Organic Chemistry:
Chemical Identification of Biomolecules
Student Guide

INTRODUCTION

Organic chemistry is a branch of chemistry that studies chemical compounds composed primarily of carbon. These compounds may contain other chemical elements (e.g. oxygen or nitrogen) but are at the base level primarily composed of carbon. As this particular field of chemistry began to emerge, it was thought that these compounds were always related to the formation and sustaining of life. As knowledge of the science grew, it was soon shown that this was a misconception. Not only was it discovered that some organic compounds were not necessarily life sustaining, but also that some inorganic substances were.

In its infancy, there was not a great deal of interest in organic chemistry, primarily for two major reasons. The first was that many of the prominent chemists at the time thought that organic substances were far too complex to understand; that being part of a living system they were the result of some sort of force (termed "vital force") that people couldn't understand. The second was, quite frankly, that there did not appear to be profit or financial gain working with these substances. To make a long story short, both of these misperceptions were shown to be false. Chemists demonstrated that it was possible to synthesize some of the compounds in the lab, without any kind of "vital force," and also that this field could generate some commercially-important materials.

The increased interest in organic chemistry also led to a deeper understanding of not only the compounds involved but the way they sometimes interacted with one another. Early in the twentieth century, some scientists began studying the organic chemistry specific to living organisms. More than merely studying the structure of the compounds, scientists began to investigate the purpose, the interactions, the changes, the cycles, etc. that these chemicals served in not only the sustenance but also the propagation of life. This sub-branch of organic chemistry came to be known as biochemistry.

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The Building Blocks: Common Biomolecules

A living organism can be incredibly complex. Even in what is considered a "simple" organism (a single-celled bacterium for example), a complex series of biochemical reactions are constantly in process, all driving the machinery that makes life possible. Every chemical, every reaction is important. Enzymes, sterols, nucleic acids, to name a few, are constantly interacting to drive life. Some compounds are more common and may be thought of as the "basic building blocks" of a living organism. These include proteins, carbohydrates, and lipids.

Proteins

Of all the biomolecules, proteins are arguably the most important. Again, all biomolecules are important but different proteins form the basis for different tissues. Proteins also are the basis for another special class of biomolecules called enzymes. Enzymes are substances that allow biochemical reactions to occur much faster than they ordinarily would. If left alone, these reactions may occur too slowly to sustain life but in the presence of an enzyme, they may occur hundreds, thousands, or even tens of thousands of times faster.

Proteins are found in an abundance of varieties. The many types vary in size, shape, structure, etc. Some scientists spend their entire lives studying nothing but proteins. Proteins are polymers. Polymer is a term for large molecules, composed of several smaller, similar molecules (called monomers). The word polymer is from the Greek polu – meaning many; and meros – meaning part. The Greek mono means one, so each monomer (one part) makes up one piece of the polymer (many parts). The monomers in this case are amino acids. A protein is made up of a variety of linked amino acids. A protein may be as small as two amino acids or may consist of tens of thousands of amino acids. Depending not only on which amino acids form the protein, but also the order of the amino acids in the chain is what helps determine the size, shape, and function of the protein.

Organisms can make many amino acids. However, there are several that can't be synthesized. These are called essential amino acids. They are necessary to sustain life and since they cannot be manufactured, they must be taken in from outside sources in order for the formation of vital proteins. In humans, for example, these amino acids are provided by eating protein-containing foods. The proteins are digested (broken down) into amino acid components that can then be used for the formation of different proteins.

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Carbohydrates

While it can be argued that proteins are the most important biomolecules, carbohydrates are the most abundant. Carbohydrates are also referred to as saccharides (from the Greek for sugar) and can be (but are not always) polymers as well. They can be rather large molecules like proteins but are generally much simpler in terms of form and structure. Carbohydrates may also be involved in the formation of structural components within an organism (especially in plants) but a large part of their function is in the generation and storage of energy.

The saccharides are the parts that make up the large polymer carbohydrates. A simple sugar molecule is referred to as a monosaccharide (one sugar) and larger molecules are referred to as polysaccharides (many sugars). Some sugars are only made of two monosaccharides and are called disaccharides (di is the prefix for two). Common table sugar, known as sucrose, is an example of a disaccharide. Of the larger polysaccharides, the two most common are starch and cellulose (found in plants).

Again, while serving a structural role in some cases, the big benefit of carbohydrates is the production and storage of energy. Many plants, for example, may produce more monosaccharides than they immediately need. The primary monosaccharide for plants is called glucose. When a plant has too much glucose, it will form a polysaccharide out of the excess molecules. The glucose molecules are linked together with chemical bonds. These chemical bonds have energy stored in them. If a human ingests these polysaccharides by eating plant material, the body will break the chemical bonds in the polysaccharide which will release and provide energy for a variety of other biochemical processes.

Lipids

Lipids are a broad category of biomolecules that cover many different types. The unifying feature of lipids is that they are hydrophobic. A hydrophobic substance is a substance that is repelled by water. An example of this can be seen by examining a mixture of oil (a lipid) and water. If added to water, the oil clumps together and forms distinct drops, or "blobs," of oil in the water. This is because the oil molecules are avoiding the water and all tend to be forced together attempting to do so. Lipids are often associated with fats. While this is true, fats are one group of lipids, there are actually many other types as well. Examples include the aforementioned oils and fats, as well as phospholipids (important in cell walls), waxes, and steroids (such as cholesterol).

Lipids serve a structural function in living organisms, primarily in the walls of cells. They also serve a role with regards to energy. When the chemical bonds that form lipids are broken down, they release more energy than either proteins or carbohydrates (over twice as much in fact). This makes lipids the most efficient method for storing energy in reserve.

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Using humans as an example again, lipids may often be ingested from outside sources. A great deal of attention has been given as of late to the negative effects of some lipids, primarily fats and cholesterol. While it is true that an overabundance of these types of lipids can cause significant problems, a certain amount is necessary. Cholesterol, a form of steroid, is absolutely vital. While it is true that too much can cause problems, too little can cause problems as well. Cholesterol aids in the formation and use of certain vitamins as well as certain hormones.

In plants, lipids serve a major role in the retention of water. A lipid layer (in the form of wax) often coats the leaves of a plant; helping to prevent water from evaporating out of the plant through the leaves. Plants do not contain the degree of fats found in animals. Animals are capable of movement, which requires a great deal of energy. Storage of energy in the form of fat can be very helpful as a source of this energy. Plants do not move and therefore do not require the added energy reserves of fats. Plants tend to obtain enough energy from other biomolecules.

Testing for Common Biomolecules

There are several ways to test for the presence of any of these biomolecules; proteins, carbohydrates, and lipids. One easy and interesting method is the use of chemical indicators. Chemical indicators are chemicals that may display a certain observable change when exposed to another chemical. Most commonly the change is in the color of the indicator but there may be other ways to observe, such as the formation of a precipitate for example.

Biuret reagent – biuret reagent is a chemical that can be used to detect proteins. It is a light blue solution that when exposed to proteins will turn violet to deep blue/violet.

Benedict's solution – Benedict's solution is a light blue solution. When combined with a monosaccharide (and most disaccharides) and heated, Benedict's reagent will change color. The color may vary and often a red precipitate is formed after heating. A green to yellow color indicates the presence of a small amount of saccharides and an orange to red color indicates the presence of more saccharides.

Iodine/potassium iodide (IKI) – Iodine/potassium iodide can be used to test for the polysaccharide starch. A yellow/brown solution, IKI will turn deep blue to black when exposed to starch.

Sudan III – Sudan III is a fat-soluble dye. If combined with a substance containing lipids, the dye will accumulate in the lipids, staining the lipids red.

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Procedure

Materials Needed per Group

4 Test tubes
Glass stirring rod

Shared Materials

Iodine/potassium iodide solution
Biuret reagent
Benedict's solution
Sudan III stain
Vegetable oil
Starch solution
Albumin solution
Glucose solution
Distilled or deionized water
Hot water bath
Assorted food items

Safety

Gloves
Goggles
Lab apron

Part I: Testing for Proteins, Starches, Sugars, Lipids

Testing for Proteins

1. Place two test tubes in a test tube rack.
2. To the first test tube, add approximately 5ml of albumin solution. To the second tube, add the same volume of distilled water.
3. Add 5 drops of biuret reagent to both test tubes.
4. Observe the contents of both tubes. Record your observations in the Data Analysis section of the lab.
5. Wash both test tubes with soap and water. Rinse thoroughly and completely dry before proceeding.

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Testing for Starches

1. Place two test tubes in a test tube rack.
2. To the first test tube, add approximately 5ml of starch solution. To the second tube, add the same volume of distilled water.
3. Add 5 drops of iodine/potassium iodide reagent to both test tubes.
4. Observe the contents of both tubes. Record your observations in the Data Analysis section.
5. Wash both test tubes with soap and water. Rinse thoroughly and completely dry before proceeding.

Testing for Sugars

1. If a hot water bath has already been set up by your instructor, proceed to step #3. If not, continue with the next step.
2. Place a large beaker of water on a hot plate. Be sure the level of water is low enough so that when tubes are placed in the water bath, water will not enter the top of the tubes. Heat the water until the temperature of the water is between 75-80°C. Adjust the heat on the hot plate to hold this temperature range.
3. Place two test tubes in a test tube rack.
4. To the first test tube, add approximately 5ml of glucose solution. To the second tube, add the same volume of distilled water.
5. Add 5 drops of Benedict's solution reagent to both test tubes.
6. Using test tube holders, place both tubes in the hot water bath. Be careful when working with the hot water bath. Place the tubes gently in the water to avoid splashing of the hot water.
7. Heat the tubes in the hot water bath for about 5 minutes. After this time, carefully remove the tubes from the water bath using a test tube holder. Place the tubes in a test tube rack to cool.
8. Observe the contents of both tubes. Record your observations in the Data Analysis section.
9. Once completely cool, wash both test tubes with soap and water. Rinse thoroughly and completely dry before proceeding.

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Testing for Lipids

1. Place two test tubes in a test tube rack.
2. To both test tubes, add approximately 5ml of distilled water.
3. Add 2 drops of Sudan III stain to both test tubes.
4. Add approximately 1-2ml of vegetable oil to the first tube. Cover the tops of both tubes with Parafilm®, or a similar item such as plastic wrap, and shake each tube vigorously for a few seconds. Place the tubes back in the test tube rack after shaking.
5. Allow both tubes to sit a couple of minutes. After a couple of minutes, observe the contents of the tubes and record your results in the Data Analysis section.
6. Wash both test tubes with soap and water. Rinse thoroughly and completely dry before proceeding.

Part II: Food Testing

1. Select a food item to test. Place a small amount of the food item in each of four test tubes in a test tube rack. Depending on the food item selected, prepare it for testing as follows:

    - If the item is a liquid (e.g. fruit juice), add about 1ml of the liquid to each tube and dilute to approximately 5ml with distilled water.
    - If the item is a solid (e.g. crackers or vegetable matter), place a small piece of the item in each test tube and add about 5ml of distilled water. Using a glass stirring rod, or similar, macerate the item in the water to release the nutrients into the liquid.

2. Using biuret reagent, repeat the protein testing procedure for testing for proteins in the first test tube. Record your results in the Data Analysis section of the lab before continuing.
3. Repeat the remaining three tests as you did in the first part of the lab using test tubes 2-4. Be sure to record your results in the Data Analysis section after each test.
4. If time permits, select a different food item and repeat the testing procedure. Test as many items as your instructor tells you.
5. Clean up and dispose of all materials according to your instructor. Wash hands with soap and water before leaving the lab.

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DATA ANALYSIS

PART 1: Testing Known Solutions

	SOLUTION	DISTILLED WATER
Biuret Reagent		
Iodine/ Potassium Iodide		
Benedict's Solution		
Sudan III Stain		

PART 2: Testing Unknown Food Items

Food Item	Protein (+/-)	Starch (+/-)	Sugar (+/-)	Lipid (+/-)

Conclusion: (Discuss results, importance of knowing presence/ absence of the 4 biomolecules in food items, etc)