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QUANTITATIVE Cu X-RAY ABSORPTION EDGE STUDIES : OXIDATION STATE AND SITE STRUCTURE DETERMINATION

LUNG-SHAN KAU, J.E. PENNER-HAHN, E.I. SOLOMON⁽¹⁾ and
K.O. HODGSON⁽¹⁾

*Department of Chemistry, Stanford University, Stanford,
CA 94305, U.S.A.*

Abstract

X-ray absorption edge studies of Cu(I) complexes with different coordination number and covalency reveal that the 8983-8984 eV feature (assigned as the $1s \rightarrow 4p$ transition) can be correlated with ligation and site geometry. These Cu(I) features have been qualitatively interpreted using a Ligand Field model, and this has been applied to analyze the polarized single crystal, pH dependent edge spectra of reduced plastocyanin. In addition, normalized difference X-ray absorption edge analysis has been used to quantitatively determine the percent of Cu(I) in several derivatives of the multicopper oxidase, laccase.

Introduction

Determination of oxidation state and geometry is essential for the interpretation of metal ion active site chemistry but can be difficult for many copper centers. This problem derives from the fact that the usual spectroscopic methods, in particular EPR, cannot probe the $3d^{10}$ cuprous configuration nor distinguish reduced copper from an antiferromagnetically coupled EPR nondetectable cupric pair. However, cuprous complexes exhibit a strong X-ray absorption edge feature at ~8984 eV which is absent in Cu(II) complexes [1]. We have found X-ray absorption edge spectroscopy to be a most useful qualitative probe of Cu(I) geometry, and can be further used to quantitate the amount of reduced copper present in multicopper enzymes [2].

Experimental

All X-ray absorption edge data were measured at Stanford Synchrotron Radiation Laboratory utilizing several different beam lines. All protein XAS edges were measured using a Si[220] double crystal monochromator and were recorded as fluorescence excitation spectra with an array of NaI(Tl) scintillation detectors. The copper model compound data were collected in transmission mode. To insure a consistent energy reference, the internal calibration method with Cu foil was used [3]. To allow proper normalization, the absorption was measured for at least 300 eV below and 200 eV above the Cu K edge. The data presented are pre-edge background-subtrated and normalized to give an edge jump of 1.0 at 9000 eV.

Result and discussion

We have systematically studied a number of copper model compounds (20 Cu(I) and 43 Cu(II)) to correlate copper X-ray absorption features with oxidation state and geometry. The Cu(I) compounds studied represent different

⁽¹⁾To whom all correspondence should be addressed

coordination numbers, geometries and degrees of covalency. Most Cu(II) complexes studied have geometries close to tetragonal but with different ligand sets and therefore covalency.

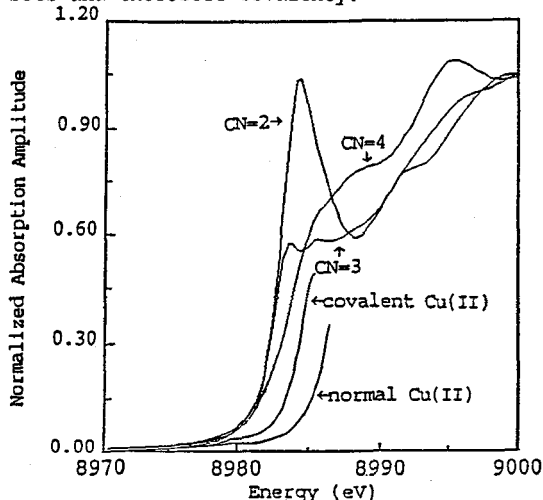


Figure 1. Absorption edge spectra of representative 2-, 3- and 4-coordinate Cu(I) complexes with those of normal and covalent Cu(II) complexes (below 8985.0 eV).

Using the energy calibration and normalization procedure described above, we find that, in all cases, Cu(II) complexes show no low energy pre-edge peak below 8985.0 eV and that their absorption intensities in this region are significantly lower than those of Cu(I) complexes, which exhibit a pre-edge maximum in the 8983-8984 eV region. As summarized in figure 1, we find that the shape, energy and intensity of the pre-edge maximum varies significantly over the different Cu(I) complexes studied. These Cu(I) pre-edge spectral changes can be correlated systematically with coordination number and geometry of the metal ion and interpreted using a simple ligand field model which predicts that the $4p_{x,y,z}$ in the free Cu(I) ion will be split differently by the ligand field associated with different geometries.

The Cu(I) model compounds with a linear CuN_2 ligation environment have the most intense pre-edge peak. Polarized single crystal studies on linear 2-coordinate Cu(I) complexes have shown that the 8983-8984 eV feature is a x,y polarized, $1s \rightarrow 4p$ electric dipole allowed transition (ligands are along the z axis) [4]. The transition from the $1s$ to the doubly degenerate $4p_{x,y}$ final state then results in an intense pre-edge peak at lower energy than that of $1s \rightarrow 4p_z$ and the covalent overlap along z axis will reduce the intensity of $1s \rightarrow 4p_z$ transition. For a 3-coordinate T-shaped complex (the third ligand along the y axis), the $4p_{x,y}$ pair would be further split with the $4p_x$ level shifted to higher energy relative to $4p_y$. In the limit of a trigonal complex ($\sim D_{3h}$, with C_3 along the x axis), $4p_x$ should be lower in energy than the degenerate $4p_{y,z}$. All 3-coordinate Cu(I) complexes have an ~ 8984 eV transition with roughly half the intensity of those with linear ligation and have a second feature on the higher energy side of the 8984 eV peak which is reasonably assigned as the $1s \rightarrow 4p_z$ transition. Finally, for the four coordinate tetrahedral Cu(I) complexes, a broad pre-edge maximum shifted to higher energy (~ 8985.5 eV) is observed in figure 1.

Most of the Cu(II) complexes studied have a very weak $1s \rightarrow 3d$ transition at 8978 eV and, in addition, many show structure on the absorption edge at energies of 8986-8988 eV. In all complexes studied, this Cu(II) peak is always observed at energies greater than 8985.0 eV. Alternatively, most Cu(II) complexes do exhibit a pre-edge low energy tail through this 8983-8984 eV region (figure 1). With six exceptions, the Cu(II) complexes still have quite low intensity over this 8983-8984 eV range. These exceptions are highly covalent Cu(II) complexes (with sulfur ligation) and the higher intensity in this low energy tail appears to be related to this increased covalency [5].

To further quantify these features, we have calculated the normalized difference absorption edge spectra (NDAES) by subtracting the normalized edge of a representative Cu(II) complex from that of a Cu(I) or from a covalent Cu(II) complex [6]. The difference of properly normalized Cu(I) and Cu(II) edge spectra has a derivative shape (figure 2) with a positive peak at 8983-8984 eV for 2- and 3-coordinate Cu(I) and at 8986 eV for 4-coordinate

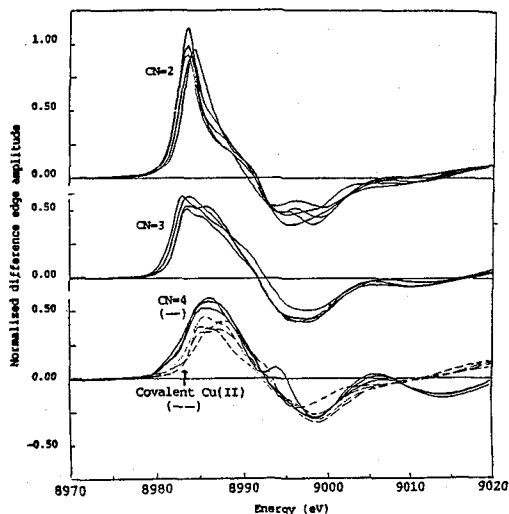


Figure 2. Representative normalized difference edge spectra for different Cu(I) and covalent Cu(II) minus normal Cu(II).

Cu(I), and a broad negative feature for all Cu(I) complexes at -8990-9000 eV. As can be seen in figure 2, the difference edge spectra of covalent Cu(II), although qualitatively similar in shape to those of 2- and 3-coordinate Cu(I), have lower intensity and in particular their maxima are shifted to significantly higher energy. We have also simulated NDAES for binuclear Cu(I) systems where each copper can have a different coordination number (2&3, 2&4 and 3&4). From the energy and shape of the NDAES maxima, we find that it is possible to distinguish 2, 3, 2&3 and 2&4 coordinate Cu(I) complexes from 4 coordinate Cu(I) and covalent Cu(II) systems. Alternatively, there is ambiguity in distinguishing between 4 coordinate Cu(I) and covalent Cu(II). Once the approximate geometry of a copper site is known, it is further possible to use the amplitude of the peak maxima of the NDAES in figure 2 to quantitate the amount of Cu(I) present.

This NDAES technique has been applied to determine the amount of Cu(I) present in several derivatives of the multicopper oxidase, laccase [2,6]. Laccase is the simplest of the multicopper enzymes, containing a total of four copper ions (one type 1 (T1) Cu, one type 2 (T2) Cu and a coupled binuclear copper center, type 3 (T3)) which together catalyze the four-electron reduction of dioxygen to water. Knowledge of the amount of reduced copper is very important in defining O_2 and H_2O_2 reactivities of the Type 2 copper depleted (T2D) and native enzymes [7]. Our X-ray absorption studies of laccase demonstrated that the T2D derivative contains a significant amount of reduced copper (~70%) and that this can be oxidized by reaction with excess H_2O_2 [6]. EPR and optical data shows that the T1 copper is fully oxidized, thus, greater than 90% of the T3 centers are reduced in T2D. In addition, from similar NDAES results, the reduced T3 site is found to be stable to oxidation by O_2 or by 25-fold protein equivalents of ferricyanide. These studies have been further extended to the native enzyme and show that ~25% of the T3 sites are reduced in the presence of O_2 but are reoxidized by peroxide [2].

This analysis of the Cu(I) edge features has been further applied to the polarized, pH dependent edge spectra of reduced plastocyanin. The pH dependent protein single crystal structure of reduced plastocyanin has been solved by Freeman et. al. [8] and, in collaboration, we have collected the polarized single crystal X-ray absorption edge spectra (figure 3) [9]. At pH =7.0, the Cu-S₉₂(met) bond is the unique, long axis of the active site with Cu-S₉₂(met) = 2.86 Å. Alternatively at pH =3.9, the Cu-N₈₇ bond becomes the long axis with Cu-S₉₂(Met) = 2.53 Å and Cu-N₈₇ = 3.19 Å. Based on the ligand field splitting of the d_{xy} orbitals, spectra taken with the E vector of the synchrotron radiation along the Cu-S₉₂(met) bond in the high pH sample would be expected to show a strong 8984 eV peak, which should be shifted to higher energy in the low pH sample. Analogously, with the E vector perpendicular to the Cu-S₉₂(met) bond (with a projection along the Cu-N₈₇ direction), a strong 8984 eV peak would be expected for the low pH but not the high pH sample. These predictions fit nicely the experimental spectra given in figure 3.

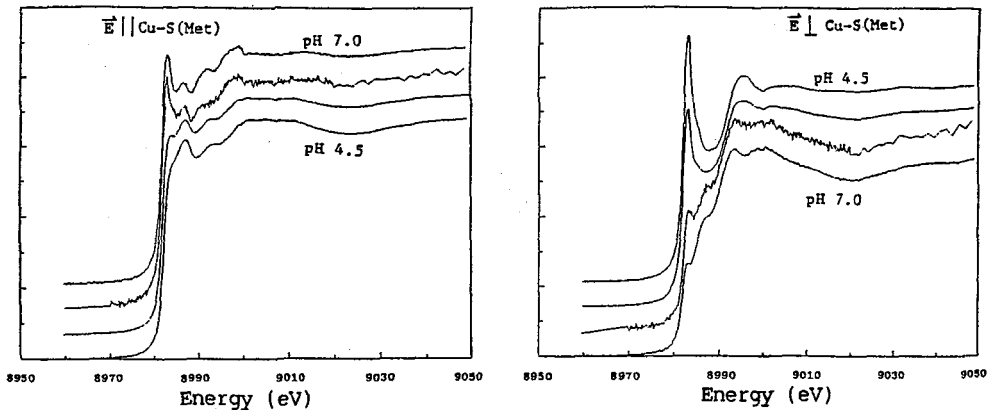


Figure 3. Cu absorption edge for reduced plastocyanin oriented with $\vec{E} \parallel \text{Cu-S(Met)}$ (left, from top, pH 7.0, 6.5, 5.5 and 4.5.) and with $\vec{E} \perp \text{Cu-S(Met)}$ (right, from top, pH 4.5, 5.5, 6.5 and 7.0).

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