Fluorescence Spectroscopy of the Tryptophan Microenvironment in
*Carcinus aestuarii* Hemocyanin

Paolo Di Muro\(^a\), Mariano Beltramini\(^{a,b}\), Peter Nikolov\(^c\), Irina Petkova\(^c\)*, Benedetto Salvato\(^{a,b}\) and Fernanda Ricchelli\(^b\)

\(^a\) Department of Biology and \(^b\) CNR Institute of Biomedical Technologies, Padova Unit, University of Padova, Padova, Italy
\(^c\) Institute of Organic Chemistry, Bulgarian Academy of Sciences, Akad. G. Bonchev str., bl. 9, 1113 Sofia, Bulgaria. Fax: 003592-700225. E-mail: iripet@orgchm.bas.bg

* Author for correspondence and reprint requests

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The steady-state and time-resolved fluorescence properties of the multitryptophan minimal subunit *Cae*SS2 from *Carcinus aestuarii* hemocyanin have been studied with the aim of probing the environment of the fluorophores within the protein matrix. Subunit \(a\) of *Panulirus interruptus* hemocyanin, whose X-ray structure is known, has been also studied. The results are compared with those collected with other two monomeric fractions (*Cae*SS1, *Cae*SS3) produced by dissociation of the native, oligomeric protein as well as with those of the hexameric aggregate. Three classes of tryptophan residues can be singled out by a combination of fluorescence quenching and lifetime measurements on the holo-Hc (the copper containing, oxygen binding form) and the apo-Hc (the copper-free derivative). One class of tryptophans is exposed to the protein surface. Some of these residues are proposed to be involved in the intersubunit interactions in *Cae*SS1 and *Cae*SS3 fractions whereas in *Cae*SS2 the protein matrix masks them. This suggests the occurrence of conformational rearrangements after detachment of the subunit from the native aggregate, which could explain the inability of *Cae*SS2 to reassociate. A second class of tryptophan has been correlative assigned, by comparison with the results obtained with *Panulirus interruptus* hemocyanin, to residues in close proximity to the active site. The third class includes buried, active site-distant, residues.