COMMUNICATION

A study of the dissociation of haemocyanin of the giant African snail, *Achatina fulica*

HUSSAIN ABDUL KAREEM

Department of Biochemistry, College of Medicine, University of Lagos, Lagos, Nigeria

Haemocyanins are highly polymerized proteins whose dissociation depends on protein concentration, ionic strength, pH etc. Svedberg & Pederson (1940) reported multiple forms of haemocyanin of *Achatina in vitro*. Aboderin & Kareem (1971) also reported two forms of *Achatina fulica* haemocyanin in *vivo*. More recently, however, Adadevoh et al. (1976) reported three homogeneous globulin-like proteins with an albumin/globulin ratio approx. 1:19 in the haemolymph of *Achatina fulica*. A re-investigation of the polymeric nature of the haemocyanin of this gastropod becomes necessary.

In the present work, the dissociation of haemocyanin molecules in the haemolymph of *Achatina fulica* was studied by using salt precipitation and gel-filtration techniques.

![Graph](https://example.com/graph.png)

**Fig. 1.** (NH$_4$)$_2$SO$_4$ precipitation of *Achatina fulica* haemocyanin on Celite (a) and rechromatography of 'peak B' material that was homogeneous when obtained from a Sepharose 6B column with 20mM ammonium carbonate, pH 9.35, as the eluting system (b).**

For general details see the text. In (b) the conditions were the same as in the experiment in which 'peak B' was first obtained except that the ionic strength was 10mM and the buffer had pH 10.5. For definition of 'peak B' see Kareem et al. (1973).

Haemolymph was collected from fresh or laboratory-fed snails as described previously (Kareem et al., 1973). Haemocyanin was precipitated by Celite (250μm mesh) and separated by centrifugation, and the protein-free supernatant was discarded. The Celite-precipitated haemocyanin was packed on a suitable column and gradient-eluted with (NH$_4$)$_2$SO$_4$. Chromatography of fresh haemolymph was performed on Sephadex G-100, Sephadex G-200 and Sepharose 6B columns under a variety of conditions, which included changes in the ionic strength and pH of the eluting solvent system.

The bulk of the protein in the haemolymph of *Achatina fulica* was precipitated by 38%-saturated (NH$_4$)$_2$SO$_4$ (Fig. 1a) and protein concentration was 1.4 ± 0.5 g/100 ml of haemolymph as determined by the method of Lowry et al. (1951). Only one protein peak was obtained with Sephadex G-100, and two unresolved peaks on Sephadex G-200. On the Sepharose 6B column and at pH 8.0 there were two protein peaks. At pH 9.0 two separate peaks, with the second occurring in a 'multiple form', were observed. At pH 9.35 there were at least three clearly defined protein peaks. The peak B (Kareem et al., 1973), which appeared to be 'homogeneous' at pH 9.35, gave multiple protein peaks (more than three) at pH 10.35 (Fig. 1b).

The protein content of 1.4g/100 ml of haemolymph agrees with values for other haemolymphs (Martin et al., 1968), and confirms my previous finding of 12-28 g of protein/1 of different samples of haemocyanin of *Achatina fulica* (Kareem, 1968).

The pattern of separation of *Achatina fulica* haemocyanin, particularly on Sepharose 6B chromatography, is reminiscent of the behaviour of the haemocyanin of *Pilla leopoldiulensis*, which yielded 98s (100%) at pH 4.9, 98s (35%) and 60s (55%) at pH 6.8, and 100s (10%), 65s (10%) and 30s (20%) at pH 8.5 (Bois D'Enghien et al., 1971).

Caution needs to be exercised in the interpretation given by Adadevoh et al. (1976) that the haemolymph of *Achatina fulica* contains three homogeneous protein molecules that are albumin and globulin and that the 'immunoglobulins are suggestive of beta myeloma'. Their data on Sephadex G-200 chromatography, electrophoresis and Ouchterlony experiments are open to other possible interpretations consistent with the polymeric nature of the haemocyanin molecules of *Achatina fulica*. Electrophoretically produced multiple peaks are characteristic of haemocyanin molecules of molluscs and arthropods (Manwell & Baker, 1963; Busselen, 1970; Ghidalia et al., 1971). The classical work of Witters & Lontie (1968), who used Ouchterlony techniques to investigate the immulogical properties of haemocyanin from four different gastropods, could have been the basis for discussion by Adadevoh et al. (1976), but this work was not even acknowledged.

The quaternary structre of molluscan and arthropod haemocyanins has already been the subject of comparative studies making use of high-resolution electron microscopy (van Bruggen et al., 1962, 1963; van Bruggen, 1968), sedimentation analysis (Bois D'Enghien et al., 1971), zone electrophoresis and light-scattering techniques (Elliot & Hoebeke, 1970). The application of these techniques to *Achatina fulica* haemocyanin is desirable.


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Kareem, H. A. (1968) Research Fellowship Monograph, Department of Physiological Sciences, University of Lagos