ULTRASTRUCTURE OF JUMBO TIGER PRAWN
(PENAEUS MONDON) OOCYTE : LATE MATURATION STAGE

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Abstract—Late maturation stage of jumbo tiger prawn (P. monodon) oocytes were collected from four different parts of ovary: the anterior thoracic, upper abdominal, middle abdominal and lower abdominal regions, respectively. All oocytes were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 at 4°C for 24 hrs. Then they were further processed for scanning and transmission electron microscopy. It was found that there was no difference in surface and internal structures of jumbo tiger prawn oocytes from four different regions. Oocytes were spherical in shape with 150-170 μm in diameter. The whole oocyte is covered by a sheath when the sheath was removed, the surface revealed evenly dispersed, pits each with central knobs. The ultrastructure of oocyte cytoplasm could be divided into two zones based on the organization of the organelles, namely, the cortex and the medulla. The cortical zone was narrow and contained numerous cortical granules, while the medulla was wider and had abundant electron-dense yolk granules. Both cortical and medullary zones surrounded the central cavity. Each oocyte were covered with flattened cells with long processes. Abundant of atypical RER and free ribosome were observed among cortical and yolk granules. A few SER, mitochondria and lipid droplets were present.

Key words : Ultrastructure, Penaeus monodon, Oocyte, Late maturation stage.

Introduction

Ultrastructural informations of oocyte is very limited in crustacean species. The investigation, undertaken in Penaeus aztecus reported the structural development of cortical granules, nucleus, and nucleolus at three early stages of oocyte maturation (1). In spider crab, Libinia emarginata. L.,(2) abundant profiles of endoplasmic reticulum have been observed in vitellogenic oocytes. It was also found that granular and agranular ER as well as the Golgi complex were active in the yolk synthesis; yolk material appeared to be derived from both intra- and extracellular sources.

In jumbo tiger prawn, (Penaeus monodon), maturation of oocyte was investigated at light microscopic level and was characterized into four stages: basing on their sizes, shape and staining properties. Immature, early maturation, late maturation and fully mature (3). However, the characterization is quite ambiguous due to scarcity of detailed structural informations, especially at ultrastructural level. Thus, this study is aimed to investigate the ultrastructures of jumbo tiger prawn oocyte at late maturation stage. Evidence obtained from this study will enhance the better understanding of oogenesis and vitellogenesis of the P. monodon egg.

Materials and methods

The female broodstocks of jumbo tiger prawn with ovary at the late maturation stage (4) were obtained from the gulf of Thailand. Their dorsal skeleton were exposed and oocytes were collected from four different portions of the ovary: the anterior thoracic, upper abdominal, middle abdominal and lower abdominal regions; respectively. All oocytes were transferred into 2.5% glutaraldehyde in 0.1 M. sodium
cacodylate buffer pH 7.4 at 4°C for 24 hr. Then, they were further processed for scanning and transmission electron microscopy (SEM and TEM) studies.

**Procedure for SEM**

In order to reveal the surface structure of the oocytes, the external translucent layer was removed by needle under dissecting microscope. Then the specimens were postfixed in 1% OsO₄ in 0.1 M sodium cacodylate at 4°C for 1 hr. and dehydrated through graded concentration of ethanol. The oocytes were then dried by criticalpoint drying apparatus Hitachi HCP-2 using liquid carbon dioxide as a transitional medium. The dried specimen were mount on the stub and shadowed with gold and examined with the Hitachi S-430 SEM, operating at 20 KV.

**Procedure for TEM**

To investigate the internal structure, TEM was used. The oocytes were further post fixed in 1% OsO₄ in 0.1 M sodium at 4°C for 1 hr. After washing in the same buffer, specimens were stained en bloc with 1% uranyl acetate in 0.1 M sodium acetate buffer pH 5.1 in the dark for 1 hr, and dehydrated through graded ethanol and finally embedded in Araldite. Semithin sections were cut and stained with 1% methylene blue, and examined under light microscope (LM). Thin section were stained with uranyl acetate respectively, and were then examined in a Hitachi H-300 electron microscope operating at 75 KV.

**Results**

**SEM observation**

It was found that there was no difference in surface structure of jumbo tiger prawn oocytes from four different regions. Jumbo tiger prawn oocytes were spherical in shape with 150-170 μm in diameter. Their surfaces were covered by a thin sheath, showing numerous shallow depressions (Figures 1, 6, 8). When the sheath was removed, deep pits with centrally-located knobs were clearly observed (Figures 2, 3). The knobs were connected with the rims of pits by thin membrane that appeared perforated (Figures 4, 5). The surface of oocytes range from those that appeared smooth and clean to those that had attaching of small particles (Figures 2, 6, 7, 8).

**LM and TEM study**

The characteristic of the oocyte cytoplasm could be divided into two regions based on the organization of the organelles; namely, the cortex and the medulla (Figures 9, 10). The cortical zone was narrow and contained numerous cortical granules, while the medullary zone was wider and had abundant large spherical electron dense granules, described as yolk granules. Both cortical and medulla zones surrounded the central cavity. External to the cortical zone was a layer of flattened cells with long processes covering all entire oocyte surface (Figures 11, 12, 13). This layer cell was separated from an oocyte membrane by the basement membrane (Figures 11, 12). The cytoplasm of each cell consisted of numerous atypical RER.

**Figure 1.** Low magnification of an oocyte, showing the sheath (S) covering its surface. Numerous pits (arrow heads) were observed, each has centrally-located knob.

**Figure 2.** Low magnification of an oocyte, showing the whole surface structure after the sheath was removed. The knobs were connected with the rims of pits by thin membrane that appeared perforated.

**Figure 3.** Medium magnification of Figure 1, the sheath (S) and pits (arrow heads) clearly observed.

**Figures 4-5.** High magnification of an oocyte surface showing the knobs (Kn) which are linked to the rest of the surface by perforated membrane.

**Figure 6.** Low magnification, showing the sheat (S) surrounding the oocyte surface

**Figure 7.** Low magnification of an oocyte, showing the surface of some oocytes which appear quite clean.

**Figure 8.** Medium magnification, showing clean pits (arrow heads) and knob structures (arrows).
**Figure 9.** Low magnification of semithin section, showing the general characteristic of oocyte cytoplasm. CZ = cortical zone; MZ = medulla zone, L = lumen.

**Figure 10.** Low magnification of an oocyte showing cortical granules (CG) and abundant yolk granules (YG).

**Figure 11.** High magnification, showing long processes of flattened cells (CP). They are separated from oocyte’s surface by a basement membrane (BM). Oocyte’s membrane (arrow heads), free ribosome (FR) and RER were also observed.

**Figure 12.** High magnification, showing the nucleus (Nu) of flattened cells, and numerous RER. BM = basement membrane; FR = free ribosome.
The prominent organelle of cortex was cortical granules. They had oval shape with about 6-8 μm in diameter. The matrix of cortical granules were mainly composed of fibrous structures each with bottle-brush appearance similar to the structure of proteoglycan molecules. These macromolecules consisted of the thick core element to which numerous thin side chains were linked, thus make them appeared as bottle-brush structure (Figures 14, 15). Cortical granule membrane was always close to oocyte membrane (Figure 14), RER, free ribosome, mitochondria and yolk granules were present between cortical granules.

The predominating organelles of the medulla was yolk granules. They were spherical in shape and had electron opaque matrix surround by a single membrane (Figure 16). Among yolk granules, abundant RER and free ribosomes were observed. A few SER, mitochondria, and lipid droplets were also present. Most RER appeared to be highly dilated and contained flocculent materials in their cisternae.

Discussion

The present study reveals for first time the detailed ultrastructure of the jumbo tiger prawn oocytes at the late maturing stage. In general, their basic structural patterns are comparable to those of other crustacean species described in previous reports (1, 2). However, there are some significant difference that indicate interspecies variations of this invertebrate class.

Oocytes of P. monodon were ensheathed with a thin single layer of elongate squamous cells. Under the scanning electron microscopic study, the sheath consists of numerous shallow pits dispersing on the entire surface of the oocyte. The indentations may represent the location of cortical granules underneath the surface, as reported in the P. aztecs (5). However, there is a striking difference in the sheath cellular structure between the two crustacean species; that of P. monodon consists of a single layer of elongate squamous-type cells having abundant profile of rough endoplasmic reticulum in their cytoplasm, while in the P. aztecs this layer is acellular and is referred to as an investment coat.

Finding in crayfish (Cambarus sp. Orconectus sp. and Procamburus sp.) also add another significant intraspecie differences (6). The outersheath of oocytes of these crayfish consist of follicle cells having numerous mitochondria, endoplasmic reticulum, Golgi complex and associated vesicles of different sizes are located on the side adjacent to the oocyte. The prominent profiles of rough endoplasmic reticulum within the sheath cells of P. monodon suggest they may synthesize and secrete some substances supporting the cortical reaction mechanism which is the mechanism responsible mainly by the cortical granules. Oocytes of P. monodon clearly lack special entry site for sperms on its surface. In contrast, this site is quite prominent in fish species (7, 8), and refered to as a micropyle, and this play significant role in preventing polyspermy.

The cortical granules were observed in the cortical layer of oocytes as an oval-shape membrane-bound structures. Under the scanning electron microscopic study, their apex were seen to protrude out at the middle of the pits. This contrasts to those of P. aztecs (5) which are rod-shape structures lying within the crypts and are seperated from the external media by an investment coat. However, the two types of cortical granules seem to contain, more or less, the same kind of content : the fibrillar structure materials which is similar in structure to the molecules of proteoglycan as desribed by Fawcett (9). The cortical granules have been found to play a major role in cortical reaction rapid expulsion and dissipate of the granules in response to seawater, these called "cortical reaction", is responsible for the formation of the jellylike layer around the oocyte which remained until the second cleavage (5). The reaction has been found to be a typical process in the oocytes of the paneid shrimp (5).

Yolk granules are round and electron dense membrane bound vesicles. They are prominent organelles observed in the medullary zone of P. monodon oocytes. Numerous rough and smooth endoplasmic reticulum and mitochondria were also observed dispersing among the yolk vesicles. This suggests that yolk formation should involve a greater degree of intraoocyte synthesis as described in
other crustacean (6) and fishes (10, 11). The absence of micropinocytotic vesicles on the ootic surface support the idea that yolk precursors of P. monodon oocytes are intraoocytic origin as reported in crayfish (8). The process is different from those of found in other crustacean species (2, 12) and insert (13) which rely on both intra and extraocytic yolk precursors. Little information is available concerning the chemical components of the yolk within the vesicle. This is a matter of further study.

References


Figure 13. Medium magnification, showing the cortical zone. Cortical granules (CG), yolk granules (YG), and RER are also present.
Figure 14. High magnification of cortical granule (CG) containing fibrous matrix inside.
Figure 15. High magnification of cortical granule, showing fibrous structure, each consisting of core element (P) linked with multiple side chains (arrow heads). Mitochondria and SER and also present
Figure 16. High magnification of the medulla, showing Yolk granules (YG), Lipid droplets (Li) RER and free ribosomes. Membrane of YG and RER with attached ribosomes are clearly seen (insertion).