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Major Yolk Protein in the Mud Shrimp, *Upogebia major* (Crustacea, Thalassinidea, Upogebiidae): Analysis of Vitellogenin Gene Expression and Vitellogenin Processing*

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Vitellogenin, the precursor of the major egg yolk protein (vitellin) in oviparous animals, is synthesized in sex- and tissue-specific manners, secreted into the blood and accumulated in developing oocytes. It is consumed as a nutrient during embryogenesis. Vitellogenin synthesis is induced in males of several aquatic species by exposing them to environmental factors such as endocrine-disturbing substances.

It was reported that a number of crustaceans such as caridean shrimps showed sequential hermaphroditism. In these species, normal eggs are formed in the male gonads (Bauer and Holt, 1998). The existence of intersex was reported in several decapodan species, in which males and females were not distinguishable because they had morphological features of both sexes (Sagi *et al.*, 1996; Pinn *et al*., 2001).

Males of the mud shrimp, *Upogebia major*, synthesize yolk-like substances that accumulate in the gonads (Ishikawa, 1891; Tucker, 1931; Oka, 1941; Kinoshita *et al*., 2003). Although males and females of *U. major* are distinguishable by their sexually dimorphic characteristics, the pair of gonads in males is each composed of a testicular part located at the anterior region and an ovarian part at the posterior region. Yolk-like substances accumulate in the "ovarian part of the testis" in males in a seasonal cycle, as in females. However, males accumulating yolk-like substances never ovulate because they lack oviducts and their sex never alters. There is limited information so far on the yolk proteins of *U. major.* In the present study, we cloned full-length vitellogenin cDNA, and analyzed the gene structure, expression and vitellogenin processing.

Full-length vitellogenin cDNA was obtained by degenerate PCR and RACE-PCR using the cDNA library constructed from the female hepatopancreas. It was 7813 bp-long and encoded 2568 amino acids in a single open reading frame. The deduced amino acid sequence had a putative signal peptide of 18 amino acid residues. The estimated molecular mass was 290.8 kDa. The deduced amino acid sequence aligned well with vitellogenins of other decapodan species. There are several RXXR sequences known as likely cleavage sites that are recognized by endoproteases belonging to the subtilisin family (Barr, 1991). The RXXR site located near the N-terminal region (RLRR) was conserved among other vitellogenins of decapodan Crustacea. Vitellogenins of *Pandalus hypsinotus* and *Penaeus semisulcatus* are actually cleaved at this site and detected as two polypeptides in the hemolymph (Tsutsui *et al*., 2004; Avarre *et al*., 2003).

Vitellogenin gene expression of *U. major* was examined by Northern blotting and reverse transcription PCR (RT-PCR). Northern blotting showed that a transcript of 8 kb was detected in the female hepatopancreas and in ovaries. RT-PCR revealed that the vitellogenin gene was expressed in the hepatopancreas of males and females, and it was also expressed in the ovaries of females and the ovarian part of testes of males. The results indicated that the *U. major* vitellogenin gene was expressed both in the hepatopancreas and the gonads of both sexes.

The major yolk proteins in eggs of *U. major* were detected as three prominent vitellin polypeptides (120 kDa, 100 kDa, and 80 kDa) on SDS-PAGE. Polyclonal antisera were raised against 100 kDa and 80 kDa polypeptides, respectively. Western blot analysis showed that anti-100 kDa antiserum reacted to the 100 kDa polypeptide and the 220 kDa polypeptide in the hemolymph. Anti-80 kDa antiserum reacted only to the 80 kDa polypeptide in the hemolymph. Similar results

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were obtained when male hemolymph was subjected to Western blotting.

These results, taken together, indicated that vitellogenin of *U. major* was synthesized in the hepatopancreas and secreted into hemolymphs after cleavage into two polypeptides of 220 kDa and 80 kDa. The 220 kDa polypeptide was then cleaved to 120 kDa and 100 kDa polypeptides in the hemolymph before being sequestered in eggs. There were no significant differences in the gene expression and processing of vitellogenin between males and females.

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