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Some studies on *Laevicaulis alte* haemocyanin in relation to starvation

K SOWJANYA and B PADMANABHA NAIDU
Department of Zoology, SV University, Tirupati 517 502

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Abstract. The copper and protein concentrations in the blood and in the purified haemocyanin of active and starved slugs were determined. During starvation there is a marked decrease in the haemocyanin concentration in the blood. However the copper protein ratio of the purified haemocyanin of the active and starved slugs remained unchanged suggesting that haemocyanin remains unaltered during starvation.

There is a significant increase of hepatopancreatic copper upon starvation. The hepatopancreas is suggested to store copper during starvation. The results are discussed in the light of the previous works on crustaceans and molluscs.

Keywords. Haemocyanin; blood; hepatopancreas; minimal molecular weight; starvation.

1. Introduction

Haemocyanin, a copper containing respiratory protein, is found dissolved in the haemolymph of many species of arthropods and molluscs (Prosser 1973). Copper will never exist in a free state in the blood. But the decapod crustaceans, isopods and amphipods are known to store copper in the hepatopancreas (Wiser 1968) often as semicrystalline or refractile granules in specific cells (Djangmah and Grove 1970). The blood copper gives a measure of the haemocyanin concentration which can be calculated based on the copper protein ratio of the purified haemocyanin and it forms the most valuable basis for the calculation of the minimal molecular weight of haemocyanin (Montgomery 1930). The molecular weights of the haemocyanins vary considerably amongst different species (Lontie and Witters 1973). Based on the copper content and the molecular weights, the haemocyanins of arthropods and molluscs have been shown to be distinctly different (Lontie and Witters 1973).

It has been shown in a decapod crustacean, *Crangon vulgaris*, that the concentration of haemocyanin in the blood decreases upon starvation (Djangmah 1970). The fall in the haemocyanin concentration in the blood of *C. vulgaris* upon starvation is related to the progressive accumulation of copper in the hepatopancreas. Djangmah (1970) has suggested that during starvation the haemocyanin is used as an energy source and the copper released during the catabolism of haemocyanin is immediately transported and translocated in the hepatopancreas.

Due to paucity of information regarding such functional role of copper in the gastropods, an attempt has been made to trace the fate of protein bound haemolymph copper and haemocyanin during starvation of the slug, *Laevicaulis alte.*

2. Materials and methods

Slugs collected from the local gardens were maintained in the laboratory in large wooden boxes filled with mud which has been kept moist by sprinkling water once in a day and the animals were fed *ad libitum* with croton leaves. These are taken as active slugs. A batch of slugs from this lot, starved for 15 days, were used for the starvation studies.

Collection of blood : An incision was made along the mid-dorsal line of the body wall through which the blood directly oozes out and it was collected into test tube kept in ice.

The purified haemocyanin was obtained by following the procedure given by Konings *et al* (1969). The haemocyanin solution was dialyzed for 24 hr against 25 mM EDTA in 0·1 M Tris-HCl buffer, pH 8·0, the normal pH of the blood, instead of pH 7·0 buffer. 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 copper content in the blood and the purified haemocyanin was estimated with sodium diethyl dithiocarbamate (Barnes and Rothschild 1950) after wet ashing with the concentrated sulphuric acid using kjeldahl flasks. The proteins were estimated by the method of Lowry *et al* (1951).

The haemocyanin content of the blood was calculated using 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 data on the copper and protein concentrations in the blood and purified haemocyanin of active and starved slugs is presented in table 1. The copper and the total protein concentrations in the blood of the active slugs are 52·78 μg/ml and 33·4 mg/ml respectively. Upon starvation there is a significant decrease in the copper and total protein levels in the blood by 61·63% and 42·18% respectively. The haemocyanin concentration decreased by 55·30% in the blood of starved animals.

From the results it is clear that starvation induced a significant decrease in the blood copper concentration in the slug and a significant 37·87% elevation in the hepatopancreatic copper. Thus there is an inverse relationship in the copper content between the hepatopancreas and blood upon starvation. Based on similar observations Djangmah (1970) has suggested that copper has been released due to the catabolism of haemocyanin and the released copper has been mobilized

Table 1. Starvation stress on the copper and protein contents in the blood and purified haemocyanin and the copper content in the hepatopancreas of *Laevicaulis alte*. The figure in the parenthesis denotes the number of observations.

Sample	Active	Starved
Blood		
Copper (μg/ml)	52·78±6·08 (15)	20·25±2·2 (15)*
Protein (mg/ml)	33·40±4·34 (15)	19·32±1·4 (15)*
% Copper	0·158	0·105
Haemocyanin (mg/ml)	21·54	9·625
Hepatopancreas		
Copper (μg/gm)	75·02±4·04 (15)	103·44±8·73 (15)
Purified haemocyanin		
Copper (μg/ml)	11·20	7·20
Protein (mg/ml)	4·57	2·94
% Copper	0·245	0·245

* $P < 0·001$

and stored in the hepatopancreas during starvation. As a result the total protein content in the blood has decreased significantly suggesting the utilization of proteins during starvation stress (Venugopal Reddy 1978). Therefore it can be concluded that the low concentration of haemocyanin in the blood of the slug during starvation may be due to the catabolism of haemocyanin, showing a significant decrease of copper and protein concentrations, suggesting that the proteins may have been used up as an energy source and the copper released has been transported and accumulated in the hepatopancreas. The low concentration of haemocyanin in the blood may be sufficient to meet the oxygen requirements of the starved slugs as evidenced by the low oxygen utilization of *Australorbis glabratus* during starvation (von Brand *et al* 1957).

The per cent copper in the purified haemocyanin of both active and starved slugs is 0·245 (table 1) a value which falls within the range reported for other molluscs (Lontie and Witters 1973). This suggests that the purified haemocyanins of both active and starved slugs may have similar physico-chemical properties. Based on the copper content of the blood, the minimal molecular weight of the haemocyanin of *Laevicaulis alte* has been found to be 25,930. Since a molecule of oxygen combines stiochiometrically with two copper atoms (Redfield 1934), the molecular weight of the functional unit of haemocyanin is 51,860. This value is readily comparable to the values of *Busycon canaliculatum*, *Helix pomatia*, *Murex trunculus* (Lontie and Witters 1973) and *Pila globosa* (Venugopal Reddy 1978) suggesting that the functional units of all the gastropod haemocyanins may be more or less the same with similar physico-chemical properties.

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