

## 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

Simon J. George,<sup>†</sup> Michael D. Lowery,<sup>‡</sup>  
Edward I. Solomon,<sup>\*,‡</sup> and Stephen P. Cramer<sup>\*,†,§</sup>

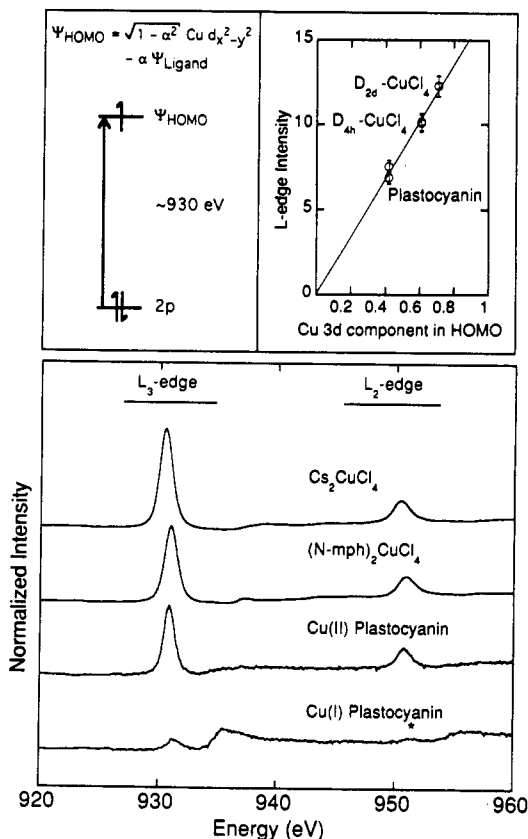
Department of Applied Science  
University of California  
Davis, California 95616  
Energy and Environment Division  
Lawrence Berkeley Laboratory  
Berkeley, California 94720  
Department of Chemistry  
Stanford University  
Stanford, California 94305

Received October 26, 1992

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  $\times$  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), square planar, D<sub>4h</sub>]<sup>10</sup> were prepared as previously documented. Finely

(7) Ellefson, W. L.; Ulrich, E. A.; Krogmann, D. W. *Methods Enzymol.* **1980**, *69*, 223–228.

(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.

(9) (a) Sharnoff, M.; Reimann, C. W. *J. Chem. Phys.* **1965**, *43*, 2993–2996. (b) Sharnoff, M. *J. Chem. Phys.* **1964**, *41*, 2203.

\* Authors to whom correspondence should be addressed.

<sup>†</sup> University of California, Davis.

<sup>‡</sup> Stanford University.

<sup>§</sup> Lawrence Berkeley Laboratory.

(1) Solomon, E. I.; Baldwin, M. J.; Lowery, M. D. *Chem. Rev.* **1992**, *92*, 521–542.

(2) (a) Chen, C. T. *Nucl. Instrum. Methods Phys. Res.* **1987**, *A256*, 595–604. (b) Chen, C. T.; Sette, F. *Rev. Sci. Instrum.* **1989**, *60*, 1616–1621.

(3) Cramer, S. P.; George, S. J.; Kraner, H.; Rogers, L.; Rescia, S.; Radeka, V.; Yocum, M.; Colaresi, J.; Tench, O.; Mullins, O. C. *X-ray Absorption Fine Structure—Proceedings of the 6th International XAFS Conference*; Hasnain, S. S., Ed.; Ellis Horwood: Chichester, 1991; pp 640–645.

(4) Cramer, S. P.; de Groot, F. M. F.; Ma, Y.; Chen, C. T.; Sette, F.; Kipke, C. A.; Eichhorn, D. M.; Chan, M. K.; Armstrong, W. H.; Libby, E.; Christou, G.; Brooker, S.; McKee, V.; Mullins, O. C.; Fuggle, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7937–7940.

(5) George, S. J.; van Elp, J.; Chen, J.; Ma, Y.; Chen, C. T.; Park, J.-B.; Adams, M. W. W.; Searle, B. G.; de Groot, F. M. F.; Fuggle, J. C.; Cramer, S. P. *J. Am. Chem. Soc.* **1992**, *114*, 4426–4427.

(6) Horsley deconvoluted platinum L-edge spectra into near-edge peaks and an edge jump in an attempt to estimate the 5d occupancy from the L-edge peak intensity. Horsley, J. A. *J. Chem. Phys.* **1982**, *76*, 1451–1458.

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.

**Acknowledgment.** This work was supported by the National Institutes of Health (GM-44380 to S.P.C.), the National Science Foundation (CHE-9217628 to E.I.S.), and LBL Exploratory Research Funds to S.P.C. The National Synchrotron Light Source is funded by the Department of Energy, Office of Basic Energy Sciences.

(10) Hitchman, M. A.; Cassidy, P. J. *Inorg. Chem.* **1979**, *18*, 1745-1754.

(11) Gewirth, A. A.; Cohen, S. L.; Schugar, H. J.; Solomon, E. I. *Inorg. Chem.* **1987**, *26*, 1133-1146.

(12) (a) Gewirth, A. A.; Solomon, E. I. *J. Am. Chem. Soc.* **1988**, *110*, 3811-3819. (b) Penfield, K. W.; Gewirth, A. A.; Solomon, E. I. *J. Am. Chem. Soc.* **1985**, *107*, 4519-4529.