

The effect of starch on the activity of amylase with ph variable

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The Effect of Starch on the Activity of Amylase with pH Variable Lab Report, Fall 2011 East Tennessee State University Department of Biological Sciences
By: Shelby Brackett Date Performed: October 10, 2011 Lab Instructor: Joseph Kusi Biology 1111, Section 018 Abstract Enzymes are very important in chemical reactions. They are used to speed up the reaction taking place. They act by binding to a specific substrate and form an enzyme-substrate complex that may put stress on chemical bonds of that substrate. In this experiment, we used the amylase as our enzyme and starch as our specific substrate.

We then used a calorimeter to measure the absorbance of our samples with the variable of pH over set periods of time. Our results indicated that at three different pH levels, the absorbance level of our samples was not the same for each one. This proved my original hypothesis to be incorrect, as I was surprised to find that the last pH buffer had no effect on the absorbance. The first two pH buffers supported my hypothesis, however. The levels of our samples kept decreasing over time. As with every experiment, it should be repeated several times to make sure your results are accurate.

Introduction Most chemical reactions must be catalyzed (sped up) by protein molecules called enzymes. Enzymes are biological catalysts that facilitate specific chemical reactions. Enzymes are three-dimensional globular proteins that fit snugly around the molecules they act on. This fit facilitates chemical reactions by stressing particular chemical bonds. The three-dimensional shape enables it to stabilize a temporary association between substrates-the

molecules that will undergo the reaction. The enzyme also lowers the activation energy required for new bonds to form.

The reaction thus proceeds much more quickly than it would without the enzyme. (Mason, 2011). The energy of activation is the energy needed to get the substrate to its transition state. KI (potassium iodide) is used to detect the presence of starch when conducting these experiments. Another thing to consider when talking about enzymes is optimal conditions. These are a set of environmental conditions at which the enzyme works at its highest rate. Some of these environmental variables are pH, temperature, and salinity.

Changes in pH may not only affect the shape of an enzyme but it may also change the shape or charge properties of the substrate so that either the substrate cannot bind to the active site or it cannot undergo catalysis. (The Effect of pH on Enzyme Activity, 2004). Increasing the temperature of an uncatalyzed reaction increases its rate because the additional heat increases random molecular movement. This motion can add stress to molecular bonds and affect the activation energy of a reaction. (Mason, 2011). When a substrate molecule is trying to bind to the active site, presence of salt could alter the rate of the reaction.

In our experiment, we used the protein amylase. Amylase is an enzyme that breaks down starch, converting it into sugar. Amylase is found in human saliva, where it begins a chemical process in digestion with the hydrolysis of starch. It is also found in the pancreas. (Brady, 2003). We used the substrate starch with the variable, pH, to measure the absorbance of our samples using a calorimeter. My hypothesis was that at each different pH buffer,

there would be more and more absorbance over time. **Materials/Methods** To execute this experiment, we did the following steps. First, you pipet 8ml of 0. % starch solution and 6ml of water into 3 test tubes and label them L, M, and H. Next, you add 1ml of pH4 buffer to L test tube; 1ml of pH7 buffer to test tube M; and 1ml of pH10 buffer to test tube H. Then pipet 2ml of water and add 3 drops of KI into 16 different test tubes (5 each behind the test tubes L, M, and H) and label them L? , M? , H?L? , M? , and H? and keep the remaining one for zeroing the calorimeter(reagent blank). Next remove 1ml of solution from L, M, and H to the test tubes L? , M? , and H? respectively. Measure their absorbance and record the values.

Make sure to zero the calorimeter before every measurement. Next, pipet 1ml of amylase solution to L, M, and H (mix) and wait for 1 minute interval. Then, remove 1ml of L, M, and H into L? , M? , and H? respectively (mix) and measure the absorbance of the samples and record the values. Repeat this last step for the rest of the samples for the same time interval. **Results** The table and graph below represent the absorbance levels that we obtained from our experiment.

Time of measurement	Reaction 1 L (pH4)	Reaction 2 M (pH7)	Reaction 3 H (pH10)
0	2. 0	0. 85	2. 00
1	1. 71	0. 53	2. 00
2	1. 46	0. 06	2. 00
3	1. 42	0. 05	2. 00
4	0. 97	0. 00	2. 00

Graph 1 **Graph 2** **Graph 3** **Discussion** In conclusion, the results from this experiment failed to support my hypothesis. My original hypothesis stated that at each different pH buffer, there would be more and more absorbance over time. Our results show that at pH4 buffer the absorbance increased by causing our readings to go down at a steady pace. From starting at Time 0,

the end reading was at 0.97. This particular reaction supported my hypothesis.

The next reaction with pH7 buffer also supported my hypothesis. There was also more absorbance over time intervals. Our numbers decreased but this time, at a faster pace. There was a jump from 0.53 to 0.06. This would cause me to believe that at pH7, this would be the optimal condition for enzyme activity for amylase. In the last reaction, I was surprised to find that there was no change at all. The pH10 buffer had no effect with the absorbance of our amylase-starch sample. This particular reaction failed to support my original hypothesis.

So, in conclusion, using the enzyme amylase and the substrate, starch, we found that the effect of pH on this solution caused a steady absorbance for pH4, a fast absorbance at pH7-which caused me to believe this is optimal pH, and no absorbance at pH10. Bibliography Brady, Matt. What is Amylase? 2003. 22 October 2011. Mason, Kenneth A., Jonathan B. Losos and Susan R. Singer. Biology. New York, NY: McGraw-Hill, 2011. The Effect of pH on Enzyme Activity. 2004. 22 October 2011.