

Distribution of Digestive Enzymes in Cockroaches

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Abstract

The digestive tract of a cockroach is a tube modified into subdivisions, which serve specialized digestive functions: food reception, conduction and storage, internal digestion, absorption, conduction, and formation of feces. The three divisions of the cockroach digestive tract are the **foregut**, **midgut**, and the **hindgut**. The enzyme reaction in the digestive tract can be determined either by determining the amount of substrates (starch and proteins) in an enzyme-reaction mixture, or measuring the presence of product present. Some of the factors that may influence the enzymatic process are pH, temperature, time, and concentration of substrate and enzyme. In this experiment, all variables are held constant except the concentration of enzymes (amylase and protease) and pH of substrates. Thus, the presence and the concentration of enzymes such as amylase and proteolytic enzymes can be studied in the gut of cockroaches.

Introduction

Animal food consists of organic material, most of which belongs to three major groups: proteins, fats and carbohydrates. Whether the food is used for fuel or for building and maintenance, the large molecules of food are first broken down into simpler units (monomers). These are then absorbed and either incorporated into the body or metabolized to provide energy. The breakdown is achieved in the digestive tract with the aid of enzymes.

Digestion can take place either within or outside of cells. In a unicellular animal, digestion is usually, of necessity, inside the cell. For example, a protozoan takes food into the digestive vacuole, and enzymes that aid in the digestion of carbohydrates, fats, and proteins are secreted into the vacuole. Extracellular digestion is usually associated with a well-developed digestive tract that allows secreted enzymes to act on the food material.

The digestive tract may have one opening, as in cnidarians, brittle stars, and flatworms. In these animals, any undigested food is expelled through the same opening that served as a mouth. In more complex animals, the digestive tract has two openings, a mouth and an anus. This permits an assembly-line type of digestion. Food is ingested in the mouth and is passed on and acted upon by a series of digestive enzymes. The soluble products of digestion are absorbed. Undigested material is expelled through the anus without interference with food intake.

The digestive tract of a cockroach is a tube modified into subdivisions, which serve specialized digestive functions: food reception, conduction and storage, internal digestion, absorption, conduction, and formation of feces. The three divisions of the cockroach digestive tract are: the **foregut**, which includes the crop and proventriculus, **midgut**, which includes the section below the proventriculus up to the caeca, and the **hindgut**, which includes the Malpighian tubules and the rectum. The products of digestion are absorbed in the midgut, especially in the more anterior parts including the caeca. Absorption also occurs in the hindgut, especially in the rectum, but there is no evidence of absorption from the foregut, which has an impermeable cuticular lining.

The enzyme reaction in the digestive tract can be determined two ways. 1) Determine the amount of substrates (starch and proteins) in an enzyme-reaction mixture. 2) Measure the presence of product present (*e.g.*, maltose for starch digestion and amino acids in protein digestion.) For example, in starch digestion, the easier of the two ways is to measure the amount of starch present. Iodine is a good indicator of presence of starch. In the presence of starch, a blue-black stain will appear. As starch is digested, the blue-black color will disappear.

Some of the factors that may influence the enzymatic process are pH, temperature, time, and concentration of substrate and/or enzyme. In this experiment, all variables are held constant except the concentration of enzymes (amylase and protease) and pH of substrates. Thus, the presence and the concentration of enzymes, such as amylase and proteolytic enzymes, can be studied in the gut of cockroaches.

An enzyme extraction procedure usually requires a method that destroys the integrity of the cell. The broken cells then release their molecular constituents including enzymes. One method that can be used to lyse (break open) cells involves treating tissue with a detergent that breaks or dissolves cell membranes. In the procedures described below, the tissue extracts will be mixed with an enzyme extraction buffer, which contains a few drops of detergent.

Objective:

The objective of this experiment is to determine the optimum pH for chymotrypsin, a proteolytic enzyme, and the presence and activity of enzymes (amylase and proteolytic) in the different divisions of the cockroach digestive system.

Materials:

- Agar powder – (10 g)
- Agar gel buffer -- 0.1M NaCl, 10 mM Tris, pH 7.4 (600 ml)
- Enzyme extraction buffer -- 20 mM NaCl, 0.02%, 4 drops of detergent, 10 mM Tris, pH 8 (100 ml)
- Starch solution- 1% (50 ml)
- Skim milk (20 ml) or 12% casein (pH=8, titrate with 6N NaOH)
- (3) large glass test tubes
- (10) 1.5-ml tubes
- 3 petri dishes per group (30)
- (18) glass Pasteur pipettes
- (10) small transfer pipettes: one pipette per student group, one for the starch solution and one for each of the amylase and chymotrypsin solutions.
- Amylase -- 4 mg/ml (10 ml)
- Chymotrypsin -- 10mg/ml- dilutions needed for various standards (10 ml)
- Pepsin -- 2mg/ml (10 ml)
- IKI solution
- Large beaker (2) to boil water
- Cockroaches, CO₂ tank
- (20) scissors and (20) forceps
- Insect saline (500 ml), and (20) petri dishes for cockroach dissection
- (3) glass tissue grinders
- Hot plates
- Acetic acid – 5% (500 ml)
- Microcentrifuge tubes
- Centrifuge
- Rulers

Procedures:*I. Removal and processing of the digestive tract of the cockroach.*

Anesthetize 20-25 cockroaches with carbon dioxide (2-3 cockroaches per students for 10 groups of student). Frozen cockroaches may be used. Cut the anus free from the body wall with scissors. Remove the complete digestive tract of the cockroach by holding the cockroach in insect saline and slowly pulling off the head and attached gut with forceps. Should the gut break between the proventriculus and midgut, open the abdomen centrally and dissect out the posterior gut portion. Refer to the diagram and **identify (do not isolate the three regions until they are rinsed)** the foregut, midgut, and hindgut.

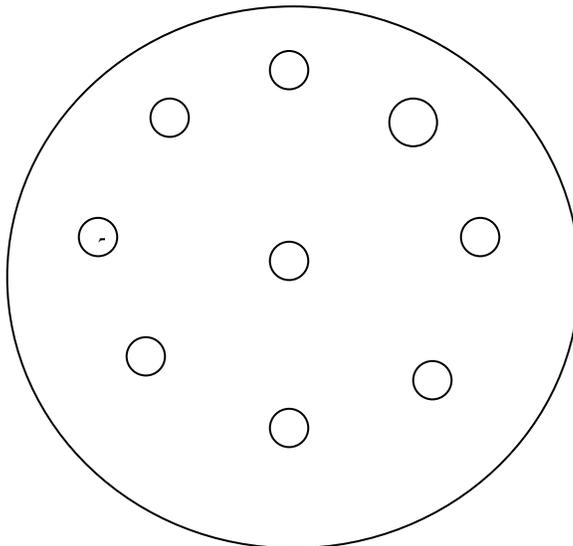
Slit open the gut and, holding it with a pair of forceps, vigorously rinse in a dish of insect saline to remove any contained food. Using a scalpel, isolate the three regions of the gut to be studied (foregut, midgut, and hindgut). Obtain four test tubes and label 1-4. Collectively, weigh the **isolated sections of** cockroach gut; be sure not to mix up the different sections of the intestine. Place the isolated sections of gut in separate **labeled** glass tissue grinders containing enzyme extraction buffer (1 to 1.5 ml of extracting buffer per gram of tissue). After grinding, allow the tissue to settle. Transfer the supernatant by Pasteur pipette into separate test tubes (1- foregut, 2- midgut, and 3- hindgut). Bring the volume of each extract to 12 ml with enzyme extraction buffer. Now pipette 1 ml of each of the extracts (1-3) into the 4th tube. Heat this combined extract for 5 minutes in a beaker of boiling water to denature the enzymes present. Cool and bring to about 3 ml with enzyme extraction buffer. This will serve as a suitable control for the enzyme tests. *Why?*

Shake the remaining three tissue extracts and divide the homogenized gut extract into 10 portions (for 10 groups of students) by placing 1 ml of each extract in a labeled 1.5-ml microcentrifuge tube. Cap and shake the tubes vigorously for 3 minutes. Centrifuge the tubes for 5 minutes and remove the supernatant fraction (the liquid) with a pipette. Place these solutions in clean labeled 1.5-ml microcentrifuge tubes. The extracted enzyme is now in these tubes.

II. Preparation of the agar gels

1. Label three large tubes, A, B and C. Dispense 18 ml of agar gel buffer into tubes A and B, and 19.5 ml to tube C. Add 0.32 g of agar to each tube.
2. Place the tubes into a boiling water bath and allow the agar suspensions to come to a vigorous boil. After boiling for 4 minutes, remove the test tubes from the bath and cool at room temperature for about 3 minutes. The solution should be clear.
3. Add 2 ml of skim milk or 1 ml of 12% casein to tubes A and B, and 0.5 ml of 1% starch solution to tube C and swirl the tube until the agar forms a suspension. Skim milk (casein) and starch will serve as the substrates for the enzymes used in this experiment. Each tube should have 20 ml of fluid.
4. Pour the melted agar into three petri dishes. One tube per dish. Label the dishes A (for casein), B (for casein), and C (for starch substrate).
5. Let the agar cool at least 15 minutes. The gels can be used or stored in the refrigerator for up to a week.
6. Make nine wells in each of the three agar plates using a Pasteur pipette.
7. Add about 5 ml of vinegar to *agar plate B*. After 15 minutes, discard the acid. The acid will denature and precipitate the casein in the milk, which will cause the protein to turn white.
8. Prepare the appropriate dilutions of chymotrypsin and amylase solutions

Pattern for arrangement of wells in the petri dish



III. Sample Application:

Using the transfer pipettes, carefully fill the wells (about 0.1 ml) of both plates as indicated below. Separate pipettes should be used to disperse the chymotrypsin and pepsin solutions.

Two petri dishes- A and B (B was treated with 5% acetic acid before extracts were placed in the wells)

<u>Well number</u>	<u>Proteolytic enzymes (A and B)</u>
1	agar gel buffer
2	Chymotrypsin (20 ug/ml)
3	Chymotrypsin (200 ug/ml)
4	Chymotrypsin (2000 ug/ml)
5	Pepsin (2 mg/ml)
6	Standard foregut extract (not boiled)
7	Standard midgut extract (not boiled)
8	Standard hindgut extract (not boiled)
9	Boiled intestinal extract

<u>Well number</u>	<u>Amylase C</u>
1	Agar gel buffer
2	Amylase- 4 ug/ml
3	Amylase- 20 ug/ml
4	Amylase- 100 ug/ml
5	Amylase- 500 ug/ml
6	Standard foregut extract (not boiled)
7	Standard midgut extract (not boiled)
8	Standard hindgut extract (not boiled)
9	Boiled intestinal extract

After all samples (about 0.1 ml) have been loaded in the wells, place the lids on the dishes. Do not over fill the wells. The dishes should not be moved at this time. The plates should remain at room temperature for 12-24 hours.

IV. Analysis of agar plates:**A. Detection of proteolytic enzyme activity (A and B):**

1. Place about 5 ml of vinegar or 4% acetic acid onto agar in plate **A**. The transparent rings around the sample wells where the casein has been degraded by the enzyme indicate protease activity on both plates A and B.
2. After 20 minutes, discard the acid on plate A, measure the diameter of the clear rings around each well.
3. The amount of chymotrypsin in the tissue extracts can be compared to the diameters of the rings around the sample wells containing known concentrations of the enzymes.

B. Detection of amylase activity (C):

1. Place about 5 ml of the Lugol's iodine solution onto the agar in the plate.
2. After 10-20 minutes, discard the iodine and fill the petri dish with water.
3. After 10-20 minutes, measure the diameter in mm of the clear rings around each well and record your results below. The amount of amylase in the tissue extracts can be compared to the

diameters of the clear rings around the sample wells containing known concentrations of the enzymes.

<u>Well number</u>	<u>Diameter of clear rings- mm</u>		Amylase activity
	<u>Plate A</u>	<u>Plate B</u>	
1			
2			
3			
4			
5			
6			
7			
8			
9			

Suggestions for Data Analysis and Discussion:

1. Determine the amount (if any) of amylase and chymotrypsin activities in the cockroach gut extracts, using Excel (diameter of ring (cm) on Y-axis, and concentration of enzyme on (log) X-axis).
2. Explain the basis for the formation of rings in the agar-starch gels.
3. What is the function of the enzyme extraction buffer?
4. Describe the effects of the heat treatment on the activity of the enzymes in the cockroach gut extract.
5. Relate the findings from the cockroach foregut, midgut, and hindgut to the site of action of proteolytic and carbohydrate enzymes in the digestive tract.

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