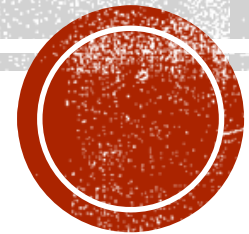


ACIDIC HYDROLYSIS OF GLYCOGEN



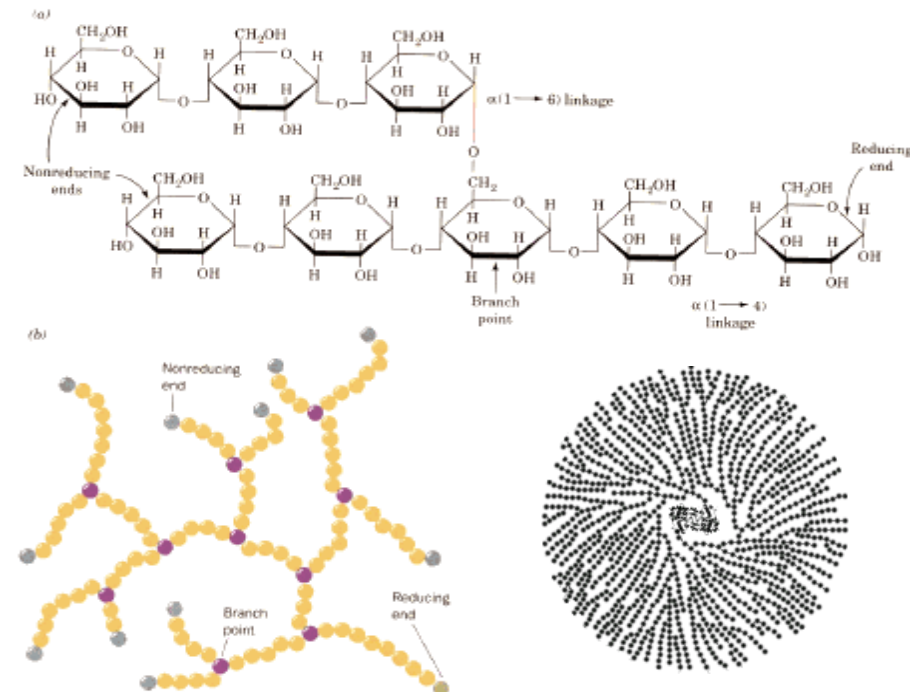
- OBJECTIVE:

- 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**.



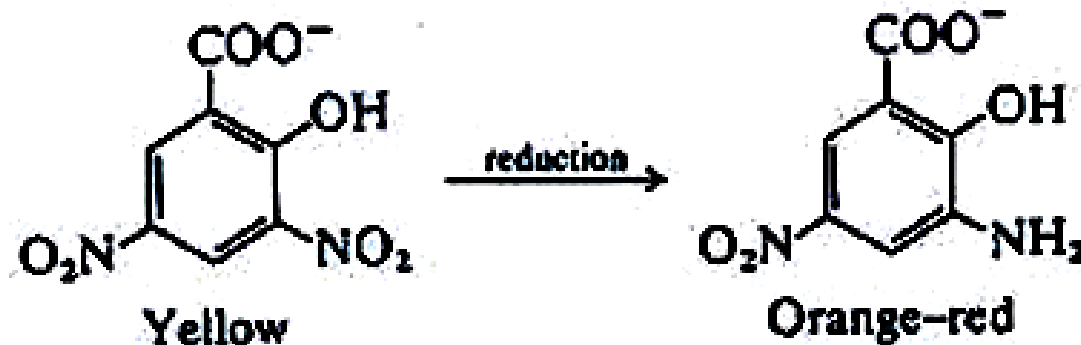
HYDROLYSIS OF GLYCOGEN:

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



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.**



ACIDIC HYDROLYSIS OF GLYCOGEN

Tubes	Diluted glycogen	0.05 mol/l PS buffer	2 mol/l HCl		Time of hydrolysis (min)	1.2 mol/l NaOH	0.05 mol/l phosphate buffer	DNS reagent		H2O
1	0.4	---	0.6	--	0	1 ml	0.5	2	Boiling water bath for 10 min ↓ Cool down	5.5
2	0.4	---	0.6	Boiling water bath in intervals of 4 min	4 min	1 ml	0.5	2		5.5
3	0.4	---	0.6		8 min	1ml	0.5	2		5.5
4	0.4	---	0.6		12 min	1 ml	0.5	2		5.5
5	0.4	---	0.6		16 min	1 ml	0.5	2		5.5
6	0.4	---	0.6		20 min	1 ml	0.5	2		5.5
7	0.4	---	0.6		24 min	1 ml	0.5	2		5.5
8	0.4	---	0.6		28 min	1 ml	0.5	2		5.5
9	0.4	---	0.6		40 min	1 ml	0.5	2		5.5
Blank	---	0.4	0.6	--	0	1 ml	0.5	2		5.5

Mix well (total volume 10 ml in each tube)



Read the absorbance at 540 nm against the blank sample (tube 10)

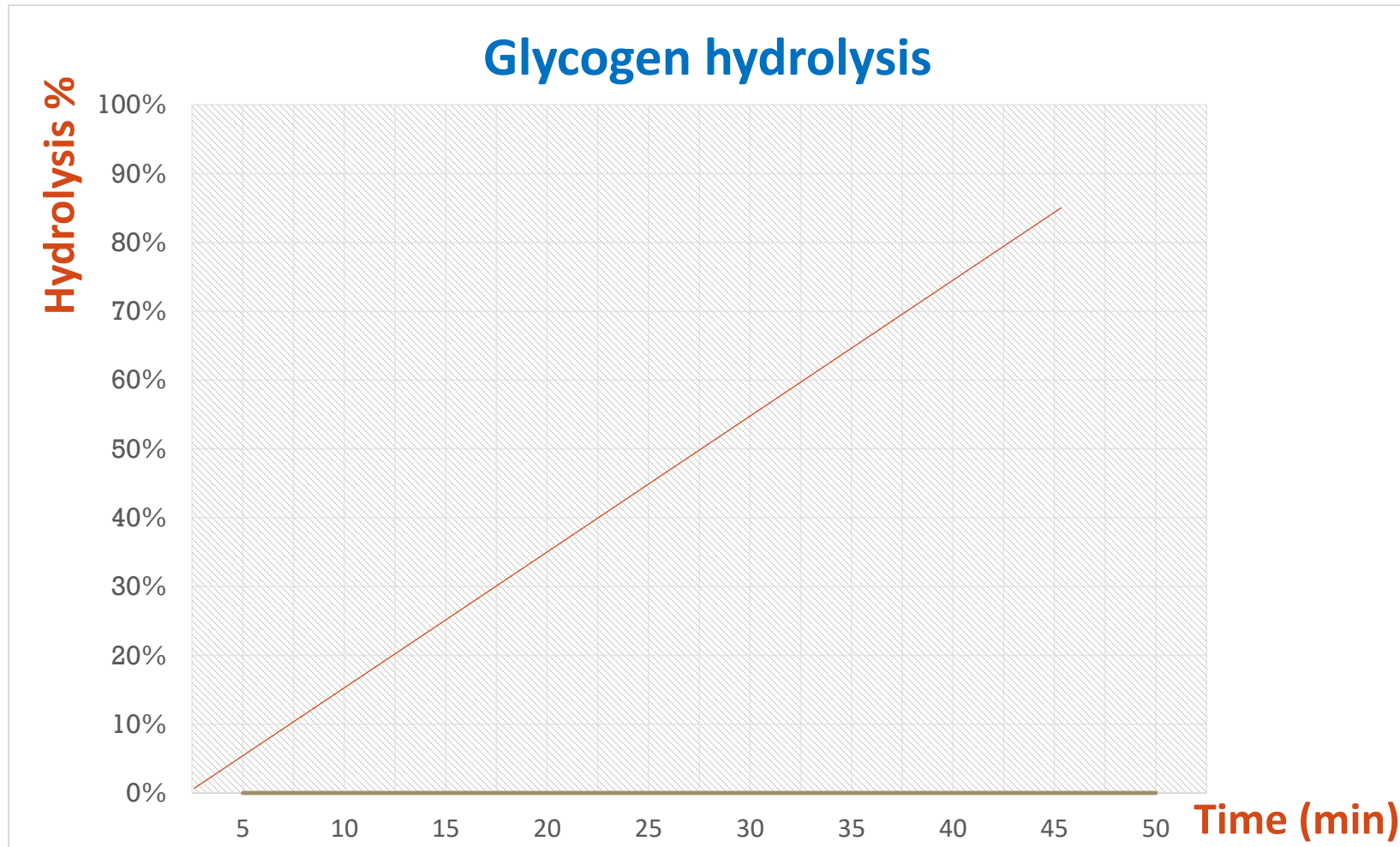
- RESULT:

Tubes	Time (min)	Abs (nm)	Hydrolysis %
2	4		
3	8		
4	12		
5	16		
6	20		
7	24		
9	28		
10	40		

- **Hydrolysis % = Abs x 100**
- **Example:**
- **Abs = 0.123**
- **Hydrolysis % = $0.123 \times 100 = 12.3$**



- RESULT:



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



- QUESTION:

Why is NaOH used in the protocol of acidic hydrolysis of glycogen?

