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Ceruloplasmin–Diethyldithiocarbamate (DTC) Interaction. Evidence of DTC Chelation to Two Non Enzymatic Cu(II)

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Ceruloplasmin (Cp) is a plasma copper oxidase containing several copper ions. Among them five are essential to enzymatic activity and are classified into three types [1]: two cupric ions are of type I (I_a and I_b [2]) or 'blue', one cupric ion of type II or 'non blue' and two cupric ions of type III which are not EPR detectable.

In this communication we report evidence suggesting the presence of two extra cupric ions that can bind chelating agents such as diethyldithiocarbamate (DTC).

The addition of DTC to Cp (pH 5.5) yields an absorption at 445 nm in the Cp spectrum. When increasing equivalents of DTC are reacted with the protein no further spectral changes are appreciable at DTC/Cp > 4 and the absorptivity at 445 nm indicates the chelation of two Cu(II) forming two $Cu(DTC)_2$. The absorption at 610 nm is not modified and the circular dichroism (CD) spectrum exhibits only a slight decrease. On the other hand the band at 445 nm due to the DTC $\rightarrow Cu^{2+}$ charge transfer transition is not optically active indicating that the $Cu(DTC)_2$ complex is not coordinated to the protein. Addition of an inhibitor, such as N_3^-, to a Cp–DTC solution gives rise to the absorption at 380 nm and to the CD spectrum characteristic of N_3^- bound to type II Cu(II). Moreover, the Cp–DTC solution exhibits the same enzymatic activity as Cp free of DTC.

From these results we can infer that the five cupric ions necessary to the enzymatic activity of Cp are not modified by the addition of DTC which instead chelates two extra cupric ions hereafter labeled X and Y.

Both X and Y Cu(II) can be removed from Cp, by centrifugation, after addition of DTC, if this is performed within ten minutes following the addition of DTC. On the other hand, when Cp is incubated for several hours with DTC a very slight precipitate appears and the solution recovers the blue color of native Cp. If DTC is then added to the supernatant the absorption at 445 nm due to both DTC-bound X and Y reappears. These results suggest that as time elapses the $Cu(DTC)_2$ complexes dissociate and that X and Y Cu(II) reenter the protein.

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The Active Site of Bovine Serum Amine Oxidase

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Bovine serum amine oxidase (BSAO) is a type II copper-containing protein. The enzyme has a molecular weight of 190 000 daltons and 2 gram atoms of copper per mol protein which is composed of two electrophoretically equivalent subunits. Native BSAO exhibits a yellowish pink color by virtue of a broad band around 476 nm (ϵ = 3800) [1]. Replacement of Co(II) [2], Ni(II) and Zn(II) for the inherent Cu(II) which is coordinated by two *cis*-arranged imidazole nitrogens and two oxygens [3] revealed that the pink color comes from the organic prosthetic group. Electronic, CD and ESR spectra of native and metal substituted BSAO treated with phenylhydrazine suggested that the unknown prosthetic group is located very close to the copper(II) ion, but probably is not directly coordinated to the metal ion. The presence of a metal ion affects sensitively the electronic state of the prosthetic group whose role is considered to bind a substrate and catalyze the subsequent deamination. At the following step, two electrons are passed from the reduced chromophore to molecular oxygen probably through the copper ion. Combined cooperation of the chromophore and copper(II) ion during the whole enzymatic process corresponds to the catalytic role of a flavin group. We are now working on the prosthetic group to clarify its structure and properties

and also on the copper(II) ion in relation to the chromophore.

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EXAFS and X-ray Crystallographic Studies of Hemerythrin

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Hemerythrin is a respiratory protein in several phyla of marine invertebrates. The active site of the protein contains a binuclear iron center which cycles from the ferrous to the ferric state upon oxygen binding, with a concomitant reduction of oxygen to peroxide [1]. The structure of the binuclear iron center in the oxidized, metazide form of hemerythrin has been revealed by X-ray crystallography [2]. In the 2.2 Å resolution structure the two iron atoms are coordinated to 2 and 3 protein histidine residues, respectively. In addition, the irons are bridged by 2 protein carboxylates and an oxo group derived from solvent. The remaining octahedral coordination site on one of the iron atoms is occupied by the exogenous ligand, azide.

The present of a μ-oxo-bridged binuclear iron center in methemerythrins was predicted on the basis of spectroscopic and magnetic behavior [1]. Additional evidence has been obtained from the EXAFS above the iron K edge [3]. For metazidohemerythrin the first coordination shell data is well fit by 5 nitrogens plus oxygens at an average distance of 2.15 ± 0.05 Å and an additional oxygen at 1.80 ± 0.05 Å. The short Fe–O bond is consistent with observed Fe–O(μ-oxo) distances in model compounds and also with the X-ray crystallographic data for metazidohemerythrin.

Oxyhemerythrin shows many similarities to methemerythrins, particularly in the strong antiferromagnetic coupling of the iron atoms comprising the binuclear iron center [1]. A comparison of the EXAFS data for oxyhemerythrin and metazidohemerythrin [3] reveals even closer similarity

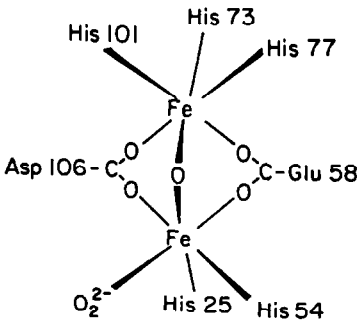

Fig. 1. Proposed structure of the binuclear iron center in oxyhemerythrin based on EXAFS and X-ray crystallographic results.

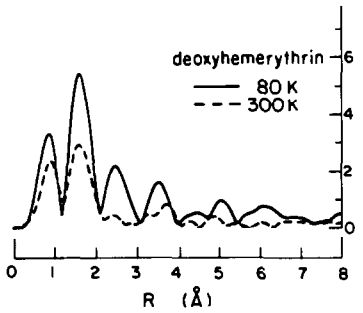

Fig. 2. Fourier transforms of the EXAFS above the iron K edge. Fe–Fe peak is at ~2.5 Å.

between the two forms than had been expected. They appear to have the same number and types of iron ligands, the same iron–ligand distances, and similar iron–iron distances. The proposed active site structure for oxyhemerythrin is analogous to that of metazidohemerythrin [2], but with peroxide in place of azide at the exogenous ligand site (Fig. 1).

Deoxyhemerythrin is distinguished by its loss of antiferromagnetic coupling between the iron atoms [1]. The decreased iron–iron interaction in deoxyhemerythrin is also apparent from the absence of the iron–iron peak in the Fourier transforms of the EXAFS data obtained at 300 K. The iron–iron peak can be observed by lowering the temperature to 80 °K (Fig. 2). The increased relative thermal motion of the two iron atoms in deoxyhemerythrin is apparently due to the absence of a μ-oxo bridge in this form of the protein.

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