The Effect of High Temperature and Inhibition (Hydroxylamine) on Enzymatic (Peroxidase) Function

Introduction

An enzyme is a biological catalyst that aims to speed up chemical reactions. They do this by drastically decreasing the energy needed for a reaction (activation energy). Enzymes are also highly specific. The active site of an enzyme bonds to, at most, a set of similar substrates(Robinson, 2015).

However, an enzyme can become dysfunctional and denatured at certain temperatures, especially high temperatures. An enzyme has an optimal range of temperature. In other words, enzymes operate the best in a specific "Goldilocks zone." As temperature increases, enzymes tend to work with increased efficiency until too high of a temperature is reached. Denaturing then occurs(Peterson, Daniel, Danson, & Eisenthal, 2007)

An enzyme can also stop functioning because of inhibitors. In this lab, a competitive inhibitor is utilized. A competitive inhibitor is a substance that competes for the active site of a substrate-enzyme complex. These substances have a very similar shape to the substrate the enzymes look to bond with(Engelking, 2015). Competitive inhibitors slow enzymatic function by occupying the active site.

In this lab, we look to find the optimal temperature range of enzymatic activity of peroxidase and the temperature at which that enzyme becomes denatured and dysfunctional. We will also look at the effect of a competitive inhibitor(Hydroxylamine) on enzymatic activity.

Methods

To test the effect of temperature on enzymatic function, we took a buffer(pH 5), Guaiacol, and Peroxidase mixture(1000uL, 250uL, 250uL, respectively) in a cuvette and put it into a SpectroVis Plus(at 500 nm). Before the cuvette was inserted into the SpectroVis Plus, the mixture was cooled or heated to a certain degree in Celsius: 0-4, 22, 32, 48, or 150.2(boiling). After the cuvette was put in the SpectroVis Plus, we added 500 microliters of hydrogen peroxide. We then measured the absorbance to determine enzymatic activity. We can use absorbance because Guaiacol changes the color of the mixture based on how well the chemical reaction occurred.

To test the effect of hydroxylamine(competitive inhibitor) on enzymatic function, we took 500 uL of peroxidase and 1000 uL of 2% hydroxylamine and mixed it. We also allowed this extract to sit for 15 minutes. To eliminate a temperature variable, the temperature was maintained at room temperature(22 degrees Celsius). The same mixture in the temperature experiment was used for one cuvette, but the other cuvette used 250 uL of the hydroxylamine extract instead of peroxidase. The same steps previously stated were followed.

We measured the absorbance of each trial for 2 minutes, noting absorbance changes at 20-second intervals. Blanks were used to calibrate SpectroVis Plus.

Hypothesis/Prediction

Temperature Experiment:

Hypothesis: Enzymatic function gradually increases until the boiling point of peroxidase is reached.

Prediction: If the temperature rises, enzymatic function will increase until the boiling point is reached.

Inhibition Experiment:

Hypothesis: Enzymatic function will decrease with the presence of a competitive inhibitor.

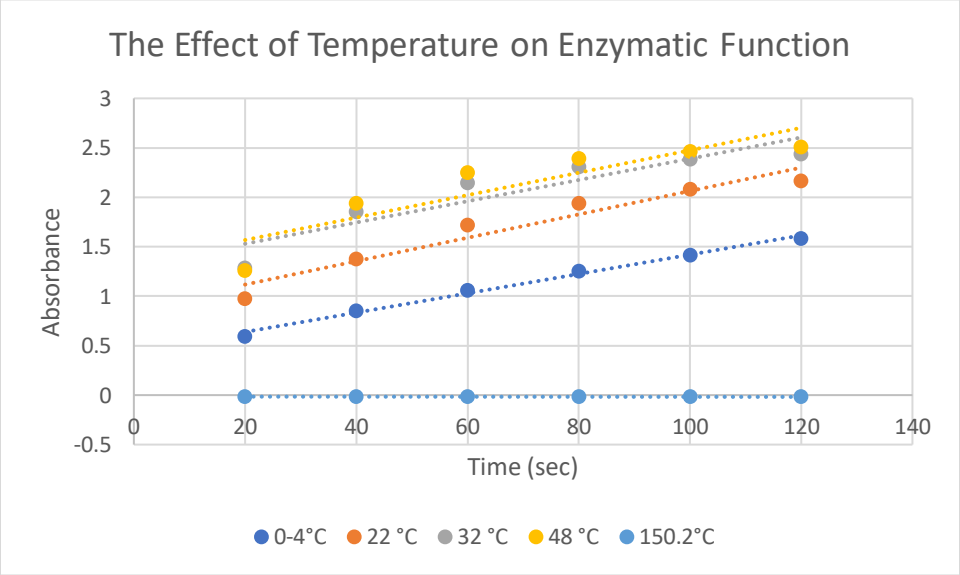
Prediction: If a competitive inhibitor is introduced to an enzyme, enzymatic function will decrease.

Results

Temperature Experiment Results:

Temp. (°C)	Cuvette	Buffer	Peroxidase	Guaiacol
22	1(blank)	1000	0	250
0-4	2	1000	250	250
22	3	1000	250	250
32	4	1000	250	250
48	5	1000	250	250
boiling	6	1000	250	250

Time (sec)	0-4°C	22 °C	32 °C	48 °C	150.2°C
20	0.595	0.974	1.282	1.259	-0.015
40	0.852	1.378	1.853	1.941	-0.016
60	1.061	1.717	2.145	2.25	-0.018
80	1.252	1.939	2.305	2.393	-0.015
100	1.417	2.081	2.383	2.461	-0.018
120	1.585	2.163	2.437	2.506	-0.018



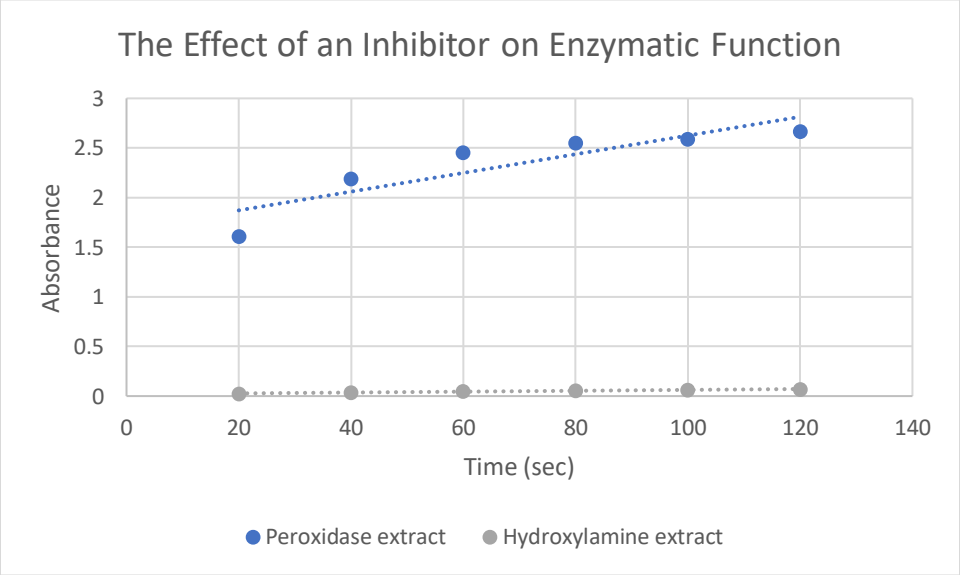
The slopes of enzymatic function between temperatures 0-48°C show a steady rise over time.

When the temperature reaches a boiling point, the slope of the line is minimal.

Inhibitor Experiment Results:

Cuvette	Buffer	Peroxidase	Hydroxylamine extract	Guaiacol
1(blank)	1000	0	0	250
2	1000	250	0	250
3	1000	0	250	250

Time (sec)	Peroxidase extract	Hydroxylamine extract
20	1.607	0.024
40	2.191	0.037
60	2.453	0.047
80	2.548	0.055
100	2.592	0.062
120	2.667	0.069



The slope of the trend of inhibition is minimal compared to that of the standard peroxidase extract.

Discussion

In the temperature experiment, we see enzymatic function increase as temperature rises. In this experiment, 48°C is considered part of the optimal temperature range, where peroxidase performs the best. It is also seen that once the boiling point is reached, the enzymatic function fails. These observations support the hypothesis.

In the inhibition experiment, the enzymatic function became minimal when the Hydroxylamine treated extract was presented. This result supports the hypothesis.

References

Robinson, P. (2015, October 26). *Enzymes: Principles and Biotechnological Applications.* Retrieved October 26, 2023, from Enzymes: Principles and biotechnological applications - PMC (nih.gov)

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