Name/Surname: İrem AŞUTKALIOĞLU DATE:16.11.2020

ID: 2284123 SECTION:2

**BIOLOGICALY IMPORTANT MOLECULES**

**AIM:**

To recognize the organic compounds which have five major type by using biochemical tests.

**INTRODUCTION:**

Macromolecules type which are carbohydrate, protein, lipids, nucleic acid are made up of smaller subunits. These small subunits come together with dehydration and break up with hydrolysis.

Carbohydrate: There are three types of carbohydrates which are monosaccharide, disaccharides, polysaccharides.

* Monosaccharides: They include one sugar unit and these unit contain some functional groups which can be hydroxyl group, aldehyde or keton group, glucose(aldehyde), fructose(keton), ribose and galactose.
* Disaccharides: They made up of condensation reaction of two monosaccharides. The structure which lead to keep together the monosaccharides is glycosidic bond. Sucrose, lactose and maltose is an example of disaccharides.
* Polysaccharides: Cellulose, starch, glycogen are the polysaccharides. Starch which have two type is the polysaccharides for plant store. One of these is amylose and, other one is amylopectin.

Protein: Amino acids are the subunit of protein. 20 different amino acids are produced in the human body. Amino acids contain amino group, carboxyl group, variable side chain. While carboxyl and amino group are attached to each other with peptide bonds (C-N interaction).

Lipids: They are soluble in organic non-polar solvent like ether acetone, but they do nor soluble in polar solvent such as water. With interaction glycerol and fatty acid, triglycerides which are type of lipids are formed.

Nucleic Acid: Nucleic acid contain subunits which are named nucleotide. Nucleotides composed of phosphate group, ribose sugar, nitrogen base. Nucleotide can be differentiated with different ribose sugar and nitrogen base.

**MATERIAL/ METHOD:**

Benedict’s test for reducing sugar:

* Get 8 tubes and labelled them with the group number or the desk number.
* 1mm indicating material is added to each tube.
* Then, 1mm Benedict’s solution is added to each tube.
* For three minutes, tubes are put in the water bath.
* Then, tubes are taken from water bath and are waited for drying.
* Colors are recorded.

All of them include reducing sugar except distilled water. However, all of them contain different rate of reducing sugar again except water. Glucose solution which is one percent contain more reducing sugar. When the percentage of glucose solution is increase, color change green to orange. Distilled water is negative control and glucose solution is positive control.

Iodine Test for Starch:

* Get 3 vials and labelled them.
* Indicating material is added to each vial.
* One drop of iodine is dripped to each vial.
* Observe the result.

Starch which is coiled polymer of glucose interacts with iodine. Then the color changes the bluish black.

Benedict’s and iodine test for salivary amylase:

* Get 6 test tube and labelled with the number.
* Indicating materials which is water, starch, amylase is added to each tube.
* Iodine and benedict’s solutions are dripped to three tubes for each solution.
* Observe the result.

Iodine is the indicator of starch and when starch is found in the solution, the color is changed. In iodine part of that experiment, tube which is contain just water is negative control and others are positive control because we know what contain is. Starch is made up of glucose. Amylase break the bonds which is found the glucoses. Therefore, benedict’s test affects the reducing sugar. If amylase is not found in starch solution, benedict’s test gives us negative result because of lack of reducing sugar. In the benedict’s test part of this experiment, two tubes are negative control. Because one of them contains just water and other one contains starch and water. Therefore, none of them contain reducing sugar. The last one is positive control because amylase break the bond and reducing sugar is found the solution. Therefore, the color of the solution is changed.

Biuret test for protein:

* Get 5 test tube and labelled which whatever substances you use.
* 1 mm NaOH which is 10% is added to each tube and shake the tubes.
* Biuret solution is added to each tube.
* Color changing is observed.

Biuret reagent contain copper 2 ions and these ions create a complex with peptide bonds. Therefore, solution color is changed from blue to dark violet.

Sudan 4 test for lipids:

* Get 2 clean test tube and labelled.
* Equal amount of water is added into each tube.
* Fat is added to one of the tubes.
* A few drops of water are added for equal liquid amount.
* 5 drops of Sudan 4 are added to each tube.
* Record the changing color.

Sudan 4 interacts to selectively absorb pigments in fat soluble dye. Sudan 4 is not soluble in water. However, it is soluble in lipids so when the lipids are found in the solution, the color changed because of interaction.

**RESULT:**

Benedict’s test for reducing sugar:

|  |  |
| --- | --- |
| **SOLUTION** | **COLOR CHANGE** |
| DISTILLED WATER | No color change stays blue |
| GLUCOSE SOLUTION 0.1% | Light green |
| GLUCOSE SOLUTION 0.25% | Light green |
| GLUCOSE SOLUTION 0.5% | Light orange |
| GLUCOSE SOLUTION 0.05% | From bright blue to blue- green |
| GLUCOSE SOLUTION 1% | Orange |
| UNKNOWN | Green |

IODINE TEST FOR STARCH:

|  |  |
| --- | --- |
| **SOLUTION** | **COLOR CHANGE** |
| DISTILLED WATER | Yellow- orange color |
| STARCH | Black-blue color |
| ORGANIC SOLUTION CONTAINING STARCH | At the bottom small drop of black-blue color |

BENEDICT’S AND IODINE TEST FOR SALIVARY AMYLASE:

|  |  |  |
| --- | --- | --- |
| SOLUTION | BENEDICT’S | IODINE |
| H2O | Blue | The color of iodine |
| H2O + STARCH | Blue | Black |
| STARCH + AMYLASE | Orange | Light black, like iodine color |

BIURET TEST FOR PROTEIN:

|  |  |
| --- | --- |
| **SOLUTION** | **COLOR CHANGE** |
| AMINO ACID SOLUTION | Light blue |
| CASEIN | Violet |
| GELATIN | Violet |
| PEPTONE | Blue |
| ALBUMIN | Violet |

SUDAN 4 TEST FOR LIPIDS:

|  |  |
| --- | --- |
| **SOLUTION** | **COLOR CHANGE** |
| WATER | Pink |
| WATER+ VEGETABLE OIL | Orange |

**DISCUSSION:**

Firstly, we should know what the positive, negative control and unknown solution are. Positive control group is a group which have expected affect (Frequently asked questions about how science works, 2020). For example, we put the bacteria in the plate and then, we put the antibiotic the same plate. We should observe antibiotic kills the bacteria. This is positive control because we know the result. Negative control group is the group which does not affect in an expected way (Frequently asked questions about how science works, 2020). For example, we put the virus to the plate then we put the antibiotic. We should the observe no change. Because antibiotic does not affect the viruses. Therefore, this is the negative control.

**Benedict’s test for reducing sugar:**

In that experiment, we use benedict’s test and water, glucose solution which have different rate, unknown solution. When we look the result, one of the tubes which is contain just water does not change color so we can say that this tube is negative control. Others have color change but have different tone because of different amount of reducing sugar. While increasing amount of reducing sugar, the tone of the solution change from blue to orange. If the solution contains how much more reducing sugar, its color will be closer to orange. In the light of this information, we understand that if solution contain enough reducing sugar, its color will be orange and it will be positive control. In the benedict’s test, we heat the solution. If we don’t heat, the color change won’t occur, the color remains blue. Because reducing sugars are capable of transferring hydrogen (electron) to other compound, that reaction is named reduction reaction (Aryal et al., 2020). Reduction reaction is endothermic reaction and heat required for occurrence of reaction. In that reason, we heat the tubes and so we observe the color change. While the tube which contains just water is negative control, the tube which contain %1 glucose solution is positive control.

**Iodine test for starch:**

In the iodine test, we have 3 different sample which are water, starch, organic solution containing sugar. Due to lack of starch in water, the color change does not happen. The color of water is just iodine color. However, when we look at the tube which contain starch, we see the changing color to black. Thanks to charge transfer complexes, the color is looked by us. Molecular iodine isn’t soluble in water so it can add as potassium iodide. The element of I is polyiodide ion and have different charge of ions. Ions which is generally negatively charge act as charge donor and iodine which is neutral is charge acceptor. Electron which is used charge- transfer complexes is alert easily by light and it provide with higher energy level. Light which is absorbed provide the complementary color and we see the black color (Goedecke, 2016). When we look at the third tube which is organic solution containing starch, we realize starch with iodine bottoming out. Firstly, we have a tube containing organic solution then we add the starch solution. Therefore, the amount of starch is less then the organic solution. With adding iodine, starch solution and iodine get together and going to bottom of tube. While the tube which contain just water is negative control, the tube which is contain starch is positive control.

**Benedict’s and iodine test for salivary amylase experiment:**

Benedict’s test is an indicator for contain reducing sugar. If solution have reducing sugar, the color of the sample will be orange. Also, iodine test is an indicator for starch. If solution have starch, the color of sample will be black. Our first sample is water. Water is pure substances and it doesn’t contain starch or reducing sugar or anything. It made up of H2O molecules. Therefore, the color of water is blue (just benedict’s solution color) and the color of other tube which is tested with iodine will be just iodine color. It isn’t change. The other solution is the mixture of starch and water. Due to lack of reducing sugar, the tube which is tested with benedict’s solution will be stay like previous test. However, when we look the other tube which is tested with iodine, we realize the color changing to black because of containing starch and iodine is starch indicator. When we look the last tubes, which contain mixture of starch and amylase, we see the different color. Amylase which is the enzyme makes starch turn into the reducing sugar. Therefore, thanks to the amylase, starch turn into reducing sugar and benedict’s solution affect the sample and its color would be orange. When we look at the other tube which test with iodine solution, the amount of starch is decrease because the amylase. Therefore, the color of the sample may be changed but it does not be black may be lighter tone. For benedict’s test, while the tubes which contain water and water- starch is negative control, the other tube which contains starch and amylase is positive control. For iodine test, while the tube which contains just water is negative control, the tube which contains water and starch is positive control.

**Biuret test for protein:**

Biuret test is the indicator for proteins. When the sample contain the protein, the color is purple. Copper ions which is found the biuret have +2 charge. When the peptide bond is found in the test tube, the charge of the copper ions will be +1. Therefore, the color of the sample changed (Aryal, 2020). When we test the amino acid solution, we expected no color change because there is no peptide bond between the amino acid. The result of that, when we look the sample, we see that there is no change. The color of the sample is blue. It is just the color of biuret. Then, the other tube contain casein. Casein is the protein which is found in milk. It has peptide bond. Therefore, the color of the sample change from blue to violet. The other sample contain gelatin which is the protein. We expected that color of gelatin sample would be violet, and our expectation is true. The color of the gelatin sample is violet. The other sample is peptone which is protein hydrolysate (Peptones - an overview | ScienceDirect Topics, 2011). Protein hydrolysates is a complex mixture which is made up of oligopeptides, peptides and, free amino acids that are produced by partial extensive hydrolysis (Protein Hydrolysates - an overview | ScienceDirect Topics, 2017). Therefore, the peptone does not contain peptide bonds, so the color of sample doesn’t change. The last tube contains albumin which is protein in the plasma of blood. We expected that the color of the albumin sample is change from blue to violet and it is true. Therefore, the color of sample is violet because of peptide bond. the tubes which contain casein, gelatin and albumin he positive controls of this experiment. The tubes which contain amino acid solution and peptone are negative control.

**Sudan 4 test for lipids:**

Sudan 4 is an indicator for lipids. When the lipid is found in the test tubes, the color change from pink to orange. In this experiment we use two different sample. One of these contain water and other one contains water and vegetable oil. Water doesn’t contain lipid; it is pure substances. It just contains H2O molecules. Therefore, when we add the Sudan 4 to water, we realize that the color change does not occur. The color of the sample would be pink. The pink color comes from the Sudan 4, not staining for being lipid. Then, we take the other tube and add vegetable oil and Sudan 4. We realize that the color of the oil changed. The top of the water where found the oil would be orange after adding Sudan 4. Everything was as expected such as color change. In that experiment, tube which is just contain water is negative control and other tube is positive control.

**REFERANCES:**

Aryal, S., Love, B., barnabas, A., Khare, N. and train, w., 2020. *Benedict's Test- Principle, Preparation, Procedure And Result Interpretation*. [online] Microbiology Info.com. Available at: <https://microbiologyinfo.com/benedicts-test-principle-composition-preparation-procedure-and-result-interpretation/#:~:text=Some%20sugars%20such%20as%20glucose,Benedicts%20reagent%20to%20change%20color.> [Accessed 18 November 2020].

Undsci.berkeley.edu. 2020. *Frequently Asked Questions About How Science Works*. [online] Available at: <https://undsci.berkeley.edu/faqs.php> [Accessed 18 November 2020].

Goedecke, C., 2016. *Why Does Iodine Turn Starch Blue? :: Education :: Chemistryviews*. [online] Chemistryviews.org. Available at: <https://www.chemistryviews.org/details/education/10128441/Why\_Does\_Iodine\_Turn\_Starch\_Blue.html#:~:text=Iodine%20Test,changes%20to%20a%20deep%20blue.> [Accessed 21 November 2020].

Aryal, S., 2020. *Biuret Test For Protein | Biochemistry | Microbe Notes*. [online] Microbe Notes. Available at: <https://microbenotes.com/biuret-test-for-protein/> [Accessed 21 November 2020].

Sciencedirect.com. 2011. *Peptones - An Overview | Sciencedirect Topics*. [online] Available at: <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/peptones#:~:text=Peptones%20are%20protein%20hydrolysates%20formed,as%20the%20source%20of%20nitrogen.> [Accessed 21 November 2020].

Sciencedirect.com. 2017. *Protein Hydrolysates - An Overview | Sciencedirect Topics*. [online] Available at: <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/protein-hydrolysates#:~:text=Protein%20hydrolysates%20are%20defined%20as,Clare%20%26%20Swaisgood%2C%202000).> [Accessed 21 November 2020].