Electronic Structure of the Mn₄Ca Cluster in the Oxygen-Evolving Complex of Photosystem II Studied by Resonant Inelastic X-Ray Scattering

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Abstract. Oxygen-evolving complex (Mn₄Ca cluster) of Photosystem II cycles through five intermediate states (Sᵢ-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 Mn₄(III₂,IV₂) for S₁ and S₂ states, while it is still controversial for the S₀ and S₃ states. We used resonant inelastic X-ray scattering (RIXS) to study the electronic structure of Mn₄Ca 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 1s2p½ 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 S₀ to S₃, and S₁ to S₂ transitions is twice as large as that during the S₂ to S₃ 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 S₀ and S₃ states also showed characteristic features which were not clear from the K-edge spectroscopy.

Keywords: photosystem II, oxygen-evolving complex, Mn₄Ca cluster, electronic structure, RIXS.
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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 H₂O to O₂ contains a Mn₄Ca 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 S₀-S₄. Upon reaching the S₄ state, the complex releases O₂ and returns to the S₀ 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 S₃ to (S₄) to S₀ 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 (L3 edges) and transitions to 655 eV correspond to J=1/2 final states (L2 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].

**S1 to S2 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 S1 state and S2 states shows that the S1 and S2 states contains a mixture of both oxidation states. The integrated cross sections along the Raman or energy transfer axis are the L3-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 S1 state contains oxidation states III and IV; thus providing confirmation for the (III2,IV2) assignment for the S1 state.

FIGURE 3. Contour plots of the 1s2p3/2 RIXS planes for three molecular complexes Mn(II)(acac)2(H2O)2, Mn(III)(acac)3, and Mn(IV)(sal)2(bipy) and PS II in the S1- and S2-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(III2,IV2) for the S1 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, (L3 edge, 2p to 3d) (left) of the contour plot shown in Fig. 3 for Mn(II)(acac)3 and Mn(IV)(sal)2(bipy) and S1-state of PS II. The spectra show that the Mn oxidation state of S1 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
interacted whether the S0 state contains any Mn(II). It is also not clear whether the S0 state contains any Mn(II).

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 S1 to S2 transition [3], in accordance with the assignment of the formal oxidation states of Mn(III) to S2. 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 S0 and S1, and between S1 and S2 is strongly delocalized. Moreover, it is not possible to exclude the formal oxidation state of Mn(II) from the S0 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 $\Delta n_{3d}^{\text{eff}}$ per change in oxidation state between the S2 and S1 states is half as much as that between between S0 and S1, and S1 and S2 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.

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