Macromolecules

Contents

Objectives

- Know the building blocks of proteins, carbohydrates and lipids
- Understand the general principle of chromatography
- Recognize appropriate reagent test for the presence or absence of molecules under study
- Make reasonable hypotheses by comparing experimen tal results

Introduction

The principal molecules found in living organisms fall into four categories: proteins, carbohydrates, lipids, and nucleic acids. The ability to determine which of these types of compounds are present and their specific composition provides important information, ranging from understanding how a single cell operates to the evolutionary history of entire groups of species.

The following exercises simulate a number of relatively quick reactions which can be used to test for the presence or absence of proteins, carbohydrates and lipids and also to tell something of the nature of those chemicals present.

Go to the Macromolecules simulation in the BiologyOne DVD.

Activity 4.1
Colorimetric Tests

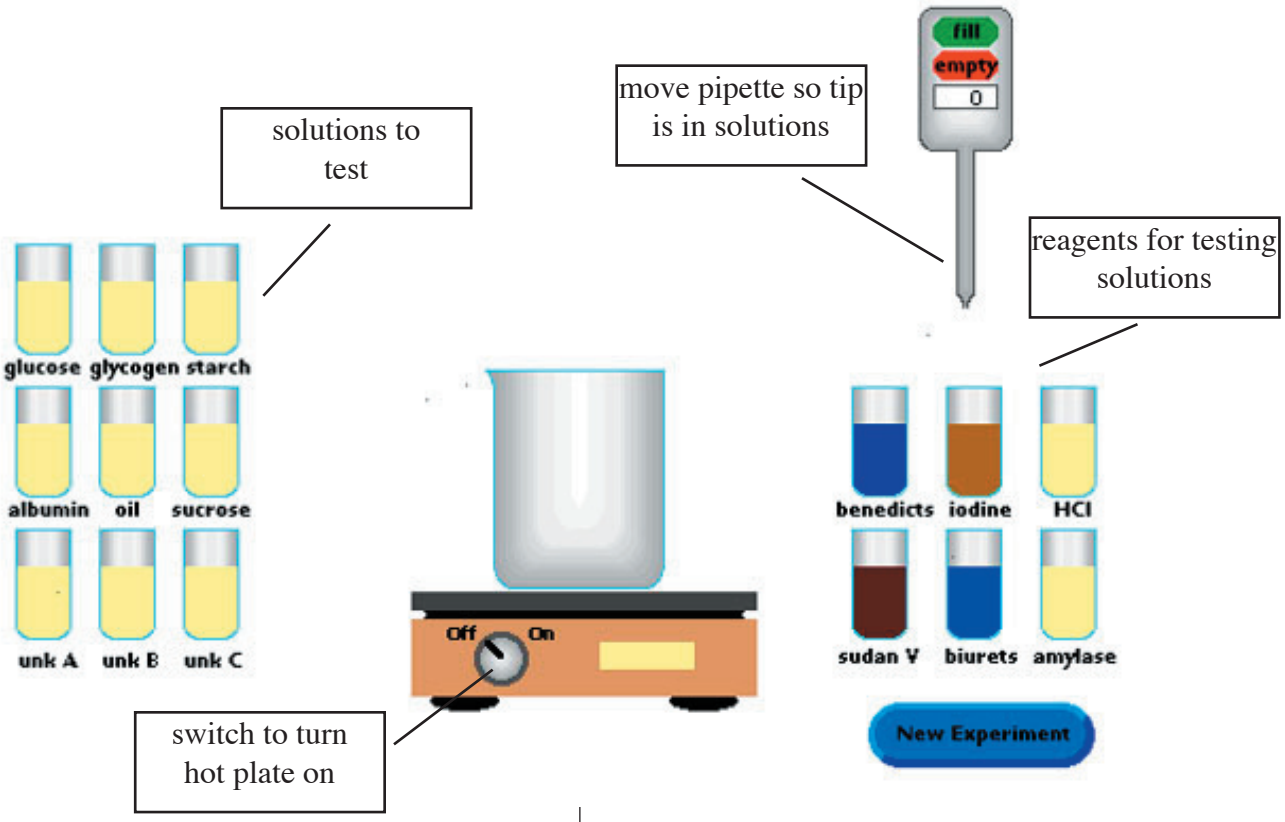
This activity simulates several chemical tests for the presence of various macromolecules. The chemical tests simulated are; the Benedicts test, the biuret test, the iodine test, and the Sudan V test. You will use these chemical tests on the six solutions containing known macromolecules and then determine which macromolecules are in an unknown solution.

The diagram below shows the screen you will be using during this portion of the simulation. Use the pipette to transfer the solution to be tested and then the testing solution into the beaker. When the tip of the pipette is 'in' the solution, click and hold on the fill button to obtain the appropriate quantity of that solution. The numbers on the pipette represent the milliliters of solution taken up. For this simulation, your volumes should be close to the values indicated but may be off slightly.

Move the pipette so that it is over the beaker and then click on the empty button to drain the pipette's contents into the beaker. If you are not over the beaker the pipette's contents will simply be drained. Click on the "new experiment" button to clean out the beaker and test different solutions.

The Benedicts Test:
The Benedicts solution tests for the presence of reducing, simple sugars such as glucose. Larger carbohydrates do not react with the Benedicts solution. To conduct the Benedicts test, transfer 15 mls of the solution to be tested into the beaker. Then add to this 5 ml of the Benedicts solution. For this test to work, the solution must be heated to near boiling, turn on the hot plate - then turn it off. A positive Benedicts test will turn the solution in the beaker orange to red. Test all six of the known solutions. Record your observations in the Results Section.

Simulation for Colorimetric Tests



The Iodine Test:
The iodine test checks for the presence of long, unbranched carbohydrates such as starch. Transfer 15 ml of the solution to be tested into the beaker. Then add to this 1 ml of the iodine solution. If starch is present, the solution will turn a dark purple. Test all six of the known solutions. Record your observations in the Results Section.

Testing the effect of HCl and amylase on carbohydrates:
Here you'll test the effect of HCl and the enzyme amylase on the carbohydrates glucose, glycogen and starch. Test each individually as described below.

Transfer 15 ml of the carbohydrate solution to the beaker. Then add 5 ml of HCl to the carbohydrate. Next add 5 ml of Benedicts solution and heat the reaction mixture. Record these observations in the Results Section. Do your results indicate the presence of reducing sugars? Are they different than when tested without the acid treatment? If so, consider the molecular structure of the carbohydrate and the effect the acid may have had on it.

Repeat this experiment with all three carbohydrates.

Repeat the above tests of the carbohydrates adding 5 ml of enzyme amylase instead of the HCl. What are your results and conclusions when using amylase? Record these in the Results Section.

The Biuret Test:
The biuret test will be positive if proteins are present. A positive test is indicated by the solution turning from a blue color to a purple color. Transfer 10 ml of the solution to be tested into the beaker. Then add 5 ml of the biuret solution. If proteins are present the solution will turn purple. Which of the solutions is a protein? What is the effect of adding HCl to the protein as you did with the starch? Do you still get a positive test? Record your observations in the Results Section.

The Sudan Test:
The Sudan V test will indicate the presence of lipids. The Sudan V complexes with the lipids to form red droplets which rise to the surface of the solution. Transfer 15 ml of the solution to be tested to the beaker. Then add 5 ml Sudan V to the beaker to test for the presence of lipids. Test all six samples. Record your observations in the Results Section.

Testing an Unknown:
Select and test one of the unknown solutions to determine what macromolecules it contains. Be sure to test with HCl and amylase to distinguish among the carbohydrates. Each unknown is a mixture of two macromolecules. Record your observations and conclusion in the Results Section.

Activity 4.2
Chromatography

This exercise simulates the paper chromatography of amino acids. In paper chromatography, chemicals (here amino acids) are placed on the paper and then the paper is placed so its bottom edge is in a chemical solvent. The solvent will move up the paper, carrying the chemicals with it.

Different chemicals, in this case amino acids, have different affinities for various solvents. One can separate and characterize these different chemicals by their affinity to a particular solvent, each chemical having a particular R_f value. The R_f value is defined as the distance the chemical moved divided by the distance the solvent moved. Consequently R_f values vary between 0 and 1.0.

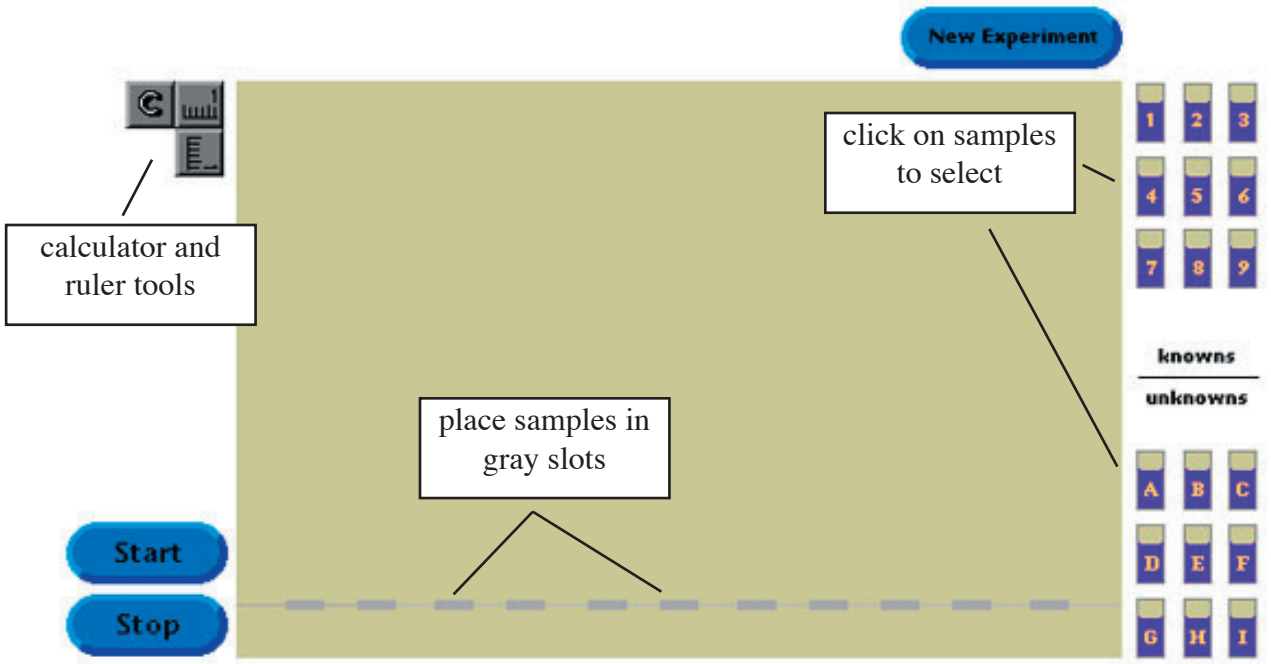
On the right hand side of the screen are 'vials' containing solutions of amino acids numbered 1 through 9. Below there are vials containing solutions of amino acids labeled A through I. Each of these contains one of the numbered amino acids. Move the cursor over one of the vials and click on it to get a sample, the cursor will change shape. Then place the sample on the chromatography paper by clicking on one of the small gray rectangles near the bottom of the 'paper'.

Record where you have placed each sample in the Results Section. When all the sample sites on the paper are filled you are ready to begin.

Click on the start button in the lower left of the screen.

A line will begin to move up the paper. This line represents the solvent front. Each sample will also move up the paper but at a slower rate. Before the solvent front line reaches the top of the paper, click on the stop button. Then you can get a 'ruler' to measure the distance the solvent front moved,distance B below (this value will be the same for all samples) and the distance each sample moved, distance

Simulation for Chromatography



A below, by clicking on the gray, vertical ruler button in the upper left. Clicking on this again will close the ruler. The ruler can be dragged around the screen. Record the distances from origin (the gray line where samples were originally placed) to the solvent front (the purple line) and to the amino acid location (the purple rectangle) in the Results Section. For each sample calculate the R_f value (a calculator can be opened on the screen by clicking on the gray 'C' button in the upper left) and record these in the Results Section.

Your task is to determine the R_f values of the numbered amino acids and then to select one of the lettered amino acids and identify the numbered vial to which it corresponds.

$$R_f = \frac{\text{distance from origin to sample}}{\text{distance from origin to solvent front}}$$

Lab Exercise 4

Name _____

Results Section

Activity 4.1
Colorimetric Tests

Benedicts Test

Substance	Observations	Conclusions
glucose		
glycogen		
starch		
albumin		
oil		
sucrose		

Iodine Test

Substance	Observations	Conclusions
glucose		
glycogen		
starch		
albumin		
oil		
sucrose		

Benedicts Test with HCL Treated Carbohydrates

Substance	Observations	Conclusions
glucose		
glycogen		
starch		

Benedicts Test with Amylase Treated Carbohydrates

Substance	Observations	Conclusions
glucose		
glycogen		
starch		

What does this tell you about the composition of the three carbohydrates and action of HCl and the enzyme amylase?

Biurets Test

Substance	Observations	Conclusions
glucose		
glycogen		
starch		
albumin		
oil		
sucrose		

Sudan Test

Substance	Observations	Conclusions
glucose		
glycogen		
starch		
albumin		
oil		
sucrose		

Testing of Unknowns Unknown: _____

Test	Observations	Conclusions
Benedicts with Unknown		
Iodine with Unknown		
Unknown & HCl with Benedicts		
Unknown & Amylase with Benedicts		
Biuret with Unknown		
Sudan with Unknown		

Conclusion: Contents of Unknown _____ is:

sample locations

1	2	3	4	5	6	7	8	9	10	11

sample	distance sample moved (A)	distane to solvent front (B)	R_f value (A/B)

Identity of Unknown _____ is amino acid number _____.