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Biochemical composition of the ovary and partial characterisation of yolk protein vitellin in *Metapenaeus monoceros* (Fabricius, 1798)

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ABSTRACT

Yolk protein vitellin in 'speckled shrimp' *Metapenaeus monoceros* was partially characterised employing polyacrylamide gel electrophoresis (PAGE). There was an increase in the level of expression of vitellin in the ovary, with the advancement of maturation. The vitellin fraction was not expressed in immature and spent ovaries. Molecular weight of vitellin was 326 kDa. Confirmation of the vitellin band by selective staining showed that the yolk protein was a lipo-glyco-carotenoprotein possessing a calcium moiety. A protein fraction with molecular weight 270 kDa, which was a lipoprotein, was expressed in ovaries of all maturity stages as well as in testis. The major biochemical constituents of mature yolk of *M. monoceros* on dry weight basis were protein (59.36±1.18%), lipid (31.23±2.98%) and carbohydrates (2.97±0.81%). Carotenoids accounted for 0.70±0.81% wet weight of yolk.

Introduction

The major gametogenic process in the female reproductive cycle of crustaceans involve the synthesis of nutritive yolk in the ooplasm, to meet the basic requirements of embryonic development independent of the maternal organism (Adiyodi and Subramoniam, 1983). In shrimps, during maturation, the weight of the ovary increases four to eight fold (Ravid *et al*., 1999). During this process, sufficient nutrients have to be accumulated in the egg yolk to allow the normal development of the embryos and pre-feeding larvae. Thus vitellin, the major yolk protein that accumulates within the ovary during vitellogenesis, functions as a source of nutrients for the successful development of an embryo independent of its mother. Unlike many other decapods, the nauplii of penaeid shrimps do not get any parental care or protection (Cook and Murphy, 1969). During the first one to two days, penaeid nauplii subsist entirely on yolk retained from the eggs. Therefore, the quality and quantity of egg yolk is crucial for early life stages of penaeid larvae.

Studies on biochemical changes in relation to ovarian maturation in invertebrates have been pioneered by Giese and Pearse, 1974. In penaeids, such studies have been done in *Metapenaeus affinis* (Sarojini *et al*., 1986*)*; *Penaeus japonicus* (Teshima *et al*., 1989) and *Penaeus aztecus* (Castille and Lawrence, 1989). The biochemical composition of mature yolk of *Penaeus indicus* and *Metapenaeus dobsoni* have been estimated by Mohamed and Diwan, (1992) and Vasudevappa (1992).

Vitellogenin, a female specific protein circulating in the haemolymph is the precursor of vitellin/lipovitellin, (Kunkel and Nordin, 1985). Ovarian vitellin has been purified and characterised from several penaeid shrimps (Vazquez-Boueard *et al*.; Tom *et al*.; 1992; Qui *et al*.; 1997).

Metapenaeus monoceros is one of the commercially important penaeid species found

along both the coasts of India. With an average annual landing of around 10,000 t, it accounts for 7-10 % of the total penaeid shrimp catch of the country (Sukumaran *et al.*, 1993). There is very good scope for this species to be taken up for semi-intensive culture practices in India due to their larger size among the *Metapenaeus* species.

Material and methods

Live specimens of *Metapenaeus monoceros* were collected during November 2001-June 2003 from trawlers operating from Kalamukku and Murikkumpadam fish landing centres of Vypeen island (Latitude 10.08° N, Longitude 76.21° E) in Kerala. Live adult shrimps of size ranging from 90 to 160 mm were transported from fishing grounds to the landing centres in 25 litre plastic bins holding aerated sea water. Shrimps were then transported live to the laboratory of Central Marine Fisheries Research Institute (CMFRI) at Cochin where they were segregated sex-wise and kept in aerated seawater (32 ppt) in 1 ton fibreglass tanks.

Maturity Stages

The stages of ovarian maturation were identified based on the external appearance of the ovary using a key modified from Rao (1989) as immature, early maturing, late maturing, mature and spent.

Vitellin characterisation

Homogenates for vitellin characterisation was prepared according to Qiu *et al.* (1997). Ovaries from female shrimps at different stages of maturation and testes from male shrimps were removed and rinsed in cold 100 mM phosphate buffer (pH 7.2) containing 0.001% phenyl methyl sulfonyl fluoride (PMSF) as a protease inhibitor. The samples were homogenized in three times the volume of the same buffer and centrifuged at 10,000 rpm for 15 min at 4°C. The floating fatty layer and the precipitate were discarded. The same process was repeated twice. Supernatants from the extracts of ovaries and testes were stored at -80°C until analysis.

Crude ovarian and testicular extracts were subjected to Polyacrylamide gel electrophoresis (PAGE) on 7.5 % slab gel using a mini electrophoresis apparatus (Genei, Bangalore) according to Davis (1964). After PAGE, glycolipoproteins were visualized by staining with 0.2 % Coomassie brilliant blue R250 for proteins, Sudan black B for lipoproteins, PAS for glycoproteins and with alizarin red 'S' for calcium bound proteins. For determination of molecular weight of lipovitellin, native PAGE molecular weight marker (Urease trimer - 272 kDa) was run along with the samples (Bryan, 1977).

Biochemical studies

Live female shrimps in mature stage (stage IV) were sacrificed and ovary dissected for determination of biochemical composition of yolk. The samples of ovary were dried at 60° C to constant weight, homogenized and dried again for 1-2 h before storing in airtight glass vials in dessicator. Dried ovarian samples from 8 shrimps were stored separately without pooling. Aliquots from these dried samples were taken for biochemical analysis. Total proteins were estimated calorimetrically by Folin – Ciocalteu phenol method (Lowry *et al*., 1951), carbohydrate by phenol - sulphuric acid method (Dubois *et al*., 1956) and total lipids by sulphophosphovanillin method (Barnes and Blackstock, 1973). The total carotenoid content of fresh ovarian samples was estimated spectrophotometrically as per the method of Olson (1979). A minimum of five ovarian samples were used for each biochemical parameter.

Results

The protein profiles of ovarian homogenates of stages I to V and testicular homogenate resolved through the native PAGE are presented in Fig. 1. From an array of many ovarian proteins, a high molecular weight protein fraction was identified as the yolk

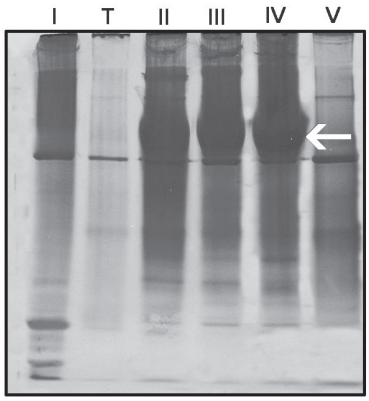

Fig. 1. Native PAGE illustrating the appearance of vitellin in ovary of females at different maturity stages. I – stage I ovary; T – testis; II – stage II ovary; III – stage III ovary; IV – stage IV ovary; V – stage V ovary; ← - vitellin

protein or vitellin. It was often possible to identify the vitellin fraction from unstained gels because of its characteristic green colouration. The expression of yolk protein vitellin was observed to be dependent on maturity stages. This protein fraction showed a steady increase in the level of expression with the maturity stages (stage II to IV). The vitellin fraction was not expressed in immature ovaries. Vitellin was absent in spent ovaries as well as in testicular homogenates (Fig. 1). Molecular weight of vitellin was found to be 326 kDa (Fig. 2).

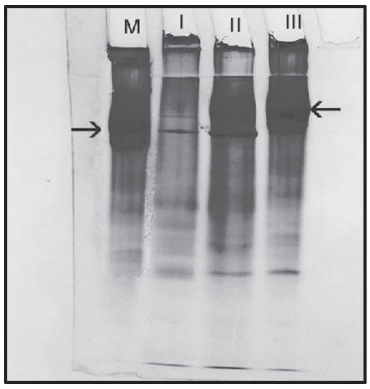

Fig. 2. Native PAGE for molecular weight determination of vitellin. M – marker; I – stage I ovary; II – stage II ovary; III – stage III ovary; ← - vitellin; → - Urease (272 kDa)

Confirmation of this protein as vitellin was made through selective staining of proteins in the gel based on the knowledge that yolk protein is a lipo-glyco-carotenoprotein possessing a calcium moiety. The results of the selective staining are shown in Figs. 3, 4 and 5. The 326 kDa band was stained with Sudan black B, periodic acid schiff (PAS) and alizarin red 'S' indicating that it is a lipo-glycoprotein with calcium.

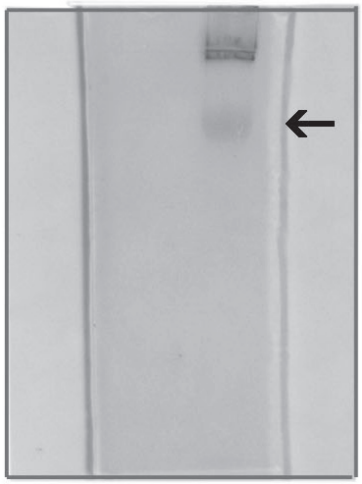

Fig. 3. Native PAGE of vitellin stained with sudan black B. ← - vitellin

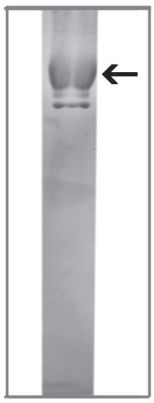

Fig. 4. Native PAGE of vitellin stained with PAS. ← - vitellin

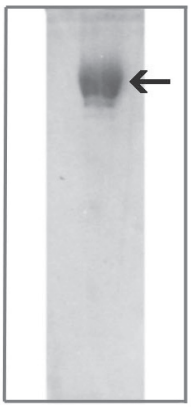

Fig. 5. Native PAGE of vitellin stained with alizarin red S. ← - vitellin

Another interesting observation from PAGE was the presence of a protein fraction with molecular weight 270 kDa that was expressed in ovaries of all maturity stages (Fig. 1). Surprisingly this protein fraction was observed in the testis samples also. This protein fraction was stained with Sudan black B and alizarin red 'S', but not with PAS indicating that it is a lipoprotein bound to calcium.

The mature yolk of *M. monoceros* had 68.3±1.4% moisture. The major biochemical constituents were protein, which formed 59.36±1.18% and lipid, which formed 31.23±2.98% of dry weight. Carbohydrates formed just 2.97±0.81% of dry weight whereas carotenoids accounted for 0.7±0.81% of wet weight of yolk.

Discussion

The molecular weight of the yolk protein vitellin in *M. monoceros* estimated in the present study is 326 kDa. Qiu *et al.* (1997) have reported a molecular weight of 350 kDa for native vitellin from *M. ensis*, after purifying the protein employing gel filtration and ion exchange chromatography. For vitellin from *P. monodon*, Quinitio *et al.* (1990) and Chang *et al*. (1993) reported molecular weights of 540 and 492 kDa, respectively. Tom *et al.* (1992) purified and characterized vitellin from *P. vannamei* and *P. semisulcatus* and reported a molecular weight of 289 kDa for the former and 283 kDa for the latter. From the above mentioned studies, it is evident that the molecular weight of native vitellin in most penaeids range from 300 kDa to 500 kDa.

Only one form of vitellin was observed in *M. monoceros*. This was in agreement with the single unit vitellins observed in *P. monodon*, *P. vannamei*, *Parapenaeus longirostris* and *Pandalus kessleri* (Tom *et al.*, 1987, 1992; Quinitio *et al.*, 1990; Chang *et al.*, 1993). Chang *et al.* (1996) isolated two forms of vitellin of 380 and 500 kDa, from the ovary of *P. chinensis.* The band of high mobility observed in native PAGE gels with the ovarian homogenates of all maturity stages (stages I to V) as well as the testicular homogenate of *M. monoceros* was found to have a molecular weight of 270 kDa and identified as a lipoprotein with calcium. Qiu *et al*. (1997) also reported a similar faster moving band in the ovarian and testicular homogenates of *M. ensis*.

Vitellogenesis is the process of hormonally regulated synthesis of yolk proteins namely vitellogenin and vitellin. Vitellogenin that circulates in the hemolymph is the precursor of vitellin, the yolk protein proper (Kunkel and Nordin, 1985). Vitellin in *M. monoceros* is of low electrophoretic mobility. On the basis of a Sudan black B staining, PAS positivity and pigment analysis vitellin has been identified as a lipo-glyco-caroteno protein (Adiyodi, 1968).

In crustaceans, yolk is a combination of proteins, lipids, sugars and some steroid hormones (Adiyodi, 1968). Protein constituted the major organic reserve in the mature ovary of *M. monoceros*, followed by lipids and carbohydrate which formed only 3 % by dry weight. In *P. indicus*, Mohamed and Diwan (1992) and in *M. dobsoni*, Vasudevappa (1992) noticed a similar trend in the concentrations of these nutrients. The major biochemical constituents are presented as a function of dry ovarian weight, which give a better picture regarding changes in the composition without being masked by changes of the moisture

content (Clarke *et al*., 1990). In crustaceans, it is generally accepted that the yolk proteins provide the basic structural material needed for tissue build up during embryonic development, while lipids serve as the major fuel component (Adiyodi and Subramoniam, 1983). The low carbohydrate concentration found in eggs of *M. monoceros* excludes this nutrient as a source of energy. Similar observations have been reported for other decapods (Clarke, 1992; Mohamed and Diwan, 1992). Carbohydrates, though in small quantities are essential during embryogenesis for the synthesis of specific compounds such as chitin for exoskeleton (Holland, 1978).

Carotenoids are present in shrimp ovary as a component of the yolk protein vitellin thus imparting the characteristic colour to the fully mature ovary. Hence in native PAGE it was possible to visualize this fraction by its green colour, even before staining the electropherograms. The increase in carotenoid concentration with ovarian maturation could be attributed to the ability of carotenoids to bind yolk protein vitellin into a lipo-glyco-caroteno protein complex which gets accumulated in the oocyte cytoplasm and serve as a source of food for the embryo (Harrison, 1990). Carotenoids, particularly astaxanthin, are also strong scavengers of free radicals which can protect the eggs from oxidative deterioration.

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