Title

Mn4Ca Cluster of the Water-oxidation Enzyme Studied by Polarized X-ray Spectroscopy of PS II Single Crystals

Authors

Yachandra, VK

Yano, J

Kern, J

et al.

Publication Date

2023-12-09

Peer reviewed

Kamiya, N. & Shen, J. R. (2003) *Proc. Natl. Acad. Sci. USA* 100: 98–103.

Kern, J., Loll, B., Lüneberg, C., DiFiore, D., Biesiadka, J., Irrgang, K.-D. & Zouni, A. (2004) *Biochim. Biophys. Acta,* in press, doi: 10.1016/j.bbabio.2004.10.007.

Michel, H. & Deisenhofer, J. (1988) *Biochemistry* 27: 1–7.

Nield, J., Kruse, O., Ruprecht, J., da Fonseca, P., Büchel, C. & Barber, J. (2000) *J. Biol. Chem.* 275: 27940–27946.

Renger, G. (2004) *Biochim. Biophys. Acta* 1655: 195–204.

Rhee, K. H., Morris, E. P., Barber, J. & Kühlbrandt, W. (1998) *Nature* 396: 283–286.

Zouni, A., Witt, H. T., Kern, J., Fromme, P., Krauss, N., Saenger, W. & Orth, P. (2001) *Nature* 409: 739–743.

MN_4CA CLUSTER OF THE WATER-OXIDATION ENZYME STUDIED BY POLARIZED X-RAY SPECTROSCOPY OF PS II SINGLE CRYSTALS

Junko Yano[1,2], Jan Kern[3], Kenneth Sauer[1,2], Johannes Messinger[4], Athina Zouni[3], Vittal Yachandra[1]. [1]Melvin Calvin Laboratory, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA. [2]Department of Chemistry, University of California, Berkeley, CA, USA. [3]Max-Volmer-Laboratorium für Biophysikalische Chemie, Technische Universität Berlin, Berlin, Germany. [4]Max-Planck Institut für Bioanorganische Chemie, Mülheim, Germany

Keywords: oxygen-evolution, PS II single crystals, X-ray Spectroscopy, Mn complex

INTRODUCTION

Polarized XAS studies of single crystals provide a method of resolving and orientationally selecting specific Mn K-edge and near-edge features and structural details from EXAFS that are frequently unresolved in the spectra of unoriented samples.

PS II membranes can be oriented on a substrate such that the membrane planes are roughly parallel to the substrate surface. This imparts a one-dimensional order to these samples; while the *z* axis for each membrane (collinear with the membrane normal) is roughly parallel to the substrate normal, the *x* and *y* axes remain disordered. Exploiting the plane-polarized nature of synchrotron radiation, spectra can be collected at different angles between the substrate normal and the X-ray ***e***-vector. The dichroism of the absorber-backscatterer pair present in the oriented samples is reflected in, and can be extracted from, the resulting X-ray absorption spectra. The EXAFS of the oriented PS II samples exhibits distinct dichroism, from which we have deduced the relative orientations of several interatomic vector directions relative to the membrane normal and derived a topological representation of the metal sites in the OEC (George et al 1989, Mukerji et al 1994, Dau et al 1995, Cinco et al 2004). However, because the samples are ordered in only one dimension, the dichroism information is available only in the form of an angle with respect to the membrane normal. For EXAFS measurements, this means that the absorber-backscatterer vectors can lie anywhere on a cone defined by the angle the vector forms with the membrane normal.

Further refinement can be performed if samples with three-dimensional order, i.e., single crystals, are examined instead of oriented membranes. The EXAFS amplitude is proportional to $\sim\cos^2\theta$, where θ is the angle between the X-ray ***e***-vector and the absorber-backscatterer vector. X-ray spectroscopy has been performed on single-crystal model complexes, and single crystals of several metalloproteins such as plastocyanin, nitrogenase and sulfite oxidase. These studies have significantly expanded the structural information available for these systems over what is gleaned from studies of isotropic samples.

The PS II structure has been solved to 3.8 Å, 3.7 Å, 3.5 Å and recently to 3.2 Å resolution (Zouni et al 2001, Kamiya & Shen 2003, Ferreira et al 2004, Biesiadka et al 2004). Examination of the orientation dependence of the EXAFS of single crystals will provide structural information about the Mn sites at a resolution higher than that will be obtainable from X-ray crystallography. Distance information between Mn atoms and between Mn and ligand atoms can be determined to an accuracy of 0.015 Å, with a resolution between distances of ~0.15 Å using EXAFS. Performing single-crystal EXAFS experiments helps to refine the low-resolution structure of the OEC. There is also a significant amount of electronic state information in the dichroism of the XANES features, especially the 1s to 3d transition.

MATERIALS AND METHODS

In collaboration with the Structural Molecular Biology group at SSRL, we have developed the methodology for collecting single-crystal XAS data from PS II. The instrumentation consists of a liquid He cryostat with one axis of rotation, a 30-element Ge detector for collecting XAS data, and a CCD or a MAR 345 imaging-plate detector placed behind the liquid He cryostat for *in situ* collection of diffraction data and determination of the crystal orientation. For inorganic crystals, that are not so radiation sensitive as PS II single crystals, the liquid He cryostat is replaced by a kappa goniometer, with three rotation axes. The instrumentation has been successfully used, and we have collected data from single crystals of PS II and inorganic Mn models.

RESULTS AND DISCUSSION

Single Crystals of Inorganic Mn Complexes. We have used XANES and EXAFS to study single crystals of several inorganic Mn complexes that are relevant to the oxygen-evolving complex. All of these complexes exhibited noticeable dichroism in the EXAFS spectra and the XANES features. The dichroism in the EXAFS spectra was fit for three orientations of the crystal using single- and multiple-scattering EXAFS theory.

As an example, Fig. 1 shows the polarized spectra of a Mn(III,IV) di-μ-oxo bridged binuclear complex. The spectra were taken at several orientations by rotating the crystal with respect to the incident polarized X-ray radiation. The Mn-Mn (2.7 Å) and Mn-O,N (1.8–2.0 Å) vectors are highly dichroic. Thus, the combination of EXAFS and the pre-edge spectra provide the relation between the 1s

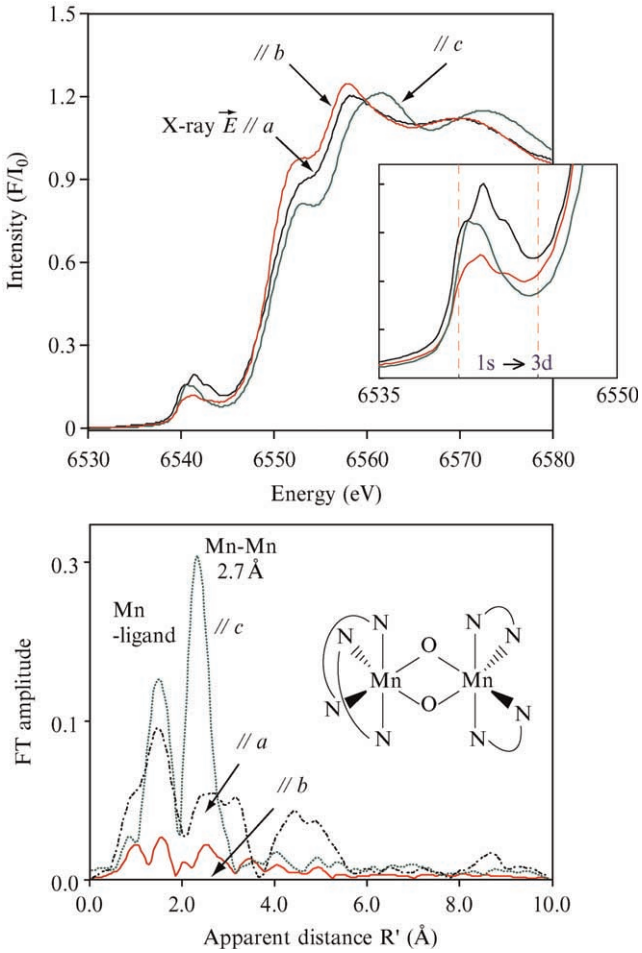

Figure 1: The Mn K-edge XANES (top) and EXAFS (bottom) of a binuclear di-μ-oxo bridged $Mn(III,IV)(phen)_4(PF_6)$ complex with the X-ray *e*-vector parallel to the a, b and c axes of the crystal. The inset on top shows the 1s-to-3d pre-edge peak, which clearly is dichroic and fits to three peaks, providing information about the electronic structure of the complex. The EXAFS is also significantly dichroic; the second Fourier peak at ~2.7 Å corresponds to the Mn-Mn interaction. We have used these spectra to refine our methodology for analyzing the data from PS II single crystals.

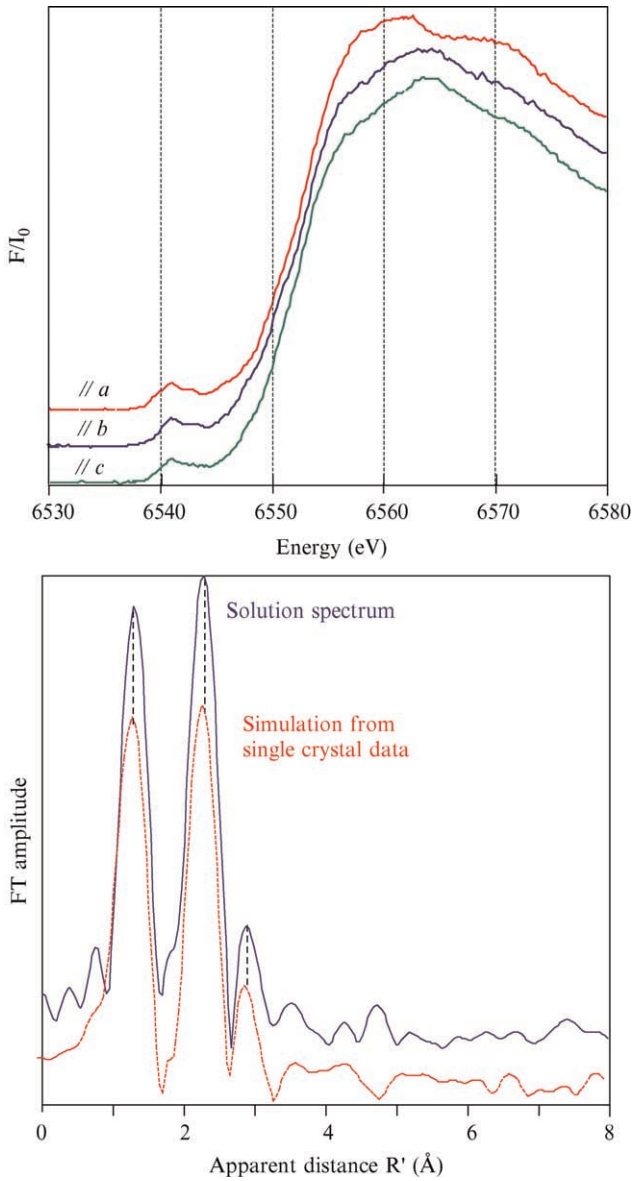

Figure 2: (Top) The PS II single-crystal XANES and EXAFS spectra with the X-ray *e*-vector parallel to the crystal a, b, and c axes. The dichroism in the XANES and the Fourier peaks is apparent (1s to 3d pre-edge is also dichroic but is not apparent in this figure). (Bottom) Simulation of solution PS II EXAFS spectra from the single-crystal data. The data were collected at 10 K with the X-ray *e*-vector parallel to the three principal axes of the crystal, using X-ray flux density that was determined not to damage the Mn complex by monitoring the Mn K-edge. This experiment was used to demonstrate that we could collect data of sufficient S/N from the crystals in different orientations without damaging them by exposure to X-rays. Our criterion of damage is different from what is used for X-ray diffraction data collection. We found that the diffraction pattern persisted subsequent to extensive Mn reduction by X-ray irradiation.

to 3d transition and the molecular orientation; namely, it is strongly correlated with the direction of the di-μ-oxo bridge.

Understanding the origins and assignments of the XANES spectra of the synthetic Mn complexes and their orientation dependence are in progress and will aid in the interpretation of the Mn XANES spectra of the OEC in random, oriented or single-crystalline forms.

Single Crystals of PS II. We have successfully collected single crystal XANES and EXAFS data from PS II in the S1 state with the X-ray *e*-vector parallel to the a, b, and c axes of the crystal. The data were collected using 10 crystals per orientation to improve the S/N. We collected data in only one orientation per crystal to minimize X-ray damage. Each crystal was exposed to X-rays for ~1.5 hours, and the Mn K-edge was monitored closely for any X-ray induced Mn reduction. Using these data we have shown that the XANES and EXAFS spectra from isotropic PS II samples can be generated from the single-crystal data (Fig. 2). This shows that the structure of the Mn cluster in the single crystal is intact and that it is similar to that seen in solution.

The XANES (Fig. 2) and EXAFS spectra (data not shown) show that the Fourier peaks are clearly dichroic, demonstrating an asymmetric Mn cluster. We are now in a position to place the vectors in the appropriate geometry in the electron density. We will also be able to determine the orientation of the 3.3 Å vector relative to the ~2.7–2.8 Å vectors in the electron density.

We have conducted a thorough study of the radiation damage to the metal site and determined the threshold of acceptable X-ray flux. We also studied the time dependence of the damage by monitoring both the K-edge and X-ray diffraction. Besides the intrinsic scientific importance of single-crystal X-ray spectroscopy in understanding the structure of the Mn site, these studies are revealing for the first time that the conditions used for structure determination by X-ray diffraction methods can be damaging to the metal-site structure as monitored by XAS. Because structure-function correlations are routinely made using the structures determined by X-ray crystallography, it is important to determine that the metal-site structures are indeed intact.

We believe the single crystal XAS studies have the potential for identifying a high resolution structure of the Mn cluster, that has eluded both Mn EXAFS and X-ray crystallography studies until now, and determine the changes in this structure during the catalytic cycle. The directions of the Mn-Mn vectors in conjunction with the electron density derived from X-ray crystallography promises to refine the structure of the Mn complex. In addition, single-crystal XANES studies will provide details about the electronic structure of the Mn complex.

ACKNOWLEDGMENTS

We thank Dr. Matthew Latimer at SSRL for invaluable help. This research was supported by the NIH grant (GM 55302), and by DOE, Director of Office of Science, BES, Chemical Sciences, Geosciences, and Biosciences Division, under Contract DE-AC03-76SF00098. Synchrotron facilities were provided by SSRL, APS and ALS, operated by DOE, Office of Basic Energy Sciences. The SSRL Biotechnology Program is supported by NIH, NCRR, Biomedical Technology Program, and by DOE, OHER. BioCAT is a NIH-supported Research Center RR-08630. JM is thankful for the support by the DFG (grants Me 1629/2-1, 2-2 and 2-3).

REFERENCES

Biesiadka, J., Loll, B., Kern, J., Irrgang, K.-D. & Zouni, A. (2004) *Phys. Chem. Chem. Phys.* (in press).

Cinco, R. M., Robblee, J. H., Messinger, J., Fernandez, C., McFarlane-Holman, K. L., Sauer, K. & Yachandra, V. K. (2004) *Biochemistry* (in press).

Dau, H., Andrews, J. C., Roelofs, T. A., Latimer, M. J., Liang, W., Yachandra, V. K., Sauer, K. & Klein, M. P. (1995) *Biochemistry* 34: 5274–5287.

Ferreira, K. N., Iverson, T. M., Maghlaoui, K., Barber, J. & Iwata, S. (2004) *Science* 303: 1831–1838.

George, G. N., Prince, R. C. & Cramer, S. P. (1989) *Science* 243: 789–791.

Kamiya, N. & Shen, J. R. (2003) *Proc. Natl. Acad. Sci. USA* 100: 98–103.

Mukerji, I., Andrews, J. C., DeRose, V. J., Latimer, M. J., Yachandra, V. K., Sauer, K. & Klein, M. P. (1994) *Biochemistry* 33: 9712–9721.

Zouni, A., Witt, H. T., Kern, J., Fromme, P., Krauss, N., Saenger, W. & Orth, P. (2001) *Nature* 409: 739–743.

^{15}N PHOTO-CIDNP MAS NMR ON REACTION CENTERS OF *RHODOBACTER SPHAEROIDES*

Shipra Prakash[1], Soek Hwa Tong[1], Alia[1], Peter Gast[2], Huub J. M. de Groot[1], Gunnar Jeschke[3], Jörg Matysik[1]. [1]Leiden Institute of Chemistry, P.O. Box 9502, 2300 RA Leiden, The Netherlands. [2]Department of Biophysics, P.O. Box 9504, 2300 RA Leiden, The Netherlands. [3]Max-Planck-Institut für Polymerforschung, Postfach 3148, 55021 Mainz, Germany

Keywords: Solid-state NMR, photo-CIDNP, *Rhodobacter sphaeroides*, special pair, electron transfer

INTRODUCTION

Photochemically induced dynamic nuclear polarisation (photo-CIDNP) can be observed in frozen photosynthetic reaction centers (RCs) under illumination by magic-angle spinning (MAS) NMR spectroscopy as strong enhancement of NMR lines. The effect has been used to study the electronic structure of the photochemical machinery of several RCs of bacteria (Zysmilich & McDermott 1994, 1996a,b, Matysik et al 2000a) and plants (Matysik et al 2000b, Alia et al 2004). Polarisation transfer from the unpaired electrons to the nuclei pushes the nuclear spin system out of its Boltzmann equilibrium. The exact mechanism of production of nuclear polarisation has been discussed recently (Jeschke & Matysik 2003). It has been proposed to be caused by two competing mechanisms. In the electron-electron-nuclear three-spin mixing (TSM) mechanism net nuclear polarization is created in the spin-correlated radical pair due to the presence of both anisotropic hyperfine interaction and coupling between the two electron spins (Jeschke 1997, 1998). In the Differential Decay (DD) mechanism a net photo-CIDNP effect is caused by anisotropic hyperfine coupling when the radical pair has different lifetimes in its singlet and triplet state (Polenova et al 1999).

Photo-CIDNP in solids has first been observed by ^{15}N MAS NMR (Zysmilich et al 1994, 1996a), whereas more recent studies applied ^{13}C MAS NMR. The latter can be obtained without isotope labelling and provides a more detailed view into the electronic structure of the aromatic ring system. On the other hand, ^{15}N photo-CIDNP MAS NMR spectra are more straightforwardly to interpret and suffer less under the overlapping of signals. Here we present high-quality ^{15}N photo-CIDNP MAS NMR spectra of uniformly ^{15}N labelled RCs of *Rhodobacter* (*Rb.*) *Sphaeroides* R26 and discuss the capacity of ^{15}N photo-CIDNP studies.

MATERIALS AND METHODS

Rb. sphaeroides R26 has been grown under anaerobic conditions in medium containing 95% ^{15}N labeled NH_4Cl from VEB Berlin Chemie (Berlin-Adlershof, Germany). The extent of ^{15}N incorporation has been determined by GC-MS to be $\sim$60%. The RCs were isolated by the procedure of Feher and Okamura (1978). Quinone depletion is reported by Prakash et al in this volume.

MAS NMR measurements are described in Matysik et al (2000a, 2001a). Chemical shifts are given relative to $^{15}NH_3$, using the response of solid $^{15}NH_4NO_3$ at $\delta = 23.5$ ppm as reference.