Experiment 1

Isolation of Glycogen from rat Liver

Amal Alamri
Figure 35: FIG-2, Liver, PAS, 100x. Note the presence of a few scattered glycogen granules (GG).
Objective

• To illustrate the method for isolating glycogen.
principle:

De proteinized homogenate of rat liver, then the glycogen is precipitated out by ethanol.
Materials and Equipments

- Trichloroacetic acid 5% and 10% TCA
- Ethanol (95% v/v)
- Diethyl ether
- Sodium chloride
- Liver (of well fed rat or other animal)
- Washed and dried sand
- Ice
- Mortar and pestle
- Refrigerated centrifuge
- Water bath at 37ºC.
- Glass rods
- Two 20 ml beakers
- One 100 ml beaker
- 100 ml graduated cylinder
- 50 ml graduated cylinder

*Caution! TCA causes severe burns; wash accidental spills on skin with plenty of running tap water for a minute.*
Sample Preparing

• The condition of the animal from which the liver is taken is important because the yield varies according to whether the animal is fed, fasted, ill etc. Therefore for a good yield the animal should be well fed before the liver is removed, the sample should be kept cold and the pH lowered with TCA.

• 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)
Sample Preparing

- When liver is ground up with tri chloroacetic acid (TCA), the larger molecules such as proteins and nucleic acids are precipitated while glycogen remains in solution with sugars and other water–soluble compounds.

- The glycogen can then be separated from the other compounds by precipitation with aqueous alcohol in which it is less soluble.
Procedure

• 1. Weigh about 5.0 g of cold liver, **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 5 min at 4°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.
Experiment 1: Isolation of Glucosylated from rat Liver

Record the weight before...
• 4. Record total volume; add twice this 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 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 weigh it.
Experiment 2: Enzymatic hydrolysis of glycogen and determination of glucose
Results

Glycogen content (g) = centrifuge tube that contain pellet - empty Centrifuge tube
• Record total yield and glycogen content per 100 g liver.

Example:
Suppose that the liver weight is 10 g and the glycogen yield was 1.5 g
Then the glycogen content per 100 g is

\[
\frac{1.5 \text{ g}}{10 \text{ g liver}} \times \frac{100 \text{ g liver}}{100 \text{ g liver}} = \frac{15 \text{ g}}{100 \text{ g liver}}
\]
Discussion

• Record total **Glycogen content (g) =**

• Record total **yield and glycogen content per 100 g liver.**
Question

Why are time, temperature and pH important in the initial stages of the isolation of glycogen, but not in the latter stages?
Enzymatic hydrolysis of glycogen and determination of glucose

Experiment 2

Glucose metabolism

Intake:

Starch

Glycogen

Disaccharides

Monosaccharides (glucose, fructose, galactose)

Storage:
Glycogen

Distribution and utilization:
Free glucose

Amal Alamri
The structure of the glycogen molecule is fan-like; with long chains of glucose residues linked by 1, 4-glycosidic bonds, with 1, 6-links at the branch points.

The whole glycogen molecule has only one free reducing end, where the C1 of a glucose residue is free (exposed). Thus the glycogen molecule is non-reducing.

Hydrolysis converts glycogen from a non-reducing substance into reducing substances.
• Hydrolysis with acid results in splitting of all glycosidic bonds giving only glucose molecules as the product.

• Enzymes are more specific in the bond type they split. Thus salivary amylase (α-amylase) will randomly split only 1, 4-glycosidic bonds and produce a mixture of products consisting of glucose, maltose and maltotriose molecules.

The increase in the number of reducing groups is determined using 3, 5-dinitrosalicylic acid (DNS) in alkaline solution.
Enzymatic Hydrolysis of glycogen by $\alpha$-amylases

- The amylases of animal origin are all $\alpha$-amylases and in the digestive system are found in saliva and in pancreatic juice.
- $\alpha$-amylases catalyze the rapid, random hydrolysis of internal $\alpha$-1, 4 bonds. They do not hydrolyze $\alpha$-1, 6 linkages, regardless of molecular size nor do they hydrolyze maltose.
- Further action of $\alpha$-amylase decreases the molecular weight of these dextrins yielding oligosaccharides. The final degradation products of the action of $\alpha$-amylase on glycogen are glucose, maltose and isomaltose.
Enzymatic Hydrolysis of glycogen by β-amylases

• β-amylases is widely distributed in plants and microorganisms. It catalyze the successive hydrolysis of the second α-1, 4 glycosidic bond from the free nonreducing ends of glucose chains, releasing maltose units.

• But β-amylases do not hydrolyze α-1, 6 bonds, nor do they hydrolyze α-1, 4 bonds of glucose chains beyond an α-1, 6 branch residues.

• the final products of the action of β-amylase on glycogen are maltose and the remaining limit dextrin.
Objective

• To examine the polysaccharide nature of glycogen and show that hydrolysis increases the number of reducing groups.
• To estimate the amount of glucose produced by hydrolysis of glycogen using DNS reagent.
Principle

In alkaline solution it is reduced to 3-amino-5- nitro salicylic acid, which is orange-red. Absorbance is determined at 540 nm.
Materials and Equipments

- Glycogen isolated in the previous experiment
- Sodium dihydrogen phosphate (NaH2PO4)
- Sodium hydroxide
- Sodium chloride
- Sodium potassium tartrate
- 3,5-Dinitrosalicyclic acid (DNS)
- HCl
- Boiling water bath
- Spectrophotometer
- Small beaker
- Big test tubes (25 ml)
- Glass cuvettes
Discussion:

Comment on the results and the concentration of glucose yield.
Question:

What would be the effect of substituting β-amylase for α-amylase?