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Abstract

This experiment investigated the effect of environmental factors on the enzyme hydrogen peroxidase. This enzyme is found in all aerobic cells and functions to decompose hydrogen peroxide into O_2(g) and H_2O (Petersen and Anderson, 2005). The specific environmental factors tested were temperature, pH, and enzyme concentration level. Homogenized cow liver supplied the hydrogen peroxidase. 1.5% H_2O_2 was used as the substrate. Timed reactions were conducted in a submerged reaction chamber. Oxygen gas produced was collected. Quantity of O_2(g) produced was used as the measure of activity of the enzyme.
The results showed a significant impact of all three factors on the activity level of the enzyme. At a temperature of 4°C, we saw little enzyme activity. Production was low and the rate was decreasing at 23°C. At 38°C production was high and showed very little decrease in production rate.

We saw steady production at all enzyme concentration levels tested. Production, however was not proportional to the concentration levels used, x, 2x and 4x. For 2x concentration we saw a 35% increase in product over x, and for 4x concentration the increase was only 58% over x. Hydrogen peroxidase showed significant sensitivity to pH levels. In an alkaline environment of pH 11, we saw almost no activity after 10 seconds. In an acidic environment of pH 0, we saw only very slight activity after 10 seconds. In a neutral pH of 7 however, high activity was maintained for the trial period. This experiment demonstrates the vital importance of temperature, pH, and enzyme concentration levels to the function of hydrogen peroxidase.

Introduction

Hydrogen peroxidase is an enzyme found in all aerobic cells, which functions to decompose toxic hydrogen peroxide (Petersen and Anderson, 2005). The products of this reaction are water and oxygen gas. Enzymes are complexes produced by living cells, which catalyze chemical reactions in organic matter. Like all catalysts, enzymes work by reducing the activation energy for a reaction, thus dramatically increasing the rate of the reaction (Petersen and Anderson, 2005). An enzyme is typically a globular protein with a specific three dimensional shape. A small part of this shape forms the active site, where the enzyme combines with the substrate. The substrate actually fits into the active site, which is why enzymes are specific to the reactions they catalyze (Campbell, et al. 2008). Because an enzymes shape is critical to its performance, environmental factors which could change this shape could have an effect on enzyme production (Campbell, et al. 2008). Factors such as temperature and pH, have been shown to have an effect on the performance of enzymes (Vishwanatha, et al. 2009). Experimental evidence shows that an "optimal level" of these factors exists (Guerfali, et al. 2009). In this experiment we will evaluate the effects on hydrogen peroxidase of three environmental factors, temperature, pH, and enzyme concentration level.

Materials and Methods

Cow liver was homogenized to provide the supply of hydrogen peroxidase. For the following

three variables tested, a reaction chamber was created with a glass bottle, stoppered with a glass tube inserted. A gas collection chamber was placed over the glass tubing. The apparatus was submerged in water so that all escaping gas from the reaction could be observed and collected. Water displacement in the collection chamber was used to measure the quantity of gas produced. Equal sized strips of filter paper were saturated with liver homogenate for each trial. The paper strip(s) were adhered to the inside of the reaction chamber so that the enzyme did not come in contact with the substrate, hydrogen peroxide, until the chamber was inverted at time 0. O_2 quantities produced were measured for 60 seconds at 5 second intervals.

Variable 1, Temperature
One paper strip of enzyme and 20 mL of 1.5% H_2O_2 at pH 7 were used for each trial. The temperature of the reaction was changed for each trial by changing the temperature of the water in which the apparatus was submerged. For each trial the reaction chamber was held in the water bath for 5 minutes before inverting it to begin the reaction. The temperatures tested were 4°C, 23°C, and 38°C.

Variable 2, Enzyme Concentration
A constant temperature of 32°C and 20 mL of 1.5% H_2O_2 at pH 7 were used for each trial. The enzyme concentration was varied by using ½ paper strip of enzyme for trial 1, 1 strip for trial 2, and 2 strips for trial 3.

Variable 3, pH.
A constant temperature of 32°C, 20 mL of 1.5% H_2O_2 at pH 7, and one paper strip of enzyme were used for each trial. One paper strip of enzyme was used for each trial. For trial 1, 10 drops of 50% HCL were added to the H_2O_2. For trial 2, 10 drops of 50% NaOH were added to the H_2O_2. The substrate's pH was measured. The data from variable 2, using one enzyme strip, was used as the neutral pH trial.

Results

Variable 1, Temperature (figure 1)
The lowest activity was seen at 4°C, the coldest temperature tested. Activity was slow and decreasing for the first 15 seconds, then continued at a very slow rate until almost leveling off at 40 seconds. The maximum yield of O_2 was 17 mL at 55 seconds. At this point production stopped. We saw better results at 23°C. O_2 was produced at an only slightly decreasing rate for 60 seconds for a yield of 42 mL at 60 seconds. Our highest yield of O_2 was the warmest temperature tested, 38°C. This temperature yielded 55mL of O_2. The activity rate decreased slightly over the trial but seemed steady at 60 seconds.

Variable 2, Enzyme Concentration (figure 2)
The lowest activity was seen with the least concentration, ½ enzyme saturated strip. O_2 was produced at a low but steady rate. Maximum yield was 34 mL at 60 sec. Moderate production was seen with 1 enzyme saturated strip. The production rate slowed slightly after 10 seconds but continued steadily, for a yield of 46 mL at 60 seconds. Highest production occurred with 2 enzyme saturated strips. O_2 was produced at the highest rate from 5 to 25 seconds, then gradually slowed slightly for a yield of 54 mL at 60 sec.

Variable 3, pH level (figure 3)
The lowest activity was seen at pH 11. Some O_2 production occurred between 0 and 25 seconds but

almost no activity was seen after that point. O_2 yield was 12 mL. At a pH of 0, higher O_2 production occurred between 0 and 15 seconds, but after that very little O_2 was produced. Highest production was seen at a pH of 7. Production occurred at a high, only slightly decreasing rate for 60 seconds. O_2 yield was 45 mL.

Discussion

The results of this experiment demonstrated that the performance of hydrogen peroxidase was greatly affected by all three factors, temperature, concentration level, and pH. Of the conditions we tested, this enzyme was most active at a temperature of 38°C, a pH of 7, and at the highest concentration tested. Because this enzyme was most active at the highest temperature we tested, it is impossible to determine its optimal temperature. Hydrogen peroxidase was relatively inactive at 4°C, and only moderately active at 23°C, with a decreasing production rate. Because it was quite active at 38°C, with only slight decrease in production rate over 60 seconds, we can assume that temperature has a significant effect on hydrogen peroxidase performance.

Enzyme concentration levels were also shown to have a significant effect on this enzyme's performance. The O_2 production rate was highest for the highest concentration tested. By doubling the enzyme concentration we saw a 35% increase in O_2 yield, and by quadrupling the concentration we achieved 58% greater yield. Thus, while production increased as enzyme concentration rose, increase in production was not proportional to increase in enzyme concentration.

Hydrogen peroxidase was shown to be highly sensitive to pH levels. In an alkaline pH a small amount of activity occurred for a few seconds, then none. In an acidic environment slightly more activity occurred in the first 15 seconds, decreasing rapidly to almost none. In a neutral pH of 7 we saw a high rate of production with little decrease after 60 sec. This suggests that the optimal pH of hydrogen peroxidase is probably close to 7.

The performance of hydrogen peroxidase is significantly affected by environmental factors. This sensitivity to the environment would help to account for the specificity of enzymes. Enzymes in various parts of an organism are subjected to a wide range of environmental conditions. For example, the human stomach, in which the enzyme pepsin is active, has a pH of 2. Other enzymes present in the human body would be denatured in that environment (Campbell, et al. 2008). Thus each enzyme requires the ability to perform in a specific range of conditions. Because an enzyme works by physically attaching to the substrate at an "active site," its shape is of vital importance. Environmental factors such as pH and temperature could cause changes in the shape of enzyme molecules, making them ineffective. Enzyme concentration levels are also of importance. Enzymes catalyze chemical reactions without being consumed themselves (Campbell et al. 2008). If the enzyme concentration level is so low that a large portion of substrate molecules do not reach an "active site," chemical reactions catalyzed by the enzyme will occur very slowly. However if the concentration is so high that many of the "active sites" are not used, reactions will be quick at first but then drop off as substrate diminishes. An optimal concentration level would keep the reaction occurring at a high but steady rate. This experiment demonstrates the vital importance of concentration levels, and the environmental factors of pH and temperature to the activity level of hydrogen peroxidase.

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