Indian J. Fish., **52**(4) : 497-500, Oct.-Dec., 2005

Note

Studies on lipovitellin in the back water shrimp *Caridina nilotica* (Roux, 1833)

M.THANGARAJ, S. G. PRAKASH VINCENT* , P.K. SUBAJA*, V. DELPHIN SAVIO* , B. VALENTIN BHIMBAC**

Vizhinjam RC of CMFRI, Vizhinjam, Kerala, India-695 521.

**Marine Biotechnology Laboratory, Centre For Marine Science and Technology, Rajakkamangalam, Tamilnadu, India-629 502.*

***Udaya School of Engineering, Vellamodi, Tamilnadu, India- 629 204.*

ABSTRACT

Based on the morphological appearance, ovary of *Caridina nilotica* was segregated as four maturation stages (I to IV). The biochemical parameters were significantly varied during maturation. Vitellin was quantified from non-denatured PAGE and noted that it positively increased during the ovarian developmental stages. This protein was considered as a lipoglyco nucleic acid binding protein based on specific staining.

In crustacean's ovaries, yolk accumulates within the oocytes during their development (oogenesis). Yolk globuli contain protein, lipid and carbohydrate (Meusy and Payen, 1988). Vitellin is the major yolk protein in the egg yolk, which has a high molecular weight of 3.5 x 10^5 Da of lipoglyco-caroteno protein (Chang *et al*., 1993). Vitellin of some penaeids like *Penaeus japonicus* (Vazquez-Boucard *et al*., 1986), *Parapenaeus longirostris* (Tom *et al*., 1987), *Penaeus vannamei* (Rankin *et al*., 1989), *Penaeus semisulcatus* (Browdy *et al*., 1990) and *P. monodon* (Quinitio *et al*., 1990) has already been studied. The polypeptide pattern for *P. vannamei* was determined from the crude ovarian extracts (Rankin *et al*., 1989). In this paper the biochemical parameters, non-denatured PAGE separation and characterization of vitellin in different stage ovaries are presented.

Female *Caridina nilotica* were collected from the Rajakkamangalam estuary of Kanyakumari district. Based on their relative shape and size were grouped into four reproductive stages. Ovaries from twenty animals of each stage were dissected out and stored immediately at -4^{0}C. These samples were homogenized with 0.5 M Tris-HCl (pH 6.8) buffer and centrifuged at 4500g for 10 min. Subsequently the middle layer was taken for biochemical estimations. Total protein of each stage ovarian sample was quantified by the method of Lowry *et al.* (1951), total glucose, lipid and triglyceride were estimated by glucose oxidase, cholesterol oxidase and GPO/POD methods respectively.

Four hundred microgram protein of fourth stage ovarian homogenate was eluted by sepharose 6B (Sigma, USA) column (100 X 1.5 cm) with Tris HCl buffer (0.1 M, pH. 6.8) at a flow rate of 0.5 ml/min. Fractions were collected in microfuge tubes and the absorbency was

measured at 280nm.

Native polyacrylamide gel (7%, 5%) was prepared. Twenty micro gram proteins with sample buffer [1% glycerol, 0.5% bromophenol blue, 0.5M Tris HCl (pH 6.8)] for each stage were loaded. The gel was run at constant current of 10mA in the presence of tank buffer (0.25M Tris-glycine, pH 8.3). After the run was over the gel was stained in a staining solution (0.02% CBB-R 250, 40% methanol, 7% acetic acid) and subsequently destained in destaining solution (40% methanol, 7% acetic acid) till clear bands appeared. The destained gel was analyzed for Relative front (*Rf*) value and each protein fraction of each lane was quantified and photographed using a gel documentation system (Syngene, U.K). Vitellin was also stained for glycogen and lipid with Periodic acid-Schiffs reagent and oilRed-O respectively. For the detection of nucleic acid, small aliquot of ethidium bromide was mixed with the homogenate during sample loading and viewed under the U.V light after completion of the run.

Stage I (previtellogenic) ovaries were thin, transparent and not visible to the naked eye through the dorsal exoskeleton. Upon dissection, it appeared as greenish white in colour. Stage II (early vitellogenic) ovaries were very thin streak, increase in size in the anterior and middle lobes, visible as thin linear band through dorsal exoskeleton. Stage III (late vitellogenic) ovaries were visible to the naked eye through the dorsal exoskeleton as a thick dark solid and linear band as it expands at the posterior thoracic and anterior abdominal region. A slight "long diamond" shape has been seen at the first abdominal segment. Stage IV (mature) ovaries were very prominent, dark green band, expands at the first abdominal segment, large and thicker.

Table 1 shows the biochemical parameters of different reproductive stage ovary. The chromatogram of matured ovarian protein is given in figure 1. By the separation procedure in sepherose 6B the ovarian protein resolved in to three proteins. From the result first peak indicates that the major protein is vitellin. The following peaks are other minor structural protein molecules. From the native PAGE result (Fig. 2) three major proteins of matured ovary are observed, with the Rf value of

Fig.1. Separation of proteins from ovary of female *C. nilotica* (stage IV) by Sepherose 6B column

TABLE 1. *Total protein, glucose, lipid and triglycerides in different reproductive stage ovary of* C. nilotica

Stage	Protein (mg/ml)	Glucose (µg/ml)	Lipid (µg/ml)	Triglyceride (µg/ml)
I	1.8 ± 0.18^{a}	21 ± 0.18^{a}	10 ± 0.17^{a}	560 ± 25^{a}
II	2.0 ± 0.22^{a}	20 ± 0.20^{b}	20 ± 0.19^{b}	840 ± 26^{b}
III	3.2 ± 0.25^{b}	10 ± 0.16^{c}	22 ± 0.20^{c}	1420 ± 26^{c}
IV	6.1 ± 0.36^{c}	10 ± 0.17^{c}	40 ± 0.25^{d}	1700 ± 30^{d}

Means with in the column sharing a common superscript are not significantly different ($P>0.05$)

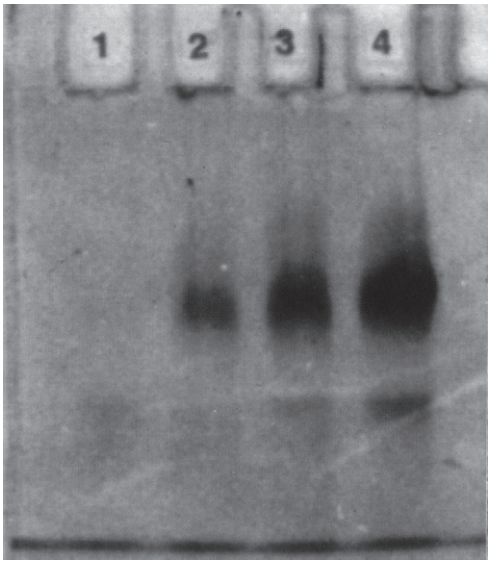

Fig.2. Non-denaturing polyacrylamide gel electrophoresis (7%) of ovarian proteins from (Stage I, II, III and IV) *C. nilotica* stained with Coomassie brilliant blue.

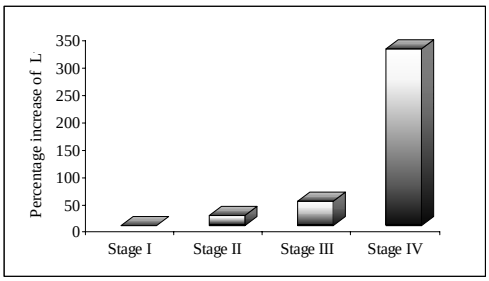

Fig.3. Percentage increase of lipovitellin (LV) in ovary of *C. nilotica* (Stage I, II, III and IV)

0.2461, 0.2804 and 0.7975. Tom *et al* (1987) considered the protein with the Rf value of 0.2461 as the vitellin. From the relative mobility, intensity of vitellin bands of each lane in the gel and total quantity of protein of corresponding stage, vitellin was densitometricaly quantified by a software system (Syngene, UK). The software analytic outputs of vitellin in four stages are illustrated in the figure 3. The native-PAGE separated mature ovarian proteins were localized with periodic acid-Schiff's (PAS) test and the major band showed a magenta colour which indicated that vitellin has glycogen moiety. In Oil red-O reagent, a red colour was formed and confirmed vitellin has lipid moiety. Under U.V illumination that band appeared slight orange in colour which prove that some times small nucleotide may be inter challate with vitellin.

This increasing feature of vitellin in developemental stages was reported earlier by Lee and Puppione (1988). In their study they found increase of vitellogenin in the haemolymph and reported oocyte development for *Callinectes sapidus*. In *Parapenaeus longirostris*, the lipovitellin of ripe ovaries was identified by comparing the electrophoretic profiles of homogenates prepared from ovaries at various stages of oogenesis (Tom *et al.*, 1987) and this has been used in the present study to identify the lipovitellins. The morphological features of ovarian development seems to have differences in the quantity of vitellin accumulation in the oocyte. The percentage of lipid in the lipovitellin has been studied by Wallace *et al*. (1967), it was varied from 28 to 33 in *Procambarus clarkii*. In the present study the matured ovarian vitellin was verified whether the lipid moiety is present or not in native gel. The egg yolk of crustaceans contains few high molecular weight lipoproteins, which also contain sugars and carotenoids (Meusy and Payen, 1988). A heavier lipoprotein which contains lipid and glycogen moiety was also fond. The amount of lipid and protein were found to increase positively towards reproductive developmental stages. The amount of vitellin also increased towards the ovarian development (0.625 to 5.383 mg/ml). From this study vitellin was identified and characterized as lipoglyco-

nucleic acid binding protein.

Acknowledgements

We thank the authorities of M. S. University for the facilities provided and Dr.K.V. Jayachandran, Fisheries College, Panangad for confirming identity of *Caridina nilotica*.

References

Browdy, C.L., M. Fainzilber, M.Tom, Y. Loya and E. Lubzens 1990. Vitellin synthesis in relation to oogenesis in *in vitro* incubated ovaries of *Penaeus semisulcatus* (Crustacea, Decapoda, Penaeidae). *J. Exp. Zool.,* **255**: 205 215.

Chang, C. F., F.Y. Lee and Y.S. Huang 1993. Purification and characterization of vitellin from mature ovaries of prawn *Penaeus monodon*. *Biochem. Physiol*., **105B**: 409- 414.

Lee, R.F and D.L. Puppione 1988. Lipoproteins I and II from the haemolymph of blue crab, *Callinectus sapidus* : lipoprotein II associated with vitellogenesis. *J. Exp. Zool*., **248**: 278-289.

Lowry, O.H., N. J. Rosenburgh, A. L. Fair and R. J. Randall 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem*., **193,** 265-275.

Mesy, J.J and G. G. Payen 1988. Female reproduction in malacostracan Crustacea. *Zool. Sci.,* **5** : 217-265.

Quinitio, E. T., A. Hara, K. Yamauchi and A. Fuji 1990. Isolation and characterization of vitellin from the ovary of *Penaeus monodon*. *Invert. Reprod. Develop*., **17**: 221-227.

Rankin, S. M., J. Y. Bradfield and L. L. Keeley 1989 . Ovarian protein synthesis in the South American white shrimp, *Penaeus vannamei*, during the reproductive cycle. *Invert. Reprod. Develop*., **15**: 27 33.

Tom, M., M.Goren and M.Ovadia 1987. Purification and partial characterization of vitellin from the ovaries of *Parapenaeus longirostris* (Crustacea, Decapoda, Penaeidae). *Comp. Boichem. Physiol.*, **87B**: 17-23.

Vazquez-Boucard, C., H. J. Cecaldi, Y. Benyamin and C. Roustan 1986. Identification, purification et caracterisation de la lipovitellin chez un crustace decapode natantia *Penaeus japonicus* (Bate). *J. Exp. Mar. Biol. Ecol.*,**97**: 37-50

Wallace, R.A., S. L. Walker and P.V. Hauschka, 1967. Crustacean lipovitellin. Isolation and characterization of the major high density lipoprotein from the eggs of decapods. *Biochem*., **6**: 1582 1590.

Date of Receipt : 22-07-2004
Date of Acceptance : 11-03-2005