Title

Calcium and Chloride cofactors of the oxygen evolving complex: X-ray absorption spectroscopy evidence for a Mn/Ca/Cl heteronuclear cluster

Authors

Yachandra, VK

Fernandez, C

Cinco, RM

et al.

Publication Date

1998

Peer reviewed

Proceedings of the XI International Congress on Photosynthesis (Garab, G., ed.) Kluwer Academic Publishers, The Netherlands, (1998) In press.

CALCIUM AND CHLORIDE COFACTORS OF THE OXYGEN EVOLVING COMPLEX - X-RAY ABSORPTION SPECTROSCOPY EVIDENCE FOR A Mn/Ca/Cl HETERONUCLEAR CLUSTER.

Carmen Fernandez,[1,2] Roehl M. Cinco,[1,2] John H. Robblee,[1,2] Johannes Messinger,[1] Shelly A. Pizarro,[1,2] Kenneth Sauer,[1,2] Melvin P. Klein,[1] Vittal K. Yachandra.[1]

[1]Physical Biosciences Division, Lawrence Berkeley National Laboratory, and [2]Department of Chemistry, University of California, Berkeley, CA 94720.

Key Words: photosystem 2, X-ray spectroscopy, O_2 evolution, manganese, calcium, chloride

1. Introduction

The oxygen-evolving complex (OEC) of photosystem II (PS II) in green plants and algae contains a cluster of four Mn atoms in the active site, which catalyzes the oxidation of water to dioxygen. Along with Mn, Cl^- and Ca^{2+} are essential cofactors for oxygen evolution (1).

Depletion of the Ca^{2+} cofactor suppresses the activity of the OEC, which can be restored by replenishing with Ca^{2+}. Partial reactivation results from addition of Sr^{2+} to Ca-depleted PS II membranes (2). Previous Mn-XAS studies from our laboratory have suggested that Ca is in close proximity to the Mn Cluster (within 3.5 Å) (3). However, there is some debate about the environment of the Ca cofactor binding site (4) and its function. Given this uncertain situation and to further test whether a Ca binding site is close to the Mn cluster, we chose a different approach: to use Sr EXAFS to probe from the Sr cofactor point-of-view for nearby Mn. This is the reverse of the previously described Mn EXAFS studies that concentrated on the Mn cluster and probed for nearby Ca or Sr neighbors. In this study, we compared the Sr EXAFS spectrum of a functional PS II sample with the Sr spectrum from an inactive sample in which the Mn complex is disrupted. These results confirm our earlier results that Sr (Ca) is proximal to the Mn complex (5).

Despite numerous EPR and XAS studies, the question of whether Cl is a ligand of Mn and the structural role of Cl cofactor has been uncertain (6). Suffice it to say that the binding of Cl^- to Mn has been a matter of controversy and there is considerable interest to gain definitive physical evidence for or against halide ligation to Mn. Recent Mn-XAS studies of PS II membranes in the S_3 state have provided the first direct evidence that Cl^- may be a ligand of Mn. Polarized Mn EXAFS measurements of samples in the S_3 state show considerable dichroism and significant structural change compared to the S_2 state. A clearly dichroic Fourier peak is observed at ~2.2 Å which fits best to a Mn-Cl vector.

2. Experimental Section

Starting from PS II-enriched membranes from spinach, Sr^{2+}-reactivated samples were prepared as described previously (3). Excess, non functional, Sr or Ca was removed by treatment with Chelex. The Chelex-treated samples, designated intact Sr-PS II, were transferred to Mylar-backed Lucite sample holders designed to fit in XAS and EPR liquid He cryostats. The inactive Sr-PS II was prepared by adding NH_2OH to the intact Sr-PS II pellet. Samples were routinely checked for O_2-evolution activity, EPR multiline signal and metals content.

PS II membranes were oriented by painting onto a mylar film using a fine brush and drying under a gentle stream of cold N_2 gas in the dark. The orientation was checked by monitoring the Y_D^+ EPR signal (7). Dark adapted samples were then subjected to saturating flash illumination either by a Xe-flash lamp or a high-powered Nd-YAG laser. The percentage of S_3 generated after two flashes was determined by measuring the decrease in the amplitude of the MLS EPR signal of a two-flash sample relative to a one-flash sample. The details are described in Roelofs et al. (8).

XAFS experiments were conducted at the Stanford Synchrotron Radiation Laboratory on Beamline VII-3 and IV-3 in unfocused mode using a Si(220) double crystal monochromator, and a Canberra Instruments 13-element Ge detector. The Fourier-isolated data in *k*-space were subjected to curve fitting, using *ab initio* phase and amplitude functions calculated using the FEFF program.

3. Results and Discussion

3.1 Ca Cofactor

After the initial characterizations by EPR, oxygen activity, and metals quantitation were completed, XAS measurements were made on the two types of (Chelex-treated) Sr-PS II: intact, and inactive samples. The Fourier transforms of the Sr EXAFS from intact Sr-PS II samples show an interaction (Peak II, Fig. 1) that is best simulated by two Mn at ~3.5 Å distance. This vector is not present in the inactive, NH_2OH-treated sample, although both types share similar first coordination shells of oxygen (Peak I, Fig. 1). Attempts to model Peak II with C (the most likely low-Z candidate, from carboxylate ligands) or other low-Z atoms (O, P, S, Cl) result in comparatively poor fits. By extensive fitting trials we have excluded light atoms as major contributors to Fourier Peak II and thus present Mn as the chemically reasonable alternative.

The Sr EXAFS results, translated back to the original Ca cofactor, support the earlier finding that Ca (Sr) is near the Mn cluster at a distance of ~3.4 Å. Such a distance indicates the presence of single-atom bridging, likely by oxygen. This bridge may be derived from carboxylate ligands (aspartate or glutamate protein residues), protein backbone carbonyl, water or hydroxide. Further studies to place constraints on the geometry and relative orientation of the Mn–Ca (Sr) vectors will rely on X-ray absorption linear dichroism of the observed Fourier Peak II. Polarized Sr EXAFS experiments on oriented Sr-PS II multilayers are in progress and preliminary results (see Cinco et al., in this issue) indicate dichroism in Peak II depending on the alignment of the membrane normal and X-ray electric field vector: maximum amplitude at 10° and suppression at 80°. These studies are continuing and should yield the average angle of the Sr–Mn vector relative to the membrane normal, and the average number of backscatterers. By acquiring these parameters, we would further refine the model of the active site. The intimate link between Ca and Mn that had been suggested in previous studies has led to the description of the catalytic center of the OEC as a "tetra-Mn/Ca cluster" (5).

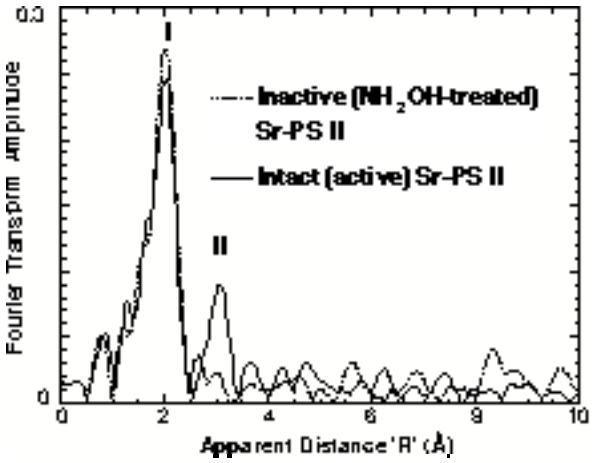

Figure 1. Fourier transforms of Sr EXAFS for intact and inactive Sr-substituted PS II samples (Chelex-treated). Fourier Peak II is present only in the intact samples and fits to Mn, confirming the proximity of Sr (Ca) to the Mn cluster.

3.2 Cl⁻ Cofactor

Our previous XAS results from isotropic samples in the S_3 state

have shown that there is considerable change in the Mn-Mn distances as the system advances from the S_2 to the S_3 state (9). The two 2.7 Å Mn-Mn distances in the S_2 state increase to ~2.8 and 2.9-3.0 Å in the S_3 state.

With the aim of determining the relative orientation of the 2.8 and 2.9-3.0 Å Mn-Mn vectors, we have initiated XAS studies of oriented S_3 state samples. The results show that the two Mn-Mn vectors are dichroic (see paper by Cinco et al in this issue). Interestingly, maybe because of the lengthening of the Mn-Mn vectors, a new Fourier peak is observed (Fig. 2) between the first and second Fourier peaks, and is indicated on the figure by an arrow. Figure 2 also shows the Fourier transform of a Mn binuclear complex with one Cl as a terminal ligand to a Mn atom. The Fourier peak corresponding to Cl backscattering is clearly evident at about the same apparent distance and is indicated by an arrow in Fig. 2. In other model compounds, the amplitude of the peak increases as the ratio of Cl/Mn increases and the peak is absent when Cl is not present as a ligand (data not shown). In the S_3 sample this Fourier peak does not fit to low Z atoms such as C, N, or O. The fit is significantly better for Cl backscattering at ~2.2 Å. The fits for the model compound are also very similar. The difference in amplitude of this Fourier peak between the S_3 sample and the model is probably due to the difference in the number of Cl backscatterers/Mn in the model and in PS II. It is likely that in PS II the ratio is only 1Cl/4Mn as compared to 1Cl/2Mn in the model compound. Dichroism studies, on seven different samples, show that the Fourier peak is more obvious at the 10 degree orientation compared to 80 degree orientation, that suggest that the Mn-Cl vector is more parallel to the membrane normal.

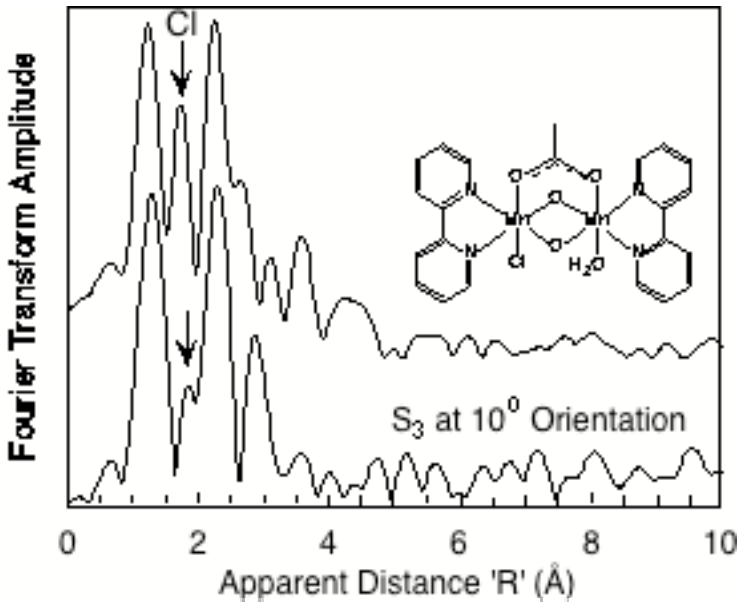

Figure 2. Fourier transform of the oriented S_3 state sample with the membrane normal at 10 degrees to the X-ray e-vector. On top is the Fourier transform from an inorganic binuclear Mn compound, $[(Mn_2(IV,IV)O_2(OAc)Cl(H_2O)](NO_3)_2$, with one Cl as a terminal ligand to Mn. The arrow is directed at the Fourier peak corresponding to Cl.

A refined model of the OEC that incorporates the Ca and Cl cofactors is shown in the article by Cinco et al in this issue.

4. Acknowledgments

This research was supported by the National Institutes of Health (GM55302), and the Director, Office of Basic Energy Sciences, Division of Energy Biosciences of the U.S. Department of Energy (DOE), under Contract DE-AC03-76SF00098. C. F. was supported by a FAPESP grant, and J. M. was partly supported by a Forschungstipendium from the DFG (Me 1629/1-1). We are grateful to Profs. G. Christou, W. H. Armstrong, V. L. Pecoraro, K. Wieghardt and J.-J. Girerd for generously providing us with the Mn model compounds. Synchrotron radiation facilities were provided by the Stanford Synchrotron Radiation Laboratory (SSRL) which is operated by the Department of Energy, Office of Basic Energy Sciences. The SSRL Biotechnology Program is supported by the NIH, National Center of Research Resources, and by the DOE, Office of Health and Environmental Research.

References

1 (a) Debus, R. J. (1992) Biochim. Biophys. Acta 1102, 269-352, (b) Britt, R. D. (1996) In Oxygenic Photosynthesis: The Light Reactions (Ort, D. R; Yocum, C. F. eds.) pp. 137-164, Kluwer Academic Publishers, Dordrecht, The Netherlands, (c) Yachandra, V. K., Sauer, K. and Klein, M. P. (1996) Chem. Rev. 96, 2927-2950.

2 Boussac, A. and Rutherford, A. W. (1988) Biochemistry 27, 3476-3483.

3 Latimer, M. J., DeRose, V. J., Mukerji, I., Yachandra, V. K., Sauer, K. and Klein, M. P. (1995) Biochemistry 34, 10898-10909.

4 (a) Booth, P. J., Rutherford, A. W. and Boussac, A. (1996) Biochim. Biophys. Acta 1277, 127-134, (b) Riggs-Gelasco, P. J., Mei, R., Ghanotakis, D. F., Yocum, C. F. and Penner-Hahn, J. E. (1996) J. Am. Chem. Soc. 118, 2400-2410.

5 Cinco, R. M., Robblee, J. H., Fernandez, C, Rompel, A, Yachandra, V. K., Sauer, K. and Klein, M. P. (1998) J. Phys. Chem. (In Press).

6 (a) Yocum C. F. (1992) In Manganese Redox Enzymes. (Pecoraro, V. L., ed.) pp. 71-83, VCH Publishers: New York. (b) Yocum C. F. (1991) Biochim Biophys Acta 1059, 1-15. (c) Homann, P. H. (1987) J. Bioenerg. Biomembr. 19, 105-123.

7 (a) Mukerji, I., Andrews, J. C., DeRose, V. J., Latimer, M. J., Yachandra, V. K., Sauer, K. and Klein, M. P. (1994) Biochemistry 33, 9712-9721, (b) Dau, H., Andrews, J. C., Roelofs, T. A., Latimer, M. J., Liang, W., Yachandra, V. K., Sauer, K. and Klein, M. P. (1995) Biochemistry 34, 5274-5287.

8 Roelofs, T. A., Liang, W., Latimer, M. J., Cinco, R. M., Rompel, A., Andrews, J. C., Sauer, K., Yachandra, V. K. and Klein, M. P. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 3335-3340.

9 Liang, W., Roelofs, T. A., Olsen, G. T., Latimer, M. J., Cinco, R. M., Rompel, A., Sauer, K., Yachandra, V. K. and Klein, M. P. (1995) In Photosynthesis: From Light to Biosphere. (Mathis, P., ed.) pp 413-416, Kluwer Academic Publishers, Dordrecht, The Netherlands.