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Arthropod (Cyamus scammoni, Amphipoda)
Hemoglobin Structure and Function

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Introduction

Respiratory protein expression in the Arthropoda generally occurs along phylogenetic lines. Hemocyanin, the blue, copper-containing respiratory protein, is found in the Chelicerata, in at least one Uniramia and in most of the Crustacea (1, 2). Hemoglobin, despite its ubiquitous appearance in both plant and animal kingdoms, is more limited in distribution amongst the Arthropoda than is Hc. The red, iron-containing Hb molecule has been described for only a few insects and four classes of Crustacea: the Branchiopoda, Ostracoda, Copepoda and Cirripedia (3–7). Thus, the occurrence of Hb in an amphipod, a more advanced crustacean belonging to the class Malacostraca, is an unexpected finding. This paper describes preliminary studies on the structure and function of Hb from Cyamus scammoni, a cyamid amphipod that is an obligate ectosymbiont on the gray whale, Eschrichtius robustus.
Figure 1. Chromatography of *Cyamus* hemolymph on 1.8 x 109 cm column of BioGel A-5m. Buffer, 0.1 ionic strength Tris-HCl, 0.1 M NaCl, 16 mM CaCl2, 8 mM MgCl2, pH 7.5. Absorbance at 280 nm (●) and 417 nm (▲). Calibrants: (A) *Helisoma trivolvis* Hb, $M_w = 1.7 \times 10^6$. (B) *Cancer magister* 25 S Hc, $M_w = 940,000$. (C) *C. magister* 16 S Hc, $M_w = 450,000$.

Materials and Methods

Live *Cyamus* were removed from the surface of several gray whales, *E. robustus*, that had just died. Hemolymph samples were obtained by microcapillary pipette from the base of the legs, the spiral gill structures and the oostegites of the brood pouch. In order to prevent gelation and proteolysis, the red hemolymph was diluted immediately in an equal volume of ice cold Tris-HCl buffer, 0.1 M NaCl, 16 mM CaCl2, 8 mM MgCl2, pH 7.5, containing 1 mM phenylmethyl sulfonyl fluoride, a protease inhibitor, and centrifuged at 2000X g for 5 min (4°C). The red color remained in the supernatant, which was then centrifuged at 10,000 g for 15 min (4°C) and used for further analysis.

Results and Discussion

Spectral analysis of the centrifuged hemolymph showed absorbance maxima at 417, 544 and 577 nm, corresponding to an oxyHb spectrum. Flushing the sample with CO shifted the maxima to 420, 542 and 577 nm, typical carboxyHb values. Thus, these amphipods contain Hb, and constitute the first example of Hb expressed in a malacostracan.