

BIOS 100 Biomolecules

Living systems are composed of millions of different types of molecules, each with their own distinct chemical composition, shape, and function. The vast majority of these molecules can be classified into four major groupings: lipids, proteins, carbohydrates, and nucleic acids. Proteins, carbohydrates, and nucleic acids are all examples of *polymers* - large molecules composed of similar subunits (monomers) covalently bonded together in a long chain. In contrast, lipids, while they can aggregate together as in your cell membranes, do not covalently bond, and therefore are not polymers. The properties of these four important classes of biomolecules are outlined below.

Carbohydrates

Carbohydrates are the most abundant biomolecule in nature. Carbohydrates are molecules composed of C, H, and O in a ratio of 1:2:1. The monomeric constituent of a carbohydrate is called a *monosaccharide*. The principle functions of carbohydrates include: cellular fuel, energy storage, and structure. Two well-known examples of monosaccharides are glucose and fructose. Sucrose, another common sugar, is a disaccharide composed of one glucose and one fructose. Examples of long carbohydrate polymers include starch, the photosynthetic product of plants, and cellulose, the principle component of wood.

Lipids

Lipids are a diverse group of fatty or oily substances characterized by their insolubility in water and their solubility in fat solvents (e.g. ether, acetone, carbon tetrachloride, etc.) Like carbohydrates, lipids are molecules composed of C, H, and O, although they contain much more C and H relative to O. This high ratio of C and H relative to O accounts for the high energy output upon oxidation of lipids. Lipids form the major component of the plasma membrane and are involved in cellular communication.

Proteins

Proteins are large molecules ranging in molecular weight from about 5000 kiloDaltons (e.g. insulin) to 40 million kiloDaltons (e.g. tobacco mosaic virus protein). A protein is a polymer composed of a long chain of nitrogen-containing monomers known as *amino acids* linked together by *peptide bonds*. Proteins often bond with other complex molecules such as lipids, carbohydrates, nucleic acids, and heme groups to form more complex structures. Proteins exhibit a virtually unlimited variety of sizes and configurations due to:

1. The large number of amino acids that enter into the manufacture of a single protein molecule (thousands in many cases).
2. The almost infinite number of combinations the different amino acids can form.
3. The reactivity of side groups of the individual amino acids.

Proteins are unparalleled in the diversity of roles assumed in the maintenance of biological systems. They constitute up to 75% of the dry weight of cells and are a large component of the plasma membrane and organelles. In addition to their role in cell structure, proteins act as biological catalysts (enzymes), regulators of cell and tissue functions, and are involved in the immune response.

Nucleic Acids

Nucleic acids are linear polymers of nucleotides which function in the storage, utilization, and transmission of genetic information. There are two types of nucleic acids: *DNA* (deoxyribonucleic acid) and *RNA* (ribonucleic acid). DNA molecules encode the "blueprints" for the manufacture of proteins. They are also involved in the continuance of genetic information from generation to generation. RNA molecules are involved in the manufacture of proteins through the processes of transcription and translation.

This lab will focus primarily on proteins and carbohydrates

A. Lugol Test for Starch

Lugol solution is an indicator solution for starch. The Iodine in the Lugol's solution forms a complex with starch molecules which is bluish black in color. No change from the reddish-orange color of the Lugol solution is an indication that starch is not present.

1. Obtain two test tubes. Label them "1" and "2"
2. Add 5ml of sucrose solution to test tube "1"
3. Add 5ml of starch solution to test tube "2"
4. Add 5 drops of Lugol solution to each tube
5. Shake and record the results

6. Now take a section of potato and add Lugol solution. Is starch present in the potato? Next try it on an onion. Is starch present in the onion?

WARNING: Lugol solution contains iodine and is very toxic. It can also cause skin burns and permanently stain clothing.

B. Benedict's Test for Reducing Sugars

All monosaccharides as well as some of the disaccharide sugars possess the capacity to reduce alkaline solutions of copper. These monosaccharides and disaccharides are called **reducing sugars**. A reducing sugar is a sugar with actual or potential aldehyde or ketone groups. Benedict's solution is a mixture of CuSO_4 , Na_2CO_3 and sodium citrate. During the reaction, a complex series of reactions occur, but the visible end result is that Cu^{2+} is reduced, causing a color change from blue to brick red (this is the positive result). The solution will remain blue if no reducing sugars are present (negative result).

0. Obtain nine test tubes. Label them "1" through "9"
1. Add 3 ml of water to tube 1. This will be your control
2. Add 3 ml of glucose solution to tube 2
3. Add 3 ml of fructose solution to tube 3
4. Add 3 ml of sucrose solution to tube 4
5. Add 3 ml of starch solution to tube 5
6. Add 3 ml of Sprite[®] into tube 6
7. Add 3 ml of Diet Sprite[®] into tube 7
8. Add 3 ml of milk into tube 8
9. Add 3 ml of fruit juice to tube 9

10. Add 3ml of Benedict's solution to each of the nine tubes.

11. Place all tubes in a block heater for about 5 minutes
WARNING: The block heater is 100°C and can cause severe burns. Be careful!

12. Check for color changes
13. Record your results

WARNING: Benedict's solution contains copper sulfate and is very toxic.

C. Identification of Amino Acids by Thin Layer Chromatography (TLC)

Ninhydrin reacts with amino acids to produce various shades of blue, purple, and yellow pigments. This reagent can be used as a qualitative test for amino acids and proteins. It was once widely used in forensics to detect fingerprints on paper, but has since been replaced by other, less toxic, chemicals.

PLATE PREPARATION

1. Put on gloves and obtain a TLC plate. Do not touch the plate with your bare hands. Do not bend, crease, or scratch the plate.
2. With a pencil, draw a line 1 cm from the bottom of the plate. Do not push hard on the pencil when drawing the line.
3. Make marks 1 cm apart along the pencil line on the bottom of the plate. Be sure that the first and last marks are *at least* 1 cm from the edge of the plate.

SPOTTING

4. Find the first amino acid. Record in your lab book that this amino acid is spotted on mark 1.
5. Dip a capillary tube in the liquid containing the amino acid.
6. While holding the capillary tube perpendicular to the plate, gently touch the tip of the capillary tube on mark 1. Let a small amount of the liquid flow onto the plate. A wet spot should appear.
7. Place the capillary tube back into the side tube and return the bottle to the appropriate station.
8. Repeat steps 4 - 7 for each of the remaining bottles (the known amino acids and one unknown). All of the marks on your plate should have a spot.
9. Let the spots dry well.

RUNNING THE PLATE

10. Gently place the plate inside of the chromatography plate with long tweezers
11. The chromatography chamber holds four plates..
12. Check the plate every few minutes. Be sure to have a pencil ready if you need to remove the plate.
13. Use the long tweezers to remove the plate from the chamber when the solvent has risen to within 1 cm from the top of the plate.
14. Immediately mark the leading edge of the solvent front. If you do not mark the solvent level, your plate will be worthless!
15. Let the plate dry.

DEVELOPING THE PLATE

16. Take the plate to the hood in the lab prep room (the room in the back of the lab).
17. Your TA will place the plates on a hot plate after soaking it with Ninhydrin - a chemical that reacts with amino acids to form a purple color (or yellow with proline).

CALCULATING R_f

18. Mark the center of each of the now visible amino acid spots.
19. Measure (in centimeters) the distance from the spot origin to the leading edge of the solvent.
20. Measure (in centimeters) the distance from the spot origin to the center of the spot.
21. Calculate R_f by dividing the distance traveled by the amino acid by the distance traveled by the solvent.

$$R_f = \frac{\text{Distance traveled by the amino acid}}{\text{Distance traveled by the solvent}}$$

22. Compare the R_f of the known amino acids to the R_f of the unknown.

Lugol Test For Starch

What was the result when you added the Lugol's solution to the following items?

Starch Solution: _____ Sucrose Solution: _____

Potato: _____ Onion: _____

Apple: _____

Benedict's Test for Reducing Sugars

Complete the chart. Indicate those solutions that contain a reducing sugar.

Solution	Color	Reducing Sugar Present or Absent?
Water		
Glucose		
Fructose		
Sucrose		
Starch		
Sprite®		
Diet Sprite®		
Milk		
Fruit Juice		

Thin Layer Chromatography

Complete the following table

Spot	Rf Value	Amino Acids Present
Histidine		
Alanine		
Proline		
Methionine		
Tyrosine		
Lysine		
Unknown - there will be three spots	spot 1. spot 2. spot 3.	