Free the role of enzyme in biological reactions report example

Environment, Water

Introduction

Biological reactions are those reactions that are involved in various activities, in the body including energy production. Reactions that produce energy are called exergonic reactions while those that use energy are called endergonic reactions. Enzymes enable chemical reactions to take place within a living system most of which are under a homeostatic constraint. In these reactions, enzymes function as a catalyst by lowering the activation energy necessary in a reaction (Cooper, 2000). Enzymes bring the reactants involved in a reaction closer together weakening the chemical bonds and this enable the reactions to occur faster compared to reactions that have no enzyme (Farabee, 2010).

Enzymes are proteins in nature, and their functioning is highly dependent of the shape of the protein structure making the enzyme. The reactants bind to a site in the enzyme known as an active site. This binding is a very specific interaction and one enzyme can only bind one or similar molecules (Cooper, 2000). Other components that facilitate the activity of the enzyme include cofactors and coenzymes. Cofactors refer to the nonproteins such as ions that are useful in the activity of the enzymes. Coenzymes, on the other hand, are nonprotein organic molecules that are bound to enzymes such as NAD (nicotinamide adenine dinucleotide) and are also helpful during an enzymatic reaction (Farabee, 2010).

There are several factors that affect the rate at which an enzymatic reaction takes place. These factors include temperature, pH, enzyme concentration, as well as the concentration of the substrate and the product. Increasing the temperature of a reaction mixture increases the rate of at which an

enzymatic reaction occurs but only to a given point. When the temperature is too high, enzymes being protein in nature become denatured. When the temperature is low, the protein nature of the enzyme causes them to be inactivated, and this reduces the rate of reaction. Increase in substrate concentration while a decrease in substrate concentration reduces the rate of an enzymatic reaction. High product concentration offers a negative feedback mechanism reducing the rate of reaction. Low product concentration, on the other hand, enhances the rate of production. Extremely low or high levels of pH result in total loss of enzyme activity. The level of pH also determines the stability of enzymes and the optimum pH is necessary for an enzyme to work effectively. Changes in pH levels reduce the rate of reaction by altering the shape of the active site (Taiji, Yasutaka, Katsuya, & Masaru, 1969).

This experiment aimed to detect the enzyme activity of α-amylase on polysaccharide starch and the effect of pH and temperature on enzyme activity.

Methods

Detecting Enzyme Activity for the polysaccharide starch of α-amylase

In this experiment, 1% (w/v) solution of polysaccharide starch was used as the substrate. A series of five reactions with different concentrations of substrate and enzyme was set up. A blank to be used to zero the spectrophotometer was also prepared using water and DNSA reagent. Six tubes were obtained and labeled 1 to 6 using masking tape and a marker pen. Labeling was done on top of the tube where the light from the

spectrophotometer would not pass through the tube. The appropriate number of drops of distilled water was transferred to the appropriate tubes. Appropriate number of drops of substrate solution was transferred to the appropriate tubes. Time was noted, and the number of drops of enzyme added appropriately. The tubes were allowed to incubate at room temperature for 10 minutes after which 5 drops of DNSA solution were added quickly to each of tube 1 to 6 to stop the α-amylase reaction. The tubes were placed in the hot block set at 80-90OC to allow the product and DNSA reagent to react developing a red color. The tubes were carefully removed from the hot block and placed in a test tube rack. Using a 5mL pipette, 5ml of distilled water to each tube and the content mixed by inverting the tubes. The absorbance of the samples was measured using a spectrophotometer at 540nm.

The Effect of pH and Temperature on Enzyme Activity

In this experiment, α-amylase was exposed to extremes of pH and temperature to determine the effect on enzyme activity. Six tubes were collected in a test tube rack and labeled 1 to 6 on top using masking tape and a marker. In tube 1 (blank), 15 drops of distilled water were transferred, and in tubes 2, 3, and 4, five drops of distilled water were added. In tubes 5 and 6, no distilled water was added. In tube 5 and 6, 5 drops of 0. 1M HCl and 0. 1M NaOH were added respectively. In tubes 2-6, 5 drops of enzyme were added. Tube 3 was placed into the ice bucket and tube 4 in a hot block set at 80-90OC for 5 minutes. The other tubes were left at the bench at room temperature for 5 minutes incubation period. After 5 minutes, time was

noted, and 5 drops of 1% starch solution added to tubes 2-6. After 10 minutes, 5 drops of DNSA solution were added to all of the tubes. All the tubes were heated for 5 minutes in the 80-90OC water bath to develop the color. The tubes were placed in a test tube rack and 3mL of distilled water placed to each tube. The content in the tubes was mixed by inverting the tubes. A piece of parafilm was placed over the mouth of the tube while inverting. Using tube 1 (blank), the spectrophotometer was zeroed at 540nm and the absorbance of each of the tubes measured.

Results

Detecting Enzyme Activity for the polysaccharide starch of α-amylase

The absorbance data from each of the tubes in the detection of enzyme activity of α-amylase on polysaccharide starch were as shown in Table 1 below. Absorbance average in blank tubes was 0, and the tubes that had no enzyme had an average absorbance of 0. 0042. The tube with a doubled amount of enzyme had an average absorbance of 0. 47. The difference between the absorbance between the tubes that had no enzyme and those that had enzymes was statistically significant ($t = 19.7$; $df = 10$; $P < 0.05$). On the effect of substrate concentration on activity of an enzyme, tubes that had no substrate has an average absorbance of 0. 033, those with a 1Xsubstrate had an average of 0. 33 and those with a 2Xsubstrate had 0. 37 average absorbance. There was a significant difference between the absorbance measured in tubes with no substrate from those that had a 1Xsubstrate ($t = 7.59$; $df = 10$; $P < 0.05$) and from those that had 2Xsubstrate content ($t = 8.67$; $df = 10$; $P < 0.05$). However, there was no

significant difference between the absorbance measured in tubes with 1Xsubstrate from those that has 2Xsubstrate (t = 0. 58; df = 10; P < 0. 05).

The Effect of pH and Temperature on Enzyme Activity

The absorbance data from each of the tubes on the effect of pH and temperature on enzyme activity of α-amylase on polysaccharide starch were as shown in Table 2 below. Absorbance average in blank tubes was 0 and the tubes that had optimum conditions had an average absorbance of 0. 33. The tube placed under low temperature had an average absorbance of 0. 19. The difference between the absorbance between the tubes that had optimum conditions and those that were under low temperature was statistically significant (t = 2. 83; df = 10; P < 0. 05). The tube placed under high temperature had an average absorbance of 0. 0438. The difference between the absorbance between the tubes that had optimum conditions and those that were under high temperature was statistically significant (t = 8. 106; df = 10; P < 0. 05). On the effect of pH level on activity of an enzyme, tubes that had low pH level had an average absorbance of 0. 012 while those that had a high pH level had an average absorbance of 0. 0132. There was a significant difference between the absorbance measured in tubes with optimum conditions from those that had a low pH level (t = 10. 4; df = 10; P < 0. 05) and from those that had low pH level (t = 10. 31; df = 10; P < 0. 05).

Discussion

Amylase refers to an enzyme that works to catalyze starch to form sugars and is mainly found in human saliva. The enzyme works best under a neutral

pH (pH6-7) and body temperature (Worthington Biochemical Corporation, 2010). Presence of inhibitors in a reaction mixture may also affect the rate at which amylase enzyme works (Koukiekolo, Desseaux, Moreau, Marchis-Mouren, & Santimone, 2001)

The experiment aimed to detect the enzyme activity for the polysaccharide starch of α-amylase and the effect of pH and temperature on enzyme activity. There was a great reduction in the amount of absorbance that was observed in the tubes that did not have an enzyme compared to those that had an enzyme. This is an indication that enzymes play a vital role in accelerating the rate of a reaction. Absence of an enzyme causes the reaction to slow down while its presence catalyzes a reaction. The tubes that had no substrate had very low absorbance since the enzyme did not have any substrate that it can work on. Addition of substrate increased the amount of absorbance detected since the enzymes were able to work on the available substrate. Increasing the amount of substrate in the reaction did not increase absorbance reading significantly. This may have been as a result of the enzyme added being the limiting factor when substrate is excess.

For an enzyme to work optimally, factors such as pH and temperature need to be at their optimum levels. The tube that had optimum temperature and pH gave very high absorbance readings. However, the tubes that were placed in ice had an absorbance that was significantly lower than the ones under optimum temperature. This may have been as a result of enzyme inactivation when placed in temperatures lower than their optimum temperature. Similarly, absorbance in tubes that were placed in hot

conditions gave low absorbance since the enzymes were denatured and thus not able to catalyze the reaction.

The tubes that were placed in low pH and high pH had lower absorbance compared to the ones under optimum pH level. High pH or low pH are known to interfere with the three dimensional configuration of the enzymes affecting the shape of the active site that is involved with the catalytic activity. This reduces the activity of the enzyme significantly.

In conclusion, this experiment showed that substrate concentration, pH level and temperature level affects the rate at which an enzyme catalyzes its reaction. It can also be concluded that the presence of an enzyme in a reaction significantly increases the rate of a reaction. Enzymes, therefore, have a significant role in biological reactions.

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