

Stepwise transition of the tetra-manganese complex of Photosystem II to a binuclear $\text{Mn}_2(\mu\text{-O})_2$ complex in response to a temperature jump: an X-ray absorption spectroscopy study

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1. Introduction

The manganese complex of photosystem II (PSII) constitutes the catalytic center of photosynthetic water oxidation. It contains four Mn atoms and, likely, one Ca. The Mn atoms are often assumed to be arranged in form of two di- μ -oxo bridged pairs connected by a mono- μ -oxo bridge. However, other arrangements are not excluded (Robblee et al., 2001, Dau et al., 2001).

We used the controlled heating at 47°C of multilayers of PSII membrane particles with the Mn-complex in the S_1 -redox state to achieve its stepwise disintegration which was assayed by oxygen polarography, EPR- and X-ray absorption (XANES, EXAFS) spectroscopy. Two Mn-Mn distances of ~ 2.7 Å were detected in the control. A ~ 3.3 Å distance between heavy atoms (Mn/Ca) was lost after only 2 min of heating, together with the oxygen evolution capability. Within 10 min of heating the loss of Mn-Mn distances of ~ 2.7 and ~ 3.1 Å occurred and two hexaquo Mn^{2+} ions were detected by EPR and EXAFS. The remaining two Mn atoms were still separated by ~ 2.7 Å.

We tentatively interpret the effects of heating as follows (Pospisil et al., 2001): The Mn complex in the S_1 -state consists of exactly two di- μ -oxo bridged Mn dimers; one Mn dimer is loosely bound and more easily released. The remaining binuclear center is a di- μ -oxo bridged Mn(III)_2 -dimer.

2. Materials and Methods

PSII membrane particles and membrane multilayers were prepared as in (Iuzzolino et al., 1998, Schiller & Dau, 2000). For the heating experiments, frozen PSII membranes were thawed for 30 min on ice and washed in a betaine-free buffer containing 10 mM NaCl, 5 mM CaCl_2 and 25 mM MES-NaOH (pH 6). Heating procedure: frozen PSII multilayer samples were thawed for 1 min at room temperature. Samples were enclosed in Eppendorf cups and exposed to 47 °C for distinct periods ranging from 0 to 180 min by immersion in a digitally-controlled thermostated water bath. Immediately after the heat treatment, the PSII multilayers were rapidly frozen in liquid nitrogen. Oxygen evolution was assayed with a Clark-type electrode under saturating continuous white-light illumination. EPR measurements were performed using an X-band EPR spectrometer (Bruker ESP 300E). XAS was performed at the ESRF (Grenoble) (Meinke et al., 2000). The excitation angle between the electric field vector

of the X-ray beam and the normal to the surface of the PSII multilayers was 55° (magic angle). A full X-ray absorption spectrum was recorded within only 8 s (scan range: 6500-7000 eV). Energy calibration was performed using a KMnO_4 standard. After averaging of ~ 45 scans, XAS spectra were processed to yield k^3 -weighted EXAFS oscillations; 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 above E_0 .

3. Results and Discussion

Fig. 1A shows the rate of oxygen evolution of PSII particles as function of the heating time. Using standard salt concentrations (5 mM CaCl_2 , solid circles), already after 2 min of heating almost total inactivation is observed. The decay of the activity follows a single exponential with a rate constant of 1 min^{-1} (Fig. 1A, straight line). It has been shown that a temperature increase induces the release of the extrinsic polypeptides (MW of 17, 23, and 33 kDa) from PSII (Enami et al., 1994) with a rate of about 1 min^{-1} which matches the deactivation rate of our heated samples. The affinity of PSII for calcium binding is reduced in PSII depleted of the extrinsic polypeptides (Seidler, 1996). We again measured the oxygen-evolution activity with a much higher CaCl_2 concentration (55 mM). Under these conditions, the loss of oxygen evolution is greatly retarded (open circles). With 50 mM of MgCl_2 plus 5 mM of Ca^{2+} no retardation is observed (triangles). This observation could indicate that the heat-induced loss of the oxygen-evolution activity is related to the rapid release of calcium.

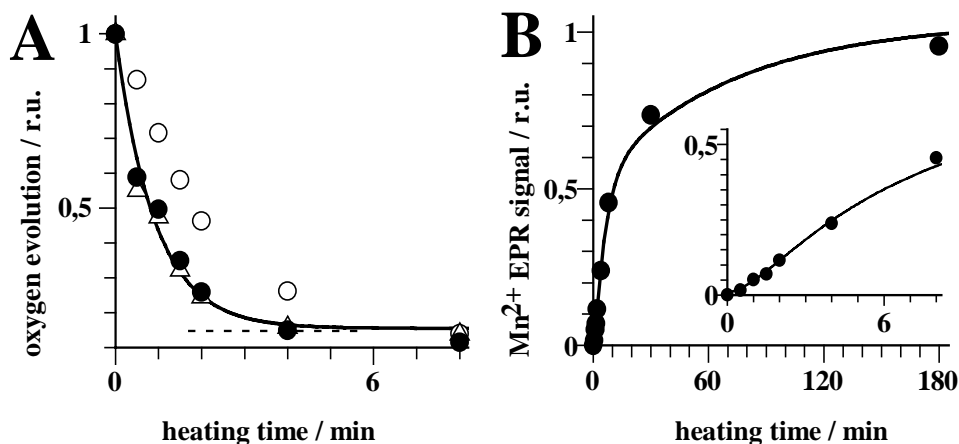


Fig. 1: (A) Oxygen evolution activity of PSII as function of the heating time at 47°C . (B) The amplitude of the six-line hexaquo Mn^{2+} EPR signal as function of the heating time. Inset in B): data points on an expanded time scale.

Mn released from its binding site is typically present in form of Mn^{2+} coordinated by six water molecules. This $[\text{Mn}(\text{H}_2\text{O})_6]^{2+}$ complex gives rise to a characteristic six-line EPR signal (not shown) which was employed to monitor the release of Mn from its binding site (Fig. 1B). The amplitude of the Mn^{2+} EPR signal as function of the heating time increases clearly biphasically. Furthermore, there is an initial lag phase (inset in Fig. 1B). The amplitudes of the fast and slow kinetic components are of equal magnitude; two of the four Mn of PSII are released more than ten times faster than the second Mn pair. A satisfying description of the data points in Fig. 1B was obtained assuming that Mn^{2+} is released in a consecutive reaction meaning that a reaction characterized by a rate constant of about 1 min^{-1} precedes the faster Mn release. The curve in Fig. 1B was calculated using rate constants of 1 min^{-1} (preceding process), 0.18 min^{-1} (fast Mn release) and 0.014 min^{-1} (slow Mn release).

EXAFS spectra of PSII multilayer samples which were heated for 0, 0.5, 1, 1.25, 2, 4, 8, 30, and 180 min were recorded. Fig. 2 shows the FTs of k^3 -weighted EXAFS oscillations recorded after 0 min (control), 4 min, and 180 min of heating. In the control, three FT peaks are visible. With increasing heating time, the peak magnitudes become gradually diminished, and one large new peak, corresponding to the aqueous Mn^{2+} ($Mn-OH_2$ distance of ~ 2.2 Å), becomes prominent.

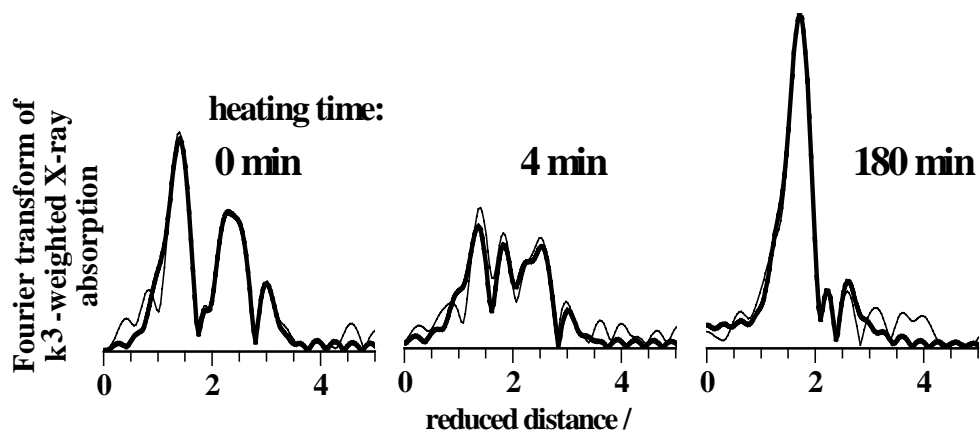


Fig. 2: FTs of k^3 -weighted EXAFS oscillations of the Mn complex of PSII multilayers heated for 0 min, 4 min, and 180 min at 47 °C. Thin lines, experimental data; thick lines, simulations.

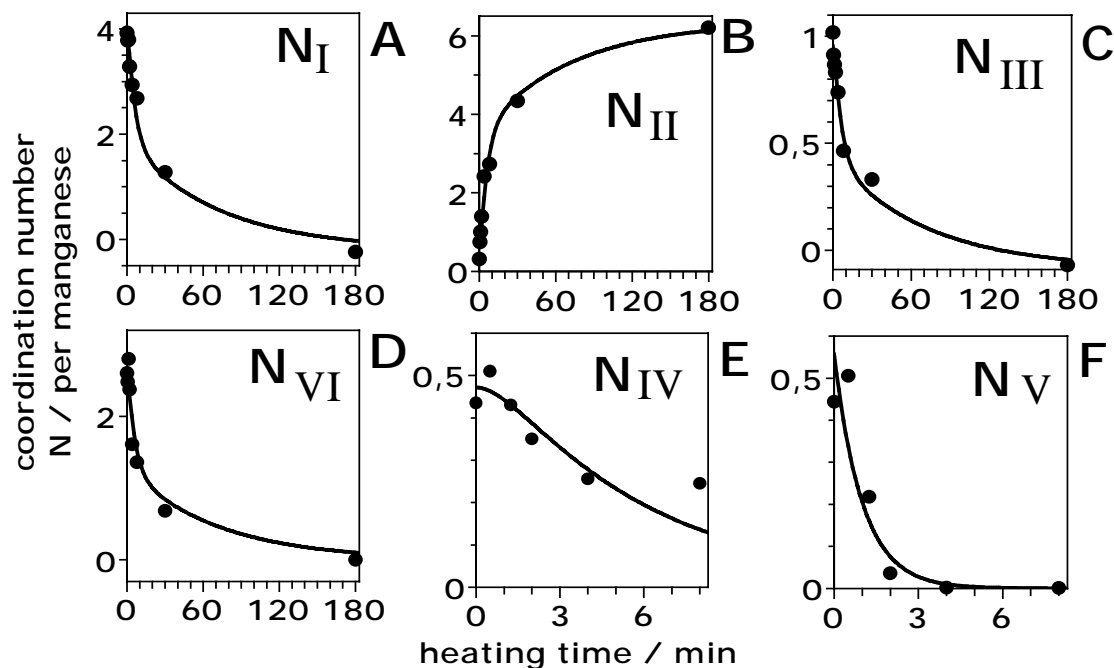


Fig. 3: Coordination numbers of six shells of backscatterers as function of the heating time resulting from a joint fit of 9 EXAFS spectra (Fig. 2). Dots, experimental data; lines, simulations using a consecutive reaction (Scheme I).

In the following we consider three shells of light atoms (O/N/C) and three shells of heavy atoms (Mn/Ca) (see also Haumann et al., these proceedings). The EXAFS spectra were simulated using a joint-fit approach (Dau et al., 1999) under the assumption that alterations as caused by the heating procedure can be modeled by changes in the coordination numbers of these shells. Nine EXAFS spectra, representing 9 different heating times, were fitted simultaneously by parameter variations and minimization of a common error-sum (R_F). Fig. 2 compares the experimental (thin lines) and simulated (thick lines) spectra. The simulation parameters are listed in Table 1; the coordination numbers as a function of the heating time are shown in Fig. 3. The R_F -value was reasonably low (around 11 %) confirming the high accuracy of the fits. Other simulation approaches yielded higher R_F -values and erratic time courses of the coordination numbers.

Fig. 3A reveals that the number of O/N atoms at ~ 1.85 Å (N_I), representing the direct ligands to Mn in the intact complex, decreases in a biphasic manner (with increasing heating time); a complementary biphasic increase of a coordination shell of O-atoms at a distance of ~ 2.16 Å (N_{II}) to Mn is observed (Fig. 3B). We attribute the 2.16 Å FT peak to released Mn^{2+} . Mn^{2+} is produced with a fast phase accounting for the release of two Mn atoms from their binding site and in a slower reaction accounting for the remaining two Mn atoms. The O/N shell at ~ 3.6 Å (Fig. 3D, N_{IV}) and the Mn-Mn distance at ~ 2.7 Å (Fig. 3C, N_{III}) show a similar biphasic behaviour. The latter is commonly attributed to two di- μ -oxo bridged Mn pairs (Dau et al., 2001). Seemingly, one pair is “destroyed” rapidly by the heating procedure, resulting in a decrease of the 2.7 Å Mn-Mn coordination number from $N_{III} \sim 1$ to $N_{III} \sim 0.5$. The second di- μ -oxo bridged Mn pair is more stable.

The coordination number of the O/N shell at ~ 3.6 Å was about halved after the release of the first Mn pair (Fig. 3D) which strongly supports our assignment of this shell to light atoms (Meinke et al., 2000). The coordination number of the shell at 3.1 Å (N_{IV}) attributed to a Mn-Mn interaction decreases to about zero (Fig. 3E) concomitantly with the release of the first two Mn atoms (in ~ 10 min) which is readily explained if we assume that the Mn-Mn

Shell	Distance vector	Coordination number / per Mn, N_i	Distance / Å, R_i	Debye-Waller-parameter / Å ² , $2\sigma_i^2$
I	Mn – O/N	3.92 ± 0.82	1.853 ± 0.003	0.0186 ± 0.0050
II	Mn – O	0.31 ± 0.35 $6.21^a \pm 0.40$	2.164 ± 0.002	0.0124 ± 0.0020
III	Mn – Mn	1.02 ± 0.06	2.709 ± 0.002	0.0046 ± 0.0020
IV	Mn – Mn	0.43 ± 0.18	3.083 ± 0.020	0.0027 ± 0.0025
V	Mn – Ca/Mn	0.45 ± 0.14	3.295 ± 0.020	0.0027 ± 0.0025
VI	Mn – O/N/C	2.86 ± 0.55	3.637 ± 0.010	0.0080 ± 0.0025

Table 1: The results of a simulation of 9 EXAFS spectra for various heating-times. The values of R_i and σ_i were coupled to yield equal numbers in all spectra (joint-fit); σ_{IV} and σ_V were fixed. The shown coordination numbers refer to the intact Mn complex (no heating) and, for Shell II, also to the state resulting from 180 min of heating (a).

vector at 3.1 Å connects one Mn atom of the loosely bound pair with one Mn of the more stable pair and this connection is lost.

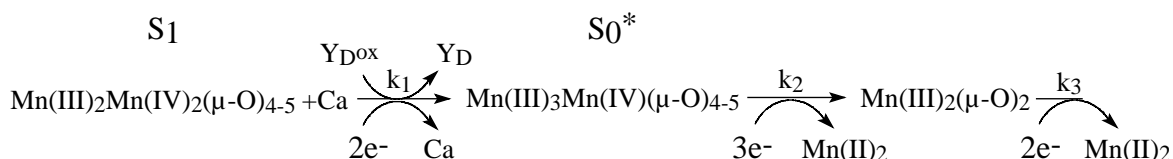
The coordination number of the 3.3 Å shell, which we attributed to a heavy atom, decreases to zero after only 2 min of heating (Fig. 3F, N_V). The coordination number of this shell is ~ 0.5 in the intact PSII (Table 1). If the 3.3 Å vector represents Ca, a single Ca ion should be located such that two different Mn atoms are both at a distance of 3.3 Å. The rate of the decay of the coordination number of the Mn-Mn/Ca interaction at 3.3 Å is the same as observed for

the loss of oxygen evolution (see Fig. 1). One explanation is that the unbinding of the extrinsic polypeptides is coupled to the loss of Ca from its binding site such that it becomes invisible in the EXAFS spectra.

4. Conclusions

We have studied the structure of the Mn complex of PSII during its stepwise disassembly induced by a sudden temperature jump to 47 °C. After only about 2 min of heat exposure, the loss of a 3.3 Å vector between heavy atoms is detected by EXAFS spectroscopy. We tentatively attribute this vector to a single Ca ion placed at the same mean distance to two Mn atoms. The release of Ca occurs concomitantly to the complete inactivation of oxygen evolution.

The joint-fit of 8 EXAFS spectra reveals the presence of (exactly) two Mn-Mn vectors of 2.71 Å in the intact Mn complex. They are attributed to two di-μ-oxo bridged Mn dimers in the S₁-state. A 3.1 Å vector is interpreted as the vector which connects two Mn atoms of different dimers. Mn is reduced and released biphasically. Two Mn atoms of the complex are released within about 10 min of heat exposure. They belong to the same Mn dimer. The remaining binuclear Mn complex still consists of a di-μ-oxo bridged unit. It takes as long as 3 hours for reduction and complete release of the second Mn pair. We summarize the effects of heating on the Mn complex in Scheme I.



Scheme I: A kinetic scheme for the stepwise reduction and disassembly of the Mn complex in response to a temperature jump. The rate constants are (at 47 °C): k_1 , 1 min⁻¹; k_2 , 0.18 min⁻¹; and k_3 , 0.014 min⁻¹. Concomitantly with the inactivation of oxygen evolution and with the putative release of a Ca ion we observed the reduction of Y_D^{ox} by EPR. The numbers of redox equivalents (e⁻) in the individual reaction steps were calculated from the observed down shifts of the Mn K-edge position on the energy scale (data here not shown, see Pospisil et al., 2001).

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