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XANES Spectroscopy for Monitoring Intermediate Reaction States of Cl$^-$-Depleted Mn Cluster in Photosynthetic Water Oxidation Enzyme

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The structural and electronic states of the Mn cluster in Cl$^-$-depleted photosynthetic water oxidation enzyme (WOE) were directly monitored by means of X-ray absorption near-edge structure (XANES) spectroscopy. Changes in XANES spectra of the Mn cluster in each intermediate reaction state induced by successive flash illumination provide direct evidence that a Mn ion is oxidized from Mn(III) to Mn(IV) after one flash, but further oxidation steps are interrupted in the absence of Cl$^-$. The normal Mn K-edge energy, however, suggests that the ligation structure of the Mn cluster does not change much after depletion of Cl$^-$.

The photosynthetic water oxidation is catalyzed by the tetranuclear Mn cluster[1] located in the photosystem II (PSII) core complex and proceeds through four stable intermediate reaction states of WOE, denoted as S$_i$ (i = 0–3), by use of the energy of four photons.[2] Our previous XANES experiments with O$_2$-evolving WOE have revealed that the energy of Mn K-edge shows a period-four oscillation depending on each intermediate S-state induced by a short laser flash.[3,4] Cl$^-$ is an indispensable inorganic cofactor for photosynthetic water oxidation and also functions in structural stabilization of the Mn cluster,[5] but the mode of Cl$^-$ action in the water oxidation has remained largely unknown. One slowly exchanging Cl$^-$ that is involved in the O$_2$ evolution exists in PSII and is released almost completely from its site by a NaCl wash.[6] In the absence of Cl$^-$, a limited number of oxidizing equivalents for water oxidation are accumulated on the donor side of PSII,[5] although their chemical identity is still a matter of debate.[7] Under these circumstances, it is necessary to probe directly the Mn cluster in order to characterize the redox reactions occurring in the Cl$^-$-depleted WOE. In this report, we adopted XANES spectroscopy to directly probe the electronic state of the Mn cluster. PSII membranes capable of O$_2$ evolution[8] were relaxed for 6 h in darkness and then subjected to a 2 M NaCl wash to facilitate the release of Cl$^-$ from WOE.[6,9] For Cl$^-$ depletion, the treated membranes were then washed three times with a Cl$^-$-free medium containing 0.4 M sucrose and 20 mM Mes-NaOH (pH 6.5) and suspended in the same medium supplemented with 20 mM Ca(OH)$_2$-Mes (pH 6.5). O$_2$ evolution activities of the control, Cl$^-$-depleted, and Cl$^-$-repleted membranes were 610, 90, and 540 μmol of O$_2$/mg of chlorophyll/h, respectively. The resulting Cl$^-$-depleted membranes were illuminated either with laser flashes or with continuous light and then immediately cooled to 77 K in liquid N$_2$.[4,5,10] XANES spectra were measured by fluorescence detection mode at the Photon Factory of the National Laboratory for High Energy Physics beam-line 4B.[4,5,10] TL was measured as described previously.[11]

K-edge XANES spectra of Cl$^-$-depleted WOE after 0–5 flashes and continuous illumination were measured (Figure 1A), and their edge energies were compared in an expanded view in the K-edge region (Figure 1B). The half-height energy in dark-adapted membranes was located at 6552.1 eV, which was almost the same value as that reported in the normal S$_1$ state with the same experimental setup.[4] This indicates that the Mn cluster is retained intact after Cl$^-$ depletion since even slight damage to the cluster is known to cause a downward shift of the K-edge energy due to the formation of Mn^{2+}. The structural intactness of the Mn cluster was also indicated by TL measurements: the Cl$^-$-depleted membranes showed the TL band with an elevated peak temperature,[11] but the TL band due to the normal S$_2$ state was restored concomitant with a normal period-four flash pattern when sufficient Cl$^-$ was added (data not shown). The half-height energies of the spectra were determined to be 6552.8, 6552.9, 6552.7, 6552.5, and 6552.6 eV after 1, 2, 3, 4, and 5 flashes, respectively, and 6552.9 eV after continuous illumination.

Figure 2 shows the flash number dependence of the half-height energy of the Mn K-edge in Cl$^-$-depleted WOE, where the flash pattern obtained in the nondepleted control WOE[4] previously measured in the presence of sufficient Cl$^-$ is shown as a reference. The K-edge energy shifted upward by 0.7 eV after 1 flash illumination of the Cl$^-$-depleted WOE. This change was comparable to that induced after 1 flash in the control WOE, indicating oxidation of Mn(III) to Mn(IV).[12] This is the first direct evidence that the Mn cluster is oxidized in Cl$^-$-depleted WOE. The K-edge energy did not undergo significant change after subsequent flashes in contrast to the control membranes, although a faint overlapping oscillation was apparent. After continuous illumination, the K-edge energy did not change much, compared with that after 1 flash. The residual oscillation exhibited a quadruple pattern as observed in the control WOE. Since the Cl$^-$-depleted WOE showed about 15% of the O$_2$ evolution activity of the control, it can be reasonably assumed that the nondepleted WOE was responsible for this residual oscillation. The closed circles in Figure 2 represent the simulated pattern obtained by assuming an upshift of the energy after 1 flash but no oscillatory behavior after subsequent flashes in 85% WOE and the period four oscillation in 15% WOE. The simulation is in good agreement with the experimentally obtained pattern, indicating that the oscillation of the K-edge energy was interrupted in the Cl$^-$-depleted WOE after 1 flash.

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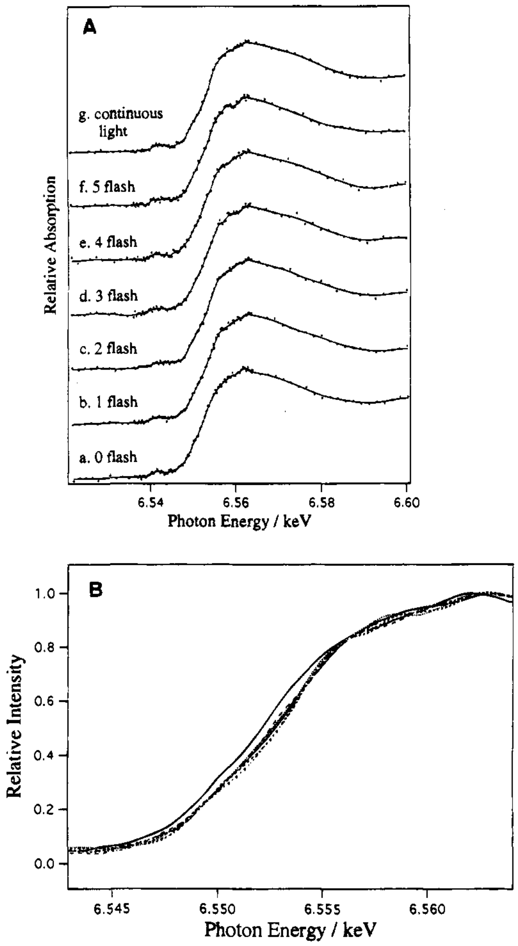

Figure 1. (A) XANES spectra of Cl^--depleted PSII membranes after a series of flashes and continuous illumination. Spectra a–f correspond to 0–5 flashes, and spectrum g is for continuous illumination at 0 °C for 1 min. (B) Effect of illumination on Mn K-edge. The membranes were illuminated with 0 (heavy solid line), 1 (light solid line), 2 (light short dash line), 3 (heavy short dash line), 4 (long dash line), and 5 (dotted line) flashes, and continuous illumination (dash-dot line). Fluorescence X-ray from the sample was detected with a solid state detector placed 10 mm from the sample. Samples were kept in darkness at 30 K during data collection. K-edge energies were calibrated using the pre-edge peak of $KMnO_4$ at 6543.3 eV measured simultaneously by absorption mode. For each spectra, 7–9 scans were averaged.

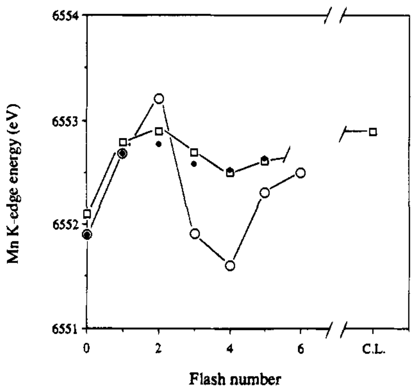

Figure 2. Flash-number-dependent changes of the half-height energy of the Mn K-edge after a series of flashes and continuous illumination (CL) in Cl^--depleted (□) and nontreated control (○) PSII membranes. The simulated pattern (●) was obtained as described in the text. The K-edge energy was obtained from the smoothed spectra shown in Figure 1. An estimated error in determination of the K-edge energy was ±0.1 eV.

In Cl^--depleted WOE, neither CW[13] nor pulsed[7] EPR detects any multiline EPR signal, but the multiline is developed by flash illumination followed by addition of Cl^- in the dark.[13] The present result provides evidence that the Mn cluster is oxidized by 1 flash, as seen in the O_2-evolving WOE, but the resulting state (Cl^--depleted S_2 state) is silent to EPR. The fact that the K-edge energies for the S_1 and S_2 states are almost the same in the normal and Cl^--depleted WOE indicate that the EPR-silent S_2 state is not ascribed to a change in the oxidation state of the Mn ion but rather to a change in antiferromagnetic and ferromagnetic coupling between Mn ions.

It is interesting to note in this context that the K-edge energy after continuous illumination was quite similar to that after 1 flash. Continuous illumination of the Cl^--depleted WOE at 0 °C leads to the formation of a broad EPR signal at $g = 2$.[14,15] The $g = 2$ EPR signal was induced by illumination in inhibited WOEs and has been thought to arise from a free radical of histidine,[16] tyrosine,[17] or partially oxidized water[18] interacting magnetically with the Mn cluster. It can, therefore, be concluded that no change in the K-edge energy is accompanied by the formation of the $g = 2$ EPR state in Cl^--depleted WOE. In contrast, the K-edge energy shifted upward by 0.9 eV concurrently with the formation of the $g = 2$ EPR state in Ca^{2+}-depleted WOE.[10] In Ca^{2+}-depleted WOE, the K-edge energy for the dark-adapted state (S_1) was lowered by 1.5 eV compared to that of the normal S_1 state, suggesting a relatively large change in the ligation structure of the Mn cluster.[10] Consistent with the marked downshift of the Mn K-edge energy, FT-IR difference spectroscopy reveals the breakage of the coordination bond by Ca^{2+} depletion between the redox active Mn ion and a certain carboxylate group that serves as a bridging ligand between the Mn cluster and Ca^{2+} in the normal S_1 state.[19] The minimal effect of Cl^- depletion on the K-edge energy may, therefore, suggest that Cl^- depletion does not result in significant structural modification of the Mn cluster. It has been proposed that Cl^- functions as a direct ligand for the Mn cluster[20] and furthermore acts as a bridging ligand between Mn atoms to stabilize the structure of the Mn cluster.[21] The present results, however, prefer the idea that Cl^- is not the first coordination ligand of the Mn cluster, although we cannot completely rule out the possibility of the direct ligation of Cl^- to the Mn cluster at present in a way that the lack of Cl^- does not cause a gross structural modification in the cluster.

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