Sample: Biochemistry - Effect of an Acidic Fluid on Enzyme Activity

Effect of acidic fluid on enzyme activity

Each enzyme has a particular optimal pH required for its functioning. Within that pH value the enzyme performs the chemical reaction at the highest rate. Further increase in pH or drop in pH results in decrease in the activity of enzyme. As a result, chemical reaction, catalyzed by the enzyme, is carried out slower.

In the current experiment I wanted to investigate the effect of pH on catalase enzyme. It is an enzyme that allows the cell to get rid of the excess hydrogen peroxide. It splits hydrogen peroxide down into oxygen and water molecule. As far as catalase is present within peroxisomes, its optimal pH should be equal to the pH value of solution inside the peroxisomes. Peroxisomes have a pH value between 6.9 and 7.1. Thus, the hypothesis was that the highest rate of enzyme activity would be observed within the neutral pH values while pH values lower than 7.0 will lead to decrease in the rate of reaction catalyzed by catalase.

First of all, solutions of different pH value were prepared. Hydrochloric acid of different concentrations was used to create acidic pH. Five flasks were used for the experiment. Concentration of each of the solutions was measured with the help of litmus papers. pH values of 3, 4, 5, 6 and 7 were reached. Each of the flasks was labelled according to the pH value of the solute inside it. It was very important to add the same amount of solute to each flask. The volume of solute in each flask was 5 cm^3.

Then, catalase was isolated from the sample. Catalase is present in the significant amounts in liver. Thus, about 1 cm cubed in size (unprocessed and uncooked) liver pieces were used for the experiment. 10 pieces of liver were ground with water (about 20 cm^3) and a little sand. This process led to the breakdown of liver cells. As a result, they released their content

and catalase was within it. The resulting mixture was filtered in order to get rid of the extracellular material and fibber. The resulting filtrate was equally divided between the test tubes with solutions of different pH values. 1cm^3 of hydrogen peroxide was added to each tube.

Then, a small apparatus was created in order to make the results not just qualitative but quantitative. Each test tube was closed with a cork containing a thin tube within it. The tube entered the solution in each tube with its one end and the second end was submerged into a box filled with water. The tube's end was present in the graduated test tube present in that water in the upside down positions. The initial water level within that tube was marked. Thus, any gas release could be detected and approximate volume of gas released could be measured. The gas was to move from the solution containing liver extract to the box with water. There, it had to cause an increase in water level within the upside down placed test tube. That increase was detected and the volume of gas released was calculated.

Gas production was caused by catalase. It broke down hydrogen peroxide present within the test tube into water and oxygen. Oxygen was released in the form of bubbles. Calculation of the amount of bubbles produced per minute could be used as a way of determining the enzyme activity.

In the result, the following was observed: high-rate bubbling took place in the test tubes containing the solutes of pH 6.0 and 7.0. Some lower rate of bubbling was observed in the test tubes with pH value of 5.0. No bubbling at all was present in the test tubes with the pH values of 4.0 and 3.0. The amount of gas released was also measured. In the case of tube with ph 7.0 the volume of gas released was 0.15cm^3. In the case of tube with pH 6.0 the volume of gas released was 0.1 cm^3. In spite of the equal high rate of gas release in tubes with pH 6.0 and 7.0,

the actual volume was different. Thus, human visual analyzer is not an all-sufficient tool in research results measurement. The change in volume was not detected in any other tube.

Thus, the highest rate of bubbling and volume of gas released in the test tube containing the solution of pH 7.0 indicate that catalase works at the highest rate in this tube. A gradual decrease in both rate of gas production and gas volume is observed in the case of decrease in pH with respect to 7.0.

Thus, enzyme activity was inhibited by the presence of acid in the solution. In order to explain the effect of acid on the rate of enzymatic reaction we should remember that enzymes are proteins. Each protein must maintain its three-dimensional shape in order to perform its functions properly. Low pH means that hydrogen ions concentration is high. Thus, hydrogen ions present in the solution may compete with the slightly positive hydrogen atoms of the protein molecule for the ability to bind with electronegative atoms of the molecule. As a result, both the secondary and tertiary structures of the protein are damaged. If the enzyme has a quaternary structure, the interaction between the subunits can be disturbed. Therefore, in the presence of hydrochloric acid the three-dimensional shape of catalase was damaged. As a result, catalase was unable to bind with its substrate – hydrogen peroxide, and perform catalysis. As a result, the rate of reaction was low in the case of acidic solution.

In addition, high concentration of hydrogen ions can disturb ionic bonds. The negatively charged carboxyl groups of some amino acids radicals can acquire the hydrogen ion and become deionized. In such condition they can't interact with the positively ionized groups of the other amino acids. Thus, the tertiary and quaternary structures of the enzyme can be damaged. Besides, sulfhydryl groups can't form the disulfide bond under such conditions. High

hydrogen ions concentration causes the sulfhydryl groups to be in the protonated condition that does not allow them to interact with each other. Thus, tertiary structure of the protein is disturbed.

Therefore, even covalent bonds can be destroyed in the case of too low pH. Thus, enzyme loses its shape completely and may even fail to perform its functions after the renewal of the normal pH value. Denaturation is said to be irreversible in that case.

Thus, my hypothesis was supported by the experimental data. The activity of catalase was the highest when pH vale was 7.0 and it gradually decreased with the decrease in pH value. Therefore, enzyme activity depends on pH and optimal pH for the enzyme functioning is determined by the environment in which it usually works. Enzyme within peroxisomes has an optimal pH value of 7.0 because such pH is present within the peroxisomes. At the same time, enzyme present in lysosomes will have a lower optimal pH value as far as lysosomes have an acidic solution.