Testing optimal conditions for pancreatic lipase

SL biology

May session,

Exploration

Research Question: How does pancreatic lipase work in the human body and what temperature and pH are optimal for the enzyme to function most efficiently?

Background information: To begin this research, we must understand what an enzyme is and how it works. An enzyme is a protein that "acts as a catalyst in all living organisms." According to Robert Gazy, a catalyst "increases the rate at which chemical reactions occur without being completely altered themselves." As a catalyst, an enzyme can create the same chemical reactions over and over again. Enzymes are made up of interconnected amino acids strung together, which compose a characteristic giving the protein its shape. The enzyme's protein specific sequence is determined in the cell's nucleus by a specific gene. Somewhere within the three dimensional shape of the enzyme lies an enzyme substrate called the active site. At the active site, only one type of chemical reaction can be catalyzed by a specific enzyme. However, it is not enough for a substrate to just bind with the active site. It must enter "with a minimum rate of motion that will provide the energy necessary for the reaction to occur." When reagents enter the active site, the enzyme can alter its original shape and satisfy the reagent. Once the reagent has "left," the enzyme will go back to its original shape. Another variable that can alter an enzyme's shape is temperature. When a change in temperature occurs it can "mess up the process of an enzyme" causing it not to work, as so much of an enzyme's activity is based on its shape.

The human body will naturally produce digestive and metabolic enzymes. Within one human cell there are "approximately 1300 enzymes" that carry energy needed for reactions in the body. The metabolic enzymes speed up chemical reactions within cells of the body for "detoxification and energy production." Each organ, tissue, and cell in the human body depends on the metabolic enzyme and thus, without it life would fail to exist.

Digestive enzymes are found within the digestive tract to help break down food molecules into nutrients and waste products. According to "Why are enzymes important" published by Healthline,

digestive enzymes are primarily found in the pancreas, stomach and small intestine. However, some salivary glands can produce digestive enzymes while chewing to break down food molecules. The digestive enzyme will break down triglycerides into monoglycerides, in which they are then transported to the stomach or into the bloodstream for energy.

Pancreatic lipase is considered the primary digestive enzyme to break down molecules in the digestive tract and “converts triglycerides to monoglycerides and fatty acids.” Triglycerides are a type of lipid stored in fat cells in the blood. This helps explain why individuals with high triglycerides are susceptible to pancreatitis, inflammation of the pancreas. In addition to pancreatitis, other health concerns caused by high triglycerides include “hardening of arteries or thickening of artery walls, increasing the chance of a stroke or heart attack.”

Purpose of investigation: The purpose of this investigation is to determine the optimal temperature and pH levels to identify when pancreatic lipase is most useful. This will determine how to speed up digestion within the human body and indicate which foods are most useful, as well. The pancreas, an organ located in the abdomen, plays a “vital role in converting the foods humans eat into energy for cells.” This research focuses on pancreatic lipase, the primary enzyme that breaks down food molecules within the digestive tract of humans. With this research, I will be able to discover what the optimal temperature and pH levels are for this enzyme to be in use. To further this investigation, I will identify foods that would also contain similar variables to improve digestion. Pancreatic lipase can also contribute to fat loss. I am incredibly interested in losing fat and building muscle which helped lead me to this research. If I am able to determine optimal conditions for pancreatic lipase to function, I will hypothetically be able to understand why certain foods are beneficial to speed up digestion. For example, foods with higher acidity levels such as lemons are suggested to help speed up digestion. Could the optimal pH level for pancreatic lipase be the same as some of those suggested foods?

Variables

Controlled:

A volume of 10mL of lipase will be controlled in each experiment. This allows the researcher to change other variables, such as temperature and pH, and be able to record the effect those variables have on the reaction rate of the enzyme.

The volume of H2O2 will also be kept the same with each test tube in the experiment. H2O2 acts as a catalyst, “substances that speed up chemical reactions without being destroyed or altered during the process” in this experiment. It is important for the hydrogen peroxide concentration to stay the same because this will allow the researcher to see the effect the temperature or pH has on the enzyme.

400 mL of water, when testing different temperature levels, will also remain the same. This allows for the test tubes to be completely submerged and the temperature of the water to remain constant. This creates more consistency and less room for error when collecting data.

5 minutes for each test tube to be submerged in the different water temperatures will remain the same. This ensures that the substance in each test tube is exposed to the same amount of time in the tub to either heat up or cool down.

Independent:

Temperature is one independent variable in this investigation. Different temperatures will be tested to find the reaction rate of the enzyme. By using a thermometer and 400mL of water in a tub, test tubes will be placed in the tub for five minutes each. Once taken out 10 drops of the enzyme will be put in the test tube. By testing temperature the researcher is able to discover how temperature affects the reaction rate of an enzyme.

The pH level is another independent variable. By testing different pH levels, the researcher is able to discover which pH is best for the enzyme involved. In this case pancreatic lipase, an enzyme found in the pancreas, would expect to have an optimal pH around 7-8. By isolating all other variables and testing pH in specific finding which pH is optimal is possible.

Dependent:

Time: Through both tests, temperature and pH, finding the time of how long the enzyme took for the reaction was tested. This allows the researcher to see how temperature and pH affects the rate of reaction on the enzyme. Times that are longer would mean the enzyme is working slower than if times were faster, which would suggest the enzyme production rate is quicker The variance in time is expected to provide an objective analysis with respect to the impact of the enzyme.

Hypothesis

Part one: Temperature affects enzymes in different ways. If the temperature is too cold, the molecules move slowly resulting in less collisions, thus, decreasing enzyme activity. The higher the temperature is raised, more collisions between molecules occur, thus increasing enzyme activity. However, if the temperature is raised too high, the enzyme could become denatured which will decrease the enzyme activity drastically.

Null: There will be no difference when changing the temperature for the rate of reaction with enzymes.

Alternative: The closer the temperature gets to human body temperature (36-37°C) the less time it will take the reaction to occur. Many enzymes in the body are working at the human body temperature, 36-37°C. Therefore, in order for the enzyme to react successfully and efficiently it should be placed in a similar environment where it works.

Part two: pH affects enzymes in a similar way temperature does. If the pH is too basic the enzyme could react slowly and not work effectively. If the pH is too acidic the enzyme could denature causing the enzyme to lose structure and not work effectively.

Null: The more acidic or basic the pH levels become, the more active the enzyme will be, resulting in a higher reaction time.

Alternative: The closer the pH level gets to 8.0 the more active the enzyme will be. This is because the pancreas has an optimum pH around “8.0-8.3.” In order for the enzyme to work at the most efficient rate it must be placed in similar conditions in which it thrives.

Materials

1. Ten 18 X 150 mm test tubes
2. Test tube holder
3. Different levels of pH (1-10)
4. pH buffers
5. enzyme suspension
6. Three dropper pipettes
7. LabPro or CBL 2 interface TI Graphing Calculator DataMate program
8. 1.5% H2O2
9. 3.0% H2O2
10. Vernier O2 Gas Sensor 400-mL beaker
11. 10-mL graduated cylinder
12. Stopwatch/timer
13. Thermometer
14. Googles (safety)
15. Gloves (safety)
16. Paper towels

Procedure

1. Obtain and wear goggles and gloves
2. “Plug the O2 Gas Sensor into Channel 1 of the LabPro or CBL 2 interface. Use the link cable to connect the TI Graphing Calculator to the interface. Firmly press in the cable ends”
3. Turn on the graphing calculator and start the program. Press clear to discard any past data
4. “Set up the calculator and interface for an O2 Gas Sensor”
    a. “Press setup.”

b. “Press enter to select ch 1”

c. “Select OXYGEN GAS from the SELECT SENSOR menu”

d. “Select percent (PCT) as the unit”

5. “Set up the data-collection mode”

    a. Select mode

    b. “Time graph” from the select mode

    c. Choose time change graph from time change setting

    d. Return to main screen

Part I- Testing reaction time of the enzyme with different temperatures

1. Set a 400mL beaker to the specified temperature being tested.

    a. Using a thermometer make sure the 400mL beaker is at the assigned temperature.

    (Temperatures are in 5 degree celsius increments.)

2. Fill each of the test tubes with “10 mL of 1.5% H2O2 and then place the test tubes in the water bath for five minutes.” Do one bath at a time with one test tube. Record the bath temperature.

3. Find the rate of enzyme reaction in minutes.

    a. Add 10 drops of the enzyme substance into the test tube.

    b. “Begin timing with the stopwatch/timer.”

    c. “Cover the opening of the test tube with a finger and gently invert the test tube two times.”

    d. “Pour the contents of the test tube into a clean 250-mL Nalgene bottle.”

    e. “Place the O2 Gas Sensor into the bottle as shown in Figure 1. Gently push the sensor down into the bottle until it stops. The sensor is designed to seal the bottle with minimal force.”

    f. Once the reaction ends, stop the timer and record the time.

    g. Repeat three times with the same temperature.

4. Repeat steps 1-3 for all ten different temperatures, for a total of 30 data points when done.
5. Take average for each temperature.

Part II- Finding the reaction time of the enzyme with different pH values

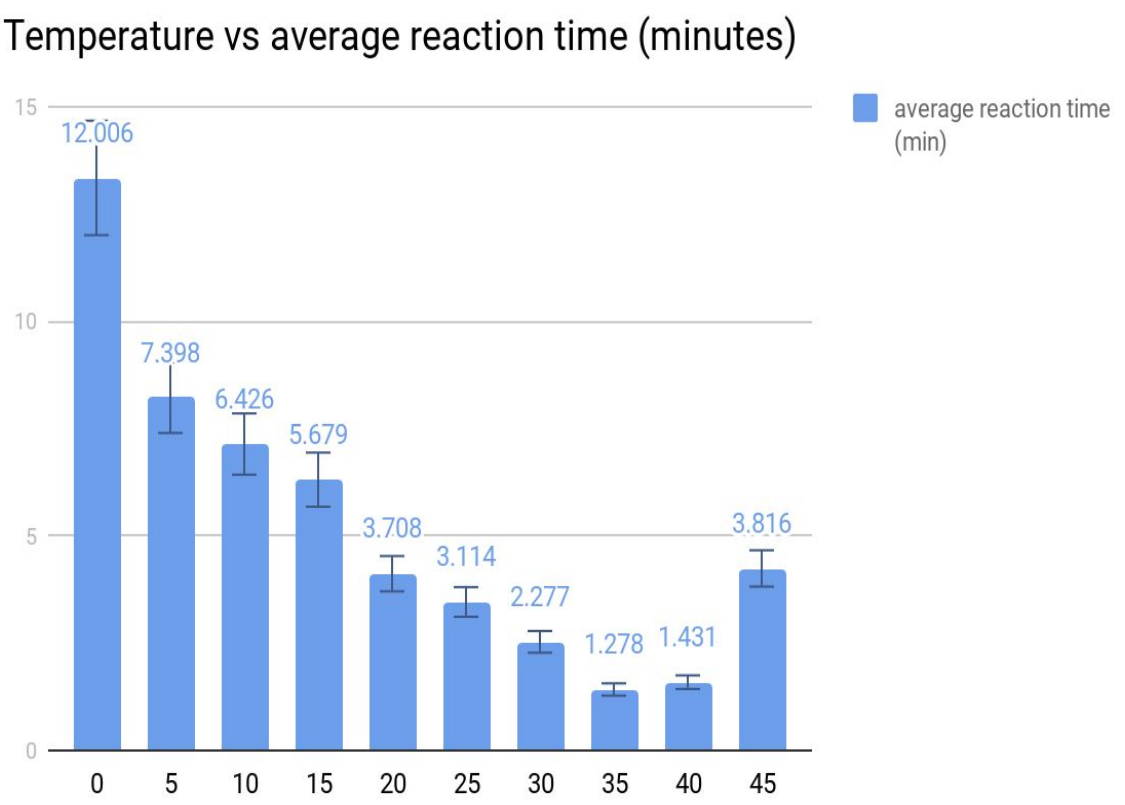
1. Wash all the test tubes with water and soap. Dry completely before proceeding.
2. Place all ten test tubes on the test tube rack.
3. Label each with the specified pH value (1-10).
4. To each test tube add 5mL of 3% H_2O_2 and 5mL of a pH buffer.
5. Do one test at a time and record data, add 10 drops of the enzyme to each test tube using a pipette.
    a. "Begin timing with the stopwatch/timer."
    b. "Cover the opening of the test tube with a finger and gently invert the test tube twice."
    c. "Pour the contents of the test tube into a clean 250-mL Nalgene bottle."
    d. "Place the O_2 Gas Sensor into the bottle as shown in Figure 1. Gently push the sensor down into the bottle until it stops. The sensor is designed to seal the bottle with minimal force."
    e. Record how long it takes (minutes) for the reaction to be completed.
    f. Repeat three times.
6. Repeat steps 1-5 for each pH value.
7. Record data- should have 30 points of data.
8. Take the average of all data for each pH value.
9. Optional- "Graph all three runs of data on a single graph. To do this:"
    a. "Select GRAPH from the main screen, then press ENTER."
    b. "Select MORE, then select L2, L3 AND L4 VS L1 from the MORE GRAPHS menu."

Data

Temperature (degrees C)	**Average reaction completed (min)**

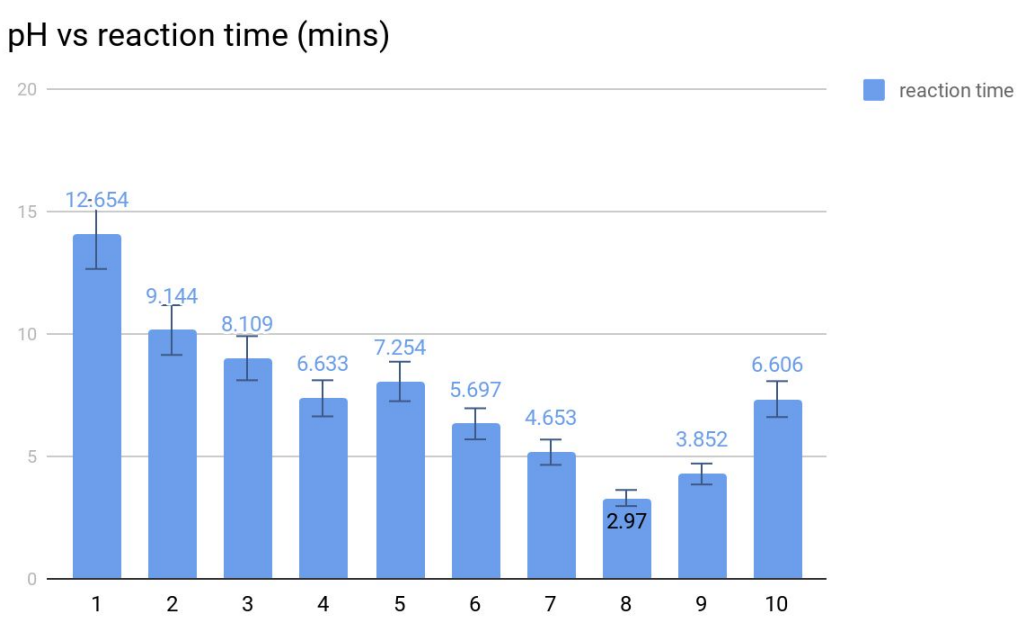
0	13.34
5	8.22
10	7.14
15	6.31
20	4.12
25	3.46
30	2.53
35	1.42
40	1.59
45	4.24

Calculation for averages shown in appendix:



Ph	Average reaction time (mins)
1	14.06
2	10.16
3	9.01

4	7.37
5	8.06
6	6.33
7	5.17
8	3.30
9	4..28
10	7.34



Analysis of data

First focus area is temperature. When looking at the averages in the table, the highlighted portion indicates the fastest time out of all the temperatures. At 35 degrees celsius, the rate of reaction to finish was 1 minute and 42 seconds. To further explain, when shown in the column chart at 35°C the smallest column is shown. This indicates how fast the reaction took place. Error bars are placed on each column on the graph to indicate the uncertainty of data. The larger the number means the larger source of error versus the smaller the number indicating less error. As seen on the graph the smallest error bar is at 35°C with an average reaction time of 1 minute and 42 seconds. With this data we are able to reject the null hypothesis in favor of the alternate. However, this is only half of the data tested.

It would have taken a college-level dissertation to acquire the materials needed to test the effect of temperature on the pH of the enzyme. Therefore, data included has been collected by Stephan Lucas at UCLA (cited below) with all processed data done by me. With the data collected shown in the line graph, when the temperature was 37°C there was the most drastic change in pH levels. This data implies that when pancreatic lipase is placed in an environment with a temperature of 35-37°C it will have the highest rate of activity within the enzyme. Further proving the alternate hypothesis.

To focus on pH, after taking the average of all data testing different pH levels, it is shown that the fastest reaction time was at the pH value of 8.0. This implies that the enzyme was working at its fastest rate, colliding with molecules and being used to its fullest potential before seeing any signs of denaturing. From this data, we are able to reject the null which states, “the more acidic or basic the pH levels become, the more active the enzyme will be, resulting in a higher reaction time..” With more basic pH, enzyme activity was not quick nor effective in its job. When the enzyme was exposed to a more acidic base the enzyme began to denature, slowing down its reaction time. This suggests that when the enzyme is exposed to a pH level of 8.0, its production rate breaking down molecules, will be most effective.

Conclusion

Overall, pancreatic lipase is the main enzyme to hydrolyse dietary fat molecules in the digestive system. The lipase will convert triglycerides substrates into monoglycerides and fatty acids. As we have identified, there are multiple factors that can help speed up the reaction rate of the enzyme. Through an extensive amount of data, we were able to determine the enzyme works most efficiently at the optimum temperature 35-37°C. The data found was able to reject the null in favor of the alternative. The alternate stated, “The closer the temperature gets to human body temperature (36-37°C) the less time it will take the reaction to occur. Many enzymes in the body are working at the human body temperature, 36-37°C. Therefore, in order for the enzyme to react successfully and efficiently it should be placed in a similar environment where it works.” This was not only expected but also proven with little source of error. To further explain how temperature can affect the enzyme, when temperature is too low, less molecules

collide resulting in little energy for the enzyme to work effectively. However, if the temperature is too hot the enzyme will denature, lose form and ability to work effectively.

The other variable tested was pH. Shown by the data, we are able to reject the null in favor of the alternate. To summarize, the null stated how lower pH would have a greater reaction rate by the enzyme, which we found to be untrue. This is because when an enzyme has a pH level that is too low, the enzyme won't have enough energy within the molecules to work effectively, resulting in lower reaction times. The alternate states how the optimal pH would be 8.0 and this was proven by the data. However , the error bars have higher values meaning there is a higher probability of error with the data.

This research comes with a lot of data which is helpful, however, that can indicate greater room for error. When looking at the raw data, times were different and had large ranges in between. This most likely occurred because when stopping the stopwatch/timer there was a time gap with my reaction to the enzyme rate being done. This is proved on the column graphs and the error bars given. Another possible source of error is the data taken by Stephan Lucas. Although his data was not primarily what I was focused on in this research, it did help prove my point and therefore could cause a source of error. I did not watch nor see the experiment done, thus, the data could be full of other errors I am unaware of. If I were to run this experiment again I would focus on one factor that affects the enzyme, not two. With that one factor I would collect more data on each point. Instead of three I would test five to limit the source of error.

Digestive enzymes are essential for a healthy body. They help break down larger molecules such as proteins, fats and carbohydrates into smaller molecules that are easier to absorb in the small intestine. This will provide energy for the human body and overall keep the digestive system clean. Digestive enzymes are available in supplement form for those who struggle with digesting foods or if the foods that will be consumed take a long time to digest. However, some foods provide natural digestive enzymes. Those foods include: bananas, avocado, papaya, ginger, kiwi, and more. These foods will naturally help improve and speed up digestion, which is why when trying to lose any type of fat these foods are suggested.

Citations

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Appendix

Calculations for averages- temperature

Temperature- time of reaction

Temperature (°C)	Trial 1	Trial 2	Trial 3	Average
0	13.22	13.52	13.31	13.34
5	8.01	7.34	8.11	7.82= 8.22
10	6.54	7.23	6.44	6.74= 7.14
15	6.12	6.45	6.37	6.31
20	4.16	4.20	4.01	4.12
25	3.52	3.35	3.52	3.46
30	2.50	2.57	2.53	2.53
35	1.53	1.30	1.44	1.42
40	1.56	2.01	2.01	1.59
45	2.37	2.65	2.36	2.37

Calculation example: (2.50+2.57+2.53)/3= 2.53

Temperature (°C)	0									
Time (min)	1	2	3	4	5	6	7	8	9	10
pH	9.09	9.09	9.09	9.08	9.08	9.07	9.06	9.04	9.04	9.02

Temperature (°C)	20									
Time (min)	1	2	3	4	5	6	7	8	9	10
pH	8.89	8.91	8.87	8.82	8.79	8.73	8.69	8.67	8.64	8.59

Temperature(°C)	35									
Time	1	2	3	4	5	6	7	8	9	10
pH	8.79	8.80	8.85	8.81	8.72	8.65	8.49	8.34	8.19	8.08

Data collected by Stephan Lucas at UCLA- analysis and data processing completely done by me.

pH

ph	Trial 1	Trail 2	Trial 3	Average (mins)
1	14.33	14.26	13.59	14.06
2	10.55	9.53	10.4	10.16
3	9.45	8.55	9.02	9.01
4	7.34	7.22	7.54	7.37
5	8.15	7.52	8.01	8.06
6	6.32	6.35	6.32	6.33

7	5.23	4.55	4.54	5.17
8	3.21	2.43	3.06	3.30
9	3.56	4.01	4.08	4.02
10	7.34	7.35	7.32	7.34

Calculation for an average

(14.33+14.26+13.59)/3= 14.06