

## Communications to the Editor

### Copper L-Edge Spectral Studies: A Direct Experimental Probe of the Ground-State Covalency in the Blue Copper Site in Plastocyanin

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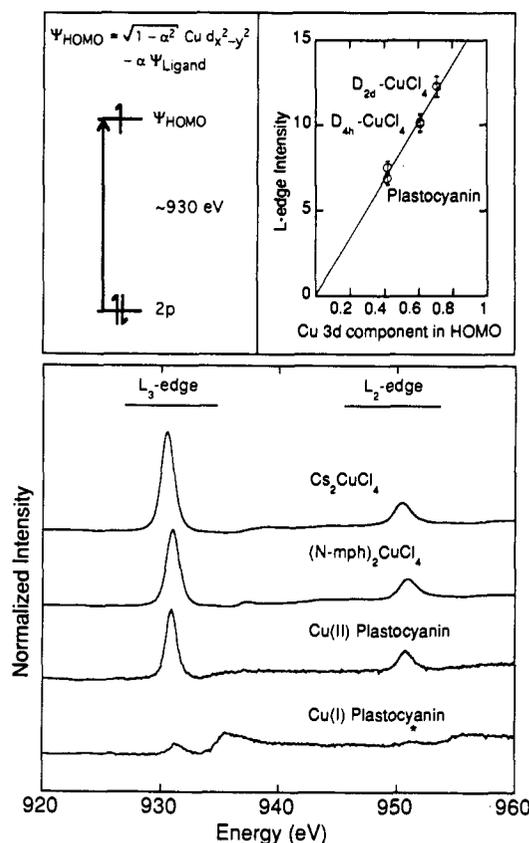
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The blue copper protein site is involved in rapid, long-range, outer-sphere electron transfer. The facility of the electron-transfer reactivity has been related to the ground-state wavefunction of the Cu(II) site, which has been found to be highly covalent as a result of a strong interaction with the thiolate sulfur of a cysteine ligand.<sup>1</sup> This bonding description has been derived from self-consistent-field X $\alpha$  scattered-wave (SCF-X $\alpha$ -SW) molecular orbital calculations. Cu L-edge X-ray absorption spectroscopy (XAS) is used here to obtain strong experimental support for this bonding description.

Over the past few years, synchrotron radiation beam lines<sup>2</sup> and array detectors<sup>3</sup> have been developed which permit measurement of soft X-ray spectra of dilute metalloproteins.<sup>3</sup> In recent publications, we have shown the chemical sensitivity of multiplet splittings of manganese L-edges<sup>4</sup> and applied this spectroscopy to the mononuclear iron center in rubredoxin.<sup>5</sup> Here, we present a new application in that we use the intensity of the copper(II) L<sub>2,3</sub> peaks, normalized to the continuum intensity and calibrated using the well-defined complex D<sub>4h</sub>-CuCl<sub>4</sub><sup>2-</sup>, to estimate the degree of covalency between a Cu(II) ion and its ligands.<sup>6</sup>

L-edge spectra were measured using AT&T Bell Labs beam line U4-B at the National Synchrotron Light Source, Brookhaven National Laboratory. Fluorescent X-rays from the protein samples were detected using a windowless 13-element germanium array detector<sup>3</sup> (Canberra Industries), while the total electron yield from model complexes was measured using a Galileo 4716 channeltron electron multiplier. The sample chamber was maintained at a vacuum of 2 × 10<sup>-9</sup> Torr or better, enabling



**Figure 1.** Intensity variation of Cu 2p to 3d  $\Psi_{\text{HOMO}}$  transition. Top left panel: Energy level diagram illustrating the observed Cu 2p to 3d  $\Psi_{\text{HOMO}}$  transition. Top right panel: Total integrated intensity of the L<sub>2</sub> and L<sub>3</sub> peaks normalized to the continuum intensity plotted as a function of estimated Cu 3d<sub>x<sup>2</sup>-y<sup>2</sup></sub> character in the HOMO; 5% error bars are included. Lower panel: Cu L-edge spectra of (from top to bottom) D<sub>2d</sub>-Cs<sub>2</sub>CuCl<sub>4</sub>, D<sub>4h</sub>-(N-mph)<sub>2</sub>CuCl<sub>4</sub>, Cu(II) plastocyanin, and Cu(I) plastocyanin. The spectra have been normalized to the continuum intensity. Features marked with an asterisk (\*) in the Cu(I) plastocyanin data arise from incomplete reduction.

windowless operation between the storage ring, sample, and detector. Spectra were calibrated using the L<sub>3</sub> peak position of CuF<sub>2</sub>, with the absorption maximum assigned as 930.5 eV. The continuum intensity was derived by a linear fit through the preedge (870–920 eV) and continuum (1000–1050 eV) regions, offset by an “edge-jump” floating parameter. Total L-edge intensity was calculated as 2L<sub>2</sub> + L<sub>3</sub>. This weighting corrects for the different degeneracies of each state. Corrections for the different fluorescence yields of the L<sub>2</sub> and L<sub>3</sub> edges were made when appropriate, but were found to have minimal effect. Fluorescence self-absorption was checked for and found to be negligible for these samples. Plastocyanin was prepared using published procedures<sup>7</sup> and partially dehydrated to form thin films.<sup>8</sup> Crystals of cesium tetrachlorocuprate(II) (D<sub>2d</sub>-distorted T<sub>d</sub>)<sup>9</sup> and bis(N-methyl-N-phenethylammonium) tetrachlorocuprate(II) [(N-mph)<sub>2</sub>, square planar, D<sub>4h</sub>]<sup>10</sup> were prepared as previously documented. Finely

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(8) Partially dehydrated thin films were made by placing about 0.1 mL of 2.00 mM protein in 10 mM phosphate buffer pH 7.0 on a silicon plate at 4 °C and evaporating under partial vacuum. Protein samples were oxidized or reduced by addition of small quantities of potassium ferricyanide or sodium dithionite, respectively.

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powdered compounds (mortar and pestle) were spread across an adhesive surface of rubber cement or double-sided adhesive tape.

Figure 1 (lower panel) presents L-edge spectra for oxidized and reduced samples of the blue copper protein plastocyanin and  $D_{2d}$ -distorted tetrahedral and square planar  $D_{4h}$  tetrachlorocuprate complexes. For the Cu(II) samples, the  $L_3$  and  $L_2$  edges are split by  $\sim 20$  eV and both edges are dominated by single strong peaks. Since the final states involve a filled 3d shell ( $2p^5 3d^{10}$ ), this splitting results from the 2p core-hole spin-orbit interaction. Reduction of the blue copper protein results in the elimination of these peaks. This is expected as Cu(I) is a  $3d^{10}$  ion, and no  $2p \rightarrow 3d$  transitions are possible. However, new features appear at 4.5-eV higher energy which can be assigned as  $2p^6 3d^{10} \rightarrow 2p^5 3d^{10} 4s^1$  transitions.

The Cu  $2p \rightarrow 3d$  transition is electric dipole allowed, and the 2p orbital is localized on the copper. Therefore, the integrated intensity of the  $L_{2,3}$  absorption peaks in the Cu(II) spectra, which involve the Cu  $2p \rightarrow \Psi_{\text{HOMO}}$  transition (Figure 1, top left), should be a direct measure of the  $3d_{x^2-y^2}$  character ( $1 - \alpha^2$ ) in the half-occupied HOMO. As seen in Figure 1, the intensity of the  $2p \rightarrow 3d$  transition in the blue copper site is reduced from that in square planar  $\text{CuCl}_4^{2-}$ , indicating that the blue copper site has less  $d_{x^2-y^2}$  character in the HOMO and is therefore more covalent. Quantitatively, spectral studies have demonstrated that the HOMO of square planar  $\text{CuCl}_4^{2-}$  has 61%  $d_{x^2-y^2}$  character.<sup>11</sup> The integrated intensity ratio of plastocyanin L-edge peaks to those of  $D_{4h}\text{-CuCl}_4^{2-}$  is 0.67, indicating that the  $d_{x^2-y^2}$  character in the HOMO of plastocyanin is  $\sim 41\%$ . This is in good agreement with SCF-X $\alpha$ -SW calculations which predict 42% Cu  $d_{x^2-y^2}$  character.<sup>12</sup> Among a variety of copper samples studied, the blue copper proteins exhibit the weakest L-edge peak intensities,

corresponding to the most covalent copper environments. It should also be noted from Figure 1 that the L-edge intensity for  $D_{2d}\text{-CuCl}_4^{2-}$  is 1.2 times larger than that of  $D_{4h}\text{-CuCl}_4^{2-}$ , indicating a less covalent  $d_{x^2-y^2}$  orbital with  $\sim 74\%$  Cu character; SCF-X $\alpha$ -SW calculations give 71%.<sup>11</sup> This trend is illustrated in the top right panel of Figure 1.

The energies of the Cu(II)  $2p \rightarrow 3d$  peaks also vary with metal site. The  $L_3$  peaks of the  $D_{2d}\text{-CuCl}_4^{2-}$ ,  $D_{4h}\text{-CuCl}_4^{2-}$ , and plastocyanin occur at 930.50, 931.05, and 930.65 eV, respectively. Shifts in the position of these peaks should approximately correspond to changes in the energy of the half-occupied  $\Psi_{\text{HOMO}}$  (Figure 1, top left), which in turn arise from changes in the ligand field. Since the ligand field splitting of the d orbitals is sensitive to geometry, the energy of the copper L-edge is thus a potential indicator of different types of structure. The half-occupied  $d_{x^2-y^2}$  level of  $\text{CuCl}_4^{2-}$  is known to be destabilized by about 0.60 eV ( $5000\text{ cm}^{-1}$ ) on distortion from  $D_{2d}$  to  $D_{4h}$  geometry.<sup>11</sup> This agrees well with the experimentally observed shift in the  $L_3$  peak of 0.55 eV. The energy of the  $L_3$  peak of plastocyanin is close to that of  $D_{2d}\text{-CuCl}_4^{2-}$ , consistent with its distorted tetrahedral structure.

In summary, the intensities of L-edge spectra of Cu(II) complexes can be used to experimentally estimate the copper character in the half-occupied HOMO. For plastocyanin, this is found to be  $\sim 41\%$ , which is in good agreement with SCF-X $\alpha$ -SW calculations<sup>11</sup> and demonstrates that the redox active orbital is highly covalent in the blue copper active site.

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