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Electrophoretic Analysis of Reproductive Tissues and Haemolymph Of the Marine Pebble Crab *Leucosia Anatum* (Herbst, 1783)

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Abstract

Elecrophoretic studies were carried out in the reproductive tissues and haemolymph of the pebble crab *Leucosia anatum* obtained from the fishermen employing trawl net on prior request from Adirampattinam sea shore. The reproductive organs like testis, vas deferens were removed; the spermatophores and seminal plasma were separated from the posterior vas deferens are used for electrophoretic study. Eleven (11) bands (peptides) were resolved in the haemolymph of *L. anatum*, whereas the testis, proximal middle and distal vas deferens, spermatophore and seminal plasma contain 3, 8, 8, 7, 6 and 3 respectively of the eleven bands. From the results, it was evident that the occurrence of homologous protein fractions suggests the possible origin of spermatophore, seminal plasma from testis, vas deferens and haemolymph.

Keywords

Leucosia anatum, Vas deferens, Spermatophore, seminal plasma.

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INTRODUCTION

The non-motile spermatozoa of decapods crustaceans are enclosed in packets called spermatophores for transmission to females during mating. The spermatophore is composed of a number of biochemical substances, which protect the flimsy and fragile spermatozoa from the damage caused, by aridness and microbial infection during their epizoic storage in the female body prior to fertilization in many groups of crustacean (Kumarasamy, 2001; Subramoniam, 1991). The seminal plasma enclosed within the spermatophore is rich in organic substances necessary not only for sperm metabolism but also provides tackiness to the sperm and mechanical support.

The seminal plasma of decapods crustaceans materialize to be mucoproteinaceous in Libinia emarginata (Hinsch & Walker, 1974) in Ocypode

platytarsis (Sukumaran, 1985) in Scylla serrta (Jeyalectumie & Subramoniam, 1991) in Clibanarius infraspinatus and Diogenes costatus (Kumarasamy, 2001) and in Parasesarma plicatum (Ganapiriya et al., 2017). Reproductive capacities of most of the insects are synchronized by accessory reproductive glands (Muse, 2002). Lange & Loughton (1984) demonstrated the presence of a variety of proteins in the accessory glands of Locusta migratoria. A molecular weight fraction of 72 kDa from the long hyaline gland of Melanoplus sanguinipes was reported to constitute a major component of viscous secretion for the formation spermatophores (Cheeseman & Gillott, 1989). Muse (2002) noticed the presence of several similar protein fractions in the seminal vesicles and accessory glands of Aractomorpha lata.

Dostal & Matousek, (1972) observed the seminal pasma proteins in the blood serum of bull. Fitzgerald & Foor, (1979) noticed the seminal plasma proteins in the body fluid of Ascaris. Homology of protein fractions have been reported by Kumarasamy (2001) in D. costatus and C. infraspinatus. The present study is carried out to explore the possible origin of proteins of spermatophore and seminal plasma from different tissues of L. anatum.

MATERIALS AND METHODS

Separation of Proteins by SDS-PAGE the protein fractions of the vasal contents were separated by polyacrylamide (slab) gel electrophoresis. Tris-HCl buffer (pH 8.8) was used for preparing the running gel (10% acrylamide), while Tris-HCl buffer with pH 6.8 was used for the spacer gel (1.5% acrylamide). Tank buffer was prepared using Tris-glycine buffer at pH 8.5. Protein from the vasal content was extracted out by homogenizing with a mixture of glycerol, Tris HCl buffer (pH 6.8), 10% SDS and mercapto ethanol. After centrifugation, the supernatant was collected and used as the extract. 75 µl of the extract was loaded in the electrophoresis apparatus. Reference standards were also loaded along with the sample. A current of 20 mA was applied and the sample was allowed to run for 10-12 hours after which the gels were stained with coommassie blue. Destaining and differentiation of the bands were done using methanol: acetic acid: water mixture (2:1:7 v/v). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 10% polyacrylamide gel according to the method of (Laemmli, 1970).

100 mg of reproductive tissues viz. testis, proximal vasdeferens (PVD), middle vasdeferens (MVD), distal vasdeferens (DVD), seminal plasma, spermatophores (separted vide) of *L. anatum* were homogenized in 0.2 ml of phosphate buffer saline the homogenates were centrifuged at 1000 rpm and the supernatant was taken as the sample for gel electrophoresis. Glass plates were rinsed and wiped with ethanol and were fixed on to the assembly using the spacers and bull dog clips. The lower edge was sealed with 1% Agar. Separating gel was prepared in a beaker (3.2 ml distilled water, 2.64 ml 30% acrylamide, 2 ml 1.5 M Tris, 0.80 ml 10% SDS, 0.08 ml 10% Ammonium persulfate (APS), 0.004 ml N, N, N, N N‟-Tri methylamine triamine (TEMED), were added just before pouring into the gel plate). The separating gel was poured between gel plates up to the required level (in a mini gel about 7.8 cm). Few ml of butanol was poured to overlay the separating gel and allow the gel to polymerize. After polymerization, the butanol was poured off and the gel was washed with distilled water. The stacking gel was prepared in a beaker (2.1 ml distilled water, 0.5 ml 30% acrylamide, 0.38 ml of 1.0 M Tris, 0.03 ml 10% SDS, 0.03 ml 10% APS, 0.003 ml TEMED APS and TEMED were added just before pouring). The comb was inserted, and the stacking gel solution was poured. After polymerization of stacking gel, the comb was removed, and the wells were washed with distilled water. The assembly was filled with distilled water. 25 µl of sample buffer was added to protein solution in an eppendorf vial and boiled for 2-3 minutes. Then it was allowed to cool. The sample wells were loaded with the sample using a Hamilton syringe. The assembly was connected to the power pack. Electrophoresis was carried out at 20 mA till the samples reach the separating gel and then it was continued at 40 mA for 2-3 hours until the dye front reaches the agar layer. The gel was removed and stained with Coommassie blue for 24 hours. After destaining the gel was photographed.

RESULTS AND DISCUSSION

Electrophoretic analysis of the male reproductive tissues of *L. anatum* viz. testis, PVD, MVD DVD, spermatophores and seminal plasma were done along with haemolymph. In the present study 11 bands (peptides) were resolved in the haemolymph (Plate1) of *L. anatum,* whereas the testis, PVD, MVD, DVD, spermatophore and seminal plasma contain 3, 8, 8, 7, 6 and 3 respectively. Homologous proteins in the hemolymph and reproductive tissues were observed in the present investigation, the protein fractions 38, 40, 68, 72 and 170 kDa of spermatophore are found in the haemolymph. Similarly, the protein fractions 85, 72 and 68 kDa of seminal plasma are found in the haemolymph.

In the present study, the protein fractions 38, 40, 68, 72, 170 kDa of the haemolymph are found in PVD, MVD DVD and spermaophore but the protein fractions 72 and 68 kDa alone are found in the seminal plasma of *L. anatum.* Proteinaceous substances involved in the reproductive processes are shown to undergo amazing changes in accordance with their utilization in various formative as well as metabolic processes. An electrophoretic analysis of the male reproductive tissues of *L. anatum* has been made to study the origin of different proteins from the reproductive tissues. It is believed that many of the reproductive activities depend on blood borne proteins for the synthetic activities (Adiyodi & Adiyodi, 1976). Hence the haemolymph of male *L. anatum* has been analysed electrophoretically along with reproductive tissues and spermatophore. The results are presented in the form of electrophorogram .

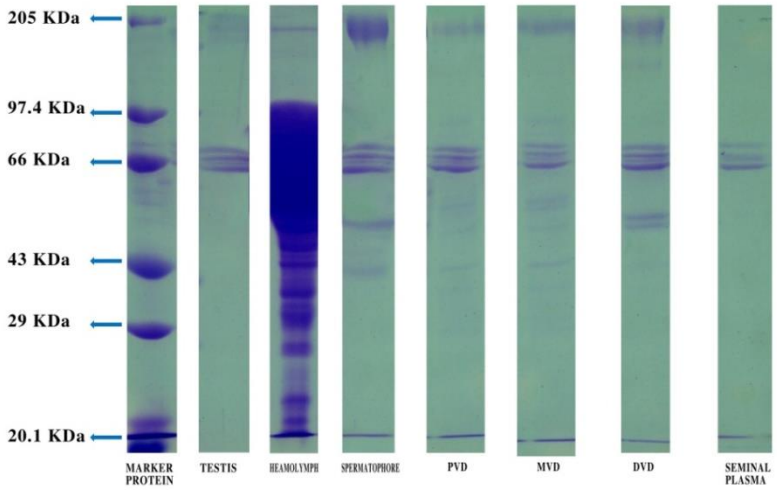
In the present study, 11 protein fractions were resolved in the haemolymph of *L. anatum.* The testis, PVD, MVD, DVD, spermatophore and seminal plasma contain 3, 8, 8, 7, 6 and 3 respectively. Homologous proteins in the hemolymph and reproductive tissues were observed in the present investigation. The protein fractions 38, 40, 68, 72 and 170 kDa of spermatophore are found in the haemolymph. Similarly, the protein fractions 85, 72 and 68 kDa of seminal plasma are also found in the haemolymph. The above findings fall in line with the result concerning the origin of the seminal plasma proteins from the testis of mammals (Koskimies & Kormano, 1973, 1975) and in an Aschelminthes worm *Ascaris suum*.

The protein fractions 38, 40, 68, 72, 170 kDa of the haemolymph are found in PVD, MVD DVD and spermaophore but the protein fractions 72 and 68 kDa alone are found in the seminal plasma of *L. anatum.* Homologous proteins in the haemolyph and reproductive tissues suggested that the proteins might have been synthesized elsewhere and they sequestered into the testes. Evidences in favour of the origin of seminal plasma proteins from the blood serum were available in mammals. Occurrence of familiar proteins in the seminal plasma, testes and body fluid has also been indicated in lower invertebrate groups (Fitzgerald & Foor, 1979; Tyler, 1946).

Hinsch & Walker (1974) observed vesicles in intercellular spaces and functional complexes in the anterior vas deferens of the spider crab *L. emarginata*. These structures are less frequent in the middle vas deferens and absent in the posterior vas deferens. They suggested that these structures are the substantial evidences of possible origin of seminal plasma proteins from haemolymph. In the present investigation the protein fractions 72, 68 and 62 kDa are found in all the reproductive tissues and spermatophore. Muse (2002) observed the 72 kDa protein in the yellow gland (accessory reproductive gland) as well as in the spermatophore of A. *lata* which was suggested to be responsible for the preservation of spermatophore. In *A. lata,* each accessory gland type produces over 40 fractions, demonstrating their heterogeneity and the ability to export mixtures of proteins during mating. The presence of identical band patterns in accessory gland types of *A. lata* was one of the reasons given for the existence of regional specialization in the synthesis of proteins in the accessory glands of *Locusta migratoria* (Laemmli, 1970). Huang *et al*. (2006) isolated a single-chain polypeptide (Scygonadin-from the gonad of *S. serrata*) with molecular weight of about 10.8 kDa, reported to have antimicrobial role, was from the seminal plasma of the mud crab, *S. serrata.*

It is quite obvious that all the bands do not represent free protein moieties, but instead, may exist in conjugated forms. In *S. serrata*, out of the 11 bands, five bands are found to be glycoproteinaceous in nature (Jayasankar and Subramoniam, 1999). Biochemical analysis of the seminal plasma of various animal groups indicates its complex nature by conjugation with the carbohydrate substance giving characteristic mucoid nature to the seminal plasma (Nevo & Rikmenspoel, 1970). Although the potential roles of the secretions of the ductal glands are considerable, it should be stressed that a complete chemical characterization of the seminal secretion would be necessary for a full understanding of their roles in reproduction.

PLATE - I



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