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Charge-Transfer Spectra of Model Cu(II) Chromophores

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Electronic 'tuning' of copper proteins for specific O_2-transport, redox, and oxygenase functions is determined by the number, nature and geometrical arrangements of the protein side-chain donor ligands which bind the copper ion(s) at the active site(s). The resulting energies and orientation of the upper occupied Cu(II) orbitals and d-vacancy in the oxidized proteins generally can be inferred from the ESR and ligand field (LF) spectra. At higher energies than those of LF absorptions, Cu(II) chromophores exhibit ligand to metal charge-transfer (LMCT) absorptions. These absorptions reflect a potentially important yet little studied aspect of electronic structure: the relative overlaps of the σ- and π-symmetry ligand donor orbitals with the Cu(II) d-vacancy. We review recent studies of the LMCT spectra exhibited by structurally unambiguous model Cu(II) chromophores containing ligation such as thiolate, thioether, imidazole, and imidazolate [1]. These results facilitate the understanding of corresponding LMCT absorptions exhibited by various copper proteins. Replacement of the Cu(II) in the model complexes and proteins by the less oxidizing Ni(II) and Co(II) ions results in characteristic blue-shifts of these LMCT absorptions, and supports an LMCT rather than MLCT assignment. Owing to the LF-dependency of the metal ion d-vacancy energy, the LMCT absorptions of planar Cu(II) and Ni(II) chromophores are blue-shifted relative to their approximately tetrahedral analogs.

Cu(II)–imidazole complexes exhibit LMCT absorptions originating from the ligand nitrogen lone pair and from two ring π orbitals, one having primarily carbon 2p character (π_1, HOMO) and the other having primarily nitrogen 2p character (π_2). The calculated (INDO/S method) orbital energy increases induced by successive ring methylations are correlated with red shifts of imidazole → Cu(II) LMCT exhibited by this series of complexes [2]. Spectroscopic effects associated with the orientation of the imidazole rings relative to the Cu(II) d-vacancy also are discussed. Imidazolate ligation results in a characteristic additional LMCT absorption at relatively low energy. The intense ($\epsilon > 1000$) near UV LMCT absorption associated with equatorial Cu(II)–thioether bonding disappears when, as for plastocyanin, the Cu(II)–thioether bond becomes elongated (apical) and is oriented normal to the d-vacancy. The syntheses and LMCT spectra of model M(II)–thiolate complexes (M = Cu, Co, Ni) are described briefly.

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Insights into Blue Copper Proteins Using Spectroscopic Methods

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By controlling the structure of the binding site, a protein can profoundly influence the properties of a metal center including its chemical potential, its kinetic reactivity and its binding constant. For example, it is evident from small molecule studies that electron transfer is much more facile in copper systems when minimal structural reorganization is required. To fill in some of the gaps in our knowledge about structural aspects of blue copper proteins, as summarized below, we have carried out a variety of physical studies on the proteins themselves as well as appropriate derivatives and relevant small molecules.

The nickel(II) derivative of azurin, Ni(II)Az, has been prepared and shown to contain two unpaired electrons which virtually rules out the possibility of a planar nickel site. This is despite the pronounced tendency of nickel(II) to prefer planar binding of an N_2S_2 donor set involving thiolate sulfurs [1]. The paramagnetism and the fact that d–d bands are found in the near infra-red argue for a pseudotetrahedral binding geometry and suggest that the metal binding

site of azurin is rather rigidly defined by the peptide moiety.

Further structural information has been obtained from NMR studies. Due to the rapid electron spin relaxation associated with tetrahedral nickel(II), we have been able to observe isotropically shifted resonances in the ^1H NMR spectrum which can be attributed to protons of the ligand moieties [2]. These resonances shift discontinuously with pH in parallel with the C2 hydrogen of histidine-35, providing direct evidence that this protonation is coupled to a conformational change. ^{113}Cd NMR studies indicate there is little difference between the binding sites of stellacyanin and azurin [3] even though stellacyanin lacks methionine which is one of the copper ligands of azurin. The NMR results are consistent with the suggestion that a closely related donor type, probably cystine sulfur, replaces methionine sulfur in the case of stellacyanin [4].

Finally, metal replacement studies have also proved useful in studies of tree laccase. By substituting mercury(II) for copper(II) in the type 1 site we have for the first time been able to isolate the EPR spectrum of type 2 copper, allowing observation of ligand hyperfine splitting in the perpendicular region [5].

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Cryo-Vibrational Spectroscopy of Blue Copper Proteins

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Resonance Raman (RR) and Fourier transform infrared (FTIR) spectra at 12 K have been obtained for *Pseudomonas aeruginosa* azurin, spinach plastocyanin, stellacyanin, and tree laccase. The temperature dependence of the azurin, plastocyanin, and stellacyanin spectra have been recorded as have the RR excitation profiles at 12 K. Room temperature RR spectra have been obtained for azurins from *Alcaligenes fecalis, Alcaligenes sp., Bortadella pretussis,* and *Bortadella bronchiseptica*; bean plastocyanin; fungal laccase, human ceruloplasmin; and zucchini squash ascorbate oxidase. Isotope studies employing $^{63}Cu/^{65}Cu$ and H/D substitution have been performed on the azurins from *Ps. aeruginosa, Alc. fecalis,* and *Alc. sp.* Principal conclusions include the following: The intense RR modes near 400 cm^{-1} include internal ligand deformations and the Cu–S(cys) stretch, rather than the Cu–S(cys) stretch and Cu–N(his-Im) stretches as previously supposed. The Cu–N(his-Im) stretches are assignable to the ubiquitous feature near 265 cm^{-1}, consistent with the frequencies of similar motions in other proteins and in model complexes. Spinach plastocyanin exhibits a frequency shift of 14 cm^{-1} in its cysteine C–S stretching frequency (*ca.* 750 cm^{-1}) upon freezing of the protein solution, suggesting that extra-protein forces (*e.g.*, solvent structure, crystallization, or substrate binding) can influence the conformation of the active site. Above the freezing point of the solvent the plastocyanin RR modes are unusually broad, suggesting either extremely facile dephasing processes, or large inhomogeneous effects due to thermally accessible conformations in the high-temperature form of the active site. No selective enhancement of either the strong or weak RR modes is observed in the S(cys) → Cu charge transfer excitation profiles of azurin, plastocyanin, or stellacyanin at 12 K. The azurin species other than *Ps. aeruginosa* exhibit an 'extra' strong RR peak near 400 cm^{-1} which is, however, seen to be related to an unresolved shoulder in the 12 K *Ps. aeruginosa* spectrum. It is therefore unnecessary to invoke higher coordination numbers than four for copper to explain the RR spectra of the azurins other than *Ps. aeruginosa.* RR peaks appear in the azurin spectrum below 200 K which may be due to methionine C–S stretching modes and Cu–S(met) stretch or methionine C–S–C angle bend. If these low-temperature features are indeed due to such motions, methionine must be