

Monitoring how ph affects the rate of reactions of barley amylase



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Abstract This experiment was carried out to monitor the ability of the Barley Amylase Enzyme to effectively break down starch in solutions that are increasing in neutral pH. To do this the experiment was carried out so that tubes containing a reaction solution of the Amylase enzyme and starch were simultaneously mixed. The reactions were then introduced to I2-KI, which stopped the reactions, at two minute intervals. Each of these trials was repeated three times to ensure proper accuracy.

After concluding the reactions they were placed into a spectrophotometer (A580) for analysis. Graphing the values of the absorbance to time for each pH it was found that the rate of reactions in the neutral pH solutions were much higher than that of the acidic solution. We concluded that this was because of the possibility of an enzyme to become denatured in acidic solution.

Introduction The study of enzymes is crucial to understanding what drives the forces that create and maintain everything that we consider living. Enzymes are biomolecules that serve as catalysts in chemical reactions with almost all of them falling into the classification of proteins. The enzyme's catalytic effect is so important because without it many reactions that maintain life functions would cease to exist.

(Holum 1968) This is due to the slow rate at which these chemical reactions function without the help of an enzyme. These enzymes can range anywhere from 62 amino acid residues to 2, 500, making them extremely specialized for each reaction. Despite their size only around 3-4 amino acids are directly involved in the catalysis. The molecules that are acted on by the enzymes are called substrates. These substances are changed by the enzymes to facilitate the reaction without permanently changing the structure of the

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enzyme. As with all catalysts, it does this by lowering the activation energy (E_a) of the reaction.

However, these enzymes lose their ability to aid in reactions when they are unfolded and inactivated, this process is called denaturalization. (Kimball 2003) The denaturalization of the barley amylase enzyme, which can be caused by heat or chemical denaturants, is the focus of this experiment. Testing the enzyme's ability to hydrolyze starch at a variety of pH levels, such as pH 4-7, and comparing the results to a control solution without the enzyme allows the effect of pH on enzyme activity to be studied. My lab group and I hypothesized that decreasing the pH will simultaneously decrease the enzyme activity. (Klein 2004) This is because the enzyme is most active at a neutral pH, in accordance with the information provided by the Worthington Biochemical Corporation.

Worthington 2008) To analyze the effect of pH on enzyme activity we used a spectrophotometer to measure the absorbance of several known enzyme concentrations in increasing pH solutions over recorded time intervals. This showed the product concentration and allowed my group to deduct the enzyme activity based on the product concentration when compared to a standardized curve. Methods Part A Establishing a standard curve 1. Set up six test tubes as explained in the table below 2. Cover tubes with parafilm and shake well. Remove parafilm before placing test tube into spectrophotometer.

. Measure the absorbance using the spectrophotometer at 580 nm using the first tube as a " blank". (Do not discard " blank" tube until the experiment is finished). 4.

Plot the absorbance at 580 nm on graph paper. Tube #Neutral Buffer (ml)0.

2 mg/ml Starch (ml)I2-KI (ml)A580Final Starch (mg/l) 13. 0*1. 000. 0 22.

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0 Part B Effect of pH on enzyme activity 1. Set up test tubes as noted in the table below, keeping all components separate until the proper mixing times.

2. Get enough reactants to repeat each trial for each pH three times. 3. Add starch to each of your trial test tubes.

4. Get ready to begin timing and add the amylase enzyme to all tubes at the same time or one after another, noting the start time of each reaction. 5. Mix thoroughly and add I2-KI to end the test tube reactions at two minute intervals for minutes 0, 2, and 4. This is the stopping point of the reactions.

6. Insert stopped reaction tubes into the spectrophotometer (A580) for analysis. 7. Plot values on graphs. Tube #Buffer pH 5 (ml)Buffer pH 6 (ml)Buffer pH 7 (ml)0. mg/ml Starch (ml)0.

5 mg/ml amylase (ml)I2-KI (ml)A580 71. 9**1. 00. 11. 0See BelowpH 5 8*1.

9*1. 00. 11. 0See BelowpH 6 9**1. 91.

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00. 11. 0See BelowpH 7 (UGA Manual 2-5) Results The results show a general increase in the rate of reactions for the hydrolysis between the starch and amylase enzyme was higher in the neutral pH solutions. When amylase reacts with starch, it separates the disaccharide maltose.

As the reaction progresses, less starch will be present and more sugar (maltose) will be present. (Bernfield 151) In lab, the activity of amylase was observed by using iodine. Remember, iodine reacts with starch to form a purple color. As amylase breaks down starch less starch is present and the color of the solution will become lighter. Discussion The hypothesis proposed by our group was supported by the observations of our experiment. The absorbencies of the more neutral pH solutions were higher, indicating a higher rate of reaction within the test tubes of those solutions.

This is supported by our hypothesis that effect of lowering the pH of the solution and increasing the acidity increases the likelihood of the enzyme becoming denatured. (Worthington 2008) This result is due to that nature of enzymes and the fact that they can easily be denatured by chemical, ionic, kinetic, and many other forces. The enzyme also exhibits high activity in acidic or near neutral conditions. More than 50% of activity was seen between pH 4.6 and 6.8, with optimal at pH 6.

0. This indicates the enzyme prefers a slightly acidic environment. (Bernfield 154) Tables and Figures X Axis- Time (minutes) Y-Axis- Absorbance (580nm)
pH = 5 Time (min) 0 24 Absorbance (580 nm) 0.7930.

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860. 0490. 002 pH = 6 Time (min)024 Absorbance (580 nm)0. 8000.

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2830. 110 pH = 7 Time (min)024 Absorbance (580 nm)1. 0350. 4700.

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4350. 236 Standard Curve Tube #Neutral Buffer (ml)0. 2 mg/ml Starch
(ml)I2-KI (ml)A580Final Starch (mg/l) 13. 0*1. 000. 0 22.

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01. 01. 03550. 0 References Division of Biological Sciences, The University of Georgia, Laboratory Manual For Principles of Biology I, 2007 Holum, J. Elements of General and Biological Chemistry, 2nd ed.

, 377, Wiley, NY (1968). Klein, S. : Barley Amylase, [http://www.chem.](http://www.chem.uwec.edu/Webpapers2005/leee/barley.html)

<http://www.chem.uwec.edu/Webpapers2005/leee/barley.html>, University of Wisconsin, (2004)

Kimball, J. : Enzyme Kinetics, [http://users.rcn.](http://users.rcn.com/jkimball)

[http://users.rcn.com/jkimball.](http://users.rcn.com/jkimball) ma. ultranet/BiologyPages/E/EnzymeKinetics.html , Harvard

College, (2003) Worthington Biochemical Corporation: Effects of pH

(Introduction to Enzymes), Lakewood, NJ, 2008 Bernfeld, P. , 1955. Amylase a and b.

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