Ultrastructural and Cytochemical Characteristics of Phagocytes in Kuruma Prawn

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Five types of phagocytes were observed in kuruma prawn Penaeus japonicus. Two of them were fixed phagocytes and three were hemocytes. Fixed phagocytes were detected in the heart and lymphoid organ. Phagocytes in the heart were observed on basal lamina covering sarcolemma of heart muscles and the cells had many lysosomal granules (0.1 μm in diameter). Phagocytes in the lymphoid organ was characterized by numerous interdigitating cell processes and few cytoplasmic granules. The hemocytes were classified into three types, hyaline cell (HC), small granular cell (SGC) and large granular cell (LGC), according to the presence of cytoplasmic deposit and size of granules. The HC had cytoplasmic deposits and a few small granules (0.2 μm in diameter). The SGC had small granules (0.2 μm in diameter) but the LGC contained large granules (1 μm in diameter). Lysosomal enzymes, such as acid phosphatase, β-glucuronidase and α-naphthyl butylate esterase, were detected in all five phagocytic cell types. Esterase activity was higher in the LGC than in any other phagocytes. Peroxidase activity was also detected in all phagocytes, although it was weak. Prophenoloxidase activity was found only in the SGC and LGC. These results indicate that the fixed phagocytes are different from the hemocytes both in their ultrastructural and cytochemical characteristics.

Key words: kuruma prawn, hemocyte, fixed phagocyte, Penaeus japonicus, hyaline cell, small granular cell, large granular cell

Phagocytosis is the most common defense mechanism among all animals including invertebrates. In decapod crustaceans, two types of phagocytes have been identified, one is the hemocyte and another is the fixed phagocyte (Johnson, 1987). The hemocytes have been generally classified in three categories, the hyaline cell, the semi or small granular cell, and the large granular or granular cell. Phagocytic hemocytes have been identified in several species (Smith and Süderhäll, 1983; Süderhäll et al., 1986; Hose and Martin, 1989; Hose et al. 1990). Kuruma prawn Penaeus japonicus also has three types of hemocytes and all types show phagocytic activity, which is especially strong in the granular cell (Kondo et al., 1992). Foreign substances such as colloidal carbon or latex beads injected into abdominal muscle have been found to be phagocytized by fixed phagocytes found in the lymphoid organ*¹ or the heart*². The purpose of this paper is to describe the ultrastructural and the cytochemical characteristics of hemocytes and fixed phagocytes in the kuruma prawn.

Materials and Methods

Animals

Kuruma prawn averaged 20 g in body weight were collected from a culture farm in Yamaguchi Prefecture and kept in aquaria containing aerated flowing seawater at 25°C.

Hemocyte smear

Hemolymph (0.5 ml) was withdrawn from the ventral sinus into a 10 ml syringe containing 9.5 ml of fixative (2% glutaraldehyde and 2% paraformaldehyde (GA/PFA) in 0.2 M cacodylate buffer (pH 7.45) containing 10% sucrose). The cells were fixed for 15 min at 5°C and washed three times in cacodylate buffer containing 10% sucrose. The smears were prepared on gelatin coated slides using an Auto Smear CF-12C (Sakura). The films were air dried, washed in distilled water and used for cytochemistry.

Frozen sections

The lymphoid organ and the heart were fixed in GA/PFA for 30 min at 5°C, washed three times, embedded in OCT compound (Miles) and frozen at -80°C. Sections were cut on a cryostat, attached to gelatin coated slides and fixed in GA/PFA for 5 min at 5°C. The slides were washed in distilled water and used for cytochemistry.

Electron microscopy

The hemocytes collected from the hemolymph were fixed for 1 h at room temperature. The cells were pelleted by centrifugation and resuspended in heat-treated (60°C, 10 min) calf serum. The suspension was centrifuged, GA/PFA was laid on the cell pellet and incubated for 1 h at room temperature to make a cell clot. The clot was washed in buffer and postfixed in 2% osmium tetroxide for 1 h at 5°C. The lymphoid organ and heart were fixed in GA/PFA for 1.5 h at room temperature, washed in buffer and post fixed in 2% osmium tetroxide for 1 h at 5°C. After post fixation, each sample was washed in buffer, dehydrated in ethanol and embedded in epon. Ultrathin sections were cut on an ultramicrotome (LKB Ultrotome) and stained with uranyl acetate and lead citrate, and examined with JEM-200CX electron microscope.

Cytochemistry

The azo coupling methods were used to determine the presence of acid phosphatase, β-glucuronidase and α-naphthyl butylate esterase. For acid phosphatase, samples were incubated in a mixture of 10 mg naphthol AS-BI phosphate and 8 mg fast garnet GBC in 50 ml 0.1 m acetate buffer (pH 5.2) for 45 min at 25°C. The incubation medium for β-glucuronidase consisted of 2.5 ml 0.028% naphthol AS-BI glucronide in 0.2 M acetate buffer (pH 5.2), 4.5 ml distilled water and 10 mg fast red ITR. Samples were incubated in this medium for 3 h at 25°C. α-naphthyl butylate esterase was demonstrated by the method of Li et al. (1973) with a slight modification of incubation (1 h at 25°C). The presence of prophenoloxidase was demonstrated using the method of Hose et al. (1987). For peroxidase activity, samples were incubated in a medium described by Graham and Karnovsky (1966).

Results

The hyaline cell (Fig. 1) was generally ovoid or spindle in shape, smaller (11.1 × 5.9 μm) than other types of hemocytes and with a high nucleocytoplasmic ratio. A small number of electron-dense granules (0.2 μm in diameter) with a striated substructure and numerous electron-dense deposits were observed in the hyaline cells. The cytoplasm also contained a few to some mitochondria, rough endoplasmic reticulum and free ribosomes.

The small granular cell (Fig. 2) also had variable number of striated electron-dense small granules (0.2 μm in diameter), but cytoplasmic deposits were not detected. The cell (14.6 × 8.7 μm) was ovoid or fusiform in shape and had low nucleocytoplasmic ratio. A varied number of mitochondria and other organelles were observed in this cell type. A well developed Golgi complex was generally present in the cytoplasm. Slightly electron-lucent granules, which seemed to be developmental stages, were often observed near the Golgi complex.

The large granular cell (Fig. 3) was largest cell observed. These cells were ovoid or fusiform in shape, had a low nucleocytoplasmic ratio and a cell size of 19.1 × 9.8 μm. The granules found in the cell were large (1 μm in diameter), electron-dense, with a striated substructure, as seen in the granules of the other types of hemocytes. Cytoplasmic organelles were generally poor in the cells which contained many granules. Electron-dense deposits observed in the hyaline cells were not detected in the large granular cells. Transitional stages of developing granules were also found in immature cells.

The fixed phagocyte in the lymphoid organ (Fig. 4) was characterized by numerous interdigitating cell processes and a small number of cytoplasmic granules. Mitochondria and other organelles were observed in the lymphoid organ phagocytes. Small vesicles were consistently present in these cells.

The fixed phagocyte in the heart (Fig. 5) was observed on basal lamina covering the sarcolemma of heart.
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This cell had many lysosomal granules (0.1 μm in diameter). The cell also contained mitochondria, rough endoplasmic reticulum, free ribosome and Golgi complex.

Positive staining for lysosomal enzymes was detected in all of three types of hemocytes and the fixed phagocytes, although the intensity of the staining reaction was variable among the different cell types (Table 1). The localization pattern of acid phosphatase (AcP) and β-glucuronidase (β-Glu) appeared to be similar in all of the cell types examined with the exception of the number of positive granules. The hyaline cell had only a few positive granules while the small granular cell and the large granular cell had a moderate number of stained granules. α-naphthyl butylate esterase (α-NBE) activity was strong in the large granular cell and found to be localized within the large granular. On the other hand, the hyaline and small granular cells had small granules.

Fig. 1. A transmission electron micrograph of hyaline cell in the hemolymph of Penaeus japonicus. The nucleocytoplasmic ratio is high. Arrows indicate the cytoplasmic deposits. Bar = 1 μm.

Fig. 2. A transmission electron micrograph of small granular cell in the hemolymph of Penaeus japonicus. The cytoplasm is filled with small dense granules. Bar = 1 μm.
positive for α-NBE.

In the fixed phagocytes of the lymphoid organ and the heart, the cytochemical staining reaction of lysosomal enzymes was observed in the small granules. AcP staining of the phagocytes in the heart was more intense than in the lymphoid organ.

A small number of prophenoloxidase positive granules were found in the small granular cells and large...
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The hyaloplasm of those cells also showed positive reaction and the reactivity in the large granular cell was much stronger than in the small granular cell. Positive reaction for prophenoloxidase was not detected in either type of the fixed phagocytes. Peroxidase activity was encountered in the small granules of all types of phagocytic cells examined, although the staining was rather faint (Table 1).

### Discussion

It has been considered that decapod crustacean has two types of phagocytes: hemocytes and fixed phagocytes. However, little has been reported about the ultrastructural and cytochemical characteristics of the phagocytes, and the origin of the fixed phagocyte has not been determined.
In decapods, hemocytes have been generally classified into three categories as mentioned before: hyaline type, small or semi granular type, and granular or large granular type. We also detected ultrastructurally three populations of hemocytes in the kuruma prawn: the hyaline cell (HC), the small granular cell (SGC) and the large granular cell (LGC). The various features of kuruma prawn hemocytes were, in some aspects, similar to those reported in other decapods. Hyaline cells of the kuruma prawn had unique electron-dense cytoplasmic deposits. The presence of the deposit only in hyaline cell of some species has been previously reported (Hose et al., 1989, 1990). However, ultrastructural study of the kuruma prawn hemocytes by Tsing et al. (1989) failed to show a cell containing this structure. The hyaline cells of decapods easily lyse and initiate coagulation of hemolymph (Omori et al., 1989). It is possible that the hyaline cells may have lysed in Tsing’s preparation, because they collected hemolymph with a pasteur pipette and then fixed the cells for electron microscopy. The chemical composition and biological function of the hyaline cell deposit remains to be analyzed.

We found that certain populations of granules present in all types of hemocytes had a specific, internal striated substructure. A similar structure has been noticed in other decapod species. Martin and Graves (1985) described that the hyaline cell of two species of shrimp contained a small number of granules with a striated substructure, but the structure was not detected in the granules of the small granule cell and the large granule cell. Tsing et al. (1989) observed the electron dense and electron lucent bands in the granules of large granule hemocytes in kuruma prawn, and the occurrence of the similar granules in granular cell were also reported in crayfish by Lanz et al. (1993). These differences in results may be due to the different physiological status of the animals at the time of examination.

We have observed by ultrastructure study immature cells of all hemocyte types, both in the hemolymph and in the hemopoietic tissues. Hyaline cells already contained the electron-dense deposits (unpublished observation). These observations suggest that most hemocytes differentiating in the hemopoietic tissues of the kuruma prawn are released in the hemolymph as immature stages and further differentiation occurs in the hemolymph. Tsing et al. (1989) classified the hemocytes in hemolymph of kuruma prawn as the undifferentiated hemocyte (UH), the small granule hemocyte and the large granule hemocyte. It is highly probable that the UH reported by these investigators may be mixed populations including immature SGC and LGC.

Electron-dense deposits and striated granules were not detected in the fixed phagocytes observed in the lymphoid organ and the heart. This morphological difference strongly suggests that the origin of the fixed phagocyte is not the hemocyte and the two cell types belong to independent lineages. On the other hand, Johnson (1987) described that the fixed phagocytes that settled on the exterior surface of hepatic arterioles in Callinectes sapidus and Homarus americanus are apparently derived from circulating hyaline hemocytes. These types of fixed phagocytes have not been found in the kuruma prawn.

We successfully showed the presence of prophenoloxidase activity in the SGC and LGC. This enzyme activity is also strong in the granular cells of other decapods, but positive sites were different among animals examined. Hose et al. (1987) described that the enzyme activity was limited to the granules of the small granule hemocytes and the large granule hemocytes. They also found this activity in the small granule hemocyte and the large granule hemocyte of the sheep crab Loxorhynchus grandis and two lobsters Homarus americanus and Panulirus interruptus. However, the positive pattern was different between the cell types: the hyaloplasm of the large granule cells also contained the enzyme activity while the staining in small granule hemocytes was confined to the granules (Hose et al., 1990). Tsing et al. (1989) found this enzyme activity only in the large granule hemocytes of the kuruma prawn. The enzyme was diffused in the whole cytosole, which showed a granular and electron dense content. Lanz et al. (1993) also found this activity only in the granular cells of the crayfish Procambarus clarki. However, in other crayfish Astacus astacus (Smith and Söderhäll, 1983) and Pacifastacus leniusculus (Johansson and Söderhäll, 1985) the enzyme activity was found to reside in the semigranular and the granular cells, most likely within vesicles. It can not be ruled out the possibility that positive staining in the hyaloplasm is mainly due to a diffusion artifact which occurred during preparation. It is worthy to note that this enzyme activity was not detected in the fixed phagocytes of kuruma prawn in our study.

Peroxidase activity was detected in all three hemocyte types and the two types of fixed phagocytes. This enzyme activity was reported by Lanz et al. (1993) in the crayfish granular cell, although Hose et al. (1987)
failed to detect peroxidase activity in the hemocytes of the ridgeback prawn.

It is a rather natural result that cytochemical activities of the three lysosomal enzymes were detected in all phagocytes examined in this study. However, there are some discrepancies with our findings and the results of other investigators in the literature; i.e. cell types which showed positive staining, the number of positive granules, and so on. We suppose that these differences may be due to various factors, such as the method of sample collection used and the physiological condition of animals. Enzymatic activity of the lysosomal enzymes may relate to the current phagocytic activity of each type of hemocyte at the time of sampling (Kondo et al., 1992).

In addition to ultrastructural differences between the hemocytes and the fixed phagocytes, we found a difference in the localization of enzyme activity within the two cell types in this investigation. These findings strongly suggest that the fixed phagocytes found in the lymphoid organ and the heart have a different precursor cell than that for the circulating hemocytes.

References


