

Polarized Range-Extended X-Ray Absorption Spectroscopy of Oriented Photosystem II Membranes in the S₁ State

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Abstract. Detailed information about the orientation of particular Mn-Mn and Mn-Ca vectors in the oxygen evolving complex (OEC) of the Photosystem II in the S₁ state provide a critical starting point for the analysis of the structural changes in the OEC along the catalytic S_n-state cycle. The method of polarized range-extended EXAFS is an important technical development, that allows: i) resolution of the 2.7 Å and 2.8 Å Mn-Mn interactions; ii) resolution of 3.2 Å Mn-Mn and 3.4 Å Mn-Ca; iii) determination of 2.7 Å, 2.8 Å, 3.2 Å Mn-Mn and 3.4 Å Mn-Ca vectors orientation relative to the membrane normal.

Keywords: XAS, Photosynthesis, Photosystem II, Oxygen Evolving Complex, (OEC), range-extended EXAFS.

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INTRODUCTION

Water oxidation by green plants, algae, and cyanobacteria is a light-driven reaction which is catalyzed by the oxygen-evolving complex (OEC) in Photosystem II (PS II). The active site of the OEC is known to be a protein-bound cluster containing four manganese and one calcium atom [1]. X-ray absorption spectroscopy has provided important insights into the structure and function of the Mn₄Ca cluster in the OEC of PS II. Recently we presented polarized Extended X-ray Absorption Fine Structure (EXAFS) measurements on PS II single crystals of the thermophilic cyanobacterium *Thermosynechococcus elongatus* that results in high-resolution structures for the Mn₄Ca cluster [2]. Combining XRD and EXAFS data, the placement of the cluster within the PSII complex was obtained taking into account the electron density of the metal site and the putative ligands. Although PS II from higher plants (spinach) has not yet been crystallized, one-dimensionally oriented samples (oriented membranes) can be prepared. The range of Mn EXAFS data that can be collected from PS II is limited by the presence of Fe in PS II. Using a crystal spectrometer with high energy resolution to

detect solely the Mn K α fluorescence, we were able to extend the EXAFS range beyond the onset of the Fe absorption edge [3]. This results in an improvement in the resolution of the Mn-backscatterer distances in PS II from 0.14 Å to 0.09 Å. This enabled us to resolve the heterogeneity in the Mn-Mn distances of solution samples of PS II from spinach in the S₁ and S₂ states, providing firm evidence for three Mn-Mn distances of ~ 2.7 Å and ~ 2.8 Å present in a 2:1 ratio [4]. Here we present the orientation of the Mn-Mn and Mn-Ca vectors with respect to the membrane normal using polarized range-extended EXAFS.

RESULTS

In Figure 1 (A) Fourier transforms (FTs) of the range-extended EXAFS for PS II isotropic solution and oriented membranes in S₁ state are compared. Peaks, labeled I, II, III corresponding to the shells of backscatterers from the Mn absorber. Peak I contains Mn-O bridging and terminal interactions, peak II corresponds to di- μ -oxo-bridged Mn-Mn moieties, peak III has information about mono- μ -oxo-bridged

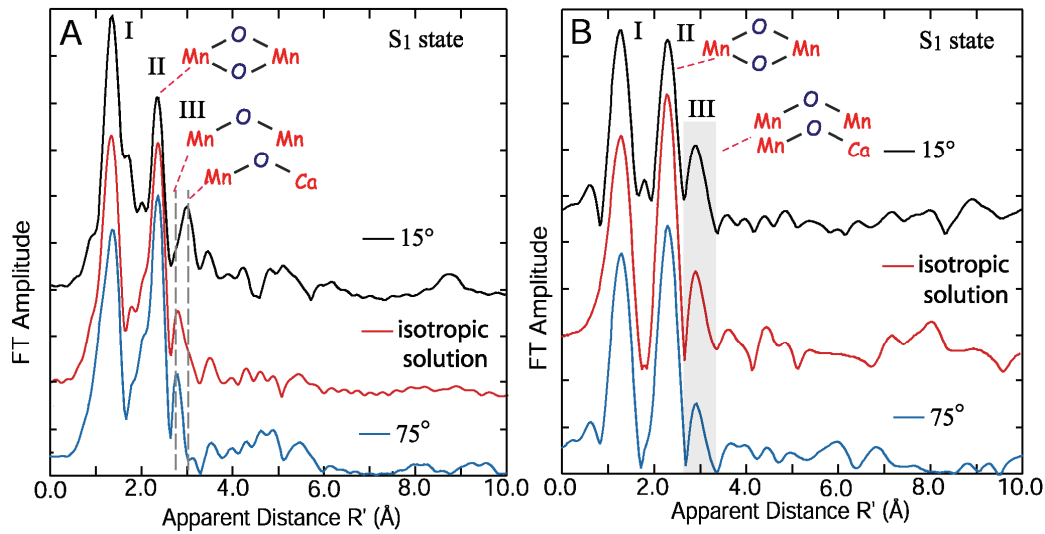


FIGURE 1. A: Fourier Transform (FT) of Mn K-edge EXAFS spectra from isotropic solution and oriented PS II membrane samples in the S_1 state obtained with a high-resolution spectrometer (range-extended EXAFS). Angles indicate orientation of the membrane normal with respect to the X-ray $\mathbf{e}$ -field vector. The k range was $3.5\text{--}15.2\text{ \AA}^{-1}$. B: Fourier transforms of the same data as in A, but the k -range was truncated $11.5\text{ \AA}^{-1}$ for comparison with polarized EXAFS data obtained with conventional EXAFS set-up published earlier [5].

Mn-Mn and Mn-Ca interactions. For comparison, the FTs of the same spectra, but truncated at $11.5\text{ \AA}^{-1}$, are shown in Figure 1 (B). A significant improvement in spectral resolution is observed for the range-extended EXAFS data, Figure 1 (A). Increased spectral resolution results in the detection of the orientation

dependence of peaks II and III. Fits in Table 1 show the results from fitting one and two Mn-Mn shells to peak II of S_1 state samples. Addition of the second Mn-Mn shell (Table 1) results in considerable improvement of the fit quality.

TABLE 1. One- and Two-Shell Fits of Fourier Peak II of PS II (S_1 state) Solution and Oriented Membranes at Angles of 15° and 75° Between the Membrane Normal and the $\mathbf{e}$ -vector of the Polarized X-ray Beam.

Fit #	Angle	Shell	$R\text{ (\AA)}$	N^{**}	$\sigma^2(\text{\AA}^2)*10^3$	ΔE_0	$\Phi(*10^3)$	$\epsilon^2(*10^5)$
One Shell, peak II isolate								
1	isotropic solution	Mn-Mn	2.72	0.98	2*	-18	0.24	0.078
2	isotropic solution	Mn-Mn	2.72	1.21	3.0	-17	0.18	0.060
3	15°	Mn-Mn	2.73	0.94	3.0	-14	0.20	0.068
4	75°	Mn-Mn	2.74	1.48	3.6	-13	0.18	0.063
Two Shell, peak II isolate								
5	isotropic solution	Mn-Mn Mn-Mn	2.72 2.83	1.08 0.33	2* 2*	-14	0.13	0.042
6	isotropic solution	Mn-Mn Mn-Mn	2.71 2.82	0.88 0.43	1* 1*	-14	0.11	0.034
7	15°	Mn-Mn Mn-Mn	2.72 2.82	0.70 0.33	1* 1*	-11	0.12	0.040
8	75°	Mn-Mn Mn-Mn	2.72 2.83	0.97 0.54	1* 1*	-11	0.11	0.036

*parameter was fixed during fitting procedure

** $N = N_{iso}$ for isotropic solution; $N = N_{app}$ for oriented samples

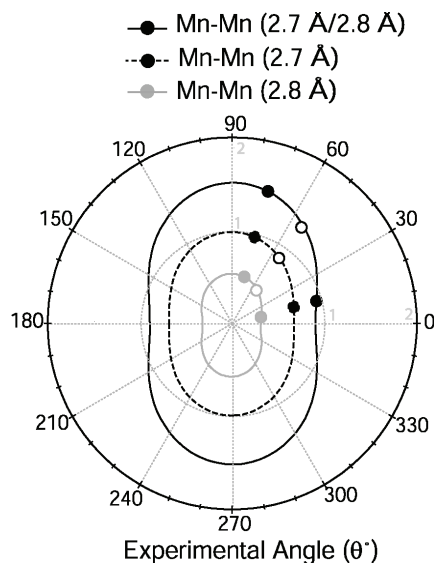


FIGURE 2. Polar plot of the X-ray absorption linear dichroism of PS II samples in the S_1 state. The N_{app} values (solid circles) for oriented membranes and N_{iso} (open circles) for isotropic solution are derived from one or two shell fits (Table 1) and are plotted with their respective detection angles (θ). The best fits of N_{app} vs. θ to the equation in the text are shown for the various Mn-Mn vectors as solid lines that take into account the experimentally determined mosaic spread of $\Omega = 20^\circ$.

The N_{app} found from EXAFS curve-fitting (Table 1) on oriented samples at particular θ is related to the coordination number of an isotropic sample, N_{iso} , by the following equation:

$$N_{app}(\theta) = N_{iso} + \frac{1}{2}N_{iso}(3\cos^2\theta - 1)(3\cos^2\phi - 1)I_{ord}$$

where I_{ord} is the order integral.

Figure 2 shows polar plots of the N_{app} derived from fits in Table 1 and plotted (solid circles) with respect to detection angle (θ). By fitting the angle dependence of the amplitude (N_{app}) to equation (1), the approximate number of backscatterers (N_{iso}) and the average orientation (ϕ) for that shell of scatterers can be determined. Analysis of the orientation dependence of the 2.72 ± 0.02 Å Mn-Mn vector results in $N_{iso} = 0.89 \pm 0.2$ (two Mn-Mn interactions) at an average angle of $\langle\phi\rangle = 61 \pm 7^\circ$, with respect to the membrane normal. The 2.83 ± 0.02 Å Mn-Mn vector has an $N_{iso} = 0.48 \pm 0.12$ (one Mn-Mn interaction) at an angle of $\langle\phi\rangle = 64 \pm 7^\circ$ with respect to the membrane normal. In this way we independently derived that the three Mn-Mn distances of ~ 2.7 Å and ~ 2.8 Å are present in a 2:1 ratio in PS II samples in S_1 state.

Range-extended EXAFS method allowed us to resolve the complex nature of the Peak III containing at least two peaks, IIIA and IIIB, having different distances of 3.2 Å and 3.4 Å. Previous Ca EXAFS

studies of native PS II [6], Sr EXAFS of Sr-substituted PS II [7], and Mn EXAFS of Ca-depleted PS II [5] have shown the contribution of both Mn-Mn (~ 3.2 Å) and Mn-Ca (~ 3.4 Å) vectors to FT peak III. The tentative assignments of peak IIIB to the Mn-Ca vector and peak IIIA to the Mn-Mn vector can be made. The dichroic behavior of the peak IIIB is strikingly similar to that reported previously for the Mn-Sr vector.

A comparison of this structural information with the latest Mn_4Ca cluster models from X-ray crystallography reveals that the models are not consistent with the EXAFS criteria. The high-resolution polarized EXAFS data imposes clear restrictions on the proposed structures, based on spectroscopic and diffraction data, and provides input for refining such structures.

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