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Bio 103

Lab E: Testing Major Organic Molecule

Procedure 1: Benedict's Test

Materials:

- Tap Water
- 250 mL Beaker
- 6 Test Tubes
- Test Tube Holder
- 100 mL Beaker
- Wax Pencil
- Benedict's Reagent
- Graduated Transfer Pipette
- 6- 1 mL samples of sugary household solutions

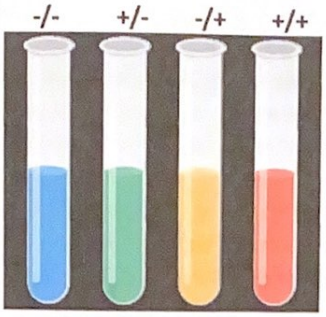
Benedict's Test is used to test for simple carbohydrates. The Benedict's test identifies reducing sugars (monosaccharide's and some disaccharides), which have free ketone or aldehyde functional groups.

Some sugars such as glucose are called reducing sugars because they are capable of transferring hydrogens (electrons) to other compounds, a process called reduction and will test positive, resulting in a brick red color. When reducing sugars are mixed with Benedicts reagent and heated, a reduction reaction causes the Benedicts reagent to change color. The color varies from green to dark red (brick) or rusty-brown, depending on the amount of and type of sugar.

Benedict's quantitative reagent contains potassium thiocyanate and is used to determine how much reducing sugar is present.
When Benedict's solution and simple carbohydrates are heated, the solution changes to orange red/ brick red. This reaction is caused by the reducing property of simple carbohydrates. The copper (II) ions in the Benedict's solution are reduced to Copper (I) ions, which causes the color change.

The red copper(I) oxide formed is insoluble in water and is precipitated out of solution. This accounts for the precipitate formed. As the concentration of reducing sugar increases, the nearer the final color is to brick-red and the greater the precipitate formed. Sometimes a brick red solid, copper oxide, precipitates out of the solution and collects at the bottom of the test tube.

1. Collect 6, 1 mL samples of household solutions into test tubes using a transfer pipette. (Examples: water, clear soda, milk, corn syrup etc.) Label each tube 1-6 using your wax pencil. List your samples in the table below:
   List your conclusion as +/+, +/- or -/- by referencing the image below.



Test Tube	Solution	Color	Conclusion
1	2% Milk	Chunky Blue	+/−
2	Snapple Juice	Beige	−/+
3	Coffee Creamer	Chunky Blue	+/−
4	Orange Juice	Yellowish Orange	−/+
5	Corn Syrup	Rusty Brown	+/+
6	Peach Wine	Beige	+/−

2. Add 2 mL of Benedict's solution to each test tube, swirl gently.
3. Add about 100 mL of tap water into a 250 mL beaker. Microwave until the water is boiling.
4. Carefully place each test tube into the beaker of hot water using a test tube holder and leave for 3 minutes.
5. Remove the test tubes from the 250 mL beaker and place them into an empty 100 mL beaker, let cool for 2 minutes. Record the color changes in the table above.
6. Using the color, or precipitate as an indicator, rank the solutions from the nonreducing sugar to the strongest reducing sugar:
   1. 2% Milk
   2. Coffee Creamer
   3. Snapple Juice
   4. Peach Wine
   5. Orange Juice
   6. Corn Syrup

Procedure 2: Iodine Test for Starch

Materials:

- Iodine Solution
- 24-well plate
- Transfer Pipette
- Dried Potato
- Tap Water

The iodine test uses iodine-potassium iodide (I2K) to test for the presence of starch. The sample turns blue-black in color when a few drops of potassium iodide solution is placed on the sample. The reaction is due to the formation of polyiodide chains from the reaction of starch and iodine. The amylose in starch forms helices where iodine molecules assemble, forming a dark blue or black color. When starch is broken down or hydrolyzed into smaller carbohydrate units, the blue-black color is not produced. Therefore, this test can also indicate completion of hydrolysis when a color change does not occur. Some carbohydrates, such as dextrin and glycogen, will produce an intermediate color reaction.

1. Procure 5 household samples to test. These can be solutions or samples of other substances, like bread, cotton, white paper ect. and use the dried potato found in your kit as a positive control. Place your 5 samples and the dried potato sample into separate wells using the 24-well plate.
   List your chosen samples in the table below:

Well Number	Sample	Color	Conclusion
A1	Stevia Sugar	Brown	Kept Iodine Color (Sugar-Covered Droplet)
B1	Bread	Black	Turned Black after few drops
C1	Cotton	Black	absorbed droplet (kinda brownish)
A2	Marshmellow	Black	Covered surface with black color
B2	Ruffle Chip	Black	Become dark overtime
C2	Dried Potato	Black	Turned black the fastest

2. Add a few drops (2-4) of Iodine to each well.
3. Which substances contained starch?
   The bread, marshmellow, potato chip, and dried potato all contained Starch.

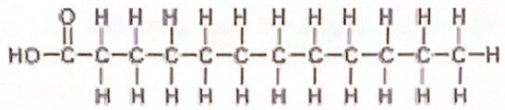
Procedure 3: Testing for Lipids

Materials:

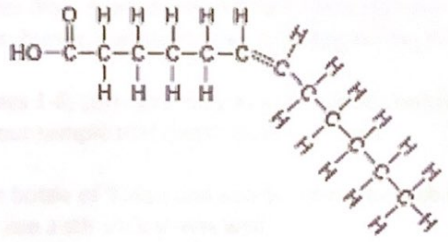
- Brown Paper
- Filter paper
- 6 test Tubes
- Sudan Stain
- Transfer Pipette
- Stir Stick

Lipids are a class of macromolecules that are nonpolar and hydrophobic in nature. Major types include fats and oils, waxes, phospholipids, and steroids. Fats are a stored form of energy and are also known as triacylglycerols or triglycerides. Fats are made up of fatty acids and either glycerol or sphingosine. Fatty acids may be unsaturated or saturated, depending on the presence or absence of double bonds in the hydrocarbon chain.

Saturated Fatty Acid



Unsaturated Fatty Acid



If only single bonds are present, they are known as saturated fatty acids. Unsaturated fatty acids may have one or more double bonds in the hydrocarbon chain. Phospholipids make up the matrix of membranes. They have a glycerol or sphingosine backbone to which two fatty acid chains and a phosphate-containing group are attached. Steroids are another class of lipids. Their basic structure has four fused carbon rings. Cholesterol is a type of steroid and is an important constituent of the plasma membrane, where it helps to maintain the fluid nature of the membrane. It is also the precursor of steroid hormones such as testosterone.

Grease-Spot Test:

This is a simple test used to identify the lipid nature of substances.

1. Using your wax pencil, draw 3 spaces on the brown paper. Example:

2. Procure 3 samples to test from your house, such as candle wax, butter, a potato chip ect. Gently rub or place a drop of liquid from the samples into one of the 3 spaces on the brown paper.

3. Let the substance and paper sit for about 15 minutes, then hold the brown paper up to a light source. Translucent spots indicate the presence of a lipid. Only need a small amount **(like size of a pea, few drops or small blob).**

Record your results below:

Substance	Result (+/-)	Conclusion
Move Over Butter	+	Had the most translucent spot
Candle Wax	+	Pretty much translucent
Ruffle Chip	+	least translucent spot

Sudan Test:

Sudan is a stain that combines with lipid molecules, forming a bright orange color. Sudan causes this reaction by forming a hydrophobic interaction with nonpolar molecules. The more vivid the color, the greater the intensity of the interaction.

1. Procure 6 samples from around your home to test. List your chosen samples in the table below. Examples: butter, margarine, egg yolk, egg white, milk ect.
2. Number test tubes 1-6, corresponding to your chosen samples in the table below. Add about 1 mL of your sample to its corresponding tube.
3. Use the dropper bottle of Sudan and add 6-7 drops to each test tube. You can gently swirl the test tube or use a stir stick to mix well.
4. Examine the color change of each tube and record your observations in the table below:

Substance	Color	Conclusion
Egg white	Pink	Doesn't mix well. Clear with Sudan particles
Egg yolk	Pink	Doesn't mix well. Same as Egg whites
Butter	Orange	Mixed very well. Turned orangish
French Onion Dip	Pink	Harder to mix but still shows pink
Milk	Pink	Easily mixed to pink
Coffee creamer	Pink	Easily mixed to pink

Procedure 4: Testing for Proteins

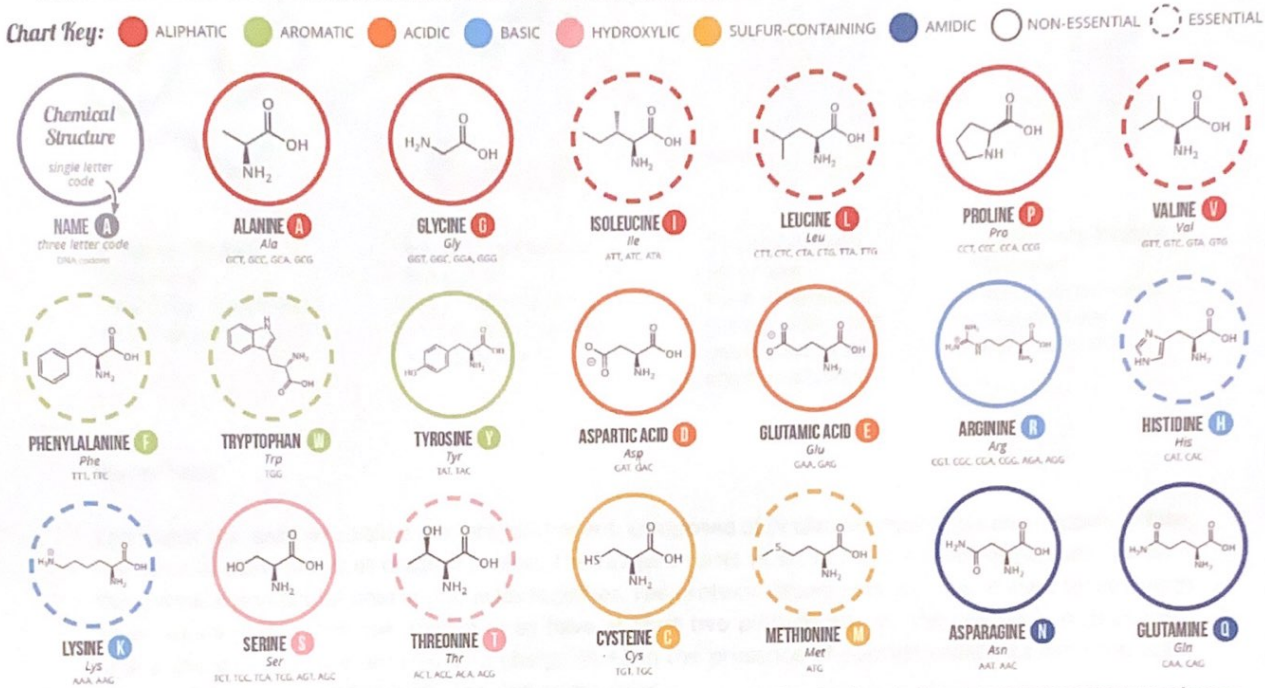
Materials:

- Biuret Reagent
- 6 test tubes
- Graduated Transfer Pipette
- Wax Pencil
- 6 household samples

Amino acids are the monomers that make up proteins. Specifically, a protein is made up of one or more linear chains of amino acids, each of which is called a polypeptide. Amino acids share a basic structure, which consists of a central carbon atom, also known as the alpha (α) carbon, bonded to an amino group (NH_2), a carboxyl group (COOH), and a hydrogen atom. Every amino acid also has another atom or group of atoms bonded to the central atom, known as the R group, which determines the identity of the amino acid. For instance, if the R group is a hydrogen atom, then the amino acid is glycine, while if it's a methyl group (CH_3), the amino acid is alanine. Twenty amino acids serve as the building blocks of life.

A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

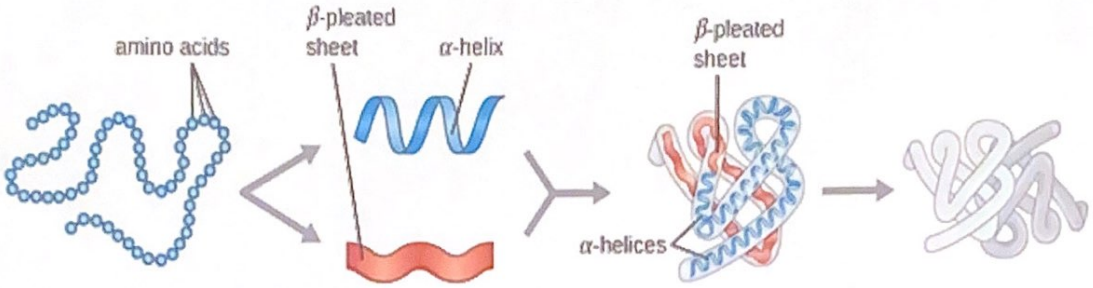


Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

Some of these amino acids can not be synthesized, so they must be obtained from diet. In humans these amino acids are:

- Histidine
- Leucine
- Lysine
- Methionine
- Phenylalanine
- Isoleucine
- Threonine
- Tryptophan
- Valine
- Arginine (in children)

Amino acids are linked together by peptide bonds. A peptide bond is a covalent bond that forms between the amino group of one amino acid, and the carboxyl group of another during a dehydration synthesis reaction. A peptide is two or more amino acids joined together by peptide bonds; a polypeptide is a chain of many amino acids; and a protein contains one or more polypeptides. Therefore, proteins are long chains of amino acids held together by peptide bonds. These long chains fold to a precise three-dimensional shape that performs a specific function. The conformation (shape) of a protein refers to the final folded arrangement of the polypeptide chain of a protein. There are four levels of protein structure: Primary structure, secondary structure, tertiary structure and quaternary structure. When heated, proteins will lose their structure and they become **denatured**.



Primary Protein Structure
Sequence of a chain of amino acids

Secondary Protein Structure
Local folding of the polypeptide chain into helices or sheets

Tertiary Protein Structure
three-dimensional folding pattern of a protein due to side chain interactions

Quaternary Protein Structure
protein consisting of more than one amino acid chain

Biuret Test:

The biuret test uses an alkaline mixture, or reagent, composed of potassium hydroxide and copper sulfate. The normal color of biuret reagent is blue. The reagent turns violet in the presence of peptide bonds -- the chemical bonds that hold amino acids together. The proteins detected must have at least three amino acids, which means that the protein must have at least two peptide bonds. The reagent's copper ions, with a charge of +2, are reduced to a charge of +1 in the presence of peptide bonds, causing the color change. The more peptide bonds, the darker the color.

1. Procure 6 samples from around your home to test. Examples: Egg white, egg yolk, vegetable oil, chicken broth etc... Add 1 mL of each sample to a test tube (labeled 1-6). List your chosen samples in the table below:

Substance	Color	Conclusion
2% Milk	No change	Stayed white with a hint of pink
Egg Yolk	No change	barely any change, hint of pink
Egg white	Pinkish	has not changed much, hint of pink
Red wine vinegar	No change	Nothing changed with the Vinegar
Beef stock	No change	Same as Vinegar, no change to color
Canola Oil	No change	No change to the oil

2. Add a few drops (3-4) of the Biuret reagent to each test tube, swirl gently or tap the test tube with your finger to mix the solution. Wait about 2 minutes for colors to develop. Record your observations in the table above.

3. Which substances were proteinaceous?

In my observations, only three samples contained protein. Those three samples are the 2% milk, Egg Yolk, and Egg white.