

Starch hydrolysis of amylase



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The purpose of experiment is to observe amylase enzyme in different environment and detect of each environment by helping colour changes.

Enzymes are biological molecules that catalyze many different chemical reactions. With few exceptions, all enzymes are proteins and each enzyme is specific to a certain chemical reaction. Enzymes must maintain a specific three dimensional structure in order to function properly. If an enzyme's structure is altered (by heat or harsh chemicals) it may not function at all.

This breakdown (denaturation) of an enzyme's structure may be fatal

Amylase Enzyme

Amylase, which is commonly found in saliva and germinating seeds. It catalyzes the breakdown of starch. When amylase reacts with starch, it cuts off the disaccharide maltose (two glucose molecules linked together). As the reaction progresses, less starch will be present and more sugar (maltose) will be present. The activity of amylase can be observed by using iodine.

Because iodine reacts with starch to form a dark brown/purple color. As amylase breaks down starch, less and less starch will be present and the color of the solution (if iodine is added) will become lighter and lighter. The color change was observed using spot-plates as illustrated on the diagram below.

Amylase activity was observed under four different treatments:

effect of temperature

effect of pH

effect of substrate concentration

effect of enzyme concentration

The Effects Of Temperature

Amylase is an important metabolic enzyme. Its function is to catalyze the hydrolysis of starch into glucose. At high temperatures, Amylase becomes denatured, denatured amylase no longer catalyzes the hydrolysis of starch into glucose.

EFFECT OF pH:

Based on these results, what is the optimal pH for amylase? Is this optimal pH considered acidic, basic/alkaline, or neutral? Why does the activity decrease when the pH is too low or too high?

APPARATUS

-Starch

-Amylase Enzyme

-KH₂P₀₄

-Na₂HP₀₄

-HCl

-Heater

-Beaker

-Falcon tube

-Spectrophotometer

<https://assignbuster.com/starch-hydrolysis-of-amylase/>

-Iodine

PROCEDURE

1. 0.27 g KH_2P_04 buffer solution PH 5 was prepared with 20ml
2. 0.27g KH_2P_04 PH6 was prepared with 20ml
3. 0.27g KH_2P_04 PH7 was prepared with 100ml
4. 0.282g Na_2HPO_4 PH8 was prepared with 20ml
5. 0.282g Na_2HP_04 PH9 was prepared with 20ml
6. 20g Starch was also prepared with 50ml cold water
7. To test amylase activity with PH difference, 5ml starch , 5ml buffer(PH5, 6, 7, 8, 9 is used each) and 1ml amylase were mixed each other.
8. 10min later, 0.5ml prepared sample was put into 5ml HCl.
9. At 620nm , the results were measured at spectrophotometer.
10. Second part temperature effect, 5ml starch , 5ml PH7 buffer and 1ml amylase were mixed.
11. Prepared sample was put into different temperature 30, 50, 70 and 90C.
12. 10 min later, 5ml HCl was put into 0.5 ml prepared sample.
13. 2-3 min later, 5ml iodine was added into 0.5ml new sample
14. Absorbance of each was measured at spectrophotometer.

OBSERVATIONS

In this experiment, we tried to create different environment to examine amylase enzyme activity. The environment differences could be provided by PH differences. Therefore we prepared different medium also different pHs. K₂HPO₄. The graph was gained from our results. One of them is a graph that related to amylase activity at different PH. The other one is related to amylase activity at different temperatures at constant PH. With K₂HPO₄ PH 5, 6 and 7 were prepared and with Na₂HPO₄ 8 and 9. Each preparation procedure was applied. 5ml starch, 5ml buffer, 1ml amylase were added each other and then waited 10 min. After 10min, 5ml HCl was added into 0.5 ml sample mixture. In a same way, the mixture for temperature observation was prepared pH 7. And added iodine to end of procedure. Absorbance results were taken from spectrophotometry. This measurement was at 620nm.

pH buffer sample with amylase

0.074

0.027

0.026

0.043

0.074

According to the results,

The smallest one can be think as a best one. How much enzyme is used is more essential point. If it is less one , it means starch can not be used adequately. High starch amount means that complex amount is also high. The opposite one shows best activity amylase at smallest concentration. The colour is more light, smaller absorbance could be think as best amylase activity.

Temperature sample with amylase

0.064

0.006

0.192

0.130

At 30C the colour is slightly orange.

At 50C the colour is extra light like iodine colour.

At 70C the colour is slightly purple.

At 90C the colour is more purple than at 30C one like orange-purple. At constant PH , the small concentration , at 50C. Because small absorbance formed by small complex. It means that amount of starch was decreased also. Best activity is 50C at constant PH.

RESULTS

Our aim is to be related to activity of amylase. To detect it, we prepared different PH from KHP04 and Na2HP04 by adding acid or base. Usage both of <https://assignbuster.com/starch-hydrolysis-of-amylase/>

them is related to interval of buffer. After preparation buffer, we measure absorbance at spectrophotometry. At different PH absorbance give also different concentration. If amylase enzyme concentration with sample is small, it means enzyme is used complex is more small so activity of enzyme is best one in there. At different PHs , smallest concentration is at PH 7. And then we did second part of experiment by using PH7. The chosen of PH7 is related to observation best amylase activity at first part. At PH7 we took sample with amylase enzyme concentration at different PHs. The smallest concentration is at 50C in second part. The concentration is 0. 006. The colour is more light like iodine colour. Starch is used with amylase and therefore complex colour is more light also. The amylase enzyme activity is best one at 50C. This measurement is done at 620nm.

DISCUSSION AND CONCLUSION

Why is measured at 620nm ? Why HCl is used for preparation ? What does Light color mean? How does more heat affect rxn? During experiment , we want to distinct purpose of experiment by answering these question. In this experiment, we related to effect of different buffer and temperature. We prepared buffers at different PH. KH_2P_04 was prepared for PH 5 , 6 , 7 and Na_2HP_04 for 8 and 9. In first part , at constant temperature (room temperature) sample with amylase concentration was measured. At PH 7 , we measured the smallest one. Small concentration means less complex less starch and enzyme is used enzyme activity is high. Our result from measurement at PH 7 is 0. 026. As a second part , constant PH, temperature was changed and then observed the effect of it. At 50 C , smallest absorbance (0. 0060) was found and the colour was extra light. It means

more less complex there. In this experiment , iodine is used to detect starch molecules by observing color change. Iodine and starch were combined and then formed complex. The another point is why HCl is used. The acid stops the enzymatic reaction and iodine reacts with starch to produce blue color. Activity of enzyme is also essential. It can be used for denaturation detection. Starch reacts with iodine which is yellow to form blue compound $\lambda_{max} = 620\text{nm}$. The intensity of the blue color can be quantified spectrophotometrically by measuring its absorbance at 620nm.