



Determination of Optimum Conditions for α - Amylase Enzyme Activity

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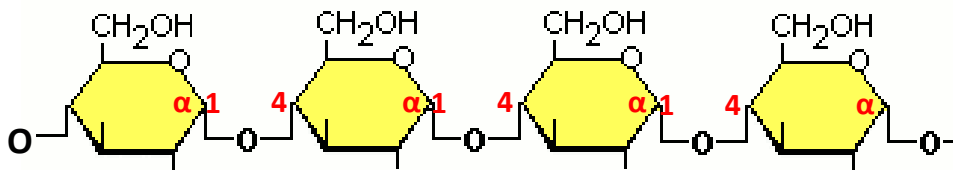


Storage Polysaccharides

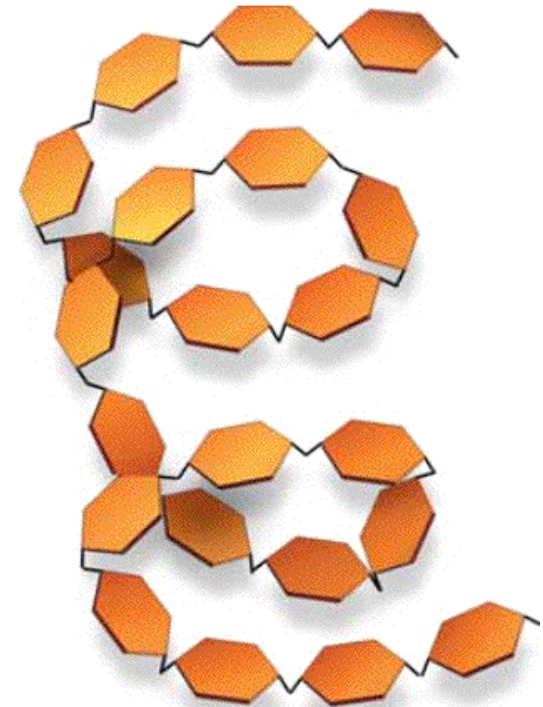
Starch: is the storage polysaccharides in plants.

- 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 \rightarrow 4) glycosidic bonds

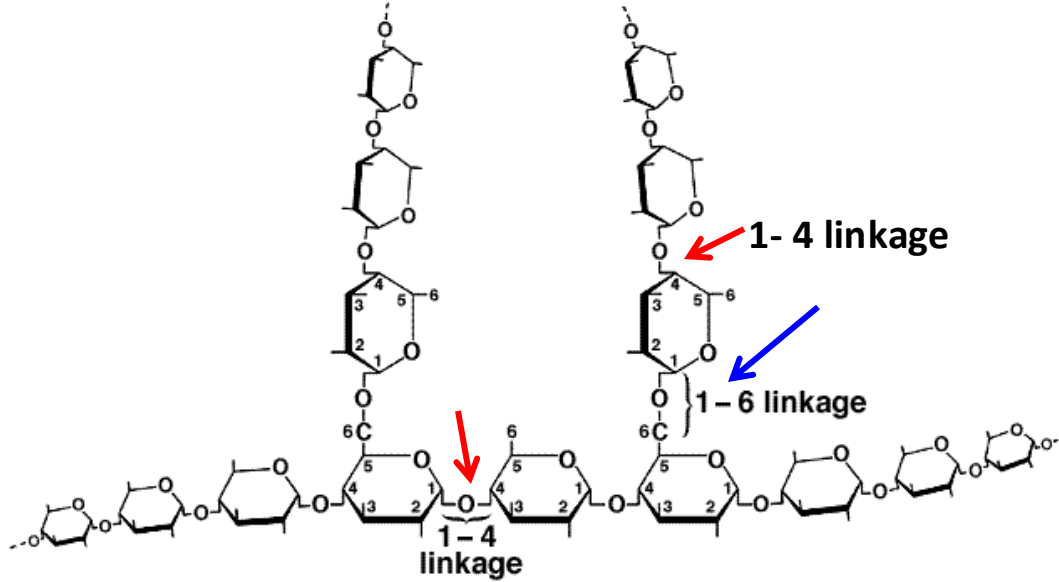


The helical structure of amylose

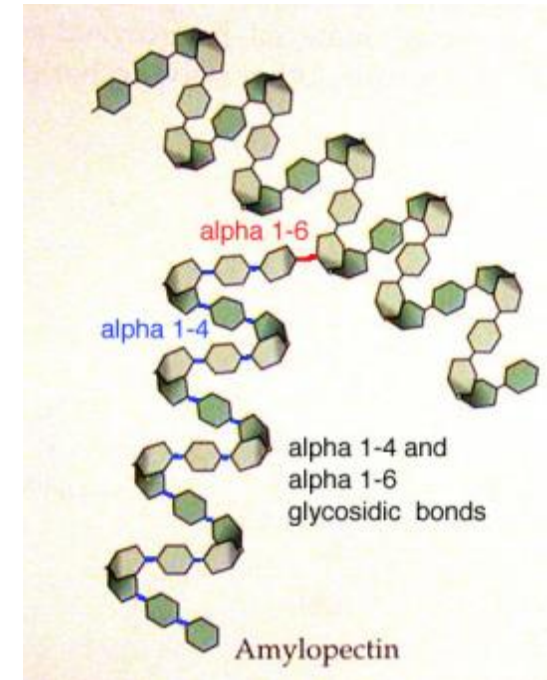
Storage Polysaccharides



branched starch



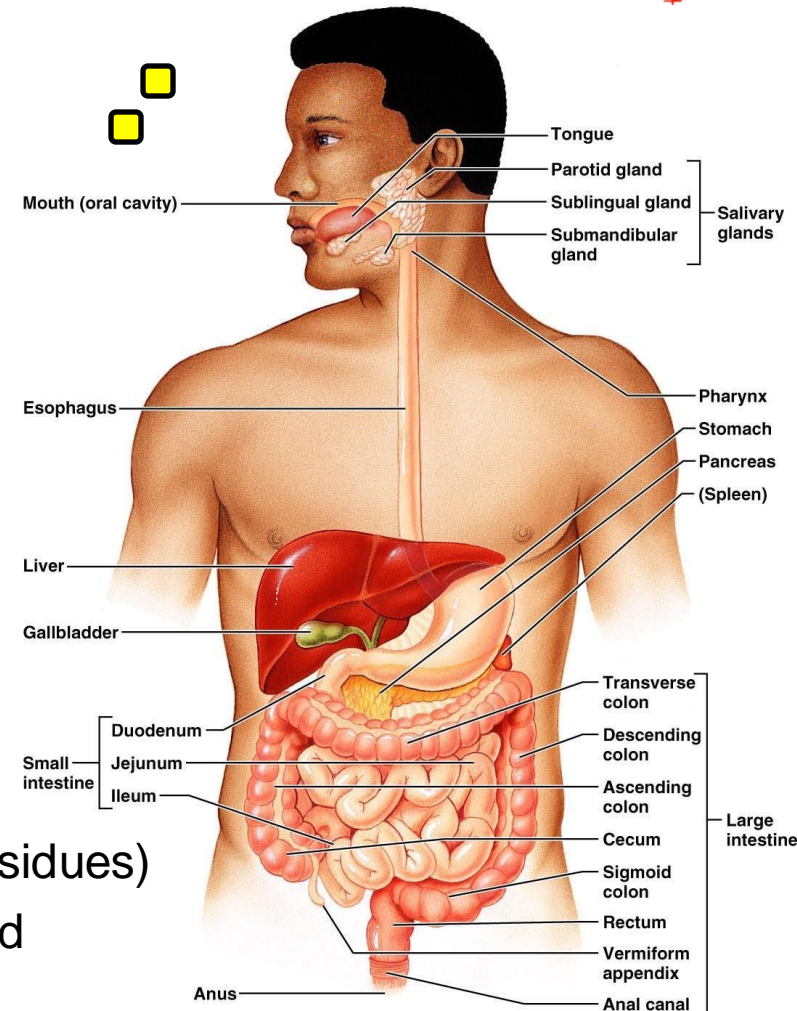
Amylopectin: α (1 \rightarrow 4) glycosidic bonds
with α (1 \rightarrow 6) at branch points



Digestion of starch



1. The salivary amylase enzyme randomly hydrolyses the α -(1 \rightarrow 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 \rightarrow 6] bond
4. The produced monosaccharides (glucose residues) are absorbed by the intestine and transported to the bloodstream



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 the starch was degraded 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)

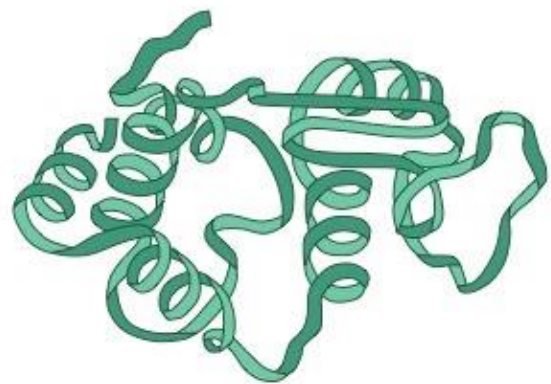
Iodine Test



α - Amylase Enzyme



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

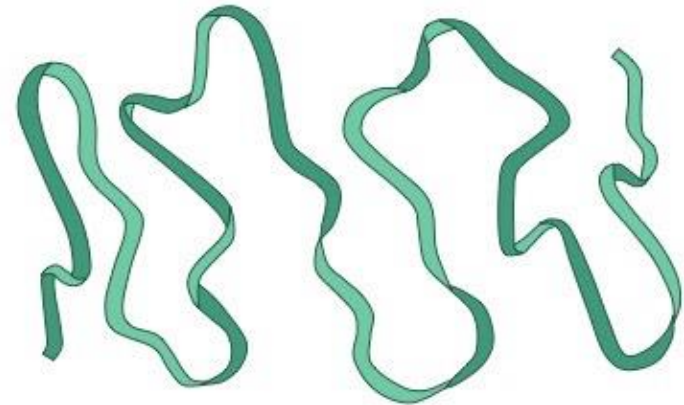


Folded Protein

DENATURATION



**Changes in
pH & Temp**



Unfolded Protein

**Loss of biological
activity**

Effect of Temperature and pH



Procedure:

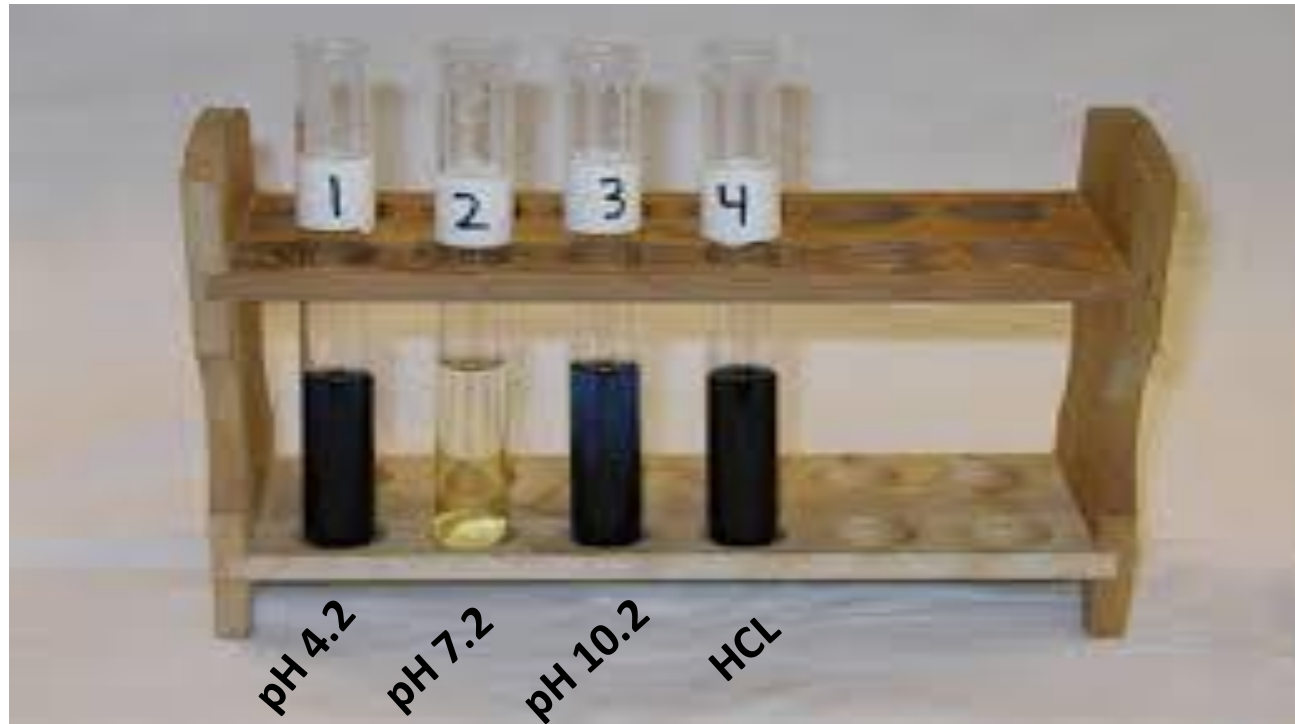
You will be provided with 0.1 U/ml of α -amylase.

Prepare the following tubes:

Component	1 (-ve)	2 (+ve)	3	4	5	6	7 (-20°C)	8 (boiling)
Starch (1%)	2 drops	2 drops	2 drops	2 drops	2 drops	2 drops	2 drops	2 drops
Distilled H₂O	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 °C (body temperature)

α - Amylase Enzyme



Iodine Test

