

## Mechanism for photosynthetic O<sub>2</sub> evolution

(water oxidation/photosystem II/manganese)

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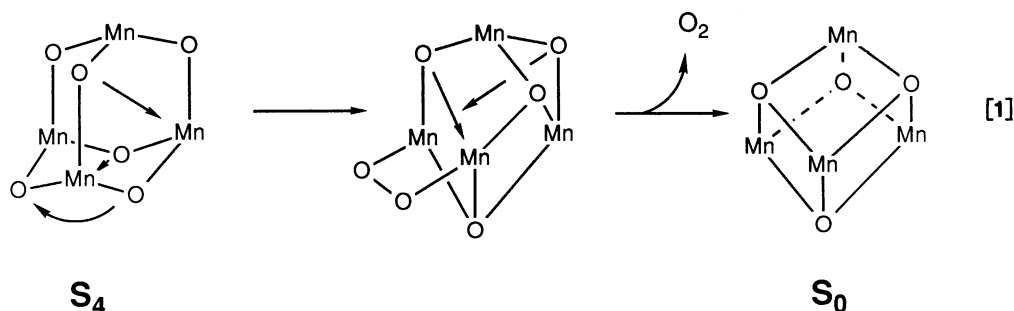
**ABSTRACT** We present a mechanism for photosynthetic O<sub>2</sub> evolution based on a structural conversion of a Mn<sub>4</sub>O<sub>6</sub> “adamantane”-like complex to a Mn<sub>4</sub>O<sub>4</sub> “cubane”-like complex. EPR spectral data obtained from the S<sub>2</sub> state of the O<sub>2</sub>-evolving complex are characteristic of a Mn<sub>4</sub>O<sub>4</sub> cubane-like structure. Based on this structure for the manganese complex in the S<sub>2</sub> state as well as a consideration of the other evidence available on the natural system and the coordination chemistry of manganese, structures are proposed for the five intermediate oxidation states of the manganese complex. A molecular mechanism for the formation of an O—O bond and the displacement of O<sub>2</sub> from the S<sub>4</sub> state is easily accommodated by the proposed model. The model is discussed in terms of recent EPR, x-ray, and UV spectral data obtained from the manganese site in the photosynthetic O<sub>2</sub>-evolving complex.

Several proposals have been made (for a recent review, see ref. 1; refs. 2–5) for the role of manganese in photosynthetic O<sub>2</sub> evolution. Recently, one of us (6) has obtained EPR evidence for an arrangement of four manganese ions at the active site in the S<sub>2</sub> state in which two strongly antiferromagnetically exchange-coupled manganese dimers are ferromagnetically exchange-coupled. The dimer of dimers arrangement of manganese ions required to account for the EPR signals from the S<sub>2</sub> state could arise from a Mn<sub>4</sub>O<sub>4</sub> “cubane”-like structure that is elongated along one of the three axes of the cube. Indeed, the combination of large antiferromagnetic and ferromagnetic exchange couplings is characteristic of cubane-like structures such as Fe<sub>4</sub>S<sub>4</sub> (7, 8) and Cu<sub>4</sub>O<sub>4</sub> (9). A Jahn–Teller distortion of a Mn<sub>4</sub>O<sub>4</sub> complex could easily give the dimer of dimers arrangement of manganese ions seen by EPR in the S<sub>2</sub> state. Cubane-like

hard *et al.* (11). The structure of this complex, which is stable in aqueous solution, has been determined crystallographically.

A consideration of the evidence available on the natural system as well as of the coordination chemistry of manganese leads us to propose the model shown in Fig. 1. We have focused on the structural aspects of manganese in our proposal. For a complete account of the involvement of polypeptides and ions in photosynthetic O<sub>2</sub> evolution, see the recent paper by Kambara and Govindjee (2). In our scheme, a tetrameric manganese site is sequentially oxidized by photosystem II. Each manganese ion in the tetrameric complex is proposed to be also coordinated to the protein via three N or O ligands to complete an octahedral coordination, although the protein-derived manganese ligands are not shown in Fig. 1. For the lower oxidation states (S<sub>0</sub>–S<sub>2</sub>), we propose that the manganese complex exists in a Mn<sub>4</sub>O<sub>4</sub> cubane structure. Upon reaching the S<sub>3</sub> state, we suggest that the manganese complex coordinates two O<sup>2-</sup> or OH<sup>-</sup> ions from water molecules and undergoes a structural rearrangement to form a Mn<sub>4</sub>O<sub>6</sub> adamantane structure.

This model has several attractive features. Because each manganese ion is proposed to be anchored to the protein matrix via three N or O ligands, one would expect that the positions of the four manganese ions would remain essentially fixed. In the proposed structural conversion between a Mn<sub>4</sub>O<sub>4</sub> structure and a Mn<sub>4</sub>O<sub>6</sub> structure, the positions of the four manganese ions need only change very little, if at all, to accommodate the change in O ligand environment. Moreover, the formation of an O—O bond and the displacement of O<sub>2</sub> from S<sub>4</sub> is easily accommodated by the proposed model (Eq. 1).



structures, such as Fe<sub>4</sub>S<sub>4</sub> (7), are known in biology but have not been proposed previously for the active site of photosynthetic O<sub>2</sub> evolution. Cubane-like structures are common in coordination chemistry, however, and such a Mn<sub>4</sub>O<sub>4</sub> structure exists in the solid-state structure of β-Mn<sub>2</sub>GeO<sub>4</sub> (10). Another relevant complex, a Mn(IV)<sub>4</sub>O<sub>6</sub> “adamantane”-like complex, has been described recently by Wieg-

In Eq. 1, the S<sub>4</sub> state is initially formed with a Mn<sub>4</sub>O<sub>6</sub> structure. The electron withdrawal by high-valent manganese from the O ligands then triggers the formation of an O—O bond. A molecule of O<sub>2</sub> is released via reduction of manganese and nucleophilic displacement of O<sub>2</sub> by the bridging O ligands.

Manganese has been shown by EPR studies to constitute the site where exogenous ligands bind (13). In addition, the finding that the Mn x-ray absorption K-edge shifts to higher energy from S<sub>1</sub> to S<sub>2</sub> indicates that manganese is oxidized during the S<sub>1</sub> to S<sub>2</sub> transition (3). These two results point to

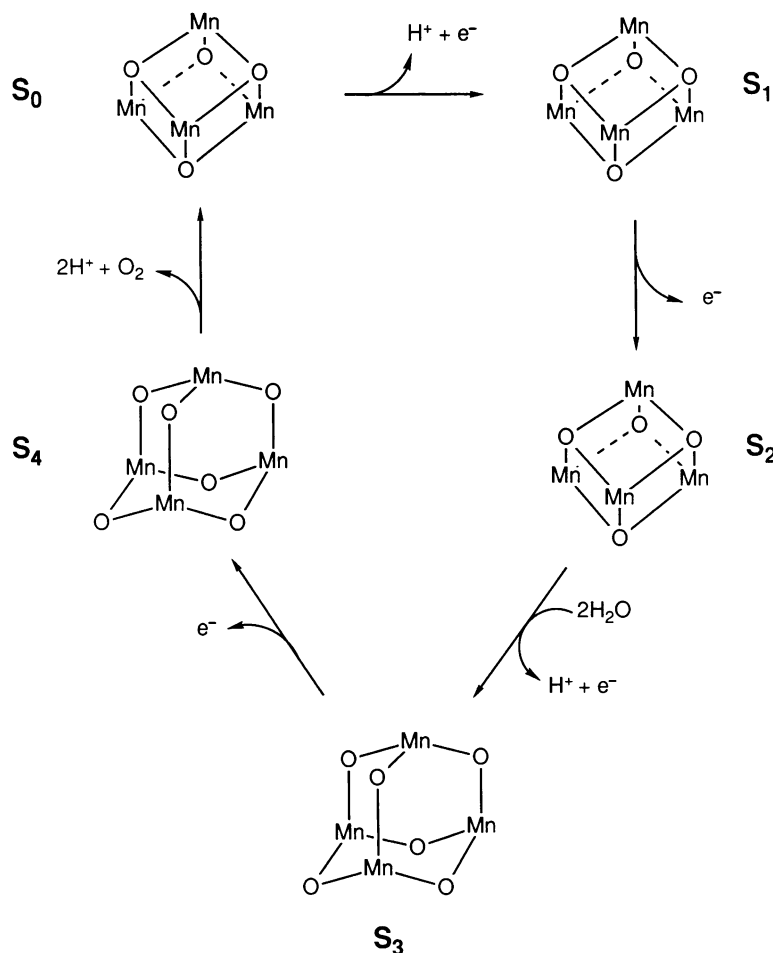


FIG. 1. Proposed scheme for the structures of manganese associated with S-state transitions. The S<sub>0</sub> to S<sub>1</sub>, S<sub>1</sub> to S<sub>2</sub>, S<sub>2</sub> to S<sub>3</sub>, and S<sub>3</sub> to S<sub>4</sub> transitions each involve the removal of one electron from the manganese complex via the light-driven turnover of photosystem II. The S<sub>4</sub> state rapidly releases O<sub>2</sub> and regenerates the S<sub>0</sub> state. It has been found that one proton is released from the O<sub>2</sub>-evolving complex in the S<sub>0</sub> to S<sub>1</sub> and S<sub>2</sub> to S<sub>3</sub> steps and two protons are released in the S<sub>3</sub> to S<sub>0</sub> step (12). In this scheme, O denotes either O<sup>2-</sup> or OH<sup>-</sup> ligands. The proton release results could be interpreted in terms of conversion of a OH<sup>-</sup> ligand to an O<sup>2-</sup> ligand, although deprotonation of protein functional groups could also account for the proton release data.

a function of manganese in storage of oxidizing equivalents and in catalysis of water oxidation. Our model, as well as all of the recent proposals for the role of manganese in O<sub>2</sub> evolution (1–5), incorporates both of these functions.

A detailed analysis of the EPR spectral data has shown that the manganese site must consist of a mixed-valence manganese tetramer in the S<sub>2</sub> state (6). This conclusion accounts for the observed stoichiometry of four manganese per photosystem II (14). Moreover, the EPR data are consistent with only three sets of oxidation states for manganese (Table 1). Although the EPR data do not directly reflect the oxidation states of individual manganese ions, the difference-UV data of Dekker *et al.* (15) show that the absorbance changes on

going from S<sub>0</sub> to S<sub>1</sub>, S<sub>1</sub> to S<sub>2</sub>, and S<sub>2</sub> to S<sub>3</sub> are all equivalent and resemble the change observed on going from a Mn(III) to a Mn(IV)-gluconate model complex. The conclusion from the UV studies was that one Mn(III) is oxidized to Mn(IV) in each of the first three S-state transitions. This observation fits naturally into the proposed scheme, if S<sub>0</sub> consists of Mn(III)<sub>3</sub>Mn(IV) (column A in Table 1), since in this case the first three S-state transitions are all Mn(III) to Mn(IV) in character.

One might argue that a Mn(III)<sub>3</sub>Mn(IV) oxidation state for S<sub>0</sub> is too oxidized to be realistic. In fact, the position of the manganese x-ray absorption K-edge for the S<sub>1</sub> state falls in the range observed for Mn(II) and Mn(III) model compounds (3), which would seem to exclude the possibility of a Mn(III)<sub>2</sub>Mn(IV)<sub>2</sub> oxidation state for S<sub>1</sub>. The position of the x-ray absorption edge, however, reflects the electron density about manganese and not the oxidation state. Interpretation of the x-ray absorption edge data in terms of specific oxidation states, therefore, cannot be done successfully until suitable model compounds are available for comparison. The ligand environment is known to profoundly modify the electron density of metal ions in metal complexes.

Considering the x-ray absorption edge data (3) in more detail, the shift in the manganese K-edge is quite large in the S<sub>1</sub> to S<sub>2</sub> transition (1.78 eV to higher energy) but smaller and in the opposite direction in the S<sub>2</sub> to S<sub>3</sub> transition (1.1 eV to lower energy). The large shift to higher energy in the x-ray absorption edge between S<sub>1</sub> and S<sub>2</sub> is rationalized by oxidation of one manganese ion without accommodation of the

Table 1. Possible manganese oxidation states

State	Possible manganese oxidation states		
	A	B	C
S <sub>0</sub>	(III) <sub>3</sub> (IV)	(II)(III) <sub>3</sub>	(II) <sub>3</sub> (III)
S <sub>1</sub>	(III) <sub>2</sub> (IV) <sub>2</sub>	(III) <sub>4</sub>	(II) <sub>2</sub> (III) <sub>2</sub>
S <sub>2</sub>	(III)(IV) <sub>3</sub>	(II) <sub>3</sub> (IV)	(II)(III) <sub>3</sub>
S <sub>3</sub>	(IV) <sub>4</sub>	(III) <sub>2</sub> (IV) <sub>2</sub>	(III) <sub>4</sub>
S <sub>4</sub>	(IV) <sub>3</sub> (V)	(III)(IV) <sub>3</sub>	(III) <sub>3</sub> (IV)

The oxidation states of manganese listed under A are in agreement with EPR (6) and UV (15) spectral data and, therefore, may be more likely. The oxidation states listed under B and C, however, are also consistent with the EPR data and cannot be excluded.

ligand environment leading to a substantial decrease in electron density about each manganese ion. The shift to lower energy in the x-ray absorption edge between  $S_2$  and  $S_3$  is rationalized by the  $Mn_4O_4$  to  $Mn_4O_6$  structural conversion shown in Fig. 1. The two extra O ligands ( $O^{2-}$  or  $OH^-$ ) present in the  $Mn_4O_6$  structure increase the electron density on each manganese ion to compensate for the oxidation step.

The  $Mn(IV)_4O_6$  complex, described by Weighardt *et al.* (11), is stable in aqueous solution. The stability of this complex in water lends support to the suggestion that  $S_4$  is composed of  $Mn(IV)_3Mn(V)$ , the  $Mn(V)$  being needed to trigger the oxidation of water. It is notable that in cytochrome P-450 model systems, oxidation of iron or manganese to  $Fe(V)$  or  $Mn(V)$ , respectively, is also required to oxidize the  $O^{2-}$  ion (16). In these cases,  $M(V)O$  is activated to abstract a hydrogen atom from a hydrocarbon, which is analogous to the present case where  $M(V)O$  is activated for O—O bond formation.

In our proposal, the sequential oxidation of manganese ions in a  $Mn_4O_4$  cubane structure leads to a sufficient electron deficiency in  $S_3$  that the manganese complex coordinates two additional water molecules and rearranges to a  $Mn_4O_6$  adamantane structure. This scenario agrees well with the known ligand binding properties of the S states.  $S_0$  and  $S_1$  appear to be inert to ligand binding (17). Recent EPR studies have shown that  $NH_3$  does not bind to the manganese site in  $S_1$  (13).  $S_2$  and  $S_3$ , however, are both reactive to ligand substitution (13, 17).  $NH_3$  has been found to bind directly to manganese in  $S_2$  (13). This reactivity in  $S_2$  and  $S_3$  is accounted for in terms of a progressive electron deficiency of the metal ions in the higher S states, which renders the site more susceptible to nucleophilic substitution and so triggers the conversion from a cubane-like structure to an adamantane-like structure.

Another attractive feature of our proposal is that it can be readily tested. Preparation of  $Mn_4O_4$  cubane and  $Mn_4O_6$  adamantane model complexes is necessary. A comparison of the spectral and chemical properties of these model com-

pounds with the natural system should provide a critical test of our proposal.

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