**The Effects of Substrate Level, Temperature, and pH on the Efficiency of the Enzyme ß-Galactosidase**

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**ABSTRACT**

The enzyme-substrate complex of ß-galactosidase (lactase enzyme) and lactose, a disaccharide found in the milk of mammals is a crucial part of human metabolism, considering that humans are agrarian, and rely sometimes on the milk of other animals as a source of protein. There are many factors that determine the efficiency of this complex. By figuring these parameters out, one can begin to set and control the conditions that make people who are lactose intolerant very sick. Human bodies do not digest lactose without the help of the lactase enzyme, so in looking at it’s behavior under controls of temperature, pH and molar concentration, the puzzle can be pieced together about what kind of environment will allow the lactase enzyme to work efficiently and effectively.

The enzyme **ß-**Galactosidase (also known as lactase) is an important enzyme in the human body involved in the digestion of milk. Lactose is a disaccharide that is made up of two smaller sugars: galactose, and glucose. Lactase, the enzyme in this lab, hydrolyzes the beta-1,4-glycosidic linkage that bonds those two sugars together, and make it possible for our stomach to digest the lactose sugar in milk (2).

Since this process in which lactase breaks down lactose is difficult to observe, for the purposes of this experiment, an alternative substrate was used. This alternative substrate is very similar to lactose, in that it is a disaccharide built with one glucose. Instead of galactose, o-nitrophenolate is produced when it is hydrolyzed by lactase. O-nitrophenolate absorbs a distinct wavelength of light at 420 nm, and produces a yellow color in solution. This makes our alternative substrate, o-nitrophenyl-ß-D-galactopyranoside (or ONPG) much easier to monitor using spectrophotometry.

**MATERIALS AND METHODS**

The first reaction to be tested was based on the production of o-nitrophenolate when the lactase enzyme was added solutions with different concentrations of ONPG. Six cuvettes were labeled (0 mM ONPG, 1.88 mM, 3.75 mM, 7.5 mM, 15 mM, and 30 mM), and 1 mL of the .1 molar phosphate buffer with a pH of 7.3 at 22˚C was used as the base for these cuvettes. One cuvette at a time, 125 µL of one of the ONPG concentrations was added. Then, 125 µL of the ß-galactosidase was added to the solution and quickly mixed. This reaction was timed for 60 seconds, to give the enzyme enough time to react with the substrate, after which the absorbance at 420 nm was recorded.

For the second test, the pH of the phosphate buffer in solution was varied for each cuvette. The three cuvettes in this test were labeled with their respective pH levels (one at pH 5.7, one at pH 7.3, and the last one at 8.0 pH). The amount of ONPG this time was a control, and was set at 30 mM. 1.0 mL of the perspective phosphate buffers and 125 µL of ONPG was added to each cuvette one at a time, and then the lactase enzyme was indroduced and mixed in. The production of ONP (o-nitrophenolate) was monitored by the absorbance of the cuvette at 420 nm after 60 seconds.

The third assay procedure was done with the intent of monitoring the effect of temperature on the production of o-nitrophenylate when a fixed amount of ONPG reacts with the lactase enzyme. 150 µL of the lactase enzyme was added to two Eppendorf tubes so that the temperatures that were to be tested could be achieved. One of the samples of the enzyme was incubated for 5 minutes at 50˚C, and another was chilled to 0˚C and left for 5 minutes. Cuvettes were labeled individually with their corresponding temperatures of the enzyme (0˚C, 22˚C, and 50˚C). Again, 125 µL of ONPG was added to the 1.0 mL of the pH 7.3 phosphate buffer in preparation for the lactase enzyme to be added to each. After the 5 minutes, 125 µL of the lactase solution at 50˚C was added to the ONPG. The substrate and the enzyme were mixed and recorded based on absorbance of 420 nm after 60 seconds.

**RESULTS**

Figure 1 shows the data gathered from the experiment that was based on substrate concentration. One can see that the more substrate is involved in the reaction, the higher the output is, but there is a sort of ceiling to the That is, the higher the concentration of ONPG that there is, the more the wavelength of 420 mM is absorbed, but the data seems to be working towards a plateau. The rate of increase of the absorbance is higher towards the beginning of the curve, and gets less dramatic as the reaction was conducted with more substrate.

Figure 1. Substrate Concentration and Product Formation at Room Temperature and pH 7.3.

The second graph, Figure 2, shows the effects of pH on the efficiency of the lactase enzyme. If any trends are clear in this graph, the higher the pH is, the more efficient the enzyme becomes.

Figure 2. pH and Product Formation at Room Temperature.

Our findings from the experiment with temperature and how that effects enzyme efficiency show that the specific 420 nm is absorbed much more by the samples at room temperature than at 0˚C or at 50˚C.

Figure 3. Effects of Temperature on Product Formation.

The enzyme substrate complex of ß-galactosidase and lactose is an important reaction in the metabolism of animals, and especially humans. In people, lactose intolerance can lead to such serious symptoms as severe as irritable bowel syndrome (IBS), eczema, asthma, and osteoarthritis when not diagnosed correctly or ignored. Mammals in general have a very low amount of lactase in their bodies, so it is important to know how different environmental factors can effect the efficiency of the enzyme to understand how the symptoms of lactose intolerance can be prevented.

**DISCUSSION**

The amount of lactase that exists in an animal can indicate how easily lactose is digested. The first part of the experiment that was done shows this trend, that a certain amount of lactose is needed to cause the reaction to occur. Without ß-galactosidase, the human body cannot break down lactose from animal milk (2). The more of the enzyme a person has, the more of the products (ONP and glucose) can be formed from the substrate. With a small amount of the enzyme, the substrate is only partially broken down. In other words, by adding more of the enzyme to the complex at the appropriate time, equilibrium can be reached, and lactose can be digested.

Based on the data that was collected in this experiment, there is also an optimal pH for this kind of reaction to take place that is higher than normal. That means that this works best in a more basic environment. Since milk, the substance that lactase primarily works in, is rich in Calcium and protein. Milk is also fairly basic, and that is likely why this enzyme is more productive in less acidic conditions. There is a different kind of ß-galactosidase that exists in Apples and Tomatoes and other fruits before they are ripened, but that is destroyed once the fruit reaches maturity. Fruit ripening is accompanied by a decrease in galactose in the cell walls of the fruit, which allows the fruit to soften. This would indicate that as the pH of the fruit becomes too high, and the fruit is ripe, the enzyme activity begins to slow (3). Our data doesn’t reflect those pH values over 8. In order for the substrate and the products to be in equilibrium, the pH must be high, but once the system goes over 8, the efficiency of the enzyme decreases dramatically.

The temperature that ß-galactosidase activates at is another very important factor in determining efficiency. The thermodynamic parameters (of ß-galactosidase) are consistent with the fact that the activated state of the enzyme-substrate complex is reached through a minimum of enthalpy change (1). This would mean that in order to increase the amount of products produced from a certain amount of substrate, the complex would have to be above a certain temperature to be successful. For example, if one were to separate the lactose in milk into galactose and glucose, one would have to execute that reaction at room temperature, and as mentioned before, at a very specific pH.

In conclusion, parameters for the environment must be met in order to maintain a proper substrate-product ratio, and in order for the activation energy of this enzyme to be met, the enzyme needs help. The closer to room temperature the reaction occurs, the better, and the closer to pH 8 the reaction occurs, the better that will be, and finally, the more of the enzyme that is in the system, the more the substrates will be digested.

**LITERATURE CITED**

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