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Enzyme Lab

Introduction

In order to perform chemical reactions fast and efficiently, a cell must use enzymes. An enzyme is a protein catalyst that performs these reactions rapidly. They are often highly regulated and highly specific to which substrate they react to (Urry 2021). Enzymes have different shapes and sizes, but there are similar parts in all of them. An enzyme has an active site which has the ability to bind to specific reactants or substrates. When a substrate binds to an active site of an enzyme, the enzyme-substrate complex is formed. The substrate molecules are forced together in a specific way. It is important to note that the activation energy of Ea is greatly reduced which makes the reaction speed up. After the reaction is completed, the substrate is converted to a product and the enzyme and the substrate separate (Urry 2021).

An enzyme that loses its structure is called denatured. Denaturation can happen in numerous ways. One way an enzyme can be denatured is due to the temperature (Urry 2021). Normally the increase of temperature speeds up the reaction of enzymes, but if the temperature reaches a certain threshold, then enzyme denature. This is why having a high fever is a problem. Your body temperature spikes and the enzymes in the cell denature (Urry 2021).

Enzyme activity is influenced by temperature as stated above, but there are other factors that affect enzyme activity. In optimal conditions, enzyme activity is maximized. The pH affects

the activity of the enzyme. Enzymes work in a specific pH range. Changes in the pH will often affect the structure of enzymes and extreme pH level- like extreme temperature- will denature the enzyme making them useless (Urry 2021).

Enzymes have many different functions. Enzymes even play a role in cell communication. Specifically, enzymes help with the G protein-coupled receptors. A signaling molecule activates the G protein-coupled receptor. Then a G protein binds to the energy-rich GTP. The G protein activates the enzyme in order to produce a reaction. This reaction is used to further cellular response usually by making second messengers like cAMP and PIP-2 (Urry 2021). After the reaction is completed, the phosphate is popped off and the G protein is deactivated. Enzymes are also important

Enzymes are also essential components in cellular respiration and photosynthesis. For instance, ATP synthase is a mitochondrial enzyme located in the inner membrane. In animals, ATP synthase is mainly used in the citric acid cycle and oxidative phosphorylation. In oxidative phosphorylation, hydrogen atoms from the electron transport chain are pumped into ATP synthase (Urry 2021). In which an ATP is formed from ADP. ATP is the energy source that cells use constantly which is why ATP synthase is essential in animals. A similar process occurs in photosynthesis. Spare hydrogen atoms from the photosystems are pumped into ATP synthase to produce ATP which will be used in the calvin cycle to make glucose (Urry 2021).

Hypothesis

Experiment 1: The effect of different substrates on the rate of enzyme reaction confirms that the catechol oxidase enzyme present in the potato extract has the specific substrate catechol because enzymes look for specific substrates.

Experiment 2: The more substrate concentration will increase the rate of enzyme reaction because the enzyme will spend less time trying to find a substrate to bind with which makes enzyme activity ultimately increase.

Experiment 3: The more enzyme concentration will decrease the rate of enzyme reaction because the enzyme will be competing with other enzymes to bind substrates which makes enzyme activity ultimately decrease.

Experiment 4: Enzyme activity will decrease as the pH becomes more acidic because the enzyme will denature making it useless.

Experiment 5: Enzyme activity will increase as temperature increases and fall off because the enzyme will faster until the temperature is high enough that it denatures the enzyme.

Methodology

The materials needed for all of the experiments are the following: One small potato, knife, cutting board, beakers , blender, cheesecloth, rubber band, test tubes, test tube racks, disposable , pipettes, deionized water, catechol solution, substrats, water bath, sodium hydroxide, hydrochloric acid, pH paper, Hot plate, five thermometers, ice, and salt.

In Experiment 1 the first step is to label and place the tubes into a water bath at 37 degrees celsius for 10 minutes. After 10 minutes, observation can be made about the color of the

tubes. The change in color is evidence of the oxidation process catalyzed by the enzyme. Rate the colors and record the data.

In Experiment 2 the first step is to label the 11 substrate test tubes. Then add water and substrate to each test tube accordingly. Then add 3 mL of deionized water into the test tubes and prepare another 11 test tubes with 10 drops of potato extract. Place all the test tubes into a water bath at 37 degrees celsius for 5 minutes. After 5 minutes, mix the potato extract into the 10 substrate tubes and put in the water bath for 10 more minutes. Remove the test tubes, and observe the color of them. Rate the color and record the data.

In Experiment 3 the first step is to label five test tubes and add water and potato extract accordingly. Then add 4 mL of deionized water into the tubes. Then prepare another five test tubes containing 10 drops of catechol solution. Place the test tubes into the water bath for five minutes. After the time expires, mix the solutions of the potato extract and return them to the water bath for 10 minutes. Remove the test tubes from the bath and compare the intensity of color. Rate the color of the tubes and record the data.

In Experiment 4 the first step is to label the test tubes and add water and HCL accordingly. Dip a disposable pipette into each tube and press onto the strip of pH paper. Record the pH for each then add 2 mL of water, eight drops of catechol, and eight drops of potato extract into all of them. Mix the solutions and place the tube into the water bath for 15 minutes. Observe the color changes and record the results.

In Experiment 5 the first step is to label five test tubes. Then place 10 drops of potato extract and 1.5mL of water into them. Then get another five test tubes and label them. Place 10 drops of catechol solution and 2 mL of water into the substrate labeled test tubes. This time, set a water bath to 0 degrees, another to 10 degrees, and another at room temperature. Then heat a

glass beaker of deionized water on a hot place to 70 degrees celsius. Place one of each tube into each of the water baths. After 15 minutes, observe the color changes. Then place all of the tubes into a room temperature water bath for 10 minutes. Observe the color of the test tubes through this process and record the data.

Data

Experiment 1: Effect of Different Substrates on Rate of Enzyme Reaction

Labeled	Type of Substrate	Intensity of Color 1-10
A	Deionized water	1
B	Cyclohexanol	1
C	Cyclohexanediol (1,4)	1
D	Cyclohexanediol (1,2)	2
E	Catechol	9
F	Resorcinol	3

Experiment 2: Effect of Substrate Concentration of the Rate of Enzyme Reaction

Labeled	Amount of Substrate	Intensity of Color 1-10
A	0	0
B	1	2
C	2	3
D	3	3
E	4	4
F	5	5

G	6	6
H	7	6
I	8	7
J	9	7
K	10	8

Experiment 3: Effect of Enzyme Concentration of the Rate of Enzyme Reactions

Labeled	Amount of Potato Extract	Intensity of Color 1-10
A	0	0
B	3	1
C	6	3
D	9	4
E	12	4

Experiment 4: Effect of pH on the Rate of Enzyme Reaction

Labeled	Recorded pH	Intensity of Color 1-10
A	0	1
B	1	1
C	5	8
D	11	7
E	14	3

Experiment 5: Effect of Temperature of the Rate of Enzyme Reactions

Labeled	Water Bath Temp in celsius	Intensity of Color 1-10	Intensity of Color after second bath
A	0	6	5
B	10	7	7
C	Room temp	8	7
D	37	5	4
E	70	1	1

Analysis

Experiment 1 explains how enzymes are substrate specific. In this case the enzymes in this experiment are specific to catechol. There is a significant decrease in enzyme activity in all of the other substrates because the color did not change much. This means that the optimal substrate was catechol because the color changed the most. Only one substrate elicited a ration with the enzyme due to enzyme specificity. In order words, the active site of the enzyme was in a specific structure for catechol.

Experiment 2 explains the effect of substrate concentration on enzyme activity. Before that I thought that more substrate concentration would increase the rate of enzyme reaction. This hypothesis is supported, because as the more drops of the substrate added, there was a greater intensity of color change. Test tube K allowed the reaction to occur the fastest because the enzyme spent less time waiting for a substrate to bind with the active site.

Experiment 3 explains the effect of enzyme concentration on the rate of enzyme reaction. I hypothesized that more enzyme concentration will decrease the rate of enzyme reaction because the enzyme will be competing with other enzymes to bind to substrates. This hypothesis is not supported as color change barely increased with more potato extract. The enzyme concentration had little to no relationship to the rate of enzyme reaction. This is due to the fast the substrate just binds the nearest enzyme while the extra enzymes just remain inactive. The rate of enzyme reaction only changed by a small margin. Although test tube E had the most enzyme activity and color change, test tube B (lowest concentration of enzyme) would eventually reach the color of test tube K. This is because the amount of substrate did not change.

Experiment 4 explains the effect of pH on the rate of enzyme reaction. I hypothesized that as the pH becomes more acidic, the enzyme activity will decrease. This hypothesis is supported because the highest pH denatured the enzymes. The optimal pH for enzyme activity is 5 because this pH made the greatest color change. At pH's other than the optimal, the enzyme activity was lowered significantly. This is due to the fact the enzyme has an optimal pH level for enzyme activity.

Experiment 5 explains the effect of temperature on the rate of enzyme reaction. I hypothesized that as the temperature increases, the enzyme activity will decrease. This hypothesis is supported because the highest temperature denatured the enzymes which caused a drop in the enzyme activity. The optimal temperature for enzyme activity is room temperature because it has the greatest color change. At temperatures other than the optimal, the enzyme activity was lowered significantly. This is due to the fact the enzymes have an optimal temperature level for enzyme activity.

Conclusion

All of the enzyme experiments have different factors that influence enzyme activity. Enzymes are essential to cell life because they speed up necessary reactions by lowering their activation energy. For instance, atp synthase is used in cellular respiration in order to make ATP. Like atp synthase, enzymes have specific substrates that can be binded to the active site. This enzyme specificity is observed in experiment 1. The amount of substrate is directly proportional to the enzyme activity. Once the amount of substrate increases, the enzyme activity decreases. Contrary to this, the amount of enzyme is not related to enzyme activity. Enzymes have multiple factors that make the enzyme activity optimal. Two of those factors are pH and temperature. If the pH or temperature of an enzyme becomes too high, the enzyme will denature and start to unfold which renders the enzyme useless. This is why there are factors that optimize the enzyme activity.

References

Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., & Reece, J. B. (2021). Campbell biology (12th ed.). https://bibliu.com/app/#/view/books/9780135858141/epub/OPS/xhtml/cover.html#