Solate and characterize macromolecules essay sample

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Macromolecules are huge highly organized molecules. These molecules carry out the activities of the cell. There are four major categories in which these molecules can be placed into. These groups include carbohydrates, lipids, proteins, and nucleic acids. Carbohydrates are organic molecules that are usually used to store chemical energy and building material for biological construction. Lipids are a group of non-polar molecules whose common property is that they are water insoluble. Proteins are polymers that are built from amino acid monomers. They carry out almost all of the cell’s activities. Proteins perform a variety of activities including the acceleration of metabolic reactions, providing of mechanical support to the cell, and have regulatory functions in areas such as hormones, growth factors and gene activators. Nucleic acids are made as long strands called nucleotides.

There are two types of nucleic acids, ribonucleic acid and deoxyribonucleic acid, and there primary use is for storage and transmission of genetic information. (Karp, 2003) In the experiment that was carried out, the macromolecules that were used were proteins and nucleic acids. To isolate the macromolecules from there original mixtures, the process of centrifugation was used. This is a technique in which the centrifugal force separates the solvent and precipitate into a supernatant and the pellet. Chromatography was used to characterize the macromolecules. The process is technique that separates mixtures into their individual components. After this process, there is an equation that is to be used. This equation is:

Rf

=

Distance (from origin) travelled by substance (cm)

Distance (from origin) travelled by solvent (cm)

This equation allows one to calculate how soluble the solvent is in the solution. The resultant figure from this simple calculation allows for the characterization of the macromolecule that is in use. (Department of Biology, 2003)

Materials and Method:

The experimental procedure used for this experiment was outlined in the Biology 130L lab manual under experiments 2 and 3. All steps were followed with no deviation in procedure.

Results:

Table 1 – Proteins

Protein

Distance Travelled by Substance (cm)

Distance Travelled by Solvent (cm)

Rf Value

Unhydrolyzed Protein

(a)

1. 4

6. 5

1. 4/6. 5 = 0. 22

(b)

3. 6

6. 5

0. 55

Hydrolyzed Protein

(a)

0. 7

6. 5

0. 48

(b)

3. 3

6. 5

0. 51

(c)

4. 9

6. 5

0. 75

Alanine

4. 5

6. 5

0. 69

Histidine

1. 5

6. 5

0. 23

Aspartic Acid

3. 2

6. 5

0. 49

Lysine

1. 9

6. 5

0. 29

Methionine

5. 3

6. 5

0. 82

Unknown

4. 5

6. 5

0. 69

Note – If protein has addition parts, it means that there were multiple separations.

These separations are written in increasing order

Table 2 – Nucleic Acids

Nucleic Acid

Distance Travelled by Substance (cm)

Distance Travelled by Solvent (cm)

Rf Value

Unhydrolyzed Nucleic Acid

0. 0

8. 3

0. 0/8. 3 = 0

Hydrolyzed Nucleic Acid

(a)

1. 1

8. 3

0. 13

(b)

2. 4

8. 3

0. 29

(c)

3. 0

8. 3

0. 36

Adenine

4. 4

8. 3

0. 53

Cytosine

3. 1

8. 3

0. 37

Uracil

4. 3

8. 3

0. 52

Adenine-Cytosine-Uracil

(a)

2. 9

8. 3

0. 35

(b)

4. 1

8. 3

0. 49

Note – If Nucleic Acid has additional parts, it means that there were multiple separations.

These separations are written in increasing order

Discussion:

The unhydrolyzed protein should have remained in its starting position instead of climbing up the chromatography paper. This indicates that the solution was not 100% unhydrolyzed. Since there were multiple substances in the solution, the molecular weight not just be of the protein and would therefore separate. In turn, this sample of protein was soluble because it still contained other substances. Because of the slight solubility with isopropanol, this indicates that the substance was somewhat polar due to the mobility across the matrix. The hydrolyzed protein results displayed three distinct separations. This is exactly what should have been expected and therefore the experiments from the previous week were a success. These three separations show that the solution of protein was indeed hydrolyzed because for it to separate, the chemical bonds had to be broken.

In terms of molecular weight, since there were three segments into which the original solution separated into, the weight was distributed between these segments and therefore the weight of each was less and was able to travel up the chromatography paper. Since this substance was quite soluble with the isopropanol, the polarity is therefore quite high. The remaining six substances that were included with the hydrolyzed and unhydrolyzed nucleic acids were alanine, histidine, aspartic acid, lysine, methionine and the unknown solution. From the results of the chromatography, the alanine, methionine, and unknown solution all appeared to have a greater displacement from the starting position compared to the results of the remaining substances.

This result of a greater displacement signifies that the molecular weight of these three substances is low. For it to move a great distance up the chromatography paper, there weights had to be quite low. In terms of solubility, since the three molecules have are high in polarity, there solubility is therefore also high. The aspartic acid was the only substance to have a displacement in a general mid-zone on the chromatography paper. This indicates that its molecular weight is lower than the weights of alanine, methionine and the unknown substance but still greater that the weight if histidine and lysine. This again indicates that the solubility and polarity is less than those substances with a greater weight and greater than those with a lower molecular weight. Finally, the histidine and the lysine were both low in molecular weight because of there little displacement from the starting position. There polarity is quite low which therefore causes there solubility to be low.

The unhydrolyzed nucleic acid should not have separated because its chemical bonds should have been intact. The results on the chromatography showed no separation at all which means that the experiment with the unhydrolyzed nucleic acid was a success. The results from the chromatography revealed there to be no displacement. This indicates that the molecular weight was high because it was unable to move. In terms of solubility, the hydrolyzed nucleic acid was insoluble because both the highly polar acetic acid and highly polar nucleic acid will not dissolve each other. The correct results from the chromatography paper should have shown the separation of hydrolyzed nucleic acid. These results are the same as what the experiment conducted expected. The chromatography paper revealed three distinct separations. For something to be hydrolyzed, the chemical bonds must be broken. Since there were three separations, the experiment was a success.

The molecular weight of the hydrolyzed nucleic acid is not high because the solution was able to climb up the matrix. Since the hydrolyzed nucleic acids moved so far up the matrix, it proved that the mixture was soluble and therefore had a high polarity. The remaining four substances that were included with the hydrolyzed and unhydrolyzed nucleic acids were adenine, cytosine, uracil, and a mixture of adenine-cytosine-uracil. The results from the chromatography showed the adenine and cytosine to have a greater displacement from the starting point compared to the others. This result signifies that the weight of these two nitrogenous bases is low which allows it to move easily up the chromatography paper. The polarity of these bases is quite high which indicates that there solubility must be high. The results of

the cytosine did not reveal a small displacement but that of medium one. Its displacement was comparable to that of aspartic acid in that it was not very high, but not very low either. This can lead one to conclude that the molecular weight of cytosine is heavier than that of the bases uracil and adenine. The polarity of cytosine is considerably lower than the polarities of adenine and uracil.

During the initial separation of the macromolecules, several errors may have occurred. One of these is that at the stage of centrifugation, the contents of the test tubes may not have been in the machine for the correct amount of time in that it could have been stopped too early or too late. Another error may have occurred when the water boiling the nucleic acids was stopped. The amount of time it had been off for was unknown and an estimate was made. Depending on the estimate, the nucleic acid containing beakers may have been left in the boiling water for a time that could be either too short or too long.

When the second set of experiments occurred, the only error that might have happened would be that when taking the hydrolyzed and unhydrolyzed proteins and nucleic acids from their tubes, some of the pellets from the base of the tubes may have also entered the solution that was to be placed on to the chromatogram. Other errors that may have occurred are general types such as when the substances had to rid themselves of a supernatant, some of the supernatant remained. Also, when pouring from tube to tube, not all of the liquid may have been transferred. These errors listed above, except for the last one, may have had a significant affect on the outcomes of the experiment.

If the results from the last week were one hundred percent successful, then certain molecules would have shown up in the hydrolyzed and unhydrolyzed proteins and nucleic acids. The results on the chromatogram for hydrolyzed protein would reveal a separation which would be the amino acids. The unhydrolyzed protein should have no substances separated because its chemical bonds have not been broken. The result should be no displacement of the solution from the line. If done correctly, the chromatogram showing hydrolyzed nucleic acid should have separations displaying the nitrogenous bases. The results of the unhydrolyzed nucleic acid portion should be just as the unhydrolyzed protein portion was, no separation. Again, the chemical bonds had not been broken and therefore the solution should have no displacement from the origin.

If the steps carried out were only partially successful for the hydrolyzed protein and nucleic acid, then the substances that could be found in hydrolyzed protein would be peptide chains containing the unbroken amino acids. In the hydrolyzed nucleic acid portion, one would be able to find nucleotides because the bonds would not have been broken.

The substances alanine, histidine, aspartic acid, lysine, and methionine fall into the category of amino acids. The adenine, cytosine, and uracil are in the group called nitrogenous bases.

References:

1. Department of Biology 2003 Cell Biology Lab Manual. University of Waterloo, Waterloo. Pages 16 – 26

2. Karp, G. 2003. Cell and Molecular Biology, 3rd Ed. Pages 43-51 and G3-G13