Review

The tetra-manganese complex of photosystem II during its redox cycle – X-ray absorption results and mechanistic implications

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Abstract

Using X-ray absorption spectroscopy (XAS), relevant information on structure and oxidation state of the water-oxidizing Mn complex of photosystem II has been obtained for all four semi-stable intermediate states of its catalytic cycle. We summarize our recent XAS results and discuss their mechanistic implications. The following aspects are covered: (a) information content of X-ray spectra (pre-edge feature, edge position, extended X-ray absorption fine-structure (EXAFS), dichroism in the EXAFS of partially oriented samples); (b) S 1-state structure; (c) X-ray edge results on oxidation state changes; (d) EXAFS results on structural changes during the S-state cycle; (e) a structural model for the Mn complex in its S 3-state; (f) XAS-based working model for the S 2–S 3 transition; (g) XAS-based working model for the S 0–S 1 transition; (h) potential role of hydrogen atom abstraction by the Mn complex. Finally, we present a specific hypothesis on the mechanism of dioxygen formation during the S 3–(S 4)–S 0 transition. According to this hypothesis, water oxidation is facilitated by manganese reduction that is coupled to proton transfer from a substrate water to bridging oxides. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Today it is generally agreed that four Mn ions bound to the protein matrix of photosystem II (PSII) are at the heart of the oxygen-evolving complex (OEC) which facilitates the use of water as an electron source for the photosynthesis of carbohydrates [1–3]. Photosynthetic water oxidation involves the light-driven cycling of this OEC through four semi-stable intermediate states denoted as S 0, S 1, S 2 and S 3. The S 3–S 0 transition is often assumed to involve a kinetically unresolved intermediate state denoted as S 4. Only the S 3–(S 4)–S 0 transition is coupled to dioxygen formation [4,5]. For elucidation of the mechanism of photosynthetic water oxidation, information on structure and oxidation states of the Mn complex is needed for all S-states. Due to the pioneering work of Melvin Klein and his coworkers, today X-ray absorption spectroscopy (XAS) can pro-
vide relevant information on structure and oxidation state of the PSII manganese complex. XAS results have contributed in the past [3,6] and certainly will contribute in the future to the joint effort to answer the question how organisms can oxidize water to dioxygen.

Here we review our recent XAS results and report considerations on mechanistic implications. An exhaustive review or in-depth discussions of controversial issues is beyond the scope of this contribution.

2. X-ray absorption spectra and S1-state results

Three sections of the X-ray absorption spectrum (measured around the Mn K-edge) are considered: (1) the pre-edge peak or shoulder at about 6540 eV, (2) the edge region (6545–6565 eV), (3) the extended X-ray absorption fine-structure (EXAFS) region ranging from 6565 to about 7100 eV.

2.1. Pre-edge feature

The pre-edge peak is related to formally forbidden 1s→3d transitions. Its magnitude depends on coordination number and symmetry (pronounced pre-edge peak in tetrahedrally coordinated Mn) and perhaps, to a lower extent, on the Mn oxidation state (e.g. the pre-edge is smaller in Mn(II)O than in Mn(IV)O2). In multi-nuclear complexes, di-μ-oxo bridges might cause a slight increase of the pre-edge magnitude of octahedrally coordinated manganese due to reduced inversion symmetry [7]. In the case of the PSII Mn complex, the peak’s small magnitude is suggestive that the complex contains exclusively five- or six-coordinated Mn.

2.2. Edge spectra

It is only insufficiently understood how properties of the metal center (oxidation state, type and arrangement of ligands) affect X-ray edge spectra. However, empirical analysis of synthetic compounds suggests a general tendency for a shift in the X-ray edge position to higher energies by 2–4 eV per unit increase in the (formal) oxidation number, provided structural changes neither counteract nor enhance the oxidation state influence [6,8–11] (see also Fig. 1). Accordingly, for a tetrnuclear Mn complex the edge shift resulting from oxidation of one out of four Mn ions is anticipated to be around 3 eV/4 = 0.75 eV. Interestingly, Mulliken charge of Mn ions as well as d-orbital occupancy appear to be almost independent of the formal oxidation state [12]. Thus, it is not surprising that edge shape and X-ray edge positions are found to depend not only on the metal’s formal oxidation state, but also on the nature of the ligands (oxygen ligands typically result in higher edge positions than nitrogen ligands) and other aspects of the structure of the metal center. One factor affecting edge position and shape seems to be the metal–ligand bond length (the shorter the bond length, the higher the edge energy, see [13]). Typically, the bond length decreases for oxidation of the central metal thus causing the X-ray edge to shift to higher energies. However, if formal oxidation of the metal center was coupled to increasing metal–ligand bond lengths, the edge shift typically observed for formal oxidation state changes might be reduced or absent. It is presently unknown how the formation of a ligand radical (e.g. a Mn-bound oxyl radical) might affect the X-ray edge.

Synthetic models with ligand types and a complex
structure close to the likely structure of the tetranuclear Mn complex of PSII are presently not available. Therefore, precise determination of absolute values for the involved Mn oxidation states by XAS is problematic. We feel that presently the accuracy of the XAS approach for oxidation state determination does not allow distinguishing between, e.g., a mean oxidation state of +3.25 and +3.5 (whereas a ‘shift’ in the oxidation state by 0.25 units may be very well detectable). The multiline electron paramagnetic resonance (EPR) signal observed for the Mn complex in its $S_2$-state is suggestive of either a Mn(III)$_3$Mn(IV)$_1$ or a Mn(III)$_1$Mn(IV)$_3$ complex. Consequently, assuming Mn oxidation upon the $S_1$–$S_2$ transition, the Mn oxidation states in the $S_1$-state are likely to be either Mn(III)$_4$ or Mn(III)$_2$Mn(IV)$_2$. It is found that the X-ray edge position is not in agreement with a mean Mn oxidation state of +3, but in good agreement with a mean oxidation state of +3.5 (see [3,11,13]). Taking into consideration both, EPR and XAS results, presently Mn(III)$_2$Mn(IV)$_2$ seems to be the most likely redox state assignment for the $S_1$-state.

It should be noted that a complicated edge shape (shoulders, several inflection points) is not indicative of a mixed-valent manganese complex. Monovalent manganese compounds are often characterized by a complex, highly structured shape of the absorption edge.

In summary, if drastic or ‘unusual’ structural changes took place, it would be extremely difficult or even impossible to judge whether the oxidation state of one Mn ion changes. However, in the absence of such structural changes, the edge position of the PSII Mn complex can be anticipated to shift by 0.5–1 eV for oxidation of one out of four Mn ions of the complex. Presently there seems to exist no alternative experimental method which might substitute for the XAS analysis of oxidation states. In the future, a deeper insight into the underlying physical principles could facilitate a less ambiguous analysis of edge spectra with respect to Mn oxidation states.

2.3. EXAFS spectra

In contrast to the edge range, the fine-structure observed in X-ray absorption spectra for energies above the edge (EXAFS) is generally well understood. Using well-established theoretical approaches (see, e.g. [14–16]), simulation of spectra is straightforward for compounds of known structure. It is the strength of EXAFS spectroscopy that analysis of spectra can also facilitate (partial) solution of the inverse problem (meaning determination of structure on the basis of spectra) in a relatively straightforward way [17]. Typically, the small oscillatory EXAFS structure is extracted by subtraction of a smooth background from the previously normalized spectra before transformation of the energy to a wavenumber scale [17]. Already the Fourier-transformed wavenumber spectra provide information on the distance between absorbing atom (here Mn) and backscattering atoms of the first, second and third coordination sphere (see Fig. 2). Fourier transforms (FTs) allow an often instructive visualization and rough, qualitative evaluation of the structural information carried by the EXAFS spectrum. Simulation of spectra (curve-fitting) is required to obtain detailed structural information.

(It should be noted that especially the magnitudes...
of the individual Fourier peaks depend on the parameters used for the transition to a wavenumber scale (energy threshold, \(k^p\)-weighting) and for calculation of the FTs (data range, window functions). The clearly different FTs shown in Fig. 2 have been obtained for the same EXAFS spectrum, but using different FT parameters. FT parameters, however, are irrelevant for data evaluation by curve-fitting of \(k\)-space data unless a Fourier isolation approach is used.

Numerous subtleties are involved in simulation of EXAFS spectra, but often average distances, \(R_i\), between the absorbing atom (here Mn) and the backscattering atoms of the first, second and occasionally also of higher coordination spheres can be determined with a precision of 0.01–0.03 Å. The precision in the determined number of backscattering atoms (coordination-number), \(N_i\), is significantly lower (about 25%) because this parameter frequently is highly correlated with the corresponding Debye–Waller parameter (\(\sigma\)), which describes the distance spread in the respective coordination sphere. In the case of highly heterogeneous backscattering shells (meaning various types of backscattering atoms or significantly non-Gaussian distance spread within one coordination sphere), it is impossible to obtain any reliable estimate of the coordination numbers without using constraints or additional assumptions (\(\sigma\) fixed to reasonable values, restriction to chemically reasonable approaches, sequence information, etc.). For the PSII Mn complex, this holds for the peak I and peak III EXAFS oscillations.

It should be noted that the choice of the simulation approach as well as the selection of structural models is necessarily guided by model chemistry. For example, in synthetic compounds, Mn–Mn distances of 2.65–2.75 Å are almost exclusively found for unprotonated \(\mu\)-oxo-bridged binuclear Mn units; in these units the Mn–O distance is around 1.8 Å [18,19]. Seemingly, Mn–Mn distances around 2.7 Å are characteristic for pairs of Mn ions connected by unprotonated \(\mu\)-oxo bridges. Spectra of the PSII Mn complex in its S\(_2\)-state show the presence of a particularly strong EXAFS interaction between absorbing Mn atoms and backscattering atoms at about 2.7 Å: the backscatrer is clearly heavier than carbon, nitrogen or oxygen. This 2.7 Å EXAFS is simulated assuming that the strong backscatterer is Mn [20–23]. This assignment results in good simulations of the experimental spectra; and it is found that there are two pairs of Mn atoms (per PSII) with Mn–Mn distances close to 2.7 Å. Therefore it has been concluded that the Mn complex contains two pairs of di-\(\mu\)-oxo-bridged binuclear Mn units (dimer-of-dimers model or ‘Berkeley model’ [9,24]), a conclusion which is strengthened by the relatively short Mn–ligand distances found by simulation of the first-peak EXAFS oscillations. This example demonstrates how the combination of EXAFS data and insights obtained by model chemistry allows formulation of detailed structural models.

In contrast to the peak II EXAFS oscillations, the peak III EXAFS is difficult to interpret. For satisfactory simulation of X-ray absorption linear dichroism spectroscopy (XALDS) data sets (unpublished results) and of room temperature EXAFS data [25], we need to assume that there are two shells of backscattering atoms with absorber–backscatter distances of \(\approx 3.1\) Å and \(\approx 3.65\) Å. However, presently an unambiguous assignment of these two backscatterer shells to Mn, Ca or lighter atoms (O, C, N) is impossible. Furthermore, relevant contributions of multiple scattering paths are conceivable. We conclude that, at backscattering distances greater than 3.0 Å, neither the presence of Ca nor of Mn is unambiguously indicated by our EXAFS data. On the other hand, the presence of Mn and/or Ca at 3.0–3.7 Å is not in conflict with the EXAFS data.

2.4. Linear dichroism of EXAFS spectra

Preparation of thick multilayers (50–500 µm) of PSII membrane particles leads to XAS-suitable samples with a preferential orientation of the membrane normal in parallel to the sample’s surface normal. Using these partially oriented samples for measurements at various angles between sample normal and electric field vector of the polarized X-rays, determination of the linear dichroism in the X-ray spectra, which carries (limited) angle and orientation information, becomes feasible. Besides providing orientation information, dichroism data may also facilitate the resolution of otherwise hidden subshells. (We call this approach XALDS. The somewhat colloquial term ‘polarized EXAFS’ is used more frequently.) Because only imperfect unidirectional orientation is
achievable, determination of the order characteristics (or ‘mosaic spread’) is mandatory for a quantitative analysis of XALDS data sets. In previous works [26–28], erroneous approaches had been used for both, determination of the disorder characteristics by simulation of EPR spectra and incorporation of the determined mosaic spread into the simulation of the EXAFS dichroism. We have corrected these subtle methodical deficiencies; consequently, we have obtained quantitatively different orientation information [29]. It should be noted that in [29] (as well as in all previous EXAFS dichroism investigations on partially ordered systems), EXAFS simulations were carried out on the basis of the plane-wave or small-atom approximation. Recently we developed an approach to account correctly for the mosaic-spread characteristic and to carry out EXAFS curve-fitting using the exact curved-wave EXAFS theory [14]. Application of this theoretically more precise approach to the dichroism data obtained for PSII results in only slightly modified results (unpublished).

2.5. Structural models for the S1-state

The existence of two 2.7 Å EXAFS interactions is highly suggestive of two di-µ-oxo bridges between Mn ions. (Removal of the two less tightly bound Mn ions seems to result in a single di-µ-oxo-bridged binuclear complex [30]. This finding does not rigorously disprove, but renders less likely speculations that the 2.7 Å EXAFS interactions of the intact complex are explainable by a chemically unprecedented, unique tetrameric structure with 2.7 Å Mn–Mn distances unrelated to di-µ-oxo bridges.) It has been proposed (on the basis of EPR results) that the two di-µ-oxo dimers are not close to each other [31]. The EXAFS results on the S1-state structure discussed below are not supportive of this proposal. Thus, the dimer-of-dimers model of Klein and coworkers (or the ‘Berkeley model’) as depicted in [9,24,28] might correspond to the structure of the S1-state complex. However, several aspects of this Berkeley model are not uniquely implied by the data and alternative structural models need to be considered seriously (as discussed, e.g., in [3]). Three distinctly different structural isomers of the Berkeley model are in agreement with the EXAFS results. Noteworthily, only one of these isomorphic forms is not in conflict with the mechanistic model proposed by Babcock and coworkers [32,33]. Furthermore, also a trimer-monomer core (as has been suggested on the basis of EPR simulations [34]) consisting of a Mn3(µ2-O)4 structure connected by a mono-µ-oxo bridge to the fourth Mn ion might be in agreement with the EXAFS data. (There are no suitable model compounds allowing definitive judgment whether the mean Mn–Mn distances of 2.72 Å are in agreement with a Mn3(µ2-O)4 unit; a trans influence of the four µ-O ligands of the intermediate Mn might result in longer Mn–Mn distances.)

3. Oxidation state changes

Due to the relation between Mn oxidation state and X-ray edge position discussed above, XAS has been used to investigate the Mn oxidation state changes for all transitions between successive semistable states of the S-state cycle [35–37]. Of particular interest is the S2–S3 transition because conflicting results have been obtained by means of various spectroscopic techniques.

All states of the Mn complex seem to be preserved at liquid nitrogen temperatures for at least 2 weeks. Therefore, S-state population by special illumination protocols followed by rapid freezing in liquid nitrogen and low-temperature XAS measurements is mostly considered to be a suitable approach [35–39]. The relevance of low-temperature data for structure and oxidation state of the Mn complex at room temperature is not a priori guaranteed. However, recently we could demonstrate for the Mn complex in its S1-state that the complex at low-temperature resembles closely the room temperature complex, at least with respect to the di-µ-oxo bridges and the Mn oxidation state [25]. Comparable control experiments for other S-states are certainly desirable (but experimentally difficult).

Almost complete population of the S1-state of the PSII Mn complex is obtained by extensive dark adaptation of PSII samples; the S2-state can be reached by continuous illumination at 200 K [40]. However, specific population of the S3- or S0-state is not easily achieved. The only way to achieve preferential population of the S1- or S0-state without using intrinsically ambiguous protocols involving ox-
idants or reductants is application of Ws or ns flashes of saturating light. These single-turnover flashes allow driving the Mn complex stepwise through its functional cycle. The resulting population of the four semi-stable intermediate states and all variations in associated observables are characterized by a period-four pattern (see Fig. 3). The flash approach does not allow preparation of pure S-states. Due to so-called misses and double hits S-state mixing is inevitable. Therefore, it is mandatory to calculate pure S-state spectra on the basis of the measured spectra and the assumed S-state population distributions. Using such a deconvolution to obtain pure S-state spectra, Ono et al. [35] reported that upon the S0→S1, S1→S2 and S2→S3 transitions the X-ray absorption edge shifts by 1.0, 0.8 and 1.2 eV. Ono et al. [35] used the periodic pattern in the investigated spectroscopic signal itself (meaning the X-ray edge positions) to determine the S-state population, an approach routinely used in the context of spectroscopic investigations on oxygen evolution. However, this approach is problematic in the case of strong S-state mixing or if spectra were collected for only a small number of flashes. In contrast to Ono et al. [35], Roelofs et al. [36] and later Iuzzolino et al. [37] determined the S-state population by an independent method. They exploited the period-four pattern in the magnitude of

![Fig. 3. Flash number dependence of the magnitude of the S2-state multiline signal. The magnitude of this EPR signal provides a measure for the extent of S2-state population. The correlation method described in [39] had been used for precise determination of the EPR signal magnitude. Simulation of the shown S-state pattern allows determination of the miss probability (M) and double hit probability (D), and thus of the relative S-state populations as obtained by application of a given number of flashes. The actual samples previously analyzed by EPR are later used for X-ray measurements.](image1)

![Fig. 4. X-ray absorption edges for pure S-states (as obtained by deconvolution). Spectra measured for angles between X-ray electric field vector and sample normal of 15° and 55° are shown in the upper and lower panels, respectively. We find the highest pre-edge magnitude in the S3-state, approximately the same intermediate magnitude in S1 and S2, and the clearly lowest magnitude in the S0-state. The broken lines in upper and lower panels indicate absorption values used for definition of edge energies. The two dotted lines in the lower panel indicate the integration boundaries for the method of edge energy determination described in [11]; using this integral method, the edge shift upon S-state transitions corresponds to the area framed by the two dotted lines and two X-ray edges. Using different methods to determine edge positions [11,37] ((1) integral method of [11], (2) half-of-norm-height, (3) half-of-peak-height and (4) inflection point approach), the following edge shift values are obtained for the individual S-state transitions (55° data set): 0.8, 1.0, 1.1 and 2.3 eV (S0→S1); 0.7, 0.8, 0.7 and 0.9 eV (S1→S2); 0.7, 0.9, 0.6 and 0.6 eV (S2→S3).](image2)
an EPR signal associated with the $S_2$-state for determination of the S-state population distribution obtained by zero, one, two and three flashes (Fig. 3). Defining the edge position as the inflection point energy, Roelofs et al. [36] found for the $S_1$–$S_2$ transition an edge shift of 1.8 eV and for the $S_2$–$S_3$ transition an edge shift of only 0.2 eV, whereas we observed that for all oxidizing S-state transitions the X-ray edge shift is of comparable magnitude [37]. Taking into consideration the likely error ranges, we concluded that the $S_2$–$S_3$ edge shift is not significantly smaller than the $S_1$–$S_2$ edge shift. Evaluation of new data sets has confirmed this conclusion. For the spectra shown in Fig. 4, some approaches to determine edge positions result in a slightly smaller shift for the $S_2$–$S_3$ transition than for the $S_1$–$S_2$ transition, but the $S_2$–$S_3$ edge shift is never significantly smaller (see legend of Fig. 4). Taking into consideration the likely error ranges, these results agree reasonably well with our previously published results [37]. Therefore, in contrast to Roelofs et al. [36] and in accordance with Ono et al. [35], we believe that XAS results are suggestive of Mn-centered oxidation upon the $S_2$–$S_3$ transition. A more detailed discussion of potential reasons for the conflicting XAS results and the relations to methodical problems can be found in [11,37].

As discussed below, the $S_2$–$S_3$ transition involves significant structural changes but no dramatic rearrangement of the complex. We suppose that due to the relatively limited extent of the observed structural changes, the relation between XAS edge energy and Mn oxidation state is not significantly affected. The type of structural change suggested by analysis of EXAFS spectra seems to be in agreement with Mn oxidation upon the $S_2$–$S_3$ transition (see below).

4. Structural changes as revealed by the EXAFS

In 1998, we have presented preliminary EXAFS results on the Mn complex in its $S_3$- and $S_0$-state [38]. In the meantime, data evaluation has been completed. In the following, exclusively these recent EXAFS results (see Figs. 5 and 6) are discussed. Other results differing with respect to the $S_1$-state EXAFS data [41] have been reviewed elsewhere ([3], see also contribution of Klein and coworkers in this issue). A discussion of potential reasons for discrepancies is beyond the scope of this minireview.

EXAFS simulations (curve-fitting) indicate that the first Fourier peak increases with increasing oxidation state of the Mn complex due to decreasing Mn–ligand distance spread. The average Mn–ligand distance is slightly diminished by each oxidizing S-state transition, a behavior explainable by Mn-centered oxidation upon all of the investigated oxidizing S-state transitions. (However, the distance shortening is observed only if constraints are used to enforce solutions which we consider chemically reasonable. This approach is problematic since there lurks the danger of misleading bias.) In the $S_0$-state, the pres-

![Fig. 5. FTs of EXAFS spectra collected at the magic angle of linear dichroism spectroscopy (~55°). Upon the $S_2$–$S_3$ transition, the magnitude of the second Fourier peak is significantly increased; an even more pronounced increase is observed at an excitation angle of 15° (see Fig. 6).](image-url)
ence of Mn–ligand distances above 2 Å is clearly indicated by the EXAFS data, a finding which is in agreement with the previously proposed presence of Mn(II) in the S₀-state complex [37,42,43]. In any event, there are no indications of ‘unusual’ changes in Mn–ligand distances that could invalidate the edge position approach for determination of Mn oxidation states.

Due to the problems associated with analysis of absorber–backscatterer distances exceeding 3 Å, presently no statement is possible with respect to changes of related structural features.

The changes in the 2.7 Å EXAFS oscillations associated with the second Fourier peak (peak II) are the most conclusive and meaningful aspect of the S-state cycle data. In S₁ and S₂, peak II is of comparable magnitude (Fig. 5). (The second FT peak is slightly smaller in the S₂-state. Curve-fitting reveals that this difference in the peak magnitude is explainable by an increased distance spread and not caused by a change in the number of backscatterers.) Preferential population of the S₃-state by application of two flashes results in an increased second Fourier peak (of the ‘convoluted’ two-flash spectra). Curve-fitting of the pure S₁-state spectra (as obtained by deconvolution) indicates that the number of 2.7 Å Mn–Mn EXAFS interactions (per tetranuclear complex) is increased from two in the S₁- and S₂-state to three in the S₃-state; the mean Mn–Mn distance is increased from ~2.71 Å in the S₁- and S₂-state to ~2.75 Å (see Figs. 5 and 6). Noteworthily, a simulation of the S₃-state data assuming the presence of only two 2.7 Å interactions fails because this approach results not only in low-quality fits but also in physically unreasonable parameters (clearly negative value for the Debye–Waller parameter describing the distance spread). The change in the EXAFS dichroism (Fig. 6) confirms that during the S₂–S₃ transition a new 2.7 Å Mn–Mn EXAFS interaction

Fig. 6. Excitation angle (θ<sub>EN</sub>) dependence of the apparent coordination numbers (N<sub>app</sub>) for the 2.7 Å EXAFS interaction. The apparent coordination numbers were obtained by simulation of the individual k-space EXAFS spectra measured at the indicated angle; Fourier isolation was not employed. The solid line is obtained by simulation of the angle dependence of apparent coordination numbers using the corrected XALDS approach described in [29]. The thus determined ‘isotropic’ coordination number, N, and the mean angle between absorber–backscatterer pairs and the membrane normal direction, θ<sub>R</sub>, are shown in the insets.
(meaning an additional vector of 2.7 Å length connecting two Mn ions) is created.

Based on these EXAFS results, we have proposed that the PSII Mn complex in its S₃-state is characterized by a sequential arrangement of Mn(IV) ions (in form of an open chain) with Mn ions pairwise connected by three di-µ-oxo bridges [38] as shown in Fig. 7. Philouze et al. reported synthesis of a tetranuclear Mn complex with a corresponding Mn₄((µ₂-O)₂)₃ core [44,45]. For this complex, the average value of the three Mn–Mn distances in the 2.7 Å range is 2.75 Å; this value is equal to the value we obtained for the Mn complex of PSII in its S₃-state. In the light of current model chemistry, the proposed Mn₄((µ₂-O)₂)₃ core structure seems to be the most probable of various alternative structural models for the manganese complex in its S₃-state; no distinctly different model agrees equally well with our EXAFS results.

In the synthetic tetranuclear complex of [45], the Mn–Mn distance is slightly longer than typically observed in binuclear di-µ-oxo-bridged complexes. This has been proposed to result from a trans effect of µ-oxygens at the two intermediate Mn ions [45]. However, also the bulky ligands of the model compound might explain the relatively long Mn–Mn distance. Less bulky terminal ligands or additional bridging carboxylates might result in shorter Mn–Mn distances for protein-bound Mn₄((µ-O)₂)₃ core. Presently we cannot exclude that in the S₃-state of the PSII Mn complex the mean Mn–Mn distance is ~2.75 Å because one out of six bridging oxides is protonated (and, consequently, one out of three Mn–Mn distances is lengthened to ~2.8 Å; see also Fig. 9B).

In the S₀-state, the height of the second Fourier peak is significantly lower than in the S₁- or S₂-state. (Using a different experimental protocol for preferential population of the S₀-state, Messinger et al. [46] obtained the same result.) In EXAFS spectroscopy, the height of a Fourier peak is proportional to the number of backscattering atoms at the respective distance. On the other hand, distance spread reduces the peak amplitude. These two determinants of the peak’s amplitude (i.e. coordination number and distance spread) affect the oscillations in k-space (wavenumber scale), which are usually subjected to curve-fitting, in distinctly different ways. Therefore, often the determination of both, coordination numbers...
and distance spread, is possible. However, discrimination between changes in the number of backscatterer versus changes in the distance spread is problematic in case the peak’s amplitude is relatively small (in comparison to neighboring Fourier peaks) or in the presence of not fully negligible noise contributions. Therefore, the S₀ results are more ambiguous than the S₁ results. For the Mn complex in its S₀-state, curve-fitting results are suggestive that increased distance spread is the more likely origin of the reduced amplitude of peak II (reduced in comparison to, e.g., the S₁-state). However, the possibility that a decreased number of backscatterers is the cause for the decreased peak magnitude is not fully excluded. For the S₀-state we find a coordination number close to one (meaning two Mn–Mn interactions per tetranuclear complex), a mean Mn–Mn distance of 2.75 Å and a σ value of ∼0.06 Å. These fit results suggest that in the S₀-state there is one 2.69 Å and one 2.81 Mn–Mn interaction (Fig. 8), a result which is in reasonably good agreement with [46]. A Mn–Mn distance of ∼2.81 Å is anticipated for a binuclear Mn structure with one μ-oxo and one μ-hydroxo bridge [47].

5. Hypotheses on the oxidizing transitions

5.1. S₂–S₃ transition

In the following, it is assumed that the S₁ and the S₂ structure resemble the Berkeley model [9,24] with respect to the number of μ-oxo bridges between Mn ions (two di-μ-oxo bridges and one mono-μ-oxo bridge). Our EXAFS data imply that upon the S₂–S₃ transition, the mono-μ-oxo bridge is transformed to a di-μ-oxo bridge. (However, as discussed above, one of the six μ-oxo bridges present in the S₁-state might be protonated.) We hypothesize that this transformation involves deprotonation of a terminally ligated hydroxide or water, and that it is facilitated by the transition from five-coordinated Mn(III) to six-coordinated Mn(IV) as depicted in Fig. 9.

This hypothesis on the S₂–S₃ transition is in full agreement with the discussed XAS data, but it is not uniquely implied by the XAS results. It was chosen because it represents a straightforward way to account for our results and results obtained by other methods. According to the above hypothesis: (1) the S₂–S₃ transition is distinguished from the other oxidizing S-state transitions between semi-stable S-states by a more significant modification of the complex structure; (2) the transition is closely related to a deprotonation event; (3) the Mn oxidation upon the S₂–S₃ transition is coupled to an increase in the coordination number of one Mn from 5–6; (4) the number of μ-oxo (or perhaps μ-hydroxo) bridges is increased in the more oxidized state of the protein-bound metal complex. These four aspects of the XAS-based hypothesis are in agreement with insights previously obtained by other means.

1. Kinetically, the S₂–S₃ transition differs clearly from the S₀–S₁ and S₁–S₂ transitions. It is characterized by the highest activation energy [48,49]. Estimates of the S₂–S₃ reorganization energy are suggestive that (only) the S₂–S₃ transition is coupled to significant structural changes [48]. It has been proposed that, in clear contrast to the ‘electron transfer-limited’ S₁–S₂ transition, the S₂–S₃ transition rate is limited by ‘molecular vibrations’ (whereas the S₀–S₁ transition seems to be an intermediate case) [49]. Furthermore, the accessibility of the Mn complex to exogenous reductants decreases by a factor of 10–20 upon the S₂–S₃ transition, suggesting a significant change in the electronic configuration and nuclear geometry [50]. We propose that upon the S₂–S₃ transition, the conversion of coordinatively unsaturated Mn(III) to coordinatively saturated Mn(IV) (see Fig. 9) explains the reduced reductant accessibility.

2. Only the S₂–S₃ transition exhibits a significant H₂O/D₂O kinetic isotope effect (on transition rate and activation energy); only the S₂–S₃ transition rate is (weakly) pH-dependent ([49] and references therein). Furthermore, only the S₂–S₃ transition is coupled to an obligatory, pH-independent release of a single proton occurring concomitantly to electron transfer to the Tyr T2 radical [49,51–53]. Consequently, it has been concluded that this S-state transition is ‘kinetically steered’ by a reaction involving a proton. In contrast to the S₁–S₂ transition, the S₂–S₃ and the S₀–S₁ transitions seem to involve no change in the charge of
the OEC [49,52,54], a finding that is explainable by deprotonation of the Mn complex concurrent with Mn oxidation.

3. Results obtained by simulation of EPR spectra [55,56] and quantum chemical calculations [12,57] indicate that the presence of five-coordinated Mn(III) in the S2-state is likely or, at least, imaginable. As discussed above, we and others have presented evidence that the S2–S3 transition involves oxidation of Mn(III) to Mn(IV). The preferred coordination mode of Mn(IV) is octahedral (with six first-sphere ligands). Thus, it is conceivable that formation of an additional μ-oxo bridge upon the S2–S3 transition is facilitated by a change from five- to six-coordinated Mn as shown in Fig. 9.

4. The tetranuclear PSII Mn complex is unique; presumably, there are no closely related protein-bound metal complexes. Nonetheless, a glance on the binuclear Mn catalase and (sulphur-free) binuclear iron complexes may be instructive. On the basis of EXAFS data, it has been proposed that the oxidized states of the Mn catalase differ from a more reduced state by the presence of additional μ-oxo bridges between Mn ions (see [13]). For binuclear iron complexes [58], oxidation state...
changes seem often to be related to changes in the bridging mode. For the diiron center of the ribonucleotide reductase, it has been demonstrated by protein crystallography that reduction of the metal center is coupled to dissolution of a μ-oxo bridge and to a decrease in the coordination number of one of the involved iron ions [59].

5.2. S₀–S₁ transition

Based on the EXAFS results, we propose that the S₀–S₁ transition involves deprotonation of a μ-oxo-μ-hydroxo bridge resulting in shortening of one Mn–Mn distance from 2.8 to 2.7 Å. Oxidation of synthetic Mn₂(μ₂-O)(μ₂-OH) complexes results in a pK shift of the μ-OH bridge by about 10 units [60]. Thus, the proposed deprotonation upon Mn oxidation comes close to be a chemical necessity. (In the context of the stimulating hydrogen abstraction model of Babcock and coworkers [32,33], it has been investigated and repeatedly discussed why Mn oxidation linked to ligand deprotonation is energetically particularly favorable [60–62].) For PSII core particles, pH-independent release of a single proton is indeed observed for the S₀–S₁ transition [52,53]. Furthermore, electrochromic absorption changes are indicative that also upon the S₀–S₁ transition no charge is accumulated by the OEC, an observation which is in agreement with deprotonation of the Mn complex [52,54,63]. However, the rate constant of the S₀–S₁ transition is almost pH-independent; and the D₂O/H₂O kinetic isotope effect is relatively weak [49]. To explain these findings, we assume that the proposed μ-oxo deprotonation is not rate limiting. It remains to clarify whether the relation between Mn oxidation and μ-hydroxo deprotonation is describable as hydrogen atom abstraction. In summary, insights obtained by model chemistry and kinetic investigations rather confirm than disprove the proposed structural changes upon the S₀–S₁ transition.

5.3. S₁–S₂ transition

As judged by EXAFS spectroscopy, the S₁–S₂ transition is not coupled to any clear structural change. (There are small, but fully reproducible differences between S₁- and S₂-state spectra. These differences are, possibly, explainable by minor changes in Mn–ligand distances resulting from oxidation of one Mn ion.) Deprotonation of di-μ-oxo bridges upon the S₁–S₂ transition can be excluded. Deprotonation of other Mn ligands or charge rearrangements involving, e.g., Cl or Ca might be invisible for EXAFS spectroscopists. The observed accumulation of a positive charge at (or near to) the OEC [52,54,63] could indicate that the S₁–S₂ transition is not coupled to any charge-compensating ligand deprotonation.

5.4. Oxidizing transitions – summary

The proposed μ-oxo deprotonation upon the S₀–S₁ transition and the proposed mechanism for formation of an additional μ-oxo bridge upon the S₂–S₃ transition are in agreement with a large body of experimental results. Further experiments are required to validate (or disprove) the presented working hypotheses. Various aspects of water oxidation at the PSII Mn complex potentially related to structural changes have not been addressed (e.g. the role of Cl and Ca) because they seem to be not closely related to the here discussed EXAFS results. Likewise, the XAS data do not allow any specific hypothesis on the coupling between electron and proton extraction (by the TyrZ) upon the oxidizing S-state transitions.

According to the structural model depicted in Fig. 7, a single tetranuclear Mn complex is present in the S₃-state (as opposed to two spatially separated binuclear Mn units [31]). The S₃-state EXAFS data are not in agreement with the proposed adamantane [64] or cubane structure [65,66]. The observation of structural changes occurring upon the S₂–S₃ and S₀–S₁ transitions is not in agreement with some aspects of the hydrogen atom abstraction model (hydrogen abstraction by TyrZ) as proposed by Babcock and coworkers [32,33].

6. The oxygen-evolving S₃–S₀ transition – a hypothesis

The EXAFS results discussed above imply that the water-oxidizing S₃–(S₄)–S₀ transition involves dissolution or modification of two μ-oxo bridges. It is
tempting to interpret these results as an indication that O–O bond formation occurs between bridging oxides as proposed, e.g., in [67]. This hypothesis, however, would be difficult to reconcile with the results of the isotope exchange experiments [68,69] indicating $S_3$-state exchange rates of $40 \text{s}^{-1}$ and $2 \text{s}^{-1}$ for the two oxygen atoms of the dioxygen released during the $S_3$–$S_0$ transition. This relatively rapid exchange is highly suggestive that the $W$-oxygens of the $S_3$-state complex are not released in the form of dioxygen. Also, results of EPR experiments using isotopically labeled water seem to exclude O–O bond formation between $W$-oxygens [70]. We hypothesize that, as discussed in the following, bridging oxides of the PSII Mn complex serve a distinctly different function.

As pointed out by Krishtalik [71], water oxidation occurring at a protein site, which is not freely accessible to the solvent, lacks the entropic free energy gain resulting from ‘dilution’ of released protons. However, strong bases immediately accepting the released protons may improve the situation significantly. The lower the $pK$ of the proton accepting bases, the higher is the free energy gain. This however leads to a dilemma: low-$pK$ bases will tend to be already protonated. Krishtalik [71] already proposed a solution. The proton accepting bases are Mn ligands, which automatically turn into strong bases when Mn becomes reduced upon the water-oxidizing transition. Taking into consideration the discussed EXAFS results on the Mn complex in its $S_3$- and $S_0$-state, we propose that, in the course of the oxygen-evolving (and Mn-reducing) $S_3$–$S_0$ transition, $W$-oxo bridges serve as proton accepting bases.

Investigations on synthetic Mn complexes indicate that upon Mn reduction the $pK_a$ of bridging oxides drops by 8–11 units [60]. Protonation of such strongly basic $W$-oxos may result in a significant gain.
free energy gain (about $pK_B \cdot 60$ meV per base; 60 meV corresponds to $\sim 1.4 \text{kcal/mol}$). Thus, the protonation of bridging oxides could improve significantly the free energy balance of the oxygen evolution step. With respect to free energy considerations, the sequence of electron and proton movements (or the extent of coupling) is irrelevant. Kinetically, however, concerted Mn oxidation and $\mu$-oxo protonation are likely to be favored. Thus, it is conceivable that the relation between Mn reduction and $\mu$-oxo protonation is best described as hydrogen atom transfer from substrate water to the core of the Mn complex.

To illustrate how $\mu$-oxo protonation might facilitate water oxidation, a specific hypothesis on the mechanism of O-O bond formation is sketched in Fig. 10. This hypothesis is based on the EXAFS results discussed above and on the insights in the character of the two substrate oxygen as obtained by isotope exchange experiments [68,69].

The terminally coordinated oxide (or oxyl) in Fig. 10 is the slowly exchanging substrate oxygen ($O_{slow}$) described in [68]. (In the S$_3$-state, this slowly exchanging oxygen might be present in the form of a terminally coordinated hydroxide.) The rapidly exchanging dioxygen ($O_{fast}$) is an outer-sphere water molecule presumably bound by multiple hydrogen bonds to the Mn complex. In the S$_4$-state, the terminally ligated substrate oxygen is assumed to have a significant radical character. The O-O bond formation involves $O_{fast}$ oxidation and Mn reduction coupled to proton transfer to $\mu$-oxygen. Conceptually this process can be considered to be a hydrogen atom transfer from substrate water to the Mn complex. The resulting outer-sphere hydroxyl radical is optimally positioned to enable O-O bond formation due to favorable orbital overlap with the singly occupied Mn-O$^*$ $\pi$-orbital [57]. The thus formed hydroperoxo ligand is transformed into a peroxy radical by reduction of Mn and the second $\mu$-oxo protonation. Subsequently or concomitantly to this second hydrogen atom transfer, oxidation of the peroxy radical by Mn results in formation and release of dioxygen. The depicted events do not necessarily occur sequentially. Concerted multi-electron and proton transfer events are conceivable because the proposed mechanism involves only a minimum of nuclear movements. (For the same reason, reasonably low activation energies are anticipated.)

Perhaps needless to say, the above specific hypothesis is meant to be an illustration of the proposed role of hydrogen atom extraction by the Mn complex during the S$_3$–S$_0$ transition. In the future, new experimental results and theoretical insights may require refinement, modification or even a total reformulation. However, taking into consideration the discussed EXAFS results and insights obtained by various other methods, we consider it likely that proton extraction by bridging oxides during the oxygen-evolving transition is a key feature of photosynthetic water oxidation.

7. Perspectives

The above discussion of recent progress demonstrates that by XAS relevant information on the active center of photosynthetic water oxidation is obtainable for four intermediate states of the functional cycle. Efforts are required to unravel the reasons for conflicting results obtained in different laboratories on the S$_3$-state and on Ca contributions to the EXAFS (assignment of the third FT peak). Collection of XALDS data of particular high quality could allow distinguishing between presently alternative structural models (e.g. different isomeric forms, presence of one singly protonated di-$\mu$-oxo bridge in the S$_3$-state).

Recently it has been demonstrated that high-quality room temperature XAS investigations on PSII particles are feasible if an appropriate experimental strategy is used [25]. Room temperature XAS investigations open a new road to gain insights in the structure–function relationships involved in photosynthetic water oxidation. Structural investigations under defined (e.g. with respect to pH) and ‘almost native’ conditions become viable; the ambiguity with respect to the relevance of low-temperature results is avoidable. In the future, time-resolved XAS investigations, which require experiments at or close to room temperature, may provide further insights in the mechanism of photosynthetic water oxidation.

In the near future, a crystallographic model of the intact PSII (including the Mn complex) may become available. Taking into consideration the size of this membrane–protein complex, the resolution may not suffice to resolve details of the Mn complex (e.g.
bridging ligands and their protonation state). However, discrimination between structural isomers and identification of amino acid ligands will allow a significantly more focused EXAFS data evaluation (for example by combination of EXAFS simulations and molecular modeling approaches). Eventually, the combination of results obtained by protein crystallography, by XAS and insights obtained by other approaches could lead to unique structural models for at least four intermediate states in the functional cycle of the PSII Mn complex.

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