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CHARACTERIZATION OF GLYCOSIDASE IN THE MID-GUT OF THE EARLY THIRD LARVAL INSTARS IN GASTEROPHILUS INTESTINALIS

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SUMMARY

Mid-gut homogenates from the early third larval instars of *Gasterophilus intestinalis* contain at least four glycosidases. Enzyme activity was found α-galctosidase and β-galactosidase . Also - α–anylase showed activity in midcythomogenates. All enzymes had acidic pH optima of 3.6-6.A drug (Banminth 12.5% pyrantel tartarate) used for the routine control of helminthes parasites of horses and donkies in Egypt was used in vitro to investigate its effect on the optimal activity of studied enzymes.

INTRODUCTION

Glycosidases are a group of enzymes that hydrolyze bonds linking a carbohydrate to a peptide, to a lipid, or to another carbohydrate. According to their site of action, they are classified as exoglycosidases or endoglycosidases.

Exoglycosidases hydrolyze only terminal non-reducing monosaccharides of poly-or oligosaccharides and are relatively specific for the sugar to be released as well as for the configuration (α or β) of the bond. Examples are α, β - galactosidase or α, β-glucosidase; they are different enzymes that release (α or β) linked galactose or glucose end groups.

Endoglycosidases are able to hydrolyze poly-or oligo - saccharides in the interior of the molecule and thus all products of the reaction are usually oligosaccharides or polysaccharides of reduced molecular weight. Examples are chitinase, which recognizes linkages of the β- 1,4- linked polymer of N - acetyl - (D) glucosamine, and lysozyme that cleaves linkages between N-acetyl - (D) - glucosamine and muramic acid in bacterial cell walls (Aronson, 1972; Flowers and Sharon, 1979).

Wigglesworth (1929) reported the presence of a weak amylase activity in insect's flies. In other Diptera, several different glycosidases have been detected in mid-gut caeca homogenates.

Ferreira and Terra (1980) have extensively studied the presence and subcellular distribution of α - and β – glucosidase, α-and β-glacatosidase, and cellobiases in *Rhynchosciara Americana.*
The activities of these enzymes exhibit acidic pH optima and are plasma membrane bound. Hori et al. (1981) reported finding several of glycosidases in the gut of hornflies, *Haematobia irritans.* Also Deloach and Spates (1984) reported acidic pH optima for all the glycosidases found in the stable fly.

Mid-gut amylases from several insects have been purified and characterized, whereas few papers deal with insect mid-gut α-glacactosidase and glucoamylases (Vonk and Western, 1984; Applebaum, 1985).

Gasterophilus intestinialis is an insect of medical importance. The third larval instars produce damage in the gastric tissue of the horse. Gastric disturbance include ulceration (Waddek, 1972;Shefstad 1978; Pandey et al., 1980), subserosal abscess formation (Waddell, 1972; Shefstad, 1978), and nodule or papilla formation (Ashizawa et al., 1972; Pandey et al., 1980).

The present study was done to investigate digestive glycosidases in the mid-gut of the early third larval instars of *G. intestinalis* to help us in understanding the physiology and consequently the nutritional process in this insect.

MATERIALS AND METHOD

Collection of larvae

Third larval instars of *Gasterophilus* were collected from the stomach of freshly slaughtered donkies and horses in the Zoo, Giza, Egypt. They were identified according to Zumpt (1965).

Preparation of mid-gut homogenate:

The selected larvae were immobilized by placing them on ice and the alimentary canals were dissected and separated in phosphate buffer saline (PBS - 0.15 M NaCl + 0.01 M sodium phosphate, pH 7.2 and 0.24gm of mid-gut were homogenized in 8 ml cold distilled water and centrifuged at 9000g for 10 minutes at 4°C (Riberio and Pereira, 1984).

Buffers used

0.2 M sodium-acetate buffer at pH 3.6, 4.6 and 5.6, also 0.2 M phosphate-citrate buffer at pH 6 were prepared.

Determination of total carbohydrate:

The total carbohydrate content was determined according to Singh and Sinha (1977)

Determination of Glycosidases activities at different pH values:

The activity of glycosidases was determined according to modified method of Ribeiro and Pereira (1984).

Substrates used were p-nitrophenyl -α-D-glucoside for α-glucosidase acting on α-glucoside linkages, p-nitrophenyl-β-D-glucoside for β- glucosidase acting on β- glucoside linkages, p-nitrophenyl-α-D-galactoside for α- galactosidase acting on β- galactoside linkages and p-nitrophenyl-β-D-galactoside for β-galactosidase acting on β-galactosidic linkages. All were prepared by dissolving 0.3 mg of each one in 1 ml distilled water and diluted with 2 ml of 0.2 M glycine: 2 M NaoH (pH 10.4).

Determination of α-amylase activities at different pH values:

The activity of amylase was determined according to modified method of Snell and Snell (1953)

Starch was used for determination of amylase activity by dissolving 50 mg in 1 ml distilled water.

The above steps were repeated by incubating equivalent amounts of 1 % of 12.5 % pyrantel tartrate at different pH levels for each enzyme.

Control samples contained all components and was under same conditions but without homogenate solution were used. The change in activity was measured at 410nm. (Using Jenway 6100 spectrophotometer).

Statistical analysis:

All analyses were performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL USA). Data were expressed as mean ± standard error of 6 replicates in each experiment. Mean values of continuous variables were compared using t-test or analysis of variance (ANOVA) followed by Duncanís multiple range test (Duncan, 1955). Correlations between variables was calculated by Pearsonís method. The significance level was set at p-value less than 0.05.

RESULTS

Glycosidase assays:

The higest activity of α-galactosidase, α-glucosidase and β-galactosidase was at pH 3.6 (Figs 1, 2, 3 respectively), while that of β-glucosidase was at pH 6 (Fig4).

The activity of amylase was high at pH 6, but with highest activity at pH 3.6 (Fig5). The drug (pyrantel tartarate) used showed that its effect on the activity of α-glucosidase and β- glucosidase was non-significant, while it was significant ($P<0.05$) for α-galactosidase and highly significant ($P<0.01$) for β-galactosidase (Fig. 6)

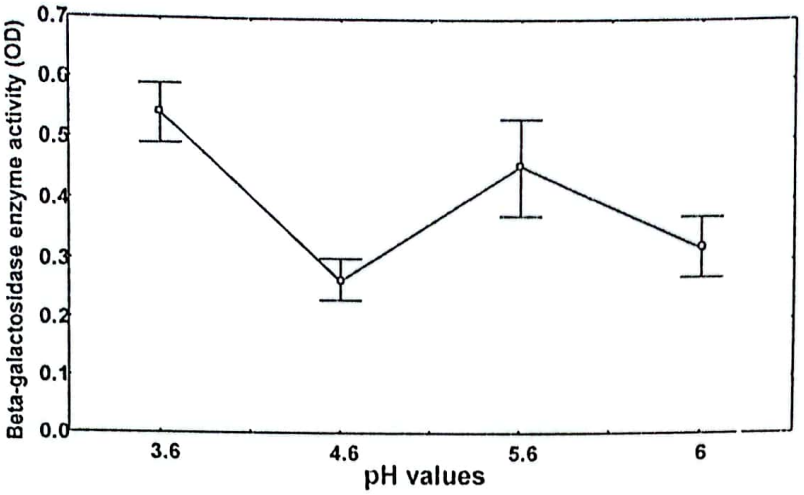

Fig. (1): Alpha-galactosidase activity at different pH values using p-nitrophenyl - α-galactoside in homogenates of mid-gut of early third larval instars of *G. intestinalis*.

Activity of enzyme expressed as O.D. / 30 min / 0.001 mg protein

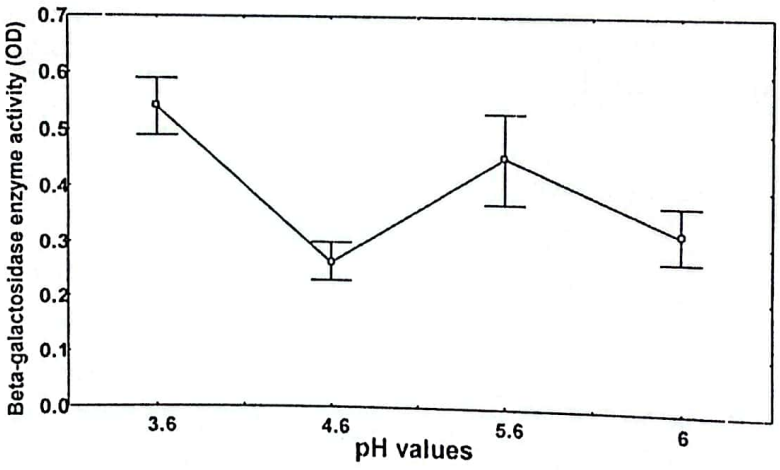

Fig. (2): Beta - galactosidase activity at different pH values using p-nitrophenyl - β -galactoside in homogenates of mid-gut of early third larval instars of *G. intestinalis*.

Activity of enzyme expressed as O.D. / 30 min / 0.001 mg protein

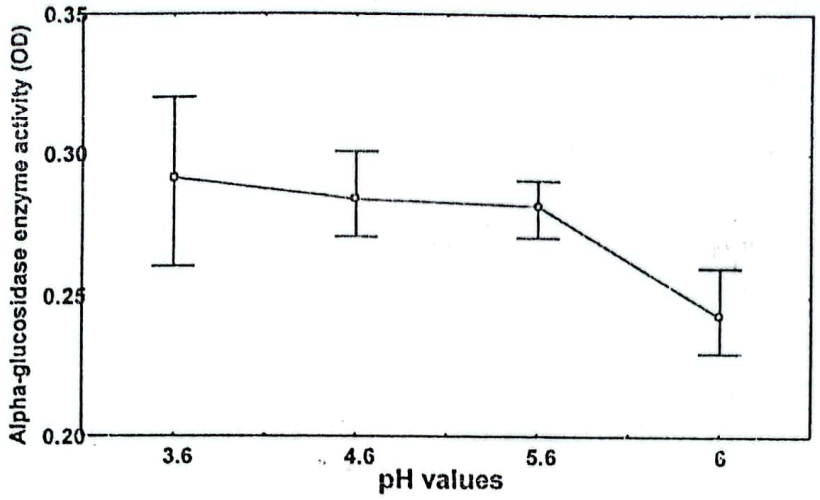

Fig. (3): Alpha - glucosidase activity at different pH values using p-nitrophenyl- α-galactoside in homogenates of mid-gut of early third larval instars of *G.intestinalis*.

Activity of enzyme expressed as O.D./30 min/0.001mg protein

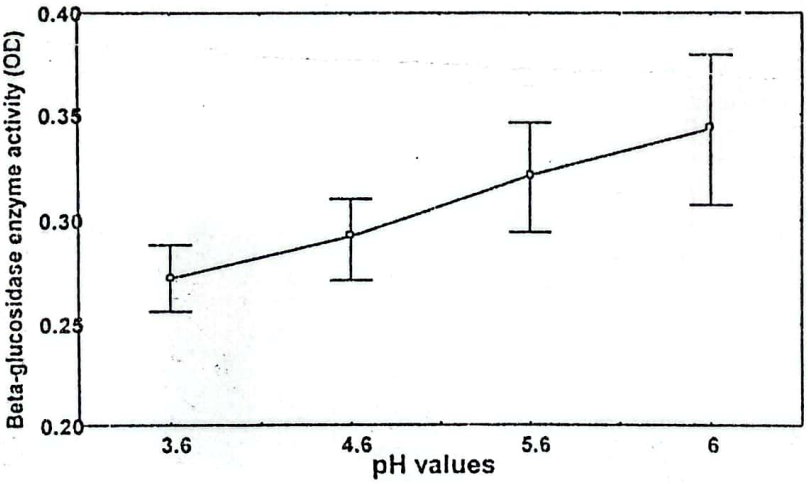

Fig.(4): Beta-glucosidase activity at different pH values using p-nitrophenyl-β-galactoside in homogenates of mid-gut of early third larval instars of *G.intestinalis*. Optimum pH at 6.

Activity of enzyme expressed as O.D. / 30 min / 0.001 mg protein

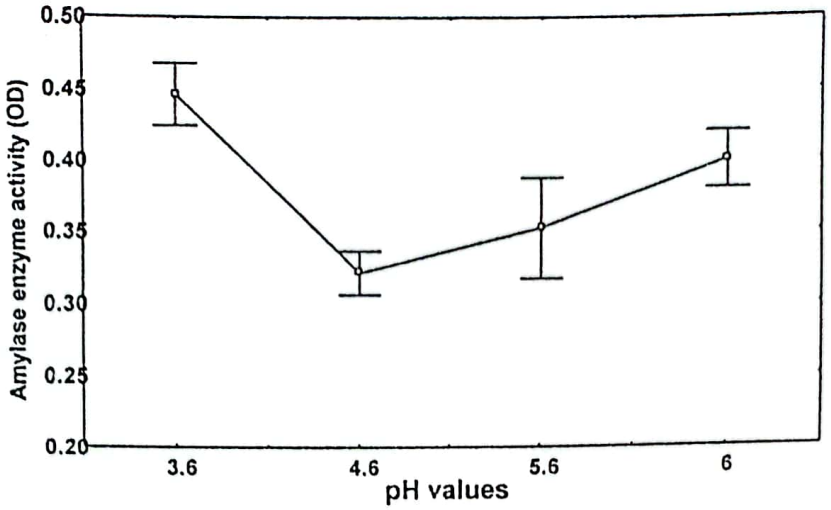

Fig. (5): Amylase activity at different pH values using starch in homogenates of mid-gut of early third larval instars of *G. intestinalis*

Activity of enzyme expressed as O.D. / 30 min / 0.001 mg protein

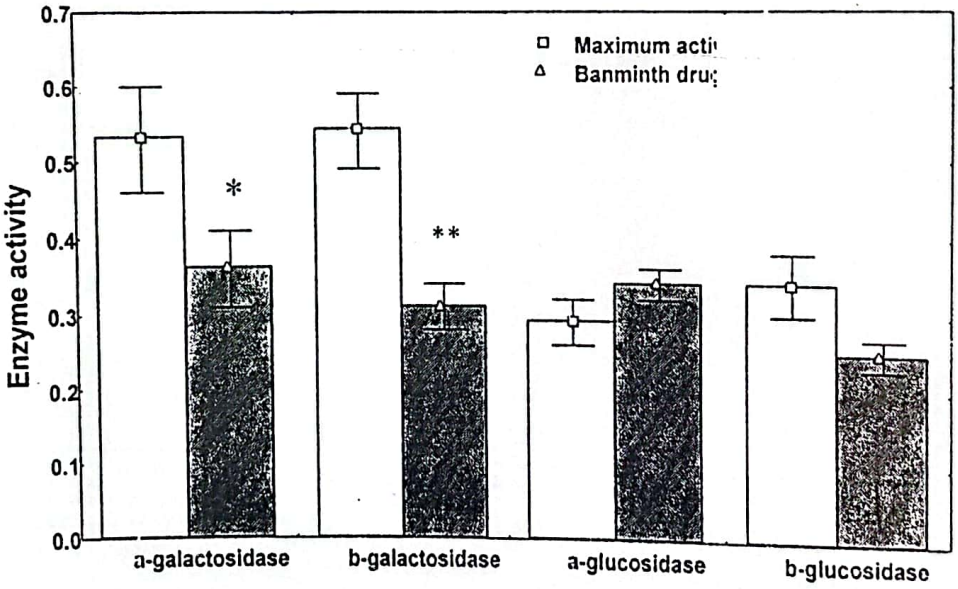

Fig. (6): Effect of Banminth drug on the maximum activity of α-galactosidase, (pH 3.6), β-- glucosidase, (pH 3.6); α-glucosidase (pH 3.6); and β - glucosidase (pH 6) in homogenates of the mid-gut of early third larval instars of *G. intestinalis*

* $P < 0.05$

** $P < 0.01$

DISCUSSION

The early third larval instars of *G. intestinalis* possessed glycosidases. The present work describes the presence of α & β - glucosidase andα & β- galactosidase in the mid-gut of the early third larval instars of *G. intestinalis*. α - glucosidases catalyze the hydrolysis of terminal, non - reducing α - 1,4 - linked glucose residues from aryl - glucosides (as p- nitrophenyl - α- D - glucoside), disaccharides or oligosaccharides with varied efficiency, thus, α - glucosidase hydrolyzes α - glucosides (e.g. maltose, sucrose, melezitose and trehalose).

The α- glucosidase in the mid-gut of the early third larval instars of *G. intestinalis* showed high pH activity in the acidic range (pH 3.6 - 6) with highest activity at pH 3.6. This pH activity is near to those pH values (5 - 6.5) found in other insects using different substrates e.g. *Apis mellifera* (Huber, 1975; Huber and Mathison, 1976) *Drosophila melanogaster* (Tanimura et al., 1979), *Stomoxys calcitrans* (Deloach and Spates, 1984), *Thaumetopoea pityocampa* (Pratviel - Sosa et al., 1986), *Rhodinus prolixus* (Ribeiro and Pereira, 1984; Terra et al., 1988), *Musca domestica* (Jordao and Terra, 1989), *Sitophilus zeamais* (Baker, 1991), and *Dysdercus peruvianus* (Silva and Terra , 1995). α - galactosidase acts on β- galactosides (e.g. melibiose and raffinose). The activity of α - galactosidase in the mid-gut of the early third larval instars of *G. intestinalis* showed high activity in the acidic range (pH 3.6- 6) with highest pH at 3.6. α- galactosidase was found in the mid-gut homogenates of *Rhenium inquisitor* (Chipoulata and Chararas, 1985), of Rhodinus prolixus (Riberio and Pereira, 1984; Ferreira et al., 1988) and of *Stomoxys calcitrans* (Deloach and Spates 1984), all with optimum acidic pH.

β - glucosidases catalyze the hydrolysis of terminal, non-reducing β- 1,4 - linked monosaccharides residues from the corresponding glycoside. Thus α-glucosidase acts on β-glucosides (e.g. cellobiose). The mid-gut of the early third larval instars of *G. intestinalis* showed high activity of β - glucosidase in the pH range (3.6 - 6) but with highest pH at 6. This pH value is more or less similar to those pH values (4.8 - 6.5) found in other insects e.g. *Phoracantha semipunctata* (Chararas and Chipoulet, 1982), *Rhynchosciara americana* (Ferreira and Terra, 1983), *Erinnyis ello* (Santos and Terra, 1985), *Thaumetopoea pityocampa* (Pratviel - Sosa et al., 1987), *Sitophilus oryzae* (Baker and Woo, 1992) and *Abracris flavolineata* (Marana et al., 1995).

β - galactosidase acts on β - galactosides (e.g. lactose). The β- galactosidase in the mid-gut of the early third larval instars of *G. intestinalis* showed high activity in the acidic pH range (3.6 - 6) with highest pH at 3.6. This pH value is near to those pH values (4.5 - 6.5) recorded in different insects, e.g. *Erinnyis ello* (Santos and Terra,

1985), *Thaumetopoea piscatorial* (Pratviel - Sosa et al., 1987), *Locusta migratoria* (Morgan, 1975), *Abracris flavolineata*, *Tenebrio molitor* (Terra et al., 1996).

In fact, all glycosidases, including lysozymes typically found in cell lysosomes. The highest acidic pH of all activities is also in accordance with its possible lysosomal origin (Aronson, 1972; Imoto et al., 1972).

Only α- amylase has been found in insects to act on long α- 1,4 - glucan chains such as starch or glycogen. In most insects, pH values of amylase are acidic; pH optima generally correspond to the pH prevailing in mid-guts from which the amylases were purified (Terra et al., 1996).

Activation of insect amylases by chloride and other anions, and displacements of the pH optima in the presence of these ions were first described in Hemiptera (Hori, 1972).

Rhynchoscriara americana mid-gut amylase binds chloride; cause a shift in the optimum pH from 6.8 to 8. Activation also occurs with anions other than chloride, such as bromide and nitrate, and it seems to be dependent on the ionic size (Terra et al., 1977).

The activity of α-amylase in the mid-gut of the early third larval instars of *G. intestinalis* was high at pH 6 but it also has another maximum peak at pH 3.6, which differ from results of Tatchell (1958) who found that amylase of mid-gut showed high activity in alkaline pH range but with optimum activity at pH 6 due to the presence of chloride ions.

The pH value of amylase in the present study is more or less similar to those pH values (4.3 - 6) found in other insects e.g. *Callosobruchus chinensis* (Podoler and Applebaum, 1971) *Locusta migratoria* (Droste and Zebe, 1974), *Tenebrio molitor* (Buonocore et al., 1976), *Sitophilus oryzae* (Baker and Woo, 1985; Baker, 1987) and *Scaptotrigona bipunctata* (Schumaker et al., 1993). Terra et al., (1979) stated that in *Rhynchosciara*, hydrolysis of starch was carried out firstly by α-amylase giving maltose molecules and perhaps small oligomaltodextrins, which will then be hydrolyzed by α-glucosidase. Also β-glucosidase hydrolyzes cellobiose molecules and probably small oligocellodextrins. This sequence may also act for the hydrolysis of carbohydrates in the mid-gut of the early third larval instars of *G. intestinalis*.

Banminth is a broad spectrum antihelminthes for control of immature and adult gastrointestinal roundworms in cattles, buffaloes, sheep, goats, horses, mules, donkies, pigs and camels. In the present study this drug showed that it decreases the activity of β- galactosidase, α- galactosidase and slightly decreases the activity of β- glucosi-

dase. It increases slightly the activity of α- glucosidase.

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