



Tishk International University
Faculty of Applied Science
Nutrition and Dietetics Department

Nutritional Biochemistry II Lab.

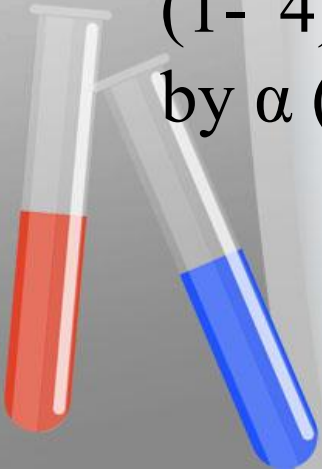
Quantitative Determination of α -amylase activity

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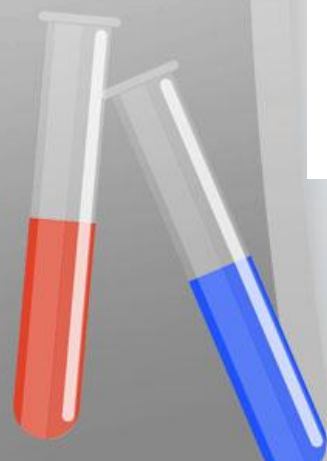
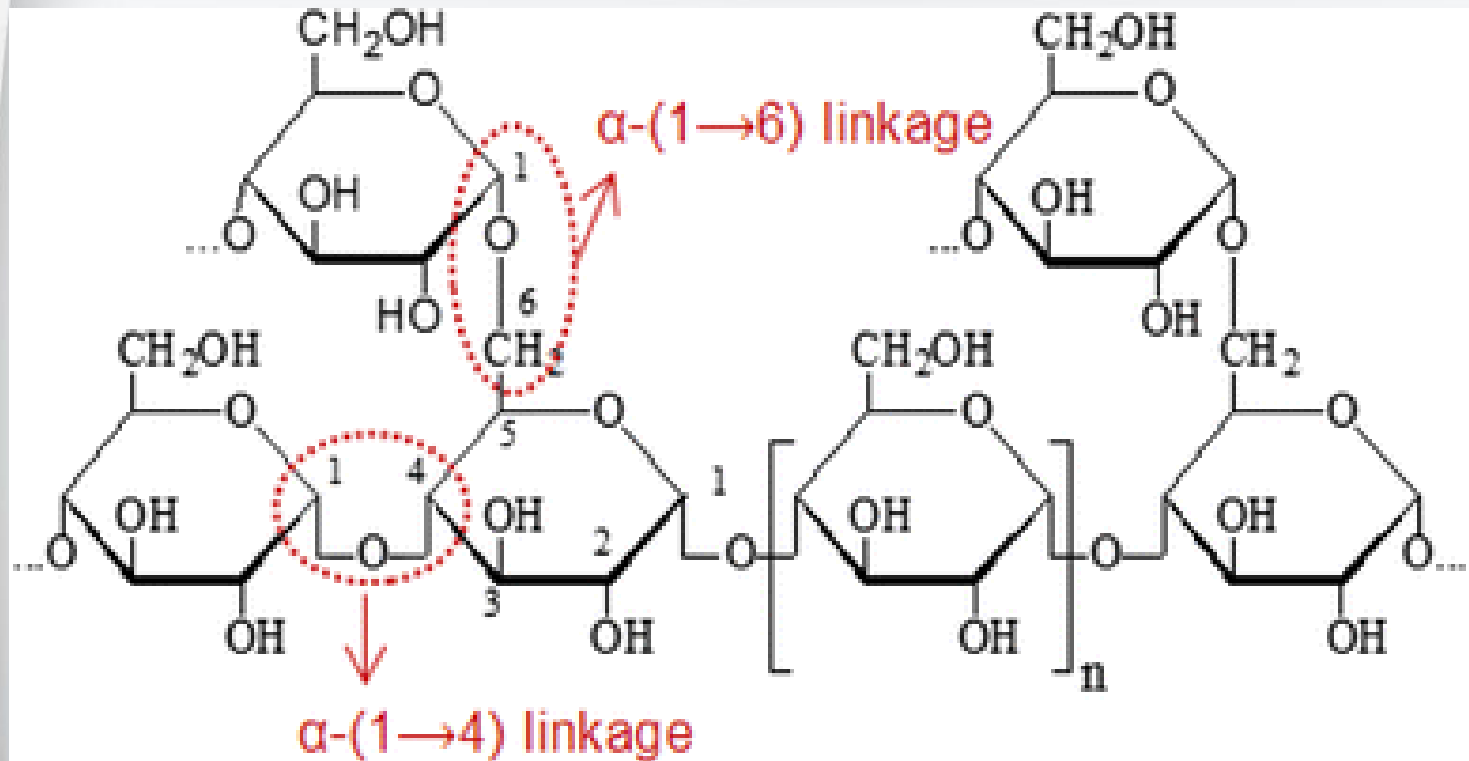
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α -amylase

- Amylase is an enzyme belonging to the class of hydrolases.
- It catalyzes (breaks down) the starch and glycogen.
- Starch consists of both **amylose** and **amylopectin**.
- Amylose comprises glucose molecules linking by α (1- 4) glycosidic bonds and amylopectin is linked by α (1 - 6) linkages at the branch points.

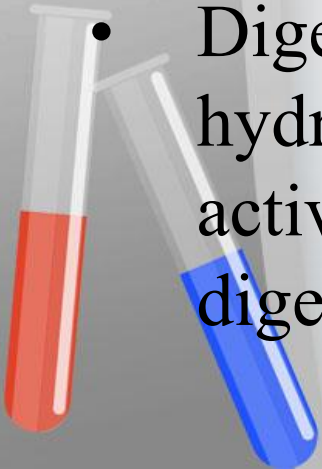


Starch structure



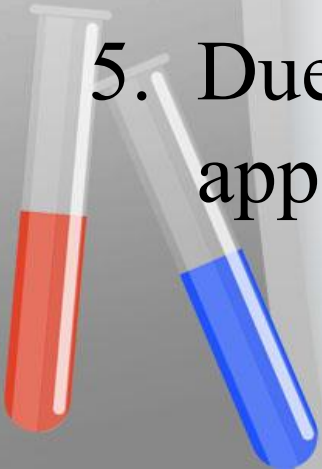
Sources of Amylase in the body;

1. Pancreas and the Salivary glands are the major tissue sources of serum amylase.
 2. Skeletal muscle , small intestine and the fallopian tubes lesser concentrations.
- Digestion of starch begins in the mouth with the hydrolytic action of Salivary , Salivary amylase activity pancreatic amylase then preform the major digestive action of starch .



Amylase properties;

1. It active in PH = 6.7 – 7.
2. It is peaceful at room temperature with one week.
3. It is active at 37 °C.
4. Activity increase with Ca_2^+ and some anions such as Cl^- , Br^- , NO_3^- , ClO_3^- , $\text{HPO}_4^{=}$.
5. Due to it's low molecular weight (50 K.D) it appears in the urine .



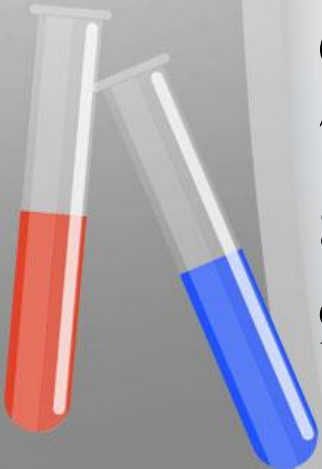
- A small amount of amylase in your blood and urine is normal.
- A larger or smaller amount may mean that you have a disorder of pancreas, an infection, alcoholism or another medical condition.
- An amylase blood test can determine whether you have a disease of the pancreas by measuring the amount of amylase in your body.
- You may have a disorder affecting the pancreas if your levels of amylase are too low or too high.



Clinical Significance;

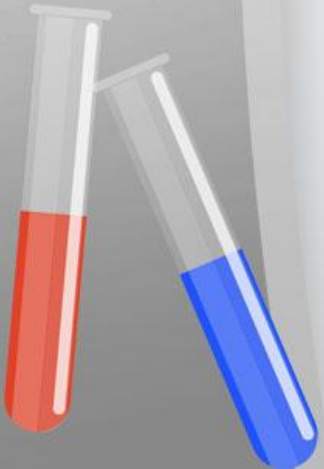
Amylase levels are increased in serum in many conditions

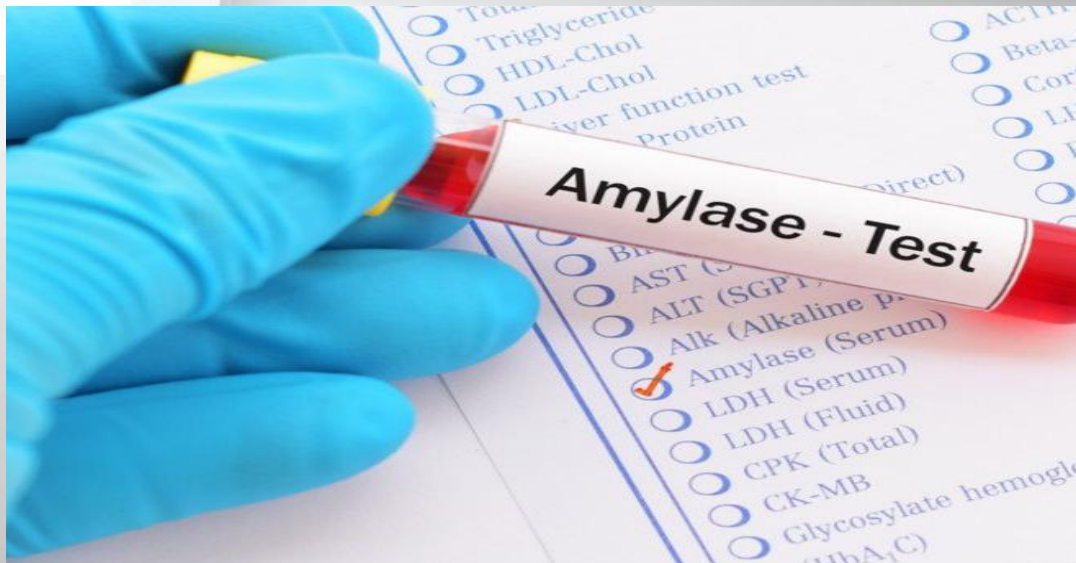
1. Pancreatic carcinoma.
2. Intestinal infection.
3. Intestinal obstruction.
4. Pancreatic trauma.
5. Hepatitis.
6. Liver cirrhosis.
7. Cholecystitis.
8. Diabetic keto acidosis.
9. Salivary gland lesions.



Expected Value:

- ✓ All ages
- ✓ Serum/Plasma
- ✓ Normal value (60-160 U/dL)
- ✓ Note: Normal value ranges may vary slightly among different laboratories.



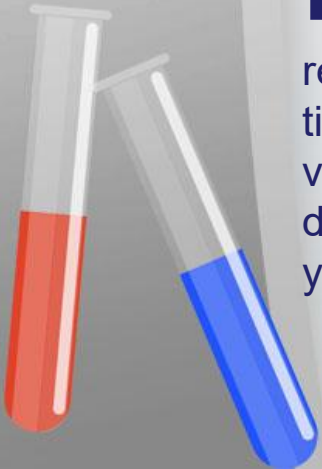


The material cleaning and drying are important factors for the stability of the reagents and to achieve accurate results.

Usage of deionized or distilled water for the preparation of reagents as well as the final glass washing .

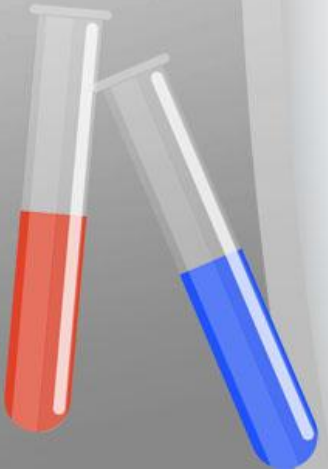
Since saliva is rich in amylase, it should be avoided the material and reagents to be contaminated with saliva.

NOTES In all enzymatic reactions, the rigorous observation of time and temperature of incubation is vital for the quality of the results. The difference of 1 min. on incubation time yields an error of 13.3% in the result.



Procedure

1. Let stand reagents and specimens at room temperature.
2. Pipette into the cuvette 1ml of the reagent.
3. Bring the to temperature 37 °C then:
4. Add 25 μ l from the specimen.
5. Mix and record the absorbance at 405 nm.
6. Record initial absorbance after 30 seconds, read the absorbance every 30 seconds during 90 seconds.



Calculation

- ✓ Calculate absorbance change per minute ($\Delta\text{Abs}/\text{min}$).
- ✓ $\text{IU}/\text{L} = (\Delta\text{Abs}/\text{min}) * 3178$

