Determination of Optimum Conditions for α- Amylase Enzyme Activity

Dr. Nesrin Mwafi
Biochemistry & Molecular Biology Department
Faculty of Medicine, Mutah University

Effect of Temperature and pH

Procedure:

You will be provided with 0.1 U/ml of α-amylase.

Prepare the following tubes:

Component	1 (-ve)	2 (+ve)	3	4	5	6	7 (-20℃)	8 (boiling)
Starch (1%)	2 drops	2 drops	2 drops	2 drops	2 drops	2 drops	2 drops	**2 drops**
Distilled H_2O	2 ml	1 mL					1 ml	**1 ml**
α-amylase		1 mL	1 ml	1 ml	1 ml	1 ml	1 ml	**1 ml**
Buffer, pH 4.2			1 ml					
Buffer, pH 7.2				1 ml				
Buffer, pH 10.2					1 ml			
HCl (1N)						**1 ml**		

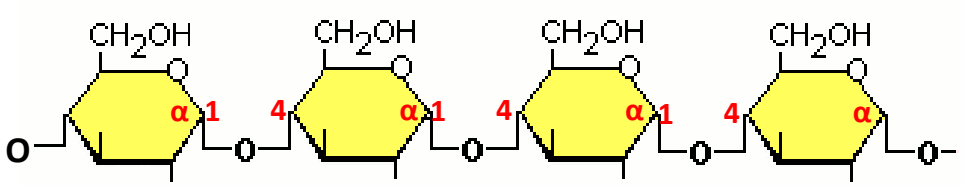
Optimum conditions: pH= 5.6-6.9 , 37 ℃ (body temperature)

Storage Polysaccharides

Starch: is the storage polysaccharides in plants.

- Polymer composed of glucose monomers
- a mixture of **amylose** (20%, water soluble) and **amylopectin** (80%, water insoluble) stored in plant cells as insoluble granules.

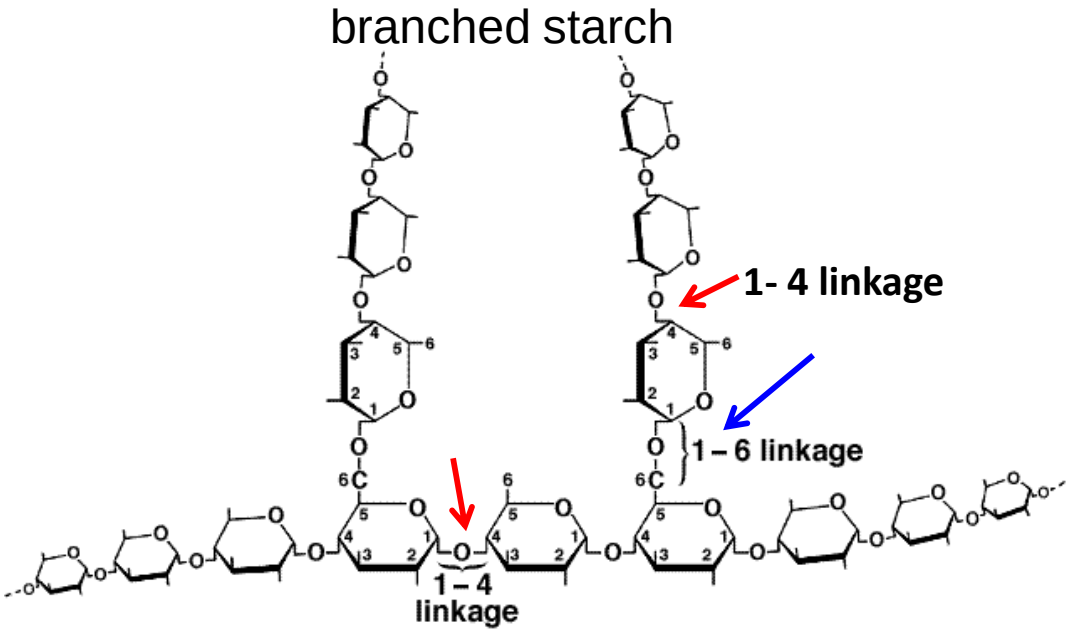
unbranched starch(linear)



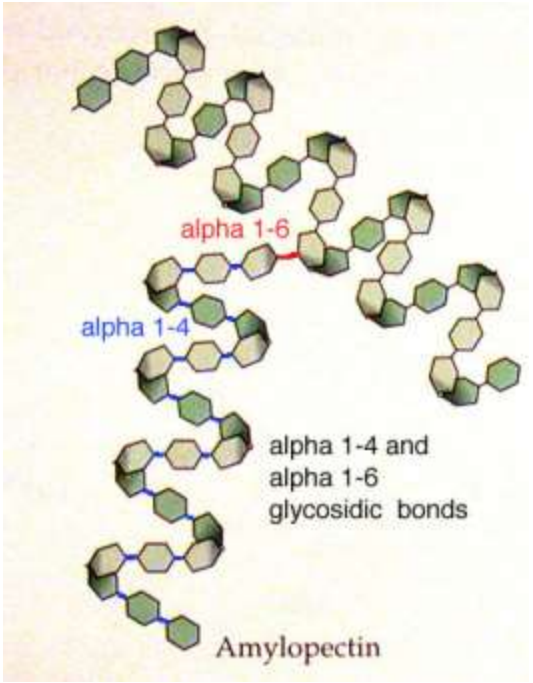
Amylose : α (1 ⟶4) glycosidic bonds

The helical structure of amylose

Storage Polysaccharides



Amylopectin: α (1 ⟶ 4) glycosidic bonds
with α (1 ⟶ 6) branch points (every 24-30 units)



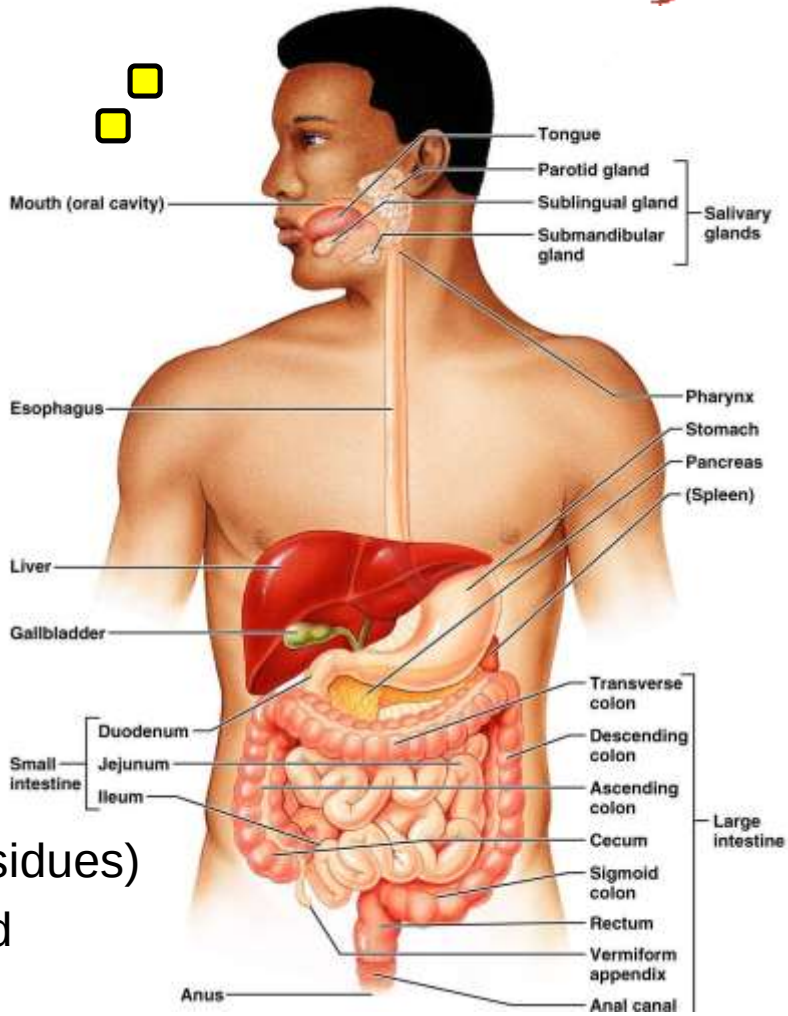
Digestion of starch

1. The salivary amylase enzyme randomly hydrolyses the α-(1⟶ 4) bonds

2. Starch digestion to small oligosaccharides continues in the small intestine by pancreatic amylase

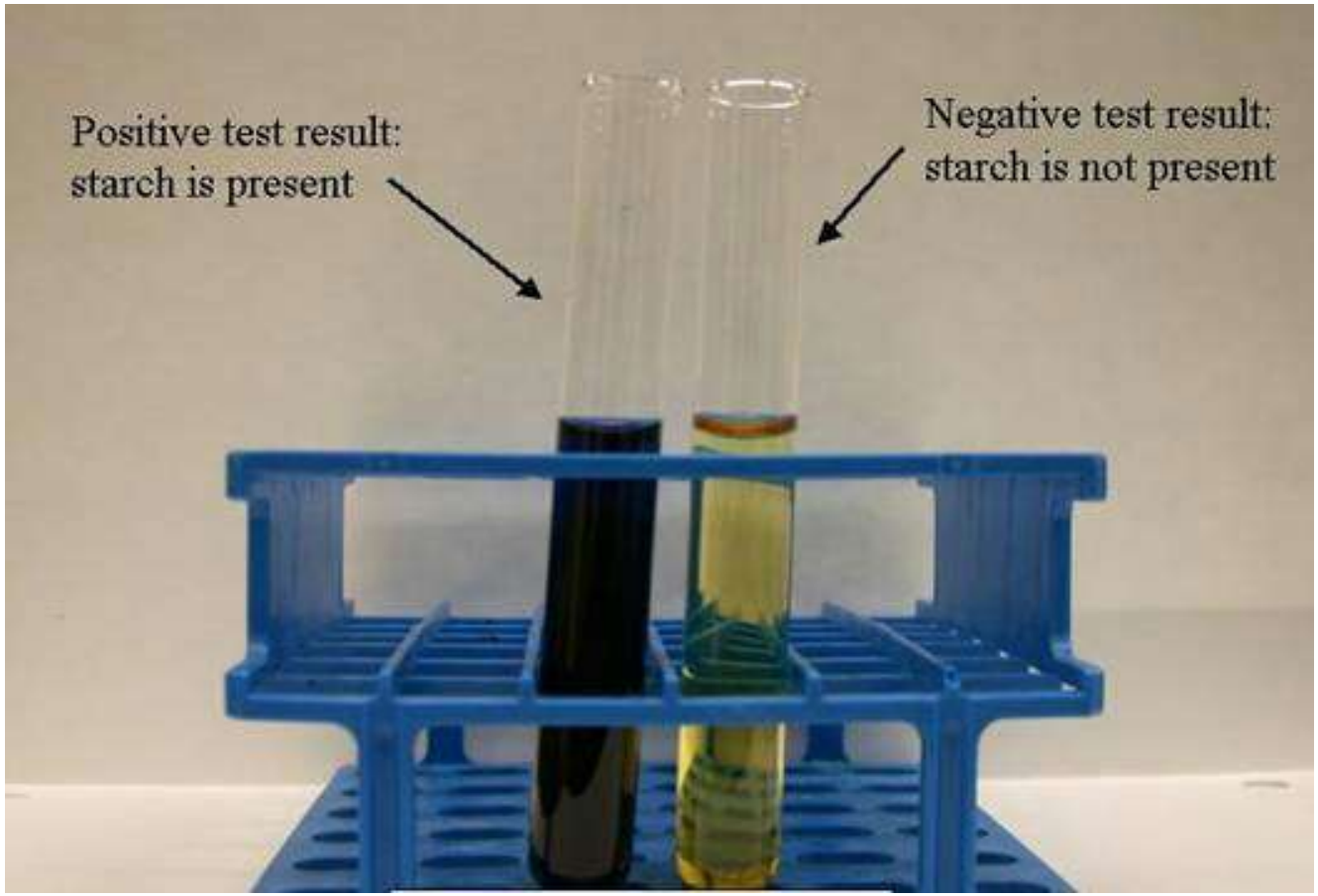
3. Further hydrolysis by α-glucosidase (which remove one glucose residue at time) and by a debranching enzyme (which hydrolyzes specifically α-[1⟶ 6] bond

4. The produced monosaccharides (glucose residues) are absorbed by the intestine and transported to the bloodstream



Iodine Test

Iodine Test

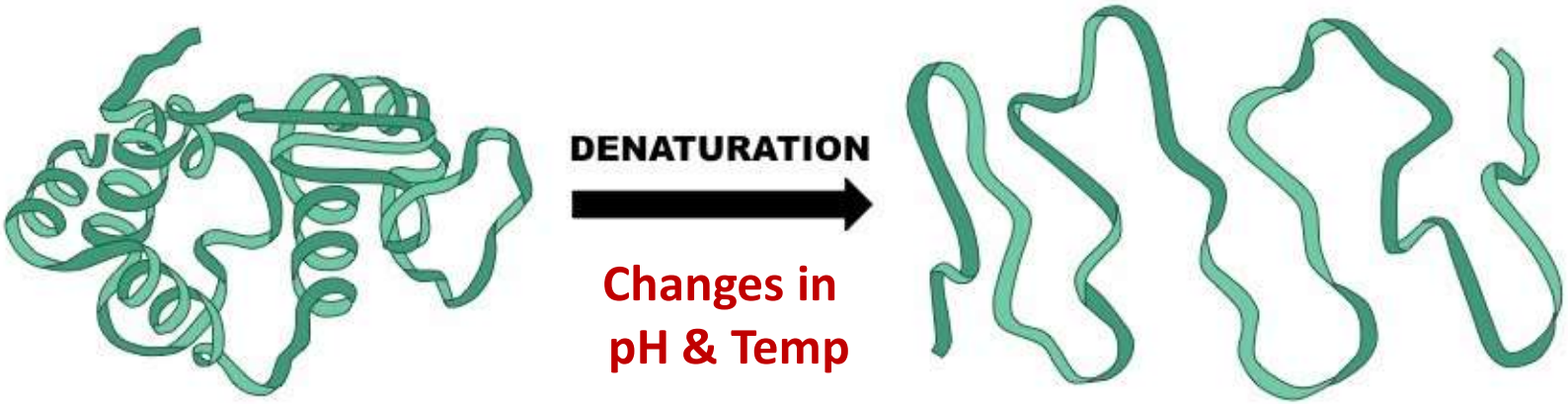

Positive test result:
starch is present
Negative test result:
starch is not present

α- Amylase Enzyme

- α -Amylase enzyme catalyzes the breakdown of starch to simple sugars
- Iodine test is used to track the digestion of starch by α – amylase enzyme
- Positive iodine test indicates the presence of starch (inactive enzyme)
- Negative iodine test indicates that the enzyme is active and degraded the starch to smaller units
- Boiling has irreversible effect on enzyme activity because high temperature causes denaturation of proteins (boiling destroys the bonds and harms the three-dimensional structure of the enzyme.
- Freezing has reversible effect on enzyme activity (inactivation of enzymes)

α- Amylase Enzyme

- Strong acids destroy the enzymes and breakdown the structure (denaturation)



Loss of biological activity

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