Lab Report: Enzymatic Action In The Digestive System

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Introduction

One of the most fundamental systems in the human body is the digestive system. Digestive enzymes aid in the breakdown of carbohydrates, lipids, and proteins in meals. “Approximately 30 g of digestive enzymes is secreted per day.” (Meisenberg & Simmons) Without these enzymes, the nutrients in your meal are lost. Food must be ingested before its nutrients can reach the cells of our bodies. It must be physically broken down into tiny particles after consumption, which must then be chemically broken down so that other molecules may absorb them. In other words, “food digestion is a necessary condition for food absorption to occur.” (Marieb, 2015).

Enzymes are known to be “hydrolytic” or “hydrolases”, hence the word “hydro”, means using water for breakdown, "thus cleaving the bonds between the chemical building blocks or monomers" (Marieb, 2015) They essentially work on organic food macromolecules as their substrates. The majority of the dietary nutrients are massive polymers that cannot be absorbed in their whole, so they must be hydrolyzed in the gastrointestinal (GI) tract. Not only do enzymes utilize water to break down large food molecules, but pH and temperature can also have a significant impact on enzyme activity! To degenerate, an ideal temperature and pH must be present for the enzyme to catalyze the reaction at a rapid rate. If the temperature or pH is too high or too low, the enzyme rate declines or even denatures.

Digestion takes place as a result of the body's natural synthesis of enzymes. “They are biological catalysts, meaning that they increase the rate of a chemical reaction without themselves becoming part of the product.” (Marieb, 2015) They’re primarily generated in the pancreas, stomach, and small intestine, synthesized in the rough endoplasmic reticulum. They

collaborate with other chemicals in the body, including stomach acid, bile, and salivary amylase, to break down food into molecules that are then used by the body for a variety of tasks.

Because macronutrients are catalyzed outside the cell before being absorbed, a set of test tubes were used to evaluate the hydro activity of digestive enzymes that break down organic nutrients. The goal of this lab activity was to evaluate how digestive enzymes interact with their substrates when it comes to the breakdown of food that's consumed on a daily basis at a molecular level. Trypsin on proteins, amylase on starch, and lipase on lipids were the enzyme-substrate pairings.

Materials

The experiment was broken into three components. For this lab, a hot plate and boiling chips were applied in each activity. The nucleation sites provided by the boiling chips ensure that the liquid boils smoothly without getting superheated. A set of parafilm was also supplied, forming a physical barrier and seal over the samples and controls used in this experiment to protect the specimen from contamination and to prevent solution spillage. An ice bath was also introduced in order to cool down hot samples throughout the experiment, as well as an incubation site to maintain samples and controls under ideal room temperature. To pour droplets into test tubes, a 250ml beaker filled with water was also utilized. Lastly, a total of 18 test tubes were utilized along with three emulsifying enzyme solutions and their four substrates. A variety of procedures were carried out in order to identify the chemical digestion utilizing the IKI and BAPNA tests. Litmus Indicator and Benedict Reagent tests were also carried out as well.

Methods

Activity 1 examines starch digestion by the enzyme Salivary Amylase. To test the activity of this enzyme, 5 gtt of water were added to three tubes labeled 1A through 3A which were the controls, to begin with. Test tube 1A was filled with 3 gtt of 1% alpha-amylase solution, tube 2A was added 3 drops of the substrate 1% starch solution, and tube 3A with 3 gtt of 1% maltose solution, formed by the enzymatic hydrolysis of starch. After being incubated at 37°C, each test tube was inspected using an Iodine/Potassium Test, commonly known as an IKI test, and another test called the Benedicts Reagent. The hue of an iodine/ potassium iodide solution in water is bright orange-brown. When it is mixed with a starch-containing sample, the hue changes to a deep blue. When Benedict's solution and simple carbohydrates are boiled together, the solution becomes orange to brick red. The reducing feature of simple carbs causes this reaction; if the color shifts to blue, then there's no glucose present. Furthermore, another 3 gtt of amylase were placed in a fresh test tube labeled 4A, the first sample. 4A was then heated for 4 minutes before 3 gtt of starch was added to the tube which was then evaluated for IKI and Benedict test under hot temperatures. New sample tubes 5A and 6A contained a combination of amylase and starch which were shaken and tested at room (37°C) and cold (0°C) temperatures to examine for any reducing sugars and starch presence.

Activity 2 focuses on protein digestion using the enzyme trypsin. To evaluate the activity of the enzyme, 5 gtt of water were introduced to two tubes designated 1T and 2T as the controls. In addition, 5 gtt of 1% trypsin solution were given to tubes 1T, 3T, 4T, and 5T. The substrate used here was Nα-Benzoyl-L-arginine 4-nitroanilide hydrochloride, also known as BAPNA, the BAPNA test measures the amount of enzyme activity is in a sample. If the substance remains

clear there's no evidence of hydrolysis of trypsin if the substance changes to yellow, hydrolysis of trypsin in BAPNA has occurred. In other words, the BAPNA test determines the amount of enzyme activity present in the test tube. In test tubes samples, 2T through 5T, 3 drops of 0.01% BAPNA solution were introduced. Tubes 1T through 4T, were evaluated for protein hydrolysis at 37°C. The test tube 5T was incubated at subzero temperatures (0°C) to be examined as well for any protein degradation.

Activity 3 focuses on bile emulsification and fat digestion using the enzyme lipase. 5 gtt of water were introduced to control tubes 1L and 2L to test this action. 3 drops of 1% pancreatin solution were added to only tube 1L, and 3 drops of the substrate, litmus cream, were added to only tube 2L. The cream utilized here serves as the fat substrate for this investigation; however, to generate litmus cream, a litmus powder indicator was added. The litmus powder detects a pH shift, which is the foundation of this test. Litmus solutions in alkaline or neutral solutions are blue but become reddish in the presence of acid. In other words, if digestion happens, the fatty acids created once fat is broken down will cause the litmus cream to turn pink rather than blue. Both tubes were studied for lipid breakdown at 37°C temperatures. In a new sample tube, 3 drops of pancreatin were added to tube 3L, which was then heated for 4 minutes before 3 drops of litmus cream had been added to it. Tube 3L was tested at boiling temperatures to see whether there was any hydrolysis. In addition, 3 drops of pancreatin and 3 drops of litmus cream were added to tubes 4L and 5L, which were also samples that were further tested at room and cold temperatures. Finally, 3 drops of litmus cream and pancreatin, together with a sprinkle of bile salts, were added to the last two fresh sample tubes 4B and 5B. The human liver secretes bile, which is not technically an enzyme, but it is essential for fat digestion due to its emulsifying

effect, which breaks down big particles into smaller ones. To identify any lipid breakdown, both tubes were inspected at room 37°C and 0°C along with their enzyme and bile salt.

Results/Data

The findings of tests performed on the three enzymes are shown in the tables below.

Activity 1: Salivary Amylase Digestion of Starch

Tube No.	**1A**	**2A**	**3A**	**4A**	**5A**	**6A**
Additives (3 gtt ea)	**Amylase, Water**	**Starch, Water**	**Maltose, Water**	**Amylase (4min boil then + starch) → Boiled Amylase, Starch**	**Amylase, Starch**	**Amylase, Starch**
Incubation Condition	37°C	37°C	37°C	37°C ↑	37°C	0°C
IKI Test (color change)	No color	Color	No color	Color	No color	Color
Result (+) or (-)	Negative	Positive	Negative	Positive	Negative	Positive
Benedict's Test (color change)	No color	No color	Color	No color	Color	Some color
Result (+) or (-)	Negative	Negative	Positive	Negative	Positive	Negative

Activity 2: Trypsin Digestion of Protein

Tube No.	**1T**	**2T**	**3T**	**4T**	**5T**
Additives (3 gtt ea)	**Trypsin, Water**	**BAPNA, Water**	**Trypsin (4min boil then +BAPNA) → Boiled Trypsin, BAPNA**	**Trypsin, BAPNA**	**Trypsin, BAPNA**
Incubation Condition	37°C	37°C	37°C↑	37°C	0°C
Color change	No color	No color	No color	Color	Some color
Result (+) or (-)	Negative	Negative	Negative	Positive	Negative

Activity 3: Pancreatic Lipase Digestion oF Fats

Tube No.	**1L**	**2L**	**3L**	**4L**	**5L**	**4B**	**5B**
Additives (3 gtt ea)	**Pancreatin, Water**	**Litmus Cream, Water**	**Pancreatin (4min boil then + Litmus Cream) → Boiled Pancreatin, Litmus Cream**	**Pancreatin, Litmus Cream,**	**Pancreatin, Litmus Cream,**	**Pancreatin, Litmus Cream, Bile Salts**	**Pancreatin, Litmus Cream, Bile Salts**
Incubation Condition	37°C	37°C	37°C ↑	37°C	0°C	37°C	0°C
Color Change	No color	No color	No color	Color	No color	Color	Some color
Result (+) or (-)	Negative	Negative	Negative	Positive	Negative	Positive	Negative

Discussion/Conclusions

Enzymes allow cell interactions to occur at normal temperatures. Many chemical reactions that occur slowly at room temperature can be accelerated by raising the temperature. High temperatures, on the other hand, can destroy live cells. (Schraer)

In the amylase digestion evaluation, tube 1A served as a control, containing solely Amylase. Because it simply included starch and water, it tested negative for both the IKI and Benedict tests. Tube 2A was a control that just contained starch; it likewise tested negative for Benedicts since no reducing sugars were discovered, resulting in a blue hue. However, due to the presence of starch, 2A tested positive for the IKI test, resulting in a dark blue tint. Tube 3A was the third control, and it contained simply maltose solution. It was positive for the Benedict exam since maltose is a byproduct of catalyzed starch, but negative for the IKI exam due to starch not being present because maltose is already a reduced sugar. Tube 4A contained amylase that had been boiled, which had denatured the enzyme and caused it to be incapable of digesting the starch that had been evenly supplied. This resulted in a positive test for the IKI test, but it seemed to be a very moderately positive test for the Benedict test owing to starch not being broken down due to boiling temperatures, hence it is considered a negative result. Tube 5A exhibited a strong positive test for the presence of starch in the Benedict test but a negative test for starch in the IKI test. This is attributed to the incubation at 37°C which is the best temperature for Amylase to react with starch and convert it to maltose. Tube 6A, on the other hand, revealed a positive test for starch and a marginally positive result for the Benedict test. This can be explained by the fact that Amylase is not as successful in digesting starch at 0°C as it is at 37°C. This causes the catalyzing reaction to work at a very slow pace causing no digestion to occur

In Activity 2 protein digestion, Tube 1T was a control that just contained trypsin, hence it did not show any color change since any color change would have to be produced by BAPNA digestion. Tube 2T, also functioned as a controller. Because it contained just BAPNA, it was unable to release the yellow dye in the solution. So in the absence of an enzyme to digest it, the solution remained transparent, resulting in no protein hydrolysis. Tube 3T was initially boiled with trypsin, which as a consequence damaged the enzyme and left it incapable of digesting the subsequently added BAPNA, resulting in a colorless solution, therefore it was marked negative. Tube 4T demonstrated a clear positive test for trypsin digestion of BAPNA. This was attributed to the incubation at 37°C which was shown to be close to the ideal temperature for trypsin to operate on proteins.

Finally, tube 5T solution developed a very light yellow hue, indicating a very moderately positive test for BAPNA digestion. This shows that trypsin is not as efficient at digesting proteins at 0°C as it is at 37°C due to the molecular mobility between the enzyme and the substrate decreasing at low temperatures, which causes them to be unable to catalyze their reactions.

Lastly, in Activity 3 fat digestion, tube 1L served as a control and it only contained pancreatin being tested at room temperature, which resulted negative for fat hydrolysis, this was due to no fat or cream being present to breakdown. An enzyme can’t degenerate itself own self, unless if it’s under boiling temperatures. Tube 2L also was introduced as a control, containing solely Litmus cream. As a result, there was no enzyme to break down the fat-containing cream. The solution stayed blue, indicating that there was no change in the pH of the solution. Tube 3L was first then boiled with just pancreatic in it, which denatured the lipase enzyme. As an

outcome, the denatured enzyme was inefficient in digesting the fat present in the cream that was later added. As a consequence, the solution stayed blue, suggesting that no pH shift change occurred in the solution, i.e., no fat hydrolysis. Tube 4L yielded a positive test result for pancreatic cream digestion with litmus cream. This was attributed to the incubation at 37°C, which is the ideal temperature for pancreatic hydrolyze fat. Furthermore, the pancreatic and litmus cream solution stayed blue with a little pink tinge in tube 5L. This suggests that fat digestion was inadequate as a result of the incubation at low temperatures and a little pH shift. In other words, pancreatic enzymes are less efficient at digesting fat at 0°C than they are at 37°C and as result, it was marked with no color and negative. Solution 4B turned into a red, suggesting a significant fall in pH caused by the full digestion of the fat-containing cream. Bile salts aided in the emulsification of fat, hence aiding digestion more efficiently at ideal temperatures. Last but not least, tube 5B solution developed a somewhat pink hue, indicating a positive test for fat digestion. Due to the presence of bile salts at 0°C, the pancreatic is still not as effective at digesting fat as it is at 37°C. There is a minor color shift owing to the bile salts, which still catalyze the fat but did not achieve its full potential, therefore this was recognized as a negative result.

In conclusion, this lab provided a fantastic learning opportunity and representation of how enzymes function. The ultimate goal of this lab was to learn about the activity of an enzyme on certain substrates and how their chemical interactions assist the human body by evaluating through a series of tubes and solutions. The human body is nearly like a machine, with hundreds of daily tasks to maintain a homeostatic environment. Lack of protein, lipid, and carbohydrate digestion can be difficult to consume all of the vitamins, nutrients, electrolytes, and minerals that

our bodies require for survival. Organs and other biological systems can malfunction and can potentially be fatal if these building components are not there. That is why it is critical to comprehend how chemical digestion works in the human digestive tract.

References

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