

**Structure of the Mn complex involved in photosynthetic water oxidation:
New insights derived from X-ray absorption spectroscopy.**

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Introduction

Many topological structures have been proposed, on the basis of EXAFS (Robblee *et al.* 2001; Dau *et al.* 2001; Penner-Hahn 1998) and EPR (Peloquin and Britt 2001, Hasegawa *et al.* 1999) studies, for the Mn cluster in Photosystem II (PS II). The issue of whether there are two, or three di- μ -oxo bridged moieties in the Mn cluster has been an open question. However, a determination of the exact number of such interactions is important for narrowing the number of options from the many that have been proposed. In this study we present evidence for the presence of three di- μ -oxo bridged motifs that are characterized by the ~ 2.7 Å Mn-Mn distance.

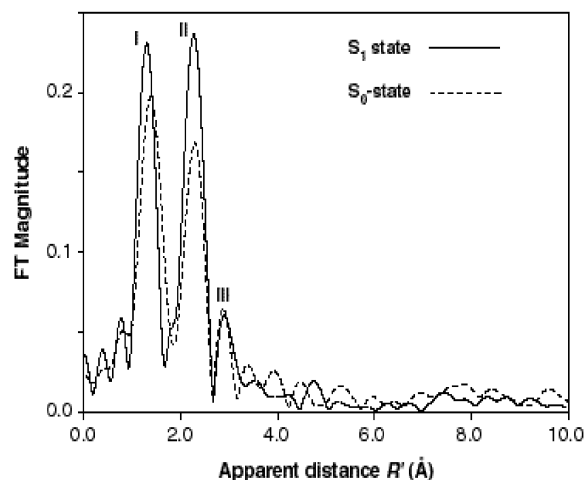
Materials and methods

PS II membranes were prepared using the BBY protocol. The S₀ samples were generated by three flash illumination followed by incubation with FCCP (Messinger *et al.* 1997; Robblee 2000). The multiline EPR spectra of the S₀ and S₂ states were used to characterize all the samples. The X-ray spectra were collected at SSRL on beamline 7-3. Eight scans were collected at each sample position (two positions per sample, six samples per S-state) and sample integrity was monitored by comparing the last two XAS scans with the first two scans at each position of the sample. There were no major differences in the X-ray spectra between the two sets. The details of the S₀ state sample preparation, deconvolution of the S₀ state spectra, and the data analysis are described in Robblee 2000.

Results and Discussion

Figure 1 shows the Fourier transforms of the k^3 -weighted EXAFS data from the S₀ and S₁ states. There are three Fourier peaks in the data from both the states. However, there are distinct differences in the Fourier peaks in the two states. Fourier peak I is backscattering from bridging and terminal ligand atoms of Mn between 1.8 - 2.2 Å, and peak II is backscattering from neighboring Mn atoms at ~ 2.7 Å. The decrease in amplitude of peak II in the S₀ state indicates either a decrease in the number of Mn-Mn interactions or the presence of Mn-Mn interactions at more than one distance. Peak III is Mn-Mn/Ca interactions at 3.3-3.4 Å. We will focus on Fourier peak II in this report.

Fig. 1. Fourier Transforms of the S_0 (dashed line) and S_1 state (solid line) of the k^3 -weighted Mn-EXAFS spectra (3.5 to 11.5 \AA^{-1}). The main Fourier peaks are labeled I, II and III. There are clear differences in amplitude and position of these peaks between the S_0 and S_1 states. The peak labeled II is from Mn-Mn interactions at ~ 2.7 \AA .



The Fourier peak II was isolated and fit to the EXAFS equation. The best fits are shown in Table 1. N is the number of Mn backscattering atoms from each Mn atom in the complex. Hence, an N of 1 translates to two Mn-Mn interactions and an N of 1.5 to three Mn-Mn interactions in a tetranuclear complex. If there is only one Mn-Mn distance, then it is possible to derive only one number for the number of such interactions. The best number that we could obtain is 1.30 for the S_0 and 1.26 for the S_1 states, at 2.73 and 2.72 \AA respectively. An N of 1.30 or 1.26 translates to between 2 and 3 Mn-Mn interactions in the tetranuclear Mn cluster. The similarity of these numbers in the S_0 and S_1 states indicates that the number of Mn-Mn interactions is unchanged between the states; however, the differences in Debye-Waller factors indicate the presence of distance heterogeneity in the S_0 state.

The bottom part of Table 1 shows the best fits with two Mn-Mn distances for the S_0 and S_1 states. Since there has to be an integral number of Mn-Mn interactions, the fits shown in Table 1 are constrained to a 1:1 and 2:1 ratio for the two Mn-Mn interactions. The fits for the S_1 state show that the two distances are separated by significantly less than what can be resolved using the data set at present, and χ^2 , the quality-of-fit parameter, does not improve between the one- and two-shell fits. However, the situation is very different for the fits for the S_0 state. The χ^2 value improves considerably on going from a one-shell to a two-shell fit, especially, for the case of the 2:1 ratio of Mn-Mn interactions. These fit results provide evidence for the presence of three Mn-Mn interactions in the Mn cluster.

Figure 2 shows the best fits to two Mn-Mn distances for the S_0 state as a function of the number of each of the Mn-Mn interactions. The contour plot graphically illustrates that the global minimum at two Mn-Mn interactions at 2.7 \AA and one Mn-Mn interaction at 2.85 \AA is very well defined, and hence gives us confidence in its reliability. We have re-examined the data from the S_0^* , the $g=4.1$ S_2 , the NH_3^- or F^- -inhibited S_2 , and the S_3 states, where we have shown that there is distance heterogeneity in the Mn-Mn vectors. In each case, the ratio of the number of the two Mn-Mn interactions was $\sim 2:1$. This leads us to think that there may be three Mn interactions at ~ 2.7 \AA in the Mn cluster in its native state, one of which is perturbed in the S_0 , F^- and NH_3^- -inhibited S_2 and the $g=4.1$ S_2 states, and all three are perturbed in the S_3 state.

We showed earlier that there are several topological models that are compatible with our EXAFS data. These models have been described in detail (DeRose *et al.* 1994; Robblee *et al.* 2001). However, only one of those options (A) is widely used as a working model, although options E, F and G (the nomenclature is from DeRose *et al.* 1994) have been shown to be preferred on the basis of EPR and ENDOR simulations (Peloquin *et al.* 2001; Hasegawa *et al.* 1999). On the basis of the present results from the S_0 state, we think it is important to consider the options that include three di- μ -oxo bridged moieties in the Mn cluster. Figure 3 shows three such options, G, I, and J, that were proposed earlier, and two newer options, L and M. Options J and I are less likely structures because J lacks a Mn-Mn interaction at 3.3 Å, and I has two such interactions. The EXAFS data

Fig. 2. The contour plot shows the fit-quality-parameter ϵ^2 for the S_0 state plotted versus the number of the two Mn-Mn interactions. The dashed lines shows the distinct minimum at one Mn-Mn vector at 2.85 Å and two Mn-Mn vectors at 2.7 Å.

Table 1: One- and two-shell simulations of Fourier peak II of the S_1 and S_0 state samples.

One Mn-Mn Shell				
Sample	Shell	R (Å)	N	ε^2 (x 10 ⁵) ^a
S ₀	Mn–Mn	2.73	1.30	0.48
S ₁	Mn–Mn	2.72	1.26	0.63

Two Mn-Mn Shells				
Sample	Shell	R (Å)	ε^2 (x 10 ⁵) ^a	
$N_I:N_2 = 1:1$				
S ₀	Mn–Mn	2.68	0.44	
	Mn–Mn	2.78		
S ₁	Mn–Mn	2.70	0.63	
	Mn–Mn	2.74		
$N_I:N_2 = 2:1$				
S ₀	Mn–Mn	2.72	0.30	
	Mn–Mn	2.85		
S ₁	Mn–Mn	2.70	0.62	
	Mn–Mn	2.77		

^a ϵ^2 is quality-of-fit parameter; ΔE_0 was constrained to be equal for all shells within a fit; N , N_1 and N_2 are the number of Mn-Mn distances; Debye-Waller factor, σ^2 , was 0.004 Å² for the S_0 and 0.002 Å² for the S_1 state for the one shell fits (not fixed), and was fixed at 0.002 Å² for all two-shell fits.

from an inorganic compound (Auger *et al.* 1990) with the motif in option J is very different from that obtained from a PS II sample (unpublished data). Options G, L and M all have three di- μ -oxo bridges and one mono- μ -oxo bridge; that is three 2.7 Å and one 3.3 Å Mn-Mn interaction.

We have not considered the presence of the Mn-Ca interaction in these models. We have obtained data using Ca/Sr X-ray spectroscopy that shows the proximity of the Ca cofactor to the Mn cluster (Cinco *et al.* this issue).

The structure of the PS II complex at a resolution of 3.8 Å was recently published based on X-ray crystallography (Zouni *et al.* 2001). The electron density from the Mn cluster has been identified.

Options G, L and M are qualitatively in agreement with the asymmetry seen in the electron density of the Mn cluster. Options L and M are also similar to the structure proposed by Siegbahn (2000) on the basis of density functional theory calculations. We are in the process

of testing the compatibility of various Mn models that satisfy the criteria set by the distance data and orientation of the various Mn-Mn/Ca vectors obtained from EXAFS with the electron density data obtained from X-ray crystallography.

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Fig. 3. Structural models for the Mn cluster with three di- μ -oxo bridged Mn moieties. Options G, I, and J are from DeRose et al., 1994, and L and M are two other viable options. One or two mono- μ -oxo bridged motifs are also present in Options G, I, L, and M. No such motif exists in option J.