Digestive System: Chemical and Physical Break Down

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The Ideology behind the Digestive System is to break down larger molecules of macromolecules that are found in food into smaller molecules, such as monomers so that nutrients can reach the cell allowing the cells to build up it's own polymers while receiving energy that is needed.When food is ingested the process starts with the salivary enzymes from saliva that helps breakdown food into smaller particles and then those smaller particles are them enzymatically digested into molecules that can be absorbed. There are many enzymes throughout our body, enzymes are proteins that function as biological catalysts that speed up the chemical reaction. In living organisms enzymes function on many substances where substrates come into

play, substrates are a substance or surface area which is functioned by an enzyme. These enzyme functions are used to help generate energy to the body, break down polymers into monomers, they help move ions across the plasma membrane, helps signal in dna etc.The enzymes found in saliva are amylase, lingual lipase, carbonic anhydrase, and lastly peroxidases. These enzymes are helpful for speeding up the rate of a chemical reaction in the body, amylase is the main enzyme in saliva that breaks down carbohydrates into smaller particles.

Lingual Lipase is an enzyme that breaks down triglycerides, which is a 3-fatty-acid, into glycerides and fatty acid components catalyzing or speeding up the digestion of lipids. The catalyst function is to increase the rate of a chemical reaction without themselves becoming part of the product. Lingual Lipase is more active in the stomach due to the lower pH, but it starts its breakdown of triglycerides into diglycerides in the oral cavity. Decomposed products of fats are absorbed by the lymphatic system and from there are transported into the systemic circulation by lymph. Carbonic anhydrase is a particular isoenzyme that regulates the pH in the oral cavity making saliva alkaline by increasing levels of hydrogen. Lastly, Peroxidases are oxidizing agents used to break down harmful substances by adding in hydrogen from a donor molecule causing reduction of oxidation which then causes the other molecule to be oxidized, enabling chemicals by producing compounds that can eliminate toxic microorganisms. After chemical digestion is when secretion occurs it causes the release of water, enzymes, acids, salts and buffers by the epithelium tissue of the digestive tract , gallbladder and glandular organs. The secretions and the digestive epithelium are a defense to nonspecific bacteria in the body causing lamina propria and other cells of the immune system to attack the bacteria that may have been swallowed with the food that was consumed.

When absorption happens the molecules are thoroughly broken down into tiny particles that can easily be absorbed into the lining of the small intestine where nutrients are absorbed by a substance called chyme. The lining of the small intestine is called villi. Its functions are to increase the surface area of the intestinal wall for the absorption process working to help pick out the nutrients such as proteins, carbohydrates, lipids etc. End product being for carbohydrates is glucose and glucose is absorbed into ATP. The end product of proteins are new proteins after the breakdown of amino acids and the pepsin enzymes are released causing the digestive process to occur and the end product of lipids are glycerol and fatty acids . The remaining indigestible waste that could not be absorbed in the large intestine would be passed through the cecum, colon, rectum and eliminated through the anus.

Objective: In this experiment the objective is to test out how each enzyme hydrolyzes on one or more substrate molecules and its reaction to specific environmental conditions that are needed for it to function smoothly. The enzyme and substrates that are being examined are amylase on starch, trypsin on protein, and lastly lipase on lipids.

Materials & Methods : The general supplies used in this experiment are hot plates used to heat up the samples, 250-ml beakers to obtain substances, Boiling chips for testing, test tubes/ rack for testing samples, wax markers for labeling, water bath set at 37°C for incubation, ice bath for testing and lastly a notebook for recording data.

In activity one Salivary Amylase Digestion of Starch, six testing tubes are needed along with dropper bottles containing the solutions," 1% alpha-amylase solution, 1% boiled starch, 1% maltose, IkI solution, benedict's solution and distilled water,(MarieB 597)". A spot plate is also used in this experiment to test out the droplets from samples. To test for the amylase digestive enzyme, Use a dropper to add 3gtts of indicated substances to each labeled tube, 3 gtts of

distilled water are only added to the tubes labeled 1A, 2A, and 3A. In tube 1A add 3 gtts of amylase solution, tube 2A add 3 gtts of starch solution, in tube 3A add 3 gtts of maltose solution, in tube 4A add 3 gtts of amylase and boil for 4 minutes using hot plate then after add 3 gtts of starch to the boil amylase, and for tubes 5A tube and 6A both have added 3gtts of amylase and 3 gtts of starch solution. After labeling and loading the tubes as indicated, place all tubes in a rack in the 37°C water bath for approximately 1 hour, shaking the rack gently from time to time. After one hour it is time to test for starch, take a spot plate, and dropper bottles for iodine and benedict's solution. Set up a boiling bath using a 250-ml beaker, hot plate and boiling chips. While that's heating, label each spot on the spot plate for each of the tubes 1A-6A, use a dropper to transfer only one drop of the sample from each tube onto each appropriate spot on the spot plate. Then place a drop of iodine solution onto the sample, if the color turns black-blue then that indicates that there's starch present. If starch is not present then the mixture will not come out blue which would mean it is negative for starch. With the remaining substances in each tube place only 3 drops of benedict's solution, after putting each tube into the beaker of boiling water for 5 minutes and if it turns a green to orange color that indicates that maltose is present and if there is no color it indicates a negative sugar test.

In activity two the trypsin digestion of protein, five test tubes are needed and a test tube rack for the experiment along with a dropper bottle of trypsin and another dropper bottle of BAPNA. To test for the activity of trypsin, use a dropper to add 3gtt to each indicated substance to each labeled tube. For tubes 1T and 2T only 3 gtts of water was added to each tube unlike the 3 other tubes. In tube 1T add 3 gtts of trypsin solution, in tube 2T add 3 gtts of BAPNA solution, In tube 3T add trypsin solution then boil with hot plate up to 4 minutes after that add BAPNA to the boiled trypsin, and lastly in tubes 4T and 5T add 3 gtts of trypsin and BAPNA in both tubes.

After property labeling and adding solutions, place all tubes in the appropriate water bath for about 1 hour. Gently shake the rack from time to time. Now after an hour exame the tubes for any trace of trypsin, the color of yellow indicates a positive hydrolysis and if the color remains clear then its negative for hydrolysis.

In activity 3 pancreatic lipases digestion of fats, for testing lipases seven test tubes are needed along with a test tube rack and one dropper bottle for each solution, those being 1% pancreatin solution, litmus cream, 0.1 NHCI, and lastly vegetable oil. Emulsified fats physically break down large fat molecules into smaller ones to provide a broader surface area for the enzymes to do its job, (Zao 600)". Label tubes 1L and 2L "L" symbolize lipase and tubes 3L,4L and 5L as well, the last two tubes are labeled as 4B and 5B symbolizing bile. In each tube add the indicated solution,for tube 1L apply 5gtts of water along with 5 gtts of pancreatin into the tube. For tube 2L apply 5 gtts of water and 5gtts of litmus cream, for tube 3L add 5gtts pancreatin boil it for 4 minutes then after the boil add 5 gtts of litmus cream to the pancreatin the 3L tube. In tube 4L and 5L apply both 5gtts of pancreatin and litmus cream to the tubes and for the last tubes 4B and 5B apply 5 gtts of pancreatin solution, 5 gtts litmus cream solution and 5gtts of bile salts into both " B" labeled tubes. Incubation condition of the tubes should be 37 degrees celsius for all tubes except tubes 5L and 5B. After applying the indicated substances to the tubes use a parafilm to cover the tops for each tube properly , then shake the mixture of contents, after being shaken remove the cover (parafilm) and place all the tubes in a rack so it can be placed in the incubation water bath for approximately 1 hour. Within that hour shake the tube rack frequently so the mixture stays mixed. After that use a notebook to record your results.

DATA : Activity 1: Salivary Amylase Digestion of Starch

Tube #'s	1A	2A	3A	4A	5A	6A

Additives (3gtt ea)	Amylase Water	Starch Water	Maltose, Water	Amylase (Boil amylase 4 min, then add 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 (-)	(-)	(+)	(-)	(+)	(-)	(+)	
Benedict's Test (Color Change)	NO COLOR	NO COLOR	COLOR	NO COLOR	COLOR	SLIGHT COLOR	
Result: (+) or (-)	(-)	(-)	(+)	(-)	(+)	(-)	

Activity 2: Trypsin Digestion of Protein

Tube #	1T	2T	3T	4T	5T
Additives (3 gtts each)	Water, Trypsin	BAPNA, water	Trypsin (boil trypsin 4 min, then add 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	COLOR	COLOR	NO COLOR
Result (+) or	(-)	(-)	(+)	(+)	(-)

(-)					

Activity 3 : Pancreatic Lipase Digestion of Fats

Tube#'s	1L	2L	3L	4L	5L	4B	5B
Additives (5gtt ea)	Pancreatin ,Water	Litmus Cream, Water	Pancreatin, (boil pancreatin 4 min, then add 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	NO COLOR
Result (-) or (+)	(-)	(-)	(-)	(+)	(-)	(+)	(-)

Conclusion/ Discussion

When testing out these three enzymes the idea is finding out the breakdown of each enzyme in body temperature 37 degrees celsius and other temperature conditions, depending on the environmental pH if it is too acidic or too much alkaline an enzyme would react a different way not allowing it to function as optimally. If the conditions are off then so are the enzymes which would no longer fit to its substrate. As temperature increases so does the enzyme and if the temperature increases too high the enzymes destruct. This experiment is to test out the hydrolytic activity to all three enzymes depicting the progressive digestion of the digestive enzymes.

In the Salivary Amylase Digestion of Starch experiment, tube 1L tested only amylase and water; it was tested negative for starch meaning that starch was not present due to the breakdown of the digestive enzyme amylase that would keep the color clear. For the benedict's test tube 1L also tested negative for no color and no trace of sugar.For test tube 2 only tested starch and water, and the temperature was also 37 degrees celsius. For the IKI test the color for test tube 2A was a dark blue color meaning it tested positive also for starch, when testing for sugar in the benedict test for 2A slight blue color became present meaning that it is negative for sugar in the sugar test and the larger molecules was successfully transitioned into smaller molecules. For test tube 3A, maltose and water were tested after incubation process testing for starch and sugar occurred leaving a positive present of sugar in the benedict test but tested negative in the IKI test meaning that this tube is present for sugar but not starch because of maltose being a type of sugar. In tube 4A it held amylase that had been boiled for only 4 minutes after the 4 minutes were over. Observation can tell the enzymes had been denatured due to the increasing temperatures when that happens then the starch is not broken down into its tiny particles so it can succeed hydrolysis. Tube 4A tested negative for the IKI test and also tested negative for no color in the benedict's test because of the denatured enzyme the enzymatic activity would not be able to occur. For tube 5A it contains amylase and starch one being a carbohydrate and another being a digestive enzyme when doing IKI test and benedict's test, for the benedict's test it came back positive for sugar meaning color change is present and for the IKI test the result was negative and no color change meaning no starch was found. Lastly , for tube 6A it contains the same substance as 5A but the difference is that one's incubation condition is lower than the other. When testing the amylase and starch in this tube for sugar and starch the test both came back positive for benedict's test and IKI test because the temperature was 0

degrees celsius instead of a higher temperature. In this tube you can tell that the starch was not able to break down into its smaller molecules and was not able to digest.

In the Trypsin Digestion of Protein experiment, tube 1T contained only trypsin and water and was incubated for 1hour at 37 degrees celsius. In the hydrolysis test for tube 1T it tested negative for any hydrolysis occurrence and also presented no color, for tube 2T contained BAPNA and water with the incubation temperature at 37 degrees celsius. In the hydrolysis test for tube 2T it tested negative for any hydrolysis occurrence and presented no color as well meaning that hydrolysis between BAPNA solution and water didn't work, for tube 3T Trypsin was only added at first to the tube and boiled for 4 minutes which causes denaturing of the enzyme. After the boiling process BAPNA was added to the tube, when testing for hydrolytic activity it is not presented in tube 3T meaning the test came back negative with no color. For tube 4T only trypsin and BAPNA were included in this tube with an incubation condition of 37 degrees celsius, the results for the test came back positive for hydrolysis occurrence meaning that color is present because a reaction occurred. In tube 5T trypsin and BAPNA were contained in this tube and incubation condition was 0 degrees celsius meaning that no reaction would occur because of the environmental condition resulting in a test for negative and no color presented for the hydrolysis test. In conclusion all tubes secreted water or under ent hydrolysis except for tube 4T.

In the Pancreatic Lipase Digestion of Fats experiment, tube 1L and 2L both contained water in their tubes except the others, but both had different solutions. Tube 1L held Pancreatin and tube 2L held Litmus Cream, when testing the both tubes the color would turn blue if the presence of the pH is alkaline and it would change pink if acidic. Tube 1L tested negative for no color and no for color change meaning digestion did not occur, for tube 2L it tested also negative

with no color change. In tube 3L it contained pancreatin at first but was boiled for 4 minutes making it denatured and adding pancreatin also to the mixture after the boiling, when testing this tube after the incubation the result came back negative and also no color change . In tube 4L and 5L both tubes held pancreatin and litmus cream but the only difference were the incubation temperatures 4L at 37 degrees celsius and 5L at 0 degrees celsius, 4L came back with a present of color also resulted in positive for digestion whereas 5L came back negative with no color change. For the two tube 4B and 5B with “b” represented as bile are both tubes at contain the same substances Pancreatin,Litmus Cream and Bile Salts for tube 4B the incubation condition was 0 degrees celsius whereas 5B was at a regular temperature of 37 degrees celsius. Tube 4B tested positive for digestion and color was presented also, for tube 5B it tested negative and a slight color was presented. In conclusion all enzymes are used well in specific temperature conditions and pH levels in the body because if it is disrupted then it can slow down in the digestive process.

Reference Page

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