

Distribution of digestive enzymes in the gut of American cockroach, *Periplaneta americana* (L.)

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Abstract- Adult male and female cockroaches, *Periplaneta americana* (L.) were assayed for the presence of digestive enzymes in the fore-, mid- and hindgut regions. Activities of α -amylase, β -amylase, γ -amylase, proteinase and lipase were detected in the three gut regions except for the absence of γ -amylase and lipase in female hindgut. The presence of these enzymes partly explains the polyphagous feeding habit of *P. americana*, enabling the insect species to digest a wide variety of food substances. In some instances, significant differences in enzyme activities were observed between sexes and among gut regions. Generally, enzyme activity was highest in the midgut followed by the fore- and hindgut in descending order. Despite this trend, a considerable level of proteinase and male lipase was observed in the hindgut suggesting that it might be necessary to give extra attention to hindgut activities in future studies.

Index Terms- Digestive enzymes, Foregut, Hindgut, Midgut, *Periplaneta americana*.

I. INTRODUCTION

Cockroaches (Blattodea) are among the most primitive and successful insects. Their omnivorous, detritophagous feeding habit and association with symbiotic bacteria (Cruden and Markovetz, 1987) obviously contributed to their survival for at least 350 million years (Thorne and Carpenter, 1992). Many modern species have retained the omnivorous nature of their ancestors and this has no doubt contributed to their success as obnoxious pests of humanity (Cruden and Markovetz, 1987). Today there are 3,500 species of cockroaches found on every continent except Antarctica. Truly representing the diversity of insects, the cockroach family provides excellent models for anatomical and physiological investigations. The American cockroach, *Periplaneta americana* (L.) (Blattidae), is the largest of the cockroaches measuring an average 4 cm in length. Adults are reddish-brown in appearance with a pale-brown or yellow band around the edge of the pronotum. The cockroach is easily, albeit unintentionally and regretfully, spread by human commerce and the species is currently cosmopolitan in distribution. It is found mainly in basements, sewers, steam tunnels and drainage systems (Rust *et al.*, 1991) making it difficult to control. *Periplaneta americana* is a voracious omnivore that feasts on almost anything such as paper, boots, hair, bread, fish, fruit, peanuts, old rice, the soft part on the inside of animal hides, dead insects and cloth, thereby causing economic loss (Bell and Adiyodi, 1981). American cockroaches can become a public health problem due to their association with human waste and disease, and their ability to move from sewers

into homes and commercial establishments. At least 22 species of pathogenic human bacteria, virus, fungi, and protozoans, as well as five species of helminthic worms, have been isolated from field-collected cockroaches (Rust *et al.*, 1991).

The digestive system functions to break down and absorb nutrients for maintenance, survival and reproduction in insects. The long and somewhat coiled digestive tube in *P. americana* could be divided into three regions: the foregut which includes the mouth, salivary glands, esophagus, crop, and the proventriculus or the gizzard; the midgut which includes the ventriculus, gastric caeca and malpighian tubules; and the hindgut comprising ileum, colon and rectum. The occurrence of a multitude of digestive enzymes in the gut of cockroaches is consistent with their omnivory and feeding adaptability. Digestive tract of *P. americana* harbours xylanase, laminaribiase, cellobiase, maltase, sucrase, α - and β -glucosidase, α - and β -glycosidase, β -fucosidase, chitinase and N-acetyl- β -glucosaminidase that attack various polysaccharides including those in the plant and fungal cell walls (Scrivener *et al.*, 1998; Genta *et al.*, 2003). Similar versatility in the digestion of proteinaceous substrates is indicated by the presence of 11 proteinases in the gut of *P. americana* (Hivrale *et al.*, 2005). Vinokurov *et al.* (2007) recorded high proteolytic and amyolytic activities in the midgut of *P. americana* with a moderate activity in the crop. Cockroaches have been the most popular group of insects for studies of lipid digestion, and a number of early studies indicated lipolytic activity in the fore- and midgut. It was also demonstrated that lipase originates in the epithelial cells of the midgut and caecae. Thus the presence of lipolytic activity in the foregut results from regurgitation of midgut contents into the foregut (Downer, 1978).

A large number of previous studies have been devoted to determining enzyme activity in the fore- and midgut. This has been as a result of the assumption that little or no digestion (but absorption) takes place in the hindgut. The present work was, therefore, designed to quantify activities of enzymes in the three gut regions of adult male and female *P. americana*. Enzymes hydrolyzing three major classes of food (carbohydrate [α -amylase, β -amylase, γ -amylase], protein [proteinases] and fat [lipases]) were assayed. Information about digestive enzymes is of fundamental importance in understanding the digestive processes, food and feeding habits of insects. Such information could also assist in formulating sustainable and effective control strategies.

II. MATERIALS AND METHODS

1. Reagents

Casein (1% w/v), Trichloroacetic acid, Lowry's reagent C, starch solution, Folin-Ciocalteu's phenol reagent, iodine mixture, Dinitrosalicylic acid and olive oil emulsion were purchased from Sigma Chemical Company, St. Louis, MO., USA. Phosphate and sodium acetate buffer, and Tris buffer were obtained from Fluka (Buchs, Switzerland). Distilled water was prepared in the laboratory. All other reagents were of analytical grade and they were obtained from either Sigma or BDH.

2. Insect culture in the laboratory

A colony of *P. americana* was raised on white bread in the Insect Physiology Laboratory, Obafemi Awolowo University, Ile-Ife at 26 ± 2 °C and $73 \pm 3\%$ RH. Newly emerged adults (1-3 days old) of both sexes were used for the experiment.

3. Enzyme bioassay

Separate bioassays were constituted for male and female cockroaches to determine activities of digestive enzymes in the foregut, midgut and hindgut. The assays were replicated three times.

A. Preparation of enzyme extract

Enzyme samples were prepared using the method described by Cohen (1993). The insects were starved for 24 h before dissection to standardize them and to allow the accumulation of digestive enzymes. The insects were placed at -20 °C for 4 min and then dissected in ice-cold 0.9% NaCl solution under a dissecting microscope. The alimentary tract was removed by placing the scissors points between the junction of the third and second to the last tergites. Two incisions were made along each laterally arranged spiracle, continuing through to the thorax. Once the tergites were freed from the underlying connective tissue they can be removed in one piece. By grabbing the head with a forceps and cutting the surrounding neck chitin, the entire digestive tract was removed by gently lifting the head and freeing the attached tract moving caudally toward the anus. The extracted digestive tubes were separated into the foregut, midgut and hindgut, and each gut region was kept in 1 ml ice-cold sodium phosphate buffer (pH 7.1). The tissues were homogenized and ultra-centrifuged at 16,000 rpm for 10 min at 4 °C. The supernatant was placed in a centrifuge tube and kept at 4 °C.

B. Proteinase assay

Proteinase activity was quantified spectrophotometrically as described by Morihara and Tsuzuki (1977) with slight modification. The reaction mixture consisted of 1 ml 1% (w/v) casein and 0.5 ml of the enzyme preparation. This was incubated in a water bath at 35 °C for 30 min. The reaction was terminated by the addition of 3 ml cold 10% (w/v) Trichloroacetic acid (TCA). The mixture was allowed to stand at 4 °C for 30 min, centrifuged at 3000 rpm for 10 min and the supernatant was collected for the determination of non-precipitated TCA protein. This was done following the Folin-Ciocalteu's phenol reagent method of Lowry *et al.* (1951). One milliliter of the TCA protein was mixed with 5 ml of Lowry's reagent C, mixed thoroughly and incubated at room temperature for 10 min. Three fold diluted

Folin-Ciocalteu's phenol reagent (0.5 ml) was added to the mixture with shaking, and incubated at room temperature for 30 min. The optical density of the mixture was read at 670 nm in a Cintra 101 spectrophotometer. The amount of non-precipitated TCA protein was estimated as tyrosine from a standard curve of known concentrations of tyrosine. One unit of protease activity is defined as the quantity that is required to produce 100 µg of tyrosine in 1 ml of TCA filtrate under the above conditions.

C. α -Amylase assay

The assay method described by Fuwa (1954) was followed with slight modification. To 0.2 ml 1% (w/v) soluble starch solution was added 0.1 ml of enzyme solution with mixing and 0.7 ml of distilled water. The mixture was incubated at room temperature for 30 min in a water bath. The reaction was terminated by the addition of 1 ml Dinitrosalicylic acid (DNSA) and mixed thoroughly; 0.1 ml of iodine mixture was added to the mixture. The optical densities of both blank and experimental samples were read at 670 nm in the spectrophotometer. One unit of the α -amylase activity is defined as the amount of α -amylase which produced a 10% reduction in the intensity of the blue colour of starch-iodine complex under the specified condition.

D. β -Amylase assay

The assay method described by Abiose *et al.* (1988) was used. The reaction mixture consisted of 0.2 ml 1% (w/v) soluble starch and 0.1 ml homogenate incubated for 5 min in a water bath at 37 °C. The mixture was boiled at 100 °C for 5 min and 8 ml of distilled water was added. The amount of reducing sugar produced from the hydrolysis was estimated following Somogyi-Nelson method (Somogyi, 1945). All tubes (experimental and control) were provided with 1 ml of combined Cu reagent and mixed thoroughly. They were incubated in a boiling water bath for 20 min, cooled in tap water for about 4 min, and then 1 ml of Arsenomolybdate colour reagent was added to each of them while shaken. Lastly, 7 ml of distilled water was added to each tube and mixed thoroughly. The optical densities of both blank and experimental samples were read at 540 nm in the spectrophotometer. One unit of the enzyme activity is defined as the amount of β -amylase which produced 1 mg of reducing sugar expressed as maltose under the experimental conditions.

E. γ -Amylase (*amyloglucosidase*) assay

The enzyme was assayed following the method described for β -amylase except that the reaction mixture was incubated at 60 °C for 30 min. One unit of the amyloglucosidase activity is defined as the amount of enzyme that produced 1 mg of reducing sugar calculated as glucose under the above conditions.

F. Lipase assay

The assay method described by Shihabi and Bishop (1971) was used with little modification. 3.0 ml of olive oil emulsion was pipetted into a cuvette and warmed to 37°C. 0.1 ml of the homogenate was added and mixed by inverting the cuvette. The change in absorbance was recorded at 340 nm for 1-min intervals till it became consistent. Lipase activity (µmol triglyceride bonds broken/min/litre of homogenate) was calculated by multiplying change in absorbance per minute ($\Delta A/\text{min}$) by the conversion

factor (F). Based on olive oil being pure glyceryl trioleate (mol wt. 880), the value of F was 5400.

4. Statistical analyses

Activities of digestive enzymes were subjected to Analysis of Variance (SAS Institute, 2002) and mean values were shown as bar charts. For each sex, standard error bars (+ s.e.) were used to compare activities of each enzyme across the three gut regions. Enzyme activities were also separated between male and female cockroaches in each gut region.

III. RESULTS

Enzyme activities within each sex differed significantly among the gut regions except in female cockroaches where mean values for proteinase were comparable (Table 1). Hydrolytic activities between male and female cockroaches were compared in each gut region as presented in Table 2. Activities of lipase ($P \leq 0.01$) and α -amylase ($P \leq 0.05$) differed between male and female *P. americana* in two regions, the midgut and hindgut. Also, sexual effect was significant in β -amylase and proteinase activities only in the foregut ($P \leq 0.05$) while γ -amylase activities varied with sex ($P \leq 0.05$) only in the hindgut.

Digestive enzymes were detected in the fore-, mid- and hindgut of *P. americana* but generally, activities were lowest in the hindgut (Fig. 1). Activities of female α -amylase were higher than those of males in the mid- and hindgut. A similar trend was observed for proteinase activity in the foregut. On the other hand, activities in male cockroaches were higher for β - and γ -amylase in the foregut and hindgut, respectively. For lipases, activity was higher in females in the midgut whereas it was higher in males in the hindgut.

A. Proteinase activity

Proteinase activity was detected in the three gut regions of adult cockroaches. The midgut and hindgut of male cockroaches had significantly higher enzyme activities than the foregut while activities were not significantly different across the gut regions of female cockroaches.

B. α -Amylase activity

In male cockroaches, activity of α -amylase was significantly higher in the foregut followed by the midgut and hindgut in descending order. On the other hand, enzyme activity was comparable in the foregut and midgut of females but significantly lower in the hindgut.

C. β -Amylase activity

A similar trend was observed in the activities of β -amylase in male and female cockroaches. Activities recorded in the fore- and midgut were comparable but significantly higher than values obtained in the hindgut.

D. γ -Amylase activity

Amyloglucosidase was detected in all the gut regions except in the hindgut of female cockroaches. Activities in the foregut and midgut of each sex were statistically equal but significantly higher than in the hindgut.

E. Lipase activity

As in the case of γ -amylase, lipase was not detected in the hindgut of female cockroaches. Level of activity was low and comparable in the foregut and midgut of male insects but it increased significantly in the hindgut. On the contrary, enzyme activity was significantly higher in the midgut of females compared to the foregut.

Table 1: Mean square values of activities of each enzyme in the gut regions of male and female *Periplaneta americana*

Enzyme	Male		Female	
	Gut region	Replicate	Gut region	Replicate
	df = 2		df = 2	
α -Amylase	86.81*	1.34	86.64**	0.94
β -Amylase	91.54*	2.76	60.81*	3.88
γ -Amylase	104.99*	15.78	126.75*	1.91
Proteinase	0.15*	0.05	0.06	0.07
Lipase	2.62*	0.24	6.40*	0.30

*, ** Significant F-test at 0.05 and 0.01 levels of probability, respectively

Table 2: Mean square values of activities of each enzyme between sexes in the fore-, mid- and hindgut of *Periplaneta americana*

Enzyme	Foregut		Midgut		Hindgut	
	Sex	Replicate	Sex	Replicate	Sex	Replicate
α -Amylase	0.73	6.11	5.90*	0.05	0.20*	0.01
β -Amylase	5.34*	1.38	1.68	12.08	0.34	0.04
γ -Amylase	5.22	1.89	12.95	25.08	8.55*	0.03
Proteinase	0.33*	0.09	0.01	0.04	0.02	0.00046
Lipase	0.00076	0.000057	0.07**	0.01	0.07**	0.0017

*, ** Significant F-test at 0.05 and 0.01 levels of probability, respectively

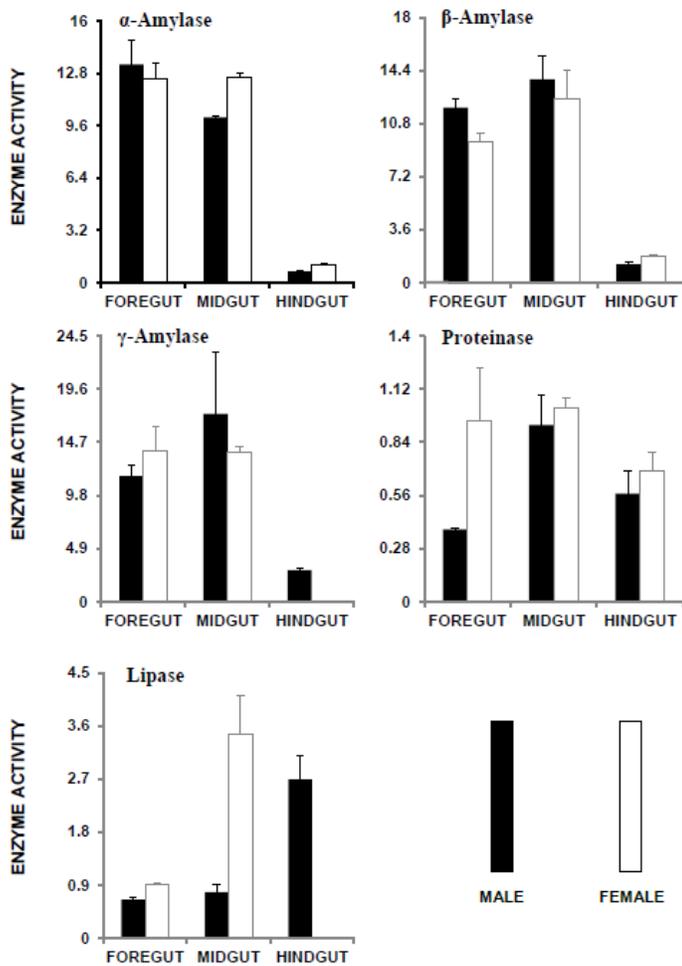


Figure 1: Activities of the amylases, proteinase and lipase in the fore-, mid- and hindgut regions of *Periplaneta americana*. Error bars compared activities within sex across the three gut regions. Lipase activity was measured in $\mu\text{mol/l/min}$ while other enzymes were measured in $\mu\text{g/ml/min}$

IV. DISCUSSION

Starch consists of two distinct fractions: amylose – linear α -1,4-linked glucans, and amylopectin – linear α -1,4-linked glucans branched with α -1,6 linkages (Ball *et al.*, 1996; Mouille *et al.*, 1996), and the enzymes responsible for hydrolysis of starch and related saccharides are called amylolytic enzymes or simply amylases. The three most known amylases (α -amylase, β -amylase and γ -amylase) were detected in the present study. This is an addition to a number of carbohydrases that had been reported earlier in *P. americana* (Scrivener *et al.*, 1998; Genta *et al.*, 2003). Unlike α - and γ -amylases, β -amylase is not produced by animals although it may be present in microorganisms contained within the digestive tract (Adachi *et al.*, 1998; Mikami *et al.*, 1999). The detection of β -amylase in the present study indicates that some symbiotic microorganisms are resident in the fore-, mid- and hindgut of *P. americana*. Zurek and Keddie (1996) reported that gut bacteria in *P. americana* played a functional role in the development and survival of the insect species. Cruden and Markovetz (1987) also reported a stoppage in the biosynthesis of cysteine and methionine and subsequent

reduction in fecundity when gut bacteria were eliminated from *P. americana*. The detection of these amylases, proteinase and lipase in the digestive tract of *P. americana* shows that the species is well equipped for polyphagous feeding habit.

The absence of lipase activity in the hindgut of females was preceded by a dramatic increase in the midgut. It is possible that female wasps made maximum use of lipase substrate in the midgut in order to meet specific reproductive requirements. Lipids, mostly triacylglycerol (TAG), and smaller amounts of phospholipids (PL) and cholesterol, make up 30-40% of the dry weight of the insect oocyte (Kawooya and Law, 1988; Briegel, 1990). Also, lipids are the main sources of energy for the developing embryo (Beenackers *et al.*, 1981) and the PL is needed for the formation of membranes. Insect oocytes synthesize TAG using fatty acids (FA) (Lubzens *et al.*, 1981; Ferenz, 1985) but since the ability of oocytes to synthesize FA *de novo* is very limited, Ziegler and Van Antwerpen (2006) concluded that nearly all the lipids must be imported, especially from ingested food. This requirement might have necessitated maximal digestion of lipase substrate in the female midgut.

Generally, enzymatic activities in each sex of *P. americana* were, more or less, higher in the midgut followed by the foregut and hindgut in descending order. This supports previous reports identifying midgut as the principal site for enzyme secretion and food digestion in insects (Klowden, 2007; Nation, 2008). However, activity of α -amylase was relatively higher in the foregut of *P. americana*. This may be due to the fact that α -amylase is secreted by the salivary glands which are situated in the anterior part of the foregut. Apart from the location of secretory organs, prevailing conditions in different regions of insect gut such as redox potential and pH may also have differential effects on digestive enzymes (Vinokurov *et al.*, 2007). This may explain in part why hydrolytic activity of each enzyme varied from one gut region to the other.

The general tendency of workers to pay little attention to the hindgut because of the assumption that negligible hydrolytic activities take place in the region may eventually rob the science world of some useful pieces of information. In the present work, a considerable level of proteinase and male lipase activities was detected in the hindgut of *P. americana*, underlying the potentials of digestive enzymes in this region. Workers are encouraged to give adequate consideration to activities in the insect hindgut in future studies.

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