The Effect Of Salt Concentration On Enzyme Activity

Abstract:

Will lower salt concentration prove to be optimal for the enzyme Turnip Peroxidase, resulting in darker shades of brown and quicker reaction rates? The purpose of this experiment was to determine the effect that salt concentration has on the enzymatic activity of Turnip Peroxidase [5]. The procedure was carried out by first preparing a control test tube by combining an enzyme and substrate tube and recording the colour change over 5 minutes and preparing serial dilutions of saline solution with 2M, 1M, 0.5M, 0.25M, and 0.125M of NaCl. We prepared six more substrate and enzyme tubes, however, we replaced the distilled water in 5 enzyme test tubes with the serial dilutions and kept one test tube similar to the control enzyme, then combined the substrate and enzyme tubes and recorded the colour change over 5 minutes. The results support our hypothesis that the solution with the highest salt concentration will be the lightest, however, the solution with no salt was lighter than solutions with salt. For example, the 2M solution was consistently among the lightest solutions, being one of the higher concentrations, but the distilled water solution ended up being the lightest at the end of the 5 minutes for each trial. This lab shows that the optimal salt concentration for the enzyme to work effectively must be low, however, no salt will result in a slow reaction.

Introduction/Background:

The purpose of this experiment was to find the relationship between salt concentration and enzyme activity. For this experiment, we used Turnip Peroxidase, which is derived from turnips, plants that have an optimal pH of 6.0 [8]. The enzyme functioned properly at pH 2.6-8.0 [7]. Enzymes are proteins that combine with substrates, or reactants, to help speed up a reaction and lower the activation energy [1]. Enzymes can be denatured when placed in higher temperatures [2], unsuitable pH levels [3], or by salt [4].

Hypothesis:

Due to salt’s ability to denature enzymes [4], the solution with the lowest salt concentration would be the darkest, indicating optimal conditions for the enzyme and more successful enzymatic activity than those with higher salt concentrations, which will be the lighter, because of the active site and substrate incompatibility [4].

Materials:

Methods:

The independent variable for this experiment was the salt concentration because the change in salinity would directly affect our dependent variable [4], enzyme activity which we measured by the colour of the solution. We were able to manipulate our independent variable through serial dilutions, allowing us to observe the effects of solutions with six different salinities. In order to measure our dependent variable, we used a color chart to determine the enzymatic activity of

solutions with various shades of brown. Different shades of brown indicate the reaction rates, for example, as the solution got lighter in colour, the enzyme was likely to have been denatured, resulting in a slower reaction [6]. Controlled variables included the temperature and volume of the solution. Our control was the combined substrate and enzyme test tube from the beginning of the experiment because it provided a baseline for us to conclude what the change in colour meant, scientifically. The control helped us determine that the unaffected reaction would produce a darker brown colour as time went on [6], which later allowed us to apply that knowledge when experimenting with different salt concentrations.

Procedure:

First, prepare a substrate test tube by adding 7 mL of distilled water using the 10 mL graduated cylinder, 0.3 mL (300 ?L) of 0.1% hydrogen peroxide using a P-1000 micropipette, 0.2 mL (200 ?L) of Guaiacol using the P-200 micropipette, and gently mix. Next, prepare an enzyme test tube by adding 6 mL of distilled water using the 10 mL graduated cylinder, 1.5 mL (2 x 750 ?L) of peroxidase using the other P-1000 micropipette, and gently mix. Combine the contents of the two test tubes, cover with parafilm, and invert twice; this is the control in a test tube in the experiment. Record the color change using the colour chart over 5 minutes at one-minute intervals. Clean out the two test tubes and begin preparing the saline solution serial dilutions. Mass 11.688 g NaCl on the electronic balance with a weigh boat, label 5 beakers as 2M, 1M, 0.5M, 0.25M, and 0.125M respectively, fold the weigh boat like a taco and pour the NaCl into the 150 mL beaker, fill the beaker up to 100 mL with distilled water, use a stirring rod to dissolve the NaCl, and pour 50 mL of 2M NaCl solution into the 1M beaker and fill up to 100 mL with distilled water. Repeat this step until the solution is distilled to 0.125 M. Label 12 tubes as “2M substrate”, “2M enzyme”, etc. Prepare six substrate tubes, just like the ones we used for our control, and 6 enzyme tubes, but for five of the enzyme tubes, replace the 6 mL of distilled water with 6 ml of serial dilutions and one tube with 6 mL of distilled water and gently mix. Combine the enzyme and substrate tubes of the same molarity, cover with parafilm, and invert twice. Record the color change using the color chart over 5 minutes at one-minute intervals.

Results/Data:

Our results showed that lower salt concentration often resulted in darker solutions, however, solutions with no salt were lighter.

Discussion/Conclusion:

The positioning of the 0.25M line supported our hypothesis, being that the lowest salinity resulted in darker shades, however, the distilled water line was an exception. To answer our initial question of whether lower salt concentration would prove to be optimal for the enzyme Turnip Peroxidase resulting in darker shades of brown and quicker reaction rates, both Figures 4 and 5 show that the 0.25M solution was the darkest, proving that less salt would lead to a darker solution. However, the distilled water solution, containing no salt, ultimately ended up being the lightest in both trials, challenging our hypothesis. Due to the comparatively low concentration of salt in the 0.25M solution, the enzyme was less likely to get denatured and slow down the reaction, allowing the solution to be darker [6]. Figures 1, 2, and 3, on the other hand, showed that more salt didn’t always slow down the reaction, as can be seen from the distilled water results. Our collaborators’ data, Figure 4, helped support our hypothesis as well.

Sources of error in this lab include inaccurate color estimates and/or timing mistakes. Fixing these errors by having a secondary source of verification, such as a fixed substrate to enzyme ratio, would provide more accurate results, which may considerably change our data. With this experiment, researchers could further the study of salt intake and its effect on enzymes in the human body, proposing new and effective dietary salt recommendations.

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