

The Effect of Temperature on Enzymes

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Introduction

Enzymes are proteins that function as biological catalysts, which increase the rate of the reaction without being destroyed by it (Campbell et al., 2008). Since not all chemical reactions are fast-acting, the slower responses need a “boost,” and that is where enzymes come in. A significant process that occurs within our bodies is the metabolic process, which tends to be a slower reaction. If the metabolic process were to happen at its usual slow rate, then many cells would die. Thus, the vital role of enzymes is critical for slow-acting processes naturally occurring in our bodies.

Enzymes are substrate-specific, meaning that only a particular substrate can react with the catalyst to make a product. The given substrate fits into a region of the protein called the active site. The active sites act as a “pocket” for the substrate. In this particular lab, the enzyme being used was tyrosinase, which is found in many organisms, including humans.

All enzymes have an active site, and a catalyst is “available” when the active place is empty. Then comes the substrate, which binds to the catalyst in the active site. With some enzyme-substrates, hydrolysis occurs, but it may not always be the case. After, the substrate is converted into products. Then, the products are released. There is a considerable turnover count for products because this cycle occurs in the thousands range per second.

The three main environmental factors that [affect enzyme activity](#) are temperature, pH, and substrate concentration. Temperature and pH are components of homeostasis, which help keep our bodies' cells in equilibrium. As the body's temperature increases, so does the enzyme activity. Then, at about 37° Celsius, enzyme activity reaches its optimum, which also happens to be the body's average temperature.

After the optimum point, the enzyme activity begins to decrease drastically. Enzymes tend to be hypersensitive to high temperatures because it is at this point that they tend to denature or “unwind.” The two main things that can cause the denaturing of enzymes are the severity and duration of the heat. Enzymes can be renatured if the temperature decreases back to body temperature.

Tyrosinase is a naturally occurring enzyme in plant cells. This particular enzyme causes the brownish coloring from bruises in fruits and vegetables. In this particular lab, the tyrosinase comes from potato cells (Couch and Beger, 2004).

The temperature was the experiment being tested by the group. There were four environmental

temperatures to which the enzymes were exposed, ranging from cold to hot water baths. The enzyme used in the experiment is tyrosinase.

The experiment tested the effect of temperature on reaction rate and enzyme activity. The hypothesis for this experiment was that the temperature of the environment determines the rate of change in a catalyzed reaction. We were able to create our interpretation because we knew that enzymes are catalysts and can help reactions occur in colder than average temperatures and also have an effect in temperatures deemed “normal” but may not have a positive outcome in warm temperatures because of the “added” heat. The first of the three predictions was that the test tube in the refrigerator would produce a positive reaction, therefore will change from its initial colouring. The second prediction was that the test tube at room temperature would create a positive response, accordingly will change from its initial colouring. The last forecast was that the test tubes in the hot water bath would not produce a positive reaction, therefore will remain the initial colour.

Results

In the temperature experiment, there were four results. The test tubes were placed in four temporal environments. The first of which was the “cold” one, which was set in the refrigerator at ten °C for approximately fifteen minutes before introducing the enzyme. The initial color for this tube was clear without the protein and remained clear with the substances. After five minutes of being returned to the original temperature, the final color continued to be clear. The second test tube, room temperature, was at 20°C; the initial color without enzyme was bright, and with them, it turned cloudy with a light yellow tint. After the last five minutes, the final color of the contents was golden translucent.

Table 1: The Effect of Temperature on Reaction Rate/ Enzyme Activity

		Initial Color		Final Color	
Test Tube	Tube Placement & Temp	<i>Without Enzyme</i>	<i>With Enzyme & Substrate</i>	<i>After 5 Mins</i>	<i>Result</i>
1	Refrigerator (10°C)	Clear	Clear	Pale/ Translucent clear	<i>Negative</i>
2	Room Temp. (20°C)	Clear	Cloudy with a light yellow tint	Golden translucent	<i>Positive</i>

3	Hot H ₂ O Bath (30° C)	Clear	Cloudy clear	Pale pink/ Translucent	<i>Positive</i>
4	Hot H ₂ O Bath (40° C)	Clear	Pale yellow tint	Golden/ Dark	<i>Negative</i>

The last two test tubes were placed in the hot-water bath at two different temperatures. The first warm temperature was at 30° C, without the enzyme it was a bright colour, and with them, it turned into pale yellow ting. The final tone of this tube was a golden, dark color. The second warm temperature was 40° C, and the primary color was bright. Then, with the enzyme, it turned into a gorgeous cloudy color. The final color was a pale pink translucent color.

Another hypothesis is that the activity of the enzyme could be affected by temperature. This is because the enzymes will have trouble getting accustomed if the environment is too hot or too cold. After this reaction, the enzymes will then stop functioning and denatured.

For this experiment, we put tyrosinase in three different test tubes and labeled them A1, A2, A3, A4, A5 and A6. Put them in six different temperatures from 20-70, respectively. Put them for ten minutes and then take them out. Add ten mL of H₂O₂ to each test tube. Record six different temperatures in Table 2, and after five minutes, measure the height of oxygen bubbles in each test tube and record them in Table 2 as well.

Table 2: The effect of temperature on an enzyme.

Tube	Temp	Foam thickness (cm)
1	20	0.18
2	30	0.22
3	40	0.36
4	50	0.25
5	60	0.15
6	70	0

Discussion

When temperature increases, the rate of reaction increases. However, the enzymes are denatured at very high temperatures. The graphs show that at approximately up to 40 degrees, the enzyme's reaction increases, and with further heat energy, the enzymes denature.

The class-generated hypothesis for the control experiment was that a specific enzyme binds to create products through a catalyzed reaction. This is somewhat reasonable given the fact that certain enzymes are substrate-specific, as stated in the Introduction. In this particular experiment, the enzyme tyrosinase was the primary focus. The lab manual also went into a brief overview as to what its purpose is.

With this given information the group was able to make a hypothesis of how the temperature would affect the activity of the enzyme. In the actual experiment testing for the effect of temperature on enzyme reactions, the results were split. There were three primary temperatures, in which one of them had a double with the different temperatures. The first test was for a refrigerator temperature reaction, and the final color was bright, which is nowhere near a yellow-brown color. This may have to do with the fact that the temperature may have been too cold, and the enzyme may have denatured, but if it were to have increased to a higher sustainable heat, it would have most likely renatured itself.

The second test tube was left at room temperature, and the resulting final color was a golden translucent color. This is because enzymes are at their optimal level when at the "right" temperature, be it the room or body temperature, depending on the particular enzyme. Because potatoes, as well as most fruits and vegetables, are usually stored at room temperature their optimal level would be room temperature.

The last two test tubes were in hot-water baths; one was at 30°C and the other at 40°C. The test tube which was at 30°C had a final colour of golden dark. It is also the only temperature that is remotely close to the room temperature of 20°C. The second test tube, which was measured at 40°C, had a final color of pale pink.

For a more significant majority of the experiment, the hypothesis and predictions were right on target for the controlled actual experiment. The only part that showed inconsistencies was the temperature experiment in the refrigerator and hot-water bath test tubes. As a group, we predicted that the refrigerator temperature would result in a positive when, in actuality, it was a negative result, which may have to do with the fact that the temperature was far too cold for the enzyme and therefore resulted in its denaturing. As for the hot-water baths, we had predicted that they would both lead to an adverse reaction, when in fact, only one of them had a negative result, and that one happened to be the one that was the warmest out of the two. This may have to do with that one denaturing as well, just the same as the refrigerator temperature one.

The experiment was a bit limited to just these four temperatures, also possible if the test tubes would

have been allowed more than five minutes in each of its moderate environmental temperatures than possibly some of the results may have differed a bit. As well as not just limiting the experiment to tyrosinase. If there had been a bit more variety in enzymes, it would have been interesting to see the results for each of the differing combinations.

References

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