A COMPARISON OF THE COPPER SITES IN ARTHROPOD AND MOLLUSC OXYHEMOCYANINS

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1. Introduction

In many arthropods and molluscs oxygen is transported by hemocyanin, a large copper-containing protein. Much information is available on the oxygen-binding site. Classical oxygen capacity experiments indicated that one oxygen molecule is bound per two copper atoms [1]. X-ray photoelectron spectroscopy (ESCA) studies on lyophilyzed oxygenated protein showed that the state of copper in oxyhemocyanin is cupric [2]. X-ray absorption studies confirmed this result and also proved the state of copper in deoxyhemocyanin is cuprous [3]. This confirmed the finding that oxygen is bound as a peroxide [4]. Spectroscopic studies have shown that the lack of an EPR signal in oxyhemocyanin [5,6] is due to antiferromagnetic coupling between the coppers via an endogenous protein bridge [7,8].

The optical spectra of oxygenated hemocyanin from arthropods and molluscs are similar. Differences have been reported in absorption band energies and their CD spectra [9]. Also, functional differences between arthropod and mollusc hemocyanin are reported, e.g. only the mollusc hemocyanin exhibits a high catalase activity [10]. Copper removal by cyanide is also different for the two types [11] as is the copper:protein ratio (0.25% for mollusc, 0.18% for arthropod). A spectroscopic comparison of five mollusc and five arthropod hemocyanins revealed the active sites in both phyla to be quite similar; however, spectral features differ quantatively. This difference was assigned to a distortion of the arthropod active site [12].

A detailed knowledge of the structures of the copper sites would aid in explaining the differences in behaviour. Unfortunately no high resolution crystal structure is yet available.

Extended X-ray absorption fine structure (EXAFS) [13] is a recent technique which can be used to determine the number, type and distance of atoms around a metal atom in a material which need not to be crystalline. Many metalloproteins have been examined by EXAFS including hemoglobin [14], rubredoxin [15, 16], xanthine oxidase [17,18], nitrogenase [19], and hemocyanin [3,20,21]. We decided to compare the EXAFS oscillations obtained from an arthropod (*Panulirus interruptus*) and a mollusc (*Helix pomatia*) hemocyanin to see whether the differences observed are reflections of different copper surroundings.

2. Experimental

Helix pomatia α-hemocyanin was isolated according to [22] and *Panulirus interruptus* according to [23]. *H. pomatia* α-hemocyanin was dialyzed, after regenerating any possible 'aged' sites with hydroxylamine [24], against 0.1 M Tris–HCl containing 10 mM $CaCl_2$, pH 8.2. *P. interruptus* hemocyanin was dialyzed against 0.1 M Tris–HCl containing 10 mM $CaCl_2$, pH 7.6. Both protein samples were pelleted at 100 000 × *g* for 4 h (*H. pomatia*) and 20 h (*P. interruptus*) respectively. The pellets were mounted in a sample holder between mylar windows. EXAFS studies were carried out on the DORIS ring at DESY, Hamburg. Fluorescence EXAFS apparatus and data-reduction procedures were as described in [25]. The spectra were calibrated in photon energy using a copper foil.

For the *H. pomatia* sample 36 spectra suitable for

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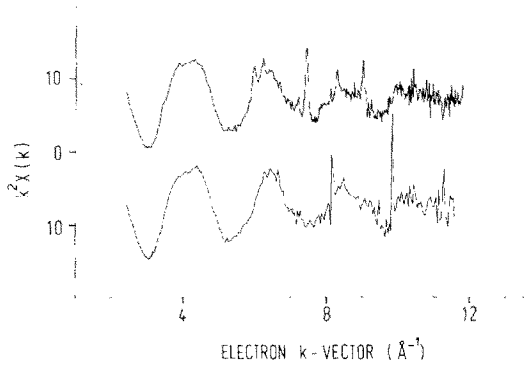

Fig.1. EXAFS oscillations of *Helix pomatia* (upper pattern) and *Panulirus interruptus* (lower pattern) oxyhemocyanins. The spectra are multiplied by k^2 to make higher k oscillations more visible. Except for monochromator 'glitches' the spectra are identical.

averaging were obtained, for the *P. interruptus*, 28. No evidence of sample decay during data collection was noted in comparing spectra taken at different times.

3. Results and discussion

EXAFS oscillations were extracted from the averaged data by standard procedures. The resulting spectra are shown in fig.1. The oscillations are virtually the same between the mollusc and the arthropod hemocyanin, demonstrating that the copper sites must be similar. The number of ligands must be the same and the copper–ligand distances are estimated to be the same to ±0.03 Å. The geometry of the sites could be different, as EXAFS provides no bond angle information, but this is unlikely. We conclude that the differences between the two kinds of hemocyanin are not the result of different copper surroundings but have to be assigned to the protein moiety.

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