Starch hyrolysis of amylase enzyme | experiment

Monosaccharides are most basic units of carbonhydrates. They are the simplest form of the sugar. Glucose, galactose, fructose and ribose are example for the monosaccharides. Disaccharide is form when two monosaccharides combined. Lactose and sucrose are example for the disaccharides.

Plants store glucose as the polysaccharide starch. Starch can be separated into two fractions–amylose and amylopectin. Natural starches are mixtures of amylose (10-20%) and amylopectin (80-90%). Amylose is a linear compound which is soluble in water. Also linked by α (1, 4) glicosidic bond. Amylopectin is branched compound which is not soluble in water. Also linked by α (1, 6) glicosidic bond. (1)

Amylase is an enzyme which present in human salvia. It breaks starch down into sugar. All amylases are glycoside hydrolases and act on α-1, 4-glycosidic bonds. It will start to denature at around 60C.

Spectrophotometer measures the transmission or absorption of liquids or solids as a function of wavelength. Spectrophotometer is used for 2 different purpose :

To determine the absorption spectrum of a pure substance in solution

To determine the concentration of a solution

% T = (I / I O) . 100 ABS = log 10 (100/ % T)

APPARATUS

Equipment

- Test tubes

- Pipettes
- Pasteur pipettes
- Beaker 250 mL
- Test tube rack
- Plastic cuvettes
- Spectrophotometer
- Heater
- Weighing dish
- Weight

Chemicals

Human salivary enzyme

Starch solution 20 g/L

HCl stopping solution, 0. 1N HCl

Iodine reagent stock solution(in aqueous solution)

Iodine : 5 g/l

KI : 50 g/l

Dilute to 1: 100

Potassium phosphate buffers

KH_2PO_4

$KH_2PO_4 . 3H_2O$

PROCEDURE

Preparation of 20 g/l starch solution

20g of soluble potato starch was mixed in approx. 50 mL of cold water.

The slurry was added to aprox. 900 mL of gently boiling water in a large beaker while stirring.

The gelatinized starch solution was mixed well and cooled to room temperature.

More water was added to bring the total volume to 1 liter.

Few drops of the starch solution was put on a glass plate. 1 drop of the iodine reagent was added and the deep blue color was seen.

Preparation of Enzyme solution

1 mL of salvia was diluted with 9 mL water. 60 mL of 0. 5 % NaCl solution was added .

Effect of the pH

0. 1 M pH buffer solutions was prepared ranging from pH= 4. 5 to pH= 9 in increments of one pH unit.

An equal volume of one of the above buffer solutions were added to 5. 0mL of the 20 g/l starch solution prepared in step 1 . The resulting solution was contained 10g/l of starch in a buffered environment.

The enzymatic digestion process was started by adding 1 mL of human salivary enzyme solution; shaked and mixed.

The hydrolysis reaction was proceeded for exactly 10 minutes at 25°C.

0. 5 mL of the reacted starch solution was added to 5 mL of the HCl stopping solution.(0. 1 N)

0. 5 mL of the above mixture was added to 5 mL iodine solution to develop color. Shaked and mixed.

The absorbance was measured with a spectrophotometer at 620nm. Buffer was used as a blank.

Effect of Temperature

The temperatures of the temporary water baths in 250 mL beakers was prepared and adjusted the temperatures ranging from 30°C to 90°C in increments for 20°C.

The starch substrate was prepared by diluting the 20g/l starch solution prepared in step 1 with an equal volume of pH= 7. 0 phosphate buffer solution. This results in a working starch concentration of 10 g/l. 5 mL of the starch solution was added to each of test tubes.

The temperature of each of the starch solutions was allowed to come to equilibrium with that of the water bath.

1 mL of human salivary enzyme solution was added to each of the thermostated test tubes to start the reaction. The raction was stopped after exactly 10 minutes and the starch content was analizied by following the procedures outlined in step 3.

CALCULATIONS AND OBSERVATIONS

DISCUSSION

In this experiment the purpose was to hydrolyze starch with amylase enzyme and observe the effect of pH and temperature on this reaction. First we prepared the starch solution, added 1 drop of the iodine reagent and saw that a deep blu color was developed. After that we prepared the enzyme solution which salvia was used. Then we looked to the effect of pH and effect of temperature. In effect of temperature buffer solutions were prepared which ranging from pH 4. 5 to pH 9. We used two different solutions because of the diffence buffering capacity of these two solutions. We used HCl and NaOH for pH. We used HCl to decrease pH and we used NaOH to increase pH also, we detected the pH by using pH meter. Each buffer with different pH values were mixed with starch solution and then salivary solution was added. Then we added iodine solution to detect whether reaction took place or not. Since if enzyme functions starch in the solution will be hydrolyzed and this will lead to have light color of the solution; absorbance will be low. Since our body is in neutral pH we expect to have light colored solution at pH 7 and dark color at pH 5, 8 and 9. The enzyme won’t work in higher temperature values that can denature it like 90, 70 and maybe 50. Protein’s absorbance values are expected to increase as the protein denaturizes. This can be explained by the surface of reflection of the light is increased. Denaturized form of protein has higher possibility to be interacted with the light from the spectrophotometer and thus absorbance will increase.