Good example of report on enzymes

Business

(City, State)

Abstract

Cellobiase is an enzyme that is involved in hydrolyzing the products of the exocellulase (cellobiose) to form individual monosaccharides. The process breaking down cellobiose is a natural process and helps most organisms produce glucose for use as a source of food. This experiment aimed to study the cellobiase by determining its rate of reaction and effects pH, temperature, concentration of the substrate or enzyme on the rate of reaction. The initial rate of a reaction catalyzed by cellobiase enzyme is 11. 25nmole/Minute with the optimum temperature being 37OC and the optimum pH was pH5. The activity of the enzyme is also affected by the substrate concentration.

Introduction

There are numerous processes that take place in a biological system. These activities are involved in different activities such as locomotion, as well as energy production. These processes occur at a very low rate in the absence of catalysts, which are known as enzymes. Presence of enzymes in a biological process acts to speed up the processes by lowering the activation energy necessary in a reaction (Cooper, 2000). The substrates involved in a reaction are brought closer together by the enzyme weakening the chemical bonds. Weakening of the bonds enables the rate of reaction to take place faster than in a reaction that is not catalyzed (Farabee, 2010).

Cellulases are enzymes that are produced mainly by bacteria, fungi, as well as protozoans and are useful in catalyzing the cellulolysis process (Peiji, 1987; Percival Zhang, et al., 2006). Other types of cellulases are produced

by other organisms, like termites and some microbes that are found in the intestines (Brune & Moriya, 2011). Some of the different types of cellulases are endocellulase that breaks the internal bones in a cellulose molecule, exocellulase involved in cleaving two to four units of glucose from the ends of a chain that has been exposed by endocellulase, cellobiase that is involved in hydrolyzing the products of the exocellulase (cellobiose) to form individual monosaccharides (Zverlov, et al., 2005). The process breaking down cellobiose is a natural process and helps most organisms produce glucose for use as a source of food. Cellobiose is the natural substrate that is acted upon by cellobiase and is made up of two beta glucose molecules. This experiment aimed to study the cellobiase by determining its rate of reaction and effects pH, temperature, concentration of the substrate and enzyme the rate of reaction.

Materials and Experimental Procedure

The experiment was conducted per the laboratory manual.

Results

Measurement of Absorbance and Recording of Standard Curve

The absorbance values for the p-Nitrophenol standards measured were recorded in Table 1 below.

The information collected was used to generate a standard curve that correlated the absorbance at 410 nm with the amount of the p-nitrophenol present as shown in Figure 1 below.

Figure 1: Absorbance of p-nitrophenol is plotted against standards with known amounts of p-nitrophenol, S1–S5

Experiment 1: Determine the Reaction Rate in the Presence or Absence of an Enzyme

The absorbance of the enzyme-catalyzed reaction cuvettes (E1–E5) and the control cuvettes (Start, End) was measured at 410 nm, and the absorbance recorded in Table 2. The recorded absorbance recorded was used to determine the amount of product, p-nitrophenol, formed in the reaction cuvettes (Table 2).

The amount of product formed was graphed at each time point (Figure 2), and the data used to calculate the initial rate of product that was formed in the presence or absence of enzyme.

Figure 2: Rate curve for the cellubiase enzyme reaction

The initial rate of reaction is given by the region where the amount of product formed increases in a linear fashion. In the graph above, this linear region was between 1 and 4 minutes and can be determined by calculating the slope of the line between the two points using the formula;

Slope of the line= Change in yChange in x

= 57. 398-23. 6484-1

= 33. 753

= 11. 25nmole/Minute

Experiment 2: Determine the Effect of Temperature on the Reaction Rate

In the determination of the amount of the product formed at different temperatures, the absorbance values for the four cuvettes were recorded in Table 3. The units of absorbance measured were converted into the amount

of product in nmol suing the standard curve in Figure 1 above and the results recorded in Table 3.

The data obtained was used to calculate the initial rate of product. The initial rate of reaction at 0OC was calculated as follows

12. 568-02-0

= 57. 1362

= 6. 284nmole/Minute

The initial rate of reaction at 22OC was calculated as follows

57. 136-02-0

= 57. 1362

= 28. 57nmole/Minute

The initial rate of reaction at 37OC was calculated as follows

99. 898-02-0

= 99. 8982

= 49. 949nmole/Minute

The initial rate of reaction at 80OC was calculated as follows

2. 057-02-0

= 2. 0572

= 1. 029nmole/Minute

Using the initial rates of product formation determined a graph of effect of temperature on the rate of the enzymatic reaction was plotted as shown in Figure 3 below.

Figure 3: Reaction rate curve for the cellubiase enzyme

Please note at 22 degrees C and 37 degrees C colour changed to lime and the rest were colourless

Experiment 3: Quantitative Analysis of the Amount of Product Formed at Different pH Levels

The absorbance values for the four cuvettes were recorded in Table 4 below. The units of absorbance were converted into the amount of the product in nmol using the standard curve and the values recorded in Table 4.

The data obtained was used to calculate the initial rate of product. The initial rate of reaction at pH3. 0 was calculated as follows

67. 625-02-0

= 67. 6252

= 33. 81nmole/Minute

The initial rate of reaction at pH5. 0 was calculated as follows

67. 739-02-0

= 67. 7392

= 33. 87nmole/Minute

The initial rate of reaction at pH6. 3 was calculated as follows

53. 591-02-0

= 53. 5912

= 26. 79nmole/Minute

The initial rate of reaction at pH8. 6 was calculated as follows

51. 375-02-0

= 51. 3752

= 25. 69nmole/Minute

Using the initial rates of product formation determined a graph of effect of pH on the rate of the enzymatic reaction was plotted as shown in Figure 4 below.

Figure 4: Reaction rate curve for cellobiase enzyme

Experiment 4: Determine the Effect of Substrate Concentration on the Reaction Rate

The absorbance values for six cuvettes measured were recorded in Table 6. Using the standard curve, the amount of p-nitrophenol formed in all of the samples was determined and the results recorded in Table 6 below.

Graphs of the amount of product produced against time for both the high substrate and the low substrate concentration reactions were plotted as shown in figure 5 below.

Figure 5: Amount of the p-nitrophenol against substrate concentration

The data obtained was used to calculate the initial rate of product. The initial rate of reaction at high substrate concentration was calculated as follows

107. 74-02-0

= 107. 742

= 53. 87nmole/Minute

The initial rate of reaction at low substrate concentration was calculated as follows

47. 91-02-0

= 47. 912

= 23. 95nmole/Minute

Discussion

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 from 0 to 37OC increased the rate of reaction of the cellobiase enzyme. However, increasing the temperature beyond the optimum temperature, which was determined to be 37OC, resulted to a rapid reduction in the rate of at which the enzymatic reaction occurred. The increased temperature may have resulted to denaturation of the enzyme. Increase in substrate concentration increases the rate of reaction while a decreased substrate concentration reduces the rate of an enzymatic reaction. The rate of reaction was also noted to be high in the reaction where the amount of substrate was high compared to the reaction where the level of substrate was low. The reduced rate of reaction at low substrate concentration results from a low number of substrates interacting with the enzyme compared to the high number of substrate that interacts with the enzyme at high substrate level. 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 efficiently. Changes in pH levels reduce the rate of reaction by altering the shape of the

active site (Taiji, et al., 1969). Cellobiase seems to work well in a weak acidic environment with the pH5 being the optimum pH.

Conclusion

The experiment aimed to study the cellobiase by determining its rate of reaction and effects of pH, temperature, substrate concentration or enzyme concentration on the rate of reaction. The initial rate of a reaction catalyzed by cellobiase enzyme is 11. 25nmole/minute with the optimum temperature being 37OC and the optimum pH is pH5. The activity of the enzyme is also affected by the substrate concentration.

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