BCH 447 Practical Metabolism

Acidic hydrolysis of glycogen

Objective

To examine the polysaccharide nature of glycogen and show that hydrolysis increases the number of reducing groups.

Structure of glycogen

- The structure of the glycogen molecule is **fan-like; with long chains of glucose residues** linked by α-1, 4 glycosidic bonds, with α-1, 6 links at the branch points.
- So, 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 **essentially non-reducing.**

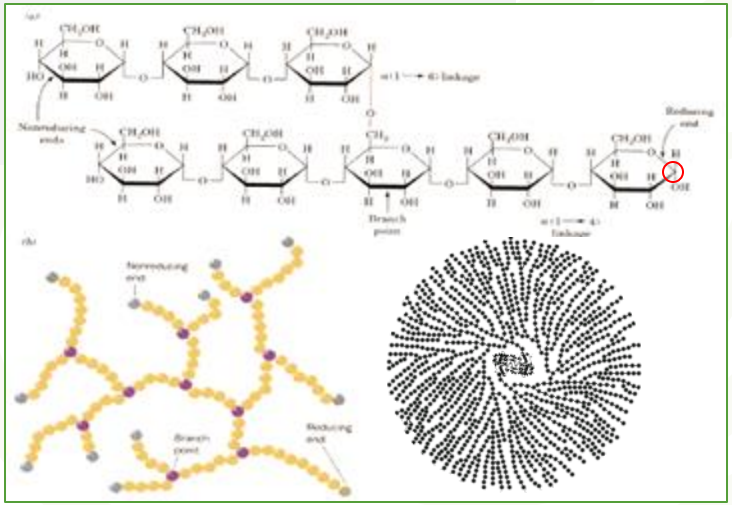


Figure 1. Glycogen structure

Hydrolysis of glycogen:

- Hydrolysis converts glycogen from a non-reducing substance into reducing substances.
- Hydrolysis of the glycogen molecule with acid results in splitting of all its glyosidic 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 α-1 ,4 glycosidic bonds and produce a mixture of products consisting of **glucose, maltose and maltotriose** molecules

Amylases

- Classified into two main groups, **α** and **β** according to the mode of their attack on the polysaccharide.
- The amylases of animal origin are all **α-amylases** and in the digestive system are found in saliva and in pancreatic juice.
- α-amylases catalyze the rapid, random hydrolysis of internal α-1, 4 bonds.
- Glycogen is initially split by α-amylase action into branched **dextrins** of medium molecular weight and only small amounts of maltose are formed.
- Further action of α-amylase decreases the molecular weight of these dextrins yielding oligosaccharides.
- The final degradation products of the action of **α-amylase** on glycogen are glucose, maltose and isomaltose.

There are many methods for measuring the **hydrolysis of glycogen** and other polysaccharides, such as measurement of reducing sugars, or change of decreasing viscosity and the loss of capacity to give a blue color with iodine

Practical part

Method:

2 **Addition of NaOH, *why ?***

Tubes	Diluted glycogen	PS buffer	HCl		Time of hydrolysis (min)	NaOH	PS buffer	DNS reagent		H2O
1	0.4 ml	---	0.6 ml	--	0	1 ml	0.5 ml	2 ml	*Boiling water bath for 10 min* ↓ *Cool down*	5.5 ml
2		---		*Boiling water bath in intervals of 4 min*	4 min					
3		---			8 min					
4		---			12 min					
5		---			16 min					
6		---			20 min					
7		---			24 min					
8		---			28 min					
9		---			32 min					
Blank	---	0.4		--	0					

1 **Acidic hydrolysis of glycogen**

Mix well (total volume 10 ml in each tube)

↓

Read the absorbance at 540 nm against blank

3 **Determination of reducing gp. no. by DNS**

Principle

- The increase in the number of reducing groups resulting from the hydrolysis is determined using 3, 5-dinitrosalicylic acid (DNS).
- In **alkaline** solution it is reduced to 3-amino-5- nitro salicylic acid, which is orange-red.
- **Absorbance is determined at 540 nm.**

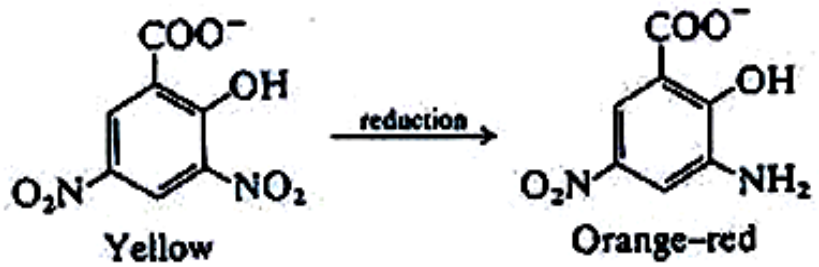


Figure 2. Chemical reaction of DNS

All reaction chemicals should be **handled with care**, you must wear **laboratory gloves mask** and **eye protection** glasses at all times

Results

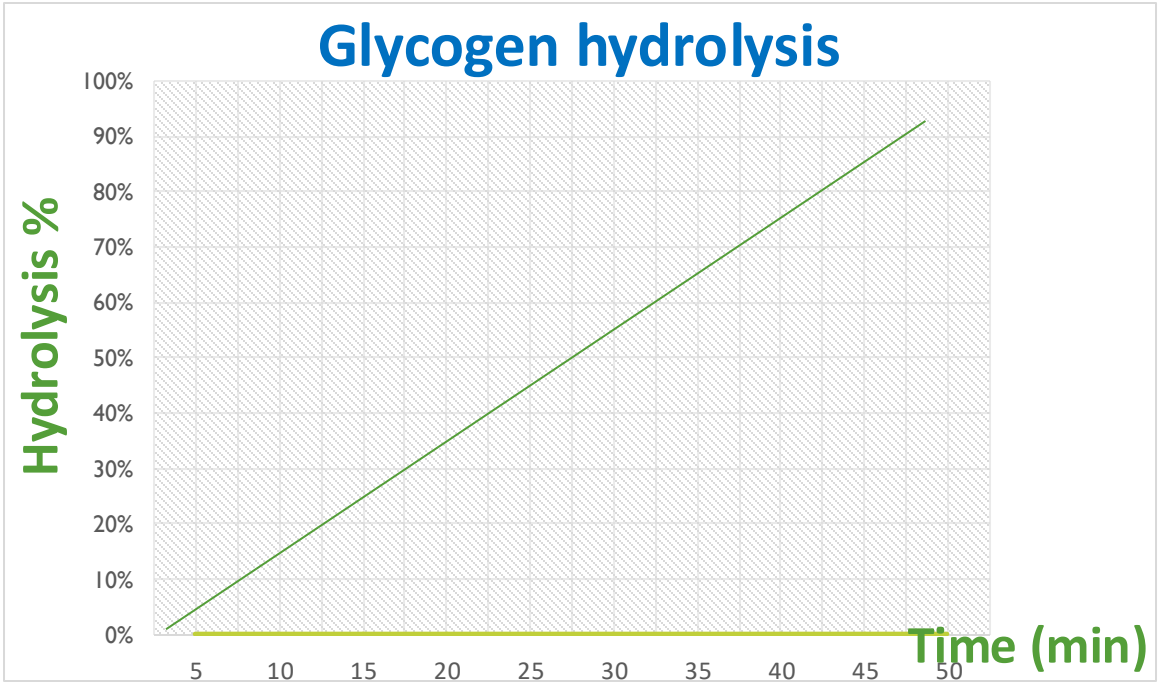
Tubes	Time (min)	Abs at 540nm	Hydrolysis %
1	0		
2	4		
3	8		
4	12		
5	16		
6	20		
7	24		
9	28		
10	32		

Hydrolysis %= (Abs of tubes (1, 2, 3, etc)/Abs of tube 10) * 100

Example:

- Abs of tube No. 10 = 2.024
- Hydrolysis % of tube No. 9 = (1.628 / 2.024) * 100 = 80.3%

Results



Note: Acidic Hydrolysis increases the number of reducing groups with increasing time

Discussion

Comment on the results and the concentration of glucose yield

Homework

1. Why is NaOH used in the protocol of acidic hydrolysis of glycogen?
   1-
   2-

2. Mention two different methods used to hydrolysis glycogen:
   1-
   2-

3. What are the factors that facilitate the hydrolysis of glycogen in this experiment ?
   1-
   2-