

Bio lab report

[Science](#), [Biology](#)



Ye Tao BISC220-13155 The Effect of Temperature on the Digestion of Starch by Activity of Enzyme α -Amylase: Observation of Rate of Starch

Disappearance through Iodine Test Introduction An enzyme is a type of protein that, through its own structure including hydrogen bonds, acts like a biological catalyst and is able to accelerate the biochemical reaction rate by lowering the activation energy of the whole process, without which cells could hardly practice any physiological functions within human bodies (Sizer, 1943).

Found in the saliva and pancreatic secretions of animals including human beings as well as the plant seeds, bacteria and fungi (Siddiqui et al. , 2010), the enzyme α -amylase that was studied during the experiment has significant impact on the hydrolysis of starch. By breaking the alpha, 1-4 glycosidic linkages in the carbohydrates, amylase hydrolyzes the starch, a polysaccharides that is stored in plants and cannot be directly digested by animal cells, into maltose, a disaccharide that later generate two units of glucose to undergo metabolisms and provides necessary energy (Slaughter et al. , 2001). The enzymatic activity of α amylase is facilitated by calcium and chloride ions during the hydrolysis (Marini, 2006 and Siddiqui et al. , 2010). The complete digestion of starch and formation of maltose and glucose can be examined through the iodine test when I₂KI reagent is added into the solution and remains brown instead of turning into dark blue, marking that all the molecules of starch have been fully hydrolyzed (Hanes, 1932). While amylase effectively activates the hydrolysis of starch, the efficiency of the catalytic process is influenced by several factors including temperature, pH level and the concentration of the substrates etc.

In this experiment, as the α -amylase is a type of protein, the efficiency of enzyme is highly related to its hydrogen bonds which are affected by the temperature. Though the enzyme is collected from the porcine pancreas, due to its structural similarities to amylase in human bodies, the behaviors of two amylases should resemble each other. Given that under extreme temperature enzymes will be denatured and unable to function and the constant temperature of pigs is around 39°C , the hypothesis of this experiment is that at 37°C amylase will catalyze the hydrolysis with the highest speed, followed by amylase at 22°C .

Amylase at 0°C will react extremely slowly due to the crystallization of hydrogen bonds and at 100°C , amylase will lose its function since it will be denatured. Materials and Methods Four test tubes were marked from A1 to A4. Then, 2mL of 1% starch solution from Carolina Biological Supply Company, 4mL of deionized water and 1mL of 6.8 hydron buffer from VWR International/ Micro Essential Laboratories were added into each tube. Another four test tubes were also labeled from B1 to B4 and added 1mL of 1% α -amylase from porcine pancreas from Sigma Aldrich. Eight tubes were paired according to the same number (A1 and B1 etc. and assigned to environments at different temperature: Tube A1 and B1 were placed into a water bath at 100°C ; Tube A2 and B2 were placed into a water bath at 37°C ; Tube A3 and B3 were placed on the tube rack (at about 22°C); Tube A4 and B4 were placed into an ice bath at 0°C . All test tubes were kept at different temperatures for 10 minutes. Meanwhile, a control group of starch solution was prepared without amylase. (Bio Lab Manual, 2013) At the same time, a

test plate was added 2 drops of I2KI reagent (1% Iodine and 2% KI) from Carolina Biological Supply Company per well.

After 10 minutes, when test tubes were still in the original environments, solutions in Tube A1 with B1 were mixed and a timer was started. At each 30-second-inteval, a drop of the mixture was released into the well on the test plate until the solution in the plate did not change into dark blue and remained brown, indicating the end of the reaction by showing no presence of starch and presence of maltose and glucose. The experiment was repeated on the tubes at other temperatures. Slow reactions were observed and recorded up to 420 seconds due to time limit.

Data were pooled from each bench and average and standard deviation were calculated. The data of the control group were also obtained. Results Figure 1 The test plate of iodine test under different temperature. Dark blue wells indicated the presence of starch while the brown ones indicate the completion of starch hydrolysis. (Upper half: 37°C; Bottom Half: 0°C) The rate of reaction was fastest at 37°C (n= 4, mean= 212. 5s, SD= 66. 1s) while the rate of reaction at 22°C was only slightly less than it (n= 4, mean= 217. 5s, SD= 61. 8s).

Though the previous two groups underwent starch hydrolysis relatively fast, the tubes at 100°C and 0°C reacted so slowly that it took more than 420 seconds for their completions (time was only recorded before 420s). There was no hydrolysis in the control group. The time of the reaction completions as the function of different temperatures was shown in the table and graph below. The Effects of Temperature| Temperature (?)| Time of Starch Disappearance(s)| | Bench 1| Bench 2| Bench 3| Bench 4| Mean| SD| Control| <https://assignbuster.com/bio-lab-report/>

> 420| > 420| > 420| > 420| 420| 0| 0| > 420| > 420| > 420| > 420| 420| 0|
2| 210| 210| 300| 150 | 217. 5| 61. 84658| 37| 270| 180| 265| 135 | 212. 5|
66. 14378| 100| > 420| > 420| > 420| > 420| 420| 0| Table 1. Time of Starch
Disappearance with Porcine Pancreatic ? -Amylase at Different Temperatures
(Time was recorded up to 420s). Graph 1. Time of 1% Starch Disappearance
with Porcine Pancreatic ? -Amylase as the Function of Different Temperature
Discussion and Conclusion As the data obtained from the experiment, all
parts of the original hypothesis were confirmed by the result. Temperature
plays an important role during the activation of ? amylase that only during
certain temperature range can the enzyme function properly to catalyze
biochemical reactions. On one hand, at 37°C the amylase showed the
greatest efficiency in catalyze the hydrolysis of starch. At the same time, the
amylase also showed considerable catalytic efficiency at 22°C. But on the
other hand, when temperature dropped or rose to extreme value such as 0°C
or 100°C, the function of amylase was inhibited and such biochemical
transformation of substances could hardly process. This result obtained is
consistent with the reality that during normal body temperature, regardless
of pig or human beings, mylase is able to catalyze the hydrolysis of starch
with the highest speed. Therefore, we may conclude that even taken out
from where it was found, the amylase still maintain its original biochemical
properties. The experiment did not show the biochemical mechanism of the
modification from temperature to amylase activity. However, according to
the scientific research done by other scientists, a temperature that ranges
from 20-50°C could make structures including weak interactions, hydrogen

bonds and disulfide bridge exist within and stabilize the enzyme molecules to maximize their activities.

At the water freezing point (0°C), the hydrogen bonds are crystallized and become more constrained and less flexible while at high temperature like 100°C, the bonds consume certain energy to become unstable and fragile, neither of which contribute to the proper functions of amylase (D'Amico et al. , 2003). While the result of the experiment perfectly matched what was expected, however, such conclusion could only be made at qualitative phase and it is obvious that weakness of this experiment existed and prevented the further understanding of amylase at quantitative level.

Several modifications to the current experimental designs could be made to enhance its accuracy. Firstly, the sample size needs to be expanded. With only four groups, the data was so limited. As a result, the data had great standard deviations of more than 60 seconds. Simultaneously, the random errors were at high possibility to take place. Therefore, with the increase of sample size, the data can be more accurate and stabilized and potential random errors could be discarded to ensure the coherence of the data.

Furthermore, even though neither the test tube at 0°C and 100°C enabled the completions of starch hydrolysis, the reasons of the two groups are not the same. Therefore, in order to detect the reason of the loss of catalytic ability, follow-up experiments need to be practiced. A possible design might be to change the test tubes from 0°C or 100°C into 37°C for another 10 minutes then redo the iodine test to see this time whether the amylase can function well or not.

This manipulation will convince the hypothesis about the reason behind the superficial phenomena that was shown in the original experiments and present the difference between denaturing of protein and crystallization of hydrogen bonds. It is important for people to thoroughly understand the amylase activity and all the factors that are potentially capable of influencing such activity through which people can understand how human bodies work as well as the physiology of other organisms. At the same time, the research in amylase activity could potentially bring economical benefits to industrialized starch products manufacturing.

And finally, the amylase activity has shown its significance in medical clinical trial that diseases including hyperamylasemia or hyperamylasuria are proven to be related to the amylase in the human serum and urines (Salt 2nd, 1976). References General Biology BISC 220 Laboratory Manual. (2013). University of Southern California. Lab2, pp33-36. D'Amico, S. , Gerday, C. , ; Feller, G. (2003). Temperature adaptation of proteins: engineering mesophilic-like activity and stability in a cold-adapted α -amylase. *Journal of molecular biology*, 332(5), 981-988. Hanes, C.

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