

closely coordinated to copper in azurin at low temperature. The RR spectra are consistent with a monotonic relationship between the force constant of the Cu-S(cys) bond and the energy of the ligand-field transitions of the various proteins. The isotope studies lead to tentative identification of the RR modes which contain significant contributions from M-L stretches and internal histidine motions. The results provide an initial basis for reliable structural interpretation of the RR spectra of the blue copper proteins.

E4

Electron Transfer Loci on Blue Copper Proteins

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The chemical properties of the Cr(II)/(III) couple have been successfully employed to affinity label electron transfer loci on redox proteins. Being strong reductants and able to exchange their ligands sphere fast, the Cr(II) ions can coordinate one or more of the surface residues on the protein while reducing it. Since the Cr(III) produced is effectively substitution inert, any protein residues in the coordination sphere of the Cr(II) during the electron transfer will remain bound to the Cr(III) product. Identification of the Cr(III) binding loci has been achieved primarily through proteolytic cleavage of the different labeled proteins. Spectroscopic methods have been useful in corroborating these assignments.

Several single blue copper proteins have been examined by the above approach. These include the bacterial electron carriers azurin derived from *Pseudomonas aeruginosa* and *Alcaligenes faecalis*. Plastocyanin (from French bean and poplar tree) which serves as an electron mediator in the photosynthetic apparatus and stellacyanin obtained from the lacquer sap of *Rhus vernicifera* have all been studied by this method. More recently it has been shown that even the multicentered blue copper oxidase-laccase can be reductively labeled by this procedure. In this latter case, while 3.3 equivalents of Cr(II)aq were required for full reduction of the protein, only 0.7 Cr(III) ions remained bound to laccase after extensive dialysis. This may indicate a single reduction locus for Cr(II) in this protein.

The cuprous ions in the Cr(III) labeled plastocyanin, azurin and stellacyanin could be fully reoxidized by inorganic or enzymatic agents. While the original, single Cr(III) ion coordinated to azurin and stellacyanin remains bound through several Cr(II) reduction and reoxidation cycles, one can label plastocyanin with at least two Cr(III) ions in two redox cycles.

In the structures of both plastocyanin and *Ps.* azurin the 'northern' end imidazol of His-87 or 117 respectively was considered to be the potential electron transfer site. The analysis of the Cr(III) labeled sites on these two proteins clearly showed that electrons can also be introduced via different loci. These were proposed to proceed via the His 35 region in azurin (Az) and the negative patch on plastocyanin (Pc).

To examine whether the latter electron transfer sites are also involved in the biochemical function of these proteins, their reactivities, in the native and Cr(III) labeled forms were compared. For Pc, photo-reduction and oxidation by chloroplasts and by photosystem I reaction centers respectively were studied. For azurin the reactions with cytochrome c_{551} and *Ps.* cytochrome oxidase were investigated. It became apparent that the Cr(III) label attenuated the reactivity of both azurin and plastocyanin with only one of their respective partners. This led to the conclusions that on both proteins: (a) There are probably two distinct and physiologically operative electron transfer sites. (b) One of these sites is centered around the respective Cr(III) labeled region. (c) By elimination, the second is at the exposed, homologous imidazol of His-87 or 117 in Pc and Az respectively.

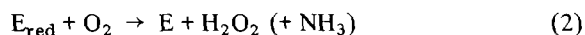
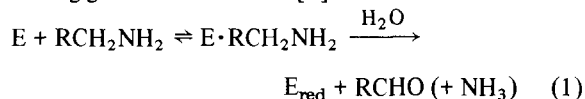
E5

Inhibition of Amine Oxidases by Cu(II) Complexes and Anions: Mechanistic Implications

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Copper-containing amine oxidases catalyze the oxidative deamination of primary amines by the following general mechanism [1]:



Our interests center on the role(s) of copper in reactions (1) and (2) and on the activation and utilization of O_2 by these enzymes. An important mechanistic question in this regard is whether the oxidation of E_{red} proceeds via sequential one-electron steps or via a single, two-electron step. One-electron oxidation would generate O_2^- as an intermediate, whereas a two-electron oxidation [2] would not. Accordingly we investigated several Cu(II) complexes, previously shown to be superoxide dismutase active, as potential