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Studies on the haemocyanin content in the blood of the freshwater field crab, *Oziotelphusa senex senex* (Fab) in relation to sex

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Abstract. The copper and protein contents have been determined in the blood and also in the purified haemocyanin of the freshwater field crab, *Oziotelphusa senex senex* (Fabricius) in relation to sex. The haemocyanin content was fairly higher in the blood of female crabs when compared to the males. However, the copper–protein ratio in the purified haemocyanin of both male and female crabs is 0·167%. Based on the copper content, the minimal molecular weight of the functional unit of the *O. senex senex* haemocyanin has been calculated as 76,120 daltons. The sex-based difference in the haemocyanin concentration has been discussed in the light of the existing literature.

Keywords. Haemocyanin ; blood ; minimal molecular weight ; *Oziotelphusa senex senex* ; sex.

1. Introduction

The respiratory pigment, haemocyanin, is found dissolved in the haemolymph of many arthropods and molluscs (Manwell 1960; Redmond 1971; Lontie and Witters 1973). It combines reversibly with oxygen in the proportion of one molecule of oxygen with two atoms of copper (Montgomery 1930).

The haemocyanin of arthropods and molluscs differs in copper content and also in the minimal molecular weights (Lontie and Witters 1973). It has been shown that the blood copper content is influenced by neither sex nor size in the shrimps (Djangmah and Grove 1970). However, an earlier investigation has revealed that the concentration of haemocyanin was influenced by sex (Horn and Kerr 1963). Therefore an attempt has been made to study the existence of any sex-based difference in the haemocyanin concentration and also some of the characteristics of the haemocyanin in the South Indian freshwater field crab, *Oziotelphusa senex senex.*

2. Material and methods

The crabs were collected from the paddy fields around Tirupati. They were brought to the laboratory and kept in glass troughs containing enough water to

keep the crabs just submerged under water. They were fed with frog muscles. Only intact and non-berried crabs were used for the experimental analysis. They were not fed for one day before the actual commencement of the experiment so as to eliminate the variations in the blood composition due to differential feeding. Crabs of similar size were used and their sex was noted.

Blood was drawn through the arthrodial membrane at the base of the fourth thoracic appendage by means of a hypodermic syringe.

The copper content was determined with sodium diethyl dithiocarbamate (Barnes and Rothschild 1950) both in the blood and in the purified solutions of haemocyanin, after wet ashing of the samples in microkjeldahl flasks. The copper content determined in the blood was taken as "total copper". "Bound" and "free copper" were estimated as per the method followed by Kazmierczak *et al* (1978).

The purified haemocyanin was obtained following the procedure given by Konings *et al* (1969). The haemocyanin solution was dialysed for 24 hr against 25 mM EDTA in 0·1 M tris-HCl buffer, pH 7·6 the normal pH of the blood. The final preparation was blue in colour and it is not denatured, since the blue colour is lost upon the addition of a pinch of sodium hydrosulphite. The protein content was determined by the method of Lowry *et al* (1951).

The haemocyanin content of the blood was calculated using the per cent copper in the purified haemocyanin. The minimal molecular weight of the haemocyanin containing one atom of copper is given by dividing the atomic weight of copper (63·54) by the fraction of protein due to this element (Montgomery 1930). The molecular weight of the functional unit of haemocyanin containing 2 atoms of copper was also calculated.

3. Results and discussion

The average contents of copper and protein in the blood and also in the purified haemocyanin of both sexes of freshwater field crab, *O. senex senex* are presented in table 1. In the same table the haemocyanin content calculated based on the copper content in the blood is also shown. From table 1, it is clear that there are significant differences in the copper, protein and haemocyanin contents in the blood of male and female crabs.

The total copper content was 16·35% higher in the blood of females than in the males and the haemocyanin content in the blood also showed a similar trend since the haemocyanin concentration is based on the copper content of the blood. As such the significant difference in the total copper content of the blood between the two sexes was shown to be due to the difference in the protein bound copper. However, the free copper content in the blood did not significantly differ with reference to sex (table 1). Thus it can be concluded from the present work that the sex influences the copper content, consequently, the haemocyanin content is higher in the blood of the female crabs. This finding is in accordance with the earlier reports showing similar trend in the sex difference that exists in the concentration of haemocyanin in the blood of the blue crab, *Callinectes* (Horn and Kerr 1963) and also in the organic and inorganic

Table 1. Copper and protein contents in the blood and in the purified haemocyanin of *O. senex senex*.

(Values are mean ± S.D. of 10 individual observations)

Sample	Normal males	Normal females	% Increase over males
Blood			
Total copper (μg/ml)	44·0 ± 8·23	52·6 ± 8·26*	16·35
Bound copper (μg/ml)	40·8 ± 7·76	48·5 ± 7·46*	15·87
Free copper (μg/ml)	3·12 ± 0·44	3·22 ± 0·41^{NS}	3·10
Total protein (mg/ml)	34·78 ± 5·14	40·29 ± 4·29*	13·67
% Copper in total protein	0·135	0·131	..
Haemocyanin (mg/ml)	26·34 ± 5·47	31·49 ± 5·29*	16·35
Purified haemocyanin			
Copper (μg/ml)	30·4	31·8	..
Protein (mg/ml)	18·2	19·0	..
% Copper	0·167	0·167	..

* $P < 0·01$. NS : Not significant.

constituents of the blood of the decapod crustaceans (Gilbert 1959a, b; Padmanabha Naidu and Ramamurthi 1961; Ambore and Venkatachari 1976).

Just like the copper content, the protein content was also higher (13·67%) in the blood of the female crabs when compared to the males (table 1), but the copper-protein ratio was almost the same in both sexes. When the haemocyanin from the blood of the male and female crabs was purified, the sex difference with regard to the copper and protein contents has disappeared and the copper–protein ratio has increased over the ratio present in the blood. From this it can be concluded that the nature of the haemocyanin molecule is same in the male and female crabs whereas sex difference exists in the amount of copper, protein and haemocyanin present in the blood.

The copper content in the purified haemocyanin of *O. senex senex* is 0·167, which agrees with other crustacean haemocyanins. The minimal molecular weight of crab haemocyanin, calculated based on the copper content, is 38,060 daltons. Since one molecule of oxygen combines stoichiometrically with two atoms of copper, the minimal molecular weight of the functional unit of the freshwater crab haemocyanin is 76,120 daltons, which falls within the range reported for other crustacean haemocyanins (Lontie and Witters 1973). The remarkable

similarity of the minimal molecular weight of the crab haemocyanin with others reveals that all the crustacean haemocyanins are similar with similar physiological properties.

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