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Manganese K-edge X-ray absorption spectra of the cyclic S-states in the photosynthetic oxygen-evolving system

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Abstract

A set of Mn K-edge XANES spectra due to the redox states S_0–S_3 of the OEC were determined by constructing a highly-sensitive X-ray detection system for use with physiologically native PS II membranes capable of cycling under a series of saturating laser-flashes. The spectra showed almost parallel upshifts with relatively high K-edge half-height energies given by 6550.9 ± 0.2 eV, 6551.7 ± 0.2 eV, 6552.5 ± 0.2 eV and 6553.6 ± 0.2 eV for the S_0, S_1, S_2 and S_3 states, respectively. The successive difference spectra between S_0 and S_1, S_1 and S_2, and S_2 and S_3 states were found to exhibit a similar peak around 6552–6553 eV, indicating that one Mn(III) ion or its direct ligand is univalently oxidized upon each individual S-state transition from S_0 to S_3. The present data, together with other observations of EPR and pre-edge XANES spectroscopy, suggest that the oxidation state of the Mn cluster undergoes a periodic change; S_0: Mn(III,III,III,IV) $\rightarrow S_1$: Mn(III,IV,III,IV) $\rightarrow S_2$: Mn(III,IV,IV,IV) $\rightarrow$ S_3: Mn(IV,IV,IV,IV) or Mn(III,IV,IV,IV)$\cdot L^+$ with L being a direct ligand of a Mn(III) ion.

Abbreviations: Chl – chlorophyll; D – tyrosine 160 on the D2 protein, an accessory electron donor in PS II; D^+ – the oxidized form of D; EDTA – ethylene-diaminetetraacetic acid; EPR – electron paramagnetic resonance; EXAFS – extended X-ray absorption fine structure; HL – py-2,6-bis[bis(2-pyridylmethyl)aminomethyl]-4-methylphenol; Mes – 2-(N-morpholino)ethanesulfonic acid; N4 – py - tris(2-pyridylmethyl)amine; OEC – oxygen evolving complex; P680 – primary electron donor of PS II; PS II – Photosystem II; Q_{400} – a high spin Fe^{3+} of the iron-quinone acceptor complex in PS II; SSD – solid state detector; XAFS – X-ray absorption fine structure; XANES – X-ray absorption near edge structure.

Introduction

A half century ago, the source of oxygen in the atmosphere was found to be H_2O, not CO_2, which was trapped by chloroplasts (Hill and Scarisbrick 1940, Ruben et al. 1941). About a quarter century later, the oscillations of period 4 in oxygen emission (Joliot et al. 1969), which were observed following illumination of the dark adapted samples by a series of saturating flashes, were interpreted by Kok et al. (1970) assuming a cyclic pathway for the OEC which includes five redox states, designated S_i (i = 0 – 4). Among the Kok's S-states, the S_1 state was later found to be the only dark stable state, and the S_4 state was defined as a transition state before emitting O_2 during the S_3–S_0 transition. This discovery opened a rich biochemical and biophysical research field with a goal to elucidate the molecular constitution of PS II and the molecular mechanism of the water-splitting reaction. Our understanding of this process has advanced significantly in the last decade, but is still insufficient at the molecular and submolecular levels. (For recent reviews, see Hansson and Wydrzynski 1990, Renger and Wydrzynski 1991, Rutherford et al. 1992, Debus 1992).

This enzymatic reaction driven by light was found to require four manganese, 1–2 calcium and several Cl^- ions (see the reviews cited above). These are thought to form the catalytic center for water oxidation. The first physical evidence for the role of Mn ions in storing positive charges came from the discovery of a low-temperature 'multiline' EPR signal due to S_2, which was ascribed to a binuclear or tetranuclear Mn complex with spin $S = \frac{1}{2}$. (Dismukes and Siderer 1981) A second broad EPR signal with a near-Gaussian form centered at $g = 4.1$ was soon found in the same S_2-state (Casey and Sauer 1984, Zimmermann and Rutherford 1984). A third signal, centered at $g = 4.8$ with a much broader near-Gaussian form, was recently detected in the S_1 state by the parallel polarization EPR experiment and was found to change its amplitude in proportion to that of the multiline signal, but with no correlation with the $g = 4.1$ signal, upon various perturbations (Dexheimer and Klein 1992). Very recently, a proper quantum mechanical theory was presented to interpret three EPR signals, i.e. the multiline signal as indicative of a trapezoid-structure $S = \frac{1}{2}$ Mn(III,IV, IV,IV) tetramer (Kusunoki 1992a), the $g = 4$ signal as a trapezoid-structure $S = \frac{5}{2}$ Mn(III,IV,IV,IV) tetramer and the S_1-state signal as an $S = 1$ Mn(III,IV,IV,III) tetramer. In addition, a minimal heterogeneous S-state model was proposed to explain them in a unified way. (Kusunoki 1992b) As yet, no detectable EPR signal has been reported from both the S_3 and the S_0 states.

X-ray absorption spectroscopy is element specific and, in principle, can provide two types of information on (1) the oxidation states of Mn ions in any S-state through XANES and (2) the local molecular structure around the Mn ions through EXAFS and XANES. In general, the Mn K-edge absorption energy increases as the metal-ligand bond length becomes shorter, mainly because a 4p-like photoelectron is excited in a larger repulsive potential of the ligands. Hence, the Mn K-edge inflection-point energy has been used to infer the valence states of Mn in the S_1 state samples. Yachandra et al. (1987) have reported the K-edge energy at 6551.3 eV, which coincides with that reported by Kusunoki et al. (1990) at 6551.4 eV, but not with those of George et al. (1989) at 6548.3 eV and Penner-Hahn et al. (1990) at 6550.2 eV. This discrepancy indicates that the samples used by the latter two groups must be contaminated with exogenous Mn(II) ions and/or contain extensively distorted Mn clusters. Such a high K-edge energy as reported by the former two groups is indicative of a rather high-valent Mn cluster without involving Mn(II) ion in the S_1 state, even when taking into account the fact that the K-edge energy somewhat varies, depending not only on the valence state but also on the surrounding ligands. The normalized XANES spectrum of a Mn cluster consisting of m Mn ions is given by the sum of each Mn contributions (normalized) divided by the number m. Hence, from the finding that the difference-normalized XANES spectrum between S_1 and S_2 rather resembles a half of that between binuclear Mn(III,IV) and Mn(IV,IV) complexes, both in the main-peak intensity and in the peak position, it was concluded that the Mn cluster contains four Mn ions, one of which is oxidized from Mn(III) to Mn(IV) upon the S_1 to S_2 transition. (Kusunoki et al. 1989) The pre-edge absorption bands due to dipole-forbidden 1s-3d transitions, which can be properly described in terms of the ligand field theory (Kusunoki et al. 1990), gave us direct information on the oxidation states of Mn ions: Mn(III,IV,III,IV) in S_1 and Mn(III,IV,IV,IV) in S_2, and, indirectly, on the number of di-μ-oxo bridges as a dominant inversion-symmetry breaker: one or, with decreased probability, two (Kusunoki et al. 1992). Main features of the Mn EXAFS data of the PS II preparations poised in the S_1 state were well simulated by assuming that each Mn atom is on average surrounded by 1.5 O atoms at 1.76 Å, 4.4 N or O atoms at 2.25 Å, 1.1 Mn atoms at 2.74 Å and 1.8 C atoms at 3.18 Å (Guiles et al. 1990a), and, based on it, 'a pair of pairs' model was proposed. (Yachandra et al. 1993).

The Mn concentration in the samples previously used for XAFS measurements was of the order of 1 mM, which was too high to ensure light saturation under illumination with single flashes. To remove this difficulty, we brought a single SSD as close as possible to the sample surface to increase the X-ray detection rate. Furthermore, physiologically native PS II membranes were used in a series of flash experiments. The results obtained were reported in a short communication (Ono et al. 1992). In this paper, we describe in detail the quality of data and report the first Mn K-edge XANES spectra of true S_0-, S_1-, S_2- and S_3-states. Furthermore, the method of difference normalized spectra will be used to obtain information on the oxidation states of Mn ions in each S-state. The new results will be discussed along with the conflicting results obtained from the PS II preparations poised in the 'S_0^*' (Guiles et al. 1990a) and 'S_3' (Guiles et al. 1990b) states.

Materials and methods

Sample preparation

BBY type PS II membranes, which integrate native oxygen-evolving complexes, were prepared from spinach as described previously (Ono and Inoue 1985), and stored in liquid N_2. After thawing, the membranes were incubated in darkness at 0 °C for 6 h. The dark adapted samples were washed under dim green safe light with a suspension of 400 mM sucrose, 20 mM NaCl, 1 or 0.5 mM EDTA and 40 mM Mes/NaOH (pH 6.5), followed by two washes with the same buffer at 4.0 mg Chl/ml. A 15.0-μl aliquot of the membranes was mounted on a membrane filter (Toyo Roshi, TM-300, 7×4 mm^2) and placed in a thin copper holder ($5 \times 8 \times 0.2$ mm^3). The dark-adapted membranes in the sample holder, which provides the zero-flash ('0F') sample, were excited by a series of saturating Nd-YAG laser flashes ($\approx$ 7 ns, 23 mJ cm^{-2} and 532 nm) at 10 °C with equal intervals of 1 s and quickly cooled to 77 K in liquid N_2 to obtain 1-flash ('1F') to 5-flash ('5F') samples.

XANES spectra measurements

Mn K-edge XANES spectra from such dilute samples (0.1 mM Mn) were measured at Photon Factory BL-4C in the National Laboratory of High Energy Physics, Tsukuba, using a sagittal-shaped Si(111) crystal monochromator and constructing a compact cryostat capable of positioning an EG and G Si(Li) SSD as close as 1.5 cm from the sample surface. The measurements were performed during steady operation with positron-beam current 360–260 mA in 12 h at 2.5 GeV. The other aspects of experimental setups were essentially unchanged (Kusunoki et al. 1990). The sample holders were kept in darkness at 80 K during data collection. Fluorescence photon counts were accumulated for 10 s per each point and data from 7 to 10 scans, each of which contains 78 points, were collected. The total SSD counts of signals were ca. 9×10^3 and the signal-to-background (S/B) ratio was ca. 0.5 around the absorption maximum. Energy calibration was carried out by measuring a sharp pre-K-edge peak at 6543.3 eV of $KMnO_4$ powder at 150–200 K before and after each data collection.

Results and discussion

Figure 1 shows the Mn K-edge XANES spectra of the oxygen-evolving complex illuminated by a series of saturating laser-flashes in the PS II preparations at a chlorophyll concentration of 4 mg/ml, which is about ten times more dilute than before (38.6 mg Chl/ml) (Kusunoki et al. 1990, Ono et al. 1991). This one-order-of magnitude decrease of Mn concentration (1 mM $\rightarrow$ 0.1 mM) was almost totally compensated by a one-order-of- magnitude increase of the solid angle to collect fluorescence X-ray from Mn, resulting in the success of laser-flash experiment. The thickness of the sample has an upper limit of the order of 0.2 mm due to saturation condition. Linear backgrounds have been subtracted from these spectra and they have been normalized by the absorption maximum. The rather low signal-to-noise (S/N) ratio compared with the previous work (Kusunoki et al. 1990) is mainly due to a 10-fold increase of the X-ray scattering component as the SSD was brought close to the sample surface. The starting sample (0F) in this series of experiments was assessed as containing ca. 25% Mn clusters with tyrosine D and ca. 75% Mn clusters with tyrosine radical D^+, both in 100% S_1 state (see Eq. (3)). The K-edge half-height energy of this spectrum (0F) is found to be 6551.7 ± 0.2 eV, which appears to be the highest value ever reported within experimental accuracy, implying that this sample would contain the least amount of extraneous Mn^{2+} ion concentration. After one-flash illumination, the spectrum showed an upshift of the K-edge by ca. 0.7 eV, which is notably somewhat smaller than that observed upon continuous-light illumination at 200 K, i.e. 1.0 eV (Guiles et al. 1990a) and 1.6 eV (Kusunoki et al. 1990). This difference may be

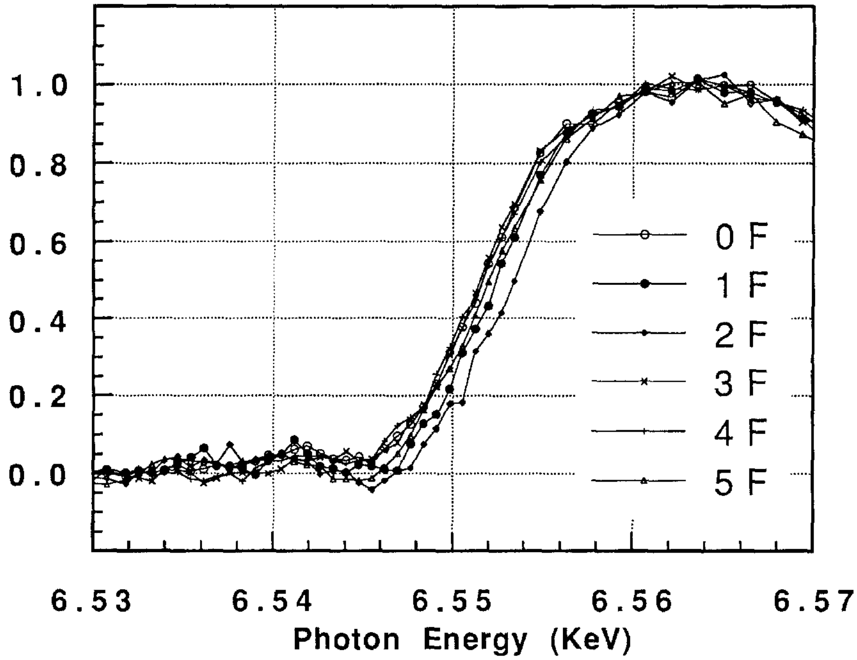

Fig. 1. Mn K-edge XANES spectra of O_2-evolving PS II membranes after a series of saturating flashes. Spectra, 0F (—O—), 1F (—●—), 2F (—◆—), 3F (—×—), 4F (—+—) and 5F (—△—), are named by the number of flashes applied to the samples at 10 °C.

interpreted as arising from the fact that, in the laser-flash experiments, the probability of double-hits is zero, while in continuous-light experiments, a finite double-hit probability exists. The spectrum undergoes a further K-edge upshift of the same order of magnitude (0.7 eV) after one more flash (compare spectra 1F and 3F).

This observation, however, contradicts an earlier report (Guiles et al. 1990b) stating that the Mn K-edge XAFS spectrum of PS II preparations poised partially in the 'S_3' states was the same as that poised in the S_2 state. Hence, we have reexamined the reported EPR data, which were used to establish the sample procedures to generate a double turnover of PS II, and propose the following reasons why there remains a possibility of no S_2-to-S_3 advancement during the illumination at 240 K (step 4): (i) The amplitude of the multiline signal may not be used as a quantitative measure of the S_2-state composition, because both the S_1 and S_2 states must be heterogeneous with respect to the total spin of the Mn cluster, e.g. S = 0 or S = 1 in the S_1 state and S = ½ or 5/2 in the S_2 state, depending upon the temperature and the duration time of sample treatment (Kusunoki 1992b); (ii) Disappearance of the EPR signals at g = 8 and g = 5.5 associated with Q_{400} was interpreted as indicating the reduction of Fe^{3+} to Fe^{2+} (Guiles et al. 1990b), but the reduction of Q_A to Q_A^- itself may eliminate these signals owing to the appearance of the Q_A^- spin being coupled with the spin of Fe^{3+}, irrespective as to whether its high spin state is changed or not; and (iii) An EPR signal which appeared at g = 1.9 shows the lineshape significantly different from that observed for Q_A^- coupled with Fe^{2+}, although Guiles et al. (1990b) assumed that this signal originates from $Q_A^-Fe^{2+}$. However, it seems to be more reasonable to assume that the Q_A^- spin coupled with the Fe^{3+} spin is the origin of this signal.

It should be emphasized that our sample poised in the S_3 state was prepared by two-flash illumination at room temperature (10 °C), which enables a physiological proton release that may be indispensable to the S_2-to-S_3 advancement. In fact, flash illuminated samples ('3F' to '5F') accomplished a period-four oscillation of the Mn K-edge energies with a remarkable down-shift of 1.5 eV to reach the minimum after three flashes, followed by reduced upshifts of 0.1 and 0.4 eV after four and five flashes, respectively. This observation was confirmed by an independent experiment using saturating Xe flashes instead of Nd-YAG laser flashes to excite the PS II membranes. As a sample assessment, we measured the flash yield sequence of O_2 produced by a series of saturating Xe flashes given to a suspension of the PS II membranes, getting a precisely normal O_2-yield pattern (data not shown).

It is now possible to derive the Mn K-edge XANES spectra for Kok's S_0-S_3 states, designated $A_i(E)$; i = 0 – 3, from those depicted in Fig. 1, designated $X_n(E)$; n = 0 – 5, by inversely transforming any choice of four among six relations:

$$X_n(E) = \Sigma_{i=0}^{3} P_{ni} A_i(E);\quad n = 0-5 \tag{1}$$

where P_{ni} represents the normalized population of the reaction centers in the S_i state in the n-flash (nF) sample, i.e. $\Sigma_i P_{ni} = 1$. These populations, P_{ni} (n = 0 – 5, i = 0 – 3), consist of two terms:

$$P_{ni} = [DS_i]_n + [D^+S_i]_n, \tag{2}$$

where $[DS_i]_n$ and $[D^+S_i]_n$ represent fractional concentrations of the Mn clusters in the DS_i and D^+S_i state after n flashes, respectively. During the preparation of BBY type PS II membranes in the light, D would be oxidized into D^+ in almost all centers and S_0, S_1, S_2 and S_3 would be evenly populated. In the course of dark adaptation, the D^+S_2 and D^+S_3 states decay into D^+S_1 and D^+S_2, respectively, by electron donation from the acceptor side and other endogenous reductants, with half-life times of tens of minutes (ca. 12 min at 0 °C in isolated thylakoids at pH 7.0, Messinger et al. 1993), and a distribution of DS-states close to 25% D^+S_0 and 75% D^+S_1 appears in accordance with the model of Kok et al. (1970). Furthermore, the reduction of D^+ advances biphasically with pH- and temperature-dependent half-times in the tens of minutes and hours time range with relative amplitudes just corresponding to the $D^+S_0 \rightarrow DS_1$ and the $D^+S_1 \rightarrow DS_1$ processes, respectively. (Vass and Styring 1991, Messinger et al. 1993) After 6 h dark adaptation at pH 6.5 / 0 °C, the former process will almost completely advance, because the half-time is estimated to be of the order of 35 min (Messinger et al. 1993), but the latter process can be ignored because it proceeds with a half-time much larger than 9 h at 21 °C (Vass and Styring 1991). Therefore, the approximate initial distribution of DS-states is given by:

$$[DS_i]_0 = 0.25\ \delta_{i,1}$$

and

$$[D+S_i]_0 = 0.75\ \delta_{i,1};\quad i = 0-3, \tag{3}$$

where $\delta_{i,1}$ and, in general, $\delta_{i,j}$ are Kronecker's δ. Illumination of dark-adapted PS II membranes at 10 °C could induce several S-state turnovers in almost all centers. The formation of DS_2 and DS_3 is followed by their conversion to D^+S_1 and D^+S_2, respectively, taking place in a time scale of seconds (Babcock and Sauer 1973, Styring and Rutherford 1987, Vass and Styring 1991, Messinger et al. 1993). These reactions, responsible for the S-state redistribution, must be added in a generalized Joliot-Kok cycle, which allows to compute the DS-distribution after flash n by knowing it after flash n–1 according to the following recurrence formulae:

$$[DS_i]_1 = \alpha[DS_i]_0 + (1-\alpha-\beta)[DS_{i-1}]_0 + \beta[DS_{i-2}]_0, \tag{4}$$

$$\begin{aligned}[D^+S_i]_1 &= \alpha[D^+S_i]_0 + (1-\alpha-\beta)[D^+S_{i-1}]_0 \\ &+ \beta[D^+S_{i-2}]_0,\end{aligned} \tag{5}$$

$$\begin{aligned}[DS_i]_n &= \alpha[DS_i]_{n-1}(1 - \delta_{i,2}\gamma_1 - \delta_{i,3}\gamma_2) \\ &+ (1-\alpha-\beta)[DS_{i-1}]_{n-1}(1 - \delta_{i-1,2}\gamma_1 - \delta_{i-1,3}\gamma_2) \\ &+ \beta[DS_{i-2}]_{n-1}(1 - \delta_{i-2,2}\gamma_1 - \delta_{i-2,3}\gamma_2)\ ;\ n > 1,\end{aligned} \tag{6}$$

and

$$\begin{aligned}[D^+S_i]_n &= \alpha\{[D^+S_i]_{n-1} + [DS_{i+1}]_{n-1}(\delta_{i,1}\gamma_1 + \delta_{i,2}\gamma_2)\} \\ &+ (1-\alpha-\beta)\{[D^+S_{i-1}]_{n-1} + [DS_i]_{n-1}(\delta_{i,2}\gamma_1 + \delta_{i,3}\gamma_2)\} \\ &+ \beta\{[D^+S_{i-2}]_{n-1} + [DS_{i-1}]_{n-1}(\delta_{i-1,2}\gamma_1 + \delta_{i-1,3}\gamma_2)\}; \\ &n > 1,\end{aligned} \tag{7}$$

with

$$\gamma_1 = C_1(1 - \exp[-k_{21}{}^{f}\tau]) + (1-C_1)(1 - \exp[-k_{21}{}^{s}\tau])\ , \tag{8}$$

and

$$\gamma_2 = C_2(1 - \exp[-k_{32}{}^{f}\tau]) + (1-C_2)(1 - \exp[-k_{32}{}^{s}\tau])\ , \tag{9}$$

where the i's are taken modulo 4, τ is the flash interval, α and β are the probabilities of misses and double-hits, respectively, that were introduced by Kok et al.(1970), and γ_1(γ_2) represents the rate of the increase of D^+ concentration during the time τ due to the $DS_2 \rightarrow D^+S_1$ ($DS_3 \rightarrow D^+S_2$) processes involving biphasic decay components; the fast one with the pH- and temperature-dependent rate constant, $k_{21}{}^f$ ($k_{32}{}^f$), at the pH-dependent concentration, C_1 (C_2), and the slow one with the $k_{21}{}^s$ ($k_{32}{}^s$) at the $1 - C_1$ ($1 - C_2$). (Styring and Rutherford 1987, Vass and Styring 1991, Messinger et al. 1993)

For BBY-particles at pH 6.5 / 21 °C, the $DS_2 \rightarrow D^+S_1$ process was reported to take the values: $\ln 2 / k_{21}{}^f = 0.4–1$ s, $\ln 2 / k_{21}{}^s = 4.1$ s and $C_1 = 0.09$ (Vass and Styring 1991). For isolated thylakoids at pH 6.5, only the dominant slow component were detected to decay with the temperature-dependent half-time: e.g. $\ln 2/ k_{21}{}^s$ is 13 s at 10 °C (Renger et al. 1992), which may lead to 5.7 s (calculated as 13*11/25 s according to Messinger et al. 1993) at 20 °C in reasonable agreement with the above 4.1 s. Assuming the same temperature-dependence for the fast component, we have roughly estimated the value of γ_1 for BBY-particles at pH 6.5 / 10 °C as $\gamma_1 = 0.095 – 0.071$. Furthermore, the literature data (Babcock and Sauer 1973, Vermaas et al. 1984, Styring and Rutherford 1987, Renger et al. 1992, Messinger et al. 1993) indicate that the $DS_3 \rightarrow D^+S_2$ process is quite similar to the $DS_2 \rightarrow D^+S_1$ process. Therefore, it is reasonable to assume that $\gamma_2 = \gamma_1 = 0.08$. Since β can be reduced to completely zero by using nanosecond flashes, the only parameter to be determined is α. The least-square method for this should involve the last two XANES spectra, $X_4(E)$ and $X_5(E)$, to minimize the mean square of their differences from theoretical ones that can be computed for a given α according to the procedures as described. The resultant value of α was 0.09, a quite reasonable value.

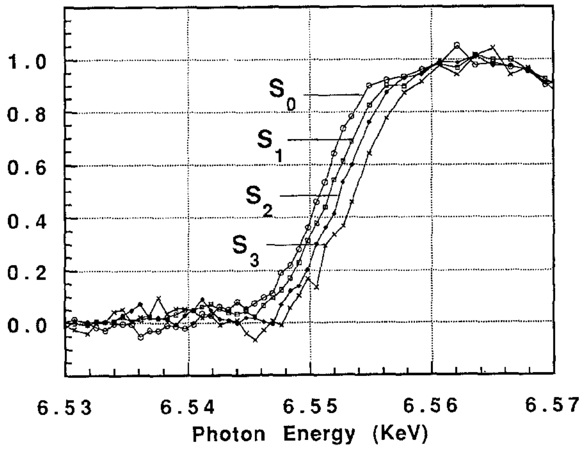

Fig. 2. Mn K-edge XANES spectra of Kok's true S_0-, S_1-, S_2- and S_3-states, whose K-edge half-height energies are found to be 6550.9 ± 0.2 eV, 6551.7 ± 0.2 eV, 6552.5 ± 0.2 eV and 6553.6 ± 0.2 eV, respectively.

Thus, we could determine the first Mn K-edge XANES spectra of Kok's S_i ($i = 0 – 3$) states $A_i(E)$ by inverse transformation of the X_n (E) values according to Eq. (1). The results obtained are depicted in Fig. 2 (Kusunoki et al. 1992). The reliability of these 'intrinsic' spectra can be checked by a comparison of theoretical and experimental XANES spectra for 4- and 5-flash samples, both of which showed an excellent agreement within noise level (data not shown). In this paper, we compare, in Fig. 3, only their K-edge energies to demonstrate the high extent of agreement obtained for both the 4- and 5-flash samples. Remarkably, the K-edges of these XANES spectra in Fig. 2 show almost parallel and stepwise upshifts of ca. 1 eV per univalent step as the S-state proceeds from S_0 to S_3. This is more clearly seen from the K-edge half-height energies at 6550.9 ± 0.2 eV, 6551.7 ± 0.2 eV, 6552.5 ± 0.2 eV and 6553.6 ± 0.2 eV in the pure S_0, S_1, S_2 and S_3 states, respectively. A more useful method to analyze this remarkable feature would be to compare the difference-normalized XANES spectra between the states S_0 and S_1, S_1 and S_2, and S_2 and S_3, respectively, with those of model Mn complexes. This is based on the deconvolution equation:

$$A_{i+1}(E) - A_i(E) \cong \frac{1}{m}\sum_{k=1}^{m} \{A_{i+1,k}(E) - A_{i,k}(E)\}; \quad i = 0, 1, 2 \qquad (10)$$

where m is the number of Mn ions per a Mn cluster and $A_{i,k}(E)$ is the normalized XANES spectrum of the k-th Mn ion in the Mn cluster of state S_i. Figure 4 reveals that these difference spectra contain a characteristic negative peak around 6552–6553 eV at the same intensity level of 0.08 ± 0.03 (or 8 ± 3%). For comparison, the normalized XANES spectra of some pairs of binuclear Mn complexes with the same ligands and the different valence states were observed to provide the model difference spectra which can be compared with Eq. (10). It was found that the difference spectra between binuclear Mn(IV,IV) and Mn(III,IV) N4-py and

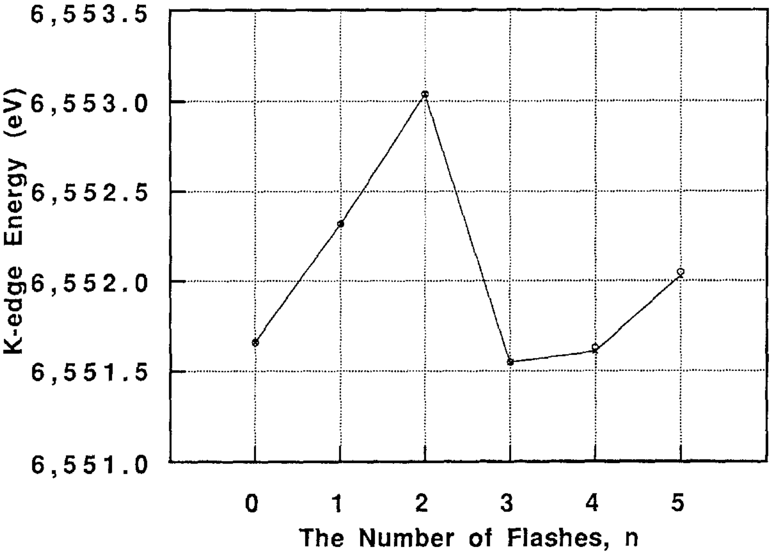

Fig. 3. Oscillatory pattern of the Mn K-edge energy in the XANES spectra of PS II membranes as a function of flash number n: The experimental one (–x–) is compared with a theoretical one (–O–) for n = 4 and 5.

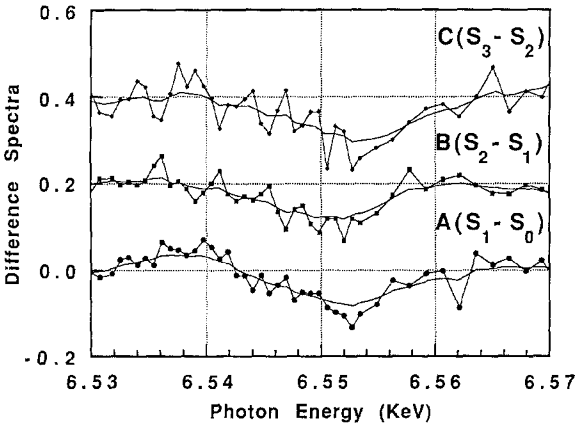

Fig. 4. Difference normalized XANES spectra between S_0- and S_1-states (spectrum A), S_1- and S_2-states (spectrum B) and S_2- and S_3-states (spectrum C). Note the similarity among these with a similar negative peak around 6552–6553 eV, which is indicative of one Mn(III) ion or its direct ligand being one-electron oxidized at each S-state transition.

between binuclear Mn(II, III) and Mn(II,II) (L-py) $(C_2H_5COO)_2$ complexes, which can be approximately deconvoluted as

$$A_{IV,IV}(E) - A_{III,IV}(E) \cong \frac{1}{2}\{A_{IV}(E) - A_{III}(E)\} \quad (11)$$

and

$$A_{II,III}(E) - A_{II,II}(E) \cong \frac{1}{2}\{A_{III}(E) - A_{II}(E)\}, \quad (12)$$

respectively, have a negative peak around 6552 eV with an intensity of 0.16 and around 6549 eV with an intensity of 0.12, respectively. (Kusunoki et al. 1989). These findings strongly suggest that the Mn cluster is a tetramer (m = 4) and one Mn(III) ion or its direct ligand in the tetramer is one-electron oxidized upon each S-state transition until three positive charges are accumulated at S_3.

Consistent with this conclusion, we have recently demonstrated, via pre-edge (1s-3d transition) XANES spectra from condensed samples, that the oxidation states of Mn ions in the S_1 and S_2 states are most likely in Mn(III,IV,III,IV) and Mn(III,IV,IV,IV), rather than in Mn(III,III,III,III) and Mn(III,III,III,IV), respectively (Kusunoki et al. 1992). According to a general belief that the first water oxidation could not occur until S_2, we conclude that the oxidation state of Mn ions in the S_0 state would be Mn(III,III,III,IV). This conclusion contradicts Guiles et al.(1990a) which is based on the Mn K-edge energy at 6549.9 eV that is significantly lower than ours at 6550.9 eV. We consider that this discrepancy would simply reflect a sample difference between our physiological S_0-state and their synthetic S_0^*-state by NH_2OH treatment, which might have caused a release of a significant amount of Mn(II) ions and/or a serious deformation of the

Mn cluster; the latter possibility may be supported by the EXAFS data themselves (Guiles et al. 1990b). A serious deformation of the Mn cluster can cause stretching of some Mn-ligand bonds which in turn down-shifts the Mn K-edge XANES spectrum. This gives rise to a similar effect as the reduction of some Mn ion. In fact, an earlier XANES study of Ca^{2+}-depleted PS II membranes revealed a similar effect. It was found that the K-edge energy in the modified S_2-state is about 0.8 eV lower than that in the normal S_2-state (Ono et al. 1991); in spite of the fact that these S_2-states exhibit similar '$g = 2$ multiline' EPR signals indicative of the same Mn(III,IV,IV,IV) oxidation states with different cluster conformations (Kusunoki 1992b).

From a mechanistic point of view, the S_3 state is most interesting. Along with the earlier theoretical considerations (Renger 1978, Kusunoki 1987), we would reserve the possibility of the first water oxidation in the S_3 state by stating that, upon the S_2 to S_3 transition, the Mn(III,IV,IV,IV)·L complex with L being a direct ligand to a Mn(III) ion will be oxidized into either Mn(III,IV,IV,IV)·L^+ or Mn(IV,IV,IV,IV)·L. Neither of these two possibilites supports the conclusion of Guiles et al. (1990b) that no oxidation of the Mn cluster took place in this step. For the former to be the case, the ligand must be bound to a special site of the Mn(III) ion such as the 6th coordination site of a haem iron, to which the K-edge is known to be very sensitive (Bianconi 1984). Indeed, the Mn cluster in the S_2 state is considered to contain only one Mn(III) ion in a di-μ-oxo bridged Mn(III,IV) dimeric subunit (Kusunoki 1992a) whose di-μ-oxo bridge plane can play the same role as the haem plane in the excitation spectrum of a photo-electron near K-edge. Such a ligand would be most likely a deprotonated water molecule.

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