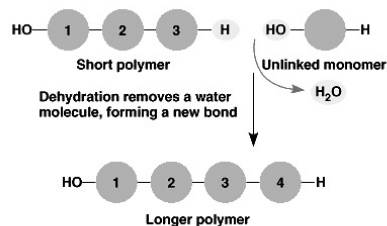


BIO 3A LABORATORY Biologically Important Molecules

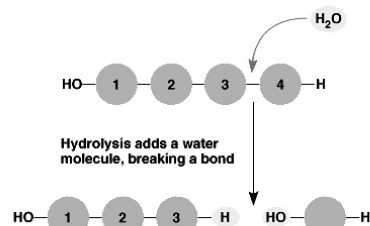
Carbohydrates, Lipids, Proteins and Nucleic Acids

Objectives

- To perform tests that detect the presence of carbohydrates, lipids, proteins, and nucleic acids in known and unknown samples
- To recognize the importance of a control in a biochemical test
- To use biochemical tests to identify an unknown compound



(a) Dehydration reaction in the synthesis of a polymer



(b) Hydrolysis of a polymer

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Figure 1. Dehydration synthesis and hydrolysis of a polymer.

Methods for Identifying Organic Compounds

There are several chemical tests available for the identification of the major types of organic compounds in living organisms. Typically these tests are used to determine the makeup of an unknown material. For instance, a forensic detective may be interested identifying a fluid found at a crime scene. The collected fluid is the *unknown*. As the tests are carried out, the detective will also use known compounds, or *controls*, for comparison. During the experiment the detective compares the unknown's response to the experimental procedure with the control's response to that same procedure.

Introduction

Organic molecules are those primarily made up of carbon, hydrogen and oxygen. The common organic compounds of living organisms are carbohydrates, proteins, lipids, and nucleic acids. Each of these macromolecules (polymers) are made of smaller subunits (monomers). The bonds between these subunits are formed by **dehydration synthesis**. This process requires energy; a molecule of water is removed (dehydration) and a covalent bond is formed between the subunits (Fig.1). Breaking this bond is called hydrolysis; it requires the addition of a water molecule and releases energy.

Each class of these macromolecules has different structures and properties. For example, lipids (composed of fatty acids) have many C-H bonds and relatively little oxygen, while proteins (composed of amino acids) have amino groups (-NH₃⁺) and carboxyl (-COOH) groups. These characteristic subunits and chemical groups impart different properties to the macromolecules. For example, monosaccharides such as glucose are polar and soluble in water, whereas lipids are non-polar and insoluble in water.

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.

A positive control contains the variable for which you are testing. It produces a positive reaction and demonstrates the test's ability to detect what you expect. A positive reaction to a positive control demonstrates that your test reacts correctly.

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 demonstrates what a negative result looks like.

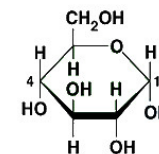
There are literally hundreds of tests available for biological molecules. In today's lab we will be using some of the more common methods to look for the presence of simple (or reducing sugars), starch, protein, lipid, and DNA.

Testing For Carbohydrates:

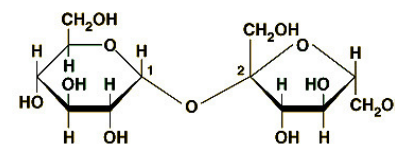
Simple Sugars

Carbohydrates are molecules made up of carbon (C), hydrogen (H), and oxygen (O) in a ratio of 1:2:1 (for example, the chemical formula for glucose is C₆H₁₂O₆). Carbohydrates may be monosaccharides, or simple sugars, such as glucose or fructose; they may be disaccharides, paired monosaccharides, such as sucrose (a disaccharide of glucose linked to fructose, Fig. 2); or they may be polysaccharides, where three or more monosaccharides form chains, such as starch, glycogen, or cellulose (Fig. 3).

As already mentioned, the linkage of subunits in carbohydrates, as well as other macromolecules, involves the removal of a water molecule (dehydration). Figure 4 depicts how dehydration synthesis is used to make maltose and sucrose, two common disaccharides.

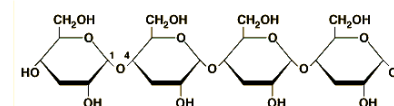


GLUCOSE

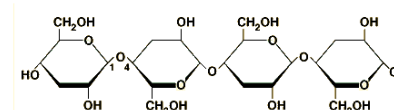


SUCROSE

Figure 2. Glucose, a monosaccharide and Sucrose, a disaccharide.



(b) Starch: 1-4 linkage of α glucose monomers



(c) Cellulose: 1-4 linkage of β glucose monomers

Figure 3. Polymers of glucose: starch and cellulose

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 Benedict's reagent. Benedict's reagent contains cupric (copper) ion complexes with citrate in alkaline solution. Benedict's test identifies reducing sugars based on their ability to reduce the cupric (Cu²⁺) ions to cuprous oxide at basic (high) pH. Cuprous oxide is green to reddish orange.

In this test, a green solution indicates a small amount of reducing sugars, and reddish orange

indicates an abundance of reducing sugars. Non-reducing sugars, such as sucrose produce no change in color (the solution remains blue).

Procedure 1. 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 (see Table 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 and mix.
3. Place all of the tubes in a boiling water-bath for three minutes and observe color changes during this time.
4. After 3 minutes, remove the tubes from the water-bath and let them cool to room temperature. Record the color of each test tube in Table 1.

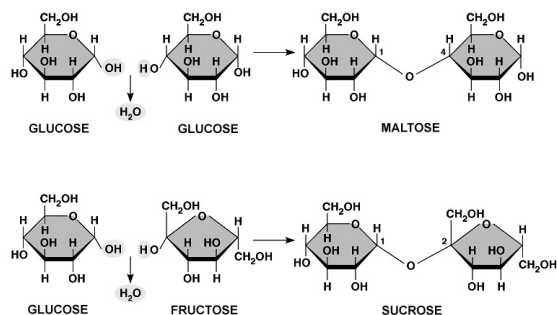


Figure 4. Dehydration synthesis of monosaccharides into disaccharides

Starches

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 2: 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 as shown in Table 1 on your worksheet. 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 and mix.
4. Record the color of the tubes' contents in Table 1.

Testing for Proteins

Proteins are remarkably versatile structural molecules found in all life-forms. Proteins are composed of amino acids (Fig. 5), each of which has an amino group (NH₃⁺) and a carboxyl group

(-COOH). The amino group on one amino acid is linked to the carboxyl group on an adjacent amino acid by a peptide bond. The peptide bond forms through dehydration synthesis (Fig. 6). This bond is the site of action for the Biuret test for protein. Biuret reagent is a 1% solution of CuSO₄ (copper sulfate). In this test, Cu²⁺ complexes with the peptide bond producing a violet color. The violet color is a positive test for the presence of protein; the intensity of color relates to the number of peptide bonds that react. A Cu²⁺ must complex with at least four to six peptide bonds to produce a color; therefore, free amino acids and very short chains do not react positively. However, free amino acids and very short chains may result in a pinkish color. Long-chain polypeptides (proteins) have many peptide bonds and produce a positive reaction.

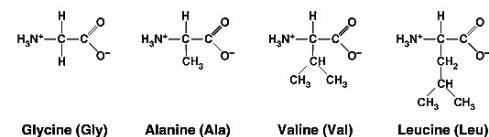


Figure 5. Structure of four common amino acids

Procedure 3: The Biuret Test for Protein

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. To each test tube add the materials listed in Table 2.
3. Add 2 ml of 2.5% sodium hydroxide (NaOH) to each tube.
4. Add three drops of Biuret reagent to each tube and mix.
5. Record the color of the tubes' contents in Table 2 on your worksheet.

CAUTION: Do not spill the NaOH-it is extremely caustic.

Testing for Lipids

Lipids include a variety of molecules that dissolve in solvents such as ether and acetone, but not in polar solvents such as water. The primary component of fat or lipid is the fatty acid. When it occurs alone it is called a free fatty acid. This molecule is composed of an even number of carbon atoms terminated in a carboxyl group (-COOH). Fatty acids are most often stored and transported as triglycerides, three fatty acids bonded to a glycerol molecule. This bond is called an ester linkage and resulted from a dehydration synthesis (Fig. 7). Tests for lipids are based on a lipid's ability to selectively absorb pigments in fat-soluble dyes such as Sudan IV.

Procedure 4: Solubility of Lipids in Polar and Non-polar Solvents: Instructor will demonstrate this

Obtain two test tubes. To one of the tubes, add 5 ml of water. To the other tube, add 5 ml of petroleum ether.

1. Add a few drops of vegetable oil to each tube.

CAUTION: Handle pet. ether carefully, it is toxic. Your instructor will demonstrate this procedure

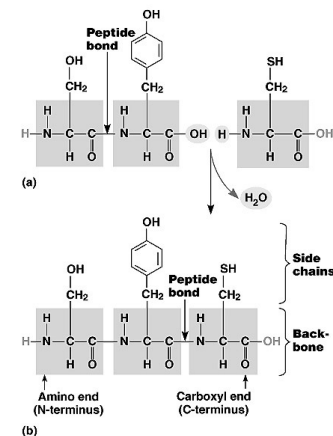
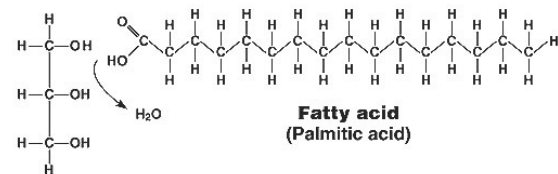


Figure 6. Dehydration synthesis of a peptide bond

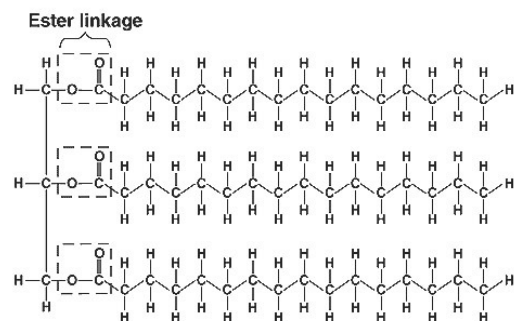
Procedure 5: The Sudan IV Test for Lipid

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. To each tube, add the materials listed in Table 3.
3. 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 3 on your worksheet.



Glycerol

(a) Dehydration synthesis



(b) Fat molecule (triacylglycerol)

Figure 7. a. Dehydration synthesis of ester linkage between free fatty acid and glycerol
b. Triglyceride.

Procedure 6: The Grease-spot Test for Lipids

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

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 4.

Testing for Nucleic Acids

DNA and RNA are nucleic acids made of nucleotide subunits (Fig. 8). One major difference between DNA and RNA is their sugar: DNA contains deoxyribose, whereas RNA contains ribose. DNA can be identified chemically with the Dische diphenylamine test. In this test, acidic conditions convert deoxyribose to a molecule that binds with diphenylamine to form a blue complex. The intensity of the blue color is proportional to the concentration of DNA.

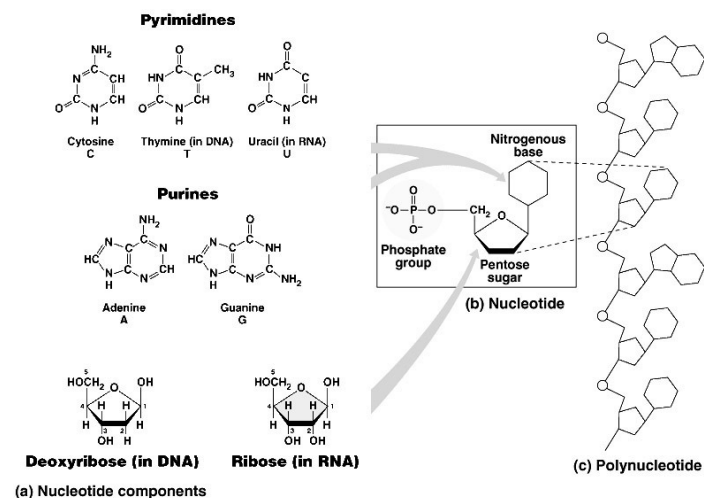


Figure 8. Structure of Nucleic acids

Procedure 7: The Dische diphenylamine test for DNA

1. Obtain four test tubes and number them 1-4. 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. Add 2 ml of the Dische diphenylamine reagent to each tube and mix thoroughly.
4. Place the tubes in a boiling water-bath to speed the reaction.
5. After 10 min, transfer the tubes to an ice bath. Gently mix and observe the color of their contents as the tubes cool. Record your observations in Table 5.

CAUTION
Handle the Dische diphenylamine reagent carefully; it is toxic.

Saddleback CSI

So now it's your turn to try your hand at identification of unknown. The Saddleback CSI team has returned with several unknowns. These fluids were collected in the food preparation area of the cafeteria where a crime has taken place. The team wants to know what these fluids are. Can you help out the team using the previously described tests?

Procedure 7:

- a. Obtain one of the unknown solutions from your laboratory instructor. Record its number in Table 6.
- b. Obtain ten clean test tubes.
- c. Label five of the tubes for the sample as S1-S5. Label the other five tubes as controls C1-C5.
- d. Place 2 ml of your unknown solution into each of tubes S1-S5.
- e. Place 2 ml of distilled water into each of tubes C1-C5. Use the procedures shown in Table 6 to detect reducing sugars, starch, protein, lipid, and DNA in your unknown.
- f. Your unknown may contain one, none, or several of these macromolecules. Record your results in Table 6.
- g. Show Table 6 to your instructor. He will give you a list of possible materials for your unknown.
- h. In the report section please write a brief report for the CSI team, indicating what your testing indicated and what you think the fluid may be. Do not forget to include why and how you eliminated or confirmed the particular item. Most good CSI reports are about half a paged typed.