Soft x-ray magnetic circular dichroism at 2 K: A tool in biological inorganic chemistry

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X-ray magnetic circular dichroism (XMCD) is the asymmetric absorption of left- and right-handed circularly polarized x rays and can be used to measure element-specific spin and oxidation states and magnetic moments. We have built an end station for XMCD spectroscopy to study transition metals located in the active sites of proteins and inorganic model compounds. The instrument is equipped with a 6 T superconducting magnet and a liquid helium cooled sample stage designed for experiments at temperatures as low as 2.2 K and beyond 160 K. Sample heating by infrared radiation is minimized using a liquid helium cooled heat shield with 100-nm-thick Al windows. We demonstrate the capabilities of the apparatus in a total electron yield study on the model compound $[(F_8{-}TPP)Fe{-}O{-}Cu(TMPA)]^+$. We show that Fe and Cu are antiferromagnetically coupled by comparing the polarity of the XMCD signal at the respective *L*-edges. We discuss the capability of the instrument to study dilute (<1000 ppm) transition metals in proteins using partial fluorescence yield. © *2004 American Institute of Physics.* [DOI: 10.1063/1.1645635]

I. INTRODUCTION

Research on metallo-proteins has become of increasing interest over the last decades. Traditionally, these proteins have been investigated by Mössbauer spectroscopy, electron paramagnetic resonance, and Fourier transform infrared spectroscopy. The focus of this research is to understand the catalytic functions which take place at the active sites of these enzymes. These active sites are often transition metal clusters (usually Fe, Ni, Mo, or Mn) with C, O, N, or S ligands. Crucial to the understanding of the reaction mechanisms is elucidating the oxidation and spin states of the transition metals involved. An important path in this research is the study of model compounds. These model compounds are aimed to reproduce function and structure of the active site of metallo-proteins. They serve as simplified models to deepen the understanding of the catalytic processes involved.

The advent of third generation synchrotron sources has led to the development of *L*-edge x-ray absorption spectroscopy (XAS) and x-ray magnetic circular dichroism (XMCD) as complementary techniques to study oxidation and spin states in metallo-proteins. Both techniques measure the change of the absorption cross section when $2p$ electrons are promoted into the $3d$ shell after absorption of x-ray photons. While *L*-edge spectroscopy in general does not utilize the polarization of the light, an XMCD experiment measures the difference in absorption cross section for left- and right-handed circularly polarized light.[1] Both techniques probe the occupation of the $3d$ shell and are for that reason extremely sensitive to the ligand environment and changes in the electronic structure of the metals of interest. Furthermore, XMCD allows studying the magnetization behavior of magnetic systems by varying magnetic field and temperature. This gives insight into ground and excited state properties.

Although XMCD is a very powerful technique it is mainly used to study ferro- and antiferromagnetic materials. To a much lesser degree it is used to probe paramagnetic transition metal materials, because low temperatures and high fields are required. To magnetically saturate transition metals in proteins or in nonmagnetically ordered compounds typically requires temperatures around 2 K and magnetic fields around 5 T.[2]

We have built an end station to perform XMCD experiments on proteins and model compounds. Since the transi-

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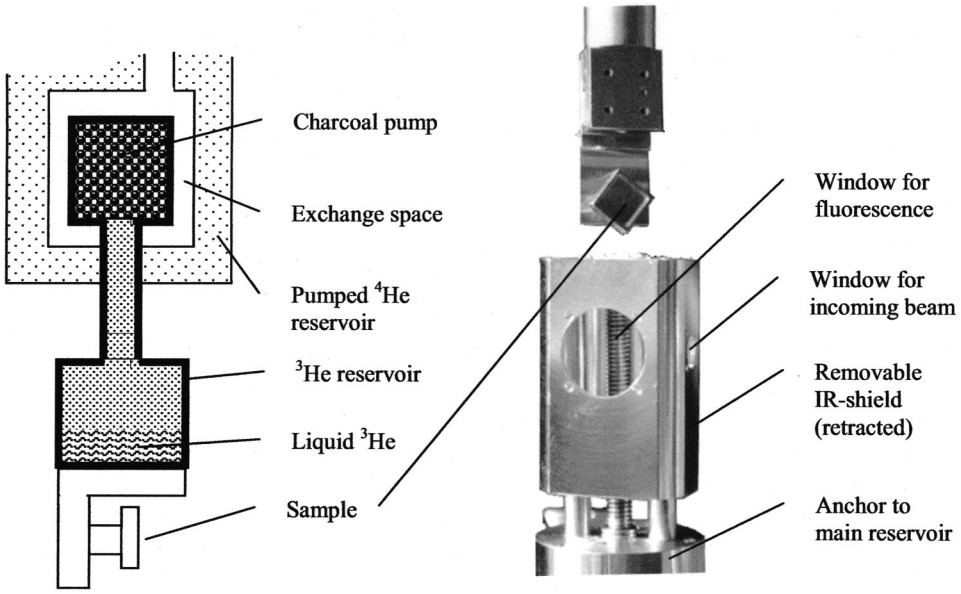

FIG. 1. The cold stage of the XMCD chamber is shown in the diagram. On the left-hand side a schematic drawing of the ^{3}He cryostat is shown, while on the right-hand side a photograph of the cryostat and the IR heat shield is shown. The sample is indicated on both sides. The stage is hosted inside the main ^{4}He reservoir of the superconducting magnet.

tion metal concentration in metallo-proteins is fairly low (100 ppm) the chamber is equipped with a 30 element solid state germanium detector to perform partial fluorescence yield (PFY) measurements. Furthermore, our experimental chamber allows total-electron yield (TEY) measurements to study model compounds. In this article we will discuss the apparatus and show experimental results obtained with our setup.

II. EXPERIMENTAL SETUP

The experiments have been performed on beam line 4.0.2 at the Advanced Light Source.[3] The beam line is equipped with an elliptically polarizing undulator and a high resolution monochromator that covers the energy range between 50 and 2000 eV for arbitrary polarization of the incoming photons. The flux of the incoming beam is 10^{12} photons/s with a resolving power between 5000 and 10 000.

The experimental setup consists of an UHV chamber with a base pressure of about 1×10^{-9} mbar which houses a commercial 6 T superconducting magnet and a sample stage attached to a liquid He bath cryostat. The temperature is measured using a calibrated germanium resistor mounted to the sample stage. To minimize heating by infrared (IR) radiation the sample stage of the cryostat is equipped with a heat shield cooled by liquid helium. The shield is movable without breaking vacuum or warming the cryostat, and provides easy access to the sample stage for sample changing and beam alignment on the sample. During measurements the IR shielding cup is put in place. The incoming beam and fluorescence radiation enter or exit through 100-nm-thick Al windows. With this setup sample temperatures of 2 K are obtainable.[2]

To allow TEY measurements the sample stage is electrically isolated from the rest of the chamber using a sapphire disk. A coaxial cable leads to the sample stage, which allows measuring the photocurrent generated by the absorption of x rays. To extract the current more efficiently the sample holder is biased to -50 V. The photocurrent has a magnitude of about 1 nA and is amplified using a Keithley picoammeter and recorded as a function of the incident photon energy or magnetic field.

For PFY measurements a 30-element germanium detector is used.[4] This detector has an energy resolution as low as 120 eV and allows the separation of the target atom fluorescence line from the background lines. The energy of the incident x-ray photons is scanned over the absorption edge of interest. For each energy point a fluorescence spectrum is recorded. To obtain an absorption spectrum the fluorescence lines of the investigated element are integrated and plotted as a function of incident energy. The resolution of the absorption spectrum is determined by the monochromator.

Figure 1 shows a detailed diagram of the cold stage used in the XMCD chamber. The IR shield is made of gold plated OFE copper and is anchored to the main ^{4}He reservoir of the superconducting magnet to ensure good thermal coupling. The base temperature of the IR shield is about 5 K. The shield itself slides on two metal rods and can be lowered or raised by turning a threaded rod from outside the chamber on a rotary *z*-stage. The stage is engaged to the threaded rod only while moving the shield to minimize heat losses.

The sample stage is attached to a ^{3}He cryostat. Compared to a pumped ^{4}He cryostat the use of a ^{3}He cryostat has several advantages. First of all with a ^{3}He cryostat a base temperature of 400 mK can be reached when required, although at the expense of a shorter hold time compared to 2.2 K operation. Furthermore, the ^{3}He cryostat offers the possibility to adjust the thermal coupling of the sample to the pumped ^{4}He reservoir for higher temperature operation.

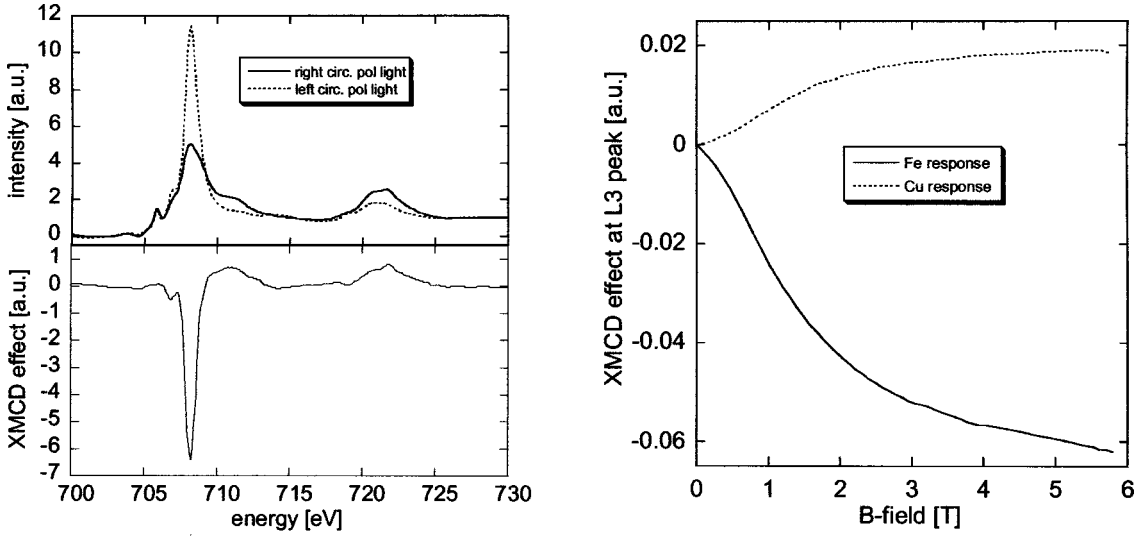

FIG. 2. (Left top) Fe L-edge absorption spectra of $[(F_8TPP)Fe^{III}-O-Cu^{II}(TMPA)](ClO_4)$ taken with right (——) and left (------) circularly polarized light. (Left bottom) The XMCD signal is given by the difference of the top two spectra. The spectra were taken at 6 T and 2.2 K. (Right) Magnetic field dependent curves taken at the energy of the maximum XMCD signal for Fe and Cu at T=2.2 K are proportional to the magnetization. Both curves were taken with the same orientation of the magnetic field. The opposite sign of the XMCD effect confirms the antiferromagnetic coupling of Fe and Cu in this compound.

The operation of the cryostat involves three different states of the system:

First, the exchange space (see Fig. 1 left panel) is evacuated and the charcoal pump is heated to about 20 K. In this state the charcoal does not absorb ^{3}He gas. Instead the ^{3}He gas comes into thermal contact with the liquid ^{4}He reservoir. If the ^{4}He reservoir is pumped, the temperature of the reservoir falls below the condensation temperature of the ^{3}He gas, which starts to liquefy at the bottom of the ^{3}He reservoir. The sample is now optimally coupled to the pumped ^{4}He reservoir by the residual ^{3}He gas. A base temperature of 2.2 K is reached.

Second, the exchange space is filled with ^{4}He gas and the charcoal pump is not heated. The charcoal pump, now at the base temperature of the pumped ^{4}He cryostat, absorbs ^{3}He gas. The thermal coupling between sample and the pumped ^{4}He reservoir drops to a minimum. As the liquid ^{3}He starts evaporating, the sample stage cools and the temperature drops to its lowest value, which is not necessarily 400 mk but depends critically on the thermal load of the cryostat.

Finally, after all liquid ^{3}He has evaporated, the cooling power is zero and the thermal coupling of the sample stage to the ^{4}He reservoir remains low. The temperature of the sample rises (due to small thermal leaks and radiative coupling) and equilibrates at about 10 K. At this point, a small heater attached to the sample stage can then be used to raise the temperature up to 160 K with a stability of 3% over the course of a XMCD scan.

III. RESULTS AND DISCUSSION

In this article we discuss the magnetic behavior of $[(F_8TPP)Fe^{III}-O-Cu^{II}(TMPA)](ClO_4)$. Fe and Cu are antiferromagnetically coupled due to a covalent linker with a total spin $S=2$. This has been confirmed using Mössbauer and nuclear magnetic resonance spectroscopy. Here we show complementary XMCD results (Fig. 2). The Fe L-edge spectra were sequentially taken with left- and right-handed circularly polarized light and were normalized to the incoming beam intensity. The Fe edge shows a rich multiplet structure and a strong XMCD effect supporting an Fe^{III} ($S=5/2$) oxidation state. The XMCD effect is negative as expected for a magnetic moment parallel to the applied magnetic field and the magnetic field antiparallel to the beam direction. The Cu L-edge spectra (not shown) were subsequently recorded under identical conditions on the same sample. The L-edge data of the Cu support a d^9 electronic configuration as expected for Cu^{II} ($S=1/2$). The XMCD effect of Cu is in this case positive. This strongly suggests that Cu is antiferromagnetically coupled to the Fe, with Fe being the majority spin oriented in the magnetic field.

The right panel of Fig. 2 shows the magnetization curves taken for Cu and Fe. For these measurements, the monochromator was set to the energy of the maximum XMCD signal at the respective L_3 edge and data points were taken alternating between left- and right-hand circularly polarized light while varying the magnetic field. From these data magnetic field dependent curves for both left- and right-handed circularly polarized light were derived. The difference between the left and right polarized curves yields the magnetization curve. The Fe and Cu magnetization curves have opposite signs, but the same shape, again confirming that Fe and Cu are antiferromagnetically coupled. The magnetization curve deviates from an $S=2$ Brillouin function, which would be expected for a pure atomic case. This indicates that higher excited states are energetically close to the ground state and can be populated. This admixture of higher excited states leads to a deviation of the pure Brillouin behavior.

The XMCD chamber has also been used to study the active sites of the proteins ACDS[5] and Ni-Azurin.[6] Both studies employed PFY measurements. Spin and oxidation states of the proteins have been determined in different states of the catalytic cycle. Another XMCD study has been performed on $ErCo_2$.[7] The L_{23} Co and M_{45} Er absorption edges

have been measured as a function of temperature up to 60 K. Below T_c the cobalt orbital moment is strongly increased.

These experiments demonstrate the strength of synchrotron-based XMCD measurements to study spin states in paramagnetic systems.

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