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Haemolymph protein profile of the edible oyster, *Crassostrea madrasensis* (Preston) exposed to different salinities

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ABSTRACT

The haemolymph total protein concentration and protein profile of *C. madrasensis* exposed to different salinities were studied. The animals were maintained at salinities 6 , 12, 24 and 36 ppt for one month. The serum total protein concentration showed significant reduction at 6 and 36 ppt. The haemolymph protein profile obtained by SDS-PAGE also varied with difference in salinities. There were four bands with molecular weight 86, 74, 31 and 26 kDa in all the four groups. The intensity of bands was higher in groups maintained at 12 and 24 ppt compared to other groups. The haemolymph of animals maintained at 6 ppt also showed three additional bands of 60, 52 and 13 kDa that were feebly expressed.

Introduction

The immune mechanisms of molluscs include cellular and humoral factors. Several workers have studied the cellular defense system of bivalves. These include studies on *Crassostrea virginica* (Cheng 1989), *Ruditapes philippinarum* (Oubella *et al.*, 1996), *Tridacna crocea* (Nakayama *et al.*, 1996), *Mytilus edulis* (Rasmussen *et al.*, 1985) etc.

Studies on humoral factors of bivalves are very limited. The haemolymph proteins of marine invertebrates are unique in composition, as they do not contain immunoglobulin or albumin like proteins and the protein composition vary in relation to physiological and functional state of the animal. This in turn may depend on exposure to pollutants and pathogens and also on environmental factors correlating with the reproductive cycle of the animal (Muromoto *et al.*, 1996).

The difference in total protein concentration in copper and cadmium stressed *C.virginica* has been studied by Cheng (1989). Chu and Peyre (1989) studied the effect of environmental factors and parasitism on haemolymph protein of *C.virginica*.

Granath *et al.* (1987) have analyzed the haemolymph of *Biomphalaria glabrata* by sodium dodecyl sulphate polyacrylamide

gel electrophoresis (SDS-PAGE). Two dimensional gel electrophoresis was used by Muramoto *et al.* (1996) to study the haemolymph composition of *Megabalanus rosa*. The change in *C. virginica* serum composition associated with parasitic infection was studied using SDS-PAGE by Ford (1986).

In the present experiment an effort was made to study the effect of variation in salinity on total protein concentration and protein profile.

Materials and methods

The edible oyster, *C. madrasensis* were collected from Cochin backwaters. They were cleaned and maintained in filtered and aerated seawater of 22 to 24 ppt salinity. About 60 animals of the same stage of gonadal development were chosen for the experiment, 15 each for each salinity group of 6, 12, 24 and 36 ppt. They were slowly acclimatized to the respective salinity during a one-week period and maintained in the same salinity at a temperature of 31°C for one month before collecting the haemolymph samples.

The haemolymph was withdrawn using a 2 ml syringe with 27-gauge needle from the adductor muscle through an adjacent notch in the shell valve without harming the animals. It was centrifuged at 5000 rpm for 10 minutes to remove cells and the supernatant serum was used for the experiment. Nine replicates for each group were taken. The total protein was estimated using the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Serum proteins were separated by SDS-PAGE as described by Laemmli *et al.* (1970). Separating gel of 11.5% was used for electrophoresis. The samples mixed with equal volume of sample buffer were loaded along with standard protein marker (Genei). Electrophoresis was carried out at 140 V for 4 to 5 hrs and the gel was stained with coomassie brilliant blue.

Results

The mean total protein concentration of haemolymph of *C. madrasensis* varied in different salinity groups (Fig. 1). In group 1 which was maintained at 6 ppt salinity, the total protein concentration varied from 0.82 to 1.12 mg/ml of

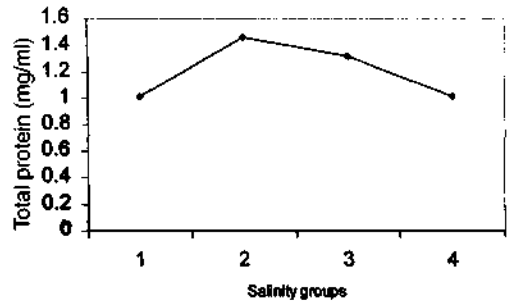

Fig.1. SDS-PAGE of *C. madrasensis* haemolymph maintained at different salinities.

haemolymph with a mean of 1.01 ± 0.09 mg/ml haemolymph. The group that was maintained in 12 ppt showed a total protein concentration ranging from 1.18 to 1.62 with a mean of 1.46 ± 0.15 mg/ml haemolymph. Group 3 showed a total protein concentration varying from 1.22 to 1.46 with a mean of 1.32 ± 0.1 mg/ml haemolymph. Group 4 had a mean total protein value of 1.01 ± 0.11 mg/ml with a range of 0.88 to 1.14 mg/ml haemolymph. The maximum total protein value was observed at 12 ppt salinity and minimum at 6 and 36 ppt. The result of total protein concentration at various salinities is given in Table 1.

The analysis of variance of the results reveals significant difference between total protein concentrations at different salinities. Students t test revealed the following facts : Group 1 and 4 behaved similarly with respect to total protein concentration. Likewise, group 2 and 3 showed similar total protein concentra-

TABLE 1. *Total protein concentration (mg/ml) of* C.madrasensis *haemolymph maintained at different salinities.*

Replications	Gr 1	Gr.2	Gr.3	Gr.4
1	1.02	1.52	1.23	1.1
2	1.06	1.44	1.24	1.12
3	0.96	1.46	1.28	0.88
4	0.96	1.36	1.26	1.14
5	0.98	1.62	1.30	0.92
6	1.12	1.60	1.22	1.06
7	1.10	1.60	1.44	1.08
8	1.06	1.18	1.45	0.89
9	0.82	1.33	1.46	0.9
Mean±SE	1.01±0.03	1.46±0.15	1.32±0.1	1.01±0.11

tions. However group 1 and 4 had a significantly lower total protein concentration when compared to group 2 and 3.

The SDS-PAGE of the proteins in the serum of *C.madrasensis* maintained at different salinities showed 4 to 7 bands. The haemolymph of animals maintained at 6 ppt, showed 5 to 7 bands of molecular weight 86, 74, 60, 52, 31, 26 and 13 kDa. In some animals 86 and 60 kDa bands were not expressed. In group 2, which was maintained at 12 ppt, and group 3 at 24 ppt only 4 bands with molecular weight 86, 74, 31 and 26 kDa were expressed. The 31 and 26 kDa bands were seen as a single zone. The haemolymph of animals maintained at 36 ppt salinity also expressed protein bands of 86, 74, 31 and 26 kDa. The 60, 52 and 13 kDa bands were feebly expressed only in group 1 at 6 ppt salinity (Fig. 2).

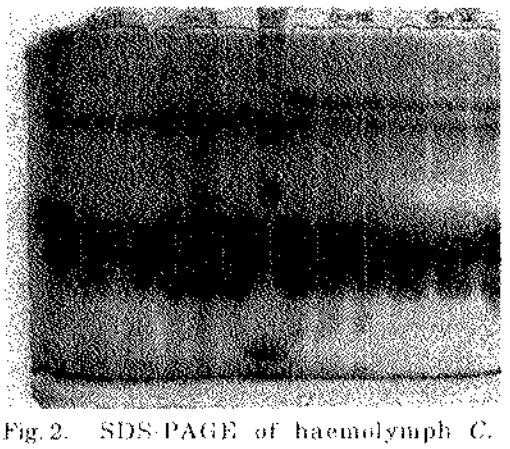

Fig. 2. SDS-PAGE of haemolymph *C. madrasensis* maintained at different salinities.

Discussion

Very limited studies have been done on the total protein concentration of bivalves. Studies by Chu and Peyre (1989) on *C.virginica* showed that the total protein concentration did not vary with salinity. The total protein value was also much higher than that of the present observation. *C. virginica* is a species which is cultured extensively in temperate zone. The difference in response to various environmental factors in these two species may be due to species variation or adaptation in different climatic conditions. The study by Ford (1986) indicated a variation in total protein value with relation to temperature and gonadal development. In the present study, both at high and low salinities, there was a significant reduction in total protein

concentration which may be due to the stress caused by these salinities. This reduction in total protein concentration in *C. madrasensis* needs further study.

The SDS-PAGE of haemolymph of *B. glabrata* (Granath *et al.*, 1987) and *C. virginica* (Ford, 1986) showed bands ranging from 10 kDa to more than 500 kDa. Though majority of bands in the above cited works were comparable to bands obtained in the present study, some bands were not at all comparable.

In the present study, we noticed differences in polypeptide bands in relation to salinity. The animals kept at 12 and 24 ppt salinity showed 4 bands of protein of molecular weight 86, 74, 31 and 26 kDa which are comparable to protein bands obtained in *C. virginica* by Ford (1986). He also noticed 12 bands having molecular weights above 500 kDa, the number of which varies seasonally. These bands were not observed in the present study. Probably this may be due to the fact that *C. virginica* were maintained in a temperate climate, which is entirely different from the climatic condition in which *C. madrasensis* were kept.

At 6 ppt salinity, three additional bands of 13, 52 and 60 kDa appeared and there was a significant reduction in 26 and 31 kDa fractions. In 36 ppt salinity also, there was a reduction in 26 and 31 kDa bands, but 13, 52 and 60 kDa protein bands were totally absent. Our study indicated that the proteins of 26 and 31 kDa are affected by the stress conditions of high and low salinities which had caused a reduction in total protein concentration. The appearance of 13, 52 and 60 kDa bands in low salinity group is a significant finding that has not been reported elsewhere. The protein fractions of *C. madrasensis* in relation to various environmental and stress condition need further study.

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