Morphology of hemocytes of *Portunus pelagicus*

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Abstract

The hemocytes of blue swimming crab (*Portunus pelagicus*) were characterized by using differential interference contrast microscopy (DIC) and transmission electron microscope (TEM), and classified as large granule hemocytes (lgh), small granule hemocytes (sgh) and hyaline hemocytes (hh). Large granule hemocytes (size 14.2 x 10.8 μm) contained numerous of type 1 electron-dense granules (G1) which scattered throughout the cytoplasm. Small granule hemocytes (size 12.0 x 8.3 μm) contained abundant of G1 that clumped to one side of the cells. Hyaline hemocytes (size 6.3 x 5.5 μm) were the main circulating blood cell type. They have few small electron-dense granules (G2) and small vesicles.

Keywords : *Portunus pelagicus*, morphology and hemocytes

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Introduction

Many investigation of hemocytes with electron microscope have been reported in various crustacean species (Bauchau,1981). Most of these reports, however, were in the hemocytes of shrimps. Crustacean hemocytes have been classified into three types: large granulocyte which is the largest cell with a small, eccentric, kidney-shaped nucleus and cytoplasm packed with membrane-bound granules; small granulocyte which is intermediate in size and has sparsely dispersed granules in the cytoplasm; hyaline granulocte, which is the smallest cell-type with a large central nucleus and few or no cytoplasmic granules (Hose *et al.*,1987; Barracco and Amirante, 1992).

Crustacean hemocytes were involved in coagulation, wound repair, phagocytosis and encapsulation of foreign particles as well as in the synthesis of lipids, proteins and carbohydrates (Sewell, 1955; Kerr,1968 ; Johnson and Davies, 1972) . Hemocytes have also been implicated in hemocyanin synthesis (Ghiretti-Magaldi *et al.*, 1973).

The blue-swimming crabs (*Portunus pelagicus*) is an important economic crustacean species whose normal habitat is along the coast line of the Gulf of Thailand. Identification and classification of crabs hemocytes were desirable to elucidate their immune function for comparison among these types of cells from different crustacean species.

The purpose of this report is to describe the morphology of hemocytes from *P. pelagicus* by DIC and TEM observations.

Materials and Methods

P. pelagicus were obtained from a local supplier (Angsila, Bangsaen, Chonburi, Thailand) at the intervals from March to July 2004. They were maintained in a flow-through water stable at ambient temperature.

Hemolymph was withdrawn from walking legs using a 26-gauge needle on a syringe containing anticoagulant solution (0.45 M NaCl, 0.1 M glucose, 30 mM sodium citrate, 26 mM citric acid, and 20 mM EDTA, pH 4.5) and a fixative solution (consisting of 1.5% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.8) (Bauchau,1981). The anticoagulant and fixative solution were mixed 1:1 (v/v) and cooled to 4 °C and the hemolymph was collected in a 9:1 proportion (anticoagulant-fixative: hemolymph). Cells in suspension were pelleted by centrifugation 10000 g for 5 min, 4 °C, then suspended in fixative for 2 hr at 4 °C, and finally washed two times in 0.1 M sodium cacodylate buffer, pH 7.8. The resultant pellet was used for cell counting and LM and TEM observations.

For LM, fixed hemocytes were examined and photographed with differential interference contrast microscope (DIC) using Olympus BX50 photomicroscope. Second, total cell counted (numbers of hemocytes per ml of hemolymph) were determined using a hemacytometer. Three counts were made from each sample. For TEM, the cells were processed for examination by transmission electron microscope in the following manner. The fixed hemocytes were pelleted by centrifugation 10000 g for 5 min, 4 °C, washed in 0.1 M sodium cacodylate (pH 7.8, contain 24% sucrose), post fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate for 1 hr at room temperature, and dehydrated with ethanol. The cells were infiltrated and embeded in Spurr' s low viscosity resin. Thin sections were cut on a Lieca ultracut R ultramicrotome. Thin sections (90 nm) were stained with uranyl acetate and lead citrate, and then examined under Phillip Tecnile 20 transmission electron microscope operating at 80 KV.

Results and discussion

The number of circulating hemocytes was about 350,000-400,000 cells/mm^3 of hemolymph. Morphologically, three main types of cells were observed in the hemocytes of *P. pelagicus* : large granular hemocytes, small granular hemocytes and hyaline hemocytes.

Large granular hemocytes (lgh) (Fig.1A, B; 2A, B)

Large granular hemocytes comprise approximately 25% of circulating hemocytes. This cell has a round or oval shape (size 14.2 x 10.8 μm). The nucleus was oval (size 7.6 x 4.1 μm) and was located toward one side of the cell. The nucleolus was very distinct and round in shape. The cytoplasm contained numerous round or oval electron-dense granules (type1, G1) (approximately1.8 μm in diameter). The granules were scattered throughout the cytoplasm. The cytoplasm also contained a few mitochondria and a Golgi complex and a small amount of RER.

Small granular hemocytes (sgh) (Fig.1A, B; 2C)

Small granular hemocytes comprised approximately 20% of the total circulating hemocytes. This cell is ovoid or fusiform size (size 12.0 x 8.3 μm) has a relatively small non-lobulated nucleus (size 6.2 x 3.9 μm). The nucleus contained a central heterochromatin mass and abundant nuclear envelope-associated heterochromatin. The cytoplasm contained fewer electron dense-granules (G1) than large granular hemocytes. These granules clumped to one side of the cells. The cytoplasm also contained mitochondria, but more RER, small vesicles and well developed Golgi complex.

Hyalin hemocytes (hc) (Fig.1A, B; 2D)

This group of cells comprised 55% of circulating hemocytes. This round or oval cell (size 6.3 x 5.5 μm) has an indented on bilobed nucleus (size 3.6 x 3.1 μm) which bored a thick peripheral band of heterochromatin. The cytoplasm contained only few small electron-dense granules (type 2, G2) approximately 0.11 μm

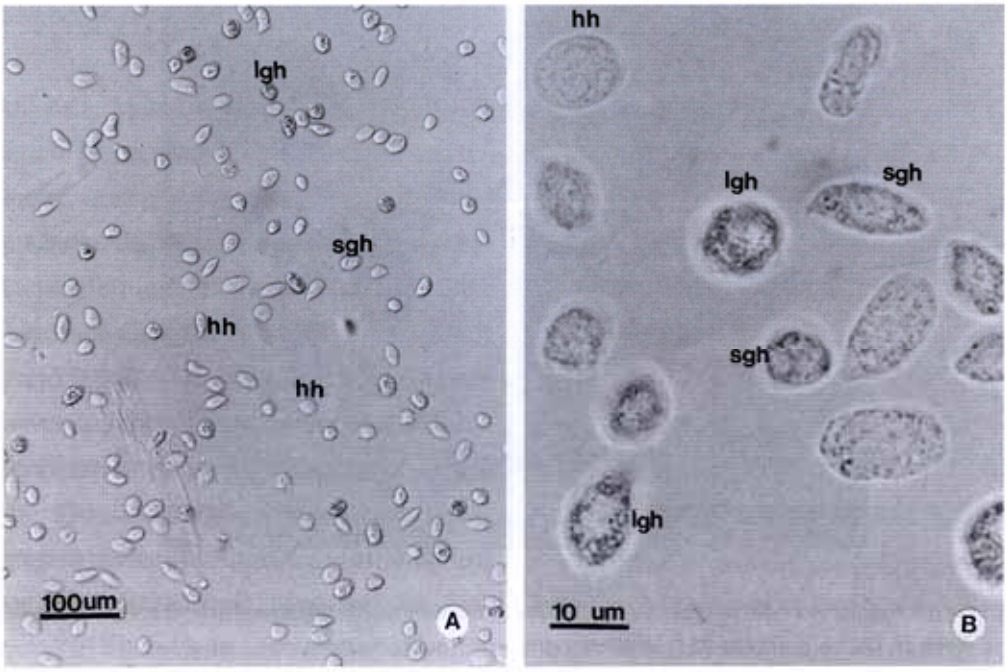

Fig.1 (A, B). DIC micrographs of hemocytes of *P. pelagicus:* large granular hemocytes (lgh), small granular hemocytes (sgh) and hyalin hemocytes (hc)

and small vesicles. The RER and several mitochondria were observed throughout the cytoplasm. The Golgi complex was poorly developed.

Our observations showed that there were three types of hemocytes in peripheral circulation of *P. pelagicus* as large granules hemocytes, small granules hemocytes and hyaline hemocytes. Our results were thus in accord with those of earlier reports on the hemocytes in ultrastructure *Callinectes sapidus* (Clare, 1994), penaeid *Penaeus paulensis* and two paiaemonids *Macrobranchium acanthurus* and *M. rosenbergii* (Tsing, 1989). The hemocytes of *P. pelagicus* closely resemble those of other crustacean studied by Clare (1994) who employed similar fixative.

The granulocytes make up 45% of hemocytes in *P. pelagicus*. They could be divided into two types: Large granule hemocytes and small granule hemocytes. The former contained more numerous G1 electron-dense granules than the latter. Since the granules in the two types of cells were essentially similar, the two cell types may actually be the same group of cells in different maturation stage. These granulocytes were responsible for the majority of the phagocytic activity as describe in other crustacean (Hose *et al.*,1987; Barracco and Amirante,1992).

Hyaline hemocytes represents the most abundant groups of hemocytes which contained few small G2 granules because of the distinct different between G1 and G2 granules, the cells should be a separate class of hemocyte. They also appear to correspond to the hyaline cells identified by Clare (1994) in *C. sapidus*. It has been suggested by Hose (1987), that the hyaline hemocytes were probably involved in the clotting of the hemolymph.

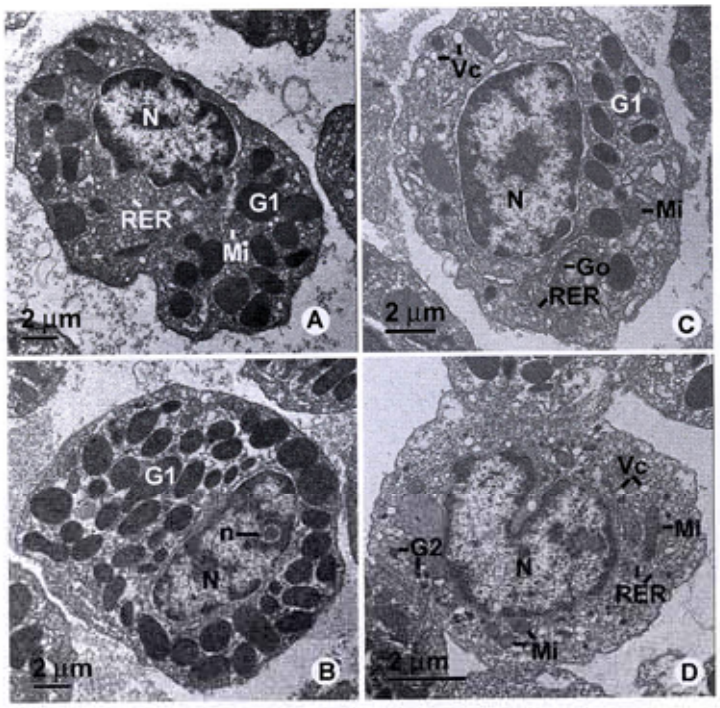

Fig.2 (A, B). Electron micrographs of large granular hemocyte showing indented nucleus (N), distinct nucleolus (n), numerous large electron-dense granules (G1), mitochondria (mi), rough endoplasmic reticulum (RER)

(C) Electron micrograph of small granular hemocyte showing small nucleus with non-lobulated nucleus (N), mitochondria (Mi), Golgi complex (Go), rough endoplasmic reticulum (RER), small vesicles (Vc) and electron-dense granules (G1).

(D) Electron micrograph of hyalin hemocyte showing bilobed nucleus (N) small electron-dense granules (G2), mitochondria (Mi), rough endoplasmic reticulum (RER) and small vesicles (Vc).

Conclusion

The hemocytes of *Portunus pelagicus* were classified as large granule hemocytes (lgh), small granule hemocytes (sgh) and hyaline hemocytes (hh). Large granule hemocytes contained numerous of type 1 electron-dense granules (G1). Small granule hemocytes contained abundant of G1. Hyaline hemocytes have few small electron-dense granules (G2) and small vesicles.

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