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Biology

Revision Guide

John Adds and Phil Bradfield

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How to use this book

Learning outcomes

When you have finished this unit, you should be able to:

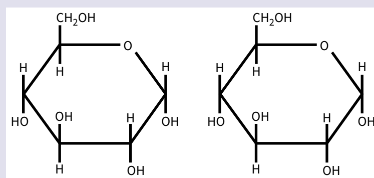
- know where phagocytes are made and describe their mode of action
- explain the terms immune response, antigen, self and non-self
- describe the modes of action of B-lymphocytes and T-lymphocytes
- explain the role of memory cells in long-term immunity
- describe and explain the effects of infections and leukaemias on white blood cell counts
- explain how the structure of an antibody molecule is related to its function
- distinguish between the different types of immunity – active and passive, natural and artificial
- describe how vaccination can control some infectious diseases
- discuss why vaccination has eradicated smallpox, but not measles, tuberculosis, malaria or cholera
- describe how autoimmune diseases occur, with reference to myasthenia gravis
- describe the hybridoma method for producing monoclonal antibodies
- outline the use of monoclonal antibodies in the diagnosis and treatment of disease.

Learning outcomes set the scene of each chapter, help with navigation through the book and give a reminder of what's important about each topic.

Worked example 2.01

Question

The diagram shows the structure of two molecules of α-glucose:



1 Copy the diagram and show how these two molecules are linked together to form a molecule of maltose.

2 Name the type of reaction involved in this process.

3 Name the type of bond formed.

Answers

1 It is important to be careful when copying the structure of the glucose molecules (e.g. by showing the positions of the –OH and –H correctly on each carbon atom). When these two molecules of glucose join to form maltose, water is lost by the removal of –OH and –H from each molecule, so you should carefully draw a circle round these on carbon atom number 1 of the first glucose molecule and carbon atom number 4 of the second glucose molecule, with water (H_2O) given off as a product. The two glucose molecules are now linked by a –O– bond between carbon atom 1 and carbon atom 4.

2 This is an example of a condensation reaction.

3 The type of bond formed is known as a glycosidic bond. (Be careful to spell this correctly!)

Worked examples provide a step-by-step approach to answering questions, guiding you through from start to finish.

Sample question 8.01

The llama is a mammal that lives in the high Andes of South America, often at an altitude of over 5000 metres above sea level. The oxygen dissociation curve for llama haemoglobin is to the left of the dissociation curves for most other mammals. Suggest why this is an important adaptation for llamas. [4 marks]

[Mark points are shown in square brackets – to a maximum of 4 marks]

At high altitudes, the partial pressure of oxygen in the air is low. [1] Because the dissociation curve is to the left of other mammals, llamas' haemoglobin will have a relatively high affinity for oxygen. This means that it will be fully saturated with oxygen [1] at low partial pressures [1]. Haemoglobin from other mammals would only be partly saturated with oxygen at high altitudes [1]. This is how llamas are adapted to life at high altitudes.

This is a good explanation of the importance of this effect. Remember that as the dissociation curve moves to the left, it implies that the haemoglobin has an increased affinity for oxygen. In other words, it will pick up oxygen and become saturated at lower partial pressures. As altitude increases, the air pressure drops which is why the partial pressure of oxygen decreases. At an altitude of 5000 metres, the partial pressure of oxygen in air is only about 50% of its value at sea level.

Sample questions contain an example of an excellent answer and an explanation of how the answer achieves this.

Progress check 5.01

Explain the difference between:

1 A chromosome and a chromatid.

2 A centromere and a telomere.

Progress check questions allow you to check your own knowledge and see how well you're getting on.

TIP

Remember the letters **PMAT**:

Prophase = **Pro** means first, or 'before'

Metaphase = chromosomes in **M**iddle

Anaphase = chromosomes move **A**part

Telophase = **T**wo nuclei

Tips contain quick suggestions to remind you about key facts and highlight important points.

Revision checklist

Check that you know:

- the structure of nucleotides, including ATP
- the structures of RNA and DNA
- the importance of base pairing between complementary bases
- semi-conservative replication of DNA
- polypeptides are coded for by genes and genes form part of a DNA molecule
- gene mutations and how a gene mutation may result in a changed polypeptide
- how the sequence of nucleotides codes for the amino acid sequence and sickle cell anaemia
- how the information in DNA is used during protein synthesis, including the roles of mRNA, tRNA and ribosomes.

Revision checklist occur at the end of each chapter so you can check off the topics as you revise them.

Exam-style questions

1 Figure 1.10 is a drawing made from an electron micrograph of an animal cell.

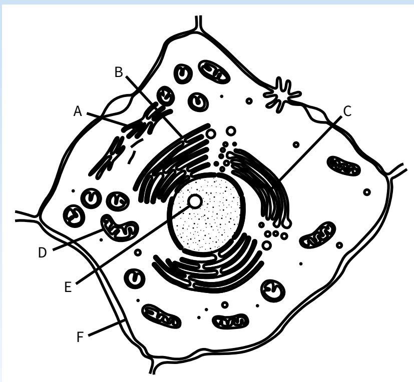


Figure 1.10 A drawing made from an electron micrograph of an animal cell.

a Copy and complete Table 1.03, name the organelles A to F and state one function of each. [6]

b Explain why an electron micrograph of this cell shows more detail than a light micrograph taken at the same magnification. [2]

c Name three other structures, not visible in Figure 1.10, which could be seen in an electron micrograph of a plant leaf cell. [3]

2 Table 1.04 lists some features of animal, plant and bacterial (prokaryotic) cells. Copy and complete Table 1.04, placing a tick (✓) in the appropriate box if the statement is correct and a cross (✗) if it is not. [8]

3 a Briefly describe the structure of a virus particle. [3]

b 'All viruses are parasitic'. Explain this statement. [2]

Exam-style questions help you to thoroughly prepare for examinations . Complete these questions and check your answers against those provided at the back of the book.

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Cell structure

Learning outcomes

When you have finished this unit, you should be able to:

- compare the structure of animal and plant cells as seen through a light microscope
- measure cells using an eyepiece graticule and stage micrometer scale
- use the units of length needed in cell studies (millimetre, micrometre and nanometre)
- calculate the magnifications of drawings and micrographs
- calculate the sizes of specimens from drawings and micrographs
- understand the difference between magnification and resolution
- interpret electron micrographs of animal and plant cells
- recognise these cell structures and know their functions:
  - cell surface membrane
  - nucleus, nuclear envelope and nucleolus
  - rough endoplasmic reticulum
  - smooth endoplasmic reticulum
  - Golgi body
  - mitochondria
  - ribosomes
  - lysosomes
  - microtubules
  - centrioles
  - chloroplasts
  - cell wall
  - plasmodesmata
  - large permanent vacuole and tonoplast of plant cells
- outline the role of ATP in cells
- compare the structure of a bacterial cell with that of animal and plant cells
- outline the main features of viruses.

The **cell** is the basic 'unit' of living organisms. There are thousands of different types of cell. Each type is adapted for a different function, but they are all recognisable as cells by the structures they contain.

1.01 The structure of animal and plant cells

You should have the opportunity to make temporary slides of suitable animal and plant cells, such as human cheek cells or cells from the leaf of a plant. Stains such as iodine solution or methylene blue are often used to show the cell contents more clearly. For example, iodine stains starch in plant cells blue-black, and colours the nuclei, cytoplasm and cell walls pale yellow. Using a light microscope only enables you to see the larger structures present in cells. From slides you can make drawings of the cells. Alternatively, a photograph of a cell as seen through a light microscope can be taken. A photograph of an image seen through a light microscope is called a light micrograph.

Using a school microscope, you can identify the structures shown in Figure 1.01.

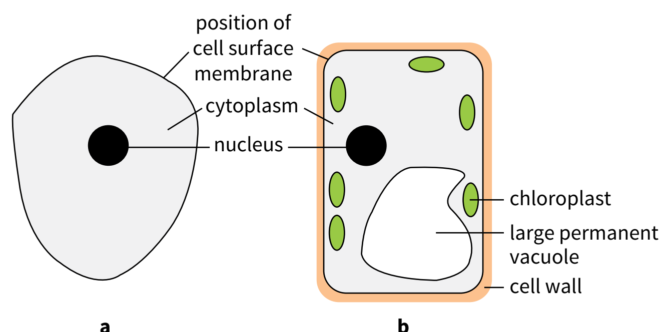


Figure 1.01 The main structures of typical animal and plant cells visible with a school microscope: **a** animal cell and **b** plant cell.

TIP

The cell surface membrane is very thin – too thin to actually be visible through a light microscope. It is better to label its location, such as 'position of cell surface membrane'.

It is possible to see one or two other cell structures through a light microscope, such as **mitochondria** and **Golgi bodies**. However, this needs a very high quality microscope and often involves special staining procedures.

Progress check 1.01

1. What structures can you see in *both* an animal cell *and* a plant cell through a light microscope?
2. What structures are visible in a plant cell but not in an animal cell?
3. Explain why stains are used when making microscope slides of cells.

1.02 Measuring cells

Ideally, to measure a cell you would place a scale or ruler on the slide alongside the specimen. This is not physically possible, but you can use a separate slide with a 'ruler' called a stage micrometer. This has a scale a millimetre in length, divided into 100 divisions (each division = 0.01 mm or 10 μm). The stage micrometer is used together with a scale in the eyepiece lens, called an eyepiece graticule. The eyepiece graticule has no measureable units such as millimetres, because the divisions will represent different lengths depending on the magnification you are using. We say that the eyepiece scale is in arbitrary units. This means that the divisions on the scale are all the same size and can be used for comparison, but if you want to know the actual length of an image, you have to calibrate the eyepiece graticule divisions using the stage micrometer.

Worked example 1.01

Question

A student placed a stage micrometer slide on the stage of his microscope and observed it using a medium power objective lens. He lined up the micrometer scale with the eyepiece graticule and noted that 100 divisions on the graticule scale measured 25 divisions on the stage micrometer (Figure 1.02).

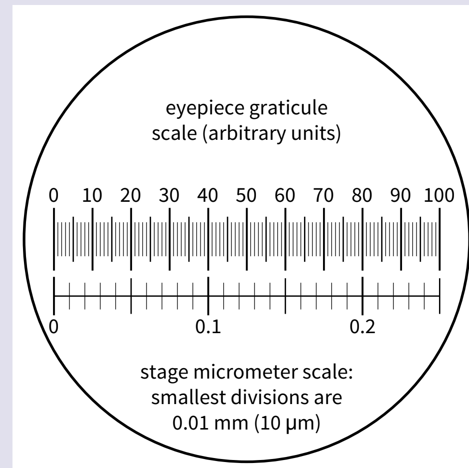


Figure 1.02 Image of a stage micrometer scale aligned alongside an eyepiece graticule scale.

The student removed the stage micrometer from the microscope and replaced it with a slide of some plant tissue. He focused on a cell using the same medium power objective lens. He noted that the cell measured 48 divisions on the eyepiece scale.

Calculate the length of the plant cell in micrometres (μm).

Answer

Step 1:

The length of 25 divisions on the stage micrometer = 25 × 10 μm = 250 μm

Therefore each eyepiece division is equivalent to:

$$\frac{250\,\mu m}{100} = 2.5\,\mu m$$

Step 2:

Using the *same magnification*, 48 divisions on the eyepiece scale are equivalent to:

48 × 2.5 μm = 120 μm

Therefore the length of the plant cell is 120 μm.

TIP

You must use the microscope on the *same magnification* when calibrating the eyepiece graticule and when using it to measure the specimen. If you need to use another objective lens, such as a high power one, you will need to re-calibrate the graticule for use with this lens.

Units of length used in cell studies

- 1 millimetre (mm) = 1/1000 of a metre, or 10^{-3} m
- 1 micrometre (μm) = 1/1000 of a mm, or 10^{-6} m
- 1 nanometre (nm) = 1/1000 of a μm, or 10^{-9} m.

Cells vary a great deal in size, but on average they are a fraction of a millimetre in diameter or length, with plant cells tending to be larger than animal ones. The plant cell in Worked example 1.01 was 120 μm in length. There are 1000 μm in a millimetre; so 120 μm is equal to 0.12 mm.

The structures within a cell are called organelles. Large organelles such as the nucleus and mitochondria are normally measured in micrometres. A typical nucleus is about 5–10 μm in diameter, while a mitochondrion is about 1 μm in diameter and up to 10 μm in length. Smaller organelles are measured in nanometres. For example, a **ribosome** is about 25 nm in diameter, while the cell surface membrane has a thickness of around 7 nm.

Progress check 1.02

1. The student in Worked example 1.01 measured the size of the nucleus of the plant cell and found it to be three divisions on his eyepiece scale, using the same medium power objective lens. What is the diameter of the nucleus in micrometres?
2. A chloroplast is 7 μm in length. What is this length in:
   - **a** millimetres
   - **b** nanometres?

1.03 Magnification

The **magnification** of a drawing or photomicrograph is the number of times larger the drawing or photomicrograph is, when compared with the actual size of the specimen. For example, the formula for the magnification of a drawing is:

$$\text{magnification} = \frac{\text{size of drawing}}{\text{size of specimen}}$$

The measurement of the drawing and that of the specimen must be in the *same units* in the formula.

A magnification is written like this: ×200 (meaning 'times 200').

Worked example 1.02

Question

A student looked at a plant cell through a microscope and measured its diameter. She found it to be 38 μm. She made a drawing of this cell and measured the diameter of the drawing with a ruler (Figure 1.03). What is the magnification of her drawing?

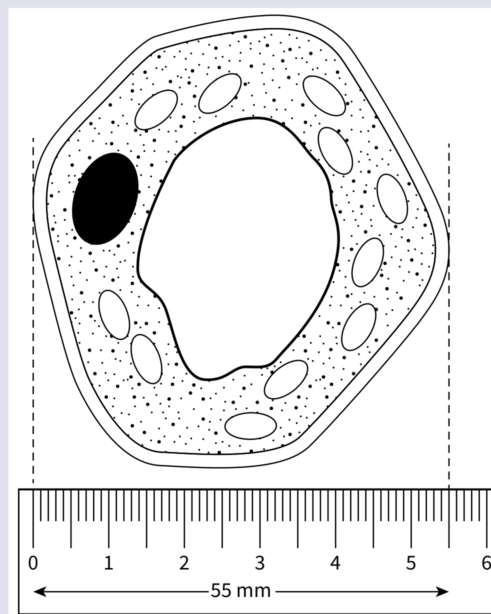


Figure 1.03 A student's drawing of a cell, with a ruler marked in millimetres alongside the drawing.

Answer

Step 1:

The width of the drawing measures 55 mm on the ruler. The drawing and the specimen must be measured in the same units. The specimen is 38 μm and the drawing is 55 mm. It is easiest to convert 55 mm into μm:

size of drawing in μm = (55 mm × 1000 μm/mm) = 55 000 μm

Step 2:

Actual size of specimen = 38 μm

$$\text{magnification} = \frac{\text{size of drawing}}{\text{size of specimen}} = \frac{55\,000\ \mu m}{38\ \mu m} = \times 1447$$

$$= \times 1400 \text{ (to two significant figures)}$$

(i.e. the student's drawing is 1400 times larger than the actual cell on the slide.)

The magnification of a microscope can be found by multiplying the power of the eyepiece by the power of the objective lens. For example, a ×10 eyepiece and a ×40 objective gives the microscope an overall magnification of 10 × 40 = ×400.

TIP

When putting a magnification on a drawing, do not be tempted to use the microscope magnification. This only tells you how much bigger the image seen through the microscope is in comparison with the specimen. The magnification of a drawing will depend on how big you make your drawing!

Drawings or photomicrographs should always show the magnification of the specimen. This can be as a number (e.g. ×800) or by using a scale bar. A scale bar is a line drawn alongside the specimen, with the length of the line labelled (Figure 1.04).

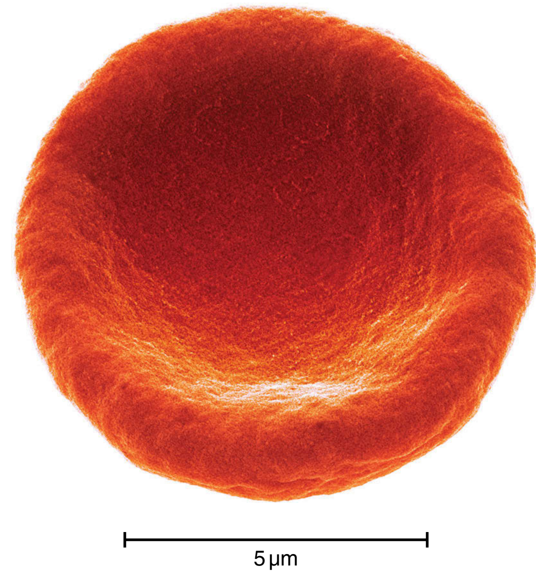


Figure 1.04 Photomicrograph of red blood cell, with a scale bar.

The scale bar can be used to find the magnification. In Figure 1.04 the scale bar is 40 mm in length, which is 40 000 μm, so:

$$\text{magnification of the scale bar (and the specimen)} = \frac{\text{size of scale bar on the photomicrograph}}{\text{real size of scale bar}} = \frac{40\,000\ \mu m}{5\ \mu m} = \times 8000$$

Magnification and resolution

A good quality light microscope can magnify objects about 2000 times (×2000), allowing us to view structures down to about 1 μm in length. However, at this magnification the 'detail' that is visible is very limited. The amount of detail is called the **resolution**. It is defined as the shortest distance between two points that can be distinguished as being separate. In a light microscope this is about 0.2 μm (200 nm). Through this microscope, two points that are closer than 200 nm will appeared blurred together and not visible as separate points.

We could take a photomicrograph and increase its magnification, 'blowing it up' so that it was the size of a poster, but this would not improve its resolution. To increase the magnification and improve the resolution of an image we have to use an electron microscope. The wavelength of a beam of electrons is much less than that

of visible light, so an electron microscope can achieve a much better magnification and resolution than a light microscope. The useful limit of a modern **transmission electron microscope (TEM)** is over a million times magnification, with a resolution of less than 1 nm.

1.04 Electron micrographs of cells

A photograph of a specimen seen through an electron microscope is called an electron micrograph. Whereas a light microscope is normally used to look at cells at a magnification of a few hundred times, most electron micrographs are taken in the approximate range ×10 000 to ×200 000. Using higher magnifications and the improved resolution, much more can be seen of the structure within a cell and within the individual organelles. This fine detail is called the ultrastructure of the cell. Figure 1.05 shows a diagram of a typical plant cell from a leaf, as seen through the electron microscope.

Much of the mass of a cell consists of membranes. As well as the cell surface membrane, the cytoplasm contains an extensive membrane system called **rough endoplasmic reticulum** (rough ER) covered with tiny organelles called **ribosomes**. There is also **smooth endoplasmic reticulum** (smooth ER), which lacks ribosomes. Other organelles such as the **nucleus, mitochondria** and **chloroplasts** are also surrounded by their own membranes. Membranes serve to isolate the processes and chemical reactions going on within the organelles. This is called **compartmentalisation**. All the membranes in a cell have a similar structure (see Unit 4). Table 1.01 shows a summary of the main organelles found in cells.

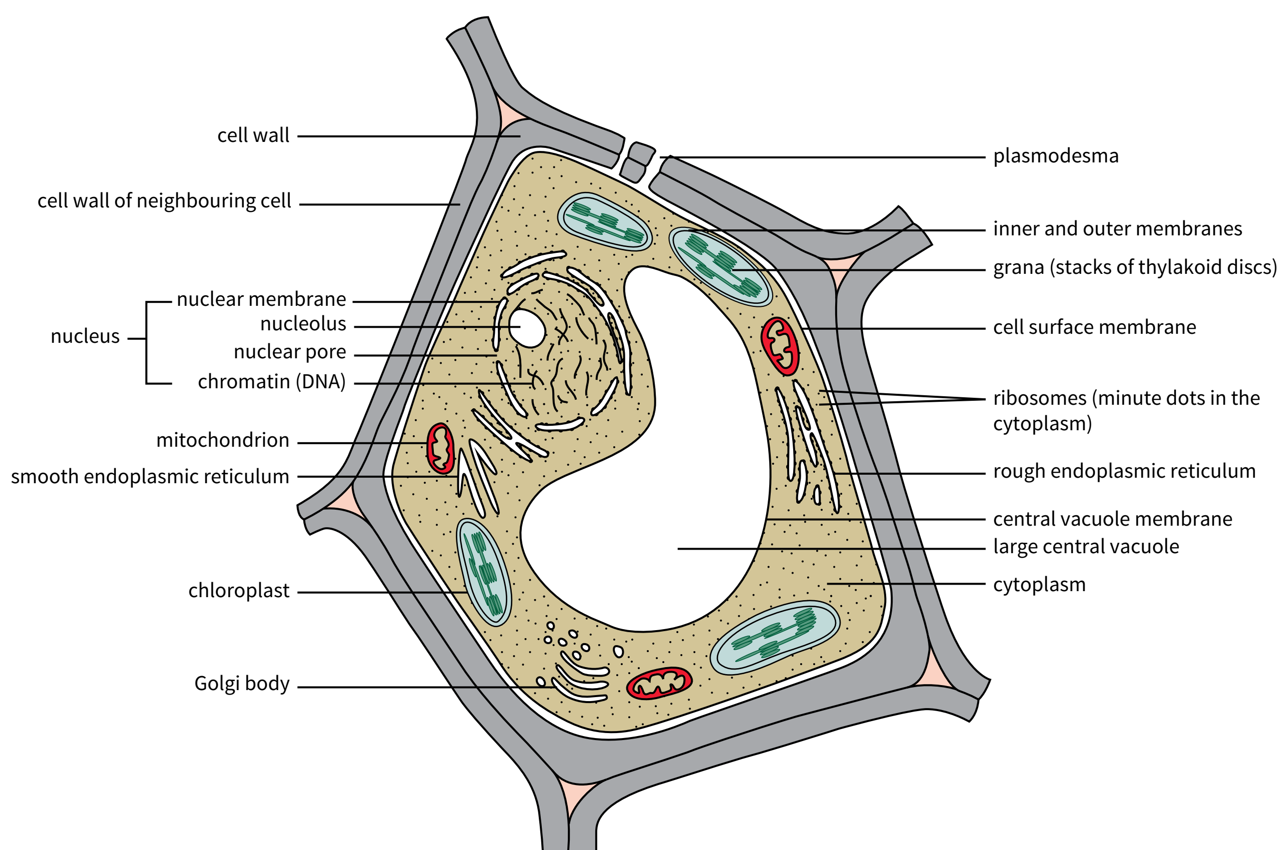


Figure 1.05 The ultrastructure of a typical plant cell from a leaf.

Organelle	Location and size	Structure and function(s)
cell surface membrane	surrounds cell (about 7 nm thick)	composed of phospholipids and protein (see Unit 4); partially permeable and controls the movement of substances into and out of the cell; allows cells to interact with each other and to respond to signals from outside the cell
nucleus	in cytoplasm, usually one per cell (about 5–10 μm in diameter)	contains the hereditary material (**deoxyribonucleic acid (DNA)**) coding for the synthesis of proteins in the cytoplasm. Surrounded by a double membrane called the **nuclear envelope**
nucleolus	one to several in nucleus (1–2 μm in diameter)	synthesises ribosomal RNA and makes ribosomes
rough ER	throughout cytoplasm (membranes about 4 nm thick)	'rough' because covered with ribosomes; membranes enclose compartments (sacs) that transport proteins synthesised on the ribosomes
smooth ER	in cytoplasm; extent depends on type of cell (membranes about 4 nm thick)	similar to rough ER but no ribosomes; synthesises and transports lipid molecules
Golgi body	in cytoplasm (variable in size and number)	synthesises glycoproteins (proteins with carbohydrate groups attached); packages proteins for export from the cell
mitochondria (singular = mitochondrion)	in cytoplasm; can be many thousands in some cells (around 1 μm diameter, up to 10 μm in length)	produce **adenosine triphosphate (ATP)** by aerobic respiration (see below and Unit 12)
ribosomes	attached to rough ER or free in cytoplasm (20–25 nm in size)	site of protein synthesis
lysosomes	in cytoplasm; variable in number (0.1–0.5 μm in diameter)	digests unwanted materials and worn-out organelles
microtubules	throughout cytoplasm (long hollow protein tubes 25 nm in diameter)	along with thinner protein filaments form the **cytoskeleton**; involved in movement of organelles
centrioles	two hollow cylinders about 0.5 μm long, present in animal cells; lie next to the nucleus in a region called the centrosome	made of protein microtubules; the centrosome is a microtubule organising centre (MTOC) and is involved with the formation of the spindle during nuclear division (see Unit 5), but the exact function of the centrioles is unknown; plant cells do not have a centrosome or centrioles, but can still form a spindle
chloroplasts	in cytoplasm of some plant cells (up to 10 μm in length)	contain **chlorophyll** and are the site of photosynthesis (see Unit 13)
cell wall	layer surrounding plant cells, variable thickness	made of the carbohydrate **cellulose** (see Unit 2); supports the plant cell and maintains its shape
plasmodesmata (singular = plasmodesma)	pores in plant cell wall (about 50 nm in diameter)	contain fine strands of cytoplasm linking a plant cell with its neighbouring cells and allowing movement of materials between cells
vacuole	large central space in plant cells (variable in size)	contains various solutes such as sugars, mineral salts and pigments; surrounded by a membrane called the **tonoplast**, which controls exchange of materials between the vacuole and the cytoplasm (note that animal cells have vacuoles, but these are small temporary structures)

Table 1.01 Summary of the main organelles present in cells.

Many of the structures in Table 1.01 will be described in more detail in later units of this book (see references in Table 1.01). For now all you need to be able to do is recognise the organelles and give an *outline* of their functions.

Figure 1.06 is a diagram of the structure of a typical animal cell, as seen through an electron microscope.

With reference to Figures 1.05 and 1.06, note these extra points:

- The nucleus is surrounded by a double membrane called the nuclear envelope. The outer membrane of the nuclear envelope is continuous with the endoplasmic reticulum.
- The nuclear envelope contains 'holes' called **nuclear pores**. These allow movement of materials between the nucleus and the cytoplasm. For example, messenger RNA (mRNA) made in the nucleus can exit to the cytoplasm, carrying the instructions for protein synthesis encoded in the DNA (see Unit 6). Substances made in the cytoplasm can enter the nucleus through the pores (e.g. ATP).
- The nucleus contains the hereditary material (DNA) within structures called **chromosomes**. These are only visible when the nucleus divides (see Unit 5). Between cell divisions the chromosomes form a loosely coiled material called **chromatin**.
- The endoplasmic reticulum forms a complex three-dimensional system of sheet-like membranes and tubes enclosing fluid-filled sacs. Rough ER is 'studded' with ribosomes. Smooth ER lacks ribosomes, and is more tubular in appearance than rough ER. Ribosomes are also found 'loose' in the cytoplasm, where they are known as free ribosomes.
- Ribosomes are the site of protein synthesis. They are composed of protein and RNA. The 'instructions' for protein synthesis are encoded in the DNA and carried out to the ribosomes by mRNA.
- Ribosomes in the cytoplasm are large (known as 80S ribosomes). There are also smaller ribosomes (70S) in mitochondria and chloroplasts.
- The Golgi body (also known as the Golgi apparatus) consists of a stack of flattened membranes enclosing hollow sacs, called **cisternae**. Small spherical membrane vesicles containing protein are continually 'pinched off' the rough ER and fuse together to

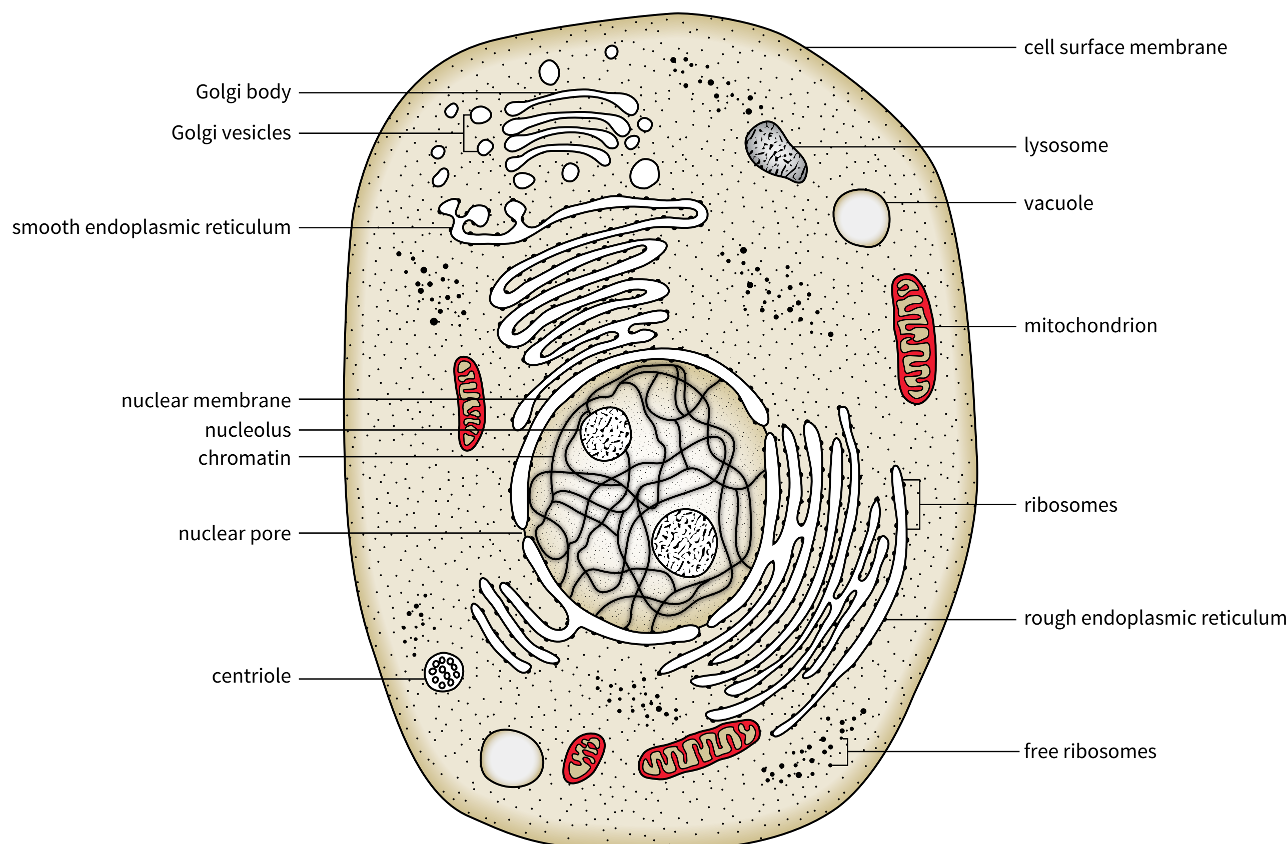


Figure 1.06 The ultrastructure of a typical animal cell.

form the Golgi body, on its side closest to the nucleus. Inside the cisternae the protein is chemically modified, such as by addition of carbohydrate to form **glycoproteins**. At the side furthest from the nucleus, vesicles containing the modified protein bud off from the cisternae and are transported to other parts of the cell. Some vesicles may fuse with the cell membrane, releasing their contents out of the cell. This secretion process is called **exocytosis** (see Unit 4). The Golgi body is also involved in making lysosomes.

- Lysosomes are found in most animal and plant cells. They are membrane-bound sacs formed when digestive enzymes are incorporated into vesicles from the Golgi body. The single membrane surrounding the lysosome keeps the digestive enzymes separate from the rest of the cell. Lysosomes can fuse with vacuoles containing unwanted structures such as old organelles. The enzymes in the lysosome then break down (digest) the unwanted material. Lysosomes are especially common in animal cells that carry out a process called phagocytosis, such as some white blood cells (see Unit 8), where they are used to digest pathogenic organisms such as bacteria.
- Chloroplasts are found in cells from the green parts of plants such as leaves and green stems. They are surrounded by a membrane and contain a complex internal system of membranes called **thylakoids**, arranged in stacks called **grana**. The membranes contain photosynthetic pigments such as chlorophyll, which absorb light energy and use it to make organic molecules such as glucose and starch (see Unit 13).

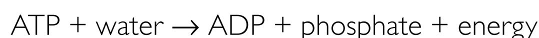
Progress check 1.03

1. Explain the difference between the magnification and the resolution of a microscope.
2. Briefly describe the location of these organelles and their functions:
   - **a** nucleolus
   - **b** lysosomes
   - **c** plasmodesmata.
3. Arrange theses organelles in increasing order of size: nucleus, chloroplast, ribosome, centriole.

1.05 Mitochondria and the role of ATP

Mitochondria are present in nearly all animal and plant cells. The number of mitochondria in a cell is directly related to its energy demands. Cells that require a lot of energy, such as a muscle cell, contain many thousands of mitochondria, whereas less active cells have fewer of these organelles. Aerobic respiration takes place inside mitochondria. This releases energy, which is used to make a substance called adenosine triphosphate (ATP). ATP is the universal energy 'currency' in cells.

During respiration, energy-rich molecules such as glucose are broken down in a series of reactions. The chemical energy contained within these molecules is used to make ATP, which is in turn used to drive all the energy-requiring processes in a cell. To extract the energy from ATP, the molecule is broken down by a hydrolysis reaction, to form adenosine diphosphate (ADP) and phosphate. A simplified equation for this is:



ATP + water → ADP + phosphate + energy

The details of the formation of ATP during respiration are described in full in Unit 12, but at this stage all you need to know is that most ATP is formed during the last stages of respiration, which take place in the mitochondria. To carry out these stages a cell needs oxygen, which is why this is called ***aerobic*** respiration.

Figure 1.07 shows the internal structure of a mitochondrion. It has a smooth outer membrane and an inner membrane that is folded into a number of shelf-like **cristae**, which increases the surface area of the inner membrane. The last two stages of aerobic respiration are called the **Krebs cycle** and **oxidative phosphorylation**. The Krebs cycle takes place in the fluid-filled **matrix** of the mitochondrion and oxidative phosphorylation (where most ATP is produced) occurs on the inner membrane.

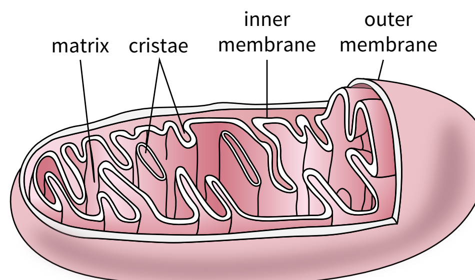


Figure 1.07 The internal structure of a mitochondrion.

Sample question 1.01

Explain the involvement of the nucleus, rough endoplasmic reticulum and Golgi body in the synthesis of glycoproteins in a cell. [10 marks]

[Mark points are shown in square brackets – to a maximum of 10 marks]

The nucleus contains the genetic material within the chromosomes, in the form of deoxyribonucleic acid (DNA) [1]. DNA carries the instructions (genetic code) needed for the synthesis of proteins [1] in the cytoplasm. These instructions are carried out to the cytoplasm by messenger RNA (mRNA) [1] through pores in the nuclear envelope [1], and enter the sacs of the rough endoplasmic reticulum (rough ER) [1], which are continuous with the nuclear envelope [1]. The rough ER is covered in small organelles called ribosomes [1], where proteins are synthesised [1]. Small vesicles containing protein are pinched off the rough ER [1] and fuse together to form the cisternae of the Golgi body [1], on its side closest to the nucleus. Inside the cisternae the protein is chemically modified by addition of carbohydrate to form glycoproteins [1]. At the side furthest from the nucleus, vesicles containing the modified protein bud off from the cisternae [1] and are transported to other parts of the cell.

This question requires you to know the location, structure and function of each of the three named organelles, and to put this information together as an account of the sequence of events taking place that result in production of glycoproteins in the cytoplasm.

The sample answer is laid out in the correct sequence and summarises the steps clearly, without including any irrelevant information.

Note that it is best to give the full names of biological terms when they are first used, such as deoxyribonucleic acid (DNA). The abbreviations can then be used in the rest of the answer.

1.06 Prokaryotic cells

The cells described so far in this unit are examples of **eukaryotic** cells. Eukaryotic means 'having a true nucleus'. Bacteria are also composed of cells, but they are much smaller than eukaryotic cells and simpler in structure. They are called **prokaryotic** cells (meaning 'before nucleus'). Bacterial cells have no nucleus or nuclear membrane. Their DNA is loose in the cytoplasm, forming a single circular loop, which is sometimes called a **bacterial chromosome**. Some bacteria also have smaller loops of DNA in the cytoplasm, called **plasmids**. Their cells lack endoplasmic reticulum and membrane-bound organelles such as mitochondria and chloroplasts.

The structure of a generalised bacterial cell is shown in Figure 1.08.

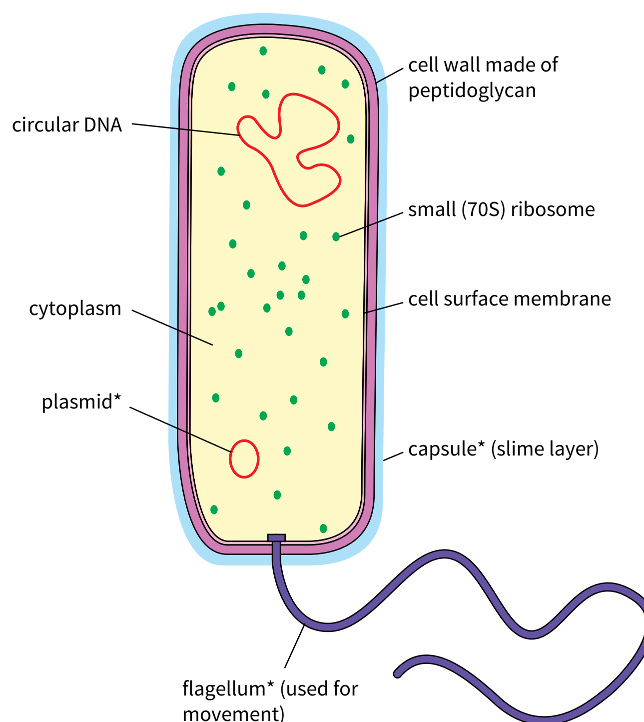


Figure 1.08 Diagram of a generalised bacterial cell. The structures marked with an asterisk are not found in all bacteria.

Eukaryotic cells	Prokaryotic cells
large (typically 10–100 μm in diameter)	small (typically 0.5–3 μm in diameter); volume as little as 1/10 000 of a eukaryotic cell
true nucleus surrounded by a nuclear membrane	no nucleus
linear DNA associated with protein, forming true chromosomes	circular DNA, not associated with proteins; may contain separate loops of DNA called plasmids
if present, cell wall made of cellulose (in plants) or chitin (in fungi)	cell wall made of **peptidoglycan** (a polysaccharide with some amino acid groups)
endoplasmic reticulum present	no endoplasmic reticulum or associated organelles such as the Golgi body
membrane-bound organelles such as mitochondria and chloroplasts present	no membrane-bound organelles (infolds of the cell surface membrane may be involved in photosynthesis and other processes)
large (80S) ribosomes attached to the rough ER and free in the cytoplasm	small (70S) ribosomes free in the cytoplasm
flagella present in some cells; they have a complex structure containing several microtubules	if present, flagella are made of a single microtubule

Table 1.02 Differences between eukaryotic and prokaryotic cells.

A comparison of the structure of eukaryotic and prokaryotic cells is given in Table 1.02.

1.07 Viruses

Viruses are tiny particles that are much smaller than bacteria. They do not consist of cells, and in many ways can be thought of as being intermediate between a chemical and a living organism. Viruses are not free-living and can only reproduce inside a host cell (i.e. they are parasites).

Viruses cause many diseases in plants and animals. For example, the tobacco mosaic virus produces brown blotches on the leaves of tobacco plants and the human influenza virus causes the symptoms we know as 'flu'. The **human immunodeficiency virus (HIV)** is the virus responsible for causing acquired immune deficiency syndrome (AIDS).

A virus particle is very simple in structure. It has no nucleus or cytoplasm, and consists of genetic material contained within a protein coat (Figure 1.09). The protein coat or **capsid** is made up of many individual protein molecules called **capsomeres**. The genetic material can be either DNA or RNA and makes up just a few genes. The genetic material, along with one or two enzymes, is all that the virus needs in order to reproduce within the host cell. The virus takes over the host cell, instructing it to make more virus particles. This normally causes the death of the host cell. Some viruses are surrounded by a membrane called an envelope. This is not part of the virus itself – it is derived from the host cell. During the life cycle of the virus, the virus particles burst out of the host cell, taking part of the surface membrane of the host cell with them.

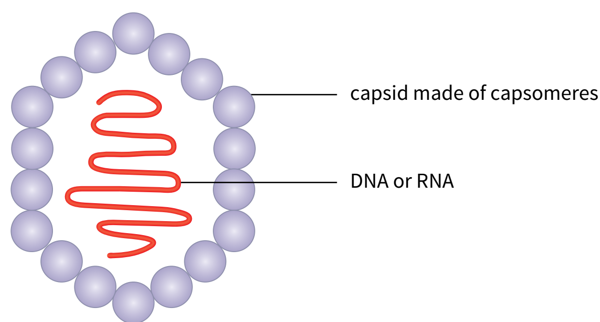


Figure 1.09 The structure of HIV.

Progress check 1.04

1. Briefly describe (in one paragraph) the main differences between a eukaryotic and a prokaryotic cell.
2. Explain why some biologists do not regard viruses as living organisms.

Revision checklist

Check that you know:

- ☐ the similarities and differences between an animal and a plant cell as seen through the light microscope
- ☐ the units of length used in cell studies (millimetre, micrometre and nanometre)
- ☐ how to calculate the magnifications of drawings, photomicrographs or electron micrographs
- ☐ how to calculate the sizes of specimens from drawings, photomicrographs or electron micrographs
- ☐ the difference between magnification and resolution
- ☐ how to describe and interpret electron micrographs of animal and plant cells
- ☐ how to recognise the following cell structures and knowing their functions:
  - cell surface membrane
  - nucleus, nuclear envelope and nucleolus
  - rough endoplasmic reticulum
  - smooth endoplasmic reticulum
  - Golgi body
  - mitochondria
  - ribosomes
  - lysosomes
  - microtubules
  - centrioles
  - chloroplasts
  - cell wall
  - plasmodesmata
  - large permanent vacuole and tonoplast of plant cells
- ☐ an outline of the role of ATP in cells
- ☐ how to compare the structure of a bacterial cell with the structure of animal and plant cells
- ☐ an outline of the main features of viruses.

Exam-style questions

1 Figure 1.10 is a drawing made from an electron micrograph of an animal cell.

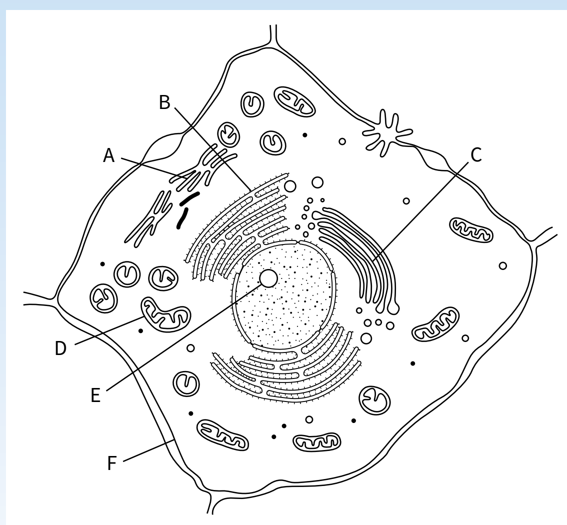


Figure 1.10 A drawing made from an electron micrograph of an animal cell.

a Copy and complete Table 1.03, name the organelles A to F and state one function of each. [6]

b Explain why an electron micrograph of this cell shows more detail than a light micrograph taken at the same magnification. [2]

c Name three other structures, not visible in Figure 1.10, which could be seen in an electron micrograph of a plant leaf cell. [3]

2 Table 1.04 lists some features of animal, plant and bacterial (prokaryotic) cells. Copy and complete Table 1.04, placing a tick (✓) in the appropriate box if the statement is correct and a cross (✗) if it is not. [8]

3 a Briefly describe the structure of a virus particle. [3]

b 'All viruses are parasitic'. Explain this statement. [2]

	Name of organelle	Function
A		
B		
C		
D		
E		
F		

Table 1.03

Feature	Animal cell	Plant cell	Bacterial cell
cell wall made of cellulose			
cell surface membrane			
rough endoplasmic reticulum			
ribosomes			
cytoskeleton			
Golgi apparatus			
chloroplasts			
mitochondria			

Table 1.04

Biological molecules

Learning outcomes

When you have finished this unit, you should be able to:

- describe how to carry out chemical tests for reducing sugars, non-reducing sugars, starch and lipids, including a semi-quantitative test for reducing sugars
- describe the ring structures of α-glucose and β-glucose
- understand what is meant by the terms monomer, polymer, macromolecule, monosaccharide, disaccharide and polysaccharide
- understand the formation of a glycosidic bond in the formation of disaccharides and polysaccharides
- describe how glycosidic bonds in disaccharides and polysaccharides may be broken
- know the structure of the polysaccharides starch, glycogen and cellulose and how the structures are related to their functions
- know the structure and formation of a triglyceride and how the structure of triglycerides is related to their functions
- know the structure of a phospholipid and understand how the structure of phospholipids is related to their functions in living organisms
- know the structure of an amino acid and how peptide bonds are formed and broken
- explain the different types of structures in proteins and the types of bonds that hold protein molecules in shape
- describe the structure of haemoglobin as an example of a globular protein and the structure of collagen as an example of a fibrous protein and relate their structures to their function
- explain how the structure and properties of water molecules are related to the roles of water in living organisms.

2.01 Testing for biological molecules

Biological molecules are organic substances found in living organisms and include **reducing sugars**, **non-reducing sugars**, **starch**, **proteins** and **lipids**. These molecules can be identified using simple chemical tests which are summarised in Table 2.01.

Biological molecule	Reagent used	Description of test
reducing sugars	Benedict's reagent	Benedict's regent contains copper (II) ions. When heated with reducing sugars, the copper (II) ions are reduced to copper (I) compounds, changing colour from blue to form a range of coloured precipitates, from green, to yellow, orange, red or brown. This test can be used semi-quantitatively, using a range of concentrations of a reducing sugar.
non-reducing sugars	Benedict's reagent, following acid hydrolysis	Sucrose is a commonly occurring non-reducing sugar. When heated with dilute acid, such as hydrochloric acid, sucrose is hydrolysed into its constituent monosaccharides, glucose and fructose, which are both reducing sugars. Excess acid is then neutralised by the addition of sodium hydrogen carbonate and the mixture is then tested with Benedict's reagent. This will show the production of reducing sugars.
starch	iodine in potassium iodide solution	Iodine solution is initially brown, but turns blue to black in the presence of starch. This test can be used quantitatively, using standard starch solutions and a colorimeter to measure the intensity of the blue colour produced.

Biological molecule	Reagent used	Description of test
proteins	biuret reagent	Biuret reagent is initially blue, but forms a violet colour when added to a solution containing proteins. This test can also be used quantitatively, using standard protein solutions and a colorimeter to measure the intensity of the violet colour produced.
lipids	ethanol	This test relies on the solubility of lipids in different solvents. A sample containing lipids is first shaken with ethanol. Lipids will dissolve in the ethanol, but when water is added, a milky emulsion is formed.

Table 2.01 Tests for biological molecules.

2.02 Carbohydrates

Carbohydrates are compounds containing the elements carbon, hydrogen and oxygen, usually in the ratio of 1 : 2 : 1. Single molecules, or simple sugars, known as **monosaccharides**, include glucose, fructose and galactose. These simple sugars each have six carbon atoms in their structure and are therefore sometimes referred to as hexoses, derived from the Greek word 'hex' meaning six. Simple sugars with five carbon atoms are known as pentoses, those with three carbon atoms are known as trioses.

Glucose exists in two forms, **α-glucose** and **β-glucose**, as shown in Figure 2.01.

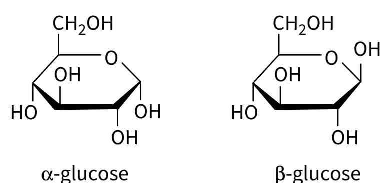


Figure 2.01 The structure of α-glucose and β-glucose.

Notice that the only difference between these two forms of glucose is the position of the –OH group on carbon atom number 1 in the ring. This small difference changes the properties of these two molecules.

Many large molecules are made up of large numbers of smaller molecules joined together in long chains. The smaller molecules are referred to as **monomers**, which join together forming **polymers**. For example, many thousands of glucose molecules can join together to form large molecules of starch or cellulose. Glucose molecules are the monomers, starch and cellulose are polymers. These polymers are sometimes referred to as **macromolecules** (very large molecules). Starch, cellulose, proteins and nucleic acids (see Unit 6) are macromolecules.

The polymers formed by many monosaccharides joining together are called **polysaccharides**.

Two monosaccharide monomers can also join together to form a **disaccharide** – a sugar consisting of two monosaccharides joined by a chemical bond. **Sucrose** is an example of a disaccharide. Three examples of disaccharides are shown in Table 2.02.

Disaccharides	Constituent monosaccharides
maltose (malt sugar)	two molecules of α-glucose joined together
sucrose (cane sugar)	α-glucose joined to fructose
lactose (milk sugar)	β-glucose joined to galactose

Table 2.02 Examples of disaccharides and their constituent monosaccharides.

Monosaccharides join together to form disaccharides and polysaccharides by the formation of **glycosidic bonds**, in a **condensation reaction** between two –OH groups on adjacent monosaccharides. Water is a product of this reaction.

Figure 2.02 shows how two molecules of α-glucose join together to form a molecule of maltose.

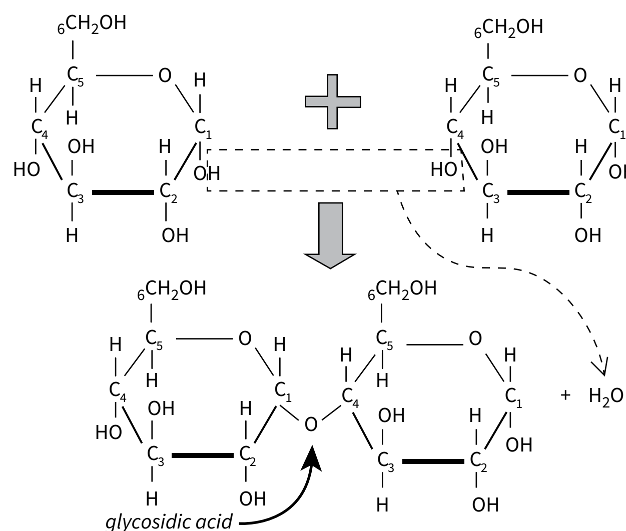


Figure 2.02 Formation of maltose.

The glycosidic bonds in disaccharides and in polysaccharides can be broken by the process of **hydrolysis**. In living organisms, disaccharides and polysaccharides are broken down to monosaccharides in the process of digestion. The chemical test for non-reducing sugars involves acid hydrolysis of glycosidic bonds.

The two forms of glucose, α-glucose and β-glucose, form different polysaccharides. **Starch** is a polymer of α-glucose and exists in two forms, known as **amylose** and **amylopectin**.

Amylose consists of many thousands of α-glucose monomers, joined by 1,4 glycosidic bonds only. The numbers 1 and 4 refer to the positions of the carbon atoms in the glucose molecules. The long chain coils to form a helix (Figure 2.03).

Amylopectin also consists of many thousands of α-glucose monomers, but it is a branched molecule. Branching occurs as a result of the formation of 1,6 glycosidic bonds (i.e. between carbon atoms 1 and 6), as illustrated in Figure 2.03. Amylose and amylopectin molecules form starch grains in many plant cells.

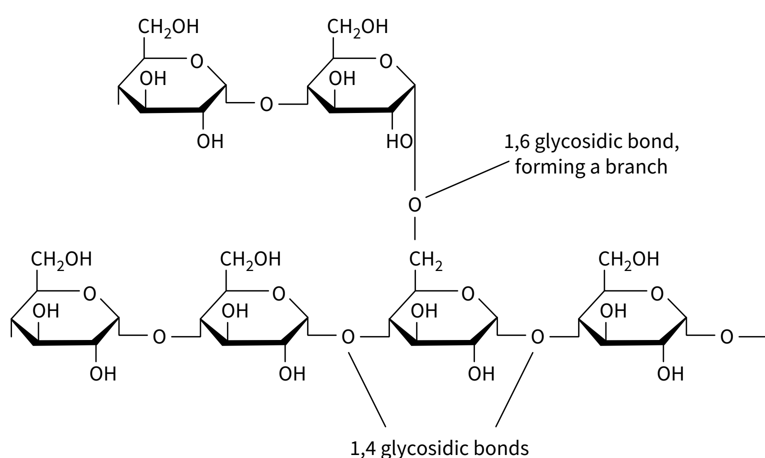


Figure 2.03 Glycosidic bonds in the structure of amylopectin

Glycogen is similar in structure to amylopectin, but is more highly branched because the 1,6 glycosidic bonds between the α-glucose monomers form more frequently. Glycogen molecules clump together to form glycogen granules in many animal cells, including liver cells.

Starch and glycogen are energy storage molecules, starch in plants and glycogen in animals. Starch and glycogen are both large, insoluble molecules and therefore do not affect the osmotic properties of cells, but they can be rapidly broken down to form glucose, which is used as an energy source.

Cellulose is a polymer of β-glucose, joined by 1,4 glycosidic bonds. The properties of cellulose are different from those of starch and cellulose is a structural polysaccharide, found in plant cell walls. Long, straight chains of cellulose molecules form bundles known as microfibrils, which in turn form cellulose fibres (Figure 2.05). The chains of cellulose fibres are held together by hydrogen bonding between projecting –OH groups. Cellulose fibres have a high tensile strength and the way in which they are arranged imparts considerable strength to plant cell walls.

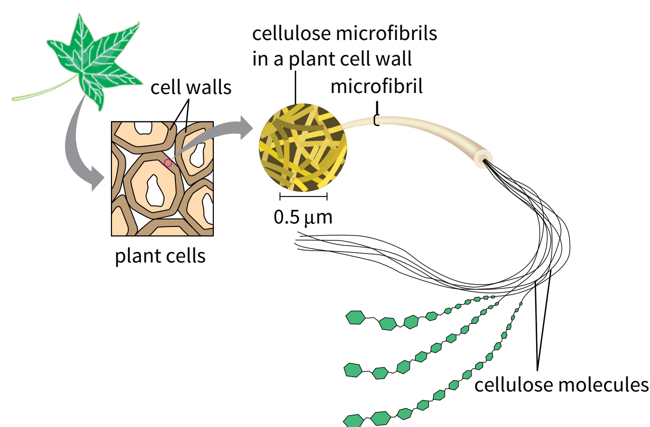


Figure 2.05 The structure of cellulose molecules, microfibrils and fibres.

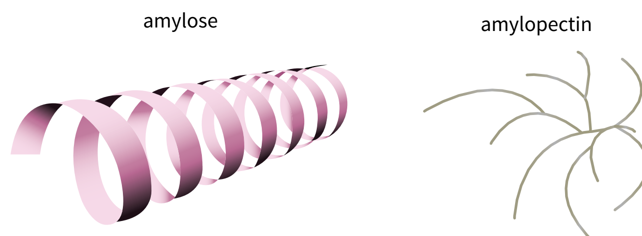
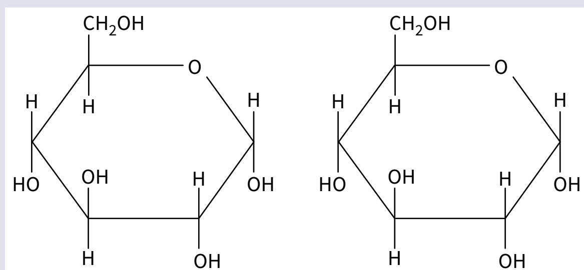


Figure 2.04 The structure of amylose and amylopectin.

Worked example 2.01

Question

The diagram shows the structure of two molecules of α-glucose:



1 Copy the diagram and show how these two molecules are linked together to form a molecule of maltose.

2 Name the type of reaction involved in this process.

3 Name the type of bond formed.

Answers

1 It is important to be careful when copying the structure of the glucose molecules (e.g. by showing the positions of the –OH and –H correctly on each carbon atom). When these two molecules of glucose join to form maltose, water is lost by the removal of –OH and –H from each molecule, so you should carefully draw a circle round these on carbon atom number 1 of the first glucose molecule and carbon atom number 4 of the second glucose molecule, with water (H_2O) given off as a product. The two glucose molecules are now linked by a –O– bond between carbon atom 1 and carbon atom 4.

2 This is an example of a condensation reaction.

3 The type of bond formed is known as a glycosidic bond. (Be careful to spell this correctly!)

TIP

Be careful to distinguish between the different forms of glucose, α-glucose and β-glucose, particularly when describing the structure and functions of the polysaccharides starch, glycogen and cellulose.

Spelling is important in words such as 'amylose', which could be confused with 'amylase'.

2.03 Lipids

Lipids, like carbohydrates, also consist of the elements carbon, hydrogen and oxygen, but in different proportions. Lipids include a variety of different compounds, such as fats, oils, steroids and phospholipids which all have the property of insolubility in water (oil and water do not mix!), but they are soluble in organic solvents such as ethanol. This forms the basis of the simple emulsion test for lipids.

Fats and oils belong to a type of lipid known as **triglycerides**. A triglyceride molecule consists of glycerol (a type of alcohol) joined to three fatty acids ('tri' means three). Each fatty acid joins to one of the –OH groups of glycerol, by means of a condensation reaction forming an **ester bond** and water. This is illustrated in Figure 2.06.

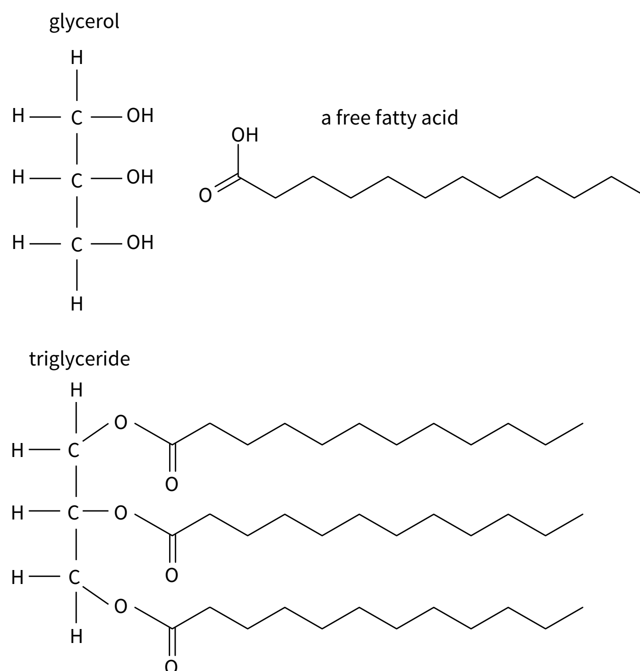


Figure 2.06 The formation of a triglyceride from glycerol and fatty acids.

Triglycerides have a number of functions in living organisms, including energy storage, insulation and they can act as a source of metabolic water. This is important in some desert mammals, such as kangaroo rats, which obtain most of their water from the oxidation of food.

The yield of energy from the metabolism of triglycerides is about twice that of the metabolism of the same mass of carbohydrates, due to the presence of more C–H bonds in triglycerides.

Phospholipids are a special type of lipid in which one of the fatty acids is replaced with a phosphate group. This phosphate group is strongly polar and will attract water molecules, but the fatty acid hydrocarbon chains are non-polar. The phosphate part of the phospholipid molecule is said to be hydrophilic, because it has an affinity for water, but the hydrocarbon chains are hydrophobic.

This property of phospholipids is important because the molecules form membranes around cells, consisting of a double layer of phospholipids, with the polar parts of the molecules on the outside and the non-polar parts on the inside (Figure 2.07). The structure and properties of the cell membrane is described in Unit 4.

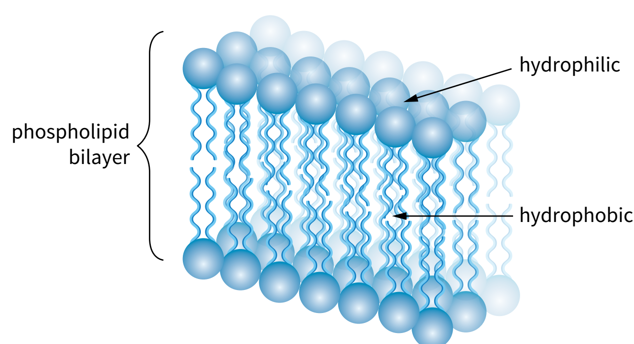


Figure 2.07 The arrangement of phospholipids in a bilayer forming the cell membrane.

Progress check 2.01

Explain how the properties of phospholipid molecules are related to the formation of a double layer in cell membranes.

2.04 Proteins

Proteins have many functions in living organisms, including structural proteins such as collagen and keratin, muscle contraction, defence against disease, transport and storage of oxygen and acting as receptors on cell surface membranes. Some hormones, including insulin and glucagon, are proteins and all enzymes are proteins.

All proteins are made of one type of monomer, **amino acids**, which join together by the formation of **peptide bonds**. This is another example of a condensation reaction and the reverse reaction, in which peptide bonds are broken, is hydrolysis. Digestion of proteins in the stomach and small intestine involves hydrolysis by enzymes.

Twenty different amino acids occur in proteins, but amino acids all have the same general structure, as shown in Figure 2.08. The R group is the variable part of the molecule.

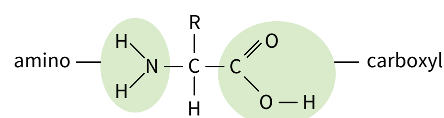


Figure 2.08 The general structure of an amino acid.

Figure 2.09 shows how two amino acids join together, in a condensation reaction, to form a peptide bond. Addition of further amino acids eventually forms a long chain, referred to as a polypeptide.

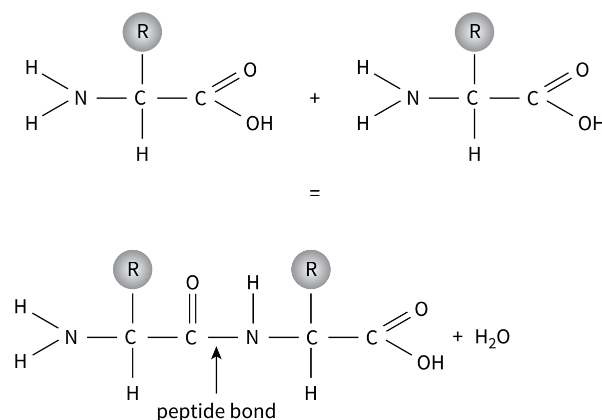


Figure 2.09 The formation of a peptide bond between two amino acids.

Once formed in the process of protein synthesis, the polypeptide chain folds in a very specific way, to form the functional protein.

We use the terms **primary structure, secondary structure**, **tertiary structure** and **quaternary structure** when describing structural features of protein molecules.

The sequence of amino acids in the polypeptide chain is referred to as the primary structure. A change is this

sequence can have significant effects on the structure and function of the final protein, as illustrated by haemoglobin A and haemoglobin S. Haemoglobin S is the type of haemoglobin which results in sickle cell anaemia.

The polypeptide chain may then coil or fold to form the secondary structure, either in the form of a coil, known as an α-helix, or a folded structure known as a β-pleated sheet. The secondary structure is held in shape by hydrogen bonds between –OH and –NH groups in the amino acids within the polypeptide.

Further folding of the polypeptide chain may then form a compact shape, referred to as the tertiary structure. This structure is held in shape by the formation of several different types of bonds between the R groups of amino acids in the polypeptide chain. These bonds include ionic bonds and covalent disulfide bonds. Some R groups are non-polar (e.g. those in the amino acids phenylalanine and leucine) and form hydrophobic interactions within the molecule. Hydrogen bonding is also present within the tertiary structure.

Some proteins consist of two or more polypeptide subunits and have a quaternary structure. For example, one haemoglobin molecule consists of four polypeptide chains, two α chains and two β chains. Each of these polypeptide chains is associated with a haem group, containing iron. The structure of a molecule of haemoglobin is shown in Figure 2.10.

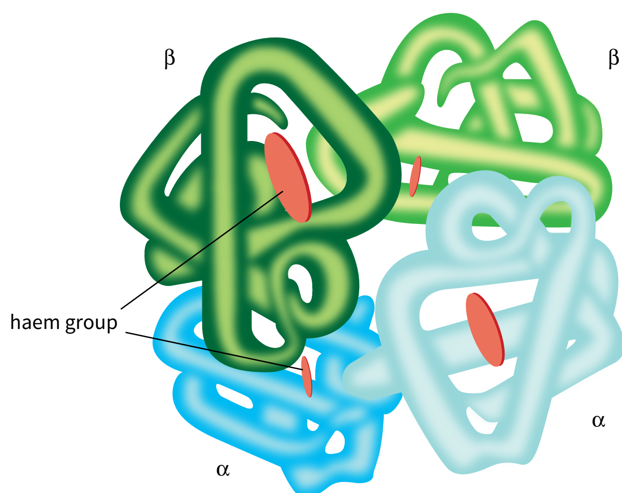


Figure 2.10 The essential structure of a molecule of haemoglobin, with two α chains and two β chains.

A molecule of haemoglobin is a compact, almost spherical shape and is an example of a **globular protein**. The iron in the haem group is able to combine reversibly with oxygen, so when fully saturated with oxygen, each molecule of haemoglobin carries four molecules of oxygen.

Collagen, a structural protein found in skin, tendons and other connective tissues, is an example of a fibrous protein. Collagen consists of long polypeptide chains, often with many glycine and proline amino acids. Each of these polypeptide chains associates with two others, forming a triple helix structure, illustrated in Figure 2.11.

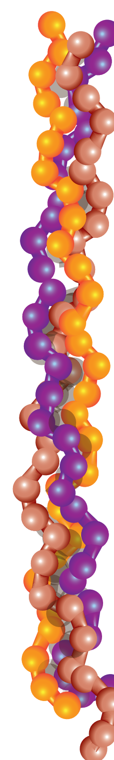


Figure 2.11 A triple helix of polypeptides forming a molecule of collagen.

Many of these collagen molecules are arranged side by side and covalently bonded to each other, forming collagen fibrils. Many collagen fibrils form larger bundles, referred to as collagen **fibres**. Collagen is flexible, but has a very tensile strength and is able to resist pulling forces without stretching.

Progress check 2.02

Table 2.03 refers to the structures of some biological molecules. If the statement is correct, place a tick (✓) in the box and if the statement is incorrect, place a cross (✗) in the box.

Statement	Starch	Cellulose	Triglyceride	Protein
contains the elements carbon, hydrogen and oxygen only				
is a polymer of β-glucose				
components are joined by ester bonds				
contains the elements carbon, hydrogen, oxygen and nitrogen				

Table 2.03

2.05 Water

Water is essential for life and water molecules have a number of special properties related to the roles of water in living organisms. Water is described as a dipolar molecule, because it has slight charges associated with the oxygen and hydrogen atoms. The oxygen atom has a slight negative charge and the hydrogen atoms have a slight positive charge. These slight positive and negative charges form weak forces of attraction between adjacent water molecules. These forces of attraction are known as **hydrogen bonding**, as illustrated in Figure 2.12.

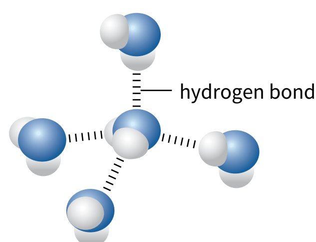


Figure 2.12 Hydrogen bonding between water molecules.

Hydrogen bonding in water is responsible for many of the properties of water that are important for living organisms.

Water acts a **solvent** because substances will dissolve in water to form solutions. Such substances are said to be hydrophilic and include polar solutes such as simple sugars and ions, including sodium ions and chloride ions. Substances that do not dissolve in water are said to be hydrophobic. These substances are non-polar and include fats and oils.

Water has a **high specific heat capacity**. This means that a relatively large amount of energy is required to increase the temperature of water and the temperature of water tends to remain about the same if the environmental temperature changes. As a result, the temperature of an organism's body tends to stay stable. Also, the temperature of large bodies of water, including lakes and ponds, stays relatively constant when the environmental temperature changes.

Water has a **high latent heat of vapourisation**. It takes a relatively large amount of energy to break the hydrogen bonds and to change water from a liquid to a gas. As a result, when water evaporates it has a cooling effect. This helps to maintain body temperature of animals that rely on sweating as a means of temperature regulation and it also has a cooling effect on leaves in transpiration.

TIP

Practise drawing the structures of glucose, an amino acid and a triglyceride and naming the characteristic bonds associated when these molecules join together or are formed.

Much of the information in this section can be summarised in the form of tables, listing the essential facts.

Sample question 2.01

You are provided with two solutions: A and B. One of these contains a reducing sugar only and the other contains a mixture of a reducing sugar and a non-reducing sugar. Giving experimental details, explain how you would identify each of the two solutions A and B. [10 marks]

[Mark points are shown in square brackets – to a maximum of 10 marks]

Put equal volumes of the solutions into two separate test-tubes and label these A and B **[1]**. Then add the same volume of Benedict's reagent to each test-tube and heat them in a water bath **[1]**. The blue Benedict's reagent will change colour in both tubes, because they both contain reducing sugar **[1]**.

Next, put the same volume of solutions A and B into another two test-tubes and add dilute hydrochloric acid to each tube **[1]**. After heating, the tubes will be allowed to cool then add sodium hydrogen carbonate to both tubes **[1]** to neutralise any remaining acid **[1]**. Heating with dilute acid will hydrolyse the non-reducing sugar and produce more reducing sugars **[1]**.

Finally, add Benedict's reagent to both tubes and heat them again **[1]**. By comparing the colour produced in the tubes **[1]**, it should be possible to determine which solution contained the mixture of reducing sugar and non-reducing sugar, because this solution will produce a deeper coloured precipitate **[1]**, or will change colour more quickly than the solution originally containing reducing sugar only **[1]**.

This is a detailed account of the experimental method and clearly explains the steps that should be taken to identify these two solutions. The answer is also set out in the correct sequence, showing good understanding of the method required to carry out a semi-quantitative Benedict's test to estimate the concentration of reducing sugars. The answer includes details of the tests for both reducing and non-reducing sugars.

Notice that the answer includes references to using the same volume of solutions A and B and the same volume of Benedict's reagent, which is necessary to make suitable comparisons and to draw valid conclusions from this experiment.

Revision checklist

Check that you know:

- [ ] chemical tests for reducing sugars, non-reducing sugars and starch
- [ ] the emulsion test for lipids and the biuret test for proteins
- [ ] the semi-quantitative test for reducing sugars using Benedict's reagent
- [ ] the structure of α-glucose and β-glucose
- [ ] how to define the terms monomer, polymer, macromolecule, monosaccharide, disaccharide and polysaccharide
- [ ] the formation of glycosidic bonds by condensation reactions
- [ ] the breakage of glycosidic bonds by hydrolysis
- [ ] the structures of starch, glycogen and cellulose
- [ ] how the structures of polysaccharides are related to their functions in living organisms
- [ ] the structure of a triglyceride and the formation of ester bonds
- [ ] how the structure of triglycerides is related to their functions in living organisms
- [ ] the structure and functions of phospholipids in living organisms

- the structure of an amino acid
- the formation and breakage of peptide bonds
- primary, secondary, tertiary and quaternary structure in proteins
- the types of bonding that hold protein molecules in shape
- haemoglobin as an example of a globular protein and collagen as an example of a fibrous protein
- how the structures of haemoglobin and collagen are related to their functions
- how hydrogen bonding occurs between water molecules
- how the properties of water are related to its roles in living organisms.

Exam-style questions

1 a Describe the structure of cellulose. [3]

b Explain how the structure of cellulose is related to its functions. [3]

Total: 6

2 a Explain what is meant by each of the following terms relating to protein structure:

i primary structure [2]

ii secondary structure. [2]

b Give three differences between the structure of haemoglobin and the structure of collagen. [3]

Total: 7

Enzymes

Learning outcomes

When you have finished this unit, you should be able to:

- explain what an enzyme is
- state where enzymes are found
- explain how enzymes work
- investigate the progress of an enzyme-catalysed reaction
- investigate and explain the effects of these factors on the rate of enzyme-catalysed reactions: temperature, pH, enzyme concentration, substrate concentration and concentration of inhibitor
- explain the effects of reversible inhibitors on the activity of enzymes
- explain how to use the maximum rate of reaction (V_{max}) to derive the Michaelis–Menten constant (K_m) to compare the affinity of enzymes for their substrates
- investigate and explain the effect of immobilisation in alginate on the activity of an enzyme.

Enzymes are protein molecules that act as biological catalysts. They take part in metabolic reactions in cells, speeding them up. When a reaction is over, each enzyme molecule is available to be used again. The substance (or substances) that the enzyme acts upon is called its **substrate**. The substance (or substances) formed is called the product.

> **TIP**
> Avoid saying 'an enzyme catalyses its substrate'. It is not possible to catalyse a *substance*. Enzymes catalyse *reactions*.

There are many different enzymes, each catalysing a different reaction. Most of them are inside cells. These are called intracellular enzymes. Some types of enzyme are released by cells, for example the enzymes secreted into the gut to digest food. These are known as extracellular enzymes.

3.01 How enzymes work

Enzymes are globular proteins (see Unit 2). There is a small region on the surface of the enzyme called the **active site**. The substrate binds to the enzyme at the active site, forming an enzyme–substrate complex. A reaction then occurs, and the product(s) leave the active site. The idea that the substrate fits exactly into the active site of the enzyme is known as the **lock and key hypothesis** (Figure 3.01).

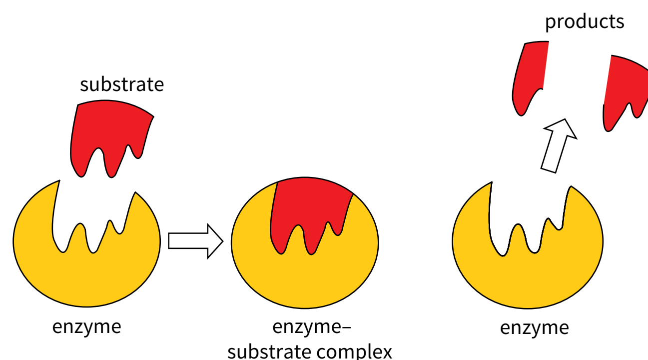


Figure 3.01 The lock and key hypothesis.

The substrate forms temporary bonds with the R groups of amino acids in the active site to form the enzyme–substrate complex. The bonds are weak interactions such as ionic and hydrogen bonds. In this model of enzyme action the substrate is the 'key', which fits into the enzyme's 'lock'.

A more recently developed model is called the **induced fit hypothesis** (Figure 3.02). This suggests that, before it enters, the substrate is not a perfect fit in the active site. When it does enter, the shape of the active site changes slightly to accommodate the substrate. This is rather like the way a glove changes shape when you put your hand in it. The induced fit

hypothesis is really a modified version of the lock and key mechanism, and is now known to be a better model of what actually takes place.

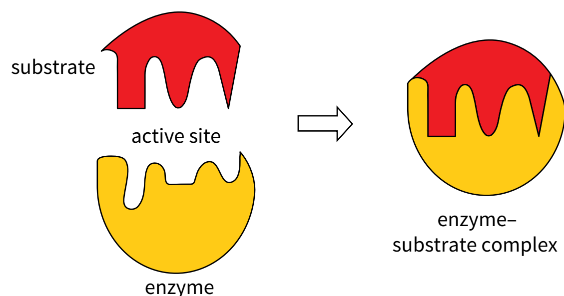


Figure 3.02 The induced fit hypothesis.

In either model, a particular substrate will only fit into the active site of a particular enzyme. We say that the shape of the active site is 'complementary' to the shape of the substrate. This means that an enzyme will only work with one substrate – it is *specific* to its substrate, so that it will only catalyse one reaction.

Specificity is important, because there are thousands of different metabolic reactions happening in cells. Which ones can take place depends on which enzymes the cell makes. In this way a cell exercises control over its metabolism.

Progress check 3.01

1. From your knowledge of protein structure (see Unit 2), explain briefly why proteins are suitable molecules to produce the specificity needed by enzymes.
2. Explain the main difference between the lock and key and the induced fit models of enzyme action.

Enzymes and activation energy

When a chemical reaction takes place, bonds in the reactants are broken and then new bonds are made in forming the products. Breaking bonds requires energy, and forming bonds gives out energy. The energy needed to break bonds in the reactants is called the **activation energy** of the reaction. In a test-tube this energy may be provided by heat, but in cells this is not possible. The temperature in cells is relatively low, so that without enzymes most metabolic reactions would take place very slowly, or not at all.

All catalysts, including enzymes, speed up reactions by lowering the activation energy of a reaction. This is easiest to show as a graph (Figure 3.03).

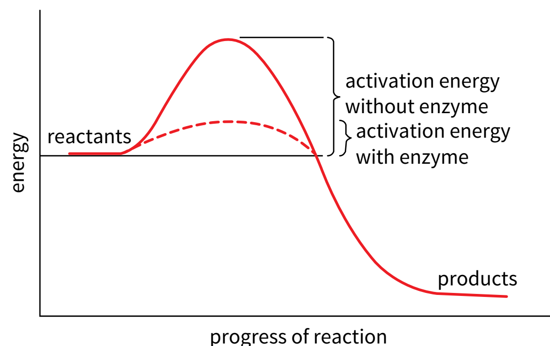


Figure 3.03 Enzymes speed up a reaction by lowering the activation energy needed to start the reaction.

Enzymes do this by forming the enzyme–substrate complex. The complex changes the shape of the substrate slightly, which reduces the energy needed to break its bonds.

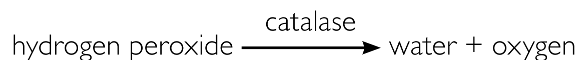
3.02 Following the progress of an enzyme-catalysed reaction

You can monitor the progress of an enzyme-catalysed reaction by either:

- measuring the rate of formation of a product
- measuring the rate at which a substrate is used up.

a Measuring the rate of formation of a product

An enzyme called catalase speeds up the breakdown of hydrogen peroxide, which is a waste product of metabolism. Hydrogen peroxide is toxic and would be harmful if it built up in cells. Catalase breaks it down into water and oxygen, which are both harmless:



The activity of catalase can be followed by measuring the rate of formation of oxygen gas.

Catalase is present in all living tissues. High concentrations of catalase can be obtained from readily available sources, such as a suspension of yeast cells or a potato tuber that has been macerated with water in a food blender. If hydrogen peroxide solution is added to one of these, the reaction mixture will produce oxygen, which you can collect in a gas syringe (Figure 3.04).

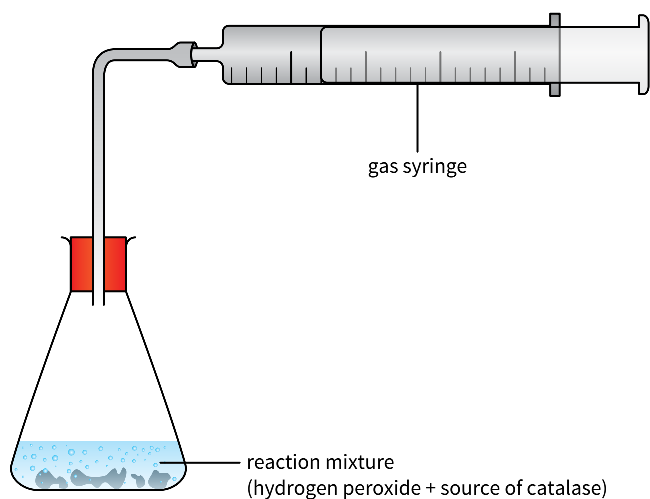


Figure 3.04 Collecting oxygen from the breakdown of hydrogen peroxide.

A graph of the volume of gas collected against time will look like Figure 3.05. Note that the rate is fastest at the start. This is called the initial rate of reaction. Over time the reaction slows. After 3 minutes the reaction stops and the curve levels off.

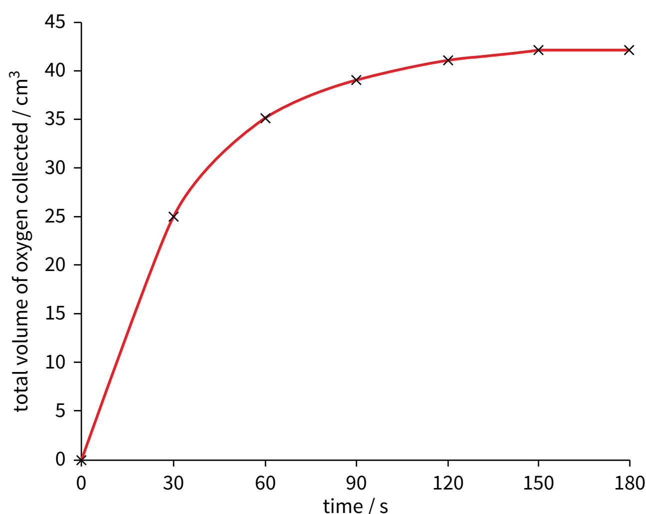
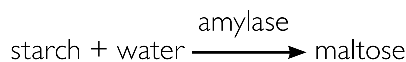


Figure 3.05 The progress of the breakdown of hydrogen peroxide, catalysed by the enzyme catalase.

The rate is fastest at the start because it depends on random collisions between enzyme and substrate molecules. As the reaction proceeds, the substrate is used up, so there are fewer collisions and the rate slows. For this reason, when we want to compare enzyme activity under different conditions, we always measure the *initial* rate of reaction.

b Measuring the rate at which a substrate is used up

The enzyme amylase catalyses the hydrolysis of starch into the reducing sugar maltose:



This reaction can be monitored by recording the disappearance of the starch, by testing for it using a solution of iodine in potassium iodide. You take samples of the reaction mixture (starch suspension + amylase) every 10 seconds and test it for starch. At first, when starch is present, the iodine solution will turn blue-black. When the starch has been broken down into maltose the iodine solution will remain yellow-brown.

To obtain continuous results over a period of time you can monitor the disappearance of the blue colour in a colorimeter. Figure 3.06 shows the expected results.

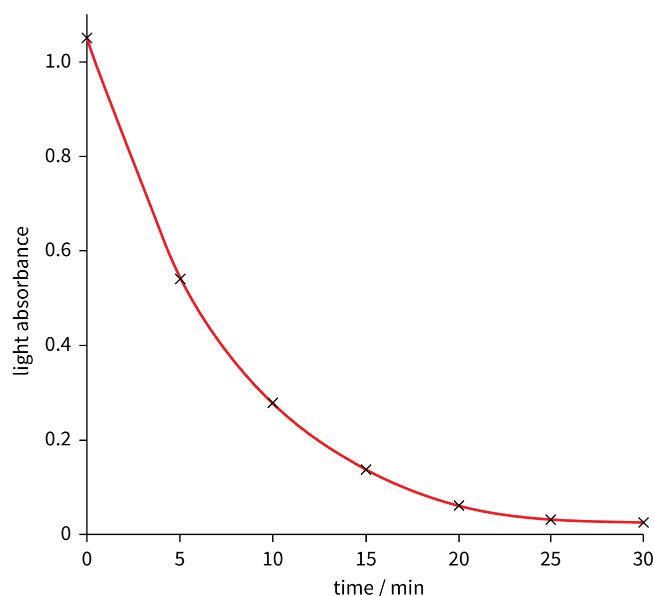


Figure 3.06 The progress of the breakdown of starch, catalysed by the enzyme amylase.

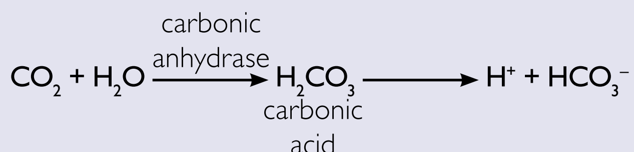
Progress check 3.02

1. Briefly explain the meaning of activation energy (of a reaction).
2. The enzyme invertase catalyses the hydrolysis of sucrose to glucose and fructose:

Suggest how you could monitor this reaction in order to measure the activity of invertase.

Sample question 3.01

Figure 3.07 shows a computer model of the enzyme carbonic anhydrase. This enzyme catalyses the conversion of carbon dioxide and water to carbonic acid (H_2CO_3). The carbonic acid then dissociates into hydrogen ions (H^+) and hydrogencarbonate ions (HCO_3^-):



The arrow shows the position of the enzyme's active site.

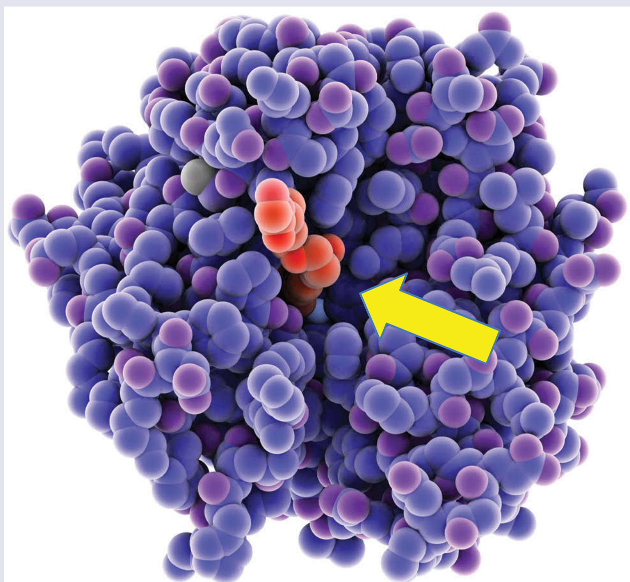


Figure 3.07

1 Suggest how you could monitor this reaction in order to measure the activity of carbonic anhydrase. [2]

2 Explain why the shape of the active site is important in the way that the enzyme works. [3]

3 Figure 3.08 shows the energy changes during the progress of an uncatalysed reaction.

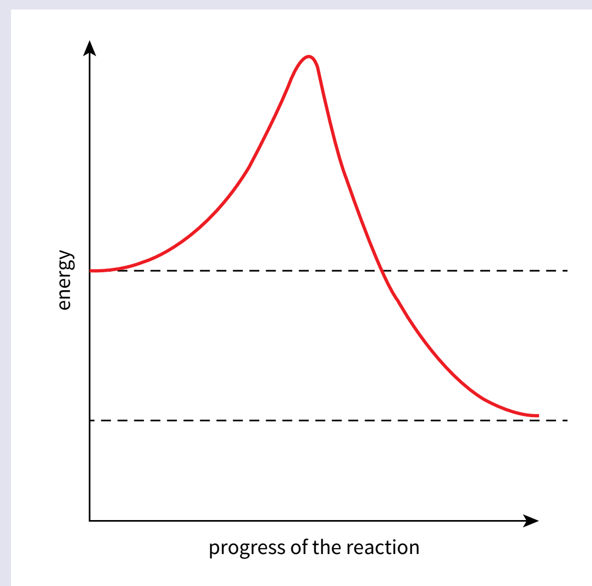


Figure 3.08

Label the activation energy of this reaction on Figure 3.08. [1]

4 On Figure 3.08, draw a curve to show the changes in energy taking place during the progress of the same reaction when it is catalysed by an enzyme. [2]

[Mark points are shown in square brackets – to a maximum of 8 marks]

1 Measure the change in concentration of a reactant / a product [1].

Measure the change in pH (because H^+ ions are formed) [1].

Use an indicator / monitor changes in colour of an indicator in a colorimeter [1].

Use a pH meter [1].

[Maximum 2]

2 Substrates / carbon dioxide and water, fit into / bind to / enter, active site [1].

(Substrates have) complementary shape [1].

Reference to specificity (of the enzyme) [1].

Lock and key / induced fit hypothesis [1].

Temporary bonds form with active site / with R groups (of amino acid residues) [1].

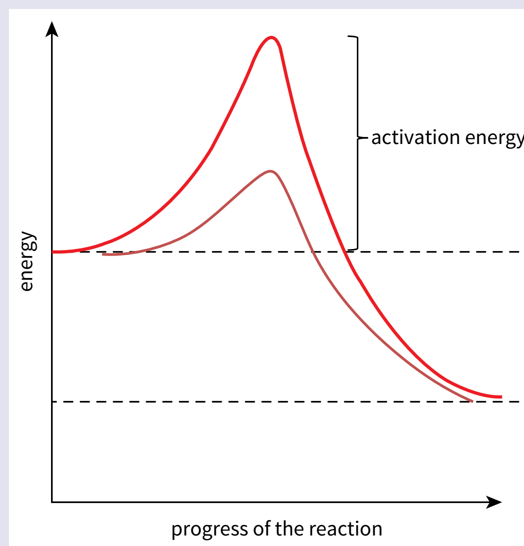
[Maximum 3]

3 Label and arrow or bracket clearly indicating height of peak above the upper dashed line [1].

4 Line with one peak, below that of uncatalysed curve [1].

Curve starts and finishes at the dashed lines [1].

The most obvious substance to measure here is the concentration of hydrogen ions (i.e. the pH). The concentration of H^+ should increase as the reaction proceeds, so the pH will fall.



If you have to label on a diagram, use a label line or bracket. Make sure the labels are clear and unambiguous, like this:

3.03 Factors affecting the rate of enzyme-catalysed reactions

There are a number of factors that affect the rate of an enzyme-catalysed reaction. These include:

- temperature
- pH
- enzyme concentration
- substrate concentration
- the presence of inhibitors.

a Temperature

An increase in temperature increases the kinetic energy of molecules. As the temperature rises, both enzyme and substrate molecules move more quickly and collide with each other more often, with higher energies. There are a greater number of successful collisions leading to conversion of substrate into product. So at first, as the temperature rises, the rate of reaction increases.

However, beyond a certain point, higher temperatures start to change the shape of the enzyme molecule. Enzymes are globular proteins with a tertiary structure that is maintained by bonds between the R groups of polypeptide chains. High temperatures cause these bonds to break, damaging the tertiary structure of the protein. This process is called **denaturation** – the protein becomes denatured.

Denaturing changes the shape of the active site, so that substrate molecules are unable to form an enzyme–substrate complex and the rate of reaction decreases.

> **TIP**
> Avoid describing enzymes as being 'killed' by heat. They are chemicals, not living things, so cannot be 'killed' ('destroyed' is acceptable).

The rate at any temperature depends on the balance between these two factors (kinetic energy and denaturation), which produces an optimum temperature, where the reaction occurs most rapidly (Figure 3.09).

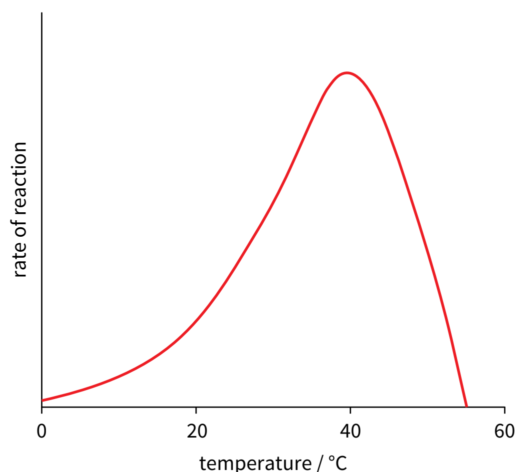


Figure 3.09 The effect of temperature on the activity of an enzyme.

Organisms have evolved enzymes that are adapted to the temperature of their surroundings. For example, human enzymes have an optimum at about body temperature (37 °C), while some bacteria that live in hot springs have evolved 'heat-resistant' enzymes, which are not denatured by temperatures as high as 100 °C.

> **TIP**
> Do not assume that all enzymes have an optimum temperature of 37 °C. A plant living at an average temperature of 20 °C will probably have evolved enzymes that work best at this temperature.

b pH

Enzymes also have an optimum pH at which they work best (Figure 3.10). A change in pH affects the concentration of hydrogen ions (H^+) around the enzyme molecule. This affects the ionisation of R groups on the amino acids of the protein. In turn this affects the shape of the active site, and the ease of formation of an enzyme–substrate complex. At the optimum pH, the shape of the active site is complementary to the shape of the substrate.

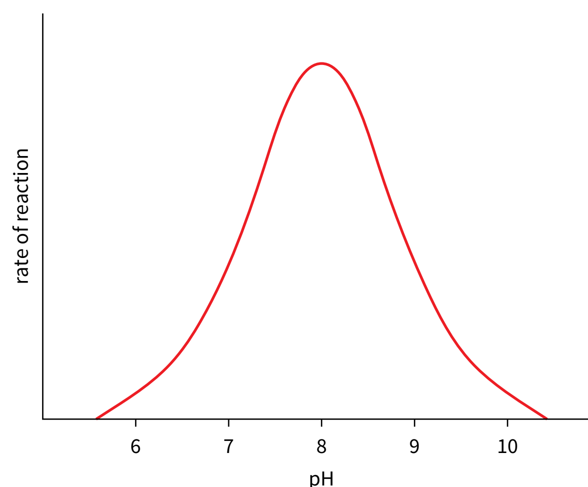


Figure 3.10 The effect of pH on the activity of an enzyme.

Most enzymes have an optimum pH near neutral (pH 7) but there are exceptions. The enzyme pepsin is found in the human stomach, and has evolved to work under the acid conditions present there. It has an optimum pH of about 2.

If the pH around an enzyme is very different from its optimum, this will result in permanent denaturing.

c Enzyme concentration

Enzyme molecules are not used up during the reactions they catalyse, so they can be used over and over again. As a result, enzymes work efficiently in very low concentrations. Usually there is an excess of substrate molecules, so the rate of reaction is limited by the concentration of the enzyme. Because of this, if the concentration of enzyme is increased it will result in an increase in collisions between enzyme and substrate molecules, and a faster rate of reaction (Figure 3.11). In theory, at high concentrations of enzyme, the concentration of substrate would become limiting and the rate level off, but this does not normally happen if there is plenty of substrate available.

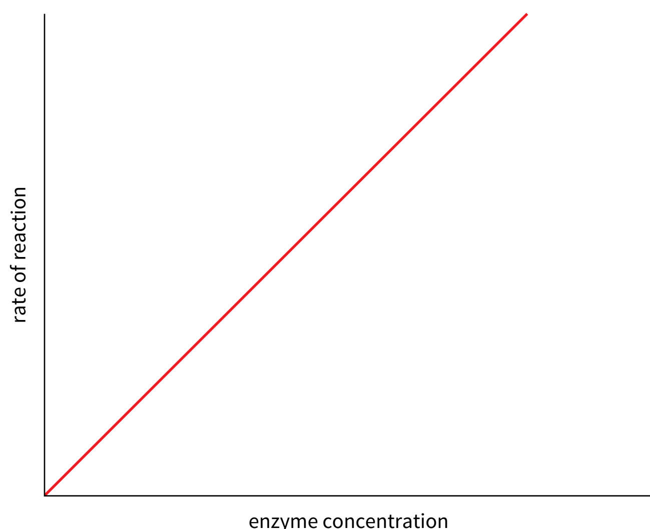


Figure 3.11 The effect of enzyme concentration on the activity of an enzyme.

d Substrate concentration

If the concentration of substrate is low, some of the active sites of the enzyme molecules will be unoccupied, and the rate of reaction will be low. If the concentration of substrate is increased, there will be more frequent collisions between enzyme and substrate molecules, more enzyme–substrate complexes are formed, producing a faster rate of reaction. However, at high substrate concentrations, the concentration of the enzyme becomes limiting – at this point the curve levels off (Figure 3.12).

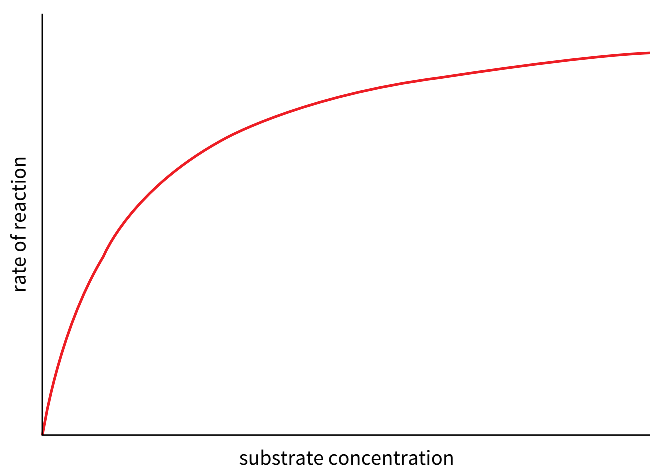


Figure 3.12 The effect of substrate concentration on the activity of an enzyme.

e The presence of inhibitors

Inhibitors are substances that reduce the rate of an enzyme-catalysed reaction. They do so by altering the shape of the active site, either directly or indirectly.

Some substances are permanent inhibitors. They cause denaturation of the enzyme and their effects are non-reversible. For example, heavy metal ions such as lead (Pb^{2+}) and mercury (Hg^{+}) fall into this category.

Other inhibitors are reversible. These only bind to the enzyme temporarily, so that their effect is not permanent. They fall into two categories: competitive and non-competitive.

Competitive inhibitors are molecules that have a shape that is similar to that of the substrate. They can fit temporarily into the active site, preventing the substrate from entering. This reduces the number of enzyme–substrate complexes and slows the reaction (Figure 3.13a). The greater the concentration of inhibitor relative to substrate in a mixture, the more effect the inhibitor will have in reducing the activity of the enzyme.

Non-competitive inhibitors do not have a shape like that of the substrate. They do not attach to the active site, but bind to other parts of the enzyme, altering the overall shape of the enzyme molecule, including the active site. This does not prevent the substrate entering the active site, but the site is no longer the correct shape to catalyse the reaction, so the enzyme–substrate complex is inactive (Figure 3.13b). However, if the concentration of substrate is increased, the enzyme will still not be able to catalyse the reaction – so the relative concentrations of inhibitor and substrate do not affect the rate. Figure 3.14 compares the effects of these two types of inhibitors as a graph.

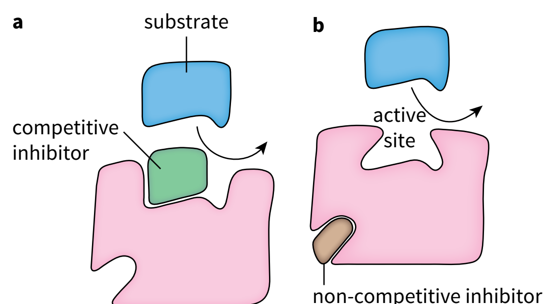


Figure 3.13 Competitive and non-competitive enzyme inhibitors.

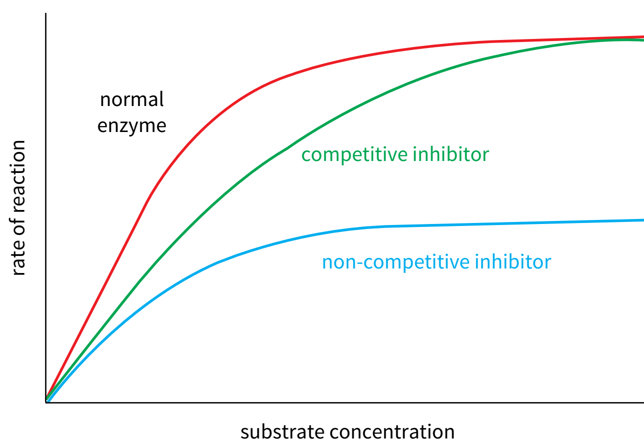


Figure 3.14 The effects of competitive and non-competitive inhibitors on the activity of an enzyme.

Progress check 3.03

1. Describe what happens to a protein when it is denatured by heat.
2. Assuming all other factors are kept constant, explain why increasing the concentration of substrate does not always increase the rate of an enzyme-catalysed reaction.

3.04 Investigating factors affecting the rate of enzyme-catalysed reactions

Many different enzymes are suitable for investigation in a school laboratory. Here we will look at the breakdown of hydrogen peroxide by catalase, which can be monitored by collecting the oxygen produced during the reaction.

Figure 3.04 shows one way this can be done using a gas syringe, but there are other methods. If you do not have a gas syringe, the oxygen can be collected over water in a measuring cylinder. You can even connect a delivery tube to the reaction vessel and count the number of bubbles of gas produced per minute from a delivery tube, although this method is not very precise, and only works if the rate of reaction is slow. Alternatively you can measure the weight decrease as the oxygen is lost, by placing the flask on a top-pan balance (Figure 3.15).

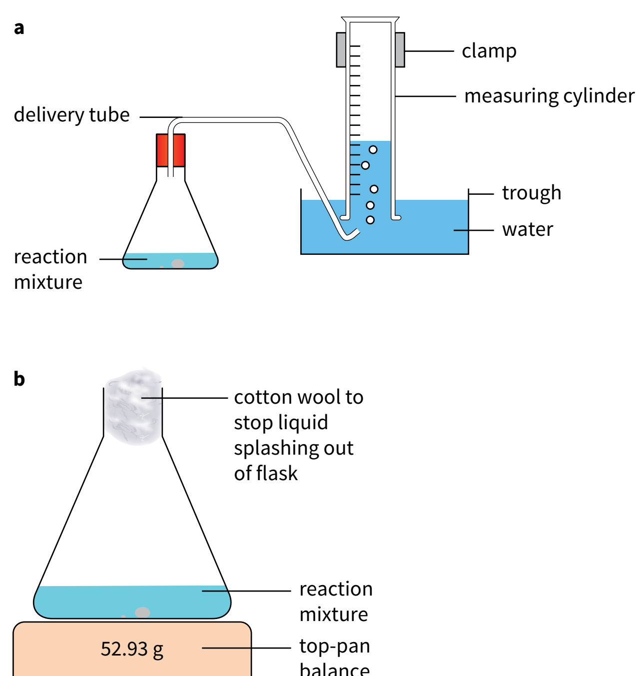


Figure 3.15 Some other ways to measure the rate of oxygen production from the breakdown of hydrogen peroxide by catalase.

A good source of catalase is a 1% suspension of yeast, which consists of living fungal cells. You can place a set volume (e.g. 100 cm^3) of 3% hydrogen peroxide solution in the flask and add a set volume (e.g. 10 cm^3) of the yeast suspension. Immediately insert the bung and delivery tube in the flask and collect the oxygen in the gas syringe, recording the volume collected at suitable intervals.

TIP

You will need to experiment to find suitable volumes to use – it will depend on the activity of the yeast, temperature used and so on.

Having decided upon a suitable method, you can select a factor to change. It is important that only one factor is changed at a time, and that any other variables that might affect the results are kept constant (controlled). This is called a 'controlled experiment'. For example, if you want to know the effect of temperature on the rate of reaction, you must keep variables such as pH, volumes of solutions, and enzyme and substrate concentration, constant.

a Temperature

A fixed temperature is achieved by placing the reaction flask or tube in a water bath. The best type to use is an electric thermostatically controlled water bath, which will maintain a temperature to within ±1 °C. Alternatively you can use a beaker of water heated over a Bunsen burner. You should try six or more temperatures over a suitable range (e.g. 20 °C to 80 °C), which might be expected to cover the optimum for catalase.

You must leave the reaction flask to reach the correct temperature (this is called 'equilibrating to temperature'). The yeast should be maintained at this temperature too. Then start the reaction as described above and measure the initial rate of production of oxygen. Repeat the procedure with the flask at the other temperatures. If you have time it is best to carry out replicate measurements at each temperature, to identify any anomalous results.

Plot a graph of the initial rate of reaction (= enzyme activity) against temperature. Your graph should look something like Figure 3.09, but note that yeast enzymes may have a relatively high optimum temperature.

b pH

Buffer solutions are solutions of salts that resist changes in pH. They can be added to a reaction mixture to maintain the pH of the mixture. You can adapt the catalase experiment by changing the pH of the mixture. Add an equal volume of buffer solution to the hydrogen peroxide solution in the flask, before adding the yeast to start the reaction. Here again, all other factors must be kept constant. This time they include temperature, which should be maintained at a constant value, close to the optimum for the enzyme (e.g. 40 °C). Various buffer solutions are available – assuming the optimum pH of catalase is likely to be near neutral (pH 7) you could use a range between pH 5 and pH 9.

When you have your results, plot a graph of the initial rate of reaction (= enzyme activity) against pH. It should look similar to Figure 3.10.

c Enzyme concentration

Make a number of dilutions of the yeast suspension that you used in the last two experiments (Table 3.01).

Volume of yeast suspension / cm^3	Volume of distilled water / cm^3	Concentration of catalase / % of original
100	0	100
80	20	80
60	40	60
40	60	40
20	80	20
0	100	0

Table 3.01 Dilutions of the yeast suspension used in the temperature and pH experiments.

You can now adapt the catalase experiment by using these different concentrations of the enzyme. Add an equal volume of the optimum pH buffer solution to the hydrogen peroxide solution in the flask. Now add the most concentrated yeast suspension to start the reaction. All other factors must be controlled. Repeat with the other concentrations of enzyme. You should be able to plot a graph of the initial rate of reaction against concentration of enzyme that looks like Figure 3.11.

d Substrate concentration

This is very similar to the way you investigate enzyme concentration, but instead of changing the concentration of catalase, keep that constant and change the concentration of hydrogen peroxide.

Sample question 3.02

The enzyme trypsin will hydrolyse the protein in a suspension of powdered milk, turning it clear. A student carried out an experiment to investigate the effect of temperature on the activity of trypsin. She incubated 10 cm^3 of trypsin solution and 10 cm^3 of a suspension of powdered milk separately in a water bath at 30 °C. After the tubes had equilibrated to temperature, she mixed the contents of the tubes and recorded the time taken for the mixture to clear. She repeated the measurements at different temperatures. Her results are shown in Table 3.02.

Temperature / °C	Time for hydrolysis of protein / min	Rate of reaction / cm³ min⁻¹
22	5.12	
30	2.28	
41	1.34	
45	1.20	
52	1.24	
57	1.41	
61	1.67	
66	3.72	
69	10.04	

Table 3.02

1 Calculate the rates of reaction by dividing the volume of milk by the times taken for hydrolysis. Write your answers in the last column of Table 3.02. Express your answer in $cm^3\ min^{-1}$. [3]

2 Plot a graph of rate of reaction against temperature. [4]

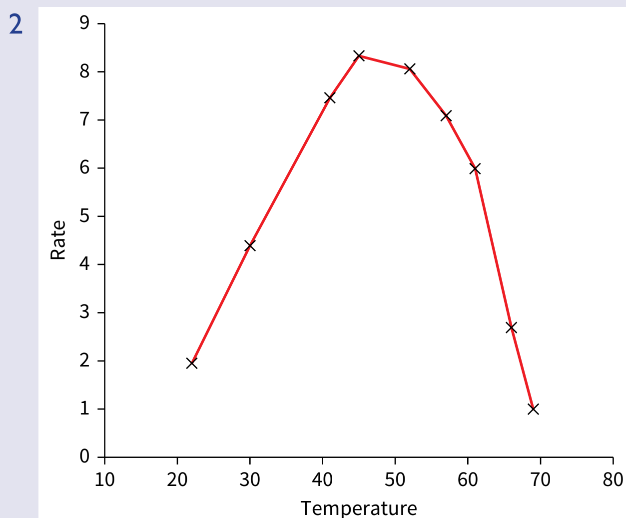
3 Explain the effects of temperature on the rate of reaction. [5]

1

Temperature / °C	Time for hydrolysis of protein / min	Rate of reaction / $cm^3\ min^{-1}$
22	5.12	1.95
30	2.28	4.39
41	1.34	7.46
45	1.20	8.33
52	1.24	8.06
57	1.41	7.09
61	1.67	5.99
66	3.72	2.69
69	10.04	1.00

The rates of reaction are rounded to three significant figures (the same number of significant figures as for the time in Table 3.02).

2



Choose a suitable scale for each axis, slightly bigger than the range of the values. Do not forget to label each axis and include units.

Axes right way round, labelled, with correct units **[1]**.

Axes drawn to a suitable scale, sensible increments **[1]**.

All points plotted correctly with suitable symbol (cross, or dot in a circle) **[1]**.

Lines joined point-to-point, with a ruler **[1]**.

3 Between 22 °C and 45 °C the rate increases due to the increased kinetic energy of the molecules/increased collisions of enzyme and substrate **[1]** resulting in more enzyme–substrate complexes forming **[1]**. Above 52 °C the rate decreases due to denaturing of the enzyme **[1]**, changing the shape of the active site **[1]**, so fewer enzyme–substrate complexes formed **[1]**. The optimum temperature is between 45 °C and 52 °C **[1]**.

It is sensible to answer part 3 by explaining the results from low through to high temperatures. Note that you do not know where the optimum is exactly – it must lie somewhere between 45 °C and 52 °C.

3.05 Enzyme affinities and the Michaelis–Menten constant

If you draw a graph of the rate of an enzyme-catalysed reaction against concentration of substrate (Figure 3.12), the curve is a parabola. There is a mathematical equation called the Michaelis–Menten equation that describes this hyperbolic curve. The rate (velocity) is given the symbol v and the concentration of substrate [S] – the square brackets mean 'concentration of'. The equation fits the results that are obtained experimentally, and predicts that at high concentrations of substrate the rate will level off and approach a theoretical maximum limit, called $\mathbf{V_{max}}$.

The equation also uses another mathematical term called the **Michaelis–Menten constant**, $\mathbf{K_m}$. K_m is the value of [S] at which the reaction rate is equal to half that of V_{max} (½V_{max}). This is shown in Figure 3.16.

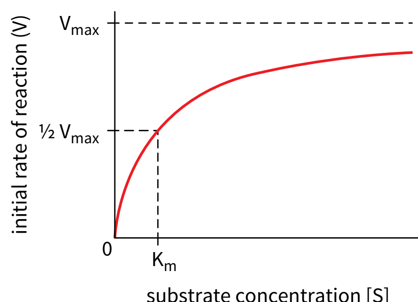


Figure 3.16 Graph of initial reaction rate V against initial substrate concentration [S] for an enzyme-catalysed reaction.

The problem with trying to calculate K_m from Figure 3.16 is that you cannot tell for certain when V_{max} has been reached – the dotted line is only an estimate drawn by eye. However it is possible to draw another graph, from which the exact values of V_{max} and K_m can be found. This is a 'double reciprocal plot' where the values of 1/v are plotted against 1/[S]. This graph produces a straight line, where the intercept on the *y*-axis is $1/V_{max}$, and the intercept on the *x*-axis is $-1/K_m$ (Figure 3.17). From these values we can calculate V_{max} and K_m.

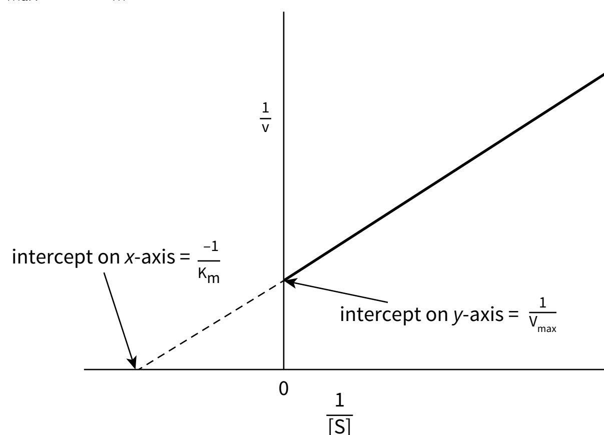


Figure 3.17 A double reciprocal plot of 1/v against 1/[S] for an enzyme-catalysed reaction.

K_m is a very useful value in enzyme kinetics. At ½V_{max} half the active sites of the enzyme are occupied by substrate, so K_m is a measure of how tightly the substrate is bound to the enzyme, which is called the affinity of the enzyme for its substrate. The lower the