AP Bio Lab #13 - Enzymes

"Toothpickase Lab"

Lab #13 10/9/14

Introduction

An enzyme acts as a biological catalyst that speeds up chemical reactions in the human body. It does this without being consumed by the reaction. Enzymes lower the activation energy needed to perform a reaction, thereby kickstarting the chemical reactions in all of the body's cells. They allow the reactants to absorb enough energy to reach a transition state, in which enough energy has been absorbed to break the bonds. Once the bonds break, products are released. The reactants are also called substrates. Once the substrate finds the enzyme, it binds to the active site, which is a pocket on the surface of the enzyme where catalysis occurs. This creates the enzyme-substrate complex. The binding of the enzyme to the substrate puts stress on the bonds of the substrate until the bond breaks. Products are then released and the enzyme is ready to be used again. This can also occur in the opposite direction, meaning the enzyme can bring substrates together to form a new product.

Enzymes generally hasten reactions that would occur much slower anyway. They are very specific to the type of reaction they perform and which substrate they bind to. Enzymes determine when and which processes happen in the body. An enzyme has no effect on the change in free energy during a reaction, however. Also, enzymes can be affected by their surrounding environment and other molecules that act on the enzyme. Temperature, pH level, and salinity must be ideal for an enzyme to function properly and prevent denaturation. Furthermore, enzyme activity can be regulated by other molecules that act as activators and inhibitors. Cofactors and coenzymes both help to catalyze, while competitive and noncompetitive inhibitors reduce enzyme productivity. In allosteric regulation, regulatory molecules bind to one site of the enzyme to control its function. In feedback inhibition, the end product also acts as the inhibitory molecule for the reaction. Without enzymes to perform and regulate chemical reactions, the human body would not function.

Students will investigate how enzymes work in this lab, by acting as enzymes themselves and recording the data they come up with. Students will effectively learn more about the effects of denaturation and inhibition on an enzyme. They will answer questions about their findings as they perform the lab.

Hypothesis

- PART 1: If toothpicks are broken by hand and the time and number of toothpicks is recorded in 10 second intervals, then the number of broken toothpicks should be constant.
- PART 2: If the thumb is taped to the palm of the hand and the time and number of toothpicks is recorded, then the number of broken toothpicks should be much less than in Part 1.
- PART 3: If paperclips are mixed in with the toothpicks and the time and number of (broken) toothpicks is recorded, then the "enzyme" will break toothpicks at a much slower rate than in Part 1.

Materials

The materials used include:

- stopwatch
- toothpicks
- tape
- paperclips

Procedure

Part 1: Reaction rates

1. Setup the stopwatch and lay the toothpicks out on the table.
2. One person acts as the enzyme and breaks the toothpicks one by one with index finger and thumb. Be sure to break the toothpicks the same way throughout the experiment.
3. Another person records the number of toothpicks broken in 10 second intervals for one minute using the stopwatch.
4. Remember not to look while breaking the toothpicks.
5. Record data in Table 1.

Part 2: Denatured Toothpickease

1. The enzyme's thumb must be taped to his/her palm.
2. The partner records how many toothpicks are broken in ten second intervals for one minute.
3. Record data in Table 2.

Part 3: Competitive Inhibition

1. Mix paperclips in with the toothpicks.
2. The enzyme breaks as many toothpicks as he/she can with untaped hands for one minute.
3. Record how many toothpicks were broken in ten second intervals in Table 3.
4. When done, switch roles.

Results (Data)

Table 1

Seconds	Broken toothpicks		
	closed	open	ice water
10	3	3	3
20	5	7	8
30	7	10	11
40	10	12	14
50	12	16	15
60	15	19	18

Table 2
denaturation

Seconds	Broken picks
10	0
20	0
30	0
40	0
50	0
60	0

Table 3
competitive inhibition

Seconds	Broken picks
10	3
20	6
30	9
40	12
50	12
60	15

Tables 1-3: one enzyme

Table 4

seconds	Broken toothpicks	
	open	ice water
10	8	6
20	15	11
30	25	17
40	32	23
50	36	27
60	40	30

Table 4: two enzymes

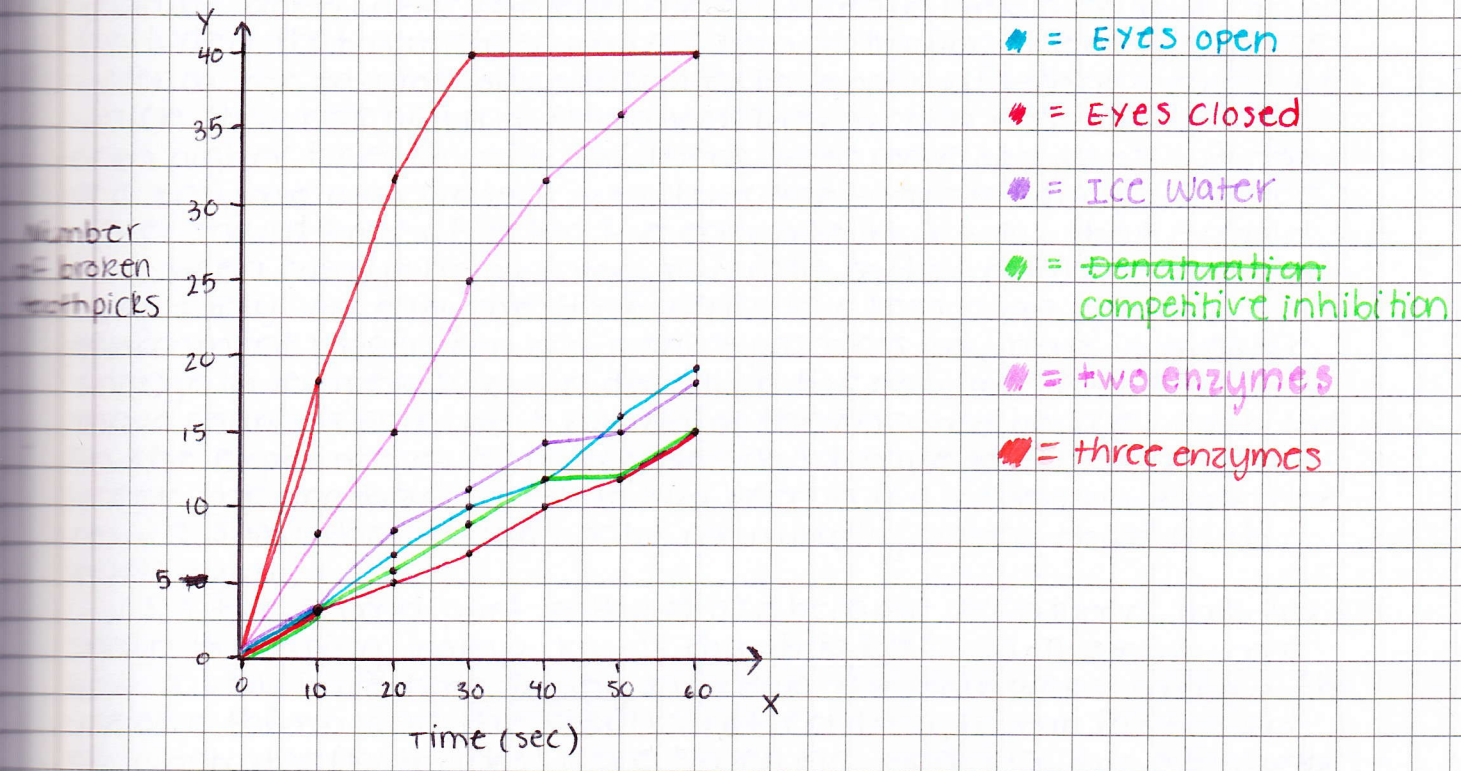
Table 5

seconds	Broken picks	
	open	ice water
10	18	13
20	32	23
30	40	38
40	–	40
50	–	–
60	–	–

~~seconds Broken picks~~

Table 5: three enzymes

Enzyme Conditions Graph



Error Analysis

Errors could include an inaccurate portrayal of a hand (enzyme) that has just been in ice water.

Students could also have made a mistake in keeping track of the exact number of toothpicks broken in the ten second intervals.

Conclusion

The experiment was successful, in the way that each hypothesis was proven mostly correct by the data. The first hypothesis was partly accurate, because the rate of reaction was constant with closed eyes, meaning only two to three toothpicks were broken every ten seconds. Performing the reaction with open eyes, however, was faster and the number of broken picks was not as evenly distributed throughout the intervals. A hypothesis on the effect of ice water ~~on~~ was not made. The second hypothesis was also correct, because the "denaturation" caused a drastic decrease in enzyme productivity. The third hypothesis stated that reaction rate would be lower due to competitive inhibition, which can be observed in the results.

The first part of the experiment included three trials; one with open eyes, one with closed eyes, and an imitation of an enzyme after it had been in ice water. Students observed how the number of toothpicks broken steadily increased over time, while the person's hand acted as the active site that catalyzed the substrate (or toothpick). After 30 seconds with closed eyes, the enzyme broke seven toothpicks, with open eyes, it broke ten, and after it had been in ice water, eleven picks were broken. The assumption was that the least toothpicks would be broken after the enzyme had been in ice water, so it can be concluded that a mistake was made in portraying the effects of ice water on the enzyme, since enzymes have optimal temperatures at which their reaction rate is highest. The reaction rate could have been higher for each part, had there been more enzymes, but only one enzyme was present, which became saturated quickly. The ice water should have affected the enzyme in a way that it could have been catalyzing at a very slow rate, or that catalysis ceased completely. An enzyme that works in a less than optimal environment usually can not produce products any more. A drastic change in temperature can denature the enzyme, meaning the shape changes and the function of the enzyme becomes inhibited. In the experiment, toothpickase should have taken longer to break the toothpicks after it had been in ice water, however the results show that it was only marginally slower than with open eyes.

In the second part, toothpickase became denatured. This was shown by taping toothpickase's thumb to ~~its~~ hand. The enzyme had a very hard time trying to break the toothpicks without the use of a thumb and the results reflect this, in the fact that no substrates (toothpicks) were turned into products. This denaturation could have resulted from a too high or low temperature, a drastic difference in pH level, or an unideal salinity. These factors can cause a change in the enzyme's conformation, which can stop it from functioning at all.

The third part of the experiment imitated competitive inhibition, since paperclips were mixed with the substrates. The amount of toothpicks broken was less than with no inhibiters, meaning the rate of reaction was lower. Competitive inhibiters (paperclips) work by binding to the enzyme and not allowing the actual substrate to bind with the enzyme. the reaction then does not occur.

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A fourth trial was also performed when more enzymes were added. First, two enzymes catalyzed the reaction and then a third person was added afterwards. The rate of reaction drastically increased with the addition of enzymes. In the trial with three enzymes, all of the toothpicks (40) were broken in 30 seconds and the enzymes were free of substrates. This shows that the addition of enzymes increases the rate of reaction until there are no substrates left or all enzymes are saturated with substrates.

The experiment provided students with a hands-on method of learning how different conditions and factors affect enzyme activity. By acting as enzymes, students were able to think about the factors that made it difficult to break toothpicks and learn how enzymes work.

AP Bio Lab #4 - Diffusion and Osmosis

Lab #4 22/9/14

Introduction

Plasma and organelle membranes are selectively permeable in cells, meaning some substances cross easier than others. They consist of a phospholipid bilayer and embedded proteins. The hydrophobic tendency of the bilayer limits water movement. Water can pass through the membrane slowly by osmosis or with the help of aquaporins, which are specialized channel proteins. These aquaporins allow water to move across the membrane much faster.

Diffusion is a simple form of movement that does not require energy input, like osmosis. The movement of solutes from an area of high concentration to one of low concentration is called diffusion. However, the movement of solutes from low to high concentration does require energy input (in the form of ATP accompanied by protein carriers called pumps) and is called active transport.

Osmosis is a form of diffusion, where water moves down its concentration gradient. This means that water moves from areas of high free water concentration and low solute concentration to areas of low free water concentration and high solute concentration (since H_2O molecules surround solute molecules).

Isotonic solutions have equal water potentials, which means that water and solute cross the cellular membrane at the same rate. However, when the surranding solution is hypertonic to the cell, water will move out of the cell and into the solution, because there is higher solute concentration and lower water potential. When the surranding solution is hypotonic, the solute concentration is lower, the water potential is higher, and water will move into the cell.

In animal cells, water moving out will cause the cell to shrivel and shrink, and water moving in will cause swelling or bursting, due to the solute concentrations inside and outside the cells.

In plant cells, there is also turgor pressure, which resists water movement into the cell, preventing it from bursting and creating pressure. If water continues to leave the cell, it could plasmolyze, when the plasma membrane shrinks away from the cell wall.

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