S10-019

Manganese oxidation states in photosystem II

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Keywords: water oxidation, S states, oxygen evolution pattern, XANES, Kβ XES

Introduction

Photosynthetic water oxidation is catalyzed by the oxygen evolving complex (OEC), which is a functional part of photosystem II. The OEC interfaces the light-induced one-electron oxidations of the PS II reaction center with the four-electron chemistry of water oxidation. The OEC has therefore two functions: a) storage of oxidizing equivalents and b) positioning and activation of the substrate water molecules. The oxidation states of the OEC are termed the S_0, S_1, S_2, S_3 and S_4 states according to the number of stored oxidizing equivalents relative to the lowest state of the redox cycle. A main constituent of the OEC is a tetranuclear Mn cluster. It is well accepted that Mn oxidations take place during the $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ transitions. In contrast, the nature of the $S_2 \rightarrow S_3$ transition is highly disputed. We present new data obtained by EPR, XANES and Kβ XES measurements that strongly support the idea of a ligand-centered oxidation during the $S_2 \rightarrow S_3$ transition. Another controversial point is the absolute calibration of the Mn redox states. $Mn_4(III_2,IV_2)$ and $Mn_4(III_4)$ are discussed as oxidation states for the dark-stable S_1 state. In the case of $Mn_4(III_4)$, the S_{-3} state would be the lowest possible oxidation state of the OEC, assuming that only Mn(IV) and Mn(III) can be reduced in a reversible fashion by exogenous reductants like N_2H_4. First results are shown which indicate the existence of the S_{-4} and S_{-5} states and thus support $Mn_4(III_2, IV_2)$ as Mn oxidation states in the S_1 state.

Materials and methods

PS II membranes were prepared using the BBY protocol. Flash samples (0F,…, 6F) were obtained by double sided laser flash illumination of PS II membranes (9.5 mg Chl/ml) containing 1 mM PPBQ. Great care was taken to minimize and monitor radiation damage during X-ray experiments. Further details are described in (Messinger et al., 2001). Oxygen flash yield measurements were obtained with a frequency doubled Nd/YAG-laser (9 ns pulse width) and a home-built Joliot-type electrode. The flash frequency was 1 Hz. Polarographic signals were stored on a computer and flash artifacts and O_2 uptake signals were subtracted from the original traces within Excel.

Results and Discussion

EPR

The S_2 EPR multiline signals (S_2 MLS) from all flash samples (109) were measured to obtain information on their S state distribution. In Figure 1A the normalized S_2 MLS amplitudes, determined from the four largest low field peaks, are plotted as a function of flash number (diamonds). Each point is the average of about 20 (0F to 3F) or 8 (4F to 6F) essentially identical individual samples. The S state composition of the samples was extracted from this S_2 MLS oscillation pattern by systematic fits with an extended Kok model. The best fit was obtained with 10.8 % miss probability, 0 % double hit probability and 5.7 % initial S_2 state population. The latter is due to the applied preflash protocol. This fit is shown in Figure 1A as solid line. It also contains about 5 % of centers which are blocked after an initial $S_1 \rightarrow S_2$ transition.

XANES

In Figure 1B, the Mn K-edge position is plotted as a function of flash number. The edge energy is determined from the first zero crossing of the second derivatives (i.e. the inflection point energy, IPE) as described by (Roelofs et al., 1996). A clear upshift in energy is observed between the 0F and 1F samples, indicating a Mn oxidation on the $S_1 \rightarrow S_2$ transition. In contrast, a much smaller shift in edge position is seen between the 1F and 2F samples. This suggests a ligand-centered oxidation during the $S_2 \rightarrow S_3$ transition. The same pattern is seen (Table 1) when the flash spectra are deconvoluted into pure S-state spectra using the EPR data discussed above. In line with a Mn oxidation on the $S_0 \rightarrow S_1$ transition, a clear edge shift is seen for this turnover.

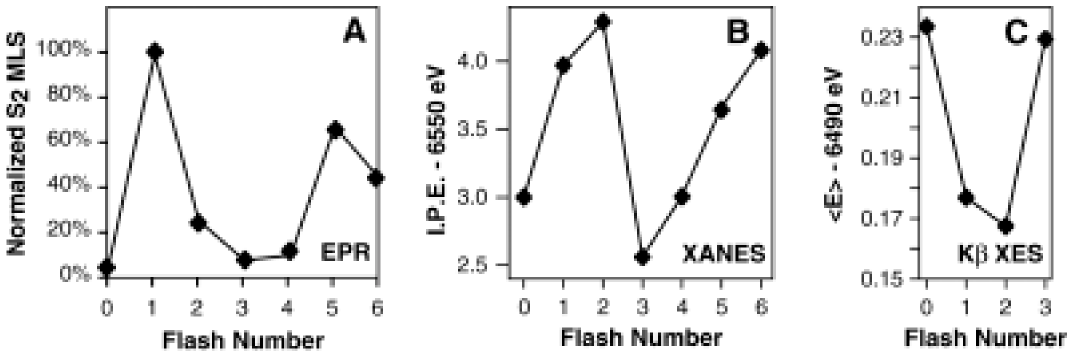

Fig. 1 (A) S_2 EPR multiline signal amplitude, (B) XANES Mn K-edge inflection point energy and (C) first moment of the $K\beta_{1,3}$ peak in high resolution X-ray emission spectra from PS II membranes as a function of flash number. All measurements were performed on the same sets of samples.

S state	IPE values (eV)
S_0	6550.8 ± 0.1
S_1	6552.9 ± 0.1
S_2	6554.0 ± 0.1
S_3	6554.3 ± 0.1

Table 1: Mn K-edge energies of PS II membrane fragments in various S-states of the OEC.

Kβ XES

It has been demonstrated that the first moments of the $K\beta_{1,3}$ peaks in Mn Kβ fluorescence emission spectra are less sensitive to structural changes than XANES edge energies (Visser et al., 2001) and that they shift to lower energies in response to Mn oxidation (Bergmann et al., 1998). The results of Figure 1C show a large downshift between the 0F and 1F samples and only a minor change between the 1F and 2F samples. This reinforces the conclusion that a ligand centered oxidation takes place during the $S_2 \rightarrow S_3$ transition (Messinger et al., 2001).

S_{-4} and S_{-5} states

In spinach thylakoids, it was shown that N_2H_4 reacts predominantly as a two-electron reductant with the OEC, i.e. $S_1 \rightarrow S_{-1} \rightarrow S_{-3}$ ($\rightarrow S_{-5}$) or $S_0 \rightarrow S_{-2}$ ($\rightarrow S_{-4}$) (Messinger et al., 1997). In this study we use *Synechococcus elongatus* thylakoids because they retain higher O_2 evolution activity during incubation with high (30-100 mM) hydrazine concentrations (data

Sample	**Fit**	**S_{-1}**	**S_{-2}**	**S_{-3}**	**S_{-4}**	**S_{-5}**	**fq x 10^5**
B	1	18.8	11.0	70.2	-	-	7.8
	2	10.5	11.1	61.9	4.6	11.9	1.0
C	3	16.7	39. 9	43.4	-	-	18.9
	4	5.7	41.3	27.5	19.7	5.8	2.7
D	5	12.7	43.0	44.3	-	-	6.5
	6	7.6	44.9	34.6	12.9	0.0	1.8

Table 2*:* Fit of O_2 oscillation patterns of *Synechococcus elongatus* thylakoids incubated with N_2H_4 (Fig.2). Sample B: 10 min incubation of dark-adapted thylakoids with 30 mM N_2H_4 on ice (+ 5 min on electrode). Sample C was enriched in S_0 by 3 flashes and then incubated 10 min with 50 mM N_2H_4 on ice. Sample D: same as B, but washed once in N_2H_4 free buffer. In Fits 1, 3 and 5 the S_{-4} and S_{-5} states were excluded. The fit quality (fq) was calculated as sum(dy_i^2)/(data points - free parameters). The O_2 yields of the first 16 flashes were included in the fits. Fixed parameters: misses 10.0 %, double hits 0.0 %, other S-states 0.0 %

not shown). Flash patterns of dark-adapted thylakoids (mostly S_1 state) were best fit with a miss parameter of 9.5 % and a double hit parameter of 0.0 % (Fig. 2A). These parameters are almost unaffected by hydrazine incubation (data not shown). The fit results in Table 2 show that the S_{-4} and S_{-5} states are required for acceptable fits of the O_2 flash patterns obtained after hydrazine incubation. It should be noted that so far we have observed the S_{-5} state only in samples that were not washed to remove N_2H_4 after incubation (Fig. 2B and fit 2 and 4, Table 2). In contrast, the S_{-4} state can still be observed (to a lesser extent) after one washing step, which decreases the N_2H_4 concentration from 50 mM to approx. 0.5 mM (Fig. 2C and fits 4 and 6, Table 2). After extensive washing (3x) S_{-4} is also lost, but S_{-3} and higher states (if present) are not affected (not shown). This could be a consequence of the expected instability of the S_{-4} and S_{-5} states. Other explanations for the high O_2 yields in the 8th and 9th flashes involving for example back reactions of the S_2 and S_3 states with N_2H_4 or Y_D within the flash train cannot be fully ruled out at the moment. However, if back reactions with N_2H_4 were involved, then the difference in S_{-4} state population would be expected to be larger between patterns C and D. In contrast, back reactions with Y_D should not be affected at all by washing steps; thus S_{-4} would not be expected to disappear after 3 washing steps.

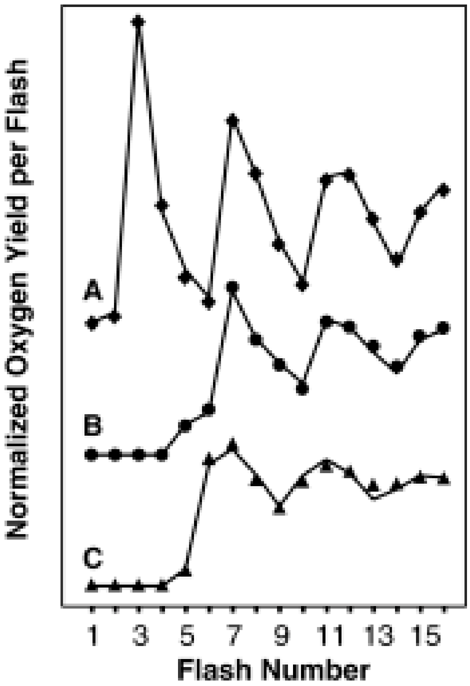

Fig. 2 Normalized oxygen flash yield patterns (symbols) and fits (lines) from control (A) and hydrazine reduced (B, fit 2; C, fit 4) from *Synechococcus elongatus* thylakoids. For details see Table 2 and text.

Summary

The results of this study are consistent with the following oxidation states of the OEC: S_0, $Mn_4(II,III,IV_2)$ or $Mn_4(III_3,IV)$; S_1, $Mn_4(III_2,IV_2)$; S_2, $Mn_4(III, IV_3)$; S_3, $Mn_4(III,IV_3)^{\bullet}$. The dot represents a ligand centered oxidation. Based on the observed lengthening of all Mn-Mn distances during the $S_2 \rightarrow S_3$ transition (Liang et al., 2000), a μ-oxo-bridge radical most likely exists in the S_3 state.

Acknowledgments

This research was supported by the National Institutes of Health Grant (GM-55302), and the Director, Office of Science, Office of Basic Energy Sciences, Division of Energy Biosciences, U. S. Department of Energy under contract DE-AC03-76SF00098. Synchrotron radiation facilities were provided by SSRL. GR gratefully acknowledges the financial support by the DFG (Sfb 498, TP C4). JM thanks the DFG for support through grants Me 1629/1-1, 2-1 and 2-2. JM and SI thank P. Fromme, A. Zouni, J. Kern and H.T. Witt for the kind gift of *Synechococcus elongatus* thylakoids.

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