

S10-013

The manganese complex of Photosystem II: A structural model for the S₁, S₂, and S₃ oxidation states derived from linear dichroism EXAFS spectroscopy at 20 K and at room temperature

M. Haumann^{1*}, M. Grabolle¹, M. Werthammer¹, L. Iuzzolino², J. Dittmer², W. Meyer-Klaucke³, T. Neisius⁴, and H. Dau¹

¹*Inst. f. Experimentalphysik, Freie Universität Berlin, Arnimallee 14, D-14195 Berlin, Germany;* ²*Philipps Universität Marburg, Lahnberge, D-35032 Marburg, Germany;* ³*EMBL Outstation, DESY, Notkestr. 85, D-22603 Hamburg, Germany;* ⁴*ESRF, BP. 220, 38043 Grenoble Cedex, France;*

*Fax. ++49 (0)30 8385 6299, Email: haumann@physik.fu-berlin.de

Keywords: Photosystem II, water oxidation, X-ray absorption spectroscopy, Mn complex, calcium

Introduction

Oxygenic photosynthesis is carried out by Photosystem II (PSII) in the thylakoid membrane. Its catalytic site contains a tetra-nuclear Mn complex which may bind the substrate, two water molecules, and likely one Ca ion. The catalytic cycle of water oxidation involves five increasingly oxidized states of the Mn complex, S₀ to S₄. S₁ is stable in the dark. Each excitation of PSII by a quantum of light induces a transition S_i→S_{i+1}. The S₄-state spontaneously decays to S₀ under release of dioxygen, thereby closing the cycle (Haumann & Junge, 1999).

The atomic structure of the Mn complex is controversially discussed. Various previously proposed models now seem to be incompatible with the recently obtained crystallographic data of PSII (Zouni et al., 2001).

We studied the structure of the Mn complex predominantly in states S₁, S₂, and S₃ by X-ray absorption spectroscopy (XAS) using partially oriented multilayers of PSII membrane particles. On basis of simulations of EXAFS spectra obtained at cryogenic temperatures (20 K) and at room temperature (291 K) at various angles between the sample normal and the incident, linearly polarized X-ray beam (polarized XAS (Dau et al., 2001)), and using a joint-fit approach we obtain a new atomic-resolution model for the arrangement of the four Mn atoms and of one Ca ion in PSII.

Materials and Methods

Highly active PSII membrane particles and membrane multilayers were prepared as in (Iuzzolino et al., 1998). XAS was performed at the EMBL (Hamburg) and the ESRF (Grenoble) (Schiller et al., 1998; Meinke et al., 2000). S-states were populated for cryo-XAS by: S₁, dark-adaptation; S₂, continuous illumination at 200 K for 1 min; S₃, 3 flashes of light (flash-lamp, FWHM 10 μs, λ > 400 nm). Samples were frozen within 1 s in liquid nitrogen thereafter (Dau et al., 1998). For room temperature XAS, states S₁, S₂, and S₃ were populated by application of 0, 1, and 2 saturating flashes from a frequency-doubled, Q-switched Nd-

YAG Laser (FWHM 5 ns, 532 nm) at the beamline. Starting 1 s after the last flash, an XAS spectrum (6450-7050 eV) was taken within 10 s. Alternatively, the monochromator was positioned at 6551.5 eV, 10 Laser flashes (spaced by 300 ms) were applied, and the changes in X-ray fluorescence were recorded. For flash experiments, samples contained 200 μ M PPBQ. After averaging (25-40) XAS spectra were processed to yield k^3 -weighted EXAFS oscillations (Dau et al., 2001); E_0 was 6546 eV. EXAFS spectra were subjected to a joint-fit using the software SimX (Dittmer, 1999). Fourier transforms (FT) of EXAFS spectra were calculated for 20-540 eV, and with cosine windows of 0.1/0.2. The 20 K spectra were deconvoluted to yield the pure spectra from states S_1 , S_2 , and S_3 on the basis of the respective amplitudes of the S_2 -state multiline EPR signal (Iuzzolino et al., 1998). 291 K data were not deconvoluted due to the near quantitative population of the S-states by the laser flashes.

Results and Discussion

Fig. 1 shows Fourier transforms (FTs) of EXAFS oscillations from the Mn complex in the states S_1 , S_2 , and S_3 (thin lines) obtained at 20 K (upper part) at angles of 15°, 35°, and 55° between the sample normal and the electric field vector of the X-ray beam, and at 291 K at 45° (lower part).

The FTs show three major peaks: Peak I is attributable to direct O/N ligands of Mn (see Dau et al., 2001). Peak II is mainly due to vectors between heavy atoms (Mn/Ca). Peak III is mainly attributable to secondary, more distant coordination shells of light atoms (O/N) but also affected by the peak-II atoms (see below).

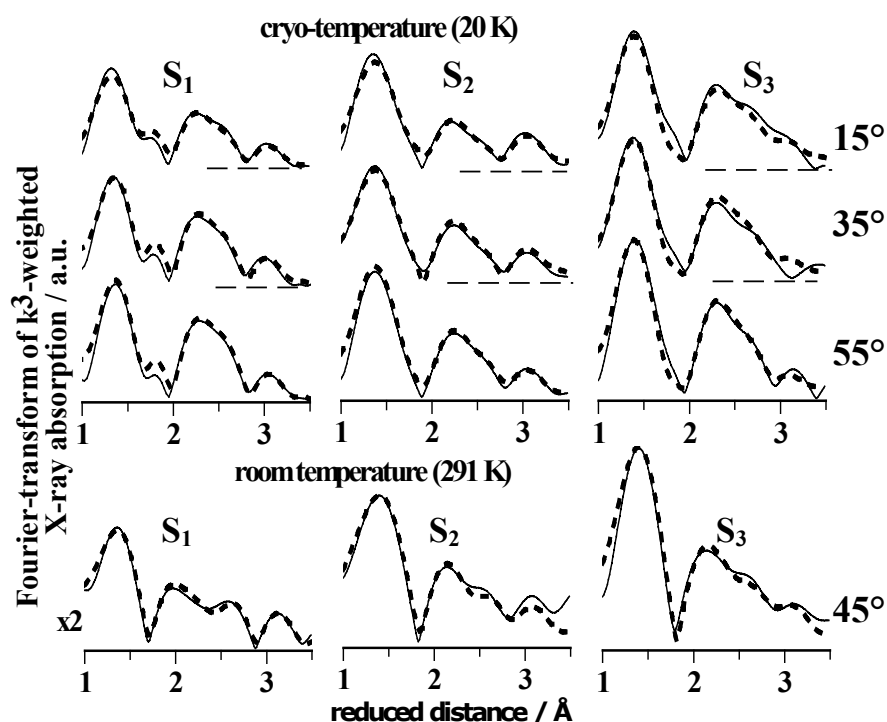


Fig. 1: Fourier transforms of k^3 -weighted EXAFS oscillations of the Mn complex of PSII predominantly in states S_1 , S_2 , and S_3 and at angles between sample normal and electric field vector of the X-ray beam as indicated.

Thin lines: experimental spectra, broken lines: simulation curves calculated with the parameters listed in Table 1. 20 K: pure spectra of the S-states obtained after deconvolution; 291 K: spectra obtained after 0, 1, and 2 Laser flashes of light.

Peak I strongly increases from S_1 to S_3 . Presumably, this behaviour reflects the increasing oxidation of the Mn complex which causes shortening of the distances between Mn and its direct ligands resulting in decreased distance spread. The extent of peak II increases from 15° to 55° which is expected for vectors between heavy atoms oriented at an angle of $>55^\circ$ with respect to the membrane normal. The magnitude of peak II is similar in S_1 and S_2 but strongly increased in S_3 .

We simulated the EXAFS oscillations obtained at 20 K using a joint-fit of spectra measured at the 3 excitation angles (Dittmer & Dau, 1998). The fit curves are shown in Fig. 1 as broken lines; the parameters are listed in Table 1. Six coordination shells of Mn, namely three shells of light atoms (O/N) and three shells of heavy atoms (Mn/Ca), were used for simulation of the S_1 -state spectrum. Only this six-shell approach has yielded satisfactory fit results (Pospisil et al., 2001).

Table 1 reveals the following features: The light atom shells (O/N) form an average angle of close to 55° with the membrane normal which is expected if these shells represent the direct ligands to Mn (1.8-2.0 Å) and O-ligands (~ 3.6 Å) of the respective opposite Mn atom in a Mn pair (see below). Two Mn-Mn vectors of 2.71 Å average length are detected in S_1 and S_2 . They are interpreted as showing the presence of exactly two di- μ -oxo bridged Mn pairs (Dau et al., 2001). Furthermore, one Mn-Mn vector of ~ 3.1 Å is present in S_1 and S_2 . This vector most likely reflects the distance between two Mn atoms from different pairs. Evidence for the latter interpretation comes from our observation that the 3.1 Å vector disappears if two Mn atoms are removed from the center by a biochemical treatment (Dörner et al., 1998) or by moderate heating of PSII (Pospisil et al., 2001). All Mn-Mn vectors form an angle of $>70^\circ$ with the membrane normal. Thus, both Mn pairs lie about in the same plane, roughly parallel to the membrane plane. A third vector between heavy atoms of ~ 3.3 Å length is present in all S-states. We tentatively attribute it to the interaction of a single Ca ion with two Mn atoms. Good evidence for the presence of Ca at such a distance to Mn has been obtained by the group of Klein, Sauer, and Yachandra (see their contributions to this proceedings). The Mn-Ca vector forms an angle of 45° with the membrane normal.

A major structural change of the Mn complex occurs upon the $S_2 \rightarrow S_3$ transition. The 3.1 Å vector is no longer detected. Instead, a third Mn-Mn vector of ~ 2.7 Å appears. We straightforwardly attribute it to the formation of a third di- μ -oxo bridge between two Mn atoms of different pairs. The average Mn-Mn distance is reproducibly larger in S_3 (2.75 Å) than in S_1 and S_2 (2.71 Å), corroborating the above notion. The average angle of the three ~ 2.7 Å vectors with the membrane normal is reduced in S_3 , indicating a significant repositioning of the Mn atoms upon $S_2 \rightarrow S_3$.

State	Shell, i	Vector	Coordination number, N_i , [per Mn]	Distance, R_i , [Å]	Debye-Waller Parameter, $2\sigma_i^2$, [Å ²]	Angle with membrane normal
S_1	I	Mn-O/N	4.5 (4.5)	1.85 (1.87)	0.016 (0.033)	62°
	II	Mn-O/N	1.0 (1.0)	2.06 (2.20)	0.004 (0.007)	45°
	III	Mn-Mn	1.0 (1.0)	2.72 (2.73)	0.004 (0.022)	71°
	IV	Mn-Mn	0.5 (0.5)	3.11 (3.11)	0.003 (0.010)	81°
	V	Mn-Ca	0.5 (0.5)	3.31 (3.39)	0.003 (0.010)	45°
	VI	Mn-O/N	3.0 (3.0)	3.65 (3.69)	0.010 (0.025)	64°
S_2	I	Mn-O/N	5.0 (5.0)	1.85 (1.88)	0.017 (0.037)	63°
	II	Mn-O/N	0.5 (0.5)	2.05 (2.27)	0.005 (0.005)	45°
	III	Mn-Mn	1.0 (1.0)	2.71 (2.71)	0.004 (0.018)	71°
	IV	Mn-Mn	0.5 (0.5)	3.17 (3.13)	0.003 (0.010)	80°
	V	Mn-Ca	0.5 (0.5)	3.35 (3.38)	0.003 (0.010)	45°
	VI	Mn-O/N	3.0 (3.0)	3.64 (3.65)	0.010 (0.011)	56°
S_3	I	Mn-O/N	5.0 (5.0)	1.85 (1.87)	0.014 (0.025)	62°
	II	Mn-O/N	0.5 (0.5)	2.01 (2.23)	0.005 (0.005)	45°
	III/IV	Mn-Mn	1.5 (1.5)	2.74 (2.74)	0.004 (0.016)	65°
	V	Mn-Ca	0.5 (0.5)	3.23 (3.23)	0.003 (0.010)	45°
	VI	Mn-O/N	3.0 (3.0)	3.61 (3.67)	0.008 (0.008)	48°

Table 1: Results of a joint-fit of EXAFS oscillations of the Mn complex predominantly in states S_1 , S_2 , and S_3 . Bold, results for 20 K data; in parenthesis, 291 K data. Restraints used in the fit procedure: The coordination numbers N_i were fixed at chemically reasonable values; $2\sigma_{IV,V}^2$ were fixed (S_1 and S_2); $2\sigma_V^2$ was fixed (S_3).

Room temperature EXAFS spectra were obtained after Laser excitation of PSII at the beamline. The Mn K-edge energy differs for all S-states (Dau et al., 2001). Therefore, we used the X-ray fluorescence changes (Fig. 2, dots) at 6551.5 eV (edge energy of S_1) to quantify the population of S-states after each flash.

The quaternary oscillation is well reproduced (line) using an initial population of S_1 of 100 % and a miss parameter of 15 %. From these numbers, we estimate the yields of S_1 , S_2 , and S_3 after flashes 0, 1, and 2 to be 100 %, 85 %, and 72 %, respectively. The near quantitative population of S-states made the deconvolution of EXAFS spectra obsolete (to a first approximation).

Good simulations (dashes) of the EXAFS spectra obtained at 291 K (Fig. 1) were obtained using the same approach as for 20 K spectra. The results are listed in Table 1 (in parenthesis). Apparently, no major structural changes occur at the Mn complex during freezing, but significantly larger Debye-Waller parameters are observed revealing a broadened distribution of the lengths between Mn and its coordination shells. Most important, the increase of the extent of peak II in the FT (Fig. 1) in S_3 at 291 K was again well simulated by a third Mn-Mn distance of ~ 2.7 Å, which we attribute to the formation of a third di- μ -oxo bridge in S_3 .

Conclusions

We summarize the distances between Mn and six coordination shells and the angles of the respective vectors in the following tentative model of the Mn complex (Fig. 3): The four Mn atoms are organized in two di- μ -oxo bridged pairs, both pairs lie about in the same plane, roughly perpendicular to the membrane normal. One Ca ion is placed at a distance of ~ 3.3 Å from two Mn atoms. This model holds for S_1 and S_2 . In S_3 , a third di- μ -oxo bridge between two Mn atoms of different pairs is formed, leading to a shortening of one Mn-Mn distance from ~ 3.1 Å to ~ 2.7 Å.

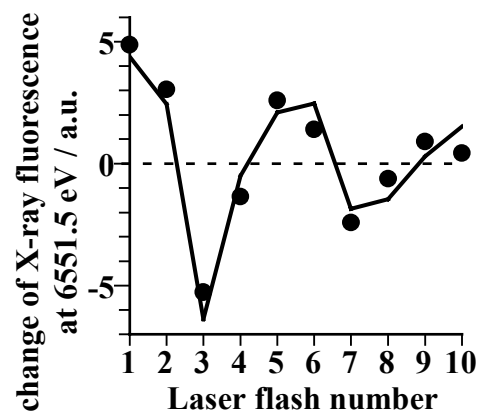


Fig. 2: Changes per flash of X-ray fluorescence at 6551.5 eV from the Mn complex induced by 10 Laser flashes at room temperature (291 K). Dots, experimental data; line, simulation.

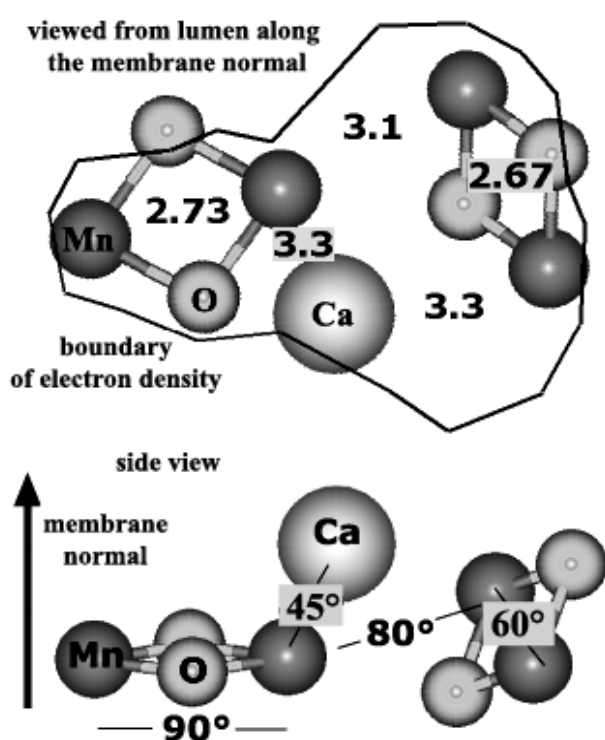


Fig. 3: A tentative atomic-resolution model of the structure of the Mn complex in the S_1 -state.

Top, viewed from lumen along the membrane normal; bottom, turned by 90° around the long axis. Only four Mn atoms, their bridging μ -Oxo atoms, and one Ca are depicted for clarity. The boundary of the electron density (solid line) was taken from (Zouni et al., 2001). The Mn atoms separated by 3.1 Å are likely bridged by a further mono- μ -oxo bridge (not shown). The structure is similar in S_2 . In S_3 a third di- μ -oxo bridge is formed between the Mn atoms separated by 3.1 Å in S_1 and S_2 . The distances between the Mn atoms of the two pairs refer to the structural results obtained by XAS at 20 K (Mukerji et al., 1994; Dittmer, 1999; Pospisil et al. 2001). The shorter Mn dimer is more loosely bound. It is first lost, e.g., by moderate heating of PSII (Pospisil et al., 2001). All distances are given in Å.

Acknowledgements

We gratefully acknowledge financial support by the German BMBF in the program “Erforschung kondensierter Materie” (grants 05SN8RMA/2 and 05KS1KEA/6). P.P. thanks the Alexander von Humboldt Stiftung for support.

References

- Dau, H., Iuzzolino, L. and Dittmer, J. *Biochim.Biophys.Acta* **1503**, 24-39, 2001.
- Dau, H., Iuzzolino, L., Dittmer, J., Dörner, W., Meyer-Klaucke, W. *In Photosynthesis: Mechanism and Effects* (Garab, G., Ed), **Vol. II**, pp. 1327-1330, Kluwer Academic Publishers, Dordrecht, 1998.
- Dittmer, J. *Ph.D. Thesis*, Christian Albrechts University Kiel, Germany, 1999.
- Dittmer, J., Dau, H. *J.Phys.Chem. B* **102(42)**, 8196-8200, 1998.
- Dörner, W., Dittmer, J., Iuzzolino, L., Dau, H. *In Photosynthesis: Mechanism and Effects* (Garab, G., Ed), **Vol. II**, pp. 1343-1346, Kluwer Academic Publishers, Dordrecht, 1998.
- Haumann, M.; Junge, W. *Biochim.Biophys.Acta* **1411**, 86-91, 1999.
- Iuzzolino, L., Dittmer, J., Dörner, W., Meyer-Klaucke, W., Dau, H. *Biochemistry* **37**, 17112-17119, 1998.
- Meinke, C., Sole, A.V., Pospisil, P., Dau, H. *Biochemistry* **39**, 7033-7040, 2000.
- Mukerji, I.; Andrews, J. C.; DeRose, V. J.; Latimer, M. J.; Yachandra, V. K.; Sauer, K.; Klein, M. P. *Biochemistry* **33**, 9712-9721, 1994.
- Pospisil, P., Haumann, M., Dittmer, J., Sole, A. V., Dau, H. *Biochemistry*, submitted, 2001.
- Schiller, H., Dittmer, J., Iuzzolino, L., Dörner, W., Meyer-Klaucke, W., Sole, V.A., Nolting, H.-F., Dau, H. *Biochemistry* **37**, 7340-7350, 1998.
- Zouni, A., Witt, H.-T., Kern, J., Fromme, P., Krauß, N., Saenger, W., Orth, P. *Nature* **409**, 739-743, 2001.