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Electrophoretic analysis of hemolymph protein of freshwater crab, *Parathelphusa hydrodromous* (Hebts.)

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Abstract

The fresh water crab plays an important role in the study on the immune system of invertebrate and their immunity of bloods. Immune system of invertebrate must rely on non-self-recognition molecules to ensure efficient defense responses against infectious pathogens that continuously threaten their survival. The aim of the present study was carried out the total protein and electrohoretic analysis of hemolymph of freshwater crab, *Parathelphusa hydrodromous* (Hebts.). The results of the total protein level in *P. hydrodromous* ranged from 4.0g % to 6.4g % with a mean value of 5.05 ± 0.72g was observed. The conclusion of the results shows hemolymph protein provides a valuable relationship among the various species of crustaceans.

Keywords: *Parathelphusa hydrodromous*, Hemolymph, Total protein and Electrophorotic analysis

Introduction

Freshwater crabs are an important component of the fauna of limnic environments (Wehrtmann *et al*.,2010). About 1300 species of freshwater crabs, distributed throughout the tropics and subtropics regions (Yeo *et al*., 2008). Freshwater crabs are significant organisms inhabiting Southeast Asian fresh waters, playing a key role in recycling nutrients through their scavenging of plant and animal materials.

In recent years blood metabolites have been investigated as a tool for monitoring physiological condition in wild or cultured crustaceans exposed to different environmental conditions (Moore et al.,2000; Rosas et al.,2004; Lorenzon et al.,2007). Blood of crab can be divided into two components, hemolymph plasma and hemocytes, which play vital roles in their defense system. These blood constituents vary depending on physiological state and hence are potential indicators of condition (Sastry and Miller, 1981).

The fresh water crab plays an important role in study on the immune system of invertebrate and their immunity of bloods. Invertebrate immune system must rely on non-self-recognition molecules to ensure efficient defense responses against infectious pathogens that continuously threaten their survival. In this present study, electrophoretic analysis of haemolymph of freshwater crab of *P. hydrodromous*(Hebts.).

Materials and Methods

The experimental animals of *Paratelphusa hydrodromous* used in the present study were collected from Tamiraparani River, Tirunelveli, Tamil Nadu, South India, by hand picking method. The live animals were brought to the laboratory, stored in cement tanks. Volume of hemolymph was calculated following the dye dilution technique described by Lee, (1961) and modified by Mullainathan, (1979). About 0.1ml of hemolymph was withdrawn by cutting the walking legs and made upto 5ml with 0.9% NaCl solution which served as a blank. Then 0.1ml of 0.1% congo red was injected into the hemocoel through the arthrodial membrane at the base of the fourth walking leg. After 45 minutes, 0.1ml of the hemolymph was collected from the same animal and made upto 5ml with 0.9% NaCl solution. The O.D was taken using Systronics Spectrophotometer at 480nm. From the O.D values, the amount of dye in the sample was determined by referring to the standard graph. From the amount of dye injected and the amount of dye present in the hemolymph sample, the blood volume was determined using the formula given by Lee, (1961).

$V = (dg\ 1 / g2) – a(\mu\ l)$

Where V = Volume of the blood; g1 = Wt (con.) of the dye injected.
g2 = Wt of the dye in sample; d = Volume of the sample.
a = Volume of saline injected with dye.

Hemolymph protein

Protein concentration of hemolymph was estimated by Biuret method (Gornall *et al.,* 1949). 80% ethylalcohol was used as deproteinising agent. For 0.05ml of hemolymph, 1ml of deproteinising agent was added. This dilution was found to be necessary for complete precitipation of protein (Kannan and Ravindranath,1980).

Analysis of protein profile

Crude hemolymph samples and fractions were studied using native and Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) according to Laemmli, (1970). Native PAGE was performed with hemolymph mixed with the sample buffer (0.125ml Tris HCl, pH 6.8) containing 0.1% bromophenol blue as tracing dye. The fraction to be analysed were diluted in 0.01ml Tris HCl buffer, pH 6.8 containing 10% glycerol and 1% SDS. Crude hemolymph and fractions were diluted respectively in 1:20 and 1:10 in buffer; this was kept in a boiling water bath for 15 minutes.

Electrophoresis was carried out with a 5% stacking gel (0.1% SDS in 0.125ml Tris HCl buffer pH 6.8) and a 7% separating gel (0.1% SDS in 0.373ml Tris HCl buffer, pH 8.6); A 0.05ml Tris / 0.384ml glycine buffer containing 0.1% SDS (pH 8.3) were used. Gels were stained with Co-massie brilliant blue R 250 for total protein. The bands obtained in the gel were scanned in densitometer (Syn Gene Tools, version 2.10.01 Umanon – MPCS).

Results

Hemolymph volume

The hemolymph volume in normal *P. hydromous* was 448µl with the THC rang of 5190 to 5295 cells/mm^3. Correspondingly the hemocyte in circulation was 23, 36,069 cells.

Hemolymph protein

Total protein level in health *P. hydrodromous* ranged from 4.0g % to 6.4g % with a mean value of 5.05 ± 0.72g %.

Electrophoretic pattern

There were six slow moving protein fractions near the point of application in the region of the cathode which were referred to as 'proximal zone'. Similarly there were four fast moving intense bands near the anodic region. This region was regarded as the 'distal zone'. The diffused bands found between proximal and distal zones were referred to as the 'middle zone'.

Sex difference in the protein concentration of the mature adults was well reflected in the electropherograms (Fig.1). The basis pattern of the electropherograms appeared somewhat similar. Major difference was seen in the proximal zone with the presence of an additional fraction (1A) in the case female. In the middle zone of the female two intensely staining fractions (1A and 1B) were seen which could not be detected in the male.

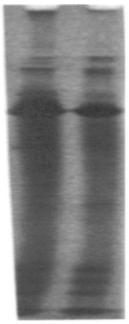

Fig.1: Electrophoretic separation of haemolymph proteins in male and female *P. hydrodromous*

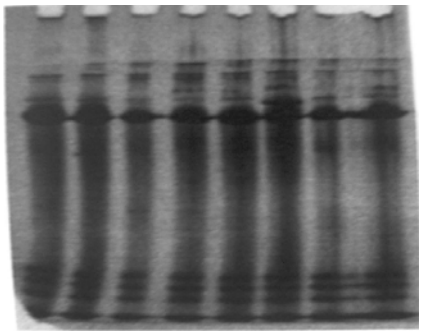

Fig.2: Electrophoretic separation of haemolymph proteins in different size of male and female *P. hydrodromous*

Not much difference in the qualitative pattern could be made out in the scan pattern of the electropherograms of the crabs of varying sizes (Fig. 2). However densitometric recording of the electrophoretic pattern of the hemolymph protein showed quantitative increase of certain protein fractions as a function of body size.

Discussion

The hemolymph of decapods has received considerable attention by a number of crustacean

physiologists because of the wide range of variability observed in its constituents. The available literature on hemolymph constituents of decapods have been periodically reviewed (Florkin, 1960; Engle and Woods, 1960; Gibert, 1972). The present study may be informative and useful since it deals with the decapod crustacean *P. hydrodromous*.

The concentration of protein in the hemolymph of *P. hydrodromous* range from 4 to 6.4g %. These values agree with the earlier reports by Engle and Wood, (1960), Florkin (1960) and Horn and Kerr, (1963). Subhashini and Ravindranath, (1981) has detected a hemolymph protein concentration of 2.6% to 17.1g % in *Scylla serrata.* They further suggest that variation in protein concentration might be due to the quantitative methods followed and due to the daily rhythm. Stewart and Dingle, (1968) has reported 8.30 ± 1.05g % in *Cancer irroratus* and 7.21 ± 1.04g % in *Hyas coarctatus.* Similarly in *Callinectes sapidus*, the protein concentration ranges from 5.24 to 6.27g % (Horn and Kerr 1963). A total protein level of 4.01 ± 0.42 to 4.94 ± 0.33g % in the hemolymph of *B. guerini* has been reported by Tulasi *et al.*, (1992). Thus a survey of hemolymph proteins in different species of decapods reveal a wide range of individual variation. Factors like sex, size, moult cycle and environment may play a vital role in the variation of hemolymph proteins as indicated in the literature.

The electrophoretic pattern of hemolymph proteins of *P. hydrodromous* reveals three distinct zones, which are evident in the running gel. The proximal zone of the electropherogram corresponds to the upper region of the classification of zones of Maguire and Fielder, (1975) who have observed three fractions in *Scylla serrata.* In the region of the electropherograms of hemolymph of *S. serrata*, Kannupandi and Paulpandian, (1975) have reported 8 fractions. The middle zone reported in this study corresponds to the middle region of the electropherograms of the hemolymph proteins reported by Maguire and Fileder, (1975). Kannupandi and Paulpandian, (1975) have identified five faint protein bands in the electropherograms of hemolymph of *Scylla serrata.* In the present study, however, distinct fractions could not be observed since the zone was diffused. Maguire and Fielder, (1975) have also noticed that the bands in this region are highly diffused.

In contrast to the upper and middle regions, the region exhibited a consistent pattern of protein bands. This has also been recognized in the work of Maguire and Fielder, (1975) and Kannupandi and Paulpandian, (1975). The above authors have reported that all the distal fractions are hemocyanins. Similarly have shown about 17 fractions in the hemolymph of *C. maenas*, out of which seven fractions were identified to be copper containing proteins.

Differences in the banding pattern of the electropherograms in the hemolymph of male and female *P. hydrodromous* may be attributed to the female limited protein, reported by a number of workers (Fielder *et al.*, 1971; Adiyodi and Adiyodi, 1972; Lee *et al.*, 1997). The presence of vitellogenin in mature females may be responsible for the additional fractions in the electropherograms. In addition to hemocyanin, other proteins like glycol or lipoproteins, globulins, coagulin may also be present in the hemolymph of crustaceans. Chan *et al.*, (1988) have reported that hemocyanin is the major protein detected in different species which accounts for 80 to 95% of the total hemolymph protein. The rest of the protein may consist of mostly free enzymes (Scheer, 1960).

Electrophoretic studies help to compare the protein fractions of *P. hydrodromous* with that of other decapods. This study of hemolymph protein provides a valuable relationship among the various species of crustaceans. Further the results of this study would provide the ground work for better monitoring of the biochemical or physiological state of the animal under various conditions.

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