Protein Digestion Design Lab

Introduction

It is known that the chemical digestion of protein occurs in the stomach and small intestine. Proteins are polymers of monomers known as amino acids (a monomer is one unit of amino acid) aligned in a genetically determined sequence. Proteins contains the elements carbon, hydrogen, oxygen, nitrogen, phosphorus and sulphur. The amino acids in a polymer are joined together by the peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. The sequence of amino acids in a protein is defined by the sequence of a gene, which is encoded in the genetic code.

Like other biological macromolecules such as polysaccharides and nucleic acids, proteins are essential parts of organisms and participate in virtually every process within cells. Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism. An enzyme is a biological catalyst made up of protein. It alters the rate of a chemical reaction without itself being chemically changed at the end of the reaction. Variables that affect enzyme activity include temperature and pH. There is a particular optimum temperature and pH which allow enzymes to perform most efficiently. This implies that since the structure of the enzyme can be affected by temperature and pH, enzymes must be proteins. When the bonds which give a protein its unique shape are broken, the protein, in this case an enzyme, is denatured.

Every enzyme functions most effectively at a specific pH. For example, pepsin, the protein-digesting enzyme secreted by the cells of the stomach lining, functions optimally at a pH of 2, depending on the substance being digested. On the other hand, trypsin, a protein-splitting enzyme secreted by the pancreas, functions optimally in an alkaline medium at a pH of 8.5, depending on the substance being digested. Most intracellular enzymes have pH optima near neutrality and do not operate successfully in an acid or alkaline medium. Furthermore, a major shift from the enzyme's optimal pH range may irreversibly inactivate the enzyme. Two protein-digesting enzymes are pepsin, secreted in the stomach, and trypsin, secreted in the pancreas. Trypsin is a component of pancreatin, along with pancreatic amylase and lipase.

To detect the amount of protein molecules or short polypeptide chains in the cooked egg white for this experiment, the biuret reagent will be used. The biuret test is a chemical test used for detecting the presence of peptide bonds. In the presence of peptides, a copper(II) ion forms a violet-colored complex in an alkaline solution. The biuret reagent is made of potassium hydroxide (KOH) and hydrated copper (II) sulfate, together with potassium sodium tartrate. The reagent turns from blue to violet in the presence of proteins, blue to pink when combined with short-chain polypeptides.

Problem

What factors allow pepsin and trypsin to have an optimum rate of enzyme activity on protein in the stomach and small intestine respectively?

Purpose

To determine at which pH and with/without the enzyme, pepsin digestion, in the stomach and small intestine, takes place the most optimally.

Hypothesis

Pepsin requires an acidic environment with an optimum pH of 2.
Trypsin requires an alkaline environment with an optimum pH of 8.

Variables

Independent Variable:

1. pH of acid/alkaline used

Dependent Variables:

1. Amount of protein digested

Control Variables:

1. Size of the egg pieces
2. Size of test tubes
3. Temperature

Materials

1. Cooked egg white
2. 8 x 150mm test tubes
3. Test tube rack
4. Standard lab glassware (eg. beakers)
5. Pepsin solution
6. Pancreatin solution
7. Hydrochloric acid (1.0M, 0.1M, 0.01M, 0.001M)
8. Sodium hydroxide (1.0M, 0.1M, 0.01M, 0.001M)
9. pH paper and pH scale
10. Thermometers
11. Incubator
12. Electronic scale
13. 10 ml granulated cylinders
14. Stir rods
15. Scoopulas
16. Eye droppers
17. Scalpels,
18. Biuret reagent
19. Hot plates
20. Distilled water

Method

1. Wash all the test tubes thoroughly
2. Cut the egg white into 8 equal pieces of 3-5mm in length

3. Put each piece of egg white into each of the 9 test tubes, number the test tubes A through B.
4. Test the egg white samples in the test tubes by adding the following substances:
   Test tube A – 5ml of distilled water
   Test tube B – 5ml of distilled water and 1ml of 0.01M sodium hydroxide
   Test tube C – 5ml of distilled water and 1ml of 0.01M hydrochloric acid
   Test tube D – 5ml of pepsin solution
   Test tube E – 5ml of pepsin solution and 1ml of 0.01M sodium hydroxide
   Test tube F – 5ml of pepsin solution and 1ml of 0.01M hydrochloric acid
   Test tube G – 5ml of pancreatin solution
   Test tube H – 5ml of pancreatin solution and 1ml of 0.01M sodium hydroxide
   Test tube I – 5ml of pancreatin solution and 1ml of 0.01M hydrochloric acid
5. Test the pH level of the solutions in all of the test tubes. Record this.
6. Set the test tubes for 24hrs in the incubator with the temperature set to 37^{o}C.
7. Add the biuret reagent to each of the test tube mixtures one drop at a time, shaking the mixtures gently in between each drop. Record any colour changes.

Data Collection and Processing

Observation Chart:

Test tube	Contents	pH	Biuret colour change

A	5ml of distilled water	7	Not tested; control
B	5ml of distilled water and 1ml of 0.01M sodium hydroxide	8	Not tested; control
C	5ml of distilled water and 1ml of 0. 1M hydrochloric acid	2	Not tested; control
D	5ml of pepsin solution	6	Violet
E	5ml of pepsin solution and 1ml of 0.01M sodium hydroxide	6	Violet
F	5ml of pepsin solution and 1ml of 0.01M hydrochloric acid	2	Pink
G	5ml of pancreatin solution	4	Violet
H	5ml of pancreatin solution and 1ml of 0.01M sodium hydroxide	6	Pink
I	5ml of pancreatin solution and 1ml of 0.1M hydrochloric acid	2	Violet

Conclusion and Evaluation

Only two test tubes had protein digestion in it, test tubes F and H. This is known because when the biuret reagent was added to both test tubes after 24 hours in an incubator, the contents both turned pink, indicating the presence of short polypeptide chains. This supports the hypothesis, as test tube F contains pepsin solution in an acidic mixture of 0.01M hydrochloric acid, which is similar to the environment inside the stomach, where gastric juice is secreted into the food to break down protein chains to short polypeptide chains. In order for the pepsin enzyme to work, it

must be in an acidic environment, confirmed in the experiment to be around 2 pH. Hence the egg white in test tube F was mostly digested, since it provided the optimum environment for pepsin to act on the protein molecules in the egg white.

This is also seen in test tube H, which contains the pancreatin solution in an alkaline mixture of 0.01M sodium hydroxide, which matches the environment inside the duodenum, whereby pancreatic juice containing trypsin is released via the pancreatic duct to break down protein chains into short polypeptide chains. In order for trypsin to work, it must be in an alkaline environment, confirmed to be around pH 6-8. Hence the egg white in test tube H was digested the most, since it provided the optimum environment for trypsin to act on the protein molecules in the egg white.

Test tubes D and E did not show any results of protein breakdown, as they did not contain the required chemicals to create an optimum environment for the enzyme pepsin in the stomach. Test tube D contained only the pepsin solution, and without the acidic environment to activate the enzymes, hence no protein digestion took place. Test tube E contained the pepsin solution and the 0.01M sodium hydroxide, and the alkaline mixture prevent the enzymes from being activated to digest the proteins, so no protein digestion took place either.

Test tubes G and I also did not show any results of protein digestion, as they did not contain the required chemicals to create an optimum environment for the enzyme trypsin in the small intestine. Test tube G contained only the pancreatin solution, and the enzymes would not be able to digest the proteins in a non-alkaline environment. Test tube I contained the pancreatin solution and the 0.1M hydrochloric acid, and the acidic environment prevented the enzymes from being activated to digest the proteins, so there was no protein digestion here too.

In conclusion, the optimal conditions for protein digestion in the stomach would be an acidic environment of pH 2; the optimal conditions for protein digestion in the small intestine would be an alkaline environment of pH 8.

Limitations of the experiment:

1. Only 1 of each trial was done
2. The experiment was based on largely quantitative results, such as based on observation of the egg white to see how much was digested, or the colour change of the biuret reagent(which can be unclear if too much reagent is added)