X-ray Magnetic Circular Dichroism Sum Rule Analysis of the Blue Copper Site in Plastocyanin. A Probe of Orbital and Spin Angular Momentum


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Received: April 30, 1998; In Final Form: July 21, 1998

Cu L edge X-ray magnetic circular dichroism (XMCD) spectroscopy has been used to study the electronic structure of the blue copper site in plastocyanin. Application of the orbital angular momentum sum rule leads to an experimental (Cu 3d specific) orbital moment \(<L>_z\) of 0.07 ± 0.02 \(\hbar/\text{Cu atom}\). This compares to 0.054 \(\hbar\) derived from the EPR measurements and 0.059 \(\hbar/\text{Cu atom}\) from a SCF-Xα-SW calculation. Application of the spin angular momentum sum rule leads to an experimental spin moment \(<S>_z\) of 0.18 ± 0.02 \(\hbar/\text{Cu atom}\), compared to 0.21 \(\hbar/\text{Cu atom}\) by SCF-Xα-SW. XMCD sum rule analysis should be a useful probe of electronic structure for many other problems in inorganic and bioinorganic chemistry.

Introduction

X-ray magnetic circular dichroism (XMCD) has recently emerged as a powerful element and site-specific probe of electronic and magnetic structure. The development of ligand field multiplet calculations allows detailed predictions to be made about orbital and spin angular momentum for particular elements and oxidation states in complex samples. Although XMCD applications are now common in materials science and especially magnetic technology, its use in biological and inorganic chemistry has been hindered by technical problems associated with the spectroscopy of dilute and paramagnetic metal centers. In this paper we present XMCD spectra for the blue Cu site in plastocyanin, recorded with a new dilution refrigerator system and 30-element fluorescence detector. Plastocyanin is an ideal system for testing fluorescence-detected XMCD sum rule analysis because its electronic structure is well understood from a variety of experimental and theoretical approaches. The half-occupied HOMO is highly covalent with only ~42% Cu d\(^{2}\) character. Our XMCD results are compared with EPR data and electronic structure calculations, and a variety of potential applications are discussed.

Experimental Section

The sample, plastocyanin, was isolated from spinach by previously published methods to a final concentration of 0.8 mM in pH 7 potassium phosphate buffer. A drop (20–50 \(\mu\)L) of the sample was placed on a gold-plated copper sample holder and allowed to dry over a period of 2 h at room temperature in air. During the experiment, the time of beam-on was controlled carefully to minimize the photoreduction of Cu(II) to Cu(I). In the last spectra taken, we estimate less than 10% Cu(I) presence in plastocyanin by monitoring the changes in the minor L edge feature (at about 938 eV). On the other hand, Cu(I) has no XMCD effect.

The XMCD experiments were performed at the SSRL bend magnet beamline 8-2 using the 1100 line/mm grating. Elliptically polarized X-rays were obtained by moving the first mirror to different positions on the two coils. Calibration experiments on polarized ferromagnetic samples, the optimum XMCD signal was obtained at a beam takeoff angle corresponding to a circular polarization of 85 ± 5%. The energy resolution is estimated to be 1 eV at the Cu L edge with these elliptically polarized X-ray beams (with both slits at 50 \(\mu\)m). The XMCD apparatus uses a 76 cm split-coil 2 T superconducting magnet surrounded by a UHV chamber maintained at \(\sim 5 \times 10^{-8}\) Torr. A 30-element windowless Ge fluorescence detector is inserted horizontally between the two coils, perpendicular to the photon beam path. The coldfinger of a He\(^{3}/\text{He}\)\(^{4}\) dilution refrigerator enters the magnet bore from the top of the chamber, and the sample is attached to the coldfinger at the center of the magnet bore. Samples were introduced into the magnet bore through a vacuum load lock and screwed into the coldfinger using a removable sample insertion device. The lowest operating temperature observed in our experiments was on the order of 400 mK, as measured by a carbon resistance thermometer and by a magnetization curve.

Individual scans were taken over the Cu L edges using 0.2 eV steps at 6 s per point integration time. One set of 20 scans was taken with right circular polarization. Every two scans, the magnetic field was switched between −2 and +2 T. A second set of 20 scans was then taken with left circular polarization, again alternating the sign of the magnetic field. The apparent XMCD effect did reverse with opposite beam polarization. For measurement of the magnetization curves, a similar procedure was followed, collecting only the L\(_2\) edge region, using between 0.1 and 2.0 T. The raw data (fluorescence signal, \(F\)) were divided by an internal \(I_0\) monitored by the intensity of oxygen K fluorescence (proportional to the beam flux).
Results and Discussion

The polarization-corrected L edge excitation spectra for Cu in plastocyanin, taken with the X-ray photon angular momentum parallel (I') and antiparallel (I") to the sample magnetization, are reported in Figure 1. Also shown is the difference in the polarization-corrected fluorescence signals (I' - I") = the XMCD spectrum. At the L1 edge peak there is a 42% decrease in fluorescence intensity between opposite polarizations, while at the L2 edge the effect is of the opposite sign. Although a 100% effect is predicted at the L2 edge for D4h molecules aligned with their 4-fold axes along the magnetic field, for randomly oriented molecules a 50% effect is predicted, similar to the current finding. Since application of the sum rules requires knowledge of the degree of sample magnetization, we also measured the field dependence of the XMCD effect (Figure 2). Least-squares fitting with a Brillouin function for a J = 1/2 system gives a sample temperature of 0.44 K, from which we deduce 99.5% magnetic saturation at 2 T.

When applied to 3d transition metal L edges, the orbital and spin sum rules relate integrated XMCD intensities to element specific projections of the 3d orbital angular momentum (Lz), the 3d spin angular momentum (Sz), and a magnetic dipole term (Tz), as summarized in eqs 1 and 2, respectively. Stöhr and König have shown that the (Tz) term angular averages to zero in "powder" samples, so this term has been omitted from our equations.

\[
\langle L_z \rangle = -2n_h \frac{\int_{I_1}^{I_2} [I' - I"] d\omega}{\int_{I_1}^{I_2} [I' + I"] d\omega} \\
\langle S_z \rangle = -\frac{3}{2}n_h \frac{\int_{I_1}^{I_2} [I' - I"] d\omega \omega - 2\int_{I_1}^{I_2} [I' - I"] d\omega}{\int_{I_1}^{I_2} [I' + I"] d\omega} \\
\]

Here nh represents the number of 3d vacancies in the metal ion, ω is the X-ray frequency, and I' refers to the absorption for X-rays linearly polarized along the magnetization direction; as is customary, we approximate this by (I' + I")/2. It is common in the XMCD literature to refer to the first integral in the numerator of eq 2 as "A" and the second integral as "B", and the denominator as "3C". The necessary integrals are illustrated in Figure 1. One can thus re-express the sum rules in the following manner, in units of n/atom:

\[
\langle L_z \rangle = -n_h \frac{2(A + B)}{3C} \\
\langle S_z \rangle = -n_h \frac{A - B}{2C} \\
\]

After normalizing the overall Cu L edge jump between 920 and 1050 eV to unity, average numerical values for A (−3.08 ± 0.3), B (1.35 ± 0.2), and C (7.27 ± 0.3) were
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obtained, where the errors refer to the degree of experimental reproducibility. The integrated total absorption intensity (C) is slightly higher than in previous work, and it leads to a value of 0.44 for $n_{s0}$, as compared to the previous value of 0.41. This value is still in good agreement with theoretical calculations that predict a highly covalent site.\textsuperscript{9,10} From eq 1, $\langle L_z \rangle$ is found to be $0.07 \pm 0.02$ h/Cu atom, while from eq 2 we derive $0.18 \pm 0.02$ h/Cu atom for $\langle S_z \rangle$.

To complement these experimental predictions for $\langle L_z \rangle$ and $\langle S_z \rangle$ values, a series of SCF-Xα-SW calculations\textsuperscript{18,19} was performed. The wave functions were orthonormalized using the Gram–Schmidt technique to generate a complete basis set. The spin–orbit interaction matrix is given by $H_{ij} = E_{ij} + \sum_{k,l} \varepsilon_{kl} L_i S_j \varepsilon_{kl}$, where the sum over $\alpha$ accounts for spin–orbit coupling for both Cu and S, and $f$ and $j$ are two basis functions, and $\lambda$ is the spin–orbit coupling constant ($\sim 828$ cm$^{-1}$ for Cu and $\sim 382$ cm$^{-1}$ for S). Diagonalizing this matrix gives the spin–orbit corrected wave functions. Calculations of $\langle L_z \rangle$ used linear combinations of the spin–orbit corrected ground-state wave functions in which $S_p$, where $q = x$, $y$, or $z$, was diagonal.\textsuperscript{19}

We first evaluated $\langle L_z \rangle$ for a hypothetical system where all orbitals are treated as having pure d character and are at the energies observed for plastocyanin.\textsuperscript{18,19} This yielded an orientation averaged $\langle L_z \rangle$ of 0.139/Cu atom, which becomes 0.058/Cu atom when scaled according to the covalency of plastocyanin. Also, on the basis of the same methodology, but starting with covalent wave functions from a converged SCF-Xα-SW calculation,\textsuperscript{18} we directly calculate a Cu 3d specific orientation-averaged $\langle L_z \rangle$ value of 0.059/Cu atom. Our XMCD result is thus in good agreement with the calculated values of $\langle L_z \rangle$, which include covalency in the wave functions.

The XMCD sum rule value of $\langle L_z \rangle$ can also be compared to an angle-averaged $\langle L_z \rangle$ determined from the deviation of the EPR g-values for plastocyanin ($g_x = 2.047$, $g_y = 2.059$, $g_z = 2.226$)\textsuperscript{20} from $g_x = 2.0023$ using $L_q = (g_q - g_x)/2$. The EPR derived value is 0.054, which reflects orbital angular momentum contributions from the entire spin system. This value is slightly less than the Cu specific experimental and calculated values, and it is consistent with the possible S ligand influence to the covalent spin system. The sum rule values for $\langle S_z \rangle$ ($0.18 \pm 0.02$ h/Cu atom) and $\langle L_z \rangle/\langle S_z \rangle$ (0.39) are also in reasonable agreement with the SCF-Xα-SW calculation ($\langle S_z \rangle = 0.21$ h/Cu atom and $\langle L_z \rangle/\langle S_z \rangle = 0.31$). In principle, the discrepancy in $\langle S_z \rangle$ could come from a number of sources, including the 3d hole measurement, fluorescence yield (FY) variation over L edge,\textsuperscript{21,22} and partial orientation of the dehydrated sample. Better XMCD spectra on a range of systems are needed to see if the accuracy of the method can be further improved.

Summary

The XMCD values for $\langle L_z \rangle$ and $\langle S_z \rangle$ for Cu(II) in plastocyanin are within 15% of those from SCF-Xα-SW calculation. Although there is some discrepancy with the results from EPR measurements, XMCD and EPR measure different average properties. These XMCD results demonstrate that sum rule analysis could be a useful method to study electronic structure in paramagnetic inorganic systems. Using X-ray spectroscopy to characterize local charge and momentum distributions should be a valuable complement to other structure determination methods in biological sciences.

Acknowledgment. This research was supported by the National Institutes of Health (GM-44380, GM-18812), the National Science Foundation (DMB-9107312, BIR-9317942, and CHE-9528250), and the Department of Energy, Office of Biological and Environmental Research. The Stanford Synchrotron Radiation Laboratory is supported by the Department of Energy, Office of Basic Energy Sciences. We also thank Dr. Mahesh G. Samant of IBM, San Jose, for his assistance in the use of beamline 8-2.

References and Notes