

## XAS of Mn in the Photosynthetic Oxygen Evolving Complex

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Nearly all of the dioxygen which animals and aerobic bacteria use during respiration originates from the photosynthetic oxygen evolving complex (OEC) which is found in the chloroplast thylakoid membranes of higher plants and algae. Four specifically bound Mn are required for OEC activity [1]. These manganese do not exhibit the six line epr spectrum which is characteristic of Mn(II) in solution, but instead, a multiline epr signal [2], indicating that at least two, and as many as four, manganese ions form a magnetically coupled cluster in the OEC. A second epr spectral feature from  $S_2$  becomes apparent at  $g_{\text{eff}}$  ca. 4.1 if preparation conditions are slightly altered [3].

In a series of studies [4], Klein, Sauer and coworkers found that the average Mn coordination environment includes an Mn-(N,O) distance of 1.75-1.8 Å, a disordered shell of oxygen or nitrogen at an average distance of 2.15 Å from the Mn, and a Mn-Mn distance of 2.7 Å. The shorter Mn-(N,O) distance was assigned to a bridging ligand and the longer distance to terminal ligands. The observed Mn-Mn distance of 2.7 Å provides additional evidence that at least two manganese ions are in close proximity.

The recently reported procedure for isolating PSII reaction centers using the detergent octylglucopyranoside in the presence of high ionic strength has permitted the preparation of highly purified, highly concentrated samples of the OEC [5]. This procedure has allowed us to prepare samples containing ca. 1.5 mM Mn, thus permitting us to obtain data having a significantly enhanced signal to noise ratio.

**EXAFS.** The  $k^3$ -weighted EXAFS data for two different OEC samples (both in the S1 form) are shown in Figure 1. The high signal/noise ratio and good reproducibility of these data is apparent. The higher quality of these data in comparison with earlier XAS studies of the OEC is attributable to the higher concentrations of Mn in our samples and the use of a solid-state detector array for monitoring x-ray fluorescence.

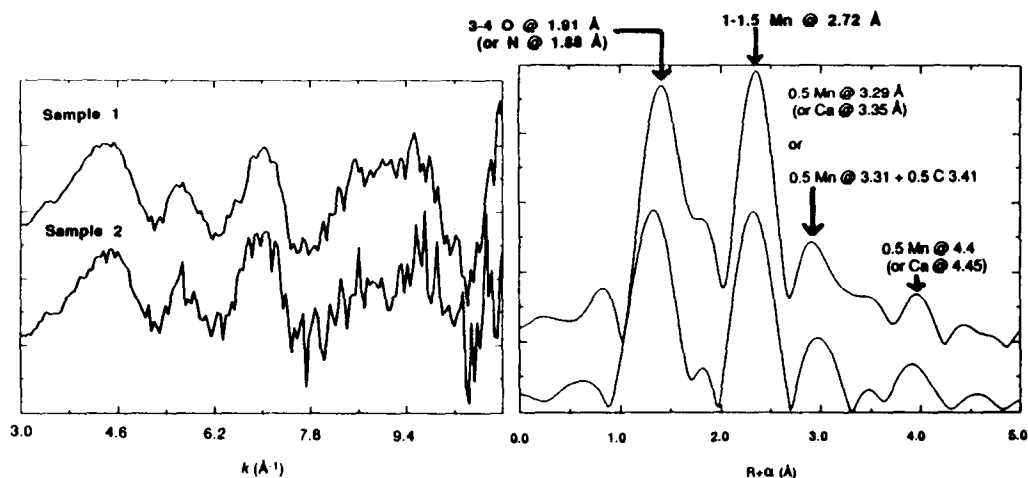
The Fourier transforms (FTs) of the data in Figure 1 are shown in Figure 2. The major features of the FTs are similar to those reported by Klein, Sauer and co-workers. We see three, and possibly four, peaks which are reproducibly present above the noise level of the data. The first two peaks, corresponding to shells of atoms at ca. 1.9 Å and 2.7 Å from the Mn, are identical to those reported in the most recent work by Yachandra *et al.* In addition to these two main peaks, we also see smaller peaks corresponding to atoms at distances of ca. 3.3 Å and 4.5 Å from the Mn.

The first shell peak can be fitted with ca. four oxygens at 1.91 Å. The low first shell coordination number likely reflects the presence of several ligands distributed over a range of longer distances, however we see no

evidence for a split first shell (1.75 + 2.1Å). The second shell peak is well modeled by 1-1.5 Mn at 2.72Å. No improvement in the fit is obtained by including a shell of carbons at ca. 2.7Å. This is not surprising, since 2.7Å Mn-C distances are generally restricted to 5 and 6 membered metallacycles (i.e. Mn(acac)<sub>3</sub>) and are thus unlikely to be present in the OEC. The third peak can be fitted with 0.5-1.0 Mn at 3.31Å. In contrast with the second shell, the fit to this shell can be substantially improved by including ca. one carbon at 3.55Å. The presence of carbon scatterers at this distance is not surprising, and is typical of distances seen for carboxylate, alkoxide and phenoxide ligation. It was not, however, possible to obtain a good fit to the third shell using carbon as the only scatterer. This suggests that there must be at least some contribution of Mn to the EXAFS at this distance, however the presence of both Mn and C significantly decreases the precision of our curve fitting analysis. There appears to be a fourth shell at ca. 4.35Å which is present in both data sets. This shell can be fitted with ca. 0.5 Mn at 4.35Å however it is not possible at present to exclude alternate interpretations for this peak.

*Structural Implications of EXAFS Results.* A variety of models have been proposed as structures for the Mn cluster(s) of the OEC. Given the complexity of the system, it is unlikely that EXAFS alone will ever provide an unambiguous description of the OEC structure. It is important however to examine these models in light of our current data with the goal of determining which, if any, possibilities can be excluded. Our finding that there is no short (1.75Å) Mn-O bond in our samples indicates that there is no unusually short oxo bridge, similar to that found in e.g. [Mn(bpy)O]<sub>2</sub>. Our results do not, however, rule out the possibility of μ-oxo bridges. In fact, the majority of the μ-oxo bridges which have been characterized for Mn complexes have Mn-O distances in the range of 1.85-1.9Å, consistent with our data. A distance of 1.75 Å would require a rather unusual structure, including at least one Mn(IV). Our results, on the other hand, are consistent with a wide range of bridging geometries. The second shell Mn-Mn distance of 2.7Å is typical of that found for oxo or alkoxy bridged species. The third shell Mn-Mn distance of 3.3Å is typical of those seen for oxo, alkoxy, phenoxy and carboxylato bridged species.

Models which invoke tetranuclear, mononuclear/dinuclear, mononuclear/trinuclear and dimers of dimers have been proposed for the OEC. The cubane model is ruled out based on the highly heterogeneous nature of both the XANES (see below) and EXAFS and by the low Mn-Mn coordination number at 2.7 Å (a similar conclusion was recently reached by Klein and co-workers). The butterfly structure is also ruled out by Mn-Mn coordination number, since the butterfly structures should have only one 2.7Å distance. The structural possibilities can be delimited further if one utilizes the EPR spectral data and interpretations of Hansson *et al.* These authors have assigned the g=4.1 signal to a mononuclear Mn(IV) ion. *If this signal does indeed come from a mononuclear center*, then the EXAFS data are only consistent with a mononuclear/trinuclear formulation for the manganese. We can state this conclusively for the following reasons. All examples of higher oxidation state manganese complexes that are separated by 2.7Å exhibit strong magnetic coupling between ions. Since the g=4.1 signal is from a magnetically isolated Mn(IV), it cannot be at a 2.7Å distance from another manganese ion. Because there is a minimum coordination number of 1-1.5 for Mn at 2.7Å, one of the remaining three manganese atoms must see *at least two* Mn atoms at 2.7Å. This necessitates a trinuclear cluster. *If such a cluster were bent*, the 3.3Å peak could be the distance between



**Figure 1** (left). EXAFS data ( $k^3$  weighted) for two OEC samples (S1 state). Top, 17 scans, bottom, 8 scans.

**Figure 2** (right). Fourier transforms of EXAFS data together with curve fitting analysis.

terminal manganese, however other interpretations of this peak are also possible.

*Near Edge Structure.* The edge energy for the OEC is consistent with an average oxidation state of Mn(III), (as noted previously by Klein and co-workers). However comparison of the OEC edge with edges for model compounds shows that the OEC edge is significantly broader. This suggests that the OEC (at least in the S1 state) contains several different Mn environments. We have investigated this observation using an analysis procedure in which the XANES of an unknown is fit by a linear combination of two or more model XANES spectra. The only variable parameters are the fraction of each XANES spectrum that is included in the fit. In calibration tests, this procedure provides oxidation state quantitation with an accuracy of  $\pm 10\%$ . Good fits to the OEC edge were only obtained when Mn(II), Mn(III) and Mn(IV) were all included as models. Regardless of the combination of models used, we consistently obtain 15-25% Mn(II), with the balance Mn(III) and Mn(IV). This supports our qualitative observation that there are several different Mn sites within the OEC, and suggests that one interpretation consistent with the data is a mixed valence II/III/III/IV composition for S1. Alternatively, structural heterogeneity might also be able to account for the breadth of the OEC edge.

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