The effects of substrate concentration, reaction time and enzyme concentration on...

Philosophy

The Effects of Substrate Concentration, Reaction Time and Enzyme Concentration on Enzyme Reactions Corey von Ellm-St. Croix Rachael Kwan ID#: 20427841 Matthew Hrycyshyn & Saeideh Mayanloo Biol 130L, Section 017 Wednesday, 9: 30am-12: 20pm, 151 November 09, 2011 A living system controls its activity through enzymes. Enzymes are made from hundreds or even thousands of amino acids connected in a very unique and specific order. Almost all enzymes are proteins, except for ribozymes. The chain of amino acids then folds into a unique shape.

That shape not only allows the enzyme to carry out specific chemical reactions but to act as a very efficient catalyst. The enzyme speeds that reaction up tremendously. Each enzyme reacts with one specific reactant called a substrate that will form its products. The purpose of the experiments is to determine the effects of substrate concentration, reaction time and enzyme concentration on the direction of an enzyme reaction. Amylase is a digestive enzyme found in both the saliva and the small intestine.

Salivary amylase is a hydrolytic reaction that breaks down starch molecules by systematically breaking off the maltose molecules from the ends of starch chains. The maltose is further broken down by another enzyme. Phosphorylase is an enzyme that systematically removes glucose molecules by consumes phosphoric acid to break the beta-1-4-glucosidic bonds in starch. The interaction of phosphate with the glucosidic bond results in the formation of glucose-1-phosphate and the loss of a chain unit in starch. In the reverse reaction the glucose part of glucose-1-phosphate is added as a new chain unit and phosphate is set free.

This reversible enzymatic polymerization occurs with little change in free energy and therefor the reaction may choose to go either way. Iodine Test is a test for the presence of starch in which the sample turns blue-black in color when a few drops of potassium iodide solution are placed on the sample. A negative iodine test is when the reaction remains yellow in colour. It is the reaction between iodine and the coiled polymer of glucose known as amylase in starch that causes the colour change. The reaction occurs when straight amylase chains form helices in which the iodine can pass inside.

Glycogen also receives a colour change because it is a glucose polymer as well but its structure differentiates from starch which therefore forms a brown colour change. The iodine test does not work for mono or disaccharides because they are too small to capture the iodine. The Benedict’s test is used to detect the presence of reducing sugars. Reducing sugars are sugars with a free aldehyde or ketone group. The free reactive carbonyl group allows all monosaccharides to be reducing sugars. The same goes for disaccharides as some also contain the free reactive carbonyl group.

The colour of precipitate formed when the Benedict’s test acquires a positive result depends on the concentration of reducing sugars present. A green colour change indicates that few reducing sugars are present. Orange indicates a higher concentration, red an even higher concentration and brown is the highest concentration colour change. A negative test for reducing sugar occurs when the Benedict’s solution remains its blue colour. Materials and Method Materials and Method found in 1119 BIOL 130, Department of Biology 2011 Cell Biology Laboratory Manual. University of

Waterloo, Waterloo. Fall 2011. p. 37-42. The procedure of the lab did not differ from that in the lab manual. Results Salivary Amylase Iodine test: Table 1 Test Tube Number| Results Through Experimentation| Control | 1 (10% salivary amylase solution) | Yellow | Negative | 2 (5% salivary amylase solution)| Yellow| Negative | 3 (2% salivary amylase solution)| Yelllow| Negative | 4 (1% salivary amylase solution)| Yellow| Negative| 5 (1% starch suspension)| Blue-Black| Positive| Table1: The table above represent the first iodine test done. Shows components of each test tube as well as the results and positive or negative control.

Dilutions were done using tap water (may contain other molecules). Corresponds to steps 5 through 10. Benedict's test: Table 2 Test Tube Number| Results Through Experimentation| Control| 1 (10% salivary amylase solution) | Brown-orange precipitate| Positive| 2 (5% salivary amylase solution)| Green precipitate | Positive| 3 (2% salivary amylase solution)| Blue | Negative| 4 (1% salivary amylase solution)| Blue| Negative| 5 (1% starch suspension)| Blue| Negative| Table 2: The table above represent the first Benedict's test done. Shows components of each test tube as well as the results and positive or negative control.

Each of the above test tubes contains 4ml Benedict's solution and were boiled for 5 minutes when determining results and are related to steps 5 through 10. Iodine test: Table 3 Test Tubes| Number of Drops Till Negative| Time Interval| Time| 9+14 (1% salivary amylase solution)| 13 | 60 seconds| 13*60= 780 seconds| 8+13 (2% salivary amylase solution)| 12| 30 seconds| 12*30= 360 seconds| 7+12 (5% salivary amylase solution)| 10| 15 seconds|

10*15= 150 seconds| 6+11 (10% salivary amylase solution)| 18| 5 seconds| 18*5= 90 seconds | 10+15 (water)| (Always positive)| N/A| N/A|

Table 3: The table above represents the time it took to reach endpoint. Shows components of each test tube, number of drops, time interval between drops and time to reach endpoint. Each of the above test tubes contains 2ml - 1% starch suspension and 2ml McIlvaine's buffer. The above test tubes were placed in a warm bath at 37 degrees Celsius and pertain to steps 11-18. Benedict's test: Table 4 Test tube| Results through experimentation| Control| 20 (water)| blue| negative| 9 (1% salivary amylase solution)| 1/3 brown -2/3 blue| Positive| 18 (2% salivary amylase solution)| 1/3 brown -2/3 blue| Positive| 17 (5% salivary amylase solution)| 1/3 brown -2/3 blue| Positive| 16(10% salivary amylase solution)| 1/3 brown -2/3 blue| Positive| Table 4: The table above represents the search for reducing sugars after endpoint. Each of the above test tubes contains 4ml Benedict's solution and were boiled for 5 minutes when determining results and are related to steps 18-20. Phosphorylase Composition of test tubes: Table 5 TEST TUBE NUMBER | CONTAINS| One| 1. 5ml of 0. 01M glucose + 1 drop of 0. % starch suspension| Two| 1. 5 of 0. 01M glucose-1-phosphate+ 1 drop of 0. 2% starch suspension| Three| 1. 5 of 0. 01M glucose-1-phosphate| Four| 1. 5 of 0. 01M glucose-1-phosphate+ 1 drop of 0. 2% starch suspension| Five| 1. 5 of 0. 01M glucose-1-phosphate + 0. 5ml of 0. 2M potassium phosphate+ 1 drop of 0. 2% starch suspension| Six| 0. 5ml of 0. 2M potassium phosphate + 1. 5ml of 0. 2% starch suspension| Seven| 0. 5ml of 0. 2M potassium phosphate+ 1. 5ml of 0. 2% starch suspension| Eight| 4ml Boiled

phosphorylase | Table 5: The above table represent the solutions present in the test tubes 1-8 from steps 2-10 Iodine test: Table 6

Test tube| Results through experimentation| Control| 1| Yellow | Negative | 2| Yellow| Negative| 3| Yellow| Negative| 4| Yellow| Negative| 5| Yellow| Negative| 6| Blue-black| Positive| 7| Blue-black| Positive | Table 6: Search for starch within test tubes 1-7. Shows components of each test tube as well as the results and positive or negative control. Composition of test tubes : Table 7 TEST TUBE NUMBER | CONTAINS| One| 1. 5ml of 0. 01M glucose + 1 drop of 0. 2% starch suspension + 2ml phosphorylase| Two| 1. 5 of 0. 01M glucose-1-phosphate+ 1 drop of 0. 2% starch suspension+ 2ml phosphorylase| Three| 1. of 0. 01M glucose-1-phosphate+ 2ml phosphorylase| Four| 1. 5 of 0. 01M glucose-1-phosphate+ 1 drop of 0. 2% starch suspension + 2ml boiled phosphorylase| Five| 1. 5 of 0. 01M glucose-1-phosphate + 0. 5ml of 0. 2M potassium phosphate+ 1 drop of 0. 2% starch suspension+ 2ml phosphorylase| Six| 0. 5ml of 0. 2M potassium phosphate + 1. 5ml of 0. 2% starch suspension+ 2ml phosphorylase| Seven| 0. 5ml of 0. 2M potassium phosphate+ 1. 5ml of 0. 2% starch suspension + 2ml boiled phosphorylase| Table 7: The above table represent the solutions present in the test tubes 1-7 from steps 11-12 Iodine Test: Table 8

Time Interval| test tube 1| Test tube 2| Test tube 3| Test tube 4| Test tube 5| Test tube 6| Test tube 7| 10: 28-10: 32| yellow| Very faint blue-black| yellow| yellow| yellow| Faint blue-black| Blue black| 10: 32-10: 36| yellow| Blue black| yellow| yellow| yellow| Very faint blue-black| Blue black| 10: 36-10: 39| yellow| Blue black| yellow| yellow| yellow| Faint blue black| Blue black| 10:

39-10: 42| yellow| Blue black| yellow| yellow| yellow| Faint blue black| Blue black| 10: 42-10: 46| yellow| Blue black| yellow| yellow| yellow| Blue black| Blue black| 10: 46-10: 49| yellow| Blue black| Very faint blue black| yellow| yellow| Blue black| Blue black| 10: 49-10: 52| Yellow| Blue black| Faint blue black| Yellow| yellow| Blue black| Blue black| 10: 52-10: 55| Yellow| Blue black| Blue black| Yellow| Yellow| Blue black| Blue black| 10: 55-10: 58| Yellow| Blue black| Blue black| Yellow| Yellow| Blue black| Blue black| 10: 58-10: 42| yellow| Blue black| Blue black| yellow| Yellow| Blue black| Blue black| Table 8: Test for the presence and synthesis of starch.

Contains the time interval from when the previous test had ended to termination of current test and the reaction result of test tubes 1-7. Figure1: above; represent the time it took each salivary amylase concentration to reach endpoint (when test for starch became negative. Discussion: Salivary Amylase The Iodine test’s control is the presence of starch. If starch is presence then the control is positive resulting in a blue-black colour change. The first iodine test or if you refer to table 1, gave a positive result for only test tube 5 which contained 1% starch suspension. Clearly starch is present based on just the component of the solution.

A negative control in an iodine test will result in maintenance of the yellow colour of iodine. According to table one the test tubes containing 10% salivary amylase solution, 5% salivary amylase solution, 2% salivary amylase solution and 1% salivary amylase solution resulted in a negative control result. This is due to the fact that all that is present is the enzyme salivary amylase and water and therefore no starch. The Benedict’s test control is the

presence of reducing sugars (sugars with a free aldehyde or ketone group). If a reducing sugar is present then a positive control reaction will occur. A positive control reaction is when a colour of the blue Benedict’s solution turns green, orange, red or brown after boiling.

Each colour represents the concentration of reducing sugars present, green being the lowest and brown the highest. Referring back to table 2, test tubes 1 and 2 resulted in a positive control reaction. Even though test tubes 1 and 2 contained only salivary amylase the tap water used to dilute the amylase solution may contain some starch which would in turn become maltose a reducing sugar. The 10% salivary amylase (test tube 1) resulted in an orange colour change due to the fact that a higher enzyme concentration would more likely produce enough reducing sugars to result in an orange colour change. The 5 % salivary amylase (test tube 2) resulted in a green colour change which describes a low concentration of reducing sugars.

This makes sense as a lower enzyme concentration would result in less reducing sugar being made through the enzymatic reaction between starch and amylase. A negative control reaction for the Benedict’s test occurs when the Benedict’s blue solution remains the same. Referring back to table 2 test tubes 3, 4 and 5 resulted in a negative control reaction. This may be due to the fact that the enzyme concentration were too low to produce enough reducing sugars from the starch found in the tap water to warrant a colour change. The starch (substrate) would for a substrate-enzyme complex with salivary amylase to produce maltose and salivary amylase. In conclusion

enzyme concentration does play a factor in the speed of an enzymatic reaction.

The results of Table 3, the second iodine test performed, is used to determine when the starch added with the different concentrations of salivary amylase has reached its endpoint and has been fully hydrolysed into maltose. The endpoint has been reached once the iodine test gives a negative control result which occurs once no starch or very few is present. According to the experimental data presented in table 3 enzyme concentrations again played a role in the speed of the reaction. 10% salivary amylase took 90 seconds where as 1% salivary amylase took 780 seconds. The starch (substrate) would for a substrate-enzyme complex with salivary amylase to produce maltose and salivary amylase.

Test tube 10 + 15 will result in a positive control reaction all the time because it is comprised of water and starch. With no salivary amylase enzymes starch will always be present which is the positive control in an iodine test. A trend was found that as the salivary concentrations were halved the time to reach endpoint was doubled, leading me to believe an inverse proportionality to be present between enzyme concentration and time to reach end point. Table 4 was another Benedict’s test performed after the each combination of test tube had reached its endpoint. The positive result in a Benedict’s test occurs once a green, orange, red or brown colour change occurs because of the presence of reducing sugars.

Test tubes 16-19, containing the different concentrations of salivary amylase, resulted in a positive control reaction because the starch

(substrate) would for a substrate-enzyme complex with salivary amylase to produce maltose and salivary amylase and due to the fact that maltose is a reducing sugar which happens to be the control for a Benedict's test, a positive control reaction will occur. The negative control reaction for a Benedict's test is when the Benedict's solution remains blue signifying the absence of reducing sugars. According to table 4, test tube 20 only contained water and the starch suspension with no amylase present a substrate-enzyme complex will not form which will not result in a reducing sugar. Phosphorylase Table 6 is another iodine test.

The positive control reaction for an iodine test is when the solution turns blue-black. The experimental data given in table 6 shows that test tubes 6 and 7 gave a positive reaction for starch because of the 1. 5 ml of 0. 2% starch found in solution. The negative control reaction is when the solution remains the colour of iodine, yellow. Test tube 1 through 5 gave negative result because they either do not contain any starch in solution or the amount of starch present is too little (starch primer) and must be in presence of phosphorylase to synthesis a larger starch chain that can be reacted with the iodine test to provide a positive result. Table 8 is once again another iodine test.

With the addition of phosphorylase some of the test tubes that gave a negative result in the previous iodine test (table 6) may now give a positive result because of the ability of the reaction between phosphoric acid and glucose to from glucose-1-phosphate and one less glucose unit in starch chain to go in either direction. Therefore a test tube with a starch primer

may use the phosphorylase to synthesis into a starch chain. The same is for the solution that gave a positive reaction may turn negative in the presence of phosphorylase to form a starch primer and glucose-1-phosphate. Referring to table 8 the test tubes that resulted in a positive control reaction were 2, 3, 6 and 7.

Because test tubes 6 and 7 were already gave positive results in previous iodine test (table 6) and did the same in this iodine test can only mean that a synthesis of a larger starch chain had occurred or the starch chain had not removed enough glucose bonds to result in a negative iodine control result. In test tube 7 the phosphorylase was boiled which would denature the enzyme so that it could not perform its task and therefore phosphorolysis could not take place and therefore test tube 7 would have to remain a positive control result. Test tubes 2 and 3 were primarily negative in previous iodine test but resulted in a positive control result when the enzyme phosphorylase was added.

Table shows that over time both solutions grew more intense in colour signifying the synthesis of a longer starch chain. Test tube 2 had the starch primer and glucose-1-phosphate to start with and therefore took less time to give a positive control result. Test tube 3 did not contain the starch primer andI believeshould not have given a positive control result. Test tube 3 did however contain the glucose-1-phosphate and perhaps may have started its own starch chain. This may have been done by having a glucose-1-phosphate and the glucose form a substrate-enzyme complex to give

phosphoric acid and a larger glucose chain. The negative results were test tubes 1, 4 and 5 each contained the starch primer.

Test tube 1 contained glucose but phosphorylase does not react with single glucose molecule and therefore test tube 1 will always give a negative control result. Test tube 4 used boiled phosphorylase and therefore the denatured enzyme would not be able to perform function which would result in an always negative control result. Test tube 5 had the right condition but perhaps never moved in one direction of the enzymatic reaction for too long resulting in a starch primer being present the whole time though it may have had potential to yield a positive control reaction. This shows that temperature do affect an enzyme. A buffer was also used in the reaction to allow for the proper pH levels to be obtained and therefore pH levels also affect enzymes.

Overall throughout the experiment it was determined that substrate concentrations, reaction time and enzyme concentration effect the direction of an enzyme reaction. Reference Pelter, W. M. , McQuade, J. (2005). BrewingSciencein the Chemistry Laboratory: A “ Mashing” Investigation of Starch and Carbohydrates. Journal of ChemicalEducation, 82(12), 1811-1812. Ophardt, E. C. , (2003). Role of Enzymes in Biochemical Reactions. Virtual Chembook, Retrieved November 06, 2011, from Elmhurst College, http://www. elmhurst. edu/~chm/vchembook/570enzymes. html. Hall, I. (2008). Benedict's Test for Reducing Sugars. Retrieved November 06, 2011, from Ohio University, http://www. biosci. ohiou. edu/introbioslab/Bios170/170_2/benedict. htm