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MINI-REVIEW

The Tetranuclear Manganese Complex of Photosystem II

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Abstract

A polynuclear manganese complex functions in Photosystem II both to accumulate oxidizing equivalents and to bind water and catalyze its four-electron oxidation. Recent electron paramagnetic resonance (EPR) spectroscopic studies of the manganese complex show that four manganese ions are required to account for its magnetic properties. The exchange couplings between manganese ions in the S_2 state are characteristic of a Mn_4O_4 "cubane"-like structure. Based on this structure for the manganese complex in the S_2 state, as well as a consideration of the known properties of the manganese complex in Photosystem II 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_2 from the S_4 state is suggested.

Key Words: Manganese; Photosystem II; polynuclear manganese complex.

Introduction

Manganese has long been known to play an important role in photosynthetic water oxidation. Nonetheless, only recently has it been possible to address the structure and function of manganese in the water oxidation process at the molecular level. The aim of this paper is to summarize the recent work that bears directly on the role of the manganese in Photosystem II and to present the current picture of the structure and function of the polynuclear manganese complex. The objective is not to present a comprehensive review of the literature; more detailed accounts of the past work are given in several recent

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reviews (Bouges-Bocquet, 1980; Sauer, 1980; Wydrzynski, 1982; Amesz, 1983; Govindjee *et al.*, 1985; Renger and Govindjee, 1985; Dismukes, 1986).

The stoichiometry of manganese in Photosystem II was first addressed by quantitating manganese released from thylakoid membranes by various treatments (Cheniae, 1970). These experiments established that manganese is specifically required for water oxidation and that four manganese ions per Photosystem II are required for optimal rates of O_2 evolution (Cheniae, 1970; Yocum *et al.*, 1981). More recently, Photosystem II preparations with high rates of O_2 evolution have been isolated from a variety of sources (Stewart and Bendall, 1979; Berthold *et al.*, 1981; Yamamoto *et al.*, 1981; Kuwabara and Murata, 1982; Clement-Metral and Gantt, 1983; Satoh *et al.*, 1985). The isolation of an O_2-evolving Photosystem II has proved to be a major step forward in both the biochemical and spectroscopic characterization of the O_2-evolving system. These preparations contain four manganese ions per Photosystem II (Murata *et al.*, 1984). Hence, the recent biochemical progress supports the earlier conclusion (Cheniae, 1970) that four manganese ions are functionally associated with each O_2-evolving center.

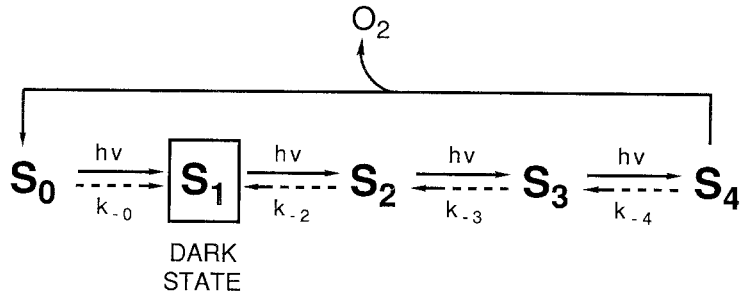
Although manganese is clearly essential for water oxidation, until very recently little concrete information was available about the structure and function of manganese in Photosystem II. In the past few years, several direct probes of functional manganese have been developed and applied to study the role of manganese in water oxidation. Electron paramagnetic resonance (EPR) spectroscopy, in particular, has proved to be an especially useful, direct probe of the manganese complex. In addition, X-ray and optical spectroscopies are being fruitfully applied to study the structure and function of manganese. These efforts are now beginning to reveal the composition and molecular structure of the manganese complex in Photosystem II. Moreover, by use of direct probes of the manganese complex, the function of manganese in water oxidation can now be addressed at the molecular level.

Photooxidation of the Manganese Complex

Kok Model for O_2 Evolution

To account for the periodicity of four in the yield of O_2 in a series of flashes (Joliot *et al.*, 1969; Kok *et al.*, 1970), Kok and co-workers proposed that an O_2-evolving center is coupled to Photosystem II and that each O_2-evolving center cycles through five oxidation states during flash illumination (Forbush *et al.*, 1971). These intermediate oxidation states, referred to as S_i states, with the subscript denoting the number of oxidizing

equivalents accumulated, are sequentially produced by charge separation in Photosystem II as shown by the equation



(1)

A large body of evidence now supports the basic model put forward by Kok and co-workers (Joliot and Kok, 1975; Wydrzynski, 1982). The S_4 state rapidly releases a molecule of O_2 and regenerates the S_0 state. The S_2 and S_3 states are reduced in the dark to the S_1 state with half-times on the order of 1 min at room temperature. It also appears that the S_0 state is oxidized in the dark to the S_1 state with a half-time on the order of 10 min at room temperature (Vermaas *et al.*, 1984). Hence, samples which are incubated in the dark for more than 30 min at room temperature contain only the S_1 state. In contrast, continuously illuminated samples contain equal fractions of states S_0 through S_3 which decay within a few minutes in the dark at room temperature to a mixture of S_0 and S_1 in a 1 : 3 ratio.

Dark adaptation not only leads to changes in the S state distribution, but also causes changes in the structure and function of the manganese site. Illumination produces an "active" state of the O_2-evolving center which decays in the dark in a first-order process to a "resting" state (Beck *et al.*, 1985). The EPR spectral properties of the manganese complex and the reactivity of the system with O_2 differ in the "active" and "resting" states of the O_2-evolving center (Beck *et al.*, 1985; de Paula and Brudvig, 1985).

The question of the molecular basis for the S states has existed since the original proposal by Kok. As first formulated, the S state designation referred to the oxidation state of the O_2-evolving center which could, in principle, include all of Photosystem II and its associated components. Indeed, there are a number of redox-active components on the electron donor side of Photosystem II in addition to the manganese complex such as D, the species that gives rise to EPR signal II_{slow}, and cytochrome b_{559}. However, the oxidation state of these species does not alter either the period-four oscillation of O_2 yields in a series of flashes, provided that the flashes are sufficiently closely spaced (Vermaas *et al.*, 1984), or the spectral properties associated

with specific S states (de Paula *et al.*, 1985). Hence, it appears that the different S states should be interpreted in terms of distinct intermediate states of the manganese complex itself.

Electron Donation in Photosystem II

The minimal model to describe electron donation in Photosystem II under physiological conditions includes only two redox active components which mediate electron transfer from water to $P680^+$ (de Paula *et al.*, 1985). These two components are the manganese site, which functions to accumulate the oxidizing equivalents associated with the S state transitions, and one electron carrier, usually referred to as Z, which functions to mediate electron transfer between the manganese site and $P680^+$. A variety of more complicated schemes have been suggested for electron donation in Photosystem II (Bouges-Bocquet, 1980), but at present there is no compelling evidence to introduce a more complex model than the minimal model above. Cases where additional complexity seems apparent can probably be attributed to heterogeneity in the sample.

The electron carrier Z has been extensively studied by EPR spectroscopy (Babcock and Sauer, 1975; Boska *et al.*, 1983; Boska and Sauer, 1984) and, more recently, by optical spectroscopy (Weiss and Renger, 1984). Z has been proposed to be a quinone species which cycles between the quinol and semiquinone cation radical states upon reduction and oxidation (O'Malley *et al.*, 1984). In addition to manganese and Z, two copies of cytochrome b_{559} (Lam *et al.*, 1983; de Paula *et al.*, 1985) and one copy of D, the EPR signal II_{slow} species (Babcock *et al.*, 1983), are present per Photosystem II. Although both D and cytochrome b_{559} can be photooxidized, neither species appears to undergo redox changes during the S–state cycle.

D appears to be oxidized by the S_2 and S_3 states (Babcock and Sauer, 1973) and D^+ may be the oxidant that leads to the oxidation of S_0 to S_1 in the dark (Vermaas *et al.*, 1984). In fact, the function of D may be to ensure that the amount of S_0 present in the dark is small, perhaps due to an undesirable reactivity of S_0 with O_2 (see Beck *et al.*, 1985) or perhaps due to a greater lability of S_0. There is some evidence that D is near the manganese complex based on observations of electron spin relaxation rate enhancements (de Groot *et al.*, 1986).

It is also possible to photooxidize cytochrome b_{559} (Knaff and Arnon, 1969), as well as chlorophyll (de Paula *et al.*, 1985) or carotenoid (Schenck *et al.*, 1982) in Photosystem II. None of these components are closely associated with the manganese complex, and their photooxidation probably only occurs when S state advancement is blocked; in this case, the powerful oxidant generated by charge separation in Photosystem II leads to oxidation of nonphysiological electron donors.

Structural Studies of the Manganese Complex

EPR Studies

Thylakoid membranes frozen rapidly following one flash of light exhibit a multiline EPR signal from a polynuclear manganese complex (Dismukes and Siderer, 1980). It is now established that this EPR signal from a manganese-containing site arises from functional manganese in the S_2 state of the O_2-evolving center (Dismukes and Siderer, 1981; Hansson and Andréasson, 1982; Brudvig *et al.*, 1983a, b). EPR spectroscopy has proven to be an especially powerful direct probe of the manganese site in Photosystem II, although at present only the S_2 state can be observed. The EPR properties of the S_2 state have been found to be variable; they depend on the temperature at which the S_1 to S_2 transition is carried out (Casey and Sauer, 1984; Zimmermann and Rutherford, 1984; Beck *et al.*, 1985; de Paula and Brudvig, 1985; de Paula *et al.*, 1985), on the presence or absence of cryoprotectants (Brudvig *et al.*, 1983a; Zimmermann and Rutherford, 1986), and on the length of dark adapatation of Photosystem II (Beck *et al.*, 1985; de Paula and Brudvig, 1985). This variability in the EPR properties of the S_2 state is due to a variability in the exchange interactions between manganese ions in the manganese complex (de Paula *et al.*, 1986), which is perhaps due to a structural flexibility of the ligand environment of manganese. Such a structural flexibility may well be significant with respect to the function of the manganese complex (see below). Moreover, the observation of several distinct S_2 state EPR signals from the manganese complex has provided a more extensive data base from which to assess the composition and structure of the manganese-containing complex.

The S_1 to S_2 transition is induced by continuous illumination of dark adapted samples in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) at temperatures above 130 K (de Paula *et al.*, 1985). Illumination of samples treated with DCMU between 130 and 160 K produces an unusual EPR signal centered at $g = 4.1$. This $g = 4.1$ EPR signal is replaced by the S_2 state multiline EPR signal upon warming in the dark to 200 K (Casey and Sauer, 1984). It was originally suggested that the $g = 4.1$ EPR signal arose from an electron carrier between the manganese complex and Z (Casey and Sauer, 1984; Zimmermann and Rutherford, 1984); in this case, the conversion of the $g = 4.1$ EPR signal into the multiline EPR signal upon incubation at 200 K in the dark would be explained by a temperature-dependent electron transfer reaction. However, the failure to detect the $g = 4.1$ EPR signal as an intermediate in the S_2 to S_3 transition does not support this idea (de Paula *et al.*, 1985). A more likely possibility is that the $g = 4.1$ and multiline EPR signals both arise from the manganese complex in the S_2 state. The conversion of the $g = 4.1$ EPR signal into the multiline EPR signal upon incubation

at 200 K in the dark can be explained by a temperature-dependent structural change in the manganese site, which alters the exchange couplings between the manganese ions (de Paula *et al.*, 1986). This explanation is supported by the observation of a temperature-dependent change of one S_2 state multiline EPR signal into an altered S_2 state multiline EPR signal that occurs in the "active" state of Photosystem II (Beck *et al.*, 1985); in this case, both EPR signals are clearly from the manganese complex.

The EPR spectral changes that occur upon warming samples after the S_1 to S_2 transition has occurred at low temperature are probably a reflection of a difference in the preferred geometry of the manganese complex in its two different oxidation states; oxidation of the manganese complex at low temperature may occur without accommodation of the structure if an activation barrier must be overcome and, upon warming, the site could relax into its preferred conformation.

The S_2 state multiline EPR signal was the first direct spectroscopic probe of manganese in the O_2-evolving center which contained a considerable amount of structural information. As a consequence, much effort has been made to translate this information into a structural model of the manganese site (Dismukes and Siderer, 1981; Dismukes *et al.*, 1982; Hansson *et al.*, 1984; de Paula and Brudvig, 1985; de Paula *et al.*, 1986). Both mixed-valence manganese dimer (Dismukes and Siderer, 1981; Hansson *et al.*, 1984) and tetramer (Dismukes *et al.*, 1982) models have been advanced to explain the S_2 state multiline EPR signal. By analyzing the several distinct EPR signals from the manganese complex in the S_2 state, we are now in a position to answer the question of the composition of the manganese-containing site observed by EPR spectroscopy. An extensive analysis of the S_2 state EPR signals leads to the conclusion that these EPR signals must arise from a mixed-valence manganese tetramer (de Paula *et al.*, 1986). A further conclusion is that a non-manganese paramagnetic species probably is not exchange coupled to manganese in the S_2 state. Three possible combinations of manganese oxidation states are compatible with the EPR data from the S_2 state: Mn(II)–$Mn(III)_3$, $Mn(III)_3$–Mn(IV), and Mn(III)–$Mn(IV)_3$.

X-ray Studies

The oxidation states and ligation of manganese in Photosystem II have been directly probed by X-ray absorption edge and extended X-ray absorption edge fine structure (EXAFS) measurements (Kirby *et al.*, 1981a, b; Goodin *et al.*, 1984; Yachandra *et al.*, 1986a, 1987). EXAFS analyses have been done for manganese in the S_1 state present in dark-adapted thylakoid membranes (Kirby *et al.*, 1981b) and in dark-adapted Photosystem II membranes (Yachandra *et al.*, 1986a). These studies indicate that each manganese ion is

probably six-coordinate and ligated to nitrogen and/or oxygen ligands. Each manganese ion also sees one neighboring manganese (or iron) ion at a distance of 2.7 Å. The EXAFS of the manganese ions in Photosystem II looks very much like that of a mixed-valence di-μ-oxo-bridged manganese dimer model compound (Cooper and Calvin, 1977), which suggests that manganese in Photosystem II also has an oxo-bridged structure.

The EXAFS data for the S_2 state look essentially the same as for the S_1 state (Yachandra *et al.*, 1987) and, therefore, it appears that the manganese site does not undergo any substantial structural reorganization during the S_1 to S_2 transition. Of note is the apparent lack of chloride in the first coordination shell of manganese in either the S_1 or the S_2 states as revealed by EXAFS studies of manganese. This observation is of particular interest because chloride is required for optimal O_2 evolution rates (Kelley and Izawa, 1978) and has been proposed to act as a bridging ligand in a polynuclear manganese complex (Sandusky and Yocum, 1983; Critchley and Sargeson, 1984). Recent EPR studies, however, suggest that chloride is not bound to manganese in the S_1 or S_2 states (Yachandra *et al.*, 1986b; Beck and Brudvig, 1986).

The energy of the X-ray absorption edge reflects the electron density about manganese which, in turn, reflects the oxidation state and ligation of manganese. The position of the X-ray absorption *K*-edge of manganese in dark-adapted thylakoid membranes (largely the S_1 state) is in the range of Mn(II) and Mn(III) model compounds (Kirby *et al.*, 1981a). The position of the X-ray absorption *K*-edge of manganese shifts to higher energy in the S_2 state and is in the range of Mn(III) and Mn(IV) model compounds (Goodin *et al.*, 1984). In light of the conclusion from the EXAFS studies that the coordination of manganese does not change in the S_1 to S_2 transition, this result demonstrates that manganese is oxidized in the S_1 to S_2 transition. However, the shift in the manganese *K*-edge is quite large in the S_1 to S_2 transition, which prompted Goodin *et al.* (1984) to suggest that two manganese ions were oxidized in this step. Such an interpretation would require that another, non-manganese species was reduced during the S_1 to S_2 transition. Moreover, the shift in the manganese *K*-edge was smaller and to lower energy in the S_2 to S_3 transition, suggesting that manganese itself was not oxidized in this step. These results seem to indicate that oxidation of manganese occurs in some, but not all, of the S state transitions and that other redox active species also are involved in the storage of oxidizing equivalents during some of the S state transitions. However, the position of the manganese *K*-edge reflects electron density about manganese and not directly the oxidation state. It is possible that the changes in the position of the manganese *K*-edge could be accounted for by a single oxidation of a manganese ion in both the S_1 to S_2 and S_2 to S_3 transitions, depending on the ligation of the manganese ions.

Structure of the Manganese Complex

The X-ray data suggest an oxo-bridged manganese dimer structure for manganese in Photosystem II (Kirby *et al.*, 1981b). However, it is known that four manganese ions are present per Photosystem II (Cheniae, 1970; Yocum *et al.*, 1981; Murata *et al.*, 1984). Hence, if the oxo-bridged manganese dimer

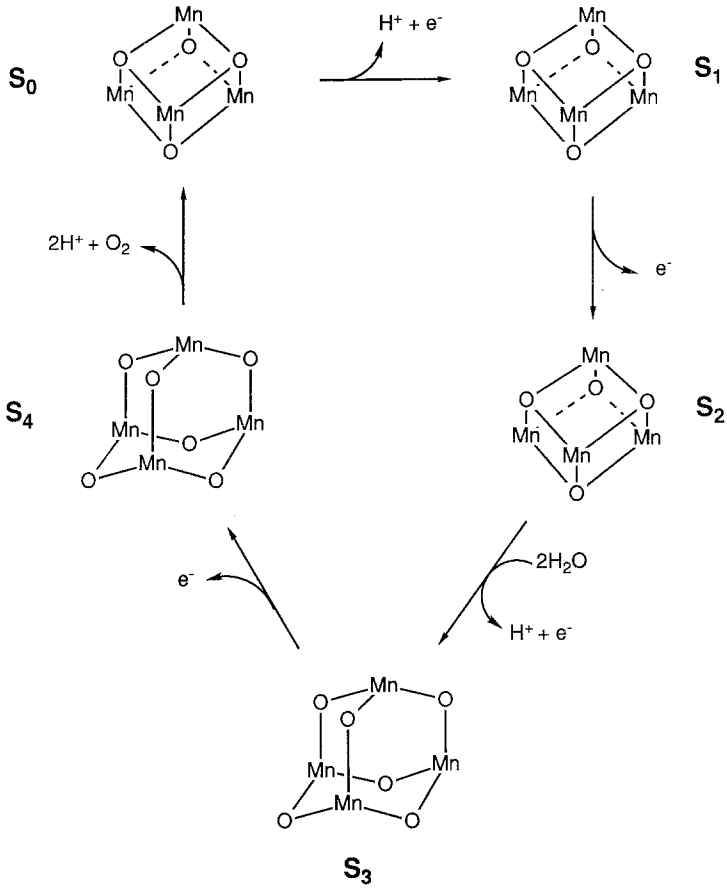


Fig. 1. A proposed scheme for the structures of manganese associated with S state transitions. It has been found that one proton is released from the O_2-evolving center in the S_0 to S_1 and the S_2 to S_3 steps and two protons are released in the S_3 to S_0 step (Förster *et al.*, 1981). In this scheme, O denotes either O^{2-} or OH^- ligands. The proton release results could be interpreted in terms of conversion of an OH^- ligand to an O^{2-} ligand, although deprotonation of protein functional groups could also account for the proton release data. Each manganese ion in the tetrameric complex is proposed to also be coordinated to the protein via three N or O ligands, as indicated by EXAFS studies of manganese in the S_1 state (Kirby *et al.*, 1981b; Yachandra *et al.*, 1986a), although the protein-derived ligands are not shown.

structure suggested by the X-ray data is correct, then there must be two such manganese dimers in Photosystem II.

A mixed-valence manganese tetramer is required to account for the S_2 state EPR signals (de Paula *et al.*, 1986). Moreover, the EPR analyses show that two antiferromagnetically exchange-coupled manganese dimers are ferromagnetically exchange coupled in the S_2 state. This unusual combination of exchange couplings has been previously observed for "cubane"-like complexes (Noodleman *et al.*, 1985; Haase *et al.*, 1983), and this analogy suggests that the tetrameric manganese complex may have a "cubane"-like structure in the S_2 state (de Paula *et al.*, 1986). A Jahn–Teller distortion of an oxo-bridged "cubane"-like manganese tetramer (see the structure of the manganese complex in the S_2 state in Fig. 1) could easily give the dimer-of-dimers arrangement of manganese ions as seen by both EXAFS and EPR in the S_2 state.

Function of the Manganese Complex

Storage of Oxidizing Equivalents

The shift of the manganese X-ray absorption K-edge to higher energy in the S_1 to S_2 transition (Goodin *et al.*, 1984) indicates that manganese is oxidized in this step. Moreover, the period-four oscillation of the intensity of the S_2 state multiline EPR signal in a series of flashes (Dismukes and Siderer, 1981; Zimmermann and Rutherford, 1984) suggests that the manganese complex undergoes redox changes during S state advancement. Both of these results indicate that one function of manganese is to store the oxidizing equivalents produced during S state advancement.

Several lines of evidence suggest that the S_0 to S_1, S_1 to S_2, S_2 to S_3, and S_3 to S_4 transitions each involve a one-electron oxidation of manganese rather than oxidation of other redox active species or bound water. Recent difference-UV spectral data of Dekker *et al.* (1984a, b, c) indicate that a chromophore associated with the S states can be monitored in the 300–350 nm range. After correcting for the absorbance changes due to redox changes of Q_A, Q_B, and Z in Photosystem II, the absorbance changes on going from S_0 to S_1, S_1 to S_2, and S_2 to S_3 are found to be all equivalent and to resemble the absorbance change on going from a Mn(III) to a Mn(IV)-gluconate model complex. The conclusion from the UV spectral studies was that one Mn(III) is oxidized to Mn(IV) in each of the first three S state transitions (Dekker *et al.*, 1984c). More recent work supports this interpretation (Saygin and Witt, 1985), although an alternate interpretation of the UV spectral data has also been proposed which invokes an absorbance increase due to the S states only in the S_1 to S_2 transition (Velthuys, 1981; Lavergne, 1986).

There is also evidence from mass spectrometric studies of the isotopic composition of O_2 evolved from Photosystem II that the water molecules to be oxidized to O_2 do not bind to the O_2-evolving center, or that they exchange readily with bulk water, in the S_0, S_1, S_2, and S_3 states (Radmer and Ollinger, 1986). This result would be difficult to accommodate if water is partially oxidized in one of the earlier S state transitions.

The picture that emerges from these studies is that a tetrameric manganese complex is sequentially oxidized four times in the S_0 to S_4 transitions and then, in a single step, two water molecules are oxidized, a molecule of O_2 is released, and the S_0 state is regenerated. With regard to the formal oxidation states of the manganese ions, the EPR analyses are consistent with either Mn(II)–$Mn(III)_3$, $Mn(III)_3$–Mn(IV), or Mn(III)–$Mn(IV)_3$ for the oxidation states of manganese in the S_2 state (de Paula *et al.*, 1986). For the interpretation (Dekker *et al.*, 1984c) of the difference-UV spectral changes in terms of sequential Mn(III) to Mn(IV) oxidations in the S_0 to S_1, S_1 to S_2, and S_2 to S_3 transitions to be consistent with the EPR data, the oxidation state of manganese in the S_2 state must be Mn(III)–$Mn(IV)_3$.

Ligand Binding

None of the studies described thus far address the question of whether the manganese complex is the active site that catalyzes the oxidation of water. A number of past studies have implicated manganese as the site where amines, and by analogy, water binds. For example, the inhibition of O_2 evolution by amines depends on the basicity of the amine (Ghanotakis *et al.*, 1983), which suggests that the amine acts as a Lewis base and, thereby, may be expected to coordinate to a Lewis acid such as manganese.

Recent EPR studies of the manganese complex have directly shown that water and water analogs bind to manganese (Beck *et al.*, 1986; Hansson *et al.*, 1986). Ammonia, but not more bulky amines, bind to the S_2 state and dramatically alter the lineshape of the S_2 state multiline EPR signal, indicating that ammonia binds directly to manganese in the S_2 state (Beck *et al.*, 1986; Beck and Brudvig, 1986). In addition, ^{17}O-labeled water appears to slightly broaden the S_2 state multiline EPR signal (Hansson *et al.*, 1986). A broadening of the S_2 state multiline EPR signal in the presence of ^{17}O-labeled water under the conditions of these experiments would indicate that exchangeable water is a ligand to manganese in the dark-adapted sample. These results substantiate the idea that manganese is, indeed, the water-binding site and is, therefore, expected to catalyze the oxidation of water.

Mechanism of Water Oxidation

Many proposals have been made for the role of manganese in photosynthetic water oxidation (for reviews see: Amesz, 1983; Renger and Govindjee,

1985). It is clear that manganese functions both to accumulate the oxidizing equivalents produced during S state advancement and to bind water and catalyze its oxidation. Unfortunately, the molecular details of the function of manganese in photosynthetic water oxidation remain far from clear. However, the results of recent EPR, X-ray, and optical studies, which directly probe the manganese complex, have increased our understanding of the function of manganese in water oxidation at the molecular level. From these studies, we are now able to address the molecular mechanism of photosynthetic water oxidation.

The key step in the formation of an O_2 molecule is the activation of a bound water in order to form an O–O bond. This step is likely to be the most demanding and it is quite reasonable that the formation of the O–O bond does not occur until the most highly oxidized state of manganese is attained. An analogy can be drawn between the cytochrome P450 chemistry and photosynthetic water oxidation; in both cases a water molecule must be activated for reaction with a nucleophile. In cytochrome P450 model systems, oxidation of iron or manganese to Fe(V) or Mn(V), respectively, is required to activate water to abstract a hydrogen atom from a hydrocarbon or to add to an olefin to form an epoxide (Sheldon and Kochi, 1981). On this basis, a Mn(V) oxidation state may be needed to trigger the oxidation of water in Photosystem II. It is noted that the only combination of oxidation states for manganese that is consistent with the interpretations of both the EPR (de Paula *et al.*, 1986) and optical (Dekker *et al.*, 1984c) spectral data is one in which the S_4 state contains $Mn(IV)_3$–Mn(V).

A number of proposals for the mechanism of O_2 formation involve the generation of a O–O bond before the S_4 state is produced (Sauer, 1980; Kusunoki, 1983; Renger and Govindjee, 1985). These proposals seem less likely based on a consideration of the requirements to activate water. Mass spectrometric data also argue against the partial oxidation of water in the lower S states (Radmer and Ollinger, 1986).

In other proposals, a redox-active species associated with the manganese complex is postulated to be alternatively oxidized and reduced during the S state cycle (Goodin *et al.*, 1984;Kambara and Govindjee, 1985). At the present time there is no clear indication that a redox-active species other than manganese is involved in storing the oxidizing equivalents associated with the S states. Moreover, analyses of EPR spectral data from the S_2 state indicate that a non-manganese paramagnetic species probably is not exchange coupled to manganese in the S_2 state (de Paula *et al.*, 1986).

A consideration of the evidence available on the natural system as well as the coordination chemistry of manganese led us to propose the model for water oxidation shown in Fig. 1 (Brudvig and Crabtree, 1986). EPR spectral data obtained from the S_2 state are characteristic of a Mn_4O_4 "cubane"-like structure (de Paula *et al.*, 1986) and EXAFS studies of manganese indicate that the manganese site does not undergo a substantial structural change

on going from S_1 to S_2 (Yachandra *et al.*, 1987). Hence, the tetrameric manganese complex is proposed to exist in a Mn_4O_4 "cubane"-like structure in the lower oxidation states (S_0 to S_2).

Studies of ammonia binding to the O_2-evolving center show that the lower oxidation states (S_0 and S_1) are inert to ligand substitution, whereas the higher oxidation states (S_2 and S_3) are reactive to ligand substitution (Velthuys, 1975; Beck *et al.*, 1986). The activation of the manganese complex for ligand substitution in the higher S states is readily accounted for in our model; the sequential oxidation of manganese without accommodation of the ligand environment leads to a progressive increase in the reactivity of the complex to nucleophilic addition with increasing S state. Upon reaching the S_3 state it is suggested that the manganese complex coordinates two O^{2-} or OH^- ions from water molecules and undergoes a structural rearrangement to form a Mn_4O_6 "adamantane"-like structure. A structural rearrangement of the manganese complex in the S_2 to S_3 transition is indicated by a change in the shape of the X-ray absorption edge of manganese on going from the S_2 to the S_3 state (Goodin *et al.*, 1984).

This scheme 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_4O_4 "cubane"-like structure and a Mn_4O_6 "adamantane"-like structure, the positions of the four manganese ions need only change very little, if at all, to accommodate the change in O ligand environment. However, the ligand environment of manganese must be rather flexible. Indeed, the variability of the S_2 state EPR signals, as noted above, suggests that a reorganization of the bridging ligands may be likely during the oxidation of water.

The formation of an O–O bond and the displacement of O_2 from the S_4 state is easily accommodated by the proposed model (Brudvig and Crabtree, 1986). The S_4 state is initially formed with a Mn_4O_6 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_2 is released via reduction of manganese and nucleophilic displacement of O_2 by the bridging ligands.

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References

Amesz, J. (1983). *Biochim. Biophys. Acta* **726**, 1–12.

Babcock, G. T., and Sauer, K. (1973). *Biochim. Biophys. Acta* **325**, 483–503.

Babcock, G. T., and Sauer, K. (1975). *Biochim. Biophys. Acta* **376**, 329–344.

Babcock, G. T., Ghanotakis, D. F., and Diner, B. A. (1983). *Biochim. Biophys. Acta* **723**, 276–286.

Beck, W. F., and Brudvig, G. W. (1986). *Biochemistry* **25**, 6479–6486.

Beck, W. F., de Paula, J. C., and Brudvig, G. W. (1985). *Biochemistry* **24**, 3035–3043.

Beck, W. F., de Paula, J. C., and Brudvig, G. W. (1986). *J. Am. Chem. Soc.* **108**, 4018–4022.

Berthold, D. A., Babcock, G. T., and Yocum, C. F. (1981) *FEBS Lett.* **134**, 231–234.

Boska, M., and Sauer, K. (1984). *Biochim. Biophys. Acta* **756**, 84–87.

Boska, M., Sauer, K., Buttner, W., and Babcock, G. T. (1983). *Biochim. Biophys. Acta* **722**, 327–330.

Bouges-Bocquet, B. (1980). *Biochim. Biophys. Acta* **594**, 85–104.

Brudvig, G. W., and Crabtree, R. H. (1986). *Proc. Natl. Acad. Sci. USA* **83**, 4586–4588.

Brudvig, G. W., Casey, J. L., and Sauer, K. (1983a). *Biochim. Biophys. Acta* **723**, 361–371.

Brudvig, G. W., Casey, J. L., and Sauer, K. (1983b). In *The Oxygen Evolving System of Photosynthesis* (Inoue, Y., Crofts, A. R., Govindjee, Murata, N., Renger, G., and Satoh, K., eds.), Academic Press, Tokyo, pp. 159–164.

Casey, J. L., and Sauer, K. (1984). *Biochim. Biophys. Acta* **767**, 21–28.

Cheniae, G. M. (1970). *Annu. Rev. Plant Physiol.* **21**, 467–498.

Clement-Metral, J., and Gantt, E. (1983) *FEBS Lett.* **148**, 307–312.

Cooper, S. R., and Calvin, M. (1977). *J. Am. Chem. Soc.* **99**, 6623–6630.

Critchley, C., and Sargeson, A. M. (1984). *FEBS Lett.* **177**, 2–5.

de Groot, A., Plijter, J. J., Evelo, R., Babcock, G. T., and Hoff, A. J. (1986). *Biochim. Biophys. Acta* **848**, 8–15.

Dekker, J. P., Plijter, J. J., Ouwehand, L., and van Gorkom, H. J. (1984a). *Biochim. Biophys. Acta* **767**, 176–179.

Dekker, J. P., van Gorkom, H. J., Brok, M., and Ouwehand, L. (1984b). *Biochim. Biophys. Acta* **764**, 301–309.

Dekker, J. P., van Gorkom, H. J., Wensink, J., and Ouwehand, L. (1984c). *Biochim. Biophys. Acta* **767**, 1–9.

de Paula, J. C., and Brudvig, G. W. (1985). *J. Am. Chem. Soc.* **107**, 2643–2648.

de Paula, J. C., Innes, J. B., and Brudvig, G. W. (1985). *Biochemistry* **24**, 8114–8120.

de Paula, J. C., Beck, W. F., and Brudvig, G. W. (1986). *J. Am. Chem. Soc.* **108**, 4002–4009.

Dismukes, G. C. (1986). *Photochem. Photobiol.* **43**, 99–115.

Dismukes, G. C., and Siderer, Y. (1980). *FEBS Lett.* **121**, 78–80.

Dismukes, G. C., and Siderer, Y. (1981). *Proc. Natl. Acad. Sci. USA* **78**, 274–277.

Dismukes, G. C., Ferris, K., and Watnick, P. (1982). *Photobiochem. Photobiophys.* **31**, 243–256.

Forbush, B., Kok B., and McGloin, M. (1971). *Photochem. Photobiol.* **14**, 307–321.

Förster, V., Hong, Y. -Q., and Junge, W. (1981). *Biochim. Biophys. Acta* **638**, 141–152.

Ghanotakis, D. F., O'Malley, P. J., Babcock, G. T., and Yocum, C. F. (1983). In *The Oxygen Evolving System of Photosynthesis* (Inoue, Y., Crofts, A. R., Govindjee, Murata, N., Renger, G., and Satoh, K., eds.), Academic Press, Tokyo, pp. 91–101.

Goodin, D. B., Yachandra, V. K., Britt, R. D., Sauer, K., and Klein, M. (1984). *Biochim. Biophys. Acta* **767**, 209–216.

Govindjee, Kambara, T., and Coleman, W. (1985). *Photochem. Photobiol.* **42**, 187–210.

Haase, W., Walz, L., and Nepveu, F. (1983). In *The Coordination Chemistry of Metalloenzymes* (Bertini, I., Drago, R. S., and Luchinat, C., eds.), D. Reidel, Holland, pp. 229–234.

Hansson, Ö., and Andréasson, L. -E., (1982). *Biochim. Biophys. Acta* **679**, 261–268.

Hansson, Ö., Andréasson, L. -E., and Vänngård, T., (1984). In *Advances in Photosynthesis Research* (Sybesma, C., ed.), Vol. 1, M. Nijhoff/Dr. W. Junk Publishers, The Hague, The Netherlands, pp. 307–310.

Hansson, Ö., Andréasson, L. -E., and Vänngård, T. (1986). *FEBS Lett.* **195**, 151–154.

Joliot, P., and Kok, B. (1975). In *Bioenergetics of Photosynethesis* (Govindjee, ed.), Academic Press, New York, pp. 387–412.
Joliot, P., Barbieri, G., and Chabaud, R. (1969). *Photochem. Photobiol.* **10**, 309–329.
Kambara, T., and Govindjee (1985). *Proc. Natl. Acad. Sci. USA* **82**, 6119–6123.
Kelley, P., and Izawa, S. (1978). *Biochim. Biophys. Acta* **502**, 198–210.
Kirby, J. A., Goodin, D. B., Wydrzynski, T., Robertson, A. S., and Klein, M. P. (1981a). *J. Am. Chem. Soc.* **103**, 5537–5542.
Kirby, J. A., Robertson, A. S., Smith, J. P., Thompson, A. C., Cooper, S. R., and Klein, M. P., (1981b). *J. Am. Chem. Soc.* **103**, 5529–5537.
Knaff, D. B., and Arnon, D. I. (1969). *Proc. Natl. Acad. Sci. USA* **63**, 956–962.
Kok, B., Forbush, B., and McGloin, M. (1970). *Photochem. Photobiol.* **11**, 457–475.
Kusunoki, M. (1983). In *The Oxygen Evolving System of Photosynthesis* (Inoue, Y., Crofts, A. R., Govindjee, Murata, N., Renger, G., and Satoh, K., eds.), Academic Press, Tokyo, pp. 165–173.
Kuwabara, T., and Murata, N. (1982). *Plant Cell Physiol.* **23**, 533–539.
Lam, E., Baltimore, B., Ortiz, W., Chollar, S., Melis, A., and Malkin, R. (1983). *Biochim. Biophys. Acta* **724**, 201–211.
Lavergne, J. (1986). *Photochem. Photobiol.* **43**, 311–317.
Murata, N., Miyao, M., Omata, T., Matsunami, H., and Kubawara, T. (1984). *Biochim. Biophys. Acta* **765**, 363–369.
Noodleman, L., Norman, J. G., Jr., Osborne, J. H., Aizman, A., and Case, D. A. (1985). *J. Am. Chem. Soc.* **107**, 3418–3426.
O'Malley, P. J., Babcock, G. T., and Prince, R. C. (1984). *Biochim. Biophys. Acta* **766**, 283–288.
Radmer, R., and Ollinger, O. (1986). *FEBS Lett.* **195**, 285–289.
Renger, G., and Govindjee (1985). *Photosynth. Res.* **6**, 33–55.
Sandusky, P. O., and Yocum, C. F. (1983). *FEBS Lett.* **162**, 339–343.
Satoh, K., Ohno, T., and Katoh, S. (1985). *FEBS Lett.* **180**, 326–330.
Sauer, K. (1980). *Acc. Chem. Res.* **13**, 249–256.
Saygin, Ö., and Witt, H. T. (1985). *Photobiochem. Photobiophys.* **10**, 71–82.
Schenck, C. C., Diner, B., Mathis, P., and Satoh, K. (1982). *Biochim. Biophys. Acta* **680**, 216–227.
Sheldon, R. A., and Kochi, J. K. (1981). *Metal-Catalyzed Oxidations of Organic Compounds*, Academic Press, New York.
Stewart, A. C., and Bendall, D. C. (1979). *FEBS Lett.* **107**, 308–312.
Velthuys, B. R. (1975). *Biochim. Biophys. Acta* **396**, 392–401.
Velthuys, B. R. (1981). In *Photosynthesis* (Akoyunoglu, G., ed.), Vol. II, Balaban International Science Service, Philadelphia, pp. 75–85.
Vermaas, W. F. J., Renger, G., and Dohnt, G. (1984). *Biochim. Biophys. Acta* **764**, 194–202.
Weiss, W., and Renger, G. (1984). *FEBS Lett.* **169**, 219–223.
Wydrzynski, T. J. (1982). In *Photosynthesis* (Govindjee, ed.), Vol. 1, Academic Press, New York, pp. 469–506.
Yachandra, V. K., Guiles, R. D., McDermott, A., Britt, R. D., Dexheimer, S. L., Sauer, K., and Klein, M. P. (1986a). *Biochim. Biophys. Acta* **850**, 324–332.
Yachandra, V. K., Guiles, R. D., Sauer, K., and Klein, M. P. (1986b). *Biochim. Biophys. Acta.* **850**, 333–342.
Yachandra, V. K., Guiles, R. D., McDermott, A., Cole, J. L., Britt, R. D., Dexheimer, S. L., Sauer, K., and Klein, M. P. (1987). *Biochemistry*, in press.
Yamamoto, Y., Doi, M., Tamura, N., and Nishimura, M. (1981). *FEBS Lett.* **133**, 265–268.
Yocum, C. F., Yerkes, C. T., Blankenship, R. E., Sharp, R. R., and Babcock, G. T. (1981). *Proc. Natl. Acad. Sci. USA* **78**, 7507–7511.
Zimmermann, J. -L., and Rutherford, A. W. (1984). *Biochim. Biophys. Acta* **767**, 160–167.
Zimmermann, J. -L., and Rutherford, A. W. (1986). *Biochemistry* **25**, 4609–4615.