QUANTITATIVE DETERMINATION OF ACID AND ALKALINE PHOSPHATASE IN DIFFERENT PARTS OF THE ALIMENTARY CANAL OF DESERT LOCUST, SCHISTOCERCA GREGARIA (FORSKAL)*

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Biochemical estimation of phosphomonoesterases was done in different parts of the alimimentary canal. Quantitative determination of phosphatases showed strong acid phosphatase activity in Malpighian tubules, posterior-midgut, fore-hindgut, caecum and anterior-midgut in decreasing order. Moderate activity was noticed in hind-hindgut, mid-hindgut, salivary glands and hind-foregut. Low activity was found in fore-foregut and mid-foregut.

Strong alkaline phosphatase activity was found in caecum, anterior-midgut and salivary glands. Moderate activity was found in hind-foregut and fore-foregut, while low in posterior midgut, mid-foregut, fore-hindgut, hind-hindgut, mid-hindgut and Malpighian tubules.

Phosphomonoesterases, have been determined biochemically and localized histochemically in higher animals and plants.[1] Most of the workers have histochemically localized these enzymes in the alimentary canal and other tissues. Phosphatases have been studied histochemically in normal organs and tissues,[2] the salivary glands of *Drosophila melanogaster*,[3] insect tissue,[4] the gut of blow-fly larva,[5,6] *Musca domestica* (L.),[7] salivary glands of the large milkweed bug,[8] and the stable fly.[9] Glycerophosphate as substrate, strong acid phosphatase activity has been reported in the midgut of silkworm.[10] Acid phosphatase activity has been studied in the digestive tract of milkweed bug, *Oncopeltus, fasciatus* (Dallas).[11] High concentration of alkaline phosphatase has been found in the intestine of silkworm, *Bombyx mori* (L.) by using the method of References 13 and 14.

While [11,12,15-22] phosphomonoesterases have been studied in the homogenate of the whole animal and specifically in holometabolous insect the present study deals with the biochemical characterzation of phosphatases, present in the alimentary canal only, of the desert locust, *Schistocerca gregaria* (Forskal), a hemimetabolous insect. This was done to find a correlation between the digestive physiology of the insect and the enzymes so that the functions of the phosphatases may be clearly traced out.

Material and Methods

Different methods have been used by different authors for the determination of phosphatases.[13,23-29] During the present investigation the methods of Bessey *et. al*[25] and Andersch and Szczypinski[27] as modified by Ashrafi[30] and Ashrafi and Fisk[31] have been employed with some modifications.

1. Reagents

(a) *Substrate.*—0.0143M stock solution of disodium *p*-nitrophenyl phosphate was prepared by dissolving 100 milligrams of sigma 104-R substrate (trade name of *p*-nitrophenyl phosphate) in 25 ml twice distilled demineralized cold water,[32] and was stored in freezing chamber at-15°C. This colorless solution represented a molarity of 0.00143 M (final) when used during experiment.

(b) *Enzyme Source.*—Adult locust (28-day old), fed on 6% glucose solution, 1 day perior to the experiment, were selected for enzyme study. Locust was dissected in cold water and the alimentary canal was transferred to a test tube containing 5 ml chilled twice distilled demineralized water. It was grounded for exactly 3 minutes in "Teflon Pyres" tissue grinder. The homogenate was filtered through a 2 mm. thick glass fibre layer in a Gooch crucible under moderate suction and the filtrate was collected in the microfilter tube placed in an ice tray. The filtrate was diluted to 10 ml by adding 5 ml of cold twice distilled demineralizer water. This represented 1/10th part of the alimentary canal per ml.

(c) *Colorimetric Standard.*—A 0.01 M stock solution of *p*-nitrophenol was prepared by dissoving 0.1391 g of *p*-nitrophenol (spectrophotometric grade) in 100.0 ml. of cold twice distilled demineralized water. This was diluted in the ratio of 1:200, to prepare working standard solution of 0.00005 M, yellow in color.

(d) *Acid Buffer Solution.*—500 ml of 0.09m citric acid solution, 180 ml of 1 N NaOH solution and 120 ml of 0.1 N HCl were mixed to prepare the

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citric acid–NaCl buffer [31] and pH was adjusted to 4.4 by adding NaOH or 0.1 N HCl as required. Few drops of chloroform were added as preservative.

(e) *Alkaline Buffer Solution.*—(Trismaleate buffer, pH 7.4, 0.2 M.) (Tris hydroxymethyl)aminomethane maleate buffer was prepared according to Reference 33 with certain modifications. 50 ml of trisacid maleate solutions were mixed with 0.2 M NaOH according the requirement for each pH and then diluted to 200 ml. The pH of each buffer solution was adjusted by Beckman Zeromatic pH metre.

2. Enzyme Assay Procedure

(a) *Acid Phosphatase.*—2 ml of twice distilled demineralized water, 2 ml of citric acid–NaCl buffer, 0.5 ml of substrate (0.0011 M final) were taken in 5 test tubes, placed in crushed ice. After 5 minutes, 0.5 ml of fresh homogenate was added and each test tube was shaken thoroughly. One ml aliquot of the reaction mixture from each test tube was transferred to another test tube containing 10 ml of 0.02 N NaOH, for zero time sample. Optical densities of zero time samples were recorded at 400 mμ. The test tubes containing the rest of the mixture were transferred to a water bath at 40°C for incubation for 35 minutes. After uniform incubation 1 ml aliquot from each test tube was taken and immediately transferred to another set of test tubes containing 10 ml of 0.02 N NaOH Optical densities were recorded at 400 mμ to get final readings. Zero time readings were substracted from final readings to obtain correct optical density, for acid phosphatase activity. These readings were converted into micromoles of *p*-nitrophenol liberated per litre per 35-minute incubation period at 40°C, with the help of standard curve. Total number of micromoles were calculated and were divided by the weight to set μmoles/mg fresh weight. The study of boiled homogenate blank and incubated water blank, indicated that this was not necessary every time. Each experiment was repeated 5 times.

(b) *Alkaline Phosphatase.*—2 ml of trismaleate alkaline buffer solution (pH 7.4 and 0.2 M), 2 ml of twice distilled demineralized cold water, and 0.5 ml of substrate (0.0006 M) were added to 5 test tubes placed in ice. After 5 minutes 0.5 ml of fresh homogenate was added and the tubes were shaken thoroughly. The reaction mixture was treated according to the procedure similar to acid phosphatase. Optimum factors for alkaline phosphatase were used during the assay 34 and the number of μmoles liberated per mg tissue weight were calculated to give comparative and quantitative results.

(c) Whole alimentary tract was divided into, fore-foregut, mid-foregut, hind-foregut, caecum, anterior midgut, posterior-midgut, Malpighian tubules, fore-hindgut, mid hindgut and hind-hindgut. Activity was divided into three categories i.e. (a) high activity 80 or above 80 μmoles (b) moderate activity 40 to 79 μ/moles and (c) low below 40 μmoles.

3. Standard Curves

(a) *Percentage Transmission Curves.*—To find out the suitable wavelength 9,18 and 45 μ moles solutions were used. The wavelength was changed from 350 to 480 mμ. The curves in Fig. 1 show 400 mμ as the best wavelength.

(b) *Concentration – Absorbance Curve.*—Different concentrations of *p*-nitrophenol working standard solution were used to prepare a standard curve for measuring the activity of enzyme as shown in Fig. 2.

Results

Experiments were performed according to the procedures mentioned and the data are given in Tables 1 and 2 for facid and alkaline phosphatases respectively. It was found that both the enzymes are present throughout the alimentary canal, Fig. 3.

Discussion

The presence of highest activity of acid phosphatase in Malpighian tubules supports the finding of Drilhon and Busnel [10] Belden [7] and Ashrafi and Fisk.[9] So the enzyme may be assigned the role of resorption of metabolites. Moderate acid phosphatase activity in salivary glands confirms the

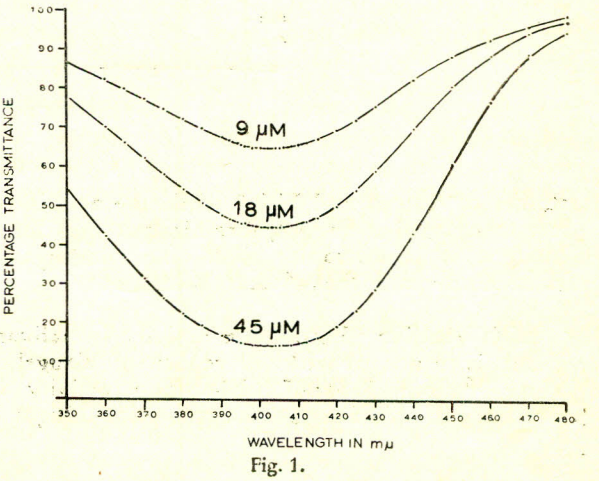

Fig. 1.

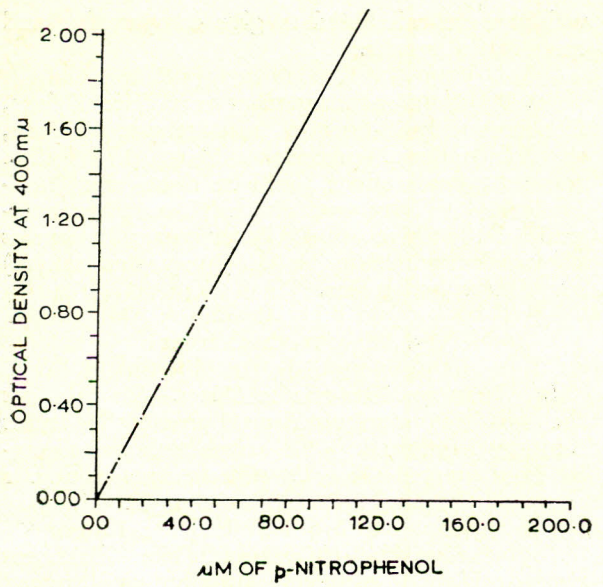

Fig. 2.

TABLE 1.—QUANTITATIVE DETERMINATION OF ACID PHOSPHATASE IN DIFFERENT PARTS OF THE ALIMENTARY CANAL.

Alimentary canal parts	Activity μ mole of *p*-nitrophenol		
	Strong	Moderate	Low
Salivary glands ..	—	78	—
Fore-foregut ..	—	—	38
Mid-foregut ..	—	—	35
Hind-foregut ..	—	70	—
Caecum ..	92	—	—
Anterior-midgut ..	90	—	—
Posterior-midgut ..	110	—	—
Malpighian tubules ..	125	—	—
Fore-hindgut ..	100	—	—
Mid-hindgut ..	80	—	—
Hind-hindgut ..	85	—	—

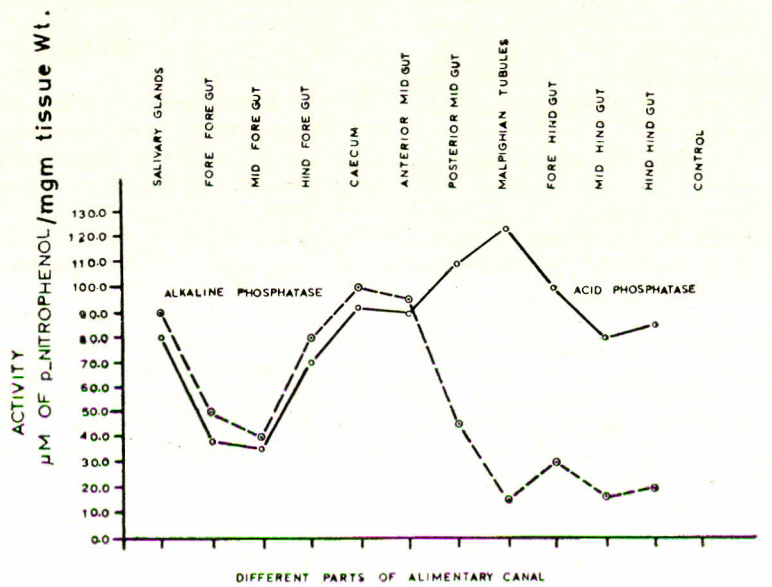

Fig. 3.

studies of Ashrafi and Fisk,[9] while the activity in fore-foregut, hind-foregut, fore-hindgut and hind-hindgut is comparable with the findings of Waterhouse and Stay,[6] and Belden[7].

High alkaline phosphatase activity in salivary glands is in accord with the reports of Krugelis,[3] Yao,[35,36] and Belden.[7] Moderate activity in fore-foregut, mid-foregut and posterior-midgut is in accord with the findings of Ashrafi and Fisk.[9] High contents of alkaline phosphatase in salivary glands, hind-foregut, caecum and anterior-midgut may be related with transformation of organic materials and cellular metabolism during the process of digestion, whereas high acid phosphatase activity in Malpighian tubules and midgut may be associated with absorption and secretion of waste materials.

TABLE 2.—QUANTITATIVE DETERMINATION OF ALKALINE PHOSPHATASE IN DIFFERENT PARTS OF THE ALIMENTARY CANAL.

Alimentary canal parts	Activity μmoles of *p*-nitrophenol		
	Strong	Moderate	Low
Salivary glands ..	90	—	—
Fore-foregut ..	—	50	—
Mid-foregut ..	—	40	—
Hind-foregut ..	80	—	—
Caecum ..	100	—	—
Anterior-midgut ..	95	—	—
Posterior-midgut ..	—	45	—
Malpighian tubules ..	—	—	15
Fore-hindgut ..	—	—	30
Mid-hindgut ..	—	—	16
Hind-hindgut ..	—	—	20

Similar activity pattern of both enzymes upto anterior-midgut indicates that both the enzymes play an important role in the digestion of food, where high energy is required to complete the metabolic processes and conversion of organic materials for tissue formation and growth. After the completion of this process the alkaline phosphatase activity declines sharply whereas acid phosphatase activity increases in posterior-mid-gut and specially in Malpighian tubules. This may be related with high rate of absorption and transfer of solute. As this process reaches completion acid phosphatase activity also starts declining.

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