Structural Changes of the Oxygen Evolving Complex in Photosystem II During the Catalytic Cycle*

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Running title: Changes in the Mn4CaO5 cluster during catalysis

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Background: Mn4CaO5-cluster catalyzes water oxidation in Photosystem II.

Results: Mn-Mn/Ca/ligand distances and changes in the structure of the Mn4CaO5-cluster are determined for the intermediate states in the reaction using X-ray spectroscopy.

Conclusion: Position of one bridging oxygen and related geometric changes may be critical during catalysis.

Significance: Knowledge about structural changes during catalysis is crucial for understanding the O-O bond formation mechanism in PSII.

SUMMARY

The oxygen-evolving complex (OEC) in the membrane-bound protein complex Photosystem II (PSII) catalyzes the water oxidation reaction that takes place in oxygenic photosynthetic organisms. We investigated the structural changes of the Mn4CaO5 cluster in the OEC during the S state transitions using X-ray absorption spectroscopy (XAS). Overall structural changes of the Mn4CaO5 cluster, based on the Mn-ligand and Mn-Mn distances obtained from this study, were incorporated into the geometry of the Mn4CaO5 cluster in the OEC obtained from polarized XAS model and the 1.9 Å high-resolution crystal structure. Additionally, we compared the S1 state XAS of the dimeric and monomeric form of PSII from T. elongatus and spinach PSII. While the basic structures of the OEC are the same for T. elongatus PSII and spinach PSII, minor electronic structural differences that affect the Mn K-edge XAS between T. elongatus PSII and spinach PSII are found, and may originate from differences in the 2nd sphere ligand atom geometry.

In nature, the water-splitting reaction takes place in Photosystem II (PSII), a multi-subunit membrane protein in plants, algae, and cyanobacteria. This sunlight driven reaction is catalyzed by an oxygen-evolving complex (OEC), which is located at the lumenal side of PSII. The OEC consists of four oxo-bridged Mn atoms and one Ca atom (Mn4CaO5) ligated to the D1 and
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CP43 subunits by carboxylate and histidine ligands (1,2). During the oxidation of water, the OEC cycles through five different intermediate states, which are known as $S_i$ states (where $i$ ranges from 0 to 4, and refers to the oxidation equivalents stored), that couple the one-electron photochemistry of the PSII reaction center with the four-electron redox chemistry of water oxidation (3) (Fig. 1).

The geometric and electronic structural changes that occur during the catalytic cycle have been studied over the last few decades using spectroscopic methods such as EPR (Electron Paramagnetic Resonance) (reviewed in (4)), FTIR (Fourier-transform infrared) spectroscopy (reviewed in (5,6)) and XAS (X-ray Absorption Spectroscopy) (reviewed in (7,8)). Among them, the information regarding the geometric structural changes comes largely from extended X-ray absorption fine structure (EXAFS) studies (9,10). The method has provided metal-to-metal (Mn-Mn and Mn-Ca) and metal-to-ligand (Mn-O/N) distances with high accuracy of ~0.02 Å and a distance resolution of ~0.1 Å. An important feature of this method is the possibility to control the X-ray dose by monitoring the Mn K-edge spectra. This allows data collection from the intact cluster, as the manganese ions are rapidly reduced to Mn(II), along with disruption of the cluster, when exposed to high X-ray doses normally used in protein crystallography (see below) (11-13).

Various EXAFS studies, including solution EXAFS, range-extended EXAFS, and single crystal polarized EXAFS, have suggested that in the $S_1$ state there are three short Mn-Mn interactions around 2.7 Å, one long Mn-Mn interaction at around 3.3 Å, and three to four Mn-Ca interactions (based on Sr XAS studies (14,15)) at around 3.4 - 3.9 Å. The combination of polarized EXAFS data from single crystals of dimeric PSIIcc with X-ray diffraction (XRD) data (16) led to three proposed models for the Mn$_4$CaO$_5$ cluster (17), of which one is shown in Fig. 2.

However, due to the limited knowledge about the accurate geometry of the Mn$_4$Ca cluster a direct correlation of the spectroscopic data to structural changes of the cluster is difficult. The recent high-resolution crystal structure from XRD reported by Umena et al. has provided a more precise geometry of the OEC structure with possibly limited effects from radiation damage (see below) (1). This high-resolution XRD structure has clearly located four Mn and one Ca positions facilitating the determination of the overall geometry of the metal cluster. Although, the similarities in the number of Mn-Mn and Mn-Ca vectors is striking between the XRD and EXAFS structural models, there are distinct differences in the distances as shown in Fig. 2. The major differences are in the Mn-Mn and Mn-O distances, which are longer by 0.1 - 0.2 Å for the Mn-Mn and ~ 0.3 Å for the Mn-O distances than determined by X-ray spectroscopy methods. We speculate that these differences in distances, summarized in Fig. 2, are caused by radiation damage during the XRD data collection and thus lead to some of the differences in the proposed models for the clusters.

Nevertheless, the 1.9 Å resolution structure of PSIIcc from Thermosynechococcus (T.) vulcanus serves as a new basis to relate our spectroscopic data with the structural changes that occur during the catalytic $S_i$ state transitions.

Until now, most of the XRD studies were performed with crystals of PSII core complexes (PSIIcc) purified from the thermophilic cyanobacteria T. elongatus and T. vulcanus, whereas spinach thylakoid membranes were used for the EXAFS studies. While a crystallographic model of PSIIcc from spinach is still missing, PSIIcc X-ray structures from cyanobacteria are available for its dimeric (1,16,18-21) as well as its monomeric form (22).

In the current study, structural changes of the Mn$_4$CaO$_5$ cluster through the $S_0$ to $S_3$ states were analyzed by means of EXAFS using the same dimeric PSIIcc preparations from T. elongatus as used for EXAFS studies. While a crystallographic model of PSIIcc from spinach is still missing, PSIIcc X-ray structures from cyanobacteria are available for its dimeric (1,16,18-21) as well as its monomeric form (22).

To investigate possible differences regarding the organization of the Mn$_4$CaO$_5$ cluster between various PSII samples, we additionally compared our EXAFS data of the $S_1$ state from dimeric PSIIcc samples with data obtained from monomeric PSIIcc samples and from spinach thylakoid membrane fragments.
EXPERIMENTAL PROCEDURES

Sample preparation. The preparation of monomeric and dimeric PSIIcc solutions from *T. elongatus* followed the protocol by Kern et al. (23). Note, that the PSII monomer fraction was not further purified in a third chromatography step as described in Broser et al. (22). The PSIIcc monomer and dimer solutions in 100 mM MES, pH 6.5, 5 mM CaCl$_2$, 0.015% β-dodecyl-maltoside were concentrated to about 15 mM chlorophyll *a* (Chl$_a$) and slowly mixed with 100% glycerol to a final concentration of 40% glycerol and 10 mM Chl$_a$.

Sample illumination. A frequency-doubled (532 nm) Nd:YAG laser was used (8 ns pulse-width) for flash illumination. To maintain maximal synchronization of the PSII centers on flash illumination, the fast recombination reaction between both the S$_2$ and S$_3$ states and the reduced form of the redox-active tyrosine residue Y$_D$ must be suppressed. This was achieved by the application of one pre-flash, followed by a 60 min dark-adaptation period at room temperature. This procedure synchronizes the PSII centers into predominantly the S$_1$Y$_D^{ox}$ state. Immediately before flashing, the electron acceptor was added to the sample (1µl of 50 mM PPBQ in MeOH per 40µl of sample). Each sample was then given zero, one, two, three, four, five, or six flashes at room temperature (0F, 1F, 2F, 3F, 4F, 5F and 6F samples), with intervals of 1.0 s between the individual flashes. The light was focused on the sample by using cylindrical lenses. After the last flash, the samples were frozen immediately (within 1 s) in liquid nitrogen. The EPR spectra were collected, and the samples were stored at 77 K for further use in the XAS experiments.

EPR spectroscopy. Low-temperature X-band EPR spectra were recorded by using a Varian E109 EPR spectrometer equipped with a model 102 microwave bridge. For the S$_2$ state multiline-signal measurements (data not shown), the sample temperature was maintained at 8 K with the use of an Air Products LTR liquid helium cryostat. Spectrometer conditions were as follows: microwave frequency, 9.21 GHz; field modulation amplitude, 32 G at 100 kHz; microwave power, 30 mW. EPR multiline-signal amplitudes were quantified by adding peak-to-trough amplitudes of the hyperfine lines down-field from g = 2. For each sample (0F to 6F), the designated S$_2$ state multiline EPR signal peaks were normalized by using the amplitude of the Fe$^{II}$ signal at g = 4.3 as an internal reference (data not shown).

We used the Kok model (Fig. 1) as described in Messinger et al. (24) to calculate the S state population for each flash number (Table 1) and have compared the calculated S$_2$ state values (normalized to be 100% for 1F amplitude) to the normalized amplitudes. The error between the calculated and measured S$_2$ state populations was minimized. Because of factors such as redox equilibrium between the cofactors in PSII, it is inevitable that some dephasing occurs while the OEC is advanced through the various S states. The possibility of double hits and miss hits as introduced by the original Kok model (3) was considered in the S state population analysis.

XAS data collection. X-ray absorption spectra were collected at the Stanford Synchrotron Radiation Lightsource (SSRL) on beamline 7-3 at an electron energy of 3.0 GeV and an average current of 300 mA. The intensity of the incident X-rays was monitored by a N$_2$-filled ion chamber (I$_0$) in front of the sample. The radiation was monochromatized by a Si (220) double-crystal monochromator. The total photon flux on the sample was limited to 1 x 10$^7$ photons per µm$^2$, which was determined to be non-damaging on the basis of detailed radiation-damage studies of PSIIcc solution samples (12). The samples were protected from the beam during spectrometer movements between different energy positions by a shutter synchronized with the scan program. The samples were kept at 8 K in a He atmosphere at ambient pressure by using an Oxford CF-1208 continuous-flow liquid He cryostat. Data were recorded as fluorescence excitation spectra by using a germanium 30-element energy-resolving detector (Canberra Electronics). For Mn XAS, energy was calibrated by the pre-edge peak of KMnO$_4$ (6,543.3 eV), which was placed between two N$_2$-filled ionization chambers (I$_1$ and I$_2$) after the sample.

The method for collecting XANES and EXAFS spectra as a function of X-ray dose is described in ref. 12.

EXAFS curve fitting procedures. Curve fitting was performed with Artemis and IFEFFIT software using *ab initio*-calculated phases and amplitudes from the program FEFF 8.2 (25,26). These *ab*
**RESULTS**

**XANES of PSIIcc dimer - S₀ to S₃ states.** Fig. 3 shows the XANES (X-ray absorption near edge structure) spectra of dimeric PSIIcc solutions from S₀ to S₃ states. Pure S state spectra were obtained by deconvoluting the spectra of flash samples (0F to 3F) using the S state distributions obtained from EPR spectroscopy (Table 1). The overall trend of the XANES edge shift is similar to that reported in spinach thylakoid membrane preparations (27). The edge position shifts to higher energy from sp inach thylakoid membrane preparations (~ 3.3 Å) as well as from Mn-Ca interactions (~ 3.4 Å). Upon the S₀ to S₁ state transition, peak I and II are shifted toward shorter apparent distances. This suggests a shortening of the Mn-ligand and the Mn-Mn distances. The S₁ to S₂ state transition does not show a detectable peak shift, while the peak intensity increases in peak I as well as peak II. The S₂ to S₃ state transition is accompanied by more substantial spectral changes; the FT peak II shifts to longer distance and the peak III region splits into two. Then upon S₃ to S₄ state transition, peak II shifts to shorter distance and peak III goes back to a single peak.

**EXAFS Curve Fitting Results of PSIIcc dimer - S₀ to S₃ states.** Mn EXAFS curve fits were carried out for the k³-weighted EXAFS spectra of the dimeric PSIIcc solutions in the S₀ to S₃ states. Table 2 summarizes the curve fitting parameters, in which R, N, and σ² are the actual distance, coordination number, and EXAFS Debye-Waller (DW) factor (in Å²), respectively. N values are defined as the total number of absorber-backscatter vectors divided by the number of absorber atoms per OEC. The R factor (R_i, in %) shows the goodness of the fit.

The ~ 3.4 Å Mn-Ca EXAFS peak in Mn XAS is concealed by the presence of the Mn-Mn interaction around ~ 3.3 Å in the peak III region. Hence, it would be ideal to carry out Ca XAS on each S state, to obtain detailed information about the Mn-Ca interactions. However, it is challenging to collect Ca XAS on PSII due to (a) the difficulties of avoiding Ca contamination during sample preparation and data collection, and (b) the higher absorption coefficient of Ca at its absorption energy (4050 eV) as compared to the Sr K-edge energy (16200 eV), that leads to faster radiation damage. Therefore, we used the 

\[ \chi(k) = \sum_{j} N_j e^{-2\sigma_{j}R_j} \sin(2\pi k R_j) \sin(2\pi k x) \]  

(1)

The neighboring atoms to the central atom(s) are divided into j shells, with all atoms with the same atomic number and distance from the central atom grouped into a single shell. Within each shell, the coordination number N_j denotes the number of neighboring atoms in shell j at a distance of R_j from the central atom. f eff(\pi, k, R_j) is the ab initio amplitude function for shell j, and the Debye-Waller term e^{-2σ_j²} accounts for damping due to static and thermal disorder in absorber-backscatterer distances. The mean free path term e^{-2λ_j²(k)} reflects losses due to inelastic scattering, where λ_j(k) is the electron mean free path. The oscillations in the EXAFS spectrum are reflected in the sinusoidal term, sin(2πkR_j + φ_j(k)) where φ_j(k) is the ab initio phase function for shell j. S₀² is an amplitude reduction factor due to shake-up/shake-off processes at the central atom(s). The EXAFS equation was used to fit the experimental data using N, R, and the EXAFS Debye-Waller factor (σ²) as variable parameters.
information of Sr EXAFS changes previously obtained from Sr-substituted T. elongatus (15) to estimate the distances and the number of Ca-Mn interactions that contribute to the Mn EXAFS in the peak III region.

The best fits are shown in Fig. 5 and the fitting parameters are summarized in Table 2 (for the detailed EXAFS fit, see supplemental Table 1).

$S_1$ state: As it was shown in previous studies with spinach thylakoid membrane fragments and Synechocystis PSII $S_1$ state (28), the predominant feature of the EXAFS spectrum of the PSII $S_1$ state arises from about six Mn-ligand interactions at an average distance of 1.87 Å, three di-µ-oxo bridged Mn-Mn interactions at ~ 2.7 Å, and one longer Mn-Mn interaction around 3.3 Å. In addition, there are two to three Mn-Ca interactions at 3.4 Å, which is confirmed by Ca XAS (29) as well as Sr XAS on Sr-substituted PSIucc and PSII membranes (15,30). In the current study, one shell fit to the peak II region shows an average Mn-Mn distance of 2.73 Å, while two shell fits prefer two distances at 2.71 Å and 2.79 Å with a slight improvement in the fit quality (supplemental Table 1, Fig. 5b). We note that the small distance heterogeneity in the Mn-Mn interactions within 2.7 to 2.8 Å (peak II) cannot be justified based only on our current conventional EXAFS data due to the limited distance resolution. We however used two shell fits in the current paper as such distance heterogeneity has been reported in the $S_0$ state and polarized EXAFS spectra of the $S_1$ state (17,28,31). Moreover, the range-extended EXAFS studies have provided evidence for the presence of distance heterogeneity (28,31).

In the previous Sr XAS study on Sr-substituted PSIucc (15), the data showed that there are two types of Sr-Mn interactions, one around 3.5 Å and the other around 3.8 Å. However, the exact ratio of these interactions was inconclusive. Just recently Koua et al. (32) published a structural model of the Mn$_n$SrO$_5$ cluster in Sr-substituted PSIucc from T. vulcanus at 2.1 Å resolution. The model shows two Sr-Mn interactions at 3.5 Å, one at 3.6 Å and one at 4.0 Å. Taking the estimated standard uncertainty of 0.21 Å into account, these results argue for a 3:1 ratio for short to long Sr-Mn interactions. In the current study, both a 2:2 or a 3:1 ratio for the two distances of the Ca-Mn interactions were tested, and only subtle differences were observed in the $R_f$ value (Table 2). This trend is true for all the $S$ states described below.

$S_2$ state: The starting parameters for the $S_2$ state EXAFS fit were taken from the best fits of the $S_1$ state. A shortening of the Mn-Mn distances was observed in the $S_1$ to $S_2$ state transition. In the $S_2$ state, the three Mn-Mn distances around 2.7 Å (peak II) become more homogeneous, which is evident by the stronger peak II with a lower DW factor. When the two shell fits were applied for peak II, the two distances are closer (2.72 Å and 2.75 Å), that is within the precision limit (~ 0.02 Å) of the conventional Mn EXAFS measurements. The one shell fit for the peak II region showed the average Mn-Mn distances to be 2.74 Å (Fig. 5c and Table 2). This result suggests that one longer Mn-Mn bond at 2.79 Å in the $S_1$ state becomes shorter in the $S_2$ state due to the oxidation state change of Mn from Mn(III) to Mn(IV).

$S_3$ state: As shown in Fig. 4, we see substantial spectral changes in the $S_2$ to $S_3$ state transition, particularly in the peak III region. Also, the intensity of peak II is weaker than in the $S_2$ state. Therefore, we have tested two possible structural models; (a) has a similar geometry to the $S_1$ and the $S_2$ states with three di-µ-oxo bridged Mn-Mn units (~ 2.7 Å, N=1.5) and a longer mono-µ-oxo bridged Mn-Mn unit (~ 3.2 Å, N=0.5), and (b) has four di-µ-oxo bridges (~ 2.7 Å, N=2) and no mono-µ-oxo bridge. In case (a), the best fit results for the $S_1$ state were used as starting parameters. The larger peak II width as compared to the $S_2$ state is reflected in the increased distance heterogeneity. In the one-shell fit (supplemental Table 1), the average Mn-Mn distance increased from 2.74 to 2.76 Å. When a two shell fit was applied, the fit quality improved with two Mn-Mn distances of 2.75 and 2.79 Å (Table 2 and supplemental Table 1). In case (b), we assumed all Mn being connected by at least two oxo bridges. The fit quality was slightly better in case (a) (supplemental Table 1). Upon a simple Mn oxidation reaction, we expect a shortening of Mn-Mn interactions due to the elimination of Jahn-Teller distortion in Mn(III). Therefore the increased Mn-Mn distances observed in both models ($S_1$ fit a and fit b) imply that in the $S_2$ to $S_3$ state transition, the structural changes we observe are not simply due to the distance change that is accompanied by the oxidation state change, but it is rather due to a fundamental geometrical change.
Possible reasons for such an elongation are described in the Discussion section.

**S<sub>0</sub> state:** Upon S<sub>3</sub> to S<sub>0</sub> state transition, the OEC goes from the most oxidized state to the most reduced state in the catalytic cycle. We observe a decrease of peak I and II intensities as well as a shortening of the average peak II distance. When two shell fits were applied, we see two Mn-Mn distances around 2.77 Å and one short distance at 2.68 Å. This 1:2 (2.7 Å : 2.8 Å) ratio is different from the results reported by Robblee et al. (33) using spinach thylakoid membrane fragments (ratio of 2:1). This difference could be due to the improved fitting protocol employed in the current study.

**Comparison of the S<sub>1</sub> state of T. elongatus dimeric and monomeric PSIIcc and spinach PSII.** A comparison of the XAS spectra of all three types of PSII samples in the dark stable S<sub>1</sub> state is shown in Fig. 6. While the XANES region (Fig. 6a) for the PSIIcc dimer and monomer samples are identical, the XANES spectrum of spinach PSII thylakoid membrane fragments shows slight differences to that of PSIIcc (Fig. 6a), that become more pronounced in the 2<sup>nd</sup> derivative spectrum (Fig. 6a, bottom). PSIIcc dimer/monomer as well as spinach PSII thylakoid membrane fragments show almost identical EXAFS spectra (Fig. 6b), confirming that the Mn<sub>4</sub>CaO<sub>5</sub> cluster has the same structure.

**Radiation damage of the S<sub>1</sub> state of dimeric PSIIcc.** Solutions of dimeric PSIIcc where exposed to various X-ray doses at 100 K, leading to reduction of 5, 10 or 25%, respectively, of the Mn in the sample from Mn<sup>III,IV</sup> to Mn<sup>II</sup>. The corresponding XANES and EXAFS spectra (recorded at 8 K) are shown in Fig. 7. A clear shift in the K-edge towards lower energies, due to the reduction of Mn to Mn(II) can be seen in Fig. 7a when comparing with the S<sub>1</sub> state spectrum. The amplitudes of the k<sup>3</sup>-weighted spectra change drastically with increasing Mn reduction (Fig 7c) and the oscillations are more damped compared to the S<sub>1</sub> state. In the k-range 8-11.5 Å<sup>-1</sup> a dephasing is clearly visible, especially at 10 and 25% damage compared to the S<sub>1</sub> state. Also, the pronounced oscillation in the range of k = 6 - 8 Å<sup>-1</sup> is reduced in amplitude in all spectra of the damaged PSIIcc solutions compared to the S<sub>1</sub> state spectrum. In the FT spectra (Fig. 7b), the intensities of all three peaks are decreased upon higher Mn reduction, with the most pronounced decrease in the peak II intensity. EXAFS curve fitting results (Table 3) show that upon increased Mn reduction the number of shorter Mn-Mn interactions are reduced and the number of longer Mn-Mn interactions are increased.

**DISCUSSION**

**XANES changes – S<sub>0</sub> to S<sub>3</sub> states.** In the current study with T. elongatus PSII core preparations, we observed a similar trend in the XANES energy shift to that seen for the spinach thylakoid membrane fragments (24). The similarity of the K-edge XANES between the cyanobacterial and spinach PSII suggests that the oxidation states proposed for spinach (for example, Mn<sup>II-III</sup>Mn<sup>IV</sup> for the S<sub>1</sub> state) are also valid for the cyanobacterial preparations. The previous study by Messinger et al. (24) showed that Mn oxidation occurs during the S<sub>0</sub> to S<sub>1</sub> and S<sub>1</sub> to S<sub>2</sub> state transitions based on the 1 – 2 eV XANES energy shifts. A much smaller shift was observed during the S<sub>2</sub> to S<sub>3</sub> state transition that is accompanied by a change in the edge shape, similar to that shown in Fig. 3 for cyanobacterial PSII. The interpretation was that the chemical changes during the S<sub>2</sub> to S<sub>3</sub> state transition are not the same as the ones during the S<sub>0</sub> to S<sub>1</sub> and S<sub>1</sub> to S<sub>2</sub> state transitions. One possible explanation for this was that a unit other than Mn (i.e. oxygen ligands) is oxidized during the S<sub>2</sub> to S<sub>3</sub> state transition. This conclusion was supported by results from X-ray Kβ<sub>1,3</sub> emission spectroscopy, which probes the occupied orbitals through 3d-3p exchange interactions and, therefore, is less affected by the ligand environment (34). The Kβ<sub>1,3</sub> peak shift was smaller in the S<sub>2</sub> to S<sub>3</sub> state transition as compared to the S<sub>0</sub> to S<sub>1</sub> and S<sub>1</sub> to S<sub>2</sub> state transitions. However, it is worth noting that the XANES edge shape changes and edge shift could be more complicated when the transition is accompanied by structural changes. Haumann et al. (10) showed that the small edge shift and the shape change could occur when one Mn coordination state changes from five to six. A similar change has been proposed by Siegbahn (35), in which an oxygen binds to a five coordinated Mn during the S<sub>2</sub> to S<sub>3</sub> state transition. As shown in the result section, the structural changes observed in the S<sub>2</sub> to S<sub>3</sub> state transition are more substantial.
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The nature of the electronic structural changes in the S$_2$ to S$_3$ transition may, therefore, require a more complete understanding of the S$_2$ and the S$_3$ states.

**Dark stable S$_1$ state structure.** The dark stable S$_1$ state structure has been studied intensely by EXAFS methods as well as XRD crystallography. The 1.9 Å crystal structure has shown that there are three shorter Mn-Mn interactions around 2.8-2.9 Å and one long Mn-Mn interaction around 3.3 Å (1). The Mn-ligand distances are distributed in the range of 1.8 to 2.6 Å, with an average value of 2.2 Å. The presence of short vs. long Mn-Mn interactions with a 3:1 ratio is in agreement with the various EXAFS studies in the past as well as with the current study. However, overall atomic distances are shorter in the EXAFS results, with Mn-ligand interactions at 1.9 Å and Mn-Mn interactions at 2.7 - 2.8 Å. This discrepancy likely arises from the distance uncertainties in both methods (XRD ~ 0.19 Å at 1.9 Å resolution, and EXAFS ~ 0.02 Å) as well as the inherent X-ray damage to Mn in the OEC during the diffraction measurement. The 1.9 Å crystal structure (1) was collected with a X-ray dose that is significantly lower than what was used in the past XRD studies (16,18-21), and under these conditions around 25% of Mn is expected to be reduced from native Mn(III)/Mn(IV) oxidation state to Mn(II).

To assess the effect of radiation damage in more detail, XANES and EXAFS spectra were collected from dimeric PSIIcc exposed to low levels of X-ray dose that induces a small amount of Mn reduction (Fig. 7). Even at the level of 5 to 10% Mn reduction clear changes in the EXAFS spectrum were observed as shown in Fig. 7.

The origin of the elongation of the atomic distances in the crystal structure has been discussed by several groups; one possibility under consideration is that the originally present S$_1$ state is reduced to the S$_0$ state by the X-rays following the catalytic pathway backwards (36). Other possibilities that have been discussed are the presence of pre-S$_0$ states such as S$_1$, S$_2$, and S$_3$ states in the crystal structure based on the observed atomic distances (36,37). As shown in Fig. 7, the 25% radiation-damaged PSIIcc EXAFS spectrum is substantially different from the intact S$_0$ state spectrum. Also, even the 5 to 10% X-ray reduced PSIIcc spectra do not match with the intact S$_1$ state nor the S$_0$ state spectrum (Fig. 7 and Table 3). These findings imply that the reduction of the metal center by X-rays does not go through the catalytic pathway. This is similar to the situation found in the case of Fe/Fe and Fe/Mn ribonucleotide reductase, where the states generated by X-ray photoreduction are significantly different from the native catalytic state of the metal center (38).

**Structural changes of the Mn$_4$CaO$_5$ cluster.** To understand the catalytic mechanism, it is critical to know the distance information within a resolution of 0.1 Å to identify the chemical nature (e.g. Mn oxidation state, nature of bridging ligands, protonation pattern of bridges) of the cluster as well as the detailed structural changes. Such distance changes are expected during the catalytic reaction, accompanied by Mn oxidation state changes as well as proton release. A proton-release pattern of 1:0:1:2 (S$_0$ to S$_1$, S$_1$ to S$_2$, S$_2$ to S$_3$, and S$_3$ to S$_0$) is supported by several studies including the most recent one using IR spectroscopy (39).

The EXAFS spectra and the curve fitting results of the PSIIcc S states presented in this study show that the structure of the Mn$_4$CaO$_5$ cluster changes during the catalytic cycle. In particular, the short Mn-Mn interactions undergo distance changes in the range of 2.7 to 2.8 Å.

Such distance changes can reflect several chemical parameters; Mn oxidation state changes, protonation state changes of bridging oxygens, ligation modes (e.g. bidentate/monodentate), as well as fundamental changes in geometry (i.e. dimeric, trimeric, or cubane-like structure). In the first case, Mn-ligand distances are shortened upon Mn oxidation from Mn(III) to Mn(IV), while Mn-Mn distances within Mn(III)/Mn(IV) and Mn(IV)/Mn(IV) multinuclear complexes strongly depend on the direction of the Jahn-Teller axis (40). When the protonation states of the bridging oxygens are changed, the di-µ-oxo bridged Mn-Mn distance changes from 2.72 Å (bis-oxo) to 2.84 Å (oxo/hydroxo) and to 2.92 Å (bis-hydroxo) (41). Also cubane-like structures show generally longer Mn-Mn distances compared to pure bis-µ-oxo dimer complexes (42). Therefore, the observed distance changes could serve as an indicator of the chemical structural changes that occur during the S state transitions.

Possible structural changes of the Mn$_4$CaO$_5$ cluster during the S state transitions are illustrated in Fig. 8. Our model incorporates the general
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ligand environment and Mn arrangement found in the 1.9 Å crystal structure (1) but builds upon EXAFS distances, FTIR and EPR results (43-51) and the distance changes extracted from the EXAFS spectra in this work. Among all S states, the S$_2$ state is spectroscopically the most studied state possessing a rich EPR signal (52). Therefore, we consider the S state structural changes starting from our model for the S$_2$ state, described in the following section.

The polarized EXAFS study of single crystals of dimeric PSIIcc (17) supports the open-cubane like structure that was also suggested by Siegbahn for the S$_1$ and S$_2$ states (35) and Neese’s group for the S$_2$ state (53). In the S$_2$ state a formal oxidation state distribution of Mn$_4$(IV,IV,IV,III) for Mn$_{1,2,3,4}$ is assumed based on $^{55}$Mn ENDOR measurements (44,54) and theoretical calculations. In our model the three short Mn-Mn distances (fitted well to a single shell at 2.74 Å) are assigned to Mn$_1$-Mn$_2$, Mn$_2$-Mn$_3$ and Mn$_3$-Mn$_4$, and the one longer interaction at 3.3 Å to Mn$_2$-Mn$_4$ (Fig. 8).

We observe a distance change in the transition from the dark stable S$_1$ state to the S$_2$ state. This shortening of one Mn-Mn interaction (~2.79 to ~2.74 Å) is likely due to the oxidation state change of one Mn (formally Mn(III) to Mn(IV)). FTIR difference spectroscopy studies indicated that the Mn atom ligated by Ala344 undergoes oxidation in the S$_1$ to S$_2$ state transition (55). Accordingly, this oxidation occurs at Mn3 in our model (Fig. 8), in agreement with Mn4 being the Mn(III)-moiety in the S$_2$ to S$_3$ state transition, unless protons from terminal water molecules are transferred to the neighboring bridging oxygens. Instead, we suggest this structural change to be the shift of the oxygen O-5 position from the Mn1 side to the Mn4 side as illustrated in Fig. 8. Such an O-5 shuffling possibility has been suggested by Pantazis et al. (48,49), but as a reason for the change from the S$_2$ low spin (S=1/2) (multiline species) to S$_2$ high spin state (S=7/2) (g=4 species for spinach and g=6-10 for T. elongatus), and by Isobe et al. (57) in the S$_2$ to S$_3$ state transition using DFT calculations. If the oxygen O-5 moves toward the Mn$_3$Ca open cubane site, a Mn$_3$CaO$_4$ closed cubane is formed in the S$_3$ state.

Thus far, Mn/Ca heteronuclear complexes have been synthesized by the Christou and Agapie groups (58,59). The Mn$_{IV}$Ca$_2$O$_4$ structure reported by Mukherjee et al. (58) has Mn-Mn interactions within the closed cubane structure with 2.73, 2.76, and 2.86 Å, with an averaged Mn-bridging oxygen distance of 1.86 Å. The Mn$_{IV}$CaO$_4$ structure reported by Kanady et al. (59) has three Mn-Mn interactions at 2.83 - 2.84 Å with an averaged Mn-bridging oxygen distance of 1.87 Å. These Mn-Mn distances are longer than what is observed in Mn dimer or trimer model complexes (40) and would resemble the observed elongation of Mn-Mn distance in the S$_2$ to S$_3$ state transition. The repositioning of oxygen O-5 could be accompanied by changes of the ligand symmetry of Mn1, changing from a five to a six coordinate geometry and leading to the 3.26 Å Mn-Mn distance for Mn1-Mn2.

We have considered two possible structural models for the S$_3$ state, if a Mn$_3$CaO$_4$ closed cubane-like moiety is formed in this state: one
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Changes in the Mn$_4$CaO$_5$ cluster during catalysis with five coordinated Mn1 upon oxygen O-5 shuffling, and the other with six coordinated Mn1 and no oxygen shuffling. In the latter case, the N number for the ~ 2.7 Å Mn-Mn interactions becomes 2, while in the former case it remains 1.5. While the EXAFS fitting result slightly prefers N=1.5, the result is not conclusive based only on the EXAFS curve fitting (Table 2 and Fig. 5d). In the first case (S$_3$ fit A), Mn1 becomes five coordinated upon the S$_2$ to S$_3$ state transition, or a new ligand, either water or carboxylate, needs to be ligated to maintain the coordination of six at Mn1. In the pre-edge region, which is generally sensitive to the ligand symmetry, the intensity tends to increase upon the S$_2$ to S$_3$ state transition. This could suggest Mn1 to be five coordinated but remains an open question at the moment. A detailed pre-edge analysis combined with theoretical calculations may give us an insight into the ligand symmetry changes, and such an approach is underway.

Upon the S$_3$ to S$_0$ state transition via the S$_4$ state, the ~ 2.7 Å Mn-Mn distances are shortened. This is counterintuitive as the Mn oxidation state changes from the most oxidized form (S$_3$) to the most reduced state (S$_0$). However, such changes could be explained if the Mn$_4$CaO$_5$ geometry upon the S$_3$ to S$_0$ state transition reverts back to a structure similar to the S$_1$ and S$_2$ states, where the Mn$_4$Ca moiety shows open-cubane like structures.

Structural changes of the Mn$_4$CaO$_5$ cluster have also been studied with Sr XAS using Sr-substituted PSII (Mn$_4$SrO$_5$ cluster) of T. elongatus. The Mn-Sr distance changes were observed during the S state transitions (15), with substantial EXAFS spectral changes in the S$_2$ to S$_3$ state transition. This result, together with the current Mn XAS, demonstrates that Ca(Sr) plays an important role during the S$_2$ to S$_3$ state transition. This is in line with the fact that the OEC does not go beyond the S$_Y$z’ state (60) when Ca (or Sr) is chemically depleted from PSII (61). More recently, Ca-depleted spinach thylakoid membrane samples were used for studying the role of Ca during the water oxidation reaction, and Lohmiller et al. showed that the depletion of Ca from the Mn$_4$CaO$_5$ core does not disturb the overall structure of the Mn$_4$ moiety on the spin-state level in the S$_1$ and S$_2$ states (62), as well as on the geometry-level (unpublished EXAFS data, T. Lohmiller). The fact that Ca can be more easily exchanged in the higher S states (63) compared to the S$_1$ and the S$_2$ state, together with the above observations, implies that the Mn-Ca binding modes are changed upon the S$_2$ to S$_3$ state transition.

In addition to the Mn$_4$CaO$_5$ core structural changes we discussed here, we expect that terminal ligands that come from carboxylates, histidine, and water/hydroxo ligands change during the catalytic states. In general, it is difficult to get detailed metal-ligand information from EXAFS studies as it only provides averaged distance information. However, it has been shown using site-directed mutant studies that some ligands have critical roles in the OEC activity (5,64). The replacement of just one His terminal ligand by a glutamate residue (D1-His332Glu) resulted in a major change in the EXAFS and XANES spectra (64). This illustrates the importance of the ligands in maintaining the active-site structure and how well-tuned the active site is by the residues surrounding the Mn$_4$CaO$_5$ cluster.

Spinach, dimeric and monomeric PSII. The existence of PSIIcc in both a monomeric and dimeric form has provided the basis for controversial discussions, concerning their contribution to the functionality of the photosynthetic apparatus (65,66). This discussion in the past included the question whether PSII always exists in a dimeric form, or whether a monomeric PSII is an equally active form. At present, the prevailing view is that the PSII dimer is the fully assembled and functionally relevant form, whereas the monomeric form is seen as an intermediate during the assembly process of all known 20 subunits of PSIIcc and the repair cycle of photo-damaged subunit D1 (67,68). The assembly of PSII is a stepwise and highly regulated process (69), that includes many auxiliary proteins which are absent in the crystallized complexes. In cyanobacteria, an intermediate Psb27-PSII complex, which has no functional Mn cluster (70), regulates the assembly of the Mn$_4$CaO$_5$ cluster and the binding of the extrinsic subunits PsbO, PsbU and PsbV (71) prior to the dimerization of the PSII core complex. Monomerization of photo-damaged PSII was suggested to be triggered by the detachment or
structural reorganization of PsbO on the lumenal side (67,68).

The monomeric form of PSIcc used in our measurements is obtained in the chromatographic purification procedure of crude PS II extract, together with PSIcc dimer, as described in Kern et al. 2005 (23). This purification protocol yields essentially equal amounts of reaction centers in the monomeric and dimeric form. The monomer:dimer ratio from 40 preparations was estimated to be 1.05 ± 0.45 based on the quantity of Chl a (22). Moreover, it was shown that the monomeric and dimeric forms of PSIcc are similar in their oxygen evolving capacity as well as in their subunit content. The crystal structure of monomeric PSIcc, albeit available at medium resolution so far, gives neither indications of a destabilization of subunit D1 nor a structural reorganization of subunit PsbO.

It is therefore not surprising that the XAS spectra of PSIcc monomer and dimer are almost identical in the dark stable S 1 state (Fig. 6). This comparison provides confidence that the monomeric PSIcc is suitable for XAS studies and for use in future investigations.

XAS spectra of PSIcc were also compared with spectra from spinach PSII. While overall spectral shapes are the same, there are some minor differences observed in the XANES 2nd derivative spectrum (Fig. 6a). This comparison provides confidence that the monomeric PSIcc is suitable for XAS studies and for use in future investigations.

CONCLUSION AND OUTLOOK

X-ray absorption spectroscopy results show that the relative position of one of the bridging oxygens in the Mn₄CaO₅ cluster may play a critical role in structural changes during the S state transitions, and might be involved in the geometric changes of the Mn₄CaO₅ cluster during catalysis. This conclusion is crucial for a detailed understanding of the O-O bond formation mechanism during the water oxidation reaction.

Note that more detailed structural changes that could provide evidence, for example, which Mn-Mn distances are shortened/elongated upon S state transition require orientational information which can be gained from oriented membrane EXAFS or single crystal polarized EXAFS studies. These studies are underway.
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FOOTNOTES

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1The abbreviations used are: Chl£­a, chlorophyll a; DW, Debye-Waller; EPR, electron paramagnetic resonance; EXAFS, extended X-ray absorption fine-structure spectroscopy; FT, Fourier-transform; FTIR, Fourier-transform infrared; OEC, oxygen-evolving complex, PPBQ, 2-phenyl-p-benzoquinone; PSII, photosystem II; PSIIcc, photosystem II core complex; XANES, X-ray absorption near-edge spectroscopy; XAS, X-ray absorption spectroscopy; XRD, X-ray diffraction.

2Note that several methods have been used for assigning the formal oxidation states of metals using XANES, that includes the half height energy of the edge jump, white line energy of the edge, as well as the zero-crossing energy of the XANES 2nd derivative spectra. None of these are perfect methods for studying the charge density changes of metals. However, the zero-crossing energy seems to be the most reliable qualitative method, when the energy shift is accompanied by the spectral shape changes. This is based on the XANES study on various model complexes.
Changes in the Mn$_4$CaO$_5$ cluster during catalysis

FIGURE LEGENDS

Figure 1. **The Kok cycle.** The classical Kok cycle with the intermediate S states in water-oxidation is shown. Proposed oxidation states for the Mn atoms are indicated. Electron and proton transfers are illustrated in red and blue, respectively.

Figure 2. **Structural models for the Mn$_4$CaO$_5$ cluster.** The structural models for the Mn$_4$CaO$_5$ cluster from (a) the polarized EXAFS and Sr EXAFS studies (15,17) and, (b) the 1.9 Å resolution XRD study (1) are shown. The Mn-Mn and Mn-O/N ligand distances from each of these studies are summarized below the respective structural model. Mn atoms are depicted in red and Ca in green.

Figure 3. **XANES spectra of dimeric PSIIcc solution in the S$_0$, S$_1$, S$_2$, and S$_3$ states.** Mn XANES (top) and their 2nd derivative spectra (bottom) of dimeric PSIIcc solution in the S$_0$ (green), S$_1$ (blue), S$_2$ (red), and S$_3$ (black) states are shown. The zero-crossing energy of the 2nd derivative spectra are 6550.94 eV for S$_0$, 6553.45 eV for S$_1$, 6554.12 eV for S$_2$, and 6554.40 eV for S$_3$.

Figure 4. **EXAFS and FT spectra of dimeric PSIIcc solutions in the S$_0$, S$_1$, S$_2$, and S$_3$ states.** (a) $k^3$-weighted EXAFS spectra and (b) their Fourier-transformed spectra of dimeric PSIIcc solution in the S$_0$ (green), S$_1$ (blue), S$_2$ (red), and S$_3$ (black) states are shown. For comparison, the spectrum of the S$_{n-1}$ state is overlaid in the S$_1$, S$_2$, and S$_3$ spectra (gray). Prominent changes between the S$_2$ and the S$_3$ state and the S$_3$ and the S$_0$ state in the peak II of the FT spectra are indicated by a dashed line. All spectra are shown in the same scale but with a vertical offset.

Figure 5. **Mn EXAFS curve fitting results of dimeric PSIIcc solution in S$_0$ to S$_3$ states.** Mn EXAFS curve fitting results for dimeric PSIIcc solution in (a) S$_0$, (b) S$_1$, (c) S$_2$, and (d) S$_3$ states. Only the best fit results are shown in the figure. The details of the fitting parameters are summarized in Table 2. The experimental data are shown in blue and the fit data in red (and green).

Figure 6. **XAS spectra of monomeric and dimeric PSIIcc solution compared with spinach PSII thylakoid membrane fragments.** A comparison of the S$_1$ state Mn XANES spectra (a) and their FT spectra (b) of monomeric (red line) and dimeric (black line) PSIIcc solution, and spinach PSII thylakoid membrane fragments (blue line) is shown.

Figure 7. **XAS spectra of dimeric PSIIcc solution sample with 0, 5, 10 and 25% reduced Mn.** A comparison of the intact PSIIcc sample in the S$_1$ and S$_0$ states with PSIIcc samples that are 5%, 10% and 25% damaged by exposure to X-rays is depicted: (a) XANES, (b) Fourier transforms of Mn EXAFS spectra, and (c) $k^3$-EXAFS spectra. Spectra in (b) and (c) are shown in the same scale, respectively, but with vertical offsets.

Figure 8. **Proposed possible structural changes during the S state transitions.** Possible structural changes during the S state transitions are illustrated. Note that the focus here is to accommodate the EXAFS distance changes, and possible protonation states (at oxo-bridging and terminal water molecules) or changes in the ligand environment (type of ligands and ligation modes) are not included in the figure. The Mn-Mn distances at ~ 2.7 Å are indicated by green arrows, ~ 2.8 Å by blue arrows and ~ 3.2 Å by red arrows. The dashed line indicates that it may not be a bond. For the S$_3$ and the S$_0$ state two possible models are presented. Mn atoms are shown in blue (Mn$^{III}$), red (Mn$^{IV}$) or magenta (Mn$^{III}$ or Mn$^{IV}$ possible), Ca in green and the surrounding ligand environment in grey.

Figure 9. **Amino acid residues within a radius of 20 Å around the Mn$_4$Ca cluster according to the structural model of PSIIcc from T. elongatus at 2.9 Å resolution (21).** The right panel shows in yellow (ribbon mode) the amino acid residues within a radius of 20 Å around the Mn$_4$Ca cluster (Mn: purple...
spheres, Ca: green sphere). Amino acids of subunit D1 different from spinach are highlighted in pink and are labeled in the enlarged view on the left (distances are given in Table S1). For better orientation, the amino acid residues Asp170, Glu189 and His332 (all of subunit D1, yellow) are labeled and are shown in stick mode. The extrinsic subunits PsbO (purple), PsbU (blue) and PsbV (light blue) are shown in cartoon mode. View is of one monomer looking onto the monomer-monomer interface along the membrane plane (tilted by 45° to the left), with the cytoplasm above and the lumen below.
Changes in the Mn$_4$CaO$_2$ cluster during catalysis

Figure 1
Changes in the Mn₄CaO₅ cluster during catalysis

Figure 2

(a) Model III from EXAFS

Mn-O/N distances: ~1.9 Å
Mn-Mn distances
AB, BC, CD: 2.7 - 2.8 Å
BD: 3.3 Å
Mn-Ca distances
2 to 3 interactions around 3.4 Å
2 to 1 interactions around 3.9 Å

(b) 1.9 Å XRD

Mn-O/N distances: ~2.2 Å
Mn-Mn distances
AB, BC, CD: 2.8 - 3.0 Å
BD: 3.3 Å
Mn-Ca distances
3 interactions around 3.4 Å
1 interaction around 3.8 Å
Figure 3
Changes in the Mn₄CaO₅ cluster during catalysis

Figure 4
Figure 5

(a) S0  
(b) S1  
(c) S2  
(d) S3
Changes in the Mn$_4$CaO$_5$ cluster during catalysis

Figure 6

(a) Changes in the normalized intensity of various states.

(b) FT Amplitude changes with apparent distance (Å).

- spinach BBY
- T. elongatus PSII cc.
- dimeric
- monomeric

S$_1$ state
Changes in the Mn₄CaO₅ cluster during catalysis

Figure 7

(a) Normalized Intensity

(b) |χ(R)| (Å⁻⁴)

(c) X(R)k³

Apparent distance (Å)

Wavevector (Å⁻¹)

X-ray reduced
S₁
S₀
5%
10%
25%

X-ray energy (eV)

Normalized Intensity

6540 6560 6580
Changes in the \( \text{Mn}_4\text{CaO}_5 \) cluster during catalysis

Figure 8

\[ \text{Mn(III)} \quad \text{or} \quad \text{Mn(IV)} \quad \text{or} \quad \text{Mn(III) or (IV)} \]

\[ \text{S}_2 \quad \text{S}_3 \quad \text{or} \quad \text{S}_0 \]

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Changes in the Mn$_4$CaO$_5$ cluster during catalysis

Figure 9
Changes in the Mn₄CaO₅ cluster during catalysis

**Table 1** S state population of flashed samples (unit: %).

| # of flashes | S₀  | S₁  | S₂  | S₃  |
|--------------|-----|-----|-----|-----|
| 0            | 3.0 | 88.0| 9.0 | 0.0 |
| 1            | 0.3 | 11.5| 80.1| 8.1 |
| 2            | 6.9 | 2.8 | 28.4| 61.8|
| 3            | 52.9| 7.5 | 15.7| 23.9|

**Table 2** EXAFS fit table for each S state.

| Path     | S₀  | S₁  | S₂  | S₃ fitA | S₃ fitB |
|----------|-----|-----|-----|---------|---------|
|          | R   | N   | σ²  | R   | N   | σ²  | R   | N   | σ²  | R   | N   | σ²  |
| MnO      | 1.91| 4.5 | 0.009| 1.86| 3.9 | 0.005| 1.84|(1.88)| 3.5 | 0.005| 1.85| 3.8 | 0.004|
| MnO/N    | 2.26| 1.5 | (1.6)| 0.009| 2.05| 2.1 | 0.005| 1.97|(2.14)| 2.5 | 0.005| 1.99|(2.00)| 2.2 | 0.004|
| MnMn     | 2.68| 0.5 | 0.002| 2.71| 1.0 | 0.002| 2.74| 1.5 | 0.002| 2.75| 1.0 | 0.002| 2.72|(2.76)| 1.0 | 0.002|
| MnMn     | 2.77| 1.0 | 0.002| 2.79| 0.5 | 0.002| 2.79| 0.5 | 0.002| 2.82| 1.0 | 0.002| 2.72|(2.76)| 1.0 | 0.002|
| MnMn     | 3.30| 0.5 | 0.007| 3.28| 0.5 | 0.002|(3.27)| 3.30| 0.5 | 0.005| 3.26| 0.5 | 0.002|
| MnC      | 3.05| (3.04)| 1.5 | 0.005| 2.99| (3.00)| 1.5 | 0.005| 2.99| 1.5 | 0.005| 2.96| 1.5 | 0.005| 3.58|(2.96)| 1.5 | 0.005|
| MnCa     | 3.36| 0.5 | (0.75)| 0.007| 3.36| 0.5 | (0.75)| 0.007| 3.36| 0.5 | (0.75)| 0.007| 3.37| (3.36)| 0.5 | (0.75)| 0.007| 3.34| (3.31)| 0.5 | (0.75)| 0.002|
| MnCa     | 3.99| 0.5 | (0.25)| 0.015| 3.99| 0.5 | (0.25)| 0.008| 3.99| 0.5 | (0.25)| 0.008| 3.99| 0.5 | (0.25)| 0.009| 3.99| 0.5 | (0.25)| 0.009|
| MnO      | 3.34| 2.5 | 0.015| 3.14| 2.5 | 0.091|(3.085)| 3.14| 2.5 | 0.086| 3.06| 2.5 | 0.015| 3.08|(3.06)| 2.5 | 0.015|

R factor (%)

\[ k = 2.4 – 11.3 \text{ (Å⁻¹)}, E₀=6561.30 \text{ eV}, S₀^2=0.85. \] The bold letters show the fixed parameters. The \( \sigma^2 \) values of shorter Mn-O and longer Mn-O/ Mn-N interactions were linked, and assumed to be the same (shown in italic letters). In the same manner the \( \sigma^2 \) values of ~ 2.7 Å Mn-Mn and ~ 2.8 Å Mn-Mn interactions were linked. The numbers in parenthesis show the results when the N ratio of the shorter vs. longer Mn-Ca interactions is fixed to 0.75:0.25.

* Mn-C interactions (Mn to C of carboxylates) and Mn-O interactions (Mn to O of carboxylates) were included in the fit, although their contribution is minor.
Table 3  EXAFS fit table for 5%, 10% and 25% damaged PSII.

| Path     | 5% damage |          | 10% damage |          | 25% damage |          |
|----------|-----------|----------|------------|----------|------------|----------|
|          | R     | N     | σ² | R     | N     | σ² | R     | N     | σ² |
| MnO      | 1.85 | 3.4 | 0.007 | 1.86 | 3.2 | 0.007 | 1.88 | 3.0 | 0.008 |
| MnO/N    | 2.03 | 2.6 | 0.012 | 2.09 | 2.8 | 0.014 | 2.12 | 3.0 | 0.017 |
| MnMn     | 2.73 | 1.1 | 0.005 | 2.73 | 0.6 | 0.003 | 2.75 | 0.2 | 0.003 |
| MnMn     | 3.28 | 1.0 | 0.004 | 3.27 | 1.2 | 0.049 | 3.26 | 1.5 | 0.011 |
| MnCa     | 3.61 | 0.75 | 0.010 | 3.61 | 0.75 | 0.010 | 3.61 | 0.75 | 0.030 |
| R factor (%) | 4.2 | 4.6 | 3.7 | ΔE=-15.0 | ΔE=-14.6 | ΔE=-14.2 |

$k = 2.4 - 11.4$ (11.5 for 25% damage) (Å⁻¹), $E_0=6561.30$ eV, $S_0^2=0.85$.

The bold letters show the fixed parameters.

Table 4  Shortest distances of amino acid residues, which are different in subunit D1 of PSII from spinach and *T. elongatus*, to the Mn₄Ca cluster.

| residue in PSIIcc from *T. elongatus* | residue in PSII from *spinach* | shortest distance to Mn₄Ca cluster [Å] |
|--------------------------------------|---------------------------------|----------------------------------------|
| Thr79                                | Ser                             | 15.4                                   |
| Val82                                | Ile                             | 12.4                                   |
| Val83                                | Ile                             | 9.8                                    |
| Ser85                                | Thr                             | 8.7                                    |
| Gln113                               | Glu                             | 14.6                                   |
| Ile116                               | Val                             | 14.0                                   |
| Phe117                               | Leu                             | 16.7                                   |
| Ser153                               | Ala                             | 16.6                                   |
| Phe155                               | Thr                             | 15.6                                   |
