The Light and Electron Microscopic Localizations of Acid Phosphatase Activity in the Mid-Gut Gland of the Juvenile Blue Crab, Portunus trituberculatus

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Histochemical and ultracytochemical examinations on the localizations of acid phosphatase (ACPase) activity were carried out in the mid-gut gland of the blue crab, Portunus trituberculatus, at the first adult-form stage. ACPase activity was detected in both lysosomal and extralysosomal sites. The extralysosomal sites were located in the plasma membranes of the apical, lateral and basal regions of R, F and B cells and, occasionally, in the cytoplasmic matrix and mitochondrial membranes of R and F cells. ACPase activity was not detected in the E cells and the secretory vacuoles of the F cells. These findings gave insight into the participation of each cell type of the mid-gut gland in the digestion and absorption of food.

Key words: blue crab; mid-gut gland; acid phosphatase; enzyme histochemistry and ultracytochemistry

The seed production of the blue crab, Portunus trituberculatus, has recently been conducted at many hatcheries in Japan and making rapid progress in the breeding of crabs. However, the results of the seed production have indeed been fluctuating due to unknown causes. Mass mortalities of the blue crab larvae have frequently occurred at many hatcheries without quick or marked changes within the environmental factors of the rearing tanks. It is of importance to elucidate the causes of the serious losses in the seed production of the blue crab, based on physiological studies on the blue crab larvae and juveniles.

Little is known about the digestive physiology of the blue crab at larval and juvenile stages, while extensive knowledge of the decapod digestive physiology has been accumulated 1). As part of a study on the digestive system of the hatchery-reared blue crab larvae and juveniles, we studied the development and ultrastructure of the mid-gut gland and light and electron microscopic localizations of ACPase activity. In this study, we observed rapid growth of the mid-gut gland in size and the existence of the four basic cell types 1): E (embryonic or undifferentiated), R (resorptive or absorptive), F (fibrillar) and B (secretory) cells which have been observed in the decapod mid-gut gland (our unpublished results). This paper describes the results of histochemical and ultrastructural examinations on the localization of ACPase activity.

Materials and Methods

Animal

The blue crab at the first adult-form stage produced at Nagasahi City Aquaculture Center (NCAC) were used.

Histochemistry

A freeze-substitution method was used for the
preparation of tissue sections. The mid-gut glands were excised from the fresh specimens, placed on aluminium foil and frozen in isopen-tane and liquid nitrogen at approximately $-160^\circ$C. The frozen tissues were then dehydrated in ethanol at about $-60^\circ$C for 4 days and embedded in paraffin. Sections of 6 $\mu$m in thickness were cut and mounted on glass slides excluding the use of water. The enzyme activity was detected by the azo dye method of Barka and Anderson$^3$ using naphthol AS-BI phosphate as the substrate. The sections were incubated in the working solution at a pH of 5.0 and at a temperature of $37^\circ$C for 30 min. In the control experiments, the sections were incubated in the substrate-free medium.

Electron microscopy

The mid-gut glands, excised from fresh specimens, were dissected and fixed in cold 2% glutaraldehyde in a buffered saline solution (1 : 9 mixture of 0.1 M cacodylate buffer, pH 7.3, and a saline solution for marine crabs containing 2.5% NaCl, 0.26% KCl, 0.23% CaCl$_2$ and 0.16% MgCl$_2$) for 1 hour. After rinsing in the cacodylate buffer containing 20% sucrose, the fixed tissues were incubated in the Gomori’s medium$^3$ at a pH of 5.0 for 30 min at room temperature. Afterwards, the tissues were dehydrated in ethanol, embedded in Spurr’s resin and cut on a Porter-Blum MT-1 ultramicrotome using glass knives. Ultrathin sections with or without staining with lead citrate$^3$ were examined using a JEOL JEM 100S electron microscope at 80 kV. In the control experiments, the fixed tissues were incubated in the substrate-free medium.

Results

Light microscopy showed that ACPase activity was observed in the striated border of the R, F and B cells, showing the weaker activity in

Fig. 1. Light micrograph of the blue crab mid-gut gland stained for ACPase using the azo dye method. Dark stain in the striated border, B cell vacuoles (B), tubule content (Tc) and lysosomes (arrow heads) shows positive reactions. Bl: basal lamina, $\times$580.
the B cells. The positive reaction was also found in the lysosomes and cytoplasm of the R and F cells, B cell vacuoles and the tubule lumen (Fig. 1). The extent of the reaction in the B cell vacuoles and tubule lumen varied considerably from crab to crab. Some crabs showed intense reactions whereas others demonstrated no positive reactions. We observed no positive reactions in the E cells.

Electron microscopy showed that ACPase activity was detected not only in the secondary lysosomes of R and F cells (Figs. 2 and 3) but also in the plasma membranes in the microvilli (Figs. 2 and 3), lateral surface (Fig. 3) and basal tubular system (Fig. 4) of the R, F and B cells. ACPase activity was also observed in the Golgi lamellae of the F and B cells (Figs. 5 and 6) and the peritrophic membranes (Fig. 7). The activity, in addition, was occasionally observed in the cytoplasmic matrix and mitochondrial membranes of the F and R cells (Figs. 3 and 8).

Secretory vacuoles of the F cells showed no positive reactions (Figs. 3 and 5).

Discussion

The localization of ACPase activity in the mid-gut gland has been studied at the light microscopic level in the crayfish, Procambarus clarkii, mud crab, Scylla serrata, and European lobster, Homarus gammarus. Miyawaki et al. observed ACPase activity in the cytoplasm of Fe cells (F cells). Monin and Rangneker observed ACPase activity in the striated border and apical cytoplasm of the R cells, B cells ("around vacuoles") and the cells lining the main duct. Barker and Gibson showed additional localizations in the supranuclear vacuoles of the F cells, B cell vacuoles and tubule lumen. In the present study, we obtained satisfactory results in the preservation of enzyme activity and cell structure at both the light and

Figs. 2-8. Electron micrographs showing ACPase activity in the mid-gut gland cells. × 30,000.
Fig. 2. Apical region of the R cell. Dense and prominent reaction products are seen in the microvilli (Mv) and secondary lysosomes (arrow heads). L: tubule lumen, M: mitochondrion, Pm: peritrophic membrane.
electron microscopic levels and showed ACPase activity in the lysosomal and extralysosomal sites mentioned above. In addition, we demonstrated no positive reaction in the secretory vacuoles of the F cells.

The participation of lysosomal ACPase in the intracellular digestion has been generally accepted. The situation appears to be the case in the mid-gut gland of the blue crab juveniles, since most of the secondary lysosomes showing the positive reaction in the R and F cells were autophagosomes. The primary function of the B cells of the blue crab is inferred to be the intracellular digestion of nutritive substances. There were two kinds of vacuoles: numerous small endocytotic vacuoles in the apical region which engulf the tubule content and an enormous central vacuole which is formed by the coalescence of the endocytotic vacuoles. ACPase activity in the B cell vacuoles may contain to
Fig. 4. Basal region of the mid-gut gland cells. Arrow heads indicate the presence of the reaction products in the basal tubular system.

Fig. 5. Supranuclear region of the F cell. Arrow heads indicate the presence of the reaction products in the Golgi lamellae. No positive reaction is seen in the secretory vacuoles.

Fig. 6. Basal region of the B cell. Arrow heads indicate positive reaction in the Golgi lamellae. Cv: central vacuole.
Fig. 7. Apical region of the B cell. The reaction products are seen in the microvilli, peritrophic membrane and some endocytotic vacuoles (V).

Fig. 8. Apical region of the R cell. The reaction products are seen in the mitochondrial membranes and cytoplasmic matrix.
some degree the tubule content. On the other hand, electron microscopy showed that the Golgi bodies of the B cells provided lysosome-like vesicles to the endocytic and central vacuoles. Thus, ACPase activity in the B cell vacuoles is considered to possess dual origin. The production of ACPase and its participation in the intracellular digestion has been demonstrated at the electron microscopic level in the mid-gut B cells of Centropages typicus\(^{10}\) (Copepoda, Calanoida).

The F cells are believed to be the digestive enzyme secreting cells\(^{11}\) although their secretory mechanisms have not yet been sufficiently elucidated. We also considered that the F cells of the blue crab larvae and juveniles are the major site of the production of digestive enzymes on the basis of ultrastructural observation (our unpublished results). In the present study, we demonstrated no positive reaction in the secretory vacuoles. This result suggests that, like in the pancreatic acinar cells of vertebrates, the secretory vacuoles are kept in the F cells as zymogen granules and digestive enzymes are activated after the discharge from the F cells.

We detected ACPase activity in the plasma membranes of the mid-gut gland cells in the present study. Such locations of ACPase in extralysosomal sites have been demonstrated in normal mammalian tissues\(^{8,11-13}\), although the physiological significance of the extralysosomal ACPase has not been sufficiently clarified\(^9\). Of interest is the difference in the substrate preference of ACPase between the mid-gut gland cells and the mammalian tissue cells when using $\beta$-glycerophosphate (used in the present study) as the substrate. ACPase was seen in the mammalian tissue cells and it was restricted to the lysosomal ACPase\(^9\).

The presence of the peritrophic membranes in the mid-gut gland was first reported in the shore crab, Carcinus maenas\(^{10}\). We showed the presence of the peritrophic membranes and the localization of ACPase activity in these membranes in the mid-gut gland of the blue crab. However, ACPase in this location may not be the membrane-bound enzyme, because the positive reactions were not consistently observed (Fig. 2). The origin and function of ACPase in this location are the subjects of future study.

We occasionally observed many sizeable autophagosomes in the R and F cells, whose cytoplasmic matrix and mitochondrial membranes showed positive reactions. Mitochondrial membrane ACPase activity was observed only in the cells whose cytoplasmic matrix showed positive reaction. In some cells, cytoplasmic matrix around the autophagosomes was intensely stained (Fig. 3). This suggested the leakage of the enzyme from the autophagosomes or associated lysosomes. We, therefore, consider such locations of ACPase activity in the R and F cells to be a degenerative change.

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References


* : in Japanese
ガザミ稚カニの中腸腺における Acid phosphatase 活性の
光顕的及び電顕的検出

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要 約

ガザミの第 1 齢稚カニの中腸腺における Acid phosphatase 活性の局在部位を光顕及び電顕レベルで検討した。
Acid phosphatase 活性はリソソーム及びこれに関連する構造物だけでなく、中腸腺の機能的細胞である R, F, B 細胞の表面、側面及び基底面の変質及や R, F 細胞の細胞基質及びミトコンドリア膜に認められた。このような非リソソーム性 Acid phos-
phatase 活性は哺乳類の組織細胞に存在することが知られているが、中腸腺細胞の酵素
基質特異性において哺乳類のそれとは性質を異にしていた。Acid phosphatase 活性
は F 細胞の分泌液胞及び未分化細胞（B 細胞）には認められなかった。
本研究で示された Acid phosphatase 活性の局在部位は、中腸腺細胞の構造と機能を
明らかにするうえで有益な示唆を与えた。