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MODELING THE Mn_4Ca CLUSTER OF THE WATER-OXIDIZING COMPLEX OF PS II

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INTRODUCTION

The water-oxidation center of PS II is a metallo-enzyme complex that is closely associated with the PS II reaction center (Sauer & Yachandra 2004). The metal cluster contains 4 Mn and 1 Ca atom (replaceable by Sr). On the basis of biochemical evidence (Debus 2001) and recent 3.2–3.7 Å resolution X-ray crystallography (Zouni et al 2001, Kamiya & Shen 2003, Ferreira et al 2004, Biesiadka et al 2004), the metal cluster is associated predominantly with the C-terminal segment of the D1 polypeptide (Psba protein).

In the absence of a high-resolution structure of PS II, information about the cluster arrangement has come largely from EPR and from Extended X-ray Absorption Fine Structure (EXAFS) measurements. Analysis of the multiline EPR signal from the S_2 state fits best to a model where the 4 Mn in the cluster are closely coupled and in a trimer/monomer arrangement of three Mn(IV) atoms and one Mn(III) (Peloquin et al 2000). The three Mn in the core are strongly antiferromagnetically coupled, and the fourth Mn at greater distance is more weakly coupled. This model is compatible also with EPR data for the S_0 and S_1 states (Britt et al 2004). EXAFS spectra of the Mn cluster in the S_1 state show evidence for the presence of Mn-O(N) bonds (vectors) of 1.8–2.0 Å length. Two or three Mn-Mn vectors in the range 2.7–2.8 Å, one or two Mn-Mn vectors at 3.3 Å and Mn-Ca(Sr) at 3.4 (3.5) Å. The 2.7–2.8 Å Mn-Mn vectors are in the range seen for di-μ-oxo bridged pairs of Mn, and the 3.3 Å Mn-Mn vectors are in the range seen in carboxylato-bridged pairs of Mn in model compounds (Yachandra et al 1996). More recently, Mn-EXAFS studies of the cluster in the S_0 state show that one of the 2.7 Å Mn-Mn vectors has lengthened to 2.85Å, providing for resolution of the overlapping component vectors (Robblee et al 2002). Fitting analysis led to the conclusion that there are three components in the 2.7Å EXAFS feature – one that has lengthened and two that remain unresolved at the shorter distance. A lengthening of the Mn-Mn vectors also in the S_3 state had been reported previously (Liang et al 2000).

RESULTS AND DISCUSSION

Information relevant to vector orientations within PS II is available from polarized X-ray absorption (dichroism) measurements. PS II preparations from spinach contain thylakoids that can be layered so as to bring the membranes nearly parallel. Samples prepared in this fashion exhibit pronounced Mn (K-edge) EXAFS dichroism at 2.7 and 3.4 Å associated with Mn-Mn vectors in the cluster (Mukerji et al 1994). The 2.7 Å peak consists of three component vectors that are oriented at an average angle approx. 60° from the membrane normal. The 3.4 Å vectors make an average angle approx. 43° from the membrane normal. Replacement of the cofactor Ca by Sr in PS II preparations from spinach, which were then layered, exhibit pronounced Sr (K-edge) dichroism at 3.5 Å associated with one or two Sr-Mn vectors in the cluster (Cinco et al 2004). Assuming that Sr replaces physiological Ca, the displacement of the Ca−Mn vectors from the membrane normal is at 0° or 23°.

Because information about Mn-Mn and Mn-Ca vector angles is now available, topological models previously discussed can be refined to include the presence of Ca and account for the dichroism data. A survey of MnO minerals has provided not only a clue as to the possible evolutionary origin of the Mn_4CaO_n cluster, but also a rich assortment of possible cluster arrangements (Sauer & Yachandra 2002). The criteria for an acceptable structure, where distances and angles are given for the S_1 state, are (1) three Mn-Mn vectors in the range 2.7–2.8 Å and one or two Mn-Mn vectors approx. 3.3 Å in length with an average 60° angle to the membrane normal, and (2) one or two Mn-Ca vectors approx. 3.4 Å in length and at 0° or 23° from the membrane normal.

The only topologically and chemically reasonable arrangement of four Mn atoms in the cluster satisfying these criteria is for three of the Mn to lie at the vertices of an approximately equilateral triangle 2.7 Å on a side. The fourth Mn is at a distance 3.3 Å from one (or two) of the other three. Each pair of Mn in the triangle is bridged by di-μ-oxo links to produce the 2.7 Å distances, and the likely arrangement is a corner-cube. This can be visualized by imagining a cubane-like structure of alternating Mn and O atoms with one of the four Mn corner atoms removed. The remaining three corner Mn atoms form the equilateral triangle; the four O atoms fall into two classes – an apical O atom that is common to the three di-μ-oxo links and three peripheral O atoms, one for each link. Each Mn can then coordinate in an approximately octahedral configuration. (See Fig. 1 for three examples.) The fourth Mn can be joined to one or two of the Mn atoms in the triangle by a mono-μ-oxo link or with additional carboxylato links in parallel.

It might be tempting to locate the Ca atom at the missing corner of the imagined cubane. Ca-O distances are typically approx. 0.4 Å longer than Mn(IV)-O distances, however. This arrangement

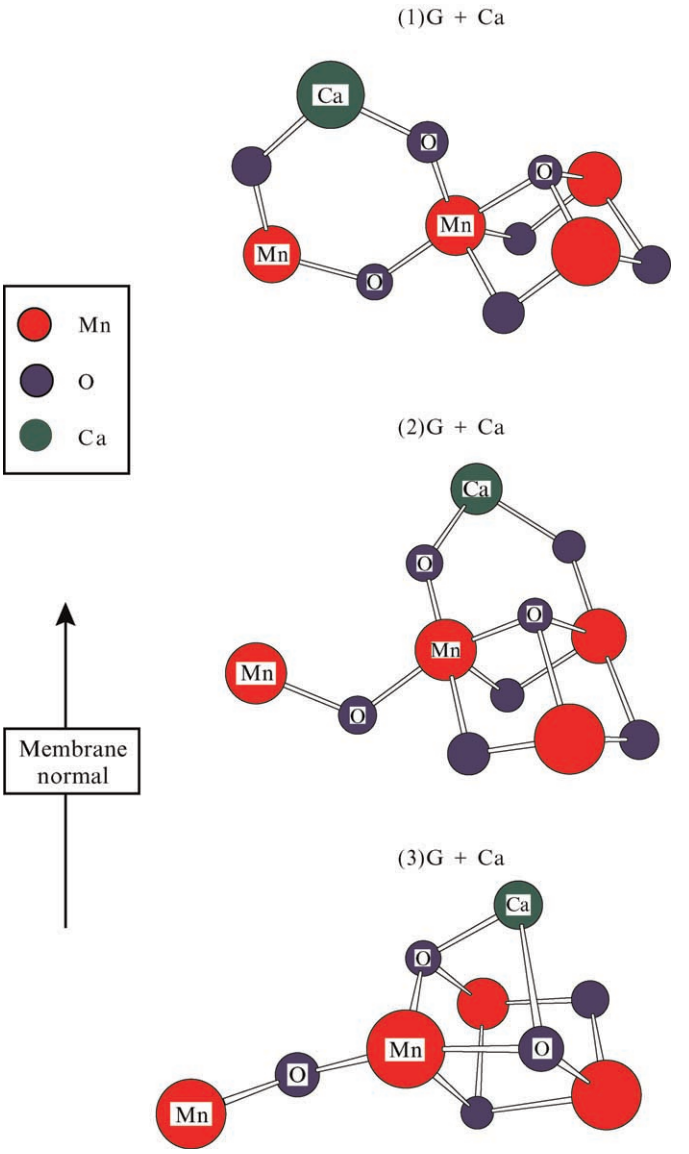

Figure 1: Models of the Mn_4Ca cluster of the water-oxidation complex of PS II that satisfy the criteria listed in the text. Three models, labelled (1) G + Ca, (2) G + Ca and (3) G + Ca, are vertically displaced and oriented with respect to the membrane normal. The models shown were adapted by adding Ca to option G from Fig. 9 of DeRose et al 1994. Other positional isomers for attaching the links from the corner cube to Ca or to the unique Mn may also satisfy the experimental criteria.

would result in three short (approx. 3.0 Å) Ca-Mn or Sr-Mn distances. Experimental measurements of both Ca (K-edge) and Sr (K-edge) EXAFS show that such short vectors are completely absent in PS II. The shortest vector lengths are 3.4 Å for Ca-Mn and 3.5 Å for Sr-Mn (Cinco et al 1998, 2002). Might a reasonable model be produced by elongating the hypothetical Ca corner? This could be accomplished only by stretching the Ca-O (or Mn-O) bonds to chemically unreasonable lengths. Breaking one of the three hypothetical Ca-O bonds relieves these distortions somewhat, but the resulting cluster models appear to contain atoms with significantly strained coordination geometry (e.g., model (3)G + Ca in Fig. 1).

The oriented-membrane dichroism studies place constraints on the relative orientations of the 2.7 Å vectors of the Mn_3 triangle, the 3.3 Å Mn-Mn vector(s), the 3.4(3.5) Å Ca(Sr)-Mn vector(s) and the membrane normal. Our published analysis of the PS II membrane dichroism at 2.7 Å showed that it could be accounted for by a single Mn-Mn vector oriented at approx. 60° to the membrane normal (Mukerji et al 1994). Given the mosaic spread of 20–25° for those samples, we calculate that the average angle for the three vectors in an equilateral triangle is also approx. 60°.

Several cluster geometries that satisfy the experimental criteria are shown in Fig. 1.

ACKNOWLEDGMENTS

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HYDROXYLAMINE IS A SUBSTRATE ANALOGUE OF WATER IN PSII

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INTRODUCTION

That Cl^- participates in water oxidation by PSII has long been established, but its role remains uncertain. Chloride depletion of PSII results in loss of O_2 evolution (Homann 1988, Oleson & Andreasson 2003), the S_2 multiline signal, and the ability of PSII centers to undergo the $S_2 \rightarrow S_3 \rightarrow S_4 \rightarrow S_0$ transitions (Ono et al 1986, Wincencjusz et al 1997). Chloride binding is pH sensitive; Cl^- is displaced from its binding site at pH > 7.5 (Homann 1988, Lindberg et al 1993). A variety of anions compete with Cl^- for its binding site: Br^-, I^- and NO_3^- activate, while F^- and CH_3COO^- inhibit (Sandusky & Yocum 1984, 1986, Ono et al 1987). Steady state kinetic experiments identified Cl^- sensitive and Cl^- insensitive PSII inhibitors. The latter were postulated to ligate PSII Mn at water specific sites. Ammonia exhibits both types of inhibition, whereas certain anions and larger amines show Cl^- sensitive behavior. This data combined with EPR experiments (Beck et al 1986, Britt et al 1989) have established NH_3 as a substrate analogue of water. Because NH_2OH-treated chloroplasts evolve N_2 upon illumination, it was proposed that NH_2OH is also a water-substrate analogue of PSII (Radmer & Ollinger 1982). However, it has been argued that NH_2OH should not be considered as a water analogue of PSII because Cl^- attenuates the NH_2OH-induced inhibition of O_2 evolution in chloroplasts and the reaction of N,N-dimethylhydroxylamine (DMHA) with polypeptide-depleted PSII (Beck et al 1986, 1987, 1988a,b). It was further argued that steric factors should exclude NH_2OH from the water site on PSII, and only molecules the size of H_2O or NH_3, or smaller, may access this site. Conversely, experiments investigating the reaction of hydroxylamines with polypeptide-depleted PSII membranes have shown that Cl^- confers protection against inhibition by methylated hydroxylamines, but not NH_2OH (Mei & Yocum 1993). In addition, ESEEM studies with deuterated alcohols have provided evidence that CH_3OH and C_2H_5OH bind directly to Mn_4, perhaps at a water site (Force et al 1998). If this is the case, then NH_2OH, which is nearly isostructural with CH_3OH, should exhibit a similar accessibility to the Mn_4 cluster. Because the substrate analogue status of NH_2OH is ambiguous, we sought to clarify the mode of NH_2OH interaction with the Mn_4 cluster in terms of Cl^- sensitivity and substrate specificity. Experiments were carried out with PSII membranes treated with NH_2OH and its methylated derivatives in which the Cl^- concentration was rigorously controlled.

MATERIALS AND METHODS

PSII membranes depleted of the 17 and 23 kDa polypeptides were prepared as previously described (Berthold et al 1981, Ghanotakis et al 1984). For Cl^- depletion, polypeptide-depleted samples were washed twice in buffer consisting of 50 mM HEPES, 400 mM sucrose at pH 7.5 (SH buffer), resuspended in SH buffer and stored at −70 °C until further use. For pH 6 experiments, washed samples were stored in buffer containing 50 mM MES and 400 mM sucrose at pH 6. Aliquots of Cl^--depleted suspensions were reconstituted with the Na^+ salts of various anions. Within 2–3 minutes of anion addition, hydroxylamines NH_2OH/H_2SO_4 (200 μM), N-methylhydroxylamine (NMHA)/HCl (400 μM) or DMHA/HCl (2.5 mM) were added and aliquots of these reaction mixtures were assayed for O_2 evolution at various time intervals. For O_2 evolution measurements, a buffer containing 50 mM MES (pH 6), 10 mM $CaCl_2$ and 350 μM DCBQ was used. Rates were compared to a freshly thawed Cl^- and polypeptide-depleted PSII sample that exhibited rates of 250 – 350 μM O_2 mg Chl^{-1} hr^{-1}.

RESULTS

Polypeptide-depleted PSII membranes that had undergone a Cl^- depletion procedure were reconstituted with varying amounts of Cl^- and then treated with 200 μM NH_2OH at pH 6. Loss of activity, which approximated first order decay, was observed (Fig. 1) and the decay rate constants were determined for each Cl^- concentration (Table 1). Addition of Cl^- accelerated the loss of activity induced by NH_2OH, because the decay rates more than doubled over the range of Cl^- concentrations tested (0 mM Cl^- to 100 mM Cl^-). Similar results were obtained at pH 7.5 (Table 1).

In contrast, when these experiments were repeated with additions of 2.5 mM DMHA, the loss of O_2 evolution caused by the inhibitor

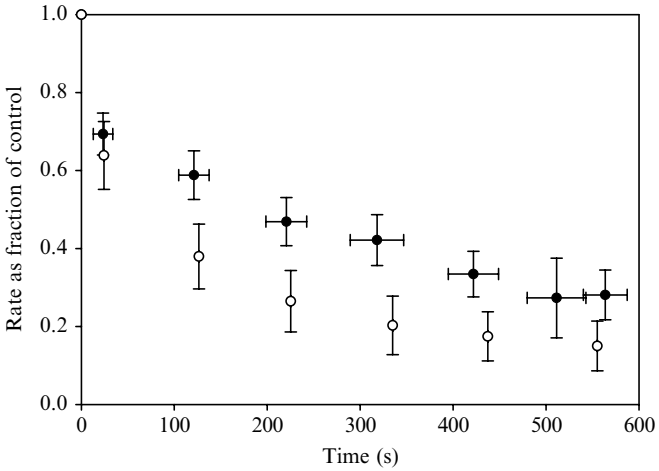

Figure 1: Time dependent loss of activity catalyzed by 200 μM NH_2OH/H_2SO_4 in polypeptide/Cl^--depleted PSII at pH 6 (closed symbols) or reconstituted with 100 mM Cl^- (open symbols).