**Optimal Conditions for the Reaction of Starch and Salivary Amylase in Regards to Enzyme Concentration, Substrate Concentration, pH, and Temperature**

Tori Ouendag

The University of Alabama

Abstract:

The optimal conditions for salivary amylase reaction were determined through the examination of enzyme concentrations, substrate concentrations, pH, and temperature. The effects of enzyme concentration and substrate concentration on the rate of reaction were tested using serial dilutions. Optimal conditions for pH and temperature were explored as well through experimentation. The overall optimal conditions for a reaction of starch and salivary amylase were found to be high enzyme concentration, low substrate concentration, a pH of 5.0, and a temperature of 36 degrees Celsius.

Introduction:

In order for a chemical reaction to take place, there needs to be an addition of energy to the molecules involved. Enzymes work to catalyze reactions by reducing the amount of energy—called the energy of activation—that the reaction needs to begin. An enzyme bonds with the substrates at its active site, lowering the activation energy. The reaction is then completed and the products released from the active site (Shefferly and Johnson, 2011). Each enzyme will catalyze only one reaction because the active sites are so specific to a particular substrate (Shefferly and Johnson, 2011).

The external conditions surrounding an enzymatic reaction affect the speed at which it takes place. Reactions proceed the most quickly when accompanied by the optimal temperature, pH level, enzyme concentration, and substrate concentration for that particular enzyme. Amylase is an enzyme found in saliva that functions in the breakdown of starch, converting large glucose chains into maltose (Shefferly and Johnson, 2011).

Scientists have concluded that a higher reaction temperature will increase the speed at which a reaction of amylase will proceed (Yadev and Prakash, 2009). Further experiments have also shown an inverse relationship between substrate concentration and reaction rates (Mussatto *et al.*, 2008). In addition, scientists have found evidence supporting the theory that the optimal pH of amylase is below seven or acidic (Hsieh *et al*., 2008; Ilori *et al*., 1997). Past studies of the amylase have examined the effects of these factors—temperature, pH, enzyme concentration, substrate concentration—on reaction rates separately, however we chose to examine the combined impact of such factors on a reaction of starch and amylase in order to determine the optimal conditions for said reaction. Based on these past findings, we predict that if a reaction of amylase and starch occurs with high enzyme concentration, low substrate concentration, higher temperatures, and an acidic pH, then the reaction rate will be quickest.

Materials and Methods:

1. Enzyme Concentration and Amylase Activity

A 2% solution of fungally derived amylase was serially diluted (Shefferly and Johnson, 2011) into concentrations of 2%, 1%, .5%, .25%, and .125% amylase solution. At the same time, we placed one drop of iodine into each of 5 wells in a well plate. One at a time, we combined each concentration of amylase with 1% starch solution in a 3:1 ratio, so that1 mL of substrate combined with 3mL of the enzyme. We quickly mixed the resulting solution and transferred to one of the prepared wells. Immediately after filling the well, we used a stopwatch to record the amount of time that elapsed between the addition of the solution to the iodine and when the mixture changed color from purple/black to yellow/clear, indicating a completion of the reaction. We then repeated this process for each concentration of amylase solution and recorded the reaction rates.

1. Substrate Concentration and Amylase Activity

We used serial dilutions on a 1% starch solution in the same manner as in Experiment 1, so that it produced concentrations of .5%, .25%, .125%, .0625%, and .03125% starch solution. A well plate was also prepared as in Experiment 1, with one drop of iodine in each well. We added each starch dilution individually to an equal amount of amylase solution, so that there was a 1:1 ratio with 2mL of each solution in every container. Once we had quickly stirred the mixture we piped it into a well in the prepared well plate. Again, we used a stopwatch to measure the amount of time it took for the substrate to disappear completely, turning the solution color from purple/black to yellow/clear. We repeated this reaction for each concentration of starch solution and recorded the resulting reaction times.

1. pH and Amylase Activity

Five mL each of buffers with varying pH—3.0, 5.0,7.0, 8.0, 10.0—were measured out and 2.5mL of 1% starch solution was added to each container, creating a 2:1 ratio of buffer to starch solution. Meanwhile, a well plate was once again prepared by adding 1 drop of iodine to each well. Two mL each of 2% amylase solution was poured into five test tubes. One at a time, we added the starch/buffer mixture to a tube of 2% amylase solution, stirred it, transferred it into a prepared well plate, and timed the reaction to completion. We started timing when we added the solution to the well plate and stopped when the mixture changed color from purple-black to yellow or clear. Once again, we repeated these steps for each additional buffer mixture, and recorded the reaction rates.

1. Temperature and Amylase Activity

Four test tubes were prepared, each containing 3mL of 2% amylase solution. We placed each test tube into a water bath of 4, 22, 36, or 60 degrees Celsius and held them there for a total of three minutes. One by one, we added 3mL of starch solution to the heated or cooled amylase and quickly stirred the solution. After transferring the new solution to a well plate prepared with one drop of iodine in each well, we timed the resulting reaction to completion by starting a timer immediately after filling the well and stopping it when the color of the solution changed from a dark purple/black to either yellow or clear. As with each of the previous three experiments, we repeated this process for each test tube mixture and recorded all reaction time results.  
Results:

The reaction rate experiments provide evidence of the optimal conditions for the reaction of salivary amylase and starch. Each of the four experiments explores the optimal temperature, pH, enzyme concentration, and substrate concentration, respectively, for a reaction involving amylase.

1. Enzyme Concentration

In each of the four starch and amylase reactions, the reaction time shows an inverse relationship with enzyme concentration. That is, the reaction time increases as enzyme concentration decreased (Fig. 1). The time it takes for the substrate to disappear completely gets exponentially longer with the halving of the reaction’s enzyme concentration. This trend is noticeable not only in the experimental data, but also in the color change of the solution as well. In the wells with a greater enzyme concentration, it is easier to distinguish the solution’s color change; the substrate dissolves easily and the solution becomes very clear. The wells with a lower concentration of amylase, however, produce cloudier solutions upon completion of the reaction because the substrate is not broken down as easily.

1. Substrate Concentration

Unlike the enzyme concentration data of Experiment 1, the substrate concentration and reaction time show a more direct correlation between the two variables (Fig. 2). As the starch concentration increases, so does the amount of time that it takes for the reaction to complete. Once again, this trend is evident in both the numerical data and the visible changes that occur in the reactions themselves. The wells that contain the solutions with the greater starch content do not produce as clear of a final product as those with lower starch concentrations. Those with the reduced starch concentrations have less leftover substrate in the bottom of the well and thus have a less cloudy final product upon the completion of the reaction.

1. pH

The pH data from Experiment 3 shows a generally direct correlation between pH and reaction time, with one exception at a pH of five (Fig. 3). The rate of reaction is quickest for a 2% amylase solution at a pH of five. If the pH moves above or below five, that is, if it increases or decreases, the reaction time begins to shift back upward. As a result, the amylase solution with a pH of 10 takes the longest time to complete the reaction at 23 seconds, while the solution with a pH of 3.0 has the second-quickest reaction time at 8 seconds.

1. Temperature

The reaction rate times from Experiment 4 indicate an inverse correlation between temperature and reaction time (Fig. 4). The quickest reaction rate is a time of less than one second at a temperature of 60 degrees Celsius, while the slowest reaction rate is at four degrees Celsius with 50 seconds.

Discussion/Conclusions:

Scientists have previously studied the optimal conditions of temperature, pH, enzyme concentration, and substrate concentration in amylase reactions; however, we made the decision to examine the collective influence of these factors on the overall optimal conditions for a reaction of starch and salivary amylase. Prior to conducting these experiments, we hypothesized that if a reaction of amylase and starch takes place with high enzyme concentrations, low substrate concentrations, high temperatures, and a pH below 7, then the rate of reaction will be at its quickest.

Overall, the data collected from these four experiments support our hypothesis regarding the optimal conditions of salivary amylase in a reaction with starch. The data from Experiment 1 maintain the prediction that a higher enzyme concentration leads to a quicker reaction rate, as the results show a strong inverse relationship between enzyme concentration and reaction time (Fig. 1). These results also make sense given the highly efficient nature of enzymes: an enzyme is able to catalyze over 1000 reactions every second (Shefferly and Johnson, 2011), so a larger number of enzymes—i.e., a higher concentration—would theoretically be able to catalyze a reaction even faster than an average number. Conversely, a larger amount of substrate in a reaction will slow the reaction down, as there is more work for the enzymes involved to perform (Mussatto *et al*., 2008). This information has support from the results of our experiments; the data from Experiment 2 displays a clearly direct relationship between substrate concentration and reaction time.

One area in which our hypothesis is incorrect is that of the optimal pH for a reaction of starch and salivary amylase. We predicted that a lower pH would lead to a faster reaction time, but this theory proved to be only partially true. The data from Experiment 3 regarding buffers of varying pH revealed that a pH below five actually slows down a reaction (Fig. 3). The numbers produced by this experiment, along with optimal pH results from other scientists’ research, identify a pH of five as the optimal condition for a reaction of salivary amylase (Fig. 3; Hsieh *et al*., 2008). An environment with a pH below five may actually alter the shape of enzyme active sites, denaturing the enzymes and slowing down or stopping the reaction (Shefferly and Johnson, 2011). Likewise, an environment with a pH greater than 7 will also begin to denature an amylase enzyme. This is because amylase is found in saliva, and thus operates in conditions typical of the human body. The optimal temperature for amylase activity also has a connection to the conditions of the human body. While our experimental data indicated that the optimal temperature for amylase activity is at 60 degrees Celsius, in reality the optimal temperature condition for salivary amylase is around 36 degrees Celsius. Thirty-six degrees is the average temperature of the human body, in which salivary amylase is found. Temperatures higher than 36 degrees Celsius actually damage and denature amylase enzymes because their increased kinetic energy causes them to collide with the active sites rather than bonding to them (Shefferly and Johnson, 2011). This data was most likely the result of experimental error and it can be avoided it in the future by paying closer attention to the process of heating the amylase and timing the rate of the reaction.

Through experimentation and examination of enzyme concentrations, substrate concentrations, pH, and temperature, we were able to determine the optimal conditions for fungally derived salivary amylase. These conditions, which include a high enzyme concentration, a low substrate concentration, a pH of around 5.0, and a temperature of 36 degrees Celsius, are the deciding factor in the determination of reaction speed for a mixture of salivary amylase and starch.

Bibliography

Hsieh M., L. Yin and S. Jiang. 2008. Purification and characterization of the amylase from a small abalone *Halitotis Sieboldi*. *Fisheries Science* 74(2): 425-432.

Ilori M.O., O.O. Amund and O. Omidiji. 1997. Purification and properties of an α-amylase produced by a cassava-fermenting strain of *Micrococcus luteus*. *Folia Microbiologica* 42(5): 445-449.

Mussatto S.I., G. Dragone, M. Fernandes, A.M.F. Milagres and I.C. Roberto. 2008. The effects of agitation speed, enzyme loading and substrate concentration on enzymatic hydrolysis of cellulose from brewer’s spent grain. *Cellulose* 15(5): 711-721

Shefferly, N. and M. Johnson. 2011. Enzymes, Pp. 67-75. University of Alabama BCS 115/118 Laboratory Manual: Fall 2011. The University of Alabama Press, Tuscaloosa.

Yadav J.K. and V. Prakash. 2009. Thermal stability of α-amylase in aqueous cosolvent systems. *Journal of Biosciences* 34(3): 377-387.