Biologically Important Molecules
Carbohydrates, Proteins, Lipids, and Nucleic Acids

Objectives

By the end of this exercise you should be able to:

1. Correctly select and perform tests to detect the presence of carbohydrates, lipids, proteins, and nucleic acids.
2. Explain the importance of a control in biochemical tests.
3. Use biochemical tests to identify an unknown compound.

Most organic compounds in living organisms are carbohydrates, proteins, lipids, or nucleic acids. Each of these macromolecules is made of smaller subunits. These subunits of macromolecules are held together by covalent bonds and have different structures and properties. For example, lipids (made of fatty acids) have many C-H bonds and relatively little oxygen, while proteins (made of amino acids) have amino groups (—NH₃⁺) and carboxyl (—COOH) groups. These characteristic subunits and groups impart different chemical properties to macromolecules—for example, monosaccharides such as glucose are polar and soluble in water, whereas lipids are nonpolar and insoluble in water.

Controlled Experiments to Identify Organic Compounds

Scientists have devised several biochemical tests to identify the major types of organic compounds in living organisms. Each of these tests involves two or more treatments: 1) an unknown solution to be identified, and 2) controls to provide standards for comparison. As its name implies, an unknown solution may or may not contain the substance that the investigator is trying to detect. Only a carefully conducted experiment will reveal its contents. In contrast, controls are known solutions. We use controls to validate that our procedure is detecting what we expect it to detect and nothing more. During the experiment we compare the unknown solution’s response to the experimental procedure with the control’s response to that same procedure.

A positive control contains the variable for which you are testing; it reacts positively and demonstrates the test’s ability to detect what you expect. For example, if you are testing for protein in unknown solutions, then an appropriate positive control is a solution known to contain protein. A positive reaction shows that your test reacts correctly; it also shows you what a positive test looks like.

A negative control does not contain the variable for which you are searching. It contains only the solvent (often distilled water with no solute) and does not react in the test. A negative control shows you what a negative result looks like.

Controls are important because they reveal the specificity of a particular test. For example, if water and a glucose solution react similarly in a particular test, the test cannot distinguish water from glucose. But if the glucose solution reacts differently from distilled water, the test can distinguish water from glucose. In this instance, the distilled water is a negative control for the test, and a known glucose solution is a positive control.

Carbohydrates

Benedict’s Test for Reducing Sugars

Carbohydrates are molecules made of C, H, and O in a ratio of 1:2:1 (e.g., the chemical formula for glucose is C₆H₁₂O₆). Carbohydrates are made of monosaccharides, or simple sugars (fig. 5.1). Paired monosaccharides
Carbohydrates consist of subunits of mono- or disaccharides. These subunits can be combined by dehydration synthesis (see fig. 5.3) to form polysaccharides.

Plant cell walls are made of cellulose arranged in fibrils and microfibrils. The scanning electron micrograph shows the fibrils in a cell wall of the green alga Chaetomorpha, 30,000X.

Form disaccharides—for example, sucrose is a disaccharide of glucose linked to fructose. Similarly, linking three or more monosaccharides forms a polysaccharide such as starch, glycogen, or cellulose (fig. 5.2).

**Question 1**

Examine figure 5.1. Shade with a pencil the reactive groups of the glucose molecule that are involved in forming a polysaccharide.

The linkage of subunits in carbohydrates, as well as other macromolecules, often involves the removal of a water molecule (dehydration). Examine figure 5.3 to become familiar with the mechanism of synthesis and hydrolysis in carbohydrates.

Many monosaccharides such as glucose and fructose are **reducing sugars**, meaning that they possess free aldehyde (—CHO) or ketone (—C=O) groups that reduce weak oxidizing agents such as the copper in Bene-
FigurE 5.3
Dehydration synthesis and hydrolysis. (a) Many biological molecules are formed by linking subunits. The covalent bond between subunits is formed in dehydration synthesis, a process that eliminates a water molecule. (b) Breaking such a bond requires the addition of a water molecule, a reaction called hydrolysis.

Table 5.1
Solutions and Color Reactions for (1) Benedict’s Test for Reducing Sugars and (2) Iodine Test for Starch

<table>
<thead>
<tr>
<th>Tube</th>
<th>Solution</th>
<th>Benedict’s Color Reaction</th>
<th>Iodine Color Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 drops onion juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10 drops potato juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 drops sucrose solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10 drops glucose solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10 drops distilled water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10 drops reducing-sugar solution</td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td>10 drops starch solution</td>
<td></td>
<td></td>
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<td>8</td>
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<td>9</td>
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</tr>
</tbody>
</table>

Benedict’s reagent. Benedict’s reagent contains cupric (copper) ion complexed with citrate in alkaline solution. Benedict’s test identifies reducing sugars based on their ability to reduce the cupric (Cu$^{2+}$) ions to cuprous oxide at basic (high) pH. Cuprous oxide is green to reddish orange.

Oxidized Benedict’s reagent (Cu$^{2+}$) + Reducing sugar (R-COH)

\[ \text{Heat} \]
\[ \text{High pH} \]

Reduced Benedict’s reagent (Cu$^{+}$) + Oxidized sugar (R-COOH)

A green solution indicates a small amount of reducing sugars, and reddish orange indicates an abundance of reducing sugars. Nonreducing sugars such as sucrose produce no change in color (i.e., the solution remains blue).

Procedure 5.1
Perform the Benedict’s test for reducing sugars
1. Obtain seven test tubes and number them 1–7.
2. Add to each tube the materials to be tested (table 5.1). Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes. Add 2 mL of Benedict’s solution to each tube.
3. Place all of the tubes in a boiling water-bath for 3 min and observe color changes during this time.
4. After 3 min, remove the tubes from the water-bath and let them cool to room temperature. Record the color of their contents in table 5.1.
Question 2
a. Which is a reducing sugar, sucrose or glucose?

b. Which contains more reducing sugars, potato juice or onion juice?

c. What does this tell you about how sugars are stored in onions and potatoes?

d. Which of the solutions is a positive control? Negative control?

Iodine Test for Starch
Staining by iodine (iodine-potassium iodide, I₂KI) distinguishes starch from monosaccharides, disaccharides, and other polysaccharides. The basis for this test is that starch is a coiled polymer of glucose; iodine interacts with these coiled molecules and becomes bluish black. Iodine does not react with carbohydrates that are not coiled and remains yellowish brown. Therefore, a bluish-black color is a positive test for starch, and a yellowish-brown color (i.e., no color change) is a negative test for starch. Notably, glycogen, a common polysaccharide in animals, has a slightly different structure than does starch and produces only an intermediate color reaction.

Procedure 5.2
Perform the iodine test for starch
1. Obtain seven test tubes and number them 1-7.
2. Add to each tube the materials to be tested (table 5.1). Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
3. Add three to five drops of iodine to each tube.
4. Record the color of the tubes' contents in table 5.1.

Question 3
a. Which colors more intensely, onion juice or potato juice?

b. What does this tell you about how these plants store carbohydrates?

c. Which of the solutions is a positive control?

Proteins
Proteins are remarkably versatile structural molecules found in all life-forms (fig. 5.4). Proteins are made of amino acids (fig. 5.5), each of which has an amino group (\(\text{NH}_3^+\)) and a carboxyl (acid) group (—COOH). The bond between these two groups found on adjacent amino acids in a protein is a peptide bond (fig. 5.6) and is identified by a Biuret test. Specifically, peptide bonds (C—N bonds) in proteins complex with Cu²⁺ in Biuret reagent and produce a violet color. A Cu²⁺ must complex with four to six peptide bonds to produce a color; therefore, free amino acids do not react positively. Long-chain polypeptides (proteins) have many peptide bonds and produce a positive reaction.

Biuret reagent is a 1% solution of CuSO₄ (copper sulfate). A violet color is a positive test for the presence of protein; the intensity of color relates to the number of peptide that react.

Question 4
Examine figure 5.5. Shade with a pencil the reactive amino and carboxyl groups on the three common amino acids shown.

Procedure 5.3
Perform the Biuret test for protein
1. Obtain five test tubes and number them 1-5.
2. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
3. Add the materials listed in table 5.2.
4. Add 2 mL of 2.5% sodium hydroxide (NaOH) to each tube.
**Figure 5.4**

Common structural proteins. (a) Fibrin. This electron micrograph shows a red blood cell caught in threads of fibrin. Fibrin is important in the formation of blood clots. (b) Collagen. The so-called “cat-gut” strings of a tennis racket are made of collagen. (c) Keratin. This type of protein makes up bird feathers, such as this peacock feather. (d) Spider silk. The web spun by this agile spider is made of protein. (e) Hair. Hair is also a protein.

**Figure 5.5**

Structures of three amino acids that are common in proteins. Each amino acid has one carbon that is bonded to both an amine group ($\text{H}_2\text{N}^+$) and a carboxyl group (−$\text{COO}^-$).
Table 5.2: Solutions and Color Reactions for the Biuret Test for Protein

<table>
<thead>
<tr>
<th>Tube</th>
<th>Solution</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 mL egg albumen</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 mL honey</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 mL amino acid solution</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2 mL distilled water</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2 mL protein solution</td>
<td></td>
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<tr>
<td>6</td>
<td></td>
<td></td>
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<tr>
<td>7</td>
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</tbody>
</table>

**Figure 5.6**
A peptide bond joins two amino acids, and peptide bonds link many amino acids to form polypeptides or proteins. The formation of a peptide bond liberates a water molecule.

**Question 5**

a. Which contains more protein (C—N bonds), egg albumen or honey?

b. Do free amino acids have peptide bonds?

**Lipids**

Lipids include a variety of molecules that dissolve in nonpolar solvents such as ether and acetone, but not in polar solvents such as water. Triglycerides (fats) are abundant lipids that are made of glycerol and three fatty acids (fig. 5.7). Tests for lipids are based on a lipid's ability to selectively absorb pigments in fat-soluble dyes such as Sudan IV.

**Question 6**

Examine figure 5.7. What are the reactive groups of the fatty acids?

**Procedure 5.4**

Perform the Sudan IV test for lipid

1. Obtain five test tubes and number them 1–5. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
2. Add the materials listed in table 5.3.
Figure 5.7

The structure of a fat includes glycerol and fatty acids. (a) An ester linkage forms when the carboxyl group of a fatty acid links to the hydroxyl group of glycerol, with the removal of a water molecule. (b) Fats are triacylglycerides whose fatty acids vary in length and vary in the presence and location of carbon–carbon double bonds.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Solution</th>
<th>Description of Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 mL salad oil + water</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 mL salad oil + Sudan IV</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 mL honey + Sudan IV</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1 mL distilled water + Sudan IV</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1 mL known lipid solution + Sudan IV</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
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<tr>
<td>7</td>
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</tbody>
</table>
3. Add 3 mL of water to each tube.
4. Add five drops of water to tube 1 and five drops of Sudan IV to each of the remaining tubes. Mix the contents of each tube. Record the color of the tubes’ contents in Table 5.3.

**Question 7**

a. Is salad oil soluble in water?

b. Compare tubes 1 and 2. What is the distribution of the dye with respect to the separated water and oil?

c. What observation indicates a positive test for lipid?

d. Does honey contain much lipid?

e. Lipids supply more than twice as many calories per gram as do carbohydrates. Based on your results, which contains more calories, oil or honey?

**Grease-spot Test for Lipids**

A simpler test for lipids is based on their ability to produce translucent grease-marks on unglazed paper.

**Procedure 5.5**

Perform the grease-spot test for lipids

1. Obtain a piece of brown wrapping paper from your lab instructor.
2. Use an eyedropper to add a drop of salad oil near a corner of the piece of paper.
3. Add a drop of water near the opposite corner of the paper.
4. Let the fluids evaporate.
5. Look at the paper as you hold it up to a light.
6. Test other food products and solutions available in the lab in a similar way and record your results in Table 5.4.

**Question 8**

Which of the food products that you tested contain large amounts of lipid?

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**Table 5.4**

<table>
<thead>
<tr>
<th>Food Product</th>
<th>Description of Grease-spot Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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<td>5</td>
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<td>6</td>
<td></td>
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</tbody>
</table>

5–8

48 **Exercise 5**
<table>
<thead>
<tr>
<th>Biochemical Test</th>
<th>Color</th>
<th></th>
<th>Unknown Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample</td>
<td>Control</td>
<td>(+/-)</td>
</tr>
<tr>
<td>Benedict's test (reducing sugars)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine (starch)</td>
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<td></td>
<td></td>
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<tr>
<td>Biuret test (protein)</td>
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<tr>
<td>Dische diphenylamine test (DNA)</td>
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<tr>
<td>Sudan IV (lipid)</td>
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</tbody>
</table>

Report: Identity of Unknown
Indicate which of the following are in your unknown:
- Reducing sugars
- Starch
- Lipid
- DNA
- Protein

Comments: