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STUDENT'S BOOK

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Chapter

2

Biological molecules

All living organisms are mainly composed of proteins, carbohydrates, lipids and nucleic acids. However, it is thought that the early conditions on planet Earth were hot and extremely hostile. Where did these organic molecules, which are so vital to life, come from?

In the 1950s, US biochemists Stanley Miller and Harold Urey tried to answer this question. They set up a laboratory experiment that attempted to model the conditions found on our planet nearly four billion years ago. Passing electrical pulses through a mixture of hot carbon dioxide, ammonia, sulfur dioxide and water vapour, they were able to produce molecules such as amino acids.

It is a big step to explain how life originated from non-living molecules. However, the work of Miller and Urey offers clues to explain how some of these fundamental ingredients may have appeared in the first place.

Prior understanding

You may remember from your study of a balanced diet that the major food types are carbohydrates, lipids and proteins. These are vital because our bodies are composed of these major groups of organic molecules. You may remember the terms ‘atom’, ‘molecule’, ‘electron’ and ‘ion’, and have a basic understanding of covalent and ionic bonding, and of molecular and structural formulae.

Learning aims

In this chapter, you will learn how the structure and properties of biological molecules are related to their functions in cells and in organisms. We will consider the roles of carbohydrates, lipids and proteins, and how they are fundamental to an understanding of many areas of biology. The importance of water to living organisms is also covered.

2.1 An introduction to biological molecules (2.2.2, 2.2.3, 2.4.1)
2.2 Testing for biological molecules (Syllabus 2.1.1–2.1.3, 2.2.4)
2.3 Carbohydrates (Syllabus 2.2.1, 2.2.5–2.2.8)
2.4 Lipids (Syllabus 2.2.9–2.2.11)
2.5 Proteins (Syllabus 2.3.1–2.3.8)
2.6 Water (Syllabus 2.4.1)

2.1 An introduction to biological molecules

As we saw at the start of this chapter, the emergence of organic molecules was probably a precursor to life on Earth. **Molecules** are the fundamental units of life. These are defined as particles with two or more **atoms** combined. Joining different atoms together in different quantities, or in different ways, produces molecules with a range of different properties. **Molecular biology** is the study of these molecules, and the ways in which they react with other particles is **biochemistry**.

In organisms, most of the atoms are of just six different kinds – hydrogen, oxygen, carbon, nitrogen, sulfur and phosphorus – with other trace elements being required in smaller quantities. As we will see during the course of this chapter, the element carbon forms a central 'skeleton' in all biological macromolecules.

MONOMERS, POLYMERS AND MACROMOLECULES

Although there is a vast number of different biomolecules, they can be grouped into different classes. These are monomers, polymers and macromolecules.

- A **monomer** is a molecule that is used as the building block for the synthesis of a polymer. Monomers are joined together by covalent bonds in **condensation reactions** to make polymers.
- A **polymer** is a giant molecule made from many similar repeating subunits, called **residues**, joined together by strong covalent bonds in a chain. Polymers can be broken into smaller subunits, often monomers, in **hydrolysis** reactions.
- A **macromolecule** is a giant molecule consisting of thousands of atoms, built from a small number of simple molecules. In this chapter, we look at the macromolecules **carbohydrates**, **lipids** and **proteins**, and describe the characteristics that make them the chemicals of life.

Link

Nucleic acids (DNA and RNA) are also macromolecules. We will look at them separately in Chapter 6.

Tip

In this chapter, all polymers are macromolecules, and all macromolecules are polymers.

Table 2.1 summarises the four main biological macromolecules and gives further details about them.

Macromolecule	Monomer	Polymer	Covalent bond
carbohydrate	sugar	starch, cellulose, glycogen	glycosidic
protein	amino acid	polypeptide	peptide
lipid	N/A	N/A	ester
nucleic acid	nucleotide	DNA, RNA	phosphodiester

Table 2.1 Carbohydrates, proteins, lipids and nucleic acids are the four most common macromolecules in living organisms. Lipids are unusual in that they do not consist of a polymer as they do not consist of repeating residues.

HYDROPHILIC AND HYDROPHOBIC MOLECULES

The properties of biological molecules are numerous and diverse. However, during this chapter, we will encounter one clear difference between them. This property is the degree to which the molecule is described as **hydrophilic** or **hydrophobic** – meaning how well it mixes with the **polar** solvent, water. There are several alternative terms that describe this property, which is due to the structure of the molecule itself. These are summarised in Table 2.2.

Molecules that mix with water are:	Molecules that do not mix with water are:
hydrophilic	hydrophobic
polar	non-polar
charged	uncharged
water-soluble	water-insoluble
lipid-insoluble	lipid-soluble

Table 2.2 One property that can differ between biological molecules is their ability to mix with water. The terms on the left describe molecules that readily mix with water; those listed on the right are used to describe molecules that do not mix well with water.

HYDROGEN BONDING

All atoms contain electrons, and an electron has a small negative charge. In many molecules, the electrons in the chemical bond are not shared equally between the oxygen and the hydrogen atoms. In water, for example, this results in the oxygen atom having a tiny negative charge, and each hydrogen atom having a tiny positive charge. These molecules are said to be polar. Although the overall charge is zero, a polar molecule has a small positive charge in some places, and a small negative charge in others. These small charges are written $\delta-$ and $\delta+$. The symbol δ is the Greek letter delta, so you can say 'delta minus' and 'delta plus'.

Negative charges and positive charges are attracted to one another. This means that some molecules are attracted to their neighbours. The negative charge on one molecule is attracted to the positive charge on another molecule. These attractions are called **hydrogen bonds**, and you will encounter them throughout this chapter. Although they are important, hydrogen bonds are much weaker than the strong **covalent bonds** that hold the atoms in molecules firmly together.

2.2 Testing for biological molecules

For a number of reasons, scientists often need to test for the presence of key biological molecules in a variety of animal or plant materials. For example, it may be important to identify the contents of mixtures of molecules in foods, or to work out the activity of digestive enzymes.

IODINE TEST FOR STARCH

To test for starch, add an orange–brown solution of iodine dissolved in **potassium iodide solution** (often abbreviated to **iodine–potassium iodide solution** or simply 'iodine solution') to the substance. A blue–black colour indicates the presence of starch (Figure 2.1).

Figure 2.1 When iodine–potassium iodide solution encounters starch, its colour changes from orange–brown to blue–black.

Tip

Stating 'no change' to record a negative result is insufficient. You should indicate what colour remains. For example, 'iodine–potassium iodide solution remains orange–brown' is an accurate observation of a negative starch test.

TESTS FOR SUGARS

Tests for sugars can distinguish two groups of sugars: the **reducing sugars** and the **non-reducing sugars**. The term 'reducing sugar' indicates that some sugars can reduce, or donate electrons to, other molecules.

The **Benedict's test** for a reducing sugar involves adding a solution containing copper sulfate called **Benedict's solution** (sometimes called **Benedict's reagent**) to the sample and heating the sample to at least 80 °C in a water bath. If a reducing sugar is present, the Cu^{2+} ions in copper sulfate are reduced by the sugar to Cu^{+} ions, resulting in a colour change.

The colour seen in a positive Benedict's test depends on the concentration of reducing sugar present in the sample (Figure 2.2). As you will see in the Experimental skills section, this can be used to estimate the concentration of reducing sugars in a sample.

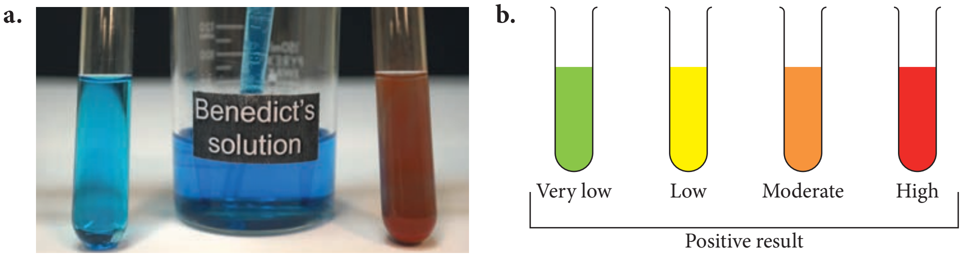

Figure 2.2 The colour change of a positive Benedict's test is caused by the precipitation of copper (I) oxide (a). The more reducing sugar present in the original sample, the more precipitate there is. Lower concentrations of reducing sugar will give a colour change from blue to green or yellow, whereas higher concentrations will give a colour change to orange or red (b).

Glucose, **fructose**, **galactose**, **maltose** and **lactose** are all reducing sugars, but **sucrose** is not – it is a non-reducing sugar. It will not give a positive test result with Benedict's solution. However, as we will see later, sucrose consists of a molecule of glucose and fructose joined together. After sucrose is boiled with dilute acid to hydrolyse (split) it into glucose and fructose, a positive result is observed (Figure 2.3). This is called **acid hydrolysis**.

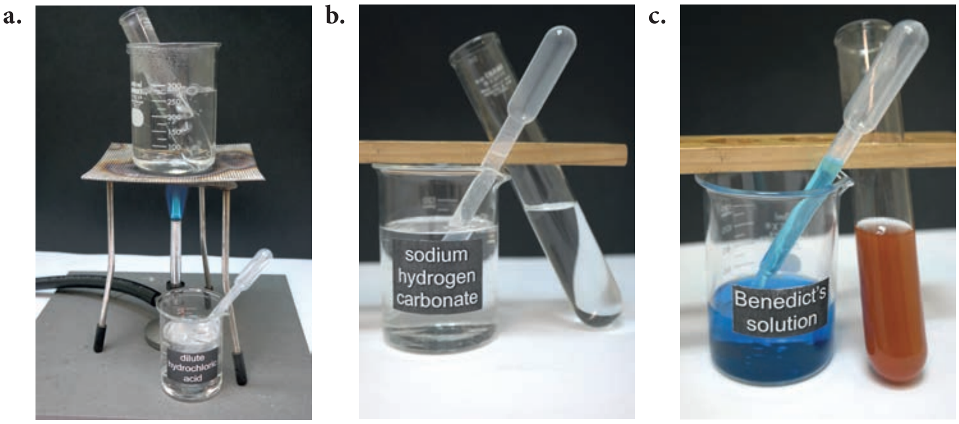

Figure 2.3 To establish the presence of a non-reducing sugar in a sample, the mixture should be heated strongly, with acid (a). Then, the sample is neutralised using sodium hydrogencarbonate (b). The Benedict's test will then give a positive result if the original sample contains a non-reducing sugar (c).

EMULSION TEST FOR LIPIDS

To test for the presence of a lipid in a sample, the sample is first crushed (if it is a solid) and then shaken with ethanol in a clean test-tube. Lipids are soluble in ethanol, so if there is any lipid in the sample, some of it will dissolve. The second step is to gently pour the ethanol into another test-tube containing water. If there is any lipid dissolved in the ethanol, it will form little droplets in the water. The mixture of these microscopic lipid droplets and water is known as an **emulsion**. The lipid droplets reduce the amount of light passing through, so the water becomes cloudy (milky white) rather than being transparent (Figure 2.4).

Figure 2.4 How to test a biological sample for the presence of lipid. An emulsion will form if lipids dissolve in ethanol when mixed with water.

Figure 2.5 The biuret test for proteins. Biuret solution is pale blue but turns pale purple in the presence of protein.

BIURET TEST FOR PROTEINS

To test a sample for the presence of protein, biuret solution is added to the biological sample and mixed gently. Unlike the Benedict's test, there is no need to heat. As shown in Figure 2.5, if protein is present, blue biuret solution will become pale purple (sometimes called lilac, or mauve).

The biochemical tests that can be carried out for biological molecules, with the positive results listed, are summarised in Table 2.3. Note that an **observation** is very different from a **conclusion**. An observation is a description of what is seen, whereas a conclusion is a statement that can be inferred from an observation. For example, 'Benedict's solution changes from blue to red' is an observation; 'the sample contains a high concentration of reducing sugar' is a conclusion.

Tip

Remember to state the original colour of the test solution. Get into the habit of stating the colour beforehand; for example, 'blue biuret solution' or 'orange–brown iodine–potassium iodide solution'.

Biological molecule	Reagent(s) used	How test is carried out	Positive result
reducing sugar (e.g. glucose)	blue Benedict's solution	add Benedict's solution to sample in a test-tube; heat in a water bath set at 80° C	colour change from blue to green, yellow, orange or red
non-reducing sugar (e.g. sucrose)	hydrochloric acid and blue Benedict's solution	once the reducing sugar test has proved negative, boil with dilute acid; add sodium hydrogencarbonate to neutralise; carry out reducing sugar test, as described above	colour change from blue to green, yellow, orange or red
starch	orange–brown iodine–potassium iodide solution	add a few drops of iodine–potassium iodide solution to the sample	colour change from orange–brown to blue–black
lipid	ethanol distilled water	shake the sample with ethanol in a test-tube tube; allow to settle; pour clear liquid into water in another test-tube	cloudy white emulsion
protein	blue biuret solution	add biuret solution to sample in a test-tube	colour change from blue to pale purple

Table 2.3 The reagents, procedures and positive results of biochemical tests for biological molecules.

Worked example

An investigation was carried out into the activity of two enzymes, sucrase and amylase. Four reaction mixtures were prepared, as shown in Table 2.4.
To answer this question you will need to know that:

- all enzymes are proteins
- amylase breaks down starch into maltose
- sucrase breaks down sucrose into glucose and fructose.

Three biochemical tests were performed after incubating each reaction mixture at 37 °C for 1 hour.

Reaction mixture contents	Benedict's test	Biuret test	Iodine test
sucrose + sucrase			
sucrose + amylase			
starch + sucrase			
starch + amylase			

Table 2.4

Complete the table by indicating which tests would give positive (✔) and negative (✘) results.

Answer

Reaction mixture contents	Benedict's test	Biuret test	Iodine test
sucrose + sucrase	✔	✔	✘
sucrose + amylase	✘	✔	✘
starch + sucrase	✘	✔	✔
starch + amylase	✔	✔	✔

- All four reaction mixtures would give a positive result for the biuret test, as they contain enzymes, which are proteins.
- The first and last reaction mixtures would give a positive result for the Benedict's test, because they contain reducing sugars which are produced as a result of the hydrolysis of sucrose or starch, respectively.
- Only the reaction mixtures that contain starch would give a positive result for the iodine test.

1. A student made the following observations when they tested a sample for three biological molecules:
- orange–brown colour when iodine–potassium iodide solution is added
- pale purple colour when biuret solution is added
- cloudy emulsion when shaken with ethanol and mixed with water.

Identify which types of macromolecule are present in the sample.

2. Boiling a disaccharide with acid hydrolyses it into monosaccharides. Explain why the Benedict's test gives a positive result after, but not before, sucrose has been heated strongly with acid. Use the information in the Worked Example to help you.

★ **3.** A scientist investigated the glucose content of a range of soft drinks to compare which had the greatest concentration of this sugar. Based on the results of the investigation, the scientist ranked the fruit juices in order, with fruit juice 1 having the highest glucose concentration, and fruit juice 5 having the lowest glucose concentration. The results are shown in Table 2.5.

Fruit juice brand	Observation with Benedict's test	Rank
A	changed from blue to orange	2
B	changed from blue to red	1
C	changed from blue to yellow	3
D	no change – remained blue	5
E	changed from blue to green	4

Table 2.5 The results of an investigation into the glucose concentration of five samples of fruit juice.

a. Suggest how the scientist was able to rank the fruit juices in order of reducing sugar concentration.

b. Describe **three** factors that must be standardised in order to make a valid comparison of the glucose concentrations of the soft drinks.

c. Suggest why estimating the concentration of glucose in soft drinks might give an underestimate of their sugar content.

Experimental skills 2.1: Diagnosing diabetes

Diabetes is a disorder in which a person cannot properly control the concentration of glucose in their bloodstream. One symptom of diabetes is the passing of urine containing glucose (Figure 2.6). Glucose is not found in the urine of a healthy person.

A scientist wanted to use Benedict's solution to develop a quick test to diagnose diabetes.

The scientist made **serial dilutions** of a 1% glucose solution. This reduces the concentration by half between each successive dilution. The scientist wanted to make 10 cm^3 of each concentration.

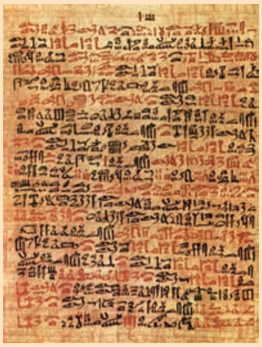

Figure 2.6 The first treatment for diabetes was recorded in Ancient Egypt on the Apers Papyrus. At around the same time, Indian physicians described how the disease led to *madhumeha*, or 'honey urine', noting that it would attract ants.

QUESTIONS

P1. With reference to Figure 2.7, show how the scientist should produce three more solutions of glucose. The values for volume that you choose should produce solutions of the concentrations shown.

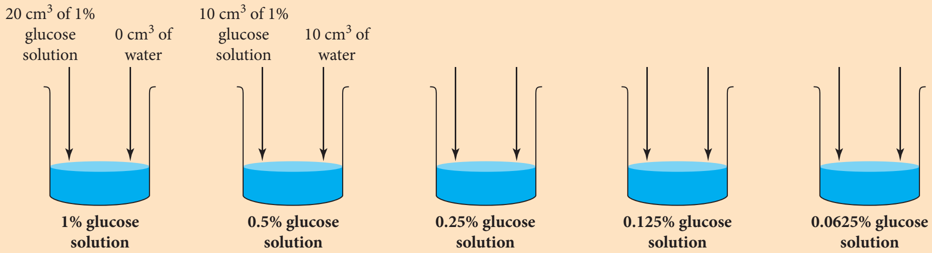

Figure 2.7 The preparation of serial dilutions using a 1% glucose solution and distilled water.

To measure the volumes of glucose solution and distilled water in their serial dilutions, the scientist used a 10 cm^3 syringe, shown in Figure 2.8.

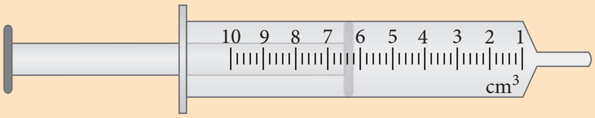

Figure 2.8 The 10 cm^3 syringe used in this investigation.