Glycogen Isolation

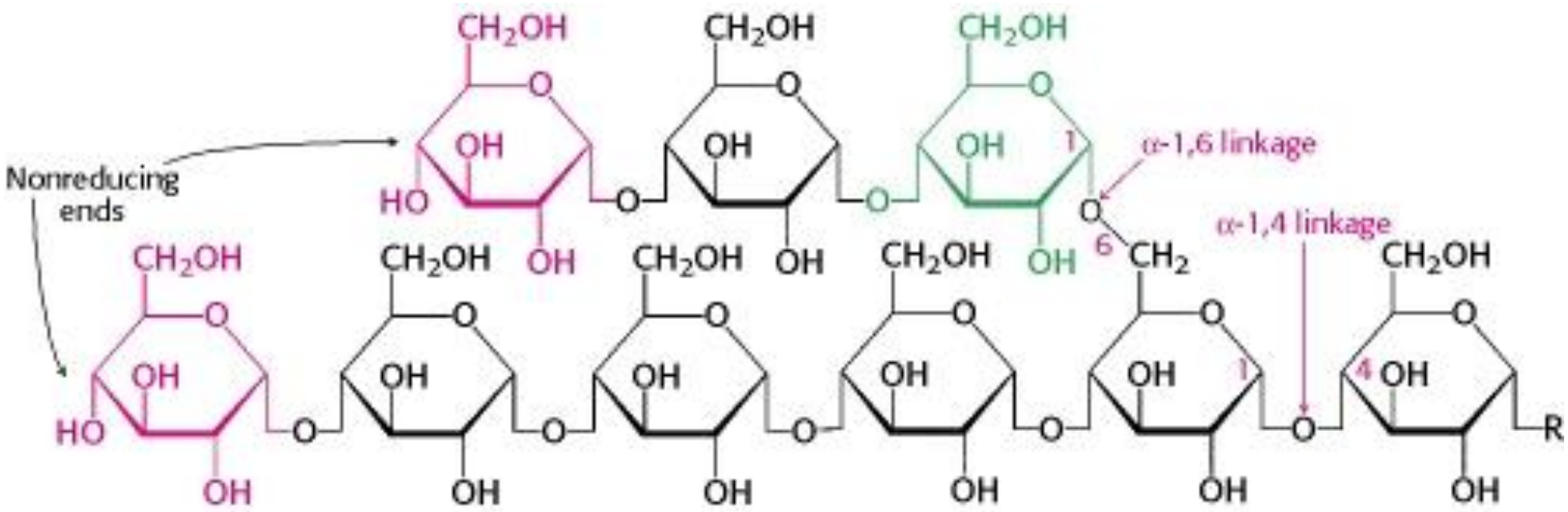
- Objective:

- To illustrate the method for isolating glycogen.

- Introduction:

- Glycogen is the *storage form of glucose* in animals and human which is analogous to the starch in plants.
- Glycogen is synthesized and stored mainly in **liver and muscles**.
- The concentration of glycogen is **higher in the liver** than in muscle.
- Various samples of glycogen have been measured at 1,700-600,000 units of glucose.
- It is a very large, branched polymer of glucose residues that can be broken down to yield glucose molecules when energy is needed.

Structure of glycogen



In this structure of two outer branches of a glycogen molecule, the residues at the non reducing ends are shown in red and residue that starts a branch is shown in green. The rest of the glycogen molecule is represented by R.

- Principle of the experiment:

Liver grounded with TCA solution

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Proteins and nucleic acids are precipitated

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Glycogen remain in solution (supernatant) with other water soluble substances

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Glycogen is separated by precipitation with alcohol, polysaccharides are less soluble than sugars in aqueous alcohol.

- Why does the body store glycogen?

- Glycogen is an *important fuel reserve* for several reasons.
- The breakdown of glycogen and release of glucose increase the amount of glucose that is available between meals.
- Hence, glycogen serves as a buffer to maintain blood-glucose levels.
- Glycogen's role in maintaining blood-glucose levels is especially important because glucose is virtually the only fuel used by the *brain*, except during prolonged starvation.
- Moreover, the glucose from glycogen is readily metabolized and is therefore a good source of energy for sudden activity.

- Unlike fatty acids, the released glucose can provide energy in the absence of oxygen and can thus supply energy for anaerobic activity.

Isolation of glycogen
(part I)

– Procedure:

1. Weigh about 5.0 g of cold liver **quickly** to the nearest 0.1 g, transfer to a mortar, cut into small pieces, grind with about 0.5 g of clean cold sand and 10%TCA (1 ml per g tissue).

2. Centrifuge homogenate at 3,000 rpm for 5min at 4 ^{o}C. Pour off supernatant into a 50 ml graduated cylinder.

3. Rinse out mortar with 5% TCA (using same volume as for 10% TCA already used). Add this rinsing fluid to the centrifuge tubes containing residue from first centrifugation. Stir up residue and re-centrifuge for another 5 min. at 3,000 rpm. Discard pellet. Add supernatant to that already collected.

4. Record total volume; add the equal volume of 95% ethanol, slowly with stirring, to supernatant. Allow to stand while precipitate settles. If it does not, add a little NaCl and warm cylinder in water bath at 37º C.

5. Centrifuge suspension at 3,000 rpm for 3 min. Discard supernatant. Dissolve pellet in centrifuge tubes in 5 ml water and re-precipitate by adding 10 ml of 95% ethanol. Re-centrifuge and discard supernatant.

6. Stir up pellet with 3 ml 95% ethanol, re-centrifuge and discard supernatant.

- Now add 3 ml of diethyl ether, stir up pellet, re-centrifuge and discard supernatant. This final pellet contains glycogen from the liver. Air -dry the glycogen in the tube and weight it.

7. Dissolve 32 mg glycogen in 4 ml phosphate buffer/NaCl.

** Measure the glycogen content by measure the empty centrifuge tube and measure the centrifuge tube that contain pellet .

Glycogen content (g) =
centrifuge tube that contain pellet - empty Centrifuge tube

Result:

Record total yield and glycogen content per 100 g liver.

Example:

Liver weight 10 g

The glycogen content 1.5 g

** the glycogen yield was 1.5 g

** the glycogen content per 100 g is

1.5 g ⟶ 10 g liver

??g ⟶ 100 g liver

= 1.5 X 100 / 10

= 15 g /100 g liver

Method:

1) Make glycogen solution :

Dissolve glycogen pellet in **phosphate buffer**
(**32mg glycogen in 4 ml phosphate buffer**)

- Example:

I have 3.52 g glycogen ??

A- 3.52g X 1000 = 3520 mg

B- 32mg ⟶ 4 ml

3520mg ⟶ ??? ml

Notes:

[1] In initial stage of isolation glycogen the Temperature should be decrease (Cold) and the PH low : because these condition important to inhibit the enzymatic hydrolysis of glycogen (to protect the glycogen structure and getting good yield)

[2] The animal should well fed to get more glycogen yield and prevent any stress for animal to prevent the consuming of glycogen .

[3] The roles of Trichloracetic acid (TCA) to precipitate large mol. and lower PH .

[4] Ethanol to precipitate glycogen.