Yolk protein profiles of three prawn (Macrobrachium) species during reproductive cycle

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Received 21 October 2004; revised 29 June 2005

Ovarian yolk protein (vitellin) of adult freshwater prawns Macrobrachium malcolmsonii, M. rosenbergii and M. lamarrei was characterized, and relationship between the gonadosomatic index (OS I) and the appearance of specific polypeptides in their ovary during different stages (early-mature, mature and spent) of reproductive cycle was studied. Mature ovaries of all the three species showed increased OSI values, compared to the respective early-mature ovaries. Protein profiles at the three stages of ovarian maturation were analysed by SDS-PAGE. Polypeptides of low molecular mass (20 to 70 kDa) were prominent in the early-mature ovary, whereas in mature ovary, 89 and 100 kDa polypeptides were predominant. Immunodiffusion studies, using antiserum raised against purified vitellin from mature ovary of M. malcolmsonii indicated antigenic similarities of vitellins among the three species.

Keywords: Yolk protein, ovary, Macrobrachium species, vitellin, gonadosomatic index, immunodiffusion

IPC Code: C07K1I26; C25B7/00

Animals are known to accumulate yolk in their oocytes during oogenesis. Yolk globuli mostly contain proteins, lipids and carbohydrates. Yolk proteins constitute an important source of nutrients for development of oocytes and embryos and also support embryos and nauplii up to several weeks after hatching. Vitellins (Vn) are lipoglycoproteins found in yolk with a molecular mass ranging 200-500 kDa. They contain up to 30% lipid and carotenoids that give oocytes of different species, a distinctive colour. Vitellins have been purified from maturing ovaries of several crustacean species.

Vitellogenin (Vg) is the precursor of major yolk protein vitellin. In crustaceans, hepatopancreas is reported to be the site of Vg synthesis. The core protein is initially synthesized in hepatopancreas, followed by glycosylation, and addition of carotenoids and lipids forming Vg, which is secreted rapidly into the hemolymph. Finally, the Vg undergoes slight structural changes and accumulates in the ovary as Vn. In the present study, an attempt has been made to characterize Vns from the ovaries of three freshwater prawn species viz., Macrobrachium malcolmsonii (H. Milne-Edwards), M. rosenbergii (De Man) and M. lamarrei (H. Milne-Edwards). In addition, relationship between the changes in gonadosomatic index (GSI) and appearance of specific polypeptides in the ovary during different stages of the reproductive cycle have been investigated. The immunological cross reactivity among the Vns of three prawn species have also been studied.

Materials and Methods

Animals and gonadosomatic index

Male and female adult prawns were collected from different local sites such as farms, ponds and dam; M. malcomsonii from the Lower Anaicut, on the river Cauvery, M. rosenbergii from farms near Thanjavur, and M. lamarrei from Gundur pond near Tiruchirappalli. Female prawns were categorized based on their ovarian stages as early-mature, mature and spent stages. GSI was calculated as gonad wt x 100/body wt at different stages of development of the ovary. The early-mature and the mature ovaries were found to occupy 1/4th and 3/4th of the carapace, respectively and the spent ovary occupied only a small portion.

Preparation of ovarian homogenates

Ovaries were dissected and homogenized in an extraction buffer containing 50 mM Tris, 1 mM EDTA and 1 mM PMSF (pH 7.4). The homogenates were centrifuged at 10,000 g for 20 min and the fat cap was removed before using the supernatant for

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Abbreviations: GSI, gonadosomatic index; PMSF, phenyl methane sulphonyl fluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulphate; Vg, vitellogenin; Vn, vitellin; SAS, saturated ammonium sulphate.
Electrophoresis

Protein content of ovaries was estimated using bovine serum albumin as standard. Samples were denatured with sodium dodecyl sulphate (SDS) sample buffer (pH 6.8) for 1 min in a boiling water bath. Equal quantities of protein (150 µg) were loaded on 7% separating gel with 4% stacking gel. The gels were stained with 0.2% Coomassie brilliant blue R-250 in 47.5% ethanol and 10% acetic acid for 6 hr and de-stained with a solution containing water:ethanol:acetic acid (66:24:10).

Purification of Vn

Ovarian tissue of mature female M. malcolmsonii was homogenized in 10 ml/g (wet tissue wt) homogenization buffer (50 mM Tris, 1 mM EDTA, 1 mM PMSF pH 7.2) and centrifuged at 4000 g for 5 min. The supernatant was collected and centrifuged again at 20,000 g for 20 min. The supernatant was combined with saturated ammonium sulphate (SAS) to produce 25% SAS solution, incubated for 1 hr, and centrifuged at 20,000 g for 10 min at 4°C. Subsequently, the supernatant was collected and sequentially combined with the SAS and made into 40, 50 and 60% solutions. The final pellet (purified Vn, 60% SAS) was collected, re-suspended in homogenization buffer solution and dialyzed overnight against three changes of 5% acetic acid. The antibodies were stained with 0.2% Coomassie brilliant blue R-250 and de-staining was carried out with several changes of 5% acetic acid. The antibodies were placed in central well, with six peripheral wells at a distance of 0.9 cm filled with samples.

Results and Discussion

GSI at various stages of the ovary during the reproductive cycle of the three species are given in Table 1. GSI of all the three species showed increase from early-mature to mature stage and decrease in the spent stage; higher GSI at mature stage ovaries of all the three species indicated their reproductive status.

In M. rosenbergii, hepatopancreas Vg mRNA levels and hemolymph Vg levels have been reported to increase gradually during the molt cycle, concomitant with an increase in GSI, suggesting a relationship between Vg synthesis and ovarian maturation. Rapid uptake of Vg from hemolymph by receptors in the ovarian membrane of M. rosenbergii, during early stages of ovarian maturation has also been reported. Subsequently, Vg is taken into the ovary, leading to ovarian maturation as reflected in the increase in GSI. Ovarian stages of M. rosenbergii are closely correlated with the GSI and the concentration of ovarian Vn. During gonad maturation in M. rosenbergii, there is a marked accumulation of Vn.

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<th>Table 1— Gonadosomatic index of various stages of ovaries during reproductive cycle in three freshwater prawns</th>
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in ovaries which increases up to ripening. Such Vn accumulation by oocytes significantly increase their diameter. The spent stage was conspicuous by the absence of Vn. It is possible that the accumulated Vn during the development of oogenesis is exhausted in the spent stage, due to utilization in egg production.

SDS-PAGE analysis of the ovary revealed differences in their polypeptide profile during different stages of the reproductive cycle (Fig. 1). In *M. rosenbergii*, early-mature ovary showed a number of polypeptides of low molecular mass (30, 33, 45, 56, 75 kDa), which were stained relatively darker and higher molecular mass polypeptides (89 and 100 kDa) stained faintly (Fig. 1, lane 4). The mature ovary exhibited two intensely stained polypeptides (89 and 100 kDa) (Fig. 1, lane 5), in addition to other polypeptides of low molecular mass, stained less intensely. These findings are consistent with earlier reports.

No polypeptide fraction was found in the spent stage ovaries of all the three species (Fig. 1, lanes 3, 6 and 9).

In *M. malcolmsonii*, the early-mature stage ovary showed a number of low molecular mass polypeptides (19, 26, 30, 34, 40 kDa) (Fig. 1, lane 1) which were stained faintly, compared to the 89 kDa and 100 kDa polypeptides of mature stage ovary (Fig. 1, lane 2). Similarly, in *M. lamarrei*, early-mature ovary (Fig. 1, lane 7) showed a number of low molecular mass polypeptides (19, 26, 30, 34, 40 kDa), while mature ovary (Fig. 1, lane 8) exhibited two intensely stained bands of 89 and 100 kDa.

Dynamics of yolk protein accumulation in brackishwater/marine prawn species, such as *Penaeus semisulcatus*, *P. longirostris*, *P. japonicus*, and *P. vannamei* have been studied in detail by comparing immature and mature ovaries using SDS-PAGE. Studies of vitellogenesis in several crustacean species have shown that the mobility of Vn subunits in SDS-PAGE is in the range of 45-200 kDa, and includes 2 to 5 subunits. The primary structure of Vg and N-terminal and partial internal sequences of Vn have already been determined. Variations in the molecular mass of Vn among marine prawns (*Penaeus*) were reported earlier. In *P. monodon*, a varying mass of 74, 83, 90, 104, 168 kDa and in *P. chinensis* 40, 58, 78, 85, 105 kDa were reported. However, only two subunits were reported in *P. semisulcatus* (86 and 95 kDa), and *P. vannamei* (61 and 69 kDa).

Immunodiffusion studies with antivitellin serum of *M. malcolmsonii* showed a positive cross reaction with crude homogenates of mature ovaries of *M. malcolmsonii* (Fig. 2, well 1, 5), *M. rosenbergii* (Fig. 2, well 2, 6), and *M. lamarrei* (Fig. 2, well 3, 4).

**Reference**

while mature ovaries of
purified vitellin (1-6)
(7) contained
male hemolymph of
Malcolmsonii (Fig. 2, well 6),
indicating that anti-vitellin serum is specific for Vn. The formation of precipitin lines in immunodiffusion
without spur indicated the antigenic similarities existing among the Vns of three prawn species. Plasma antigenic species-specific reactivity of Vg is reported earlier in rosary barb. The present study of ovarian stages in three Macrobrachium species further supports the view that Vn accumulation is closely correlated with ovarian maturation and GSI. In addition, the 89 and 100 kDa fractions of Vn of all the three Macrobrachium species were found to be identical in nature with antigenic similarities.

Acknowledgement

The authors wish to thank Prof. Amir Sagi, Ben-gurion University of Negev, Israel and Brian Tsukimura, Assoc Prof., California State University, USA for their valuable suggestions. A special thanks to Prof. Philip. A. Thomas, Head, Department of Ocular Microbiology, Joseph Eye Hospital, India for critical evaluation of the manuscript.

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