Electronic Structure of the Mn_4Ca Cluster in the Oxygen-Evolving Complex of Photosystem II Studied by Resonant Inelastic X-Ray Scattering

Junko Yano,[1] Yulia Pushkar,[1] Johannes Messinger,[2] Uwe Bergmann,[3] Pieter Glatzel,[4] Vittal K. Yachandra[1]

[1]Melvin Calvin Laboratory, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA, [2]Max-Planck-Institut für Bioanorganische Chemie, Mülheim an der Ruhr, Germany, [3]Stanford Synchrotron Radiation Laboratory, 2575 Sand Hill Road, Menlo Park, CA 94025, USA, [4]European Synchrotron Radiation Facility, BP 220, F-38043 Grenoble Cedex, France

Abstract. Oxygen-evolving complex (Mn_4Ca cluster) of Photosystem II cycles through five intermediate states (S_i-states, i =0-4) before a molecule of dioxygen is released. During the S-state transitions, electrons are extracted from the OEC, either from Mn or alternatively from a Mn ligand. The oxidation state of Mn is widely accepted as $Mn_4(III_2,IV_2)$ and $Mn_4(III,IV_3)$ for S_1 and S_2 states, while it is still controversial for the S_0 and S_3 states. We used resonant inelastic X-ray scattering (RIXS) to study the electronic structure of Mn_4Ca complex in the OEC. The RIXS data yield two-dimensional plots that provide a significant advantage by obtaining both K-edge pre-edge and L-edge-like spectra simultaneously. The second energy dimension separates the pre-edge (1s to 3d) transitions from the main K-edge (1s to 4p), and thus more precise analysis is possible. The 1s2p RIXS final state electron configuration along the energy transfer axis is identical to conventional L-edge absorption spectroscopy and the RIXS spectra are therefore sensitive to the metal spin state. We have collected data from PS II samples in the each of the S-states and compared them with data from various inorganic Mn complexes. The spectral changes in the Mn $1s2p_{3/2}$ RIXS spectra between the S-states are small compared to those of the oxides of Mn and coordination complexes. The results indicate strong covalency for the electronic configuration in the OEC, and we conclude that the electron is transferred from a strongly delocalized orbital, compared to those in Mn oxides or coordination complexes. The magnitude for the S_0 to S_1, and S_1 to S_2 transitions is twice as large as that during the S_2 to S_3 transition, indicating that the electron for this transition is extracted from a highly delocalized orbital with little change in charge density at the Mn atoms. The RIXS spectra of S_0 and S_3 states also showed characteristic features which were not clear from the K-edge spectroscopy.

Keywords: photosystem II, oxygen-evolving complex, Mn_4Ca cluster, electronic structure, RIXS.
PACS: 87.64.Gb

INTRODUCTION

Most of the oxygen in the atmosphere which supports aerobic life on earth is generated by plants and cyanobacteria by the photo-induced oxidation of water to dioxygen. The oxygen-evolving complex (OEC), of the photosynthetic apparatus that catalyzes the oxidation of H_2O to O_2 contains a Mn_4Ca cluster [1]. Water oxidation in photosystem II (PS II) is a stepwise process wherein each of 4 sequential photons absorbed by the reaction center powers the advance of the OEC through the S-state intermediates S_0-S_4. Upon reaching the S_4 state, the complex releases O_2 and returns to the S_0 state (Fig. 1).

A promising approach to study the Mn oxidation states in the native S-states is to step samples through the S-state cycle by the application of saturating single-turnover flashes and to characterize these samples by X-ray spectroscopy.

A key question for the understanding of photosynthetic water oxidation is whether the four oxidizing equivalents generated by the reaction center are accumulated on the four Mn ions of the OEC during S-state turnover, or whether a ligand-centered oxidation takes place, especially, before the formation and release of molecular oxygen during the S_3 to (S_4) to S_0 transition. It is crucial to solve this problem, because the Mn redox states form the basis for any mechanistic proposal. Mn K-edge XANES has been

the traditional X-ray spectroscopic method for determining the oxidation states. The description of the Mn OEC in the various S-states in terms of the formal oxidation states is very useful, but it is also important to determine a detailed view of the electronic structure of the Mn cluster.

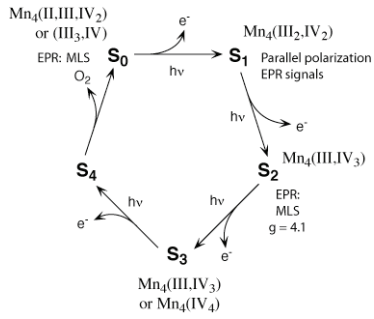

FIGURE 1. S-state Scheme for Oxygen Evolution, with EPR signals and proposed Mn oxidation states.

We have addressed these questions by using Mn K-edge XANES (1s-4p absorption), Kβ XES (3p-1s emission) [2] and the recently introduced resonant inelastic X-ray scattering spectroscopy (RIXS) (1s to 3d/4p absorption followed by 2p-1s Kα emission) to obtain L-edge-like spectra (2p-3d absorption).

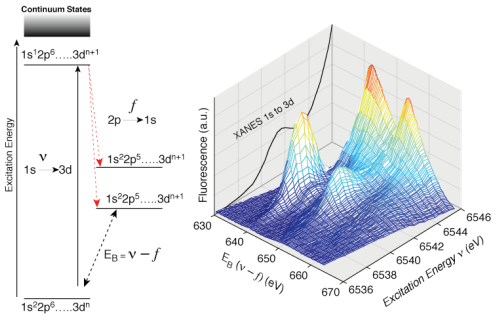

FIGURE 2. A two-dimensional plot showing the RIXS spectrum from a Mn(II)acetylacetonate complex. The 1s-3d K-edge spectrum is plotted in the back in black. A cross-section of the 2D plot parallel to the Y-axis yields L-edge like spectra, the more intense feature at 640 eV corresponds to transitions to J=3/2 like states (L_3 edges) and transitions to 655 eV correspond to J=1/2 final states (L_2 edges). Cross sections parallel to the energy transfer axis sort the spectrum according to the final state.

Resonant Inelastic X-ray Scattering (RIXS)

We have started using the new technique of X-ray resonant Raman or X-ray inelastic scattering spectroscopy (RIXS) to study the electronic structure of the Mn cluster in the OEC. The RIXS data is collected by scanning the incident energy (1s to 3d/4p absorption followed by 2p-1s emission) to yield two-dimensional plots that can be interpreted along the incident energy axis or the energy transfer axis, which is the difference between the incident and emission energies (Fig. 2) [3].

S_1 to S_2 Transition

The 2D data are best shown as contour plots (Fig. 3). The comparison of Mn(II), Mn(III), Mn(IV) and PS II in the S_1 state and S_2 states shows that the S_1 and S_2 states contains a mixture of both oxidation states. The integrated cross sections along the Raman or energy transfer axis are the L_3-like edge (2p to 3d) (Fig. 4, left). The integrated cross sections along the incident energy are the K-edge (1s to 3d) transition (Fig. 4, right). It is clear from Figs. 3 and 4 that Mn in the S_1 state contains oxidation states III and IV; thus providing confirmation for the (III_2,IV_2) assignment for the S_1 state.

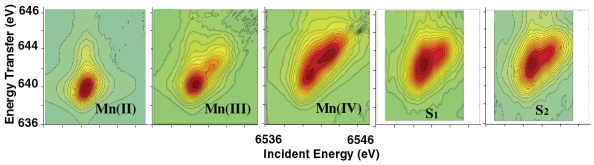

FIGURE 3. Contour plots of the $1s2p_{3/2}$ RIXS planes for three molecular complexes $Mn^{II}(acac)_2(H_2O)_2$, $Mn^{III}(acac)_3$, and $Mn^{IV}(sal)_2(bipy)$ and PS II in the S_1- and S_2-state. One axis is the excitation energy and the other is the energy transfer axis. The L-edge like spectra are along the energy transfer axis and the 1s to 3d transition is along the excitation energy. The assignment of Mn(III_2,IV_2) for the S_1 state is apparent in these spectra.

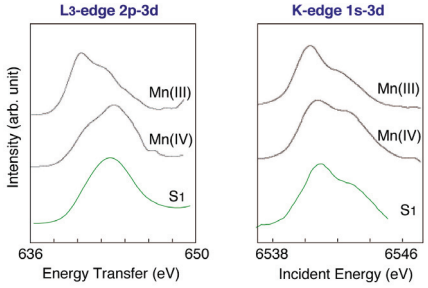

FIGURE 4. Integrated spectra along the incident energy axis, (K-edge, 1s to 3d) (right), and energy transfer axis, (L_3 edge, 2p to 3d) (left) of the contour plot shown in Fig. 3 for $Mn^{III}(acac)_3$ and $Mn^{IV}(sal)_2(bipy)$ and S_1-state of PS II. The spectra show that the Mn oxidation state of S_1 is a combination of (III) and (IV).

We have focused on the 1s to 3d aspect of the RIXS spectra, where line splittings have been

interpreted within a ligand field multiplet model. The spectral changes in the Mn 1s to 3d transition between the oxides, coordination complexes and PS II in the S_1 and S_2 states have been compared.

The results (see below) indicate strong covalency for the electronic configuration in the OEC and we conclude that the electron is transferred from a strongly delocalized orbital for the S_1 to S_2 transition [3], in accordance with the assignment of the formal oxidation states of $Mn(III_2,IV_2)$ to the S_2 state.

S_2 to S_3 and S_0 to S_1 Transitions

Although there has been general agreement with respect to the increasing oxidation of Mn in the cluster during S_0->S_1 and S_1->S_2, there is a lack of consensus concerning Mn oxidation during S_2->S_3. It is also not clear whether the S_0 state contains any Mn(II).

The XANES results showed that there is a larger shift in the inflection point energy between the S_0 to S_1 and S_1 to S_3 transitions compared to that occurring between the S_2 to S_3 transition [3]. The Kβ emission spectroscopy results based on the shifts, or lack thereof, of the 1st moments, also showed that a Mn-centered oxidation does not occur during the S_2->S_3 transition [2]. At the same time it is worth noting that this need not be an all-or-none situation. If the Mn is not oxidized, presumably some other species (a protein side-chain ligand or bound water) is oxidized. The delocalization of a small amount of electronic charge from a Mn atom to the ligand could account for the small residual changes seen in the X-ray energies [3].

RIXS data from the S_0 and S_3 states have been collected in order to identify the nature of the oxidations occurring between the S_0 to S_1 and S_2 to S_3 transitions. The spectra were processed in a manner similar to those from the S_1 and S_2 states. The 1st moment of the spectrum integrated along the incident energy axis (see Fig. 4) was calculated for all the S-states and compared with the 1st moments obtained from Mn oxides and Mn coordination compounds in formal oxidation states of (II), (III) and (IV).

The changes per oxidation state in the first moment positions are more pronounced between the Mn oxides than between the Mn coordination complexes. This observation is consistent with a stronger covalency in the coordination complexes. We furthermore observe a Mn oxidation between the S_1 and S_2 states of PS II even though the change in the Mn electronic structure is less pronounced than in the oxides. The spectral change per Mn ion between $Mn^{III}(acac)_3$ and $Mn^{IV}(salicylate)_2(bipy)$ is by a factor of 2 more pronounced than between S_1 and S_2. In other words, the orbital population change Δn_{3d}^{eff} per change in oxidation state is largest between the Mn oxides and smallest between S_1 and S_2. The reason is an increased covalency or delocalization of the Mn valence orbitals. We thus find that the electron that is transferred from the OEC in PS II between S_0 and S_1, and between S_1 and S_2 is strongly delocalized. Moreover, it is not possible to exclude the formal oxidation state of Mn(II) from the S_0 state as the resonance at lower energy is present in both Mn(II) and Mn(III) complexes, although the assignment of the resonance is different in both the cases [2].

The orbital population change Δn_{3d}^{eff} per change in oxidation state between the S_2 and S_3 states is half as much as that between that between S_0 and S_1, and S_1 and S_2 transitions indicating that the electron is removed from a more covalent form or a more delocalized orbital.

These results are in agreement with the earlier qualitative conclusions derived from the shifts in the XANES inflection point energies and the Kβ emission peaks from the S-states.

ACKNOWLEDGMENTS

This work was supported by the NIH Grant GM-55302, and by the Director, Office of Science, Basic Energy Sciences (BES), Division of Chemical Sciences, Geosciences, and Biosciences of the Department of Energy (DOE) under Contract DE-AC02-05CH11231. We thank Prof. Stephen Cramer at LBNL for providing access to the crystal analyzer (NIH grant GM-65440, NSF grant CHE-0213592, and OBER, DOE to SPC). JM was supported by the DFG (Me 1629/2-3). Synchrotron facilities were provided by SSRL, Stanford supported by DOE, BES. The SSRL Structural Molecular Biology Program is supported by the DOE, OBER, and by the NIH, NCRR, Biomedical Technology Program, and at the BioCAT, APS, Argonne supported by DOE, BES under contract W-31-109-ENG-38. BioCAT is a NIH-supported Research Center RR-08630.

REFERENCES

1. T. Wydrzynski and S. Satoh. *Photosystem II: The Light-Driven Water:Plastoquinone Oxidoreductase*, Springer, Dordrecht, 2005.
2. P. Glatzel, U. Bergmann, J. Yano, H. Visser, J. H. Robblee, W. W. Gu, F. M. F. de Groot, G. Christou, V. L. Pecoraro, S. P. Cramer and V. K. Yachandra *J. Am. Chem. Soc.* **126**, 9946-9959 (2004).
3. J. Messinger, J. H. Robblee, U. Bergmann, C. Fernandez, P. Glatzel, H. Visser, R. M. Cinco, K. L. McFarlane, E. Bellacchio, S. A. Pizarro, S. P. Cramer, K. Sauer, M. P. Klein and V. K. Yachandra. *J. Am. Chem. Soc.* **123**, 7804-7820 (2001).