Macromolecule Analysis

UW ID:
Partner:
TA:
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Introduction

The purpose of this lab experiment is to identify the types of macromolecules in the solutions supplied. Using the results of the known substances, the unknown may be predicted. This experiment also familiarize with the concept of treatment controls which are used to check if the experimental conditions and procedures were conducted correctly. A positive control displays that the procedure that is very similar to the actual experimental test. The experimental result is that which is hypothesized and done many times before. A negative control is a result which does not change the normal state or negative result. (Johnson and Besselsen, 2002) In this lab, we dealt with three biochemical processes including Iodine test, Benedict's test, and Biuret test. The Iodine test reacts with starch forming a colour change. Starch is a polymer of glucose which is made up of two polymers, amylase and amylopectin. Glycogen is also a polymer of glucose which is made up of amylopectin. Amylose is an unbranched, helical molecule whose sugars are joined by α (1 → 4) linkages. Amylopectin is a branched helical molecule. The amylose forms helices where iodine molecules assemble, forming a dark blue/black colour. The amylopectin forms much shorter helices and iodine molecules are unable to assemble, forming a brown/orange colour. (Karp 2010) Benedict's test is used to detect reducing sugars. Benedict's reagent is clear blue but when combined and heated to boiling with a substance containing glucose in a chain form, the cupric ions are reduced to a cuprous form (Cu^{+}) and then oxidized to form copper oxide (Cu_2O). Copper oxide is a brownish-orange substance that is insoluble in water. A positive reaction in a Benedict's Test is the change of the clear light blue solution to an opaque orange-brown solution in a boiling water bath. This color change indicates the presence of glucose in a given solution. A high concentration of reducing sugars generates the formation of a red precipitate; a lower concentration produces a yellow precipitate. Biuret test detects the

presence of peptide bonds. The peptide bonds bond with the copper ions to form a violet Cu^{2+} peptide complex. All of these tests are considered semi-quantitative since there results are in a range of colour. In a positive test, a copper(II) ion is reduced to copper(I), which forms a complex with the nitrogens and carbons of the peptide bonds in an alkaline solution. A violet color indicates the presence of proteins. Quantitative is concerning something which can be measured. The yields from this experiment are only an approximation of the quantity of the substance and lack the precision of a quantitative result. (Griggs and Bray 2001)

Materials and Methods

The protocol and materials used for the experiment were completed as written in the Fall term 2010 Biology 130L Lab Manual, pages 16 to 20.

Results

1) Iodine Test for Starch and Glycogen

Substance	Observation	Experiment Result
1% glucose	Yellow	Negative
0.3 % glucose-1-phosphate	Yellow	Negative
1% maltose	Yellow	Negative
Honey	Yellow	Negative
1% sucrose	Dark yellow	Negative
1% lactose	Dark yellow	Negative
1% glycogen	Reddish brown	Positive
1% starch	Blue-black	Positive
Protein	Dark yellow	Negative
Beer	Dark yellow	Negative
Distilled water	Yellow	Negative
Unknown 225	Blue-black	Positive

This table shows the results of the substances reacting to the iodine test. Three of the twelve substances have a positive control. As you can see there is a range of colours which indicates that this is a semi-quantitative test.

2) Benedict's Test for Reducing Sugars

Substance	Observation	Experiment Result
1% glucose	Red	Positive
0.3 % glucose-1-phosphate	Clear Blue	Negative
1% maltose	Red	Positive
Honey	Orange/brown	Positive
1% sucrose	Clear blue	Negative
1% lactose	Orange	Positive
1% glycogen	Clear blue	Negative
1% starch	Clear blue	Negative
Protein	Clear dark blue	Negative
Beer	Orange/brown	Positive
Distilled water	Clear blue	Negative
Unknown 225	Brown	Positive

This table shows the results of the substances reacting with Benedict's solution. Half of the substances show a positive control. As you can see there is a range of colours which indicates that this is a semi-quantitative test.

3) Biuret Test for Protein

Substance	Observation	Experiment Result
1% glucose	N/R	Negative
0.3 % glucose-1-phosphate	N/R	Negative
1% maltose	N/R	Negative
Honey	Clear Yellow	Positive
1% sucrose	N/R	Negative
1% lactose	N/R	Negative
1% glycogen	N/R	Negative
1% starch	N/R	Negative
Protein	Protein	Positive
Beer	Dark Yellow	Positive
Distilled water	N/R	Negative
Unknown 225	N/R	Negative

This table shows the substances reacting with the NaOH and copper ions. Three of the substances show a positive control. As you can see there is a range of colours which indicates that this is a semi-quantitative test.

Discussion

In the first biological test, the starch turns blue/black due to the amylose and amylopectin which form helices where iodine assemble. Glycogen forms a reddish-brown colour in iodine is due to the fact that it has many branches. Its structure is similar to that of amylopectin except for the fact that it is more branched. Another substance which had a positive result is the unknown. This means that we can predict that there is either amylose or amylopectin in the unknown sample. The negative results are due to a lack of starch or glycogen. For example, beer which is made of made of a high starch plant has no starch. The reason for this is that the starch is converted into sugars. In the Benedict's test, glucose, maltose, honey, lactose, and beer showed a positive control. The reason for this is that Benedict's test identified the presence of reducing sugars. This has to do with the structure and position of the glycosytic bonds. Reducing sugars are sugars with a free aldehyde or ketone group. All monosaccharides are considered reducing sugars because they all have a free reactive carbonyl group. Some disaccharides such as the maltose have exposed carbonyl groups and are also reducing sugars. While other disaccharides like the sucrose are non-reducing sugars and will not react with Benedict's solution because they do not have these exposed groups.(Karp 2010) This explains why sucrose, glucose-1-phosphate, starch protein and water didn't react. The positive results came in a range of colours since the copper sulfate in the Benedict's solution reacts with electrons from the aldehyde or ketone group of the reducing sugar to form cuprous oxide, a red-brown precipitate. (Karp 2010) Depending on the amount of the cuprous oxide, the colour change. The reddish/brown colour is the highest amount of reducing sugar, red is second, followed by orange. The unknown turned brown which means that it contains a high amount of reducing sugars. The clear blue represents no reducing sugars. This range of different colour is an indication of a semi-quantitative test. Finally for Biuret test, the honey, protein test showed a positive result. Before the experiment I was

expecting honey and beer to not react however after researching I noticed that they do contain traces of protein. That explains the clear yellow colour which is not purple but still changed slightly from the negative results. Protein had the darkest colour. The reason is that the liquid used for this test forms a complex with the nitrogen and carbons of the peptide bonds in the solution. The darker purple colour indicates that there is a high concentration of purple (copper-peptide complex). The unknown remained unchanged which shows that it has no peptide bonds. The negative results did not react because they do not have any peptide bonds to create the copper- peptide complex which is the indicator. I am predicting that the unknown is cellulose. The reason for this is that it contains reducing sugars, starch, and no peptides.

References

Department of Biology 2010 Introductory Cell Biology Laboratory Manual. University of Waterloo, Waterloo. pp. 16 – 20.

Griggs, Jeremy, and Dennis Bray. "Cell Macromolecules." *ENCYCLOPEDIA OF LIFE SCIENCES*. 2001. Print.

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