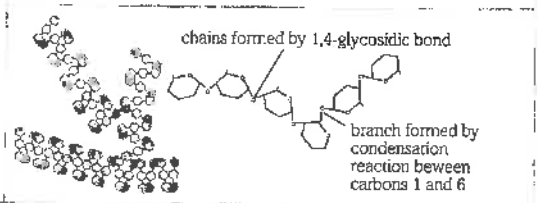
Type of polysaccharide	Detailed structure	
Amylose (in plants): this molecule is a long chain of α-glucose molecules. Like maltose, it has glycosidic bonds between carbons 1 and 4.	Amylose coils into a spiral shape, with hydrogen bonds holding the spiral in place. Hydroxyl groups on carbon 2 are situated on the inside of the coil, making the molecule less soluble and allowing hydrogen bonds to form to maintain the coil's structure.	
Amylopectin (in plants): this is like amylose, with glycosidic bonds between carbons 1 and 4 but in addition it has branches formed by glycosidic bonds between carbons 1 and 6.	Amylopectin also coils into a spiral shape, held together with hydrogen bonds, but with branches emerging from the spiral.	
Glycogen (in animals): this molecule is like amylopectin with glycosidic bonds between carbon 1 and 4, and branches formed by glycosidic bonds between carbon 1 and 6.	The 1–4 bonded chains tend to be smaller than in amylopectin, so glycogen has less tendency to coil. However, it does have more branches, which makes it more compact. And it is easier to remove monomer units as there are more ends.	

Table 1 Energy storage in plants and humans.

DID YOU KNOW?

- The core of a glycogen or amylopectin molecule is resistant to hydrolysis by enzymes. Such a unit is called a limit dextrin, and is one of the major components of mucus.
- Glycogen is stored in the liver and in muscle cells. It may form up to 7% of the mass of the liver.

LEARNING TIP

Remember that glucose is an energy source when used in respiration, and any polymer of glucose therefore provides an energy store.

Questions

1. Copy and complete the following table:

	Amylose	Amylopectin	Glycogen
Do you find it in humans or plants?			
Where in the organism is it stored?			
Is it a form of starch?			
What is the monomer?			
Is it branched?			
Is it soluble?			
Which glycosidic bonds does it have: 1–4 or 1–6?			
Is it spiralled?			
Are hydrogen bonds important in holding the structure in place?			

2. Glycogen is more branched than starch. Explain why this difference is important to animals.
3. Why is it important that hydroxyl groups on carbon 2 of glucose are found on the inside of the amylose spiral?
4. What kind of bonding holds the coiled structure of amylose together?
5. What are the advantages of a polysaccharide having a branched chain? Explain your answer.

2.2 5 Carbohydrates 3: Polysaccharides as structural units

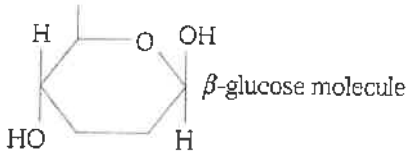
By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:

- **the structure of starch (amylose and amylopectin), glycogen and cellulose molecules**
- **how the structures and properties of glucose, starch, glycogen and cellulose molecules relate to their functions in living organisms**

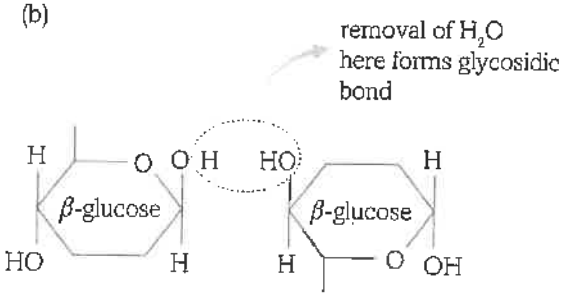
Cellulose

Cellulose is found in plants, forming the cell walls. It is a tough, insoluble and fibrous substance. Cellulose is a homopolysaccharide made from long chains of up to 15 000 β-glucose molecules, bonded together through **condensation** reactions to form glycosidic bonds.

(a)

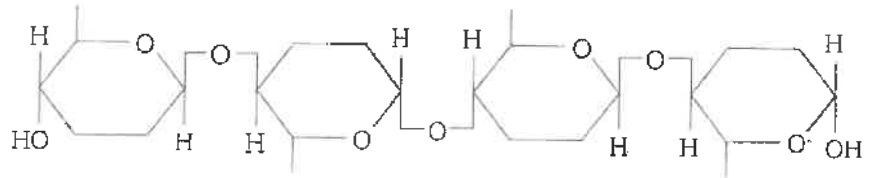


(b)



second β-glucose molecule is rotated forwards by 180° compared to first – as if it is doing a handstand

(c)



chains of β-glucose joined by condensation reactions are straight

Figure 1 Cellulose is made up of a chain of β-glucose molecules.

DID YOU KNOW?

Cellulose is the most common polysaccharide in the world.

Rather than spiralling like chains of α-glucose, cellulose chains are straight and lie side by side. This difference in structure is a direct result of bonding:

- Hydrogen and hydroxyl groups on carbon 1 are inverted in β-glucose (as compared with α-glucose). This means that every other β-glucose molecule in the chain is rotated by 180 degrees. This and the β-1–4 glycosidic bond help to prevent the chain spiralling.
- Hydrogen bonding between the rotated β-glucose molecules in each chain also gives the chain additional strength, and stops it spiralling.
- Hydrogen bonding between the rotated β-glucose molecules in different chains gives the whole structure additional strength. The hydroxyl group on carbon 2 sticks out, enabling hydrogen bonds to be formed between chains.

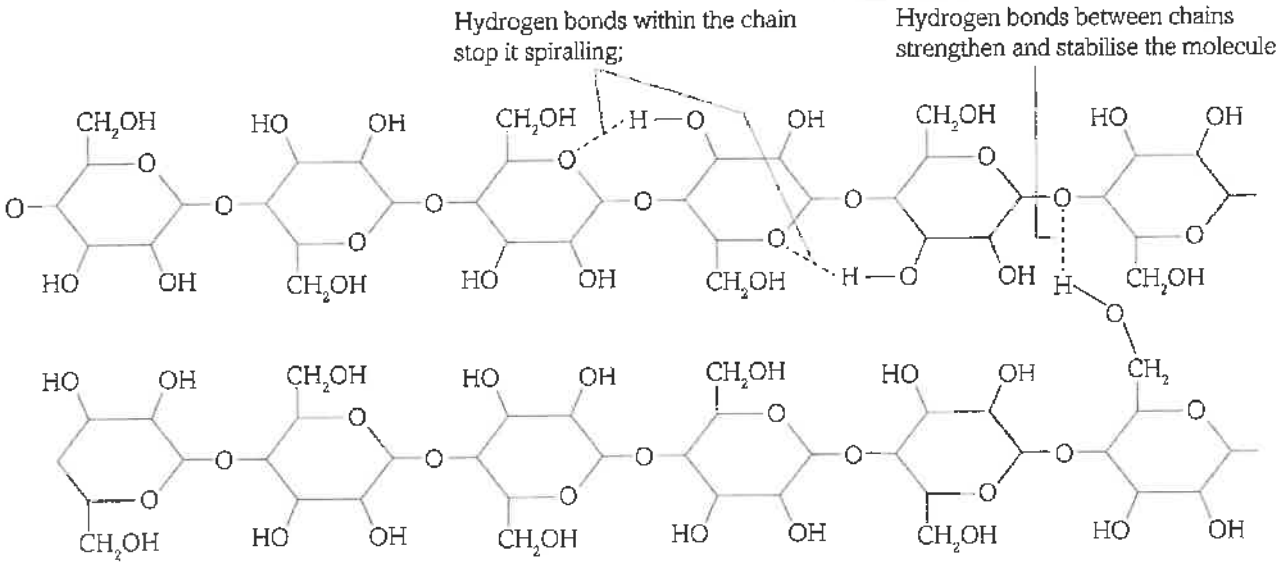


Figure 2 Straight cellulose molecules lie side by side. Hydrogen bonding within and between the chains of β-glucose molecules gives the structure strength.

When 60 to 70 cellulose chains are bound together in this way, they form microfibrils, which are 10–30 nm in diameter. These then bundle together into macrofibrils containing up to 400 microfibrils, which are embedded in pectins (like glue!) to form plant cell walls.

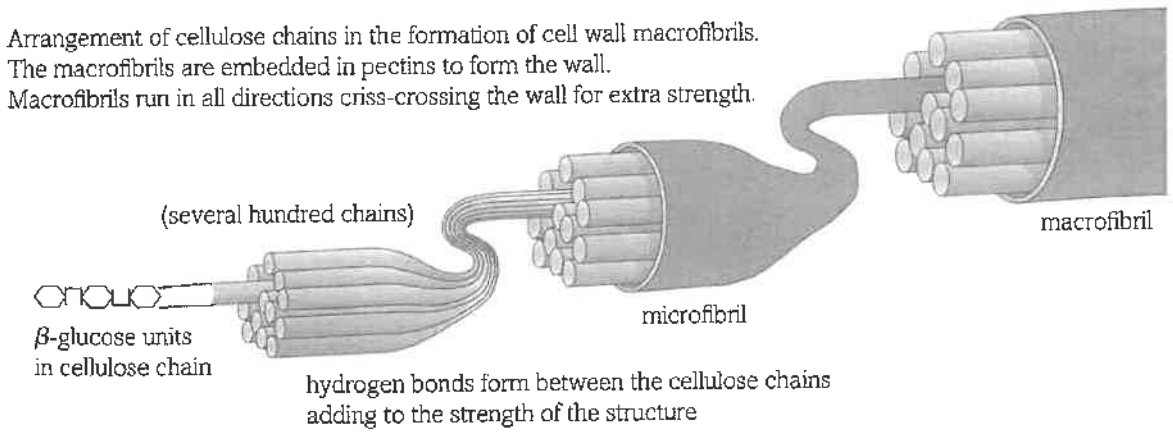


Figure 3 Relative structures of cellulose chains, microfibrils and a macrofibril (fibre).

Structure and function of plant cell walls

Cellulose is an excellent material for plant cell walls:

- Microfibrils and macrofibrils have very high tensile strength, both because of the strength of the glycosidic bonds but also because of the hydrogen bonds between chains. Macrofibrils are stronger than steel wire of the same diameter.
- Macrofibrils run in all directions, criss-crossing the wall for extra strength.
- It is difficult to digest cellulose because the glycosidic bonds between the glucose molecules are less easy to break. Indeed, most animals do not even have an enzyme to catalyse the reaction.

These key features help the plant cell wall to do its job:

- Because plants do not have a rigid skeleton, each cell needs to have strength to support the whole plant.
- There is space between macrofibrils for water and mineral ions to pass on their way into and out of the cell. This makes the cell wall fully permeable.

2.4 1 Enzymes – biological catalysts

By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:

* the role of enzymes in catalysing reactions that affect metabolism at a cellular and whole organism level
* the role of enzymes in catalysing both intracellular and extracellular reactions

KEY DEFINITIONS

active site: indented area on the surface of an enzyme molecule, with a shape that is complementary to the shape of the substrate molecule.
catalyst: chemical that speeds up the rate of a reaction and remains unchanged and reusable at the end of the reaction.
extracellular: outside the cell.
intracellular: inside the cell.
metabolic, metabolism: the chemical reactions that take place inside living cells or organisms.
product: molecule produced from substrate molecules, by an enzyme-catalysed reaction.
substrate: molecule that is altered by an enzyme-catalysed reaction.

Enzymes are called biological **catalysts** because they speed up **metabolic** reactions in living organisms. Their actions affect both structure and function within cells, tissues and organs.

- Catalysts speed up chemical reactions and remain unchanged at the end of the reaction, able to be used again.
- A small amount of catalyst can catalyse the conversion of a large number of **substrate** molecules into **product** molecules.
- The number of reactions that an enzyme molecule can catalyse per second is known as its *turnover number*.

Why are enzymes so remarkable?

Whereas chemical catalysts usually need very high temperatures, increased pressures and extremes of pH, enzymes speed up metabolic reactions by up to 10^{12} times at lower temperatures, often at neutral pH and at normal pressures. Hence, as biological catalysts, they are able to function in conditions that sustain life.

Enzymes are also *more specific* than chemical catalysts. They do not produce unwanted by-products and rarely make mistakes. The cells in which they are made and/or act can also regulate their production and activity to fit the needs of the cell or organism at the time.

Enzymes are therefore remarkable molecules, and in this chapter we shall see how they work.

Enzyme structure determines function

As with all biological molecules, the structure of enzymes enables them to carry out their functions.

- For enzymes to catalyse some reactions, they may need help from **cofactors** (see topic 2.4.2).
- The instructions for making enzymes are encoded in genes. If the gene has a mutation that alters the sequence of amino acids in the protein, then this may alter the enzyme's tertiary structure and prevent it from functioning.
- If an enzyme that catalyses a metabolic reaction is deficient, then a metabolic disorder results.
- Enzymes also catalyse the formation of the organism's structural components, such as collagen in bone, cartilage, blood-vessel walls, joints and connective tissue. Some genetic disorders cause malformations of connective tissue and can be very harmful, such as 'stone man syndrome' (see the Thinking Bigger spread in Chapter 2.6).

DID YOU KNOW?

Many metabolic disorders are caused by deficient or non-functioning enzymes, for example if the active site is misshapen. The genetic disorder phenylketonuria, PKU, results when the enzyme phenylalanine hydroxylase does not function and cannot convert the essential amino acid, phenylalanine, to another amino acid, tyrosine. As a result, sufferers cannot make melanin (which is made from tyrosine), and the accumulation of phenylalanine in their blood impairs brain development, leading to severe mental impairment.
Because this enzyme deficiency is so severe, all new-born babies are screened for PKU, so that if the result is positive their diet can be adjusted to include only very small amounts of phenylalanine, to prevent the irreversible brain damage.

The active site

Enzymes are large molecules with a specific area, an indentation or cleft on the surface of the molecule, called the **active site**. This consists of just a few – often about 6 to 10 – amino acids.

- The tertiary structure of the active site is crucial, as its shape is complementary to the shape of the substrate molecule.
- So, each type of enzyme is highly specific in its function, as it can only catalyse a reaction involving the particular type of substrate molecule that fits into its active site.

- The shape of the enzyme's active site, and hence its ability to catalyse a reaction, can be altered by changes in temperature and pH, as these affect the bonds that hold proteins in their tertiary structure.

LEARNING TIP

The active site is part of the enzyme molecule, not part of the substrate molecule.

LEARNING TIP

Most enzymes catalyse a reaction in either direction depending on the cell's needs. Hence ATPase can catalyse the formation of ATP or the hydrolysis of ATP. Sometimes an enzyme catalyses two reactions, because it is really a large enzyme complex and has more than one active site. So, the same enzyme complex may be known by different names.

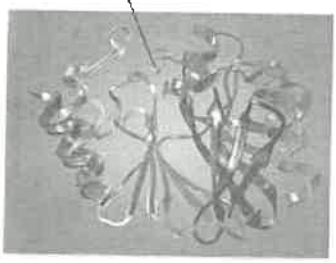


Figure 1 Structure of an enzyme molecule. The protein, made from a specific sequence of amino acids (the primary structure) has some pleats and coils (secondary structure) and is folded into its tertiary structure. The substrate molecule is shown attached to the cleft that forms the active site. This region, consisting of just a few amino acids, has a specific shape that is complementary to that of the substrate molecule.

Where enzymes work

Enzymes catalyse a wide range of **intracellular** as well as **extracellular** reactions.

Intracellular enzymes

In any cell, and within its organelles, there may be up to 1000 metabolic reactions going on at the same time, each being catalysed by a different enzyme. Some of these reactions are part of a metabolic pathway (see Figure 2).

- Each metabolic pathway in a living cell is one of a series of consecutive reactions, every step catalysed by a specific enzyme that produces a specific product.
- The various reactants and intermediates act as substrates for specific enzymes.
- The reactants, intermediates and products are known as metabolites.
- In some metabolic pathways, described as *catabolic*, metabolites are broken down to smaller molecules and release energy.
- In other metabolic pathways, described as *anabolic*, energy is used to synthesise larger molecules from smaller ones.
- Respiration and photosynthesis are examples of complex metabolic pathways, with many enzymes involved.

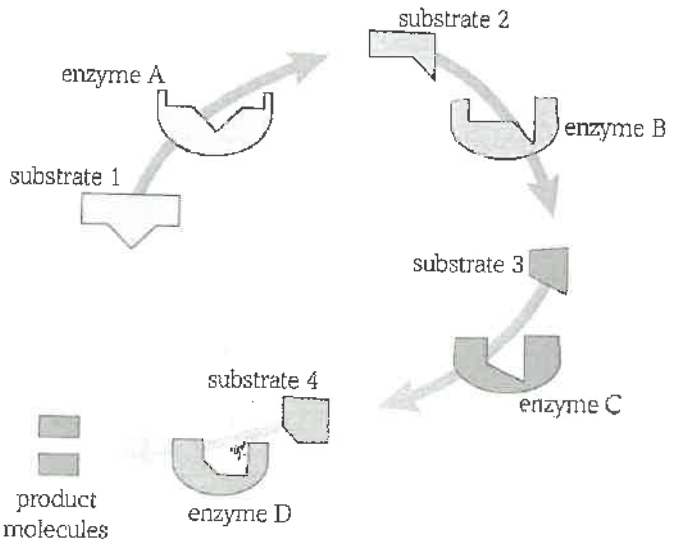


Figure 2 A metabolic pathway. Each step is catalysed by a different enzyme. If one of the enzymes cannot function, then the metabolic pathway cannot run.

Catalase is found in nearly all living organisms that are exposed to oxygen. It is a very important enzyme, as it protects cells from damage by reactive oxygen by quickly breaking down hydrogen peroxide, a potentially harmful by-product of many metabolic reactions, to water and oxygen.

- Catalase consists of four polypeptide chains and contains a haem group with iron (see topic 2.4.2).
- It is the fastest-acting enzyme, having the highest turnover number known, of about 6 million per second.
- In eukaryotic cells, catalase is found inside small vesicles, called peroxisomes.
- When white blood cells ingest pathogens they use catalase to help kill the invading microbe.

The optimum pH for human catalase is around pH 7, but for other species it varies between pH 4 and 11. The optimum temperature also varies with species. For humans this is 45 °C and for some thermophilic archaea it is 90 °C.

DID YOU KNOW?

Some people lose hair pigment and 'go grey' earlier in life than others. One reason may be that these people have lowered levels of catalase, and so more hydrogen peroxide bleaches their hair shafts from the inside.

Extracellular enzymes

Some enzymes are secreted from the cells where they are made and act on their substrates, extracellularly. Fungi, such as the bread mould *Mucor*, release hydrolytic enzymes from their thread-like hyphae. The enzymes digest carbohydrates, proteins and lipids in the bread, and the products of digestion – glucose, amino acids, glycerol and fatty acids, are absorbed into the fungal hyphae for use in respiration and growth.

In our digestive system many enzymes are secreted, from cells lining the alimentary canal, into the gut lumen. There they extracellularly digest the large molecules, such as proteins, lipids, carbohydrates and nucleic acids, found in food. The products of digestion are then absorbed, via epithelial cells of the gut wall, into the bloodstream in order to be used for respiration, growth and tissue repair.

- Amylase is produced in the salivary glands, and acts in the mouth to digest the polysaccharide starch to the disaccharide maltose. It is also made in the pancreas, and acts to catalyse the same reaction in the lumen of the small intestine.
- Trypsin is made in the pancreas, and acts in the lumen of the small intestine to digest proteins into smaller peptides by hydrolysing peptide bonds. Its optimum pH is between 7.5 and 8.5.

DID YOU KNOW?

If you had to wait for a meal to be digested in your alimentary canal, without any enzymes, it would take several years. Hence, without enzymes, the speed of chemical reactions inside living organisms could not sustain life. Humans produce about six times as much amylase as chimpanzees do. Chimpanzees eat some meat and a lot of fruit, but very few starchy vegetables.

Questions

1. Explain why enzymes (a) are specific in their action, and (b) are described as biological catalysts.
2. Peptidases are enzymes that catalyse the breaking of peptide bonds within small peptide molecules. Suggest names for the following enzymes: (a) those that catalyse the breaking of glycosidic bonds (between glucose residues in polysaccharides) and (b) those that catalyse the breaking of ester bonds (in triglycerides).
3. Explain what is meant by the 'turnover number' of an enzyme.
4. Explain why a change to the sequence of DNA base triplets in a gene can result in an enzyme that cannot function properly.
5. Suggest why a non-functioning enzyme may cause a serious disease.

2.4 3 The mechanism of enzyme action

By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:

- **the mechanism of enzyme action**

KEY DEFINITIONS

enzyme-product complex: enzyme molecule with product molecule(s) in its active site. The two are joined temporarily by non-covalent forces.

enzyme-substrate complex: enzyme molecule with substrate molecule(s) in its active site. The two are joined temporarily by non-covalent forces.

The lock-and-key hypothesis

You have already seen (in topic 2.4.1) that a specific indented area on the surface of the enzyme molecule, called the active site, is where the substrate molecules fit. They fit because the tertiary structure of the enzyme's active site gives it a shape that is *complementary* to that of the substrate molecule – rather like the way in which only a specific key will fit into a particular lock. This idea about how enzymes work is described as the lock-and-key hypothesis (see Figure 1). The lock is the enzyme's active site, and the key is the substrate molecule.

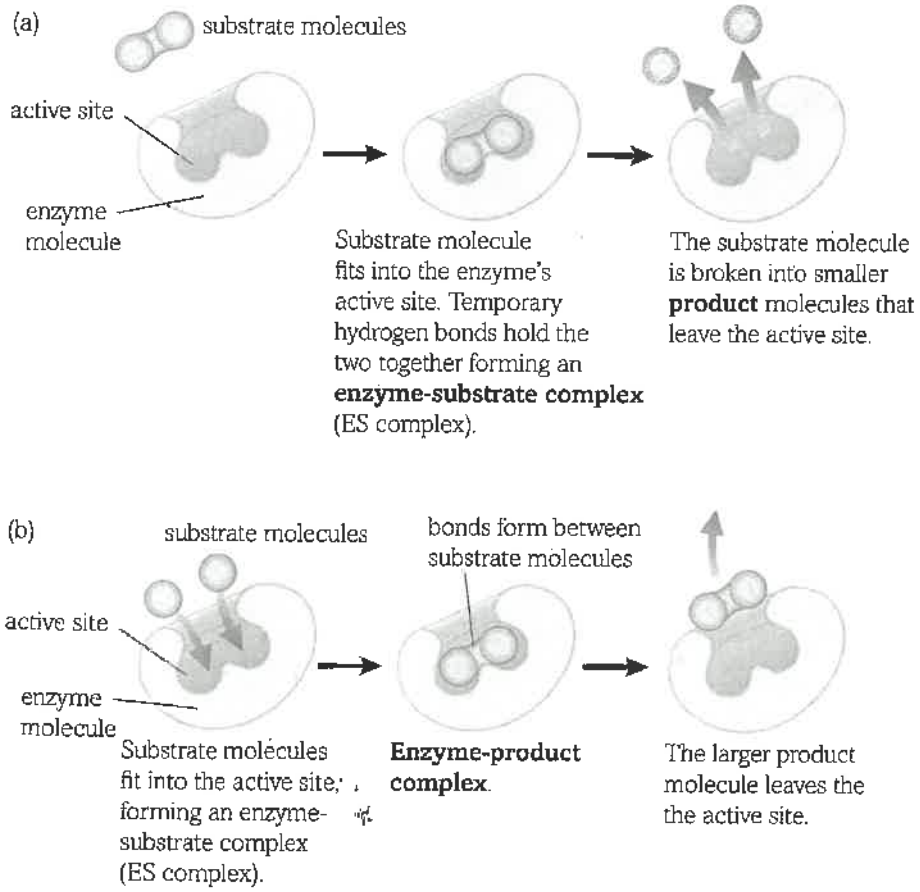


Figure 1 Lock-and-key mechanism of enzyme action. (a) A large substrate molecule is split into smaller product molecules that are then released from the active site. (b) Two small substrate molecules attach to the active site and bonds form between them, making a larger product molecule, which then leaves the active site. In each case, at the end of the reaction, the enzyme is able to form an ES complex with another substrate molecule and catalyse another reaction.

- The substrate molecules and enzyme molecules each have kinetic energy and are constantly moving randomly.
- If a substrate molecule successfully collides with an enzyme molecule, then an **enzyme-substrate complex** (ES complex) forms as the substrate molecule fits into the complementary-shaped active site on the enzyme molecule.
- The substrate molecules are either broken down or built up into the product molecule(s), and these form an **enzyme-product complex** whilst still in the active site.
- The product molecules leave the active site.
- The enzyme molecule is now able to form another enzyme-substrate complex.
- A small number of enzyme molecules can therefore convert a large number of substrate molecules into product molecules.

DID YOU KNOW?

A scientist, Emil Fischer, discovered in 1894 that glycolytic (sugar-splitting) enzymes could distinguish between sugar molecules that have the same molecular formula but slight differences in the geometry of how the atoms are arranged within the molecules (think of a left-hand glove and a right-hand glove). This led him to form his lock-and-key hypothesis, which explains enzyme specificity.

DID YOU KNOW?

Although scientists have known about enzymes since the late 19th century, it was only in the 1930s that it was established that enzymes are proteins. The first enzyme to have its amino acid sequence worked out was bovine pancreatic ribonuclease, in 1963. In 1965, the first 3D structure of an enzyme, hen egg-white lysozyme, was worked out using X-ray diffraction. Since then, the structures of many enzymes have been elucidated. In the 1990s, scientists discovered that some types of RNA have catalytic properties in cells.

The induced-fit hypothesis

Although the lock-and-key hypothesis explains enzyme specificity, it does not explain how the transition state – namely the ES complex – is stabilised.

In 1958, Daniel Koshland modified the lock-and-key hypothesis by suggesting that the active site of the enzyme is not a rigid fixed structure, but that the presence of the substrate molecule in it *induces* a shape change, giving a good fit (see Figure 2).

He suggested that:

- When the substrate molecules fit into the enzyme's active site, the active site changes shape slightly to mould itself around the substrate molecule. Think of putting on a glove – it will only accept a hand-shaped object, but when you insert your hand the glove moulds around and fits your hand perfectly.

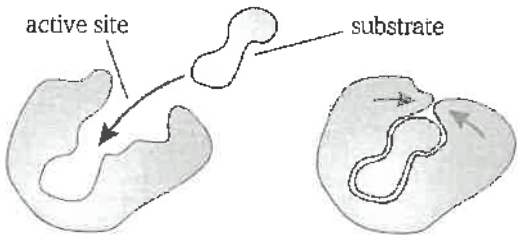


Figure 2 The induced fit hypothesis. Binding of the substrate molecule (yellow) to the active site of the enzyme (blue) results in a change of shape in the enzyme's active site so that it fits around the substrate molecule more closely to give the enzyme-substrate (ES) complex. When the product molecules are still attached to the active site this gives an enzyme-product complex.

- The active site still has a shape complementary to the shape of the substrate molecule. But, on binding, the subtle changes of shape of the side chains (R-groups) of the amino acids that make up the active site give a more precise conformation that exactly fits the substrate molecule.
- This moulding enables the substrate to bind more effectively to the active site.

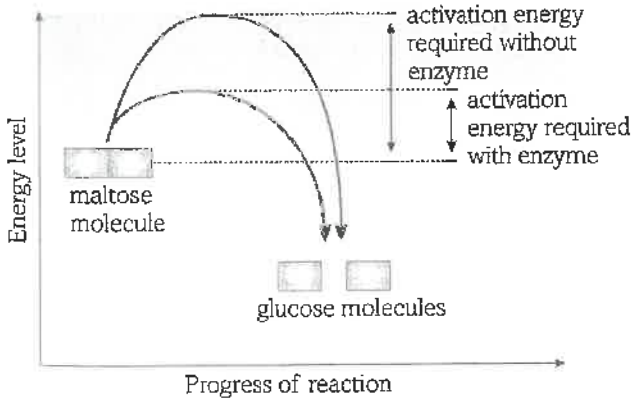
- An enzyme-substrate complex is formed, and *non-covalent* forces such as hydrogen bonds, ionic attractions, van der Waals forces and hydrophobic interactions, bind the substrate molecule to the enzyme's active site.
- When the substrate molecules have been converted to the product molecules and these are still in the active site, they form an enzyme-product complex.
- As the product molecules have a slightly different shape from the substrate molecule, they detach from the active site.
- The enzyme molecule is now free to catalyse another reaction with another substrate molecule of the same type.
- The equation below outlines how an enzyme catalyses a reaction:

Enzyme + Substrate	→	Enzyme-substrate complex	→	Enzyme-product complex	→	Enzyme + Product
E + S	→	ESC	→	EPC	→	E + P

Enzymes lower the activation energy of a reaction

Chemical reactions need energy to activate or begin them. Many chemicals can be heated to provide this activation energy and make them react together. This increases the kinetic energy of the molecules so that they move about more, in a random fashion, and are more likely to successfully collide and then react together.

In a living cell, the temperature cannot be raised too much or the proteins within it would denature and lipids would melt. Because enzymes have an active site specific to *only* the substrate molecules, they bring the substrate molecules close enough together to react, without the need for excessive heat. Therefore they lower the activation energy and hence speed up metabolic reactions (see Figure 3).



Adding the enzyme maltase reduces the amount of activation energy required for the reaction to take place

Figure 3 How the enzyme maltase lowers the activation energy needed to hydrolyse maltose to glucose.

LEARNING TIP

Digestion means to break down. Large molecules in food, such as proteins and starch, are digested by being broken down by hydrolysis to smaller molecules, such as amino acids and glucose. Therefore the words 'digestion' and 'hydrolysis' may be used synonymously.

INVESTIGATION

Enzyme specificity

Wear eye protection.

Yeast, *Saccharomyces cerevisiae*, is a single-celled fungus. It is a facultative anaerobe, which means it can respire with or without oxygen. If oxygen is present, then it will respire aerobically, but if oxygen is absent then it can respire anaerobically.

When yeast respires anaerobically it produces ethanol and carbon dioxide. The carbon dioxide produced during a unit time, such as 10 minutes, can be collected and its volume measured.

Yeast may respire different sugars. Enzymes are specific and each type will catalyse a specific sugar. You can investigate whether yeast can metabolise the monosaccharide sugars glucose, fructose and galactose.

Questions

Use information gained from previous chapters, as well as the information in this chapter, to help you answer these questions.

1. Explain how enzymes lower the activation energy and therefore enable metabolic reactions to proceed quickly at the relatively low body temperatures of living organisms.
2. Explain how a cell can carry out many different types of metabolic reaction at the same time.
3. Describe how the lock-and-key hypothesis of enzyme action differs from the induced-fit hypothesis.
4. Some organisms grow in extreme environments. For example, some archaea and bacteria grow in very hot springs. Suggest how the structure of their enzymes, and other proteins, enables them to withstand such high temperatures.
5. Explain how an enzyme-substrate complex differs from an enzyme-product complex.
6. Are the enzymes involved in respiration intracellular or extracellular? Explain your answer.
7. Explain why a large amount of substrate can be converted by a small amount of enzyme.

2.4 4 The effect of temperature on enzyme activity

By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:

- the effect of temperature on enzyme activity
- practical investigations into the effect of temperature on enzyme activity

KEY DEFINITION

Q_{10}: temperature coefficient, calculated by dividing the rate of reaction at $(T + 10)$°C by the rate of the reaction at T°C.

Heat and kinetic energy

All molecules have kinetic energy and can continuously move around randomly. In so doing, molecules will collide with one another.

If a substance is heated, then:

- The extra energy, in the form of heat, causes the molecules to move faster.
- This increases the rate of collisions between molecules.
- It also increases the force with which they collide, as they are moving faster.

Collisions between enzyme and substrate molecules

You have already seen in topic 2.4.3 that an enzyme-catalysed reaction only takes place if a substrate molecule successfully collides with the active site of an enzyme molecule, forming an enzyme-substrate complex.

If the reactant mixture containing enzyme and substrate molecules is heated, then:

- both types of molecule will gain kinetic energy and move faster
- this will increase the rate (number per second) of successful collisions
- therefore the rate of formation of ES complexes increases, and the rate of reaction increases, increasing the number of enzyme-product complexes per second, up to a point
- at a particular temperature, called the enzyme's **optimum temperature**, the rate of reaction is at its *maximum*.

Heat makes molecules vibrate

As well as making molecules move faster, increasing the temperature also makes them vibrate.

- This may break some of the weak bonds, such as hydrogen and ionic bonds, that hold the tertiary structure of the enzyme's active site.
- As the active site shape begins to change, the substrate molecules will not fit into it so well and the rate of reaction begins to decrease.
- As more heat is applied, the shape of the enzyme's active site completely and *irreversibly* changes so that it is no longer complementary to the shape of the substrate molecule.
- The reaction cannot proceed at all.
- The enzyme is **denatured** (see Figure 1).

Heat does not break the peptide bonds between amino acids, so the enzyme's *primary structure* is not altered.

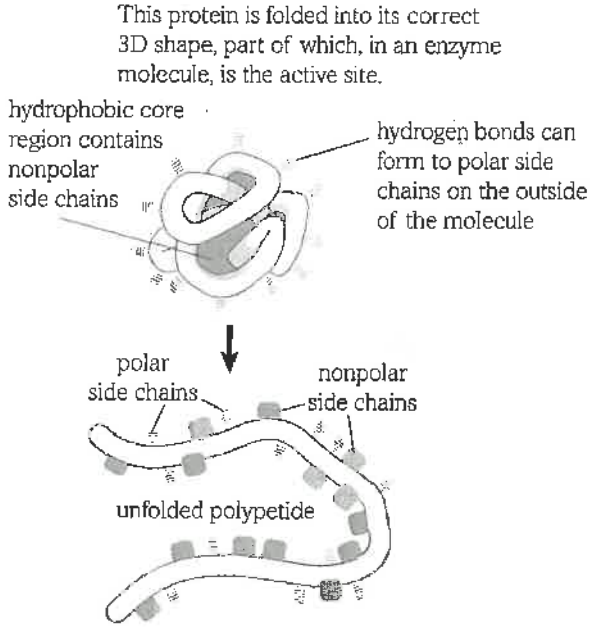


Figure 1 Denaturation.

Optimum temperature

This is the temperature at which the enzyme works *best*. It is the temperature at which the enzyme has its *maximum* rate of reaction.

LEARNING TIP

Enzymes are biological molecules but they are not organisms. They are *not* killed, they are denatured.

Some enzymes work best at cool temperatures. Bear in mind that some organisms are adapted to living in cold places. For example, there are *psychrophilic* bacteria (see Figure 2(a)), which live in very cold conditions. Their enzymes can work at really low temperatures.

Some organisms, such as *thermophilic* bacteria in hot springs, live at very high temperatures (see Figure 2(b)). Their enzymes (as well as their other proteins) are heat stable. They have more disulfide bonds that do not break with heat and keep the shape of the protein molecules stable. Their enzymes will have high optimum temperatures. You will learn in the second year of your course about an enzyme obtained from a thermophilic bacterium, *Thermophilus aquaticus*, called Taq polymerase, that is used at high temperatures in the polymerase chain reaction (PCR) to amplify fragments of DNA for forensic gene-screening analysis or cloning.

(a) 

(b) 

Figure 2 (a) Red algae growing in ice. Their enzymes function at cold temperatures. (b) Thermophilic bacteria in a hot pool. The enzymes of these bacteria function well at high temperatures.

The graph in Figure 3 shows the effect of temperature on the rate of an enzyme-catalysed reaction. In this case, the reaction is the digestion (hydrolysis) of cooked egg-white (the protein albumen) by the protease enzyme pepsin, at pH 2. The rate of reaction was measured by the time taken for the end point (a clear solution) to be reached.

$$\text{Rate of reaction} = \frac{1}{\text{time taken to reach end point}}$$

The units for rate of reaction are s^{-1}

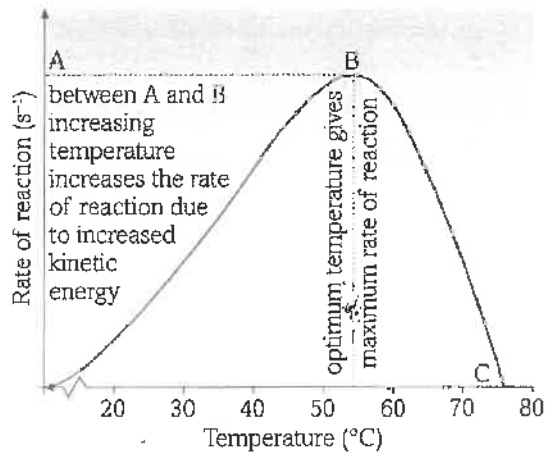


Figure 3 The results of an investigation into the effect of temperature on the action of the protease enzyme pepsin. This enzyme has an optimum temperature of around 50–60 °C, which is higher than the mammalian body temperature.

Temperature coefficient, Q_{10}

The temperature coefficient here refers to the increase in the rate of a process when the temperature is increased by 10 °C.

It is given by the equation:

$$Q_{10} = \frac{\text{rate of reaction at } (T + 10)\,^{\circ}\text{C}}{\text{rate of reaction at } T\,^{\circ}\text{C}}$$

For chemical reactions in a test tube, Q_{10} is approximately 2, which means that for every 10 °C rise in temperature the rate of reaction is doubled.

For metabolic reactions catalysed by enzymes, between temperatures of 0 °C and approximately 40 °C, the rate of these reactions is also roughly doubled for every 10 °C rise in temperature. This is because the increase in temperature provides more kinetic energy, so enzyme and substrate molecules move faster and collide more often.

For enzymes at temperatures above their optimum temperature, the value of the temperature coefficient, Q_{10}, drops. This is because the higher temperatures alter the structure of the active sites of the enzyme molecules so that they are no longer complementary to the shape of the substrate molecules.

WORKED EXAMPLE

The table below shows the results of starch hydrolysis in a solution of Linmer's (soluble) starch and amylase enzyme.

Temperature (°C)	Mass of starch hydrolysed in 10 minutes (mg)
25	50
35	108
45	100

Q_{10} (between 25 and 35 °C) is $\frac{108}{50} = 2.16$

Q_{10} (between 35 and 45 °C) is $\frac{100}{108} = 0.93$

INVESTIGATION

There are many enzymes that you can use to investigate the effect of temperature change. One is phosphatase enzyme. This enzyme breaks down organic phosphates in cells in order to maintain the pool of phosphate ions for use by cells to make chemicals such as ATP, ribulose bisphosphate and NADP.

Effect of temperature on phosphatase enzyme

Wear eye protection.

In this investigation, a chemical substrate phenolphthalein phosphate, (PPP), is used. Phosphatase enzyme breaks this down, liberating free phenolphthalein. When sodium carbonate, which is alkaline, is added in excess, the free phenolphthalein produces a deep-pink colour. The intensity of the colour is proportional to the concentration of free phenolphthalein. This intensity can be measured using a colorimeter with a green filter. If each reaction tube has been given the same length of time, the intensity of the colour gives an indication of the rate of reaction – the darker the pink, the more molecules of PPP were hydrolysed by the enzyme in the set period of time.

Questions

1. Explain why the rate of an enzyme-controlled reaction is lower at 10 °C than it is at 30 °C.
2. Explain why an enzyme solution kept in the fridge for a week will catalyse a reaction at 40 °C, whereas an enzyme solution that has been boiled does not catalyse a reaction at 40 °C.
3. Explain why the rate of an enzyme-catalysed reaction does not keep on increasing as the temperature is increased above 40 or 50 °C.
4. Explain why using living tissue, such as bean sprouts, as a source of enzyme may lead to difficulties in controlling the enzyme concentration.
5. (a) Explain the difference between the independent variable and the dependent variable.
   (b) Explain why tubes of reactants and separate tubes of enzyme solutions are placed in the water-bath for 5–10 minutes before they are mixed and the reaction allowed to proceed.
   (c) Explain why, in an investigation about enzyme activity, the enzyme solution is always added last.

2.4 5 The effect of pH on enzyme activity

By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:

- **the effect of pH on enzyme activity**
- **practical investigations into the effect of pH on enzyme activity**

What is pH?

The pH indicates whether a substance is acidic, alkaline or neutral.

pH values of 0–6 are acidic, pH 7 indicates neutral, and pH 8–14 indicates that the solution is alkaline.

Acids such as hydrochloric acid and sulfuric acid dissociate into protons and a negatively charged ion.

$HCl \rightarrow H^+ + Cl^-$

$H_2SO_4 \rightarrow H^+ + HSO_4^-$

Organic acids are also proton donors:

- lactic acid dissociates into H^+ and lactate
- pyruvic acid dissociates into H^+ and pyruvate

Buffers

In biology, a **buffer** is something that resists changes in pH. There are certain chemicals in your blood that help resist changes in pH so that the blood pH remains within fairly narrow limits close to pH 7.4. These chemicals can donate or accept hydrogen ions. Some proteins, such as haemoglobin, can also donate or accept protons and so act as buffers.

In laboratory investigations, you will use buffer solutions to maintain the desired pH for investigating enzyme action at different pH values or to keep the pH constant whilst you investigate another factor.

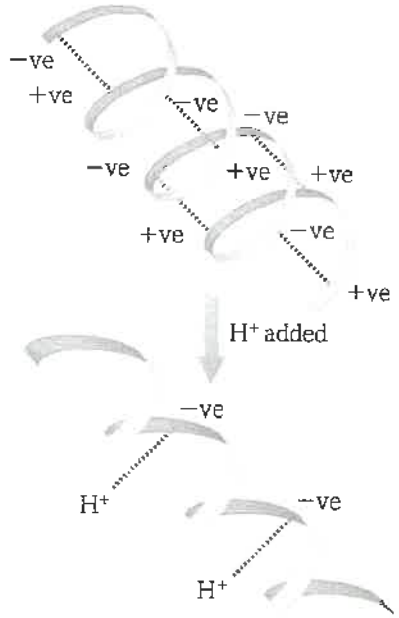
LEARNING TIP

- Remember that pH is worked out by the formula $\log \frac{1}{[H^+]}$, so a large concentration of hydrogen ions gives a small value of pH. If the acidity increases, then the pH decreases.
- Remember also that hydrogen ions are protons.

How changes in pH affect bonds within molecules

- A hydrogen ion, which is a proton, has a positive charge, so it is attracted towards negatively charged ions, molecules or parts of molecules. Figure 1 shows how hydrogen bonds and ionic forces between amino acids hold the tertiary structure of an enzyme molecule, particularly the active site, in the correct shape so that the substrate molecule will fit into it.
- Excess hydrogen ions will interfere with these hydrogen bonds and ionic forces, and so the active site of the enzyme molecule will change shape. If the substrate molecule does not fit well into the active site, then the rate of the reaction that the enzyme catalyses will be lowered.
- Increasing the concentration of hydrogen ions will also alter the charges on the active site of enzyme molecules, as more protons will cluster around negatively charged groups (for example amino acid R-groups – see topic 2.2.8) in the active site. This interferes with the binding of the substrate molecule to the active site.

Hydrogen bonds hold structures like an α helix in place in protein molecules.



As H^+ is increased in concentration, the positive charges are attracted to the negative charges on the α helix and so 'replace' the hydrogen bonds.

Figure 1 How protons interfere with the hydrogen bonds holding the tertiary structure of an enzyme molecule.

Enzymes work within a narrow range of pH.

- *Small* changes of pH, either side of the **optimum pH**, slow the rate of reaction, because the shape of the active site is disrupted (see Figure 2).
- However, if the normal optimum pH is restored, the hydrogen bonds can re-form and the active site's shape is restored.
- At extremes of pH, the enzyme's active site may be permanently changed. When the enzyme is thus denatured, it cannot catalyse the reaction.

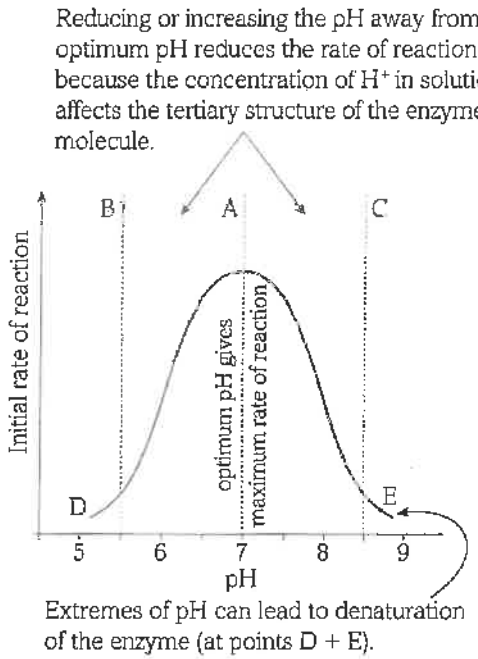


Figure 2 The effect of pH on an enzyme-catalysed reaction.

Not all enzymes have the same optimum pH

Enzymes that work intracellularly have an optimum pH that is close to pH 7.

- Enzymes that work extracellularly may have optimum pH values different from pH 7. During digestion, for example, food is first taken into your mouth, and the amylase enzymes that digest starch to maltose there work best at a pH of 6.8.
- As food passes into your stomach, hydrochloric acid is secreted, giving a very low pH. One of the acid's functions is to kill bacteria and other pathogens in the food. The protease enzyme, pepsin, in the stomach works best at very low pH values, of between 1 and 2. It digests large protein molecules into smaller peptide molecules.
- As the partly digested food moves into the small intestine, salts in bile made in the liver neutralise it and raise the pH to around 7.8. This is optimal for the protein-digesting enzymes, trypsin and enterokinase, that catalyse further digestion of peptides to amino acids in the small intestine (Figure 3).

DID YOU KNOW?

It is important that your blood pH is maintained within quite narrow limits, between 7.35 and 7.45. There are many plasma proteins in blood. Changes in blood pH can also cause vasodilation or vasoconstriction. In the blood plasma there are buffers that resist changes in pH. For example, potassium dihydrogenphosphate can donate hydrogen ions. Some proteins act as buffers, as they can donate or accept protons. Hydrogencarbonate ions also act as buffers.

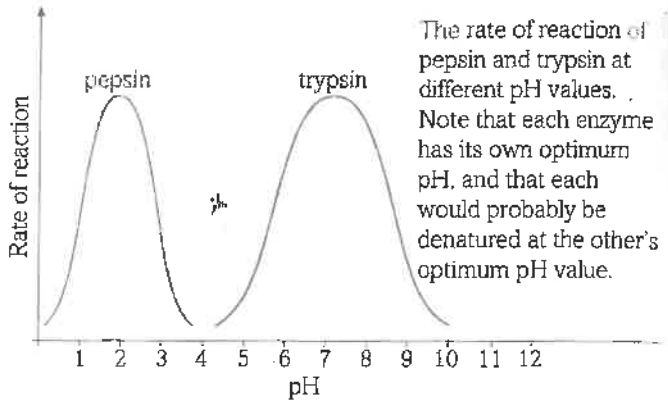


Figure 3 Pepsin and trypsin – rates of reaction at different pH values.

INVESTIGATION

The effect of pH changes on the rate of an enzyme-catalysed reaction

Design an investigation to find the effect of changing pH on the activity of the enzyme phosphatase. You can then carry out similar experiments with the enzymes pepsin and trypsin.

In each case, write a plan for your investigation. Include a clearly stated testable hypothesis; a list of apparatus needed; the independent and dependent variables; control variables with details of how and why you need to control them; and how you will deal with your data.

Questions

1. Explain why enzymes only work within narrow ranges of pH.
2. Suggest why blood pH is one of the factors monitored closely in some hospital patients.
3. Explain what is meant by 'a buffer solution'.
4. Explain why both increasing and decreasing the pH away from the optimum pH for a specific enzyme reduces its rate of reaction.
5. Dogs produce a much greater concentration of stomach acid than do humans. Hence they are able to eat food that would make us ill. How would you expect the optimum pH of canine pepsin to differ from that of human pepsin? How could you investigate this experimentally?
6. The proteases pepsin and trypsin are both produced in an inactive form – pepsinogen and trypsinogen, respectively. The stomach acid changes pepsinogen to the active pepsin, and an enzyme enterokinase changes trypsinogen to trypsin. Suggest why these enzymes are first secreted in an inactive form.

2.4 6 The effect of substrate concentration on the rate of enzyme-catalysed reactions

By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:

- the effects of substrate concentration on enzyme activity
- practical investigations into the effects of substrate concentration on enzyme activity

KEY DEFINITION

concentration: number of molecules per unit volume.

The effect of changing substrate concentration

If there is no substrate present, then an enzyme-catalysed reaction (see topic 2.4.3) cannot proceed. This is because there are no substrate molecules to fit into the enzyme molecules' active sites, and so no enzyme-substrate complexes can be formed.

As substrate is added and its **concentration** increases, the rate of reaction increases (see Figure 1).

- This is because more enzyme-substrate (ES) complexes can form.
- As a result, more product molecules are formed.
- Substrate concentration is limiting the reaction, because, as it increases, the rate of reaction increases.
- Substrate concentration is the **limiting factor**.

As the concentration of substrate is increased even further, the reaction will reach its *maximum* rate.

- Adding more substrate molecules to increase the substrate concentration will *not* increase the rate of reaction.
- This is because all the enzymes' active sites are occupied with substrate molecules.
- If more substrate molecules are added, then they cannot successfully collide with and fit into an enzyme's active site.

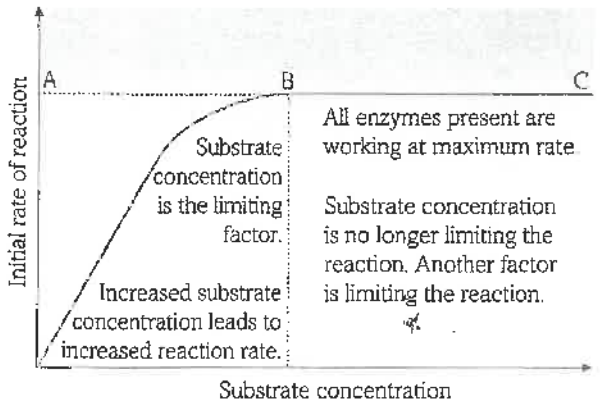


Figure 1 The effect of increasing substrate concentration on the rate of an enzyme-controlled reaction.

INVESTIGATION

The effect of increasing substrate concentration on the rate of an enzyme-catalysed reaction

Urease is a hydrolytic enzyme found in some bacteria, fungi and plants. It breaks the C–N bonds in amides, such as urea.

$$(NH_2)_2CO + H_2O \rightarrow 2NH_3 + CO_2$$

This releases ammonia, which can be used by the organisms as a source of nitrogen.

As ammonia is released, the pH of the solution is increased. The indicator phenol red changes from yellow to pink when alkaline (see Figure 2). Universal indicator solution may also be used to detect the change of pH due to the evolution of ammonia. The solution will change from green to blue.

Colorimetry can be used to measure absorbance and hence depth of colour, and therefore the amount of ammonia produced in a set time. This gives an indication of the rate of reaction.

The independent variable is concentration of urea. The dependent variable is rate of reaction as measured by absorbance.

Control variables:

- concentration of enzyme solution
- volume of enzyme and substrate solutions
- temperature (use a thermostatically controlled water-bath at a specified temperature within the range 30–40 °C)
- time for reaction, e.g. 20 minutes
- stirring/shaking of reactants
- wavelength of light/filter in the colorimeter.

Wear eye protection.

You will have a stock solution of urea of 0.1 M concentration. Make up a range of urea concentrations as follows:

Volume of 0.1 M urea solution (cm^3)	Volume of distilled water (cm^3)	Concentration of urea (M)
10	0	0.10
9	1	0.09
8	2	0.08
7	3	0.07
6	4	0.06
5	5	0.05
4	6	0.04
3	7	0.03
2	8	0.02
1	9	0.01
0	10	0.00

2.4 7

The effect of enzyme concentration on the rate of reaction

By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:

* the effects of enzyme concentration on enzyme activity
* practical investigations into the effects of enzyme concentration on enzyme activity

Enzyme availability in cells

In a laboratory practical, you may be able to change the concentration of a solution of a particular enzyme. In living cells, the enzyme concentration or availability depends on the rate of synthesis of the enzyme and its rate of degradation.

Each of these rates is directly controlled by the cell.

Enzyme synthesis

Depending on the cell's needs, genes for synthesising particular enzymes can be switched on or off.

Enzyme degradation

The protein component of living cells is constantly being turned over. Cells are continuously degrading old enzyme molecules to their component amino acids and synthesising new enzyme molecules from amino acids.

This might appear to be rather wasteful, but there are advantages:

- the elimination of abnormally shaped proteins that might otherwise accumulate and harm the cell
- the regulation of metabolism in the cell by eliminating any superfluous (surplus to requirements) enzymes.

Therefore, for a cell to regulate its metabolism properly, the control of enzyme degradation is equally as important as the control of enzyme synthesis.

DID YOU KNOW?

One of the longest-lived proteins in humans is haemoglobin, found inside erythrocytes. Each haemoglobin molecule lasts for about 120 days.

Increasing the enzyme concentration in an enzyme-controlled reaction

As enzyme concentration increases (see Figure 1):

- more active sites on the enzyme become available
- more successful collisions between the enzyme and substrate occur
- more enzyme-substrate (ES) complexes can form per unit time, so the rate of reaction increases
- enzyme concentration is the limiting factor – as it increases, so does the rate of reaction.

If substrate concentration is fixed or limited, all the substrate molecules will be occupying an active site, or will have occupied an active site and been released as product molecules. The reaction is at its maximum rate for the fixed substrate concentration, so:

- if the enzyme concentration is increased further, then there will be no increase in the rate of reaction because the active sites of the extra enzyme molecules will not be occupied by substrate molecules
- the enzyme concentration is no longer the limiting factor; as enzyme concentration increases, the rate of reaction does *not* increase
- substrate concentration is now the limiting factor. Lack of substrate molecules is preventing the rate of reaction from increasing.

LEARNING TIP

When you study a graph, describe what is happening in each region and explain why these changes are occurring. Refer to both axes and quote data figures if you can.

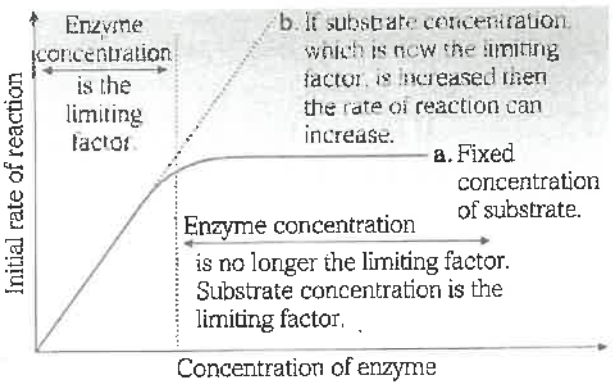


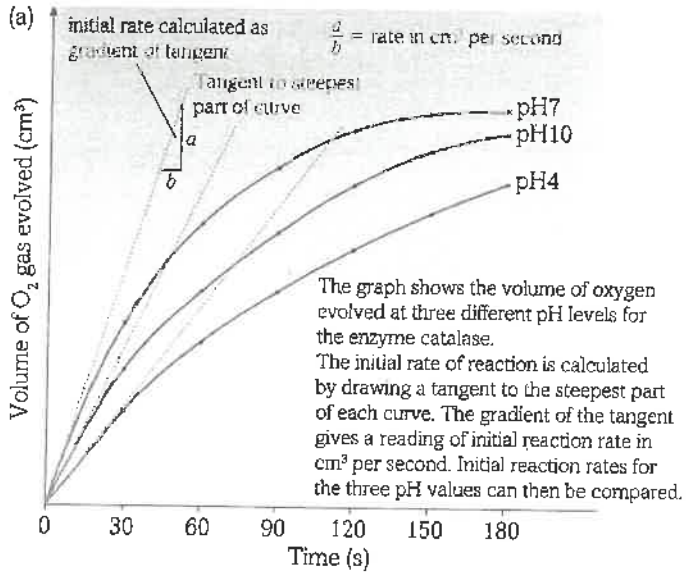
Figure 1 Effect of enzyme concentration on the rate of an enzyme-controlled reaction.

Initial rate of reaction

In any reaction, the initial rate of reaction between the reactants is fastest. The same is true of an enzyme-catalysed reaction.

- At the beginning of the reaction, when enzyme and substrate molecules are first mixed and are then moving randomly, there is a great chance of a substrate molecule successfully colliding with an enzyme's active site.
- As the reaction proceeds, substrate molecules are used up as they are converted to product molecules, so the concentration of substrate drops.
- As a result, the frequency of successful collisions between enzyme and substrate molecules decreases because some enzymes may collide with product molecules, and so the rate of the reaction slows down.
- Thus, the initial reaction rate gives the maximum reaction rate for an enzyme under a particular experimental situation.

(a)



(b)

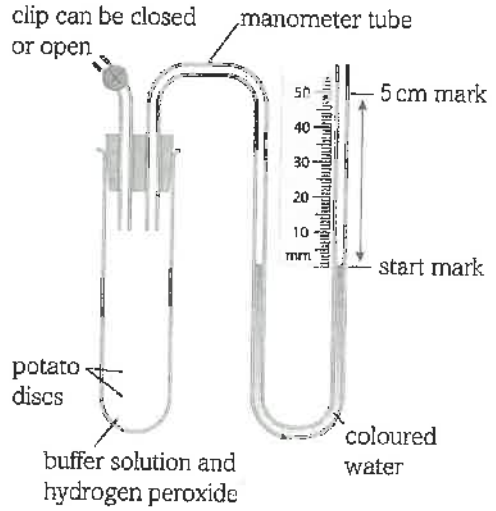


Figure 2 (a) Comparing the initial rates of reaction, at different pH values, for the breakdown of hydrogen peroxide catalysed by the enzyme catalase, using the apparatus shown in (b).

INVESTIGATION

The effect of enzyme concentration on an enzyme-catalysed reaction

Wear eye protection.

You have carried out an investigation into the effect of changing the substrate (urea) concentration on the activity (rate of reaction) of the enzyme urease.

Now think about how changing the enzyme (urease) concentration will affect the rate of the same reaction – the breakdown of urea catalysed by urease.

Write a plan for the investigation to include the following:

1. State your hypothesis and explain it.
2. State your independent and dependent variables.
3. Decide which concentration of substrate (urea) you will use. Do you want the substrate to be in excess?
4. Decide on a suitable range of enzyme concentrations and how you will make up these solutions. Which strength of stock solution of urease will you need?
5. Which variables will you need to control? How will you control them? Why do they need to be controlled?
6. Draw up a table showing the apparatus that you will need and explain why you need each piece.
7. Carry out a risk assessment.
8. Carry out your investigation, repeating each reading so you have three sets of data at each enzyme concentration and can find the mean value. This will also help you identify anomalies and repeat those anomalous readings if necessary.
9. Graph your data.
10. Write a conclusion.
11. Discuss sources of errors and limitations, and suggest improvements to your method.

Questions

1. Suggest why cells regulate the amount of enzymes in them.
2. When cells make new enzymes they may need more of specific types of amino acids than have been made available by enzyme degradation. What is the source/origin of the amino acids that have not come from the degradation of enzymes?
3. Suggest why the number of enzyme molecules within a living cell is kept fairly low.
4. Explain why, if you want to demonstrate the effect of any independent variable on the rate of an enzyme-catalysed reaction, it is best to compare the initial reaction rates at different values for the independent variable.