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Communication:

Changes in oviduct structure in the black tiger shrimp, *Penaeus monodon*, during ovarian maturation

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Objective: To examine the structure of the oviduct of the shrimp *Penaeus monodon*. Methods: The oviducts of *P. monodon* with three different major groups of ovarian development (Group (Gr.) 1: Stages I & V; Gr. 2: Stages II & III; and Gr. 3: Stage IV) were examined by light, transmission electron, and scanning electron microscopies, respectively. Results: The epithelium of the oviduct in Gr. 1 was composed of tall simple columnar cells with their basal nuclei located on the basement membrane and its thick collagen fibers. In Gr. 2, the oviduct seemed to produce some substances and their epithelial cells became transitional with centrally located nuclei and formed some vacuoles. Obviously, the epithelial cells in Gr. 3 (at Stage IV) were disorganized, disrupted, and shed accumulated spherical secretory substances including some cellular contents into the lumen. Conclusions: The structural changes of the *P. monodon* oviduct were related to ovarian maturation stages (Grs. 1–3). Prior to spawning, only the oviduct epithelium at ovary Stage IV produced and secreted a number of spherical secretion substances into the lumen. These substances may act as the oviductal lubricants to facilitate the spawning process.

Key words: Oviduct, Ovarian maturation, Black tiger shrimp, *Penaeus monodon*, Spherical secretory substances

1 Introduction

The ovary and other organs of Decapoda have been extensively documented (Bell and Lightner, 1988; Zhao *et al*., 1998; Deng, 2000), while the morphology and function of the shrimp oviduct have not been well studied. The oviduct of penaeid shrimp is considered to play a role not only in carrying the mature eggs from the ovary to the gonopore during spawning but also in the secretion of some molecules (Talbot and Helluy, 1995; Yu and Lu, 2006). The possible substances secreted from the oviduct may play various physiological roles in the female reproductive system, such as lubrication of the oviduct, participation in oocyte maturation, and induction of capacitation of sperm stored in the female seminal receptacle.

Unlike those of vertebrate animals, the oviducts of penaeid shrimp are short, narrow, simple tubes that continuously connect to the lateral lobes of the ovary. Few histological studies of the shrimp oviduct have been reported, e.g., *Macrobrachium nipponense* (Lu *et al*., 2006), *Jasus frontalis* (Elorza and Dupre, 2000), and *Penaeus sertiferus* (King, 1948). In the shrimp *Penaeus setiferus*, the wall of the oviduct has been described as being made of three layers (connective tissue, basal lamina, and a layer of columnar epithelial cells) without a muscular layer (King, 1948). The oviductal wall of *P. setiferus* (King, 1948) is folded in

some areas, similar to that of *M. nipponense* (Lu *et al*., 2006). In *Penaeus monodon*, with 2 g body weight (BW), the oviductal epithelium also consists of a layer of tall simple columnar cells with a basal nucleus (Bell and Lightner, 1988), whereas with 80–100 g BW, possessing a mature ovary (Stage IV), the oviductal epithelial cells become disorganized. In contrast to the oviducts of penaeid shrimp, the oviductal walls of lobsters *Jasus lalandii* (Silberbauer, 1971), *Homarus americanus* (Talbot and Helluy, 1995), and *J. frontalis* (Elorza and Dupre, 2000) consist of criss-crossed muscular fibers, which also cover the ovarian wall, suggesting an active participation in the extrusion of oocytes from the ovarian follicles and the translocation of the oocytes to the oviduct (Elorza and Dupre, 2000). It has been suggested that the oviductal epithelium of lobsters undergoes cyclical changes with ovarian development and spawning (Herrick, 1909). It is believed that the tall columnar epithelial cells lining the oviductal wall secrete a lubricating fluid to facilitate the passage of the mature eggs along the oviduct (King, 1948; Talbot and Helluy, 1995; Lu *et al*., 2006), although lubricating substances have never been characterized. However, it has been proposed that substances secreted from the shrimp oviduct may be involved in fertilization during spawning (King, 1948; Talbot and Helluy, 1995; Lu *et al*., 2006).

Herein, we report the structural changes of the oviduct of the shrimp *P. monodon* during ovarian maturation. We also demonstrate the presence of spherical secretion substances in the oviduct before the spawning process.

2 Materials and methods

2.1 Animals

Adult female black tiger shrimp, *P. monodon* (120–150 g BW at one year of age) possessing different stages of ovarian development were kindly provided by the Bangkok Aquaculture Farm Company (BAFCO), Nakhon Sri Thammarat, Thailand. The ovarian maturation stages of *P. monodon* can be classified into five stages as described by Rao *et al*. (1995): Stage I (immature), Stage II (early maturing), Stage III (late maturing), Stage IV (mature), and Stage V (spent), respectively. Based on visibility of the ovary by the naked eye through the shrimp dorsal exoskeleton, they were further reclassified into three major groups: Group (Gr.) 1, Stages I & V; Gr. 2, Stages II & III; and Gr. 3, Stage IV. These shrimp were stocked in hatchery maturation tanks under seawater with 30 practical salinity unit (PSU).

2.2 Preparation of samples

The female shrimp were anaesthetized under ice, and then cut through the thoracoabdominal segment. The head part of shrimp was further cut along the midline. The oviducts were situated laterally to the inner side of the body wall. The oviduct descends latero-ventrally from the tips of the 5th lateral lobes and ends at gonopores that are located in coxapods of the 3rd walking leg. To understand the morphology of the shrimp oviduct, the structure of oviducts based on the ovarian development in *P. monodon* was examined by light microscopy (LM) and transmission electron microscopy (TEM) as described below.

2.3 Light microscopy (LM)

Female *P. monodon* at different stages of ovary maturation was anaesthetized under ice, and then immediately perfused with Davidson's fixative (prepared from 330 ml 95% ethyl alcohol (EtOH)+220 ml 37% formalin+115 ml glacial acetic acid+335 ml distilled water) by injecting into the cephalothorax area and the thelycum. Then, the entire length of oviduct was dissected free from the ovary and gonopore. The oviducts were cut into small pieces, and further immersed in the same fixative at room temperature for 24 h. Thereafter, these specimens were dehydrated in ascending concentrations of EtOH (50%, 70%, 80% and 90% EtOH, 1 h each, and 2 changes of 100% EtOH, 1 h each), cleared in xylene (2 changes, 1 h each), and infiltrated in paraffin using an automated tissue processor. Specimens embedded in paraffin were sectioned at 4–6 μm thick and stained with hematoxylin and eosin (H & E). All images were captured by a Nikon light microscope equipped with a DXM1200 digital camera.

2.4 Transmission electron microscopy (TEM) and scanning electron microscopy (SEM)

Shrimp at the same stages as above were perfused with 2% (v/v) glutaraldehyde plus 4% (v/v) paraformaldehyde in artificial seawater (ASW) (9.3 mmol/L $CaCl_2$, 423 mmol/L NaCl, 9 mmol/L KCl, 23 mmol/L $MgCl_2$, 9.3 mmol/L $MgSO_4$, 2.1 mmol/L $NaHCO_3$, pH 7.8) into the same area as described above. The oviducts were dissected free from other tissues, cut into small pieces (~1 mm^2) and fixed with 4% glutaraldehyde in seawater overnight at 4 °C. Subsequently, the specimens were washed with ASW 5–6 times and post-fixed with 1% (0.01 g/ml) osmium tetroxide (OsO_4) in ASW for 1 h at 4 °C. Then they were dehydrated in increasing concentrations of EtOH (50%–100%, 30 min each), cleared in two changes of acetone, infiltrated in a mixture of acetone and Spurr resin at the ratio of 1:1 (v/v) for 45 min, 1:4 for 30 min, and pure Spurr overnight. They were embedded in pure Spurr and allowed to polymerize at 70 °C for 10–15 h. The resin blocks of specimens were sectioned at 0.3–0.5 μm thick (semithin) by ultramicrotome. Semithin-resin sections were stained with basic fuchsin for LM observations. The ultrathin sections (50–70 nm) mounted on 200-mesh-copper grids were counterstained with lead citrate and uranyl-acetate and viewed under a Hitachi H-300 TEM controlled at 75 kV.

After perfusion with the TEM fixative described above, the oviducts were cut along the longitudinal plane, fixed in the same fixative, washed in ASW, and further fixed with 1% OsO_4 for 30 min. Fixed oviducts were then dehydrated in an increasing series of ethanol solution and critically point-dried (CPD) with CO_2. Subsequently, the dehydrated oviducts were mounted on stubs, coated with 25 nm of platinum and gold, and then viewed under SEM.

3 Results

3.1 Structure of shrimp oviduct at different stages of ovarian maturation

The fresh oviduct in the Gr. 1 (Stages I & V) was very thin and transparent without secretory products (Fig. 1a, small photograph). Histologically, the epithelium of this early stage of oviduct was composed of tall simple columnar cells with their basal nuclei located on the basement membrane and its thick collagen fibers (Fig. 1a). In the Gr. 2 (Stages II & III), it is likely that the oviducts produce some substances (fresh yellowish; Fig. 1b small photograph), and the epithelial cells became transitional having centrally located nuclei and formed some vacuoles (Fig. 1b). Interestingly, the disgustingly yellowish product was abundant at Stage IV and it also appeared along the entire oviduct extending to the ovary (Fig. 1c, small photograph and Figs. 2a and 2b). In contrast to Gr. 1 and Gr. 2, Fig. 1c shows that the epithelial cells at Stage IV (Gr. 3) were disorganized and shed the accumulated secretory substances including their cellular content into the lumen (Fig. 1c).

The female *P. monodon* with fully developed ovary (Gr. 3) was perfused with the fixative and dissected gently by approaching the anterio-thoracic region to investigate the gross anatomy of the shrimp oviduct before spawning. Paired oviducts of *P. monodon* descend latero-ventrally from the 5th or 6th lateral lobe of the ovary and end at the external genital apertures called gonopores located at the coxopods of the 3rd pair of walking legs (Figs. 2a and 2b). In addition, the oviduct penetrating the ovary at Stage IV contained large amounts of accumulated secretory substances in the oviductal lumen (Fig. 2c).

3.2 Structure of shrimp oviductal epithelium at Stage IV of ovarian maturation before spawning

Because the oviduct in the Gr. 3 (Stage IV) showed disorganized epithelium and accumulated secretory substances at the LM level (Fig. 1c) as compared to Gr. 1 and Gr. 2 (Figs. 1a and 1b), both TEM and SEM techniques were used to define the structural changes of the oviduct and also illustrate the secretory process of spherical secretory products particularly at Stage IV (Fig. 3). The disorganized epithelial cells lay on the basement membrane and they seemed to secrete the spherical secretory substances (Figs. 3a and 3b). Figs. 3c and 3d show the secretion and shading of various sizes of spherical secretory substances produced by the oviductal epithelium into the lumen. This secretory process was confirmed with TEM micrograph (Figs. 3a and 3b).

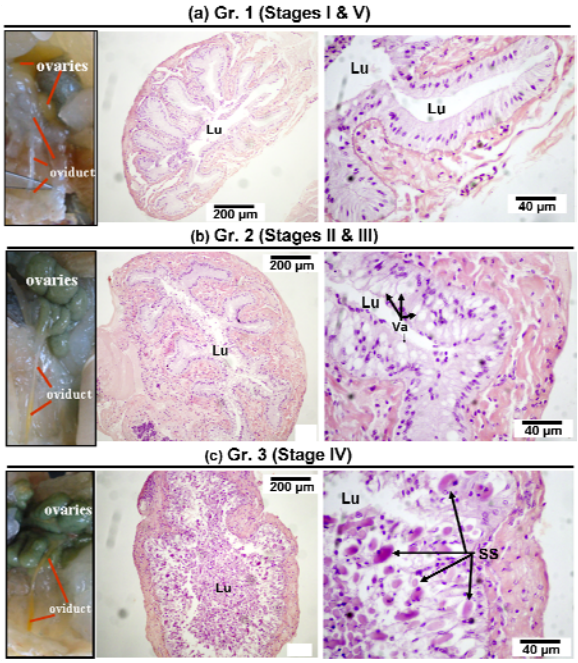

Fig. 1 Structure of the oviduct of black tiger shrimp, *P. monodon*, before spawning, corroborated with its ovarian development stages: (a) Stages I & V (Gr. 1), (b) Stages II & III (Gr. 2), and (c) Stage IV (Gr. 3), respectively

Left photographs show gross anatomy of shrimp oviduct connecting to the ovary (ventral aspects). Hematoxylin and eosin (H & E) stained sections: Lu, lumen; Va, vacuole; SS, secretory substance

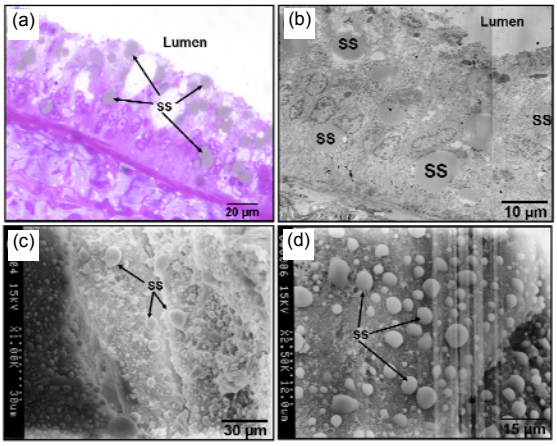

Fig. 3 Structure of the oviductal epithelium (basic fuchsin-stained semithin (a), TEM (b), and SEM (c, d) micrographs) of black tiger shrimp, *P. monodon* at Stage IV before spawning

(b) TEM micrograph of the oviduct epithelium illustrating the secretory process of spherical secretory products and other cell contents into the lumen; (c, d) SEM luminal aspect showing the secretion of various sizes of spherical secretory products being shed into the lumen. SS: secretory substance

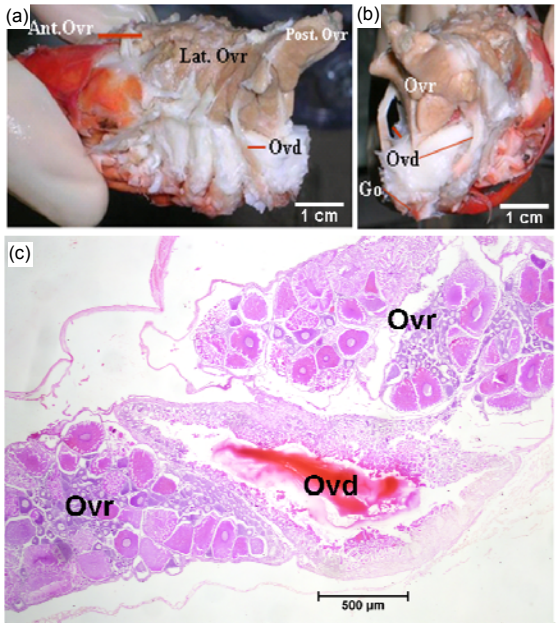

Fig. 2 Photographs showing the gross anatomy of the shrimp oviduct connecting to the ovary (Gr. 3)

The female *P. monodon* with the ovary at Stage IV was fixed and dissected gently by approaching the anterio-thoracic region (a, b) and histology (c; H & E stained section) of the shrimp oviduct connecting to the ovary Stage IV of black tiger shrimp, *P. monodon*. (a) Lateral aspect; (b) Latero-dorsal aspect. Ant.Ovr: anterior lobe of ovary; Lat.Ovr: lateral lobe of ovary; Post.Ovr: posterior lobe of ovary; Go: gonopore; Ovr: ovary; Ovd: oviduct

4 Discussion

The oviducts of the shrimp *P. sertiferus* (King, 1948), *J. frontalis* (Elorza and Dupre, 2000), and *M. nipponense* (Lu *et al*., 2006) have been examined histologically. It is considered that the oviductal wall of lobsters undergoes cyclical changes with ovarian development (Talbot and Helluy, 1995). Additionally, the epithelial cells of the shrimp oviduct are believed to secrete a lubricating fluid to protect the mature eggs from damage during the transit in the oviduct before spawning (King, 1948; Talbot and Helluy, 1995; Yu and Lu, 2006; Lu *et al*., 2006). However, the changes in oviduct structure of *P. monodon* have never been documented. Herein, the morphological study of the shrimp *P. monodon* oviducts (Grs. 1–3 or Stages I–V) showed shedding of epithelial cells from their base to lumen. This corresponded to changes in the five ovarian maturation stages (Figs. 1c, 2, and 3).

These histological findings suggest that the changes of oviductal epithelium correspond to individual stages of ovarian maturation in *P. monodon.* In addition, the production of various spherical substances by the oviductal epithelial cells was shown to take place when the ovary underwent the maturation process (Figs. 1c, 2, and 3). As shown in Figs. 1 and 3, the secretions of oviductal substances are dynamic and can be described as follows: Gr. 1 (Stages I & V), the tall simple columnar epithelial cells with the basal neuclei line the inner surface of the oviductal wall; Gr. 2 (Stages II & III), nuclei of oviductal epithelial cells move from the base to the center of the cells and some vacuoles are produced; and Gr. 3 (Stage IV), a number of spherical substances are produced and secreted into the oviductal lumen. In addition, self-dispersion of the epithelial cells also results in the release of cytoplasmic content into the oviductal lumen. The production of the substances increases at Stage IV and the epithelial cells appear to be initially disorganized. All of these results support the idea that the shrimp oviduct undergoes cyclical changes with ovarian maturation (Munuswamy and Subramoniam, 1985; Talbot and Helluy, 1995; Yu and Lu, 2006). This study has shown that oviductal epithelial cells at Stage IV self-disperse, indicating that no epithelial cells line the oviductal wall after the completion of spawning. It is currently not known how the new oviductal epithelial cells reform on the wall. There are two possibilities regarding the significant roles of *P. monodon* secretion substances. Firstly, the spherical secreted-substances (Fig. 3) may play an important role in lubrication of the oviduct during the ovulation and spawning because these substances secreted from the epithelial cells show vacuolar form and contain highly-glycosidic proteins. However, the biochemical properties of these vesicles need to be further elucidated to confirm this possibility. Secondly, the secreted substances including other cytosolic molecules from self-dispersion of the oviductal epithelium may play a significant role in egg/sperm maturation and probably in sperm-egg interaction. These substances likely belong to the oviduct, since the oviductal epithelial cells at Stage IV seem to release their cytoplasmic components by self-dissolution of the epithelial cells (Fig. 3). We assume that the spherical substances may be produced and secreted to lubricate the oviduct during spawning, although the mucin-like properties of these vesicles need to be further demonstrated.

In conclusion, the dynamic renewal of the oviductal epithelial cells is likely to relate to the cyclical change during ovarian maturation. At Stage IV, a number of the spherical substances are massively secreted into the oviductal lumen together with self-dissolution of the oviductal epithelial cells.

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