Temperature Dependance of Maltase's Enzymatic Activity

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Abstract

Maltase is an enzyme which is responsible for the breakdown of maltose, a disaccharide, into two glucose monomers. Temperature is an important factor which largely determines the function of maltase and its ability to catalyze reactions. Thus, the goal of our study was to determine the effects of temperature on the enzymatic activity of maltase. We hypothesized that the temperature at which maltase is exposed to would have a significant effect on the enzymatic activity of the enzyme. The enzymatic activity of maltase exposed to maltose-containing solutions at various temperatures were tested: 30℃, 40℃, 50℃, and 60℃. The glucose concentration of the resulting solutions were measured every minute for 5 minutes. The mean rates of change of glucose in the 30℃, 40℃, 50℃, and 60℃ samples were 6.00 +/- 30.66, 6.26 +/- 11.96, 3.93 +/- 7.88, and 5.09 +/- 18.00 mmol/L, respectively. A one-way ANOVA test determined that the rate of change of glucose at different temperatures was not statistically significant, thus our data did not support our hypothesis. While there were noticeable variations in the data, there was a general decrease in the enzymatic activity across all temperatures within the first minute. For future studies, it is recommended that the enzyme-containing maltose solution is vortexed prior to measuring the glucose concentration in order to ensure proper distribution of the enzyme throughout the sample.

Introduction

Maltose, a disaccharide sugar composed of two linked glucose monomers, is derived from starch, a polysaccharide of glucose chains. Maltose can be introduced into the human body through consumption of foods such as honey and muesli, where it is naturally present, or through the breakdown of starch during digestion (Sugar Nutrition Resource Centre, 2022). The breakdown of maltose in the body is accomplished by the enzyme maltase. Maltase is an enzyme which is secreted by the intestinal epithelial cells and is responsible for the degradation of the disaccharide into two glucose monomers (Dashty, 2013). The role of the maltase is of extreme importance as it results in glucose generation, which is a primary source of energy for the body (Hantzidiamantis & Lappin, 2022). Improving our knowledge on the enzyme's optimal conditions can help enhance scientists' understanding of the complex mechanisms underlying

nutrient digestion and energy metabolism in the human body. One factor which has an important effect on the structure and thus functioning of the maltase enzyme is temperature. The temperature sensitivity of enzymes strongly influences their ability to carry out catalytic functions (Somero, 1978). Additionally, the interaction and binding between the enzyme and its target substrate can be strongly influenced by temperature (Somero, 1978). In general, enzyme activity tends to increase with temperature up until a certain point, after which higher temperatures will result in the loss of enzyme activity due to structural changes, or denaturation (Daniel & Danson, 2013). A study which looked at the optimal temperature of maltase functioning determined that it performed best in the range of 48℃ - 50℃ (McWethy & Hartman, 1979). Another study found similar results, with the highest activity of maltase being performed at 45℃, and also observed a 90.01% decrease in activity at a temperature of 60℃ (Nawaz, M, et al. 2019).

We hypothesized that the rate of change in enzymatic activity of maltase will vary significantly depending on the temperature of the solution which the enzyme is exposed to. Specifically, we predicted that the enzymatic activity of maltase will increase with temperature until 50℃, and then rapidly decrease once this temperature is surpassed due to denaturation of the enzyme. To test our hypothesis, we monitored the enzymatic activity of the maltase at 4 various temperatures: 30℃, 40℃, 50℃, and 60℃. In order to monitor how the enzymatic activity changes with temperature, we will measure the changes in glucose concentrations of our various enzyme-containing maltose samples.

Materials and Methods

This experiment began by first measuring 12 ml of a Tris Buffer Solution in a graduated cylinder, then transferring this amount into a beaker containing a stir bar. 8 maltase pills, each containing 3mg of maltase, were cracked open and poured into the beaker. The beaker was then placed on a stir plate at maximum setting for a few minutes for the clumps of maltase to dissolve. 3 ml of a pure maltose solution was pipetted into 12 test tubes. For each test tube, it was first placed into a water bath set to the designated temperature for 5 minutes. Then, 0.25ul of the maltase solution was pipetted into the maltose test tube. Glucose readings were taken just before the enzyme was added, then every minute for a total of 5 minutes as the test tube remained inside the water bath. For a glucose reading, a glucose meter was selected which could measure a minimum amount of 1.1 mmol/L and a maximum of 33.3 mmol/L. The glucose test strip was inserted into the glucose meter and the tip of the strip tested the glucose concentration. For each reading, 20ul of the solution was pipetted from the test tube onto a piece of parafilm for the glucose meter to provide a reading. Data was recorded onto a spreadsheet.

Figure 1- Maltase containing supplements used.

Results

The mean rate of change of glucose was determined to be 6.00 +/- 30.66, 6.26 +/- 11.96, 3.93 +/- 7.88, and 5.09 +/- 18.00 mmol/L at 30℃, 40℃, 50℃, and 60℃ respectively. A one way ANOVA test was performed using R to determine the significance of the results. A P-value of 0.98 (>0.05) was obtained, therefore the data is not statistically significant.

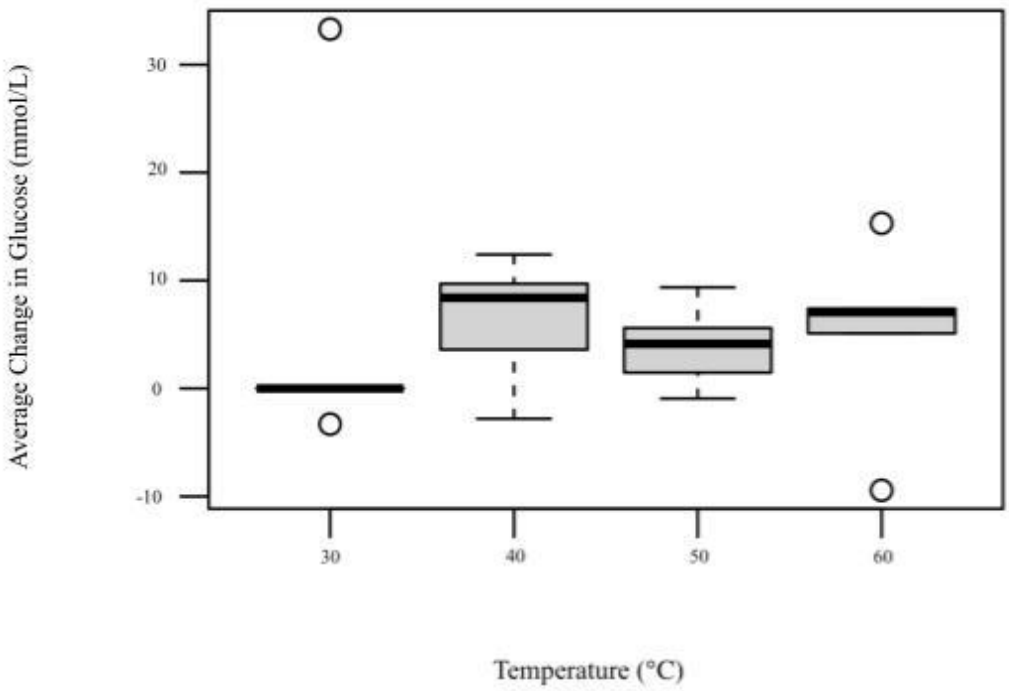

Figure 2 - Enzymatic activity of maltase (rate of change of glucose) at 30℃ (n=3), 40℃ (n=3), 50℃ (n=3), and 60℃ (n=3). Box plots represent the 25th and 75th interquartile ranges. Each error bar represents a 95% confidence interval. Outliers are represented by individual dots. No significant difference as determined by a one-way ANOVA test (P = 0.98).

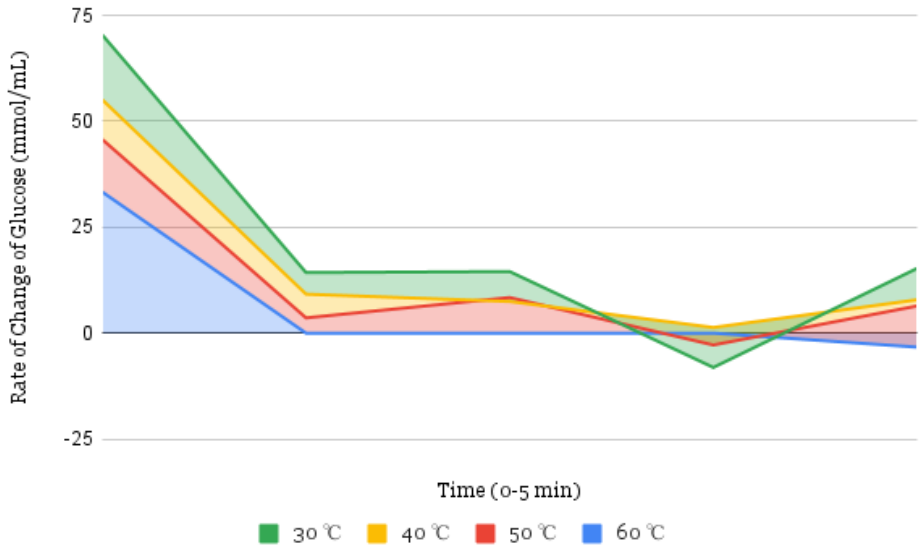

Figure 3 - Enzymatic activity of maltase (rate of change of glucose) at 30℃ (n=3), 40℃ (n=3), 50℃ (n=3), and 60℃ (n=3). Line graphs represent the rate of change of glucose at 5 one minute intervals (0-5mins).

Discussion

The experiment aimed to determine differences in the enzymatic activity of maltase at different temperatures. Our results concluded no significant difference in enzymatic activity between any of the four tested temperatures: 30℃, 40℃, 50℃, and 60℃. A large variance in data was observed with an overall decrease in activity after one minute in all temperatures. This is likely due to the high ratio of maltase to maltose used. The hypothesis that a significant difference will be observed between the temperatures was not supported. Previous research has concluded the temperature for highest maltase activity is 48-50℃ (McWethy *et. al*). Other studies have expanded, finding maltase activity rapidly decreases after this point because of denaturation of the enzyme's tertiary structure (El-Shora *et. al*). This was not observed in this experiment. No significant decline occurred after 50℃. After 3 minutes, a negative change in glucose was

observed in multiple treatments. Because both maltose and maltase are denser than the tris buffer solution, the majority of the solutes would have sunk towards the bottom of the test tubes. When pipetting was performed every minute, the depth of the collected sample was not constant. Due to the short amount of time between sampling, no vortexing was performed. Thus, the negative change is likely caused by pipetting from a higher depth than the previous sample. A sample size of 3 per treatment was used, more accurate future data should involve a larger number of samples in order to obtain a more comprehensive average. An important limitation of this study is the capacity of the glucose reader. The monitor gave values between 1.1 and 33.3 mmol/L. Values outside of this range were displayed as “less than 1.1 mmol/L” and “greater than 33.3 mmol/L”. These values were recorded as 0 and 33.3 mmol/L respectively, though this introduced potential for error if values were much greater than 33.3 mmol/L. Further research using constant vortexing, a larger sample size, and a constant depth of sampling should be conducted to obtain more reliable results. As maltase is an important digestive enzyme in the human body, understanding the temperature dependence is key and allows further understanding of enzymatic function in the context of the human body.

Conclusion

The enzymatic activity of maltase was tested in maltose-containing solutions with varying temperatures: 30℃, 40℃, 50℃, and 60℃. The glucose concentrations every minute, for a total of five minutes, in each solution was plotted on a graph to determine the rate of change of glucose concentration. A one-way Anova test determined that there was no significant statistical difference in the enzymatic activity of maltase between the different temperature solutions. Our

hypothesis which stated that temperature would have a significant effect on the enzymatic activity of matlase was thus not supported.

Acknowledgments

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Appendix

	Average Change in Glucose Concentration (mmol/L)			
Time Period	30℃	40℃	50℃	60℃
0-1 min	33.3	12.4	9.367	15.3
1-2 min	0	3.6	5.6	5.1
2-3 min	0	8.4	-0.93333	7.0667
3-4 min	0	-2.8	4.13333	-9.4333
4-5 min	-3.3	9.7	1.46667	7.4
Average	6.00	6.26	3.93	5.09
Standard Deviation	15.33	5.98	3.94	9.00