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RESEARCH ARTICLE

Studies on muscle albumin proteins of the deep water mud shrimp *Solenocera melantho* (De Man, 1907) by polyacrylamide gel electrophoresis (PAGE)

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Abstract

Muscle albumin proteins of males and females of *Solenocera melantho* were studied electrophoretically with water and Tris- HCl buffer at five concentrations (0.1M to 0.5M) against three marker proteins - Catalase (240 kDa), Bovine Serum Albumin (67 kDa) and Ovalbumin (14 kDa). Protein fractions of 3, 7, 5, 3, 4 & 3 protein fractions were observed in males with water, 0.1M, 0.2M, 0.3M, 0.4M and 0.5M-Tris HCl extractions respectively whereas in females 6 protein fractions with water, 6 with 0.1M, 4 with 0.2M, 5 with 0.3M, 4 fractions each with 0.4M and 0.5M Tris-HCl were resolved. The molecular weights of the albumin fractions ranged from 50.65kDa to 219.46 kDa in both sexes. Significant difference was observed between the sexes though there was some similarity in some of the band pattern.

INTRODUCTION

Shrimps are generally identified by their morphological characteristics like rostral spines, sulci, carina and spines on the carapace, petasma and thelycum etc. (Kubo, 1949) The characteristics expressed by different species are species specific, since they are the products of the genes. Proteins are also of such characteristics or traits, which may vary with species and sex both qualitatively and quantitatively and hence such variations, are often used as biochemical markers. Protein constitutes about 20% of the muscle and is subjected to change depending on factors such as the availability of food, moult condition, spawning and migration. The muscle is composed of different types of proteins such as albumins, myosin, actin, actomyosin, tropomyosin and are extractable with solvents of different ionic strengths of salt solutions, organic solvents and acids (Viswanathan and Suseela Mathew, 2000).

Muscle albumins are the proteins of sarcoplasm and other interstitial fluids. Albumins are soluble in water, coagulated by heat and precipitated in salt solutions of low ionic strength. Albumins include water-soluble myoalbumins and water-insoluble myogens. The albumin fractions constitute about 16-22% of the total muscle proteins and can be extracted with the salt solutions of low ionic strengths *i.e.*, < 0.5M (Viswanathan and Suseela Mathew, 2000). Different types of albumin proteins are soluble in different ionic strengths of salt solutions below 0.5M. The albumin proteins impart specific taste and flavour to the muscle and different species possess different types of albumins. The accumulation of these proteins may vary with sex, size and season, since they are of primarily genetic origin and secondarily of the diet (Viswanathan and Suseela Mathew, 2000). In penaeid shrimps, protein is the major constituent of muscle. Its composition varies with species, sex, size and season (Shaikhmuhmad and Magar, 1957).

Electrophoresis is the movement of charged particles under the influence of an electric field and is the main method for analysis of the biochemical systematics in various taxa (Avise, 1975). All proteins carry surface electric charge which is determined by their amino acid composition and the pH of the medium. Each species is identified by a specific number of proteins by means of high-resolution starch or polyacrylamide gel and isoelectic focusing which forms species-specific band patterns of different proteins and the protein is a translated phenotypic expression of a genetic code and the variations in the genotype usually result in change of the structure and function of proteins

(Bye and Ponnaiah, 1983). The relative mobility of proteins at an electric field depends on their molecular weight, conformation and surface electric charge (Mc Laughlin *et al.*, 1982). Studies on muscle proteins can be dated back to Roth (1947), who has demonstrated that the classical muscle protein fractions of fresh water fish resemble those of rabbit or frog. The other notable works on muscle albumin proteins are those of Connell (1953), Chakravarty *et al* (2009) and Chakravarty *et al* (2014). An attempt is made to study the electrophoretic separation of muscle albumin proteins at different concentrations of Tris- HCl up to 0.5M against marker proteins.

Material and methods

Shrimp specimens of *S. melantho* were collected from the trawl catches off Visakhapatnam in the coastal waters of the Bay of Bengal. The specimens were kept in insulated ice-box and brought to the laboratory where they were separated as per sex. The abdominal muscles of both the sexes were taken and blotted on a filter paper. The wet weight of the muscle of the individual specimen was recorded and after drying in hot air oven for about 48 hrs at $55^{o} – 65^{0}$c, dry weight was taken. The dried tissue was finely powdered in a mortar and was used for electrophoretic studies (PAGE) following the method of Sambrook and Russell (1988). The albumin proteins of muscles were isolated with water and Tris- HCl buffer (0.1M, 0.2M, 0.3M, 0.4M and 0.5M). The marker proteins (MP) used were MP-1 Catalase (240 kDa), MP-2 Bovine Serum Albumin (67 kDa) and MP-3 Ovalbumin (14 kDa).

Results

Water soluble albumins

In males three albumin bands were identified with the molecular weights of 155.54 kilo Daltons (kDa), 138.48 kDa and 93.27 kDa whereas in females six albumin proteins with molecular weights ranging from 50.65 kDa to 155.54 kDa were found. Of the six proteins in females three were different from males with molecular weights 64.90 kDa, 59.53 kDa and 50.65 kDa (Table 1; Fig.1a & b). The common bands observed in both males and females were intense and the other three bands in females were faint (Table 2). The zonal-wise dispersion of protein bands males showed two intermediate and one faint while in females the intermediate, fast and very fast were of two each (Table 3).

Albumins at 0.1M Tris -HCl

Albumins were found as seven bands in males and six in females. The relative mobility in males ranged from 0.33 to 0.81 and the molecular weights from 50.65 kDa to 155.54 kDa. In females the relative mobility and molecular weights of protein bands were in the order of 0.33 and 155.54kDa, 0.40 and 138.48kDa, 0.59 and 93.27kDa, 0.73 and 64.90kDa, 0.76 and 59.53kDa and 0.81 and 50.65kDa. The male differed from female in having the protein band with a relative mobility of 0.68 and with a molecular weight 65.9kDa (Table 1; Fig.1a & b). Males and females showed three intense and three medium bands. The extra band observed in males was intense (Table 2). With respect to dispersion of zones, males showed two intermediate, three fast and two very fast whereas in females they were of two intermediate, two fast and two very fast (Table 3).

Albumins at 0.2M Tris -HCl

At this concentration, five bands in males and four in females were observed with four similar albumins in both the sexes i.e. albumins with the relative mobilities of 0.33, 0.40, 0.59 and 0.73 and the molecular weights of 155.54 kDa, 138.48 kDa, 93.27 kDa and 64.90 kDa. Male differed from female in protein with a relative mobility of 0.81 and molecular weight of 50.65 kDa (Table 1; Fig.1a & b). Males showed three intense and two medium bands. In females three intense and one faint band were observed (Table 2). The zonal-wise dispersion showed two intermediate and two fast bands in males and two intermediate, two fast and one very fast band in females (Table 3).

Albumins at 0.3M Tris-HCl

In males three albumin bands were found with relative mobilities of 0.14, 0.33 and 0.44 and molecular weights of 219.46 kDa, 155.54 kDa and 138.48 kDa. Females showed five bands at this concentration with relative mobilities of 0.14 to 0.68 and molecular weights ranging from 65.19 kDa to 219.46 kDa. Females differed from males in having three different protein bands of molecular weights 130.62 kDa, 106.79 kDa and 65.19 kDa (Table 1; Fig.1a & b). Two medium and one intense band were observed in males and in females all the bands were intense (Table 2). The males showed one slow and two intermediate bands in zonal- wise dispersion whereas in female's one slow, two intermediate and two fast bands were observed (Table 3).

Albumins at 0.4M Tris-HCl

In both males and females four protein bands with relative mobilities of 0.14, 0.33, 0.43 and 0.68 and molecular weights of 219.46 kDa, 155.54 kDa, 130.62 kDa and 65.19 kDa respectively were observed. There was no sex- based difference (Table 1; Fig.1a & b). In males, three medium and one intense band were observed and in

females all bands were intense (Table 2). In zonal- wise dispersion of protein bands also both males and females showed one slow, two intermediate and one fast bands (Table 3).

Albumins at 0.5M Tris-HCl

Males showed three bands and females four whose relative mobilities ranged from 0.14 to 0.68 and the molecular weights from 65.19 kDa and 219.46 kDa. Proteins with molecular weight 65.19kDa present in female was absent in male (Table 1; Fig.1a & b). Three medium bands in males and four intense bands in females were found (Table 2). In males one slow and two intermediate bands were observed whereas in females they were one slow, two intermediate and one fast band in the zonal-wise dispersion of bands (Table 3).

Table 1 Relative mobility and molecular weights(kDa) of muscle albumins with water and at 0.1M, 0.2M, 0.3M, 0.4M & 0.5M Tris- HCl in relation to marker proteins of males and females of *S. melantho*.

Marker proteins	Molecular Weights	Relative mobility's of marker proteins
Catalase	240	0.13
Bovine serum albumin	67	0.66
Ovalbumin	43	0.90

S.No.	Rm Valuesof different muscle albumins	Molecular weight(kDa) of the muscle albumins	Water soluble		0.1M		0.2M		0.3M		0.4M		0.5M	
			Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	0.14	219.46	-	-	-	-	-	-	+	+	+	+	+	+
2	0.33	155.54	+	+	+	+	+	+	+	+	+	+	+	+
3	0.40	138.48	+	+	+	+	+	+	+	-	-	-	-	-
4	0.43	130.62	-	-	-	-	-	-	-	+	+	+	+	+
5	0.53	106.79	-	-	-	-	-	-	-	+	-	-	-	-
6	0.59	93.27	+	+	+	+	+	+	-	-	-	-	-	-
7	0.68	65.19	-	-	+	-	-	-	-	+	+	+	-	+
8	0.73	64.90	-	+	+	+	+	+	-	-	-	-	-	-
9	0.76	59.53	-	+	+	+	-	-	-	-	-	-	-	-
10	0.81	50.65	-	+	+	+	+	-	-	-	-	-	-	-
Total No. of Bands			3	6	7	6	5	4	3	5	4	4	3	4

+ Presence - Absence

Table 2 Relative mobility, molecular weights (kDa) and band intensities of muscle albumins with water and at 0.1M, 0.2M, 0.3M, 0.4M & 0.5M Tris- HCl of males and females of *S. melantho.*

S.No.	Rm values	Molecular weight(kDa)	Band Intensity											
			Water soluble		0.1M		0.2M		0.3M		0.4M		0.5M	
			Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	0.14	219.46	-	-	-	-	-	-	M	I	M	I	M	I
2	0.33	155.54	I	I	I	I	I	I	M	I	M	I	M	I
3	0.40	138.48	I	I	I	I	I	I	I	-	-	-	-	-
4	0.43	130.62	-	-	-	-	-	-	-	I	I	I	M	I
5	0.53	106.79	-	-	-	-	-	-	-	I	-	-	-	-
6	0.59	93.27	I	I	I	I	I	I	-	-	-	-	-	-
7	0.68	65.19	-	-	I	-	-	-	-	I	M	I	-	I
8	0.73	64.90	-	F	M	M	M	F	-	-	-	-	-	-
9	0.76	59.53	-	F	M	M	-	-	-	-	-	-	-	-
10	0.81	50.65	-	F	M	M	M	-	-	-	-	-	-	-
Total No. of Bands			3	6	7	6	5	4	3	5	4	4	3	4

I = Intense M = Medium F = Faint

Table 3 Zonal - wise protein band dispersion of muscle albumins with water and at 0.1M, 0.2M, 0.3M, 0.4M & 0.5M Tris- HCl of males and females of *S. melantho.*

Zone	Number of bands present in											
	Water soluble		0.1M		0.2M		0.3M		0.4M		0.5M	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Slow (0.10 – 0.25)	-	-	-	-	-	-	1	1	1	1	1	1
Intermediate (0.26 – 0.50)	2	2	2	2	2	2	2	2	2	2	2	2
Fast (0.51 – 0.75)	1	2	3	2	2	2	-	2	1	1	-	1
Very fast (0.76 – 1.0)	-	2	2	2	1	-	-	-	-	-	-	-
Total No. of Bands	3	6	7	6	5	4	3	5	4	4	3	4

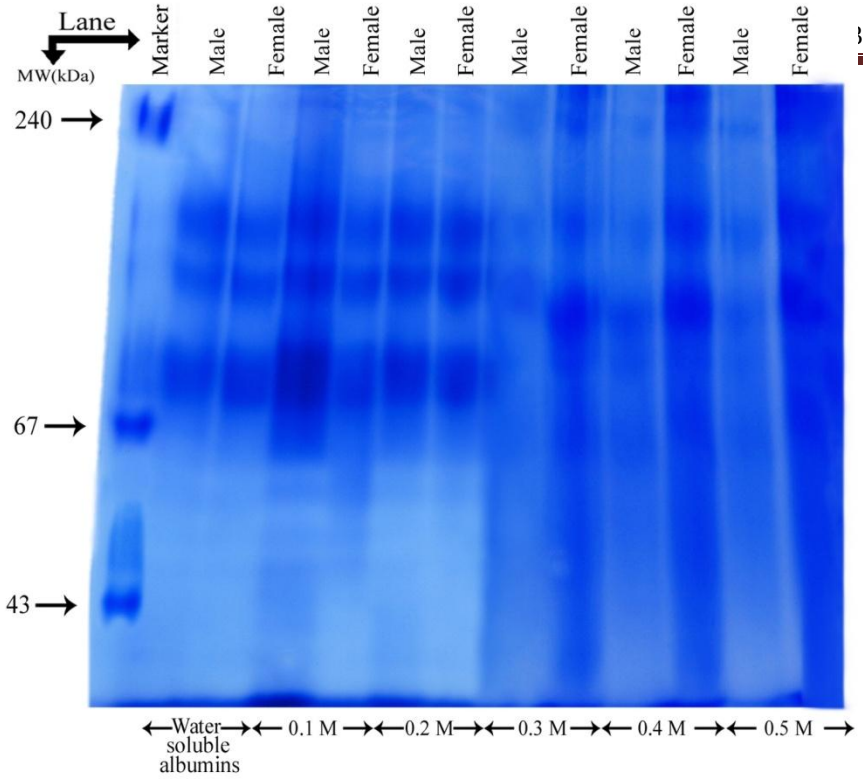

Fig. 1 (a) Electropherogram of muscle albumins with water and at 0.1M, 0.2M, 0.3M, 0.4M and 0.5M Tris- HCl in males and females of *S. melantho*.

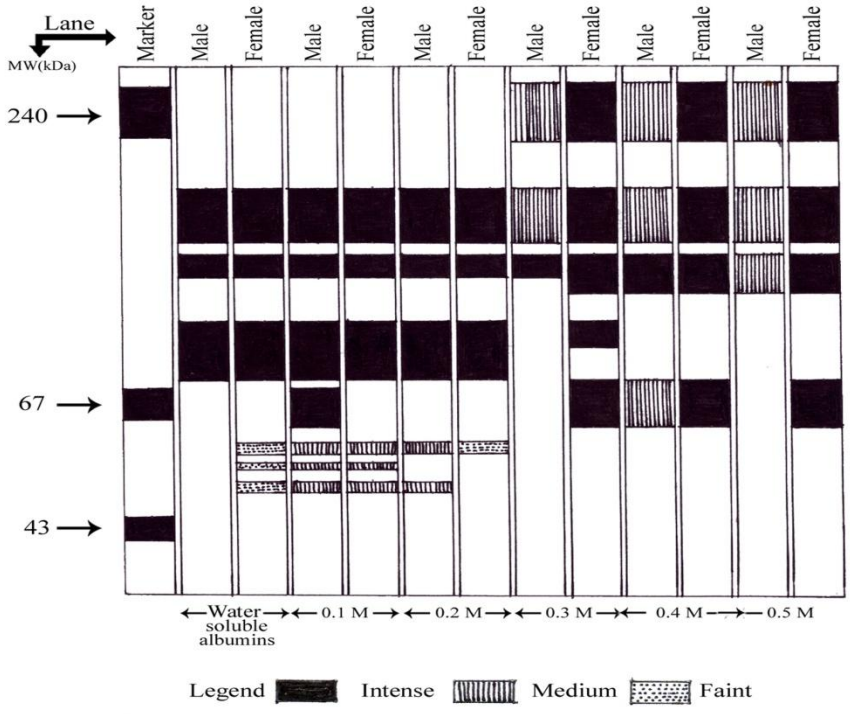

Fig. 1(b) Digrammatic representation of electropherogram of muscle albumins with water and at 0.1M, 0.2M, 0.3M, 0.4M and 0.5M Tris- HCl in males and females of *S. melantho*.

Discussion

Muscle albumins are one of the most important of the fish proximate principles found in the aqueous system of the muscle *i.e.*, sarcoplasm and myoplasm extractable with water or dilute salt solutions (Lovell, 1989). Electrophoretic separation of albumin proteins of the muscle by PAGE with water and salt solutions of low ionic strengths (0.1M to 0.5M Tris-Hcl) reveals the difference between the sexes of the deep water mud shrimp *S. melantho* in terms of presence or absence of a particular protein fraction and its relative mobility against marker proteins, staining intensity and molecular weights. Significant difference has been observed between the sexes though there is some similarity in some of the band patterns. In the present study males have shown 3, 7, 5, 3, 4 & 3 protein fractions in the water soluble, 0.1M, 0.2M, 0.3M, 0.4M and 0.5M-Tris HCl extractions respectively whereas in females 6 protein fractions in water soluble, 6 fractions in 0.1M, 4 fractions in 0.2M, 5 fractions in 0.3M, 4 fractions each in 0.4M and 0.5M Tris-HCl. The albumin fractions resolved were ranged from 50.65kDa to 219.46 kDa.

Connell (1953) has established the electrophoretic mobility of the codling, *Gadus callarias* skeletal muscle extracts- globulin X, myogen & myoalbumin at different ionic strengths such as 0.05I, 0.1I and 0.2I. In *Penaeus kerathurus*, Cuzon and Ceccaldi (1972) have identified the presence of seventeen protein fractions by PAGE. Lester (1979) has documented low level of genetic heterogeneity for *Penaeus aztecus*, *Fenneropenaeus duorarum* and *Penaeus setiferus* in the Gulf of Mexico. Kulkarni *et al* (1980) have observed 2,5,4,3 and 1,4,4,3 in males and females respectively in penaeid shrimp species of *Metapenaeus affinis, M. monoceros, Parapenaeopsis hardwickii* and *P. stylifera.* According to them 2 bands in males and 1 in females in *M. affinis*, 3 in males and 4 in females in *M. monoceros*, 2 in males and 2 in females in *P. hardwickii* and 1 in both male and femle of *P. stylifera* are thick.

Thirty seven genetic loci coding for soluble proteins and enzymes in six species of *Metapenaeus* and seven species of *Penaeus* commonly found in Australian waters have been reported by Mulley and Latter (1980). They have observed polymorphism in *P. monodon* at three loci and monomorphism at single loci. Thomas (1981) has studied the electrophoretic separation of abdominal muscle tissue of four commercially important species of penaeid prawns *viz., Fenneropenaeus indicus*, *Metapenaeus dobsoni*, *M. monoceros* and *M. affinis* by PAGE. According to him the number of the protein bands varied from 7 to13, indicating the distinct nature of the species investigated. Low level of genetic heterogeneity in Southern Australian population of *P.latisulcatus* has been observed by Richardson (1982). Lester (1985) has found a low level of genetic variation with little geographic differentiation within species among wild stocks of *Penaeus aztecus, P. setiferus, P. stylirostris* and *Litopenaeus vannamei* from American waters. Ko *et al* (1985) have observed a very low genetic diversity among thirteen populations of seven species of penaeid shrimps.

Philip (1987) has observed 8, 9, 10 proteins with three common fractions in *Parapenaeopsis stylifera, P. sculptilis* and *P. hardwickii* respectively from the West coast of India. Chan *et al* (1988) have observed no changes in the major polypeptides but in case of less abundant polypeptides, one small (32kDa) and one high molecular weight (175kDa) polypeptide have shown variation in relative abundance in *Litopenaeus vannamei* during the moulting cycle. Murthy (1991) has observed eight fractions in *Metapenaeopsis stridulans*, six in *M. barbata* and with two common fractions in the muscle extracts. Albores *et al* (1993) have identified a protein with 180kDa in the native state, 85 kDa under non-reducing and 41 kDa under reducing condition in shrimp haemagglutinin of *Penaeus californiensis.*

Sriraman *et al* (1995) have recorded a total of 12 protein fractions of which, the fractions 6, 7, 8 and 11 are common in four species of the genus *Penaeus i.e P. indicus, P. monodon, P. merguiensis* and *P. semisulcatus* and the fractions 5, 7, 10 and 12 are common in *Metapenaeus monoceros* and *M. dobsoni* showing their genetic affinity, whereas the fractions 6, 7 and 11 are common to *P. stylifera* and *P. maxillepedo* referring to their affinity. The presence of fraction 7 in both *Solenocera crassicornis* and *Metapenaeopsis stridulans* establishes their relationship with other species of the genera *Penaeus, Metapenaeus* and *Parapenaeopsis*. According to Lubzens *et al* (1997) the peptide of high density lipoprotein I (HDL-I) of male consists of 110 kDa whereas the female HDL is composed of two proteins- one is identical to that of male and the other, vitellogenin with molecular weights 200kDa, 120kDa and 80kDa in *Penaeus semisulcatus*.

Xu *et al* (2001) have reported six to eleven electrophoretic bands of proteins by PAGE in the muscular proteins of six species of shrimps *Macrobrachium rosenbergii*, *Aristeus virilis*, *Penaeus penicillatus*, *P. monodon*, *Metapenaeus japonicus*, and *M. joyneri*. According to them the protein analysis was consistent with the extent of taxonomy of the shrimp group, confirming the phylogenetic relationship. Vazquez Boucard *et al* (2002) have identified a major protein of 500kDa molecular weight by PAGE from the ovary extract of vitellogenic shrimp *Fenneropenaeus indicus*. Kruevaisayawan *et al* (2007) have characterized major protein bands ranging from 35-230kDa in the cortical rods of *Penaeus monodon*. Kazuo Shiomi *et al* (2008) have purified a new sarcoplasmic calcium binding protein (SCP) crustacean allergin with 20kDa from the muscles of the black tiger shrimp *Penaeus*

monodon. Chakravarty *et al* (2009) have found 11 fractions in *T. sedili* and *T. pescadorensis* and 7 fractions in *T. curvirostris* with 6 common fractions at 0.1M Tris-HCl. At 0.2M Tris- HCl, 6 fractions in *T. curvirostris* and *T. pescadorensis* and 5 fractions in *T. sedili*. Chakravarty *et al* (2014) have reported no bands in *Apolectis niger* and maximum albumins with water and at 0.1M, 0.2M and 0.3M Tris-HCl in *Pampus argentius* and *P. chinensis*.

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