QUANTITATIVE Cu X-RAY ABSORPTION EDGE STUDIES: OXIDATION STATE AND SITE STRUCTURE DETERMINATION
Lung-Shan Kau, J. Penner-Hahn, E. Solomon, K. Hodgson

To cite this version:

HAL Id: jpa-00226215
https://hal.archives-ouvertes.fr/jpa-00226215
Submitted on 1 Jan 1986

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
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-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 3d⁰ 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 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-subtracted 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.

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 \( \rightarrow \) 3d transition 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 \( xy \) polarized, \( 1s \rightarrow 4p \) electric dipole allowed transition (ligands are along the \( z \) axis) [4]. The transition from the \( 1s \) to the doubly degenerate \( 4p \) final state then results in an intense pre-edge peak at lower energy than that of \( 1s \rightarrow 4p \) transition. For a 3-coordinate \( T \)-shaped complex (the third ligand along the \( y \) axis), the \( 4p_y \) pair would be further split with the \( 4p_y \) level shifted to higher energy relative to \( 4p_z \). In the limit of a trigonal complex (-D\(_3\)), \( 4p_y \) should be lower in energy than the degenerate \( 4p_x \). 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 \( 1s \rightarrow 4p \) 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 substituting 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).

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 O2 and H2O2 reactivities of the Type 2 copper depleted (T2D) and native enzymes [7]. 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 H2O2 [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 O2 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 O2 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-S (met) bond is the unique, long axis of the active site with Cu-S (met) = 2.86 Å. Alternatively at pH = 3.9, the Cu-N7 bond becomes the long axis with Cu-S2 (Met) = 2.53 Å and Cu-N7 = 3.19 Å. Based on the ligand field splitting of the 3d orbitals, spectra taken with the E vector of the synchrotron radiation along the Cu-S2 (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-S2 (met) bond (with a projection along the Cu-N7 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.
Acknowledgment

We thank Dr.'s Hans Freeman and Darlene J. Spira-Solomon for their contributions to this research and useful discussions. The work reported herein was supported by NIH AM 31450 (EIS) and CHE 85:12129 (KOH). Synchrotron beam time was provided by the Stanford Synchrotron Radiation Laboratory which is supported by U.S. Department of Energy and the Division of Research Resources of the National Institute of Health.

References

8. Freeman, H.C., personal communication.