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LACK OF HEMOCYANIN IN ORIENTAL PLECOPTERA AND MULTIFUNCTIONALITY OF THE PROTEIN IN LARVAE

VALENTINA AMORE, BRUNELLA GAETANI & [1]ROMOLO FOCHETTI*
Dipartimento di Scienze Ambientali, Università della Tuscia
Romolo Fochetti, Dipartimento di Scienze Ambientali, Università della Tuscia, snc, Largo dell'Università 01100, Viterbo, Italy.
[1]Email: fochetti@unitus.it

ABSTRACT. Thus far, data on the hemocyanin are available only for European Plecoptera, present study reports on this from the Oriental fauna. Six species of the family Perlidae and one species of Peltoperlidae from Thailand studied did not reveal mRNA hemocyanin sequences, confirming that not all Plecoptera species universally have mRNA hemocyanin sequences. All species show hexamerins similar to the ones previously found in European species of the same families. Results also suggest that the presence of hemocyanins, at least in quantitative terms, does not depend on the body size, life cycle or trophic role, as had been hypothesized in the past. Also, the specific role of this protein in Plecoptera is yet to be completely understood. The hemocyanin expression pattern obtained across the entire order could be explained also by hypothesizing its other functions besides respiratory.

Key words: cDna, respiratory proteins, hemocyanins, stoneflies.

Introduction

Hemocyanins are copper containing, multisubunit proteins freely dissolved in the hemolymph of many mollusks and arthropods (Linzen, 1989; Markl & Decker, 1992; van Holde et al., 2001). Together with hemerythrins and hemoglobins, hemocyanins are considered metal containing animal respiratory proteins with the function of reversibly binding O_2 for its transport or storage. They are composed of six identical or similar subunits with molecular mass of around 75 kDa each (van Holde & Miller, 1995; Salvato & Beltramini, 1990). The active site, in domain II of each subunit, is a highly conserved region able to bind an O_2 molecule via two Cu^+, coordinated by three histidines in two distinct binding sites. These proteins have been studied thoroughly in Crustacea Malacostraca and Chelicerata (Mangum, 1980, 1983a, 1983b, 1985; Volbeda & Hol, 1989), but in the last few years hemocyanins have been reported in many Hexapoda like Collembola, Diplura, Archaeognatha, Zygentoma, Plecoptera, Orthoptera, Phasmida, Dermaptera, Isoptera and Blattodea (Burmester & Hankeln, 2007; Pick et al., 2009). Moreover, copper less proteins resembling hemocyanins in structure and sequence have been identified in all insects investigated thus far. They are referred to as hexamerins (Telfer & Kunkel, 1991; Beintema et al., 1994).

The recent finding of hemocyanin in many insect orders raises doubts about the paradigm of the tracheal system and respiratory proteins. The widely accepted notion was that the tracheal system is sufficient for insect respiration (Brusca & Brusca, 2002). Starting from the first report of hemocyanin in the stonefly *Perla marginata* (Panzer, 1999) (Hagner-Holler et al., 2004), our research has been aimed at better understanding the presence, functional significance and role of this protein in Plecoptera. We have analyzed many species of both European superfamilies (Eulognatha and Systellognatha) and the mRNA expression of hemocyanin in different stages of their life cycle (Fochetti et al., 2006; Amore et al., 2009; Amore &