DALTON
PERSPECTIVE

A mechanistic and structural model for the formation and reactivity of a Mn^V=O species in photosynthetic water oxidation

Julian Limburg,[a] Veronika A. Szalai[b] and Gary W. Brudvig*[a]

[a] *Department of Chemistry, Yale University, PO Box 208107, New Haven, CT 06520-8107, USA. E-mail: gary.brudvig@yale.edu*

[b] *Department of Chemistry, University of North Carolina at Chapel Hill, CB# 3290, Chapel Hill, NC 27599-3290, USA*

Received 29th September 1998, Accepted 15th January 1999

Photosynthetic water oxidation is carried out by a tetranuclear Mn cluster contained in the membrane-bound protein complex photosystem II (PSII). The mechanism of PSII catalysed water oxidation is unknown; however, several current models invoke a high-valent Mn=O species as a key intermediate in O–O bond formation. In part, these proposals are based on biophysical studies of the protein which suggest that the redox-active tyrosine residue, Y_Z, abstracts hydrogen atoms directly from substrate water molecules bound to the Mn_4 cluster. In this paper, we consider organic oxidation and O–O bond-forming reactions catalysed by biomimetic Mn and Ru model complexes that are believed to proceed *via* M=O intermediates. We also interpret biophysical data concerning the roles of Ca^{2+} and Cl^- in photosynthetic water oxidation, proposing that they are involved in a hydrogen-bonded network between the Mn_4 cluster and Y_Z. Connecting the observed reactivities of model complexes containing M=O groups to spectroscopic information on the environment of the Mn_4 cluster in the protein leads us to favour an O–O bond-forming step in photosynthetic water oxidation that occurs through nucleophilic attack of a calcium-bound hydroxide ligand on the electrophilic oxygen atom of a Mn=O intermediate. In addition, a new role for Cl^- is proposed in which Cl^- tunes the nucleophilicity of the calcium-bound hydroxide.

1.0 Introduction

The mechanism of photosynthetic water oxidation by photosystem II (PSII) remains, for the most part, an unresolved problem. In this review, we discuss recent results on the reactivity patterns of high-valent metal-oxo species and consider them in the context of current biophysical studies of PSII in order to propose a mechanistic model of the O–O bond-forming step of photosynthetic O_2 evolution. The model extends previous proposals and includes a new mechanistic role for Ca^{2+} and Cl^- which are required cofactors for water oxidation.

The oxygen-evolving complex (OEC) of PSII, Fig. 1, contains a tetranuclear manganese cluster and a redox active tyrosine, Y_Z.[1–4] While the precise structure of the Mn_4 cluster is unknown, interpretations of EPR and X-ray absorption data have allowed for some fairly detailed proposals,[3,5–9] some of which are shown in Fig. 2. A key structural element indicated from EXAFS studies is the di-μ-oxo Mn dimeric unit, and there are numerous examples of oxo-Mn cluster model complexes (reviewed in refs. 6 and 7). To date, no manganese cluster has been synthesised that closely matches the spectroscopic properties of the OEC.

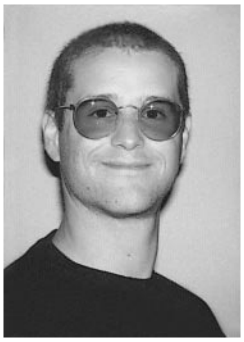
Water oxidation is a four-electron process, and the OEC has been shown to cycle through five intermediate oxidation states from S_0 to S_4 (Fig. 3).[10] Each S-state advance is associated with

---

Gary Brudvig received his bachelor's degree in chemistry from the University of Minnesota in 1976 and then went on to gain his Ph.D. in chemistry from the California Institute of Technology in 1980. His graduate work at Caltech concerned cytochrome c oxidase and was performed under the direction of Sunney Chan. From 1980–82, he was a Miller Fellow at the University of California, Berkeley studying with Kenneth Sauer. He joined the faculty at Yale University in 1982, where he is now a Professor of Chemistry, and where he applies both biophysical and bioinorganic techniques to the study of photosystem II.

Julian Limburg was born in London in 1972. He earned his bachelor's degree in chemistry from the University of East Anglia in 1993. His undergraduate studies included an exchange year at the University of Massachusetts, Amherst (1991–1992). Since 1993 he has been studying for a Ph.D. at Yale University under the direction of Gary Brudvig and Robert Crabtree working on bioinorganic model systems for photosynthetic water oxidation.

Gary W. Brudvig



Julian Limburg

Veronika A. Szalai

Veronika Szalai received her bachelor's degree in chemistry from Bryn Mawr College in 1988. Before entering graduate school, she was employed as a research assistant at EniChem America, Inc. She recently completed her Ph.D. in chemistry at Yale University using electron paramagnetic resonance to study the active site for water oxidation in photosystem II. Currently a Lineberger Comprehensive Cancer Center post-doctoral researcher in Dr. H. Holden Thorp's laboratory at the University of North Carolina at Chapel Hill, she is interested in biophysical and bioinorganic chemistry.

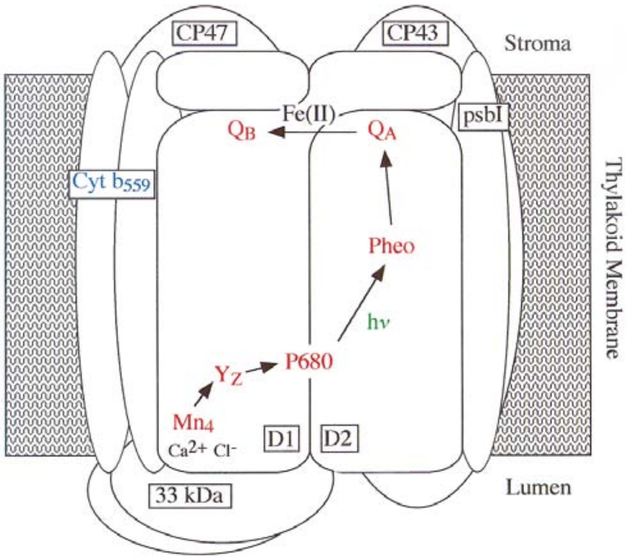


Fig. 1 Model of photosystem II in the thylakoid membrane. The arrows show the direction of electron-transfer reactions. After initial photoexcitation, the special chlorophyll called P680 transfers an electron to a pheophytin (Pheo) molecule. The electron is rapidly passed to Q_A, a tightly-bound plastoquinone, and ultimately reduces an exchangeable plastoquinone in the Q_B-binding site. $P680^+$ is reduced by tyrosine Z (Y_Z). Oxidized Y_Z is reduced by a tetranuclear manganese cluster, Mn_4. Calcium and chloride are required and expected to be in close proximity to the Mn_4 cluster.

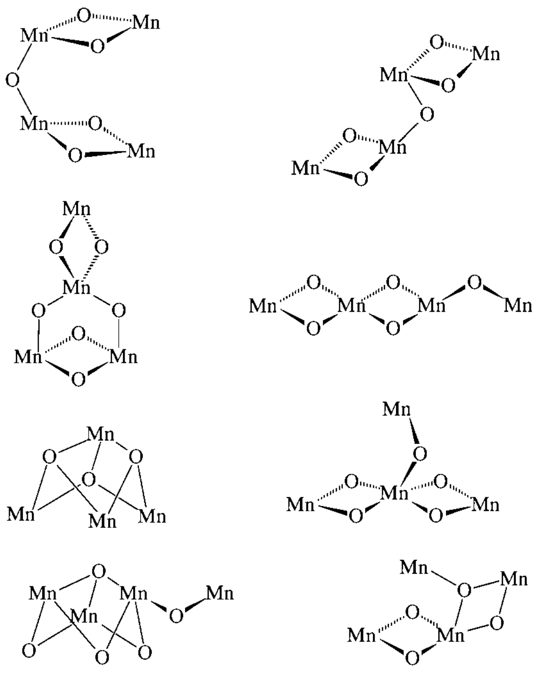


Fig. 2 Structural models of the OEC based on X-ray absorption spectroscopic data of PSII. Adapted from ref. 3.

light-induced charge separation at the chlorophyll-containing pigment P680 to form the strong oxidant $P680^+$. The tyrosyl radical, $Y_Z^\bullet$, formed upon reduction of $P680^+$ oxidises the manganese cluster which, in turn, is reduced by electrons stripped from water. Flash-induced UV[11] and XANES[12–14] data have been interpreted as showing that the Mn_4 cluster is oxidised on each S-state transition. However, there is a current controversy over whether Mn or an associated ligand is oxidized in the S_2 to S_3 step.[15]

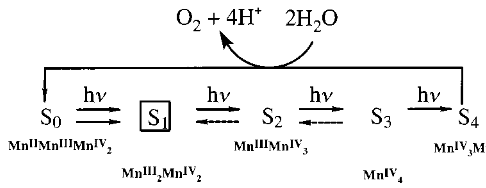


Fig. 3 The S-state cycle proposed by Kok *et al.*[10] The solid arrows represent light-driven reactions and the dashed arrows represent dark reactions. The box around S_1 denotes that this is the dark-stable state. The Mn oxidation states are based on UV and X-ray absorption studies.

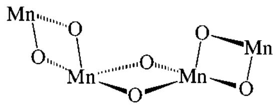


Fig. 4 Structural core of the complex of Blondin *et al.*[25]

The manganese tetramer in PSII exhibits EPR signals from the odd-electron S_0 and S_2 states.[16–19] A combination of EPR[8,20] and XANES[3,14,21,22] data has led to the assignment of S_2 as $Mn^{III}Mn^{IV}_3$ although $Mn^{III}_3Mn^{IV}$ cannot be ruled out.[23,24] Assignment of a $Mn^{III}Mn^{IV}_3$ configuration for the S_2 state of the OEC has recently been supported by Blondin *et al.*[25] who reported a mixed-valence manganese tetramer (Fig. 4) with an $S = 1/2$ ground state, formed by gamma irradiation of the corresponding EPR-silent Mn^{IV} tetramer. The EPR spectrum so obtained is the closest match from a model complex to the S_2-state multiline EPR signal of the OEC seen thus far.

Dioxygen is released during the transition from S_3 to S_0, and there have been a number of proposals on the nature of the active species involved in O–O bond formation.[3,5,26–41] Many of these mechanisms have not been developed with the chemistry of Mn=O groups in mind. The most current mechanistic models take into account the point-dipole distance between $Y_Z^\bullet$ and the Mn_4 cluster, estimated to be 7–10 Å.[42–45] Spectroscopic studies of Y_Z indicate that its proton is released to a nearby histidine residue[46–49] upon oxidation to generate the neutral tyrosyl radical, $Y_Z^\bullet$,[50] which suggests that proton movement is an important component of Y_Z reactivity. The O–H bond-dissociation energy of water terminally ligated to Mn(III) or Mn(IV) has been estimated to lie between 78 and 89 kcal mol^{-1}.[27,51,52] This is comparable to the O–H bond-dissociation energy of tyrosine, estimated at 86.5 kcal mol^{-1},[53,54] allowing for H-atom abstraction from a bound water to be exothermic and so provide extra driving force relative to a purely outer-sphere oxidation of the Mn_4 cluster by $Y_Z^\bullet$. It has been argued on the basis of studies of inhibited PSII that $Y_Z^\bullet$ cannot remain a competent oxidant of the Mn_4 cluster above the S_2 state unless proton-coupled electron-transfer from the Mn_4 cluster to $Y_Z^\bullet$ is invoked.[55] As a result, an attractive possibility is that the S_4 state contains a Mn=O species, formed by $Y_Z^\bullet$ abstracting hydrogen atoms from a water bound to manganese.[26,27,30,32–34,41] The proposal for a high-valent Mn=O-containing intermediate assumes that the manganese cluster is oxidized on each S-state advance.

Both Ca^{2+} and Cl^- are required for maximal rates of O_2 production by PSII[2,56,57] and, primarily, these cofactors have been assigned structural or electrostatic charge-balance roles.[12,26,40,57–61] Most mechanistic proposals of water oxidation attempt to include all four relevant cofactors—Ca^{2+}, Cl^-, the Mn_4 cluster and Y_Z—because they are believed to be in close proximity to one another in the protein matrix.

Removal of calcium or chloride produces a state of the OEC that exhibits a "split" radical EPR signal[62] that has been interpreted as arising from a tyrosyl radical interacting with the Mn_4-cluster in its S_2 state, and has been formulated as $S_2Y_Z^\bullet$.[43,45,55,63–67] Chloride can be competitively replaced by acetate,[63,68] to produce an $S_2Y_Z^\bullet$ state, although the effects of acetate inhibition do not mimic all the effects of chloride depletion.[63,69,70] Importantly, acetate-inhibited samples display characteristics of both chloride-depleted and calcium-depleted PSII preparations,[63] suggesting that the roles of chloride and calcium are related. Spectroscopic studies have demonstrated that bound acetate is close to both Y_Z and the Mn_4 cluster,[68,71] and could be bridging between them in a similar manner to that observed in Concanavalin A.[63,72]

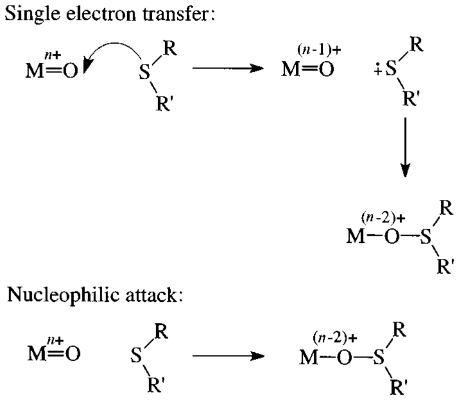
The body of biophysical data on the OEC provides evidence for a mechanism in which $Y_Z^{\bullet}$ is involved in H-atom abstraction from Mn-bound substrate water molecules ultimately leading to the formation of a Mn=O species. In section 3, we propose a model that explains these effects by assigning chloride as a bridging ligand between calcium and the Mn_4 cluster, with calcium being involved in an H-bonded network between the Mn_4 cluster and Y_Z as well as being a site for substrate binding. The participation of these cofactors in hydrogen bonds and proton movement is supported by the observation that Mn-, Ca^{2+}- or Cl^--depleted PSII samples cannot undergo S-state transitions and/or Y_Z oxidation at the same temperatures as intact PSII.[1,70,73–80] In this way, Ca^{2+} and Cl^- may be intimately involved in the formation of a Mn=O species in PSII.

This review begins with a discussion of oxygen-atom transfer reactions involving M=O (M = Ru or Mn) species, emphasizing the factors which affect the reactivity of the oxo group, especially toward nucleophilic attack. Although there is a vast amount of literature on the reactivity of Fe-based oxidation catalysts as cytochrome P450 models (for leading references see 81–83), none of the Fe-based catalysts has been implicated in O–O bond formation leading to O_2 production so they are not discussed in any detail here. The review concludes with a model for photosynthetic water oxidation that combines the biophysical and model chemistry results. We present our proposal of the S-state cycle which involves a Mn^V=O as a key intermediate in O–O bond formation. In addition, we suggest roles for both Ca^{2+} and Cl^- and consider why these cofactors are specifically required by the OEC. Finally, we support the proposal that the key step in photosynthetic water oxidation may be nucleophilic attack of a Ca^{2+}-bound hydroxide/water on an electrophilic Mn^V=O.[26,41]

2.0 Inorganic oxidation catalysts

The role of activated metal-oxo species in oxidation chemistry is well established in both biological and inorganic model systems. Such species are capable of oxidizing a wide variety of substrates including phosphines, amines, sulfides, alkenes and alkanes.[84–91]

Scheme 1 shows a simplified view of how a metal-oxo species may react with a potential nucleophile, using a sulfide as an example. The first example, with a one-electron rate-determining step, requires a radical intermediate and the second example, with a two-electron rate-determining step, can be thought of as an S_N2 reaction involving nucleophilic attack on an electrophilic oxo. The mechanism of a particular oxidation is strongly dependent on the nature of both the oxidant and the substrate. In this review, we argue that in the case of water oxidation by a Mn=O, a two-electron, or S_N2-like, mechanism would predominate.



Scheme 1 Reactions of M=O with nucleophiles highlighting the differences between one- and two-electron processes.

In the first part of this section, we discuss oxidations involving Ru=O complexes. These are very well characterized and so serve as excellent models for M=O reactivity. In the second part, we look at reactions that are thought to proceed *via* Mn=O intermediates and discuss their mechanisms by analogy to the better understood Ru chemistry.

2.1 Ruthenium

Despite their reactivity, a number of Ru=O complexes with a variety of coordination environments have been isolated and structurally characterized.[92] Ru=O complexes (Ru = +4 to +7) can oxidize organic substrates[87,88,92] and, most importantly for our interests, have been identified as key intermediates in homogeneous catalysis of water oxidation.[93]

Studies on electronic effects governing the reactivity of Ru oxidation catalysts have shown that as the basicity of the ligands to the Ru decrease, the oxo group becomes more reactive.[94,95] A simplified way of considering this is that the positive charge residing on the metal increases and so the Ru–O bond becomes more polarized.[92]

Acquaye *et al.*[95] studied the oxidation of thioanisoles and methyl phenyl sulfoxides by $(O)(P(p\text{-}C_6H_5R)_3)Ru^{IV}$ complexes, varying the basicities of both the phosphines on the Ru and of the substrates by changing the *para* substituents on the phenyl rings. When the substituents on the phosphines were varied, the order of the rates of reactivity was CF_3 > F > H > Me > OMe, with an approximately ten-fold rate difference across the entire range for both sulfide and sulfoxide oxidation. These results clearly indicate that better donor ligands decrease reactivity, presumably by stabilizing the M=O complex. The authors also showed that the reaction rates increased linearly with an increase in the $E_{\frac{1}{2}}$ of the $Ru^{IV/III}$ couple. Similar results were observed for substrate reactivity, *i.e.* the more basic the substrate, the faster it reacted.

A comparison of sulfide and sulfoxide reactivity led to some interesting conclusions. Thioanisoles reacted with the Ru=O complexes approximately 100 times faster than the corresponding sulfoxides. For instance, the second-order rate constant for thioanisole oxidation was 2.30 M^{-1} s^{-1} compared to 0.06 M^{-1} s^{-1} for methyl phenyl sulfoxide, and this is expected as sulfides are more basic than sulfoxides. However, detailed kinetic analyses of the reactions suggested that the two substrates react *via* different mechanisms. A deuterium-isotope effect of $k_H/k_D = 1.14$ was measured comparing thioanisole and methyl-d_3 phenyl sulfide *vs.* a $k_H/k_D = 0.64$ for the equivalent sulfoxides. This result is consistent with a single-electron transfer mechanism for sulfide oxidation and an S_N2-like mechanism for sulfoxide oxidation. The assignment was further supported by Hammett plots on the rates of oxidations when the basicities of the phosphine ligands and substrate were varied. One interpretation of these results is that the rate-determining step in sulfoxide oxidation involves nucleophilic attack on the oxo group on the metal. This change in mechanism for oxidation of sulfides *vs.* sulfoxides would then be explained by the unfavourable energetics of the one-electron oxidation of sulfoxides (for the one-electron oxidation *vs.* SCE: $E^0_{\text{thioanisole}} = 1.45$ V,[95] $E^0_{\text{methylphenylsulfoxide}} = 2.30$ V[96]). An important conclusion is that the mechanism—nucleophilic attack *vs.* radical formation—is decided by the redox properties of the substrate.

2.1.1 O–O bond formation: O_2 evolution. The most characterized system for catalytic homogeneous water oxidation is $[(bpy)_2(H_2O)RuORu(H_2O)(bpy)_2]^{4+}$ (bpy = 2,2′-bipyridine), which was first reported by Gersten *et al.*[97] in 1982, and which

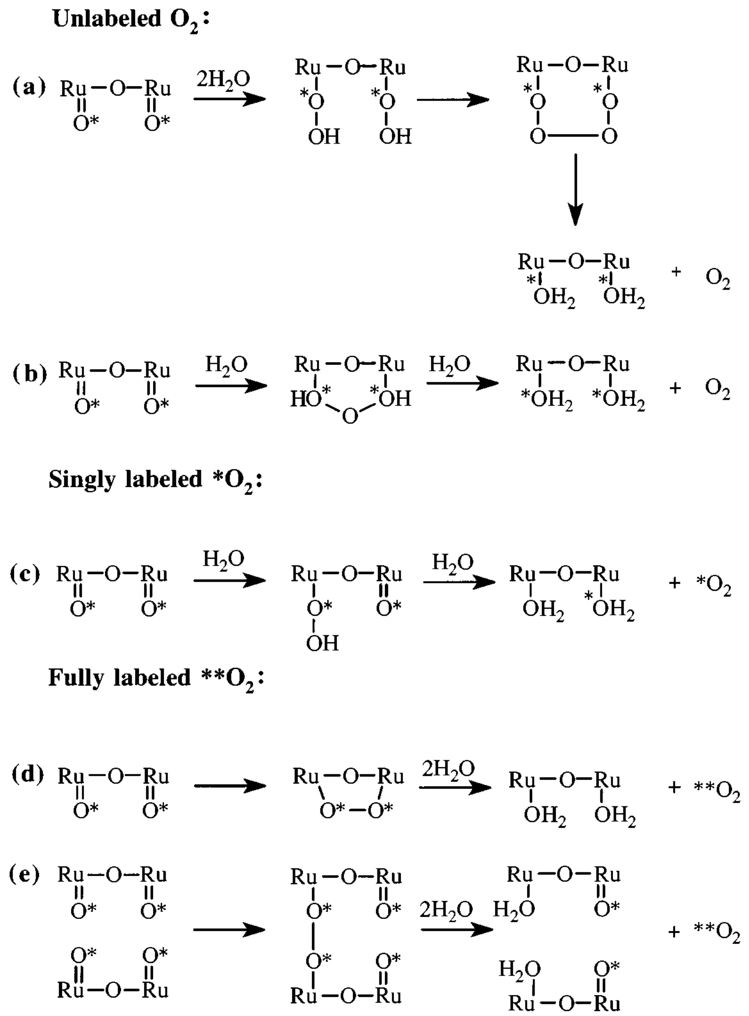


Fig. 5 Mechanisms to explain ^{18}O incorporation into $[(bpy)_2(H_2O)RuORu(H_2O)(bpy)_2]^{4+}$. Adapted from ref. 5.

can oxidize water both electrochemically,[97] and chemically, using Co^{3+} or Ce^{4+} as primary oxidants.[93,98] Subsequent studies have shown that the key intermediate involved in O–O bond formation is a Ru^V=O species.[93]

Single-turnover labeling studies using $H_2{}^{18}O$-Ru and unlabeled solvent water, where the O_2 formed was analysed using mass spectrometry, were inconclusive as to the precise mechanism of O–O bond formation. Geselowitz and Meyer[98] reported a $^{36}O_2$: $^{34}O_2$: $^{32}O_2$ ratio of 13 : 64 : 23, whereas a similar study by Hurst *et al.*[93] gave a $^{34}O_2$: $^{32}O_2$ ratio of approximately 50 : 50, and no $^{36}O_2$, and also showed that the aqua ligands on the Ru(III) complex exchanged slowly on the timescale of the catalysis. It should be noted that the experiments were run under different conditions. Fig. 5 shows possible mechanisms that are consistent with the results of the labeling studies,[5,37,93,98] and multiple pathways must be invoked to account for all the products. Importantly, $^{34}O_2$ formation requires that one oxygen atom originate from the solvent and the other from a ligand to Ru, and this is consistent with a pathway involving an attack of solvent water on a terminal oxo ligand.

2.2 Manganese

In contrast to the Ru chemistry, the detection of Mn=O catalytic intermediates has been a challenge. Manganates excepted, there are only four structurally characterized Mn=O complexes,[99–102] only one of which has shown reactivity.[102] The reactive system was developed by engineering a ligand that could coordinate cations close to the Mn(V)=O, thereby tuning the electrophilicity of the terminal oxo.[102] For instance, adding 5 equivalents of Sc^{3+} to the complex increased the rate of Ph_3P oxidation by a remarkable three orders of magnitude compared to the system in the absence of an added cation.

There have been a few reports of spectroscopic characterization of Mn=O intermediates. The first was published by Groves and Stern who synthesized three porphyrin Mn^{IV}=O complexes, (TMP)Mn(O), [(TMP)Mn(O)(OH)] and (TPP)-(Mn)(O) (TMP = 5,10,15,20-tetramesitylporphyrin, TPP = tetraphenylporphyrin),[103,104] and spectroscopically characterized them. The Mn=O group is generated by reacting the appropriate Mn^{III} complex with *m*-CPBA (*meta*-chloroperoxybenzoic acid). The presence of Mn=O was confirmed with ^{18}O labeling and vibrational spectroscopy. A transient species with a strong visible absorbance at approximately 420 nm which cannot be attributed to either Mn^{IV}, Mn^{III} or a Mn^{IV}=O-porphyrin radical cation has been seen in the *m*-CPBA systems, as well as in reaction mixtures using potassium peroxomonosulfate as the oxidant.[105] Accordingly, this has been assigned as a Mn^V=O. The presence of Mn=O's in manganese-porphyrin oxidation chemistry has also been inferred from incorporation of ^{18}O into the product from labeled water in olefin epoxidation,[105–108] proposed to proceed by the mechanism shown in Fig. 6.

$[(salen)Mn^{III}]^+$ (salen = *N*,*N*′-bis(salicylidene)ethylenediamine di-anion) Catalysed epoxidations are thought to require a Mn^V=O species analogous to that seen in the porphyrin systems.[109,110] Recently, Feichtinger and Plattner[111] used ES–MS to study the reaction between $[(salen)Mn^{III}]^+$ and iodosobenzene, and saw a *m*/*z* peak that was attributable to a Mn^V=O complex. This is the first direct evidence for this intermediate in the salen system.

Both the salen and porphyrin systems have been well studied, and their reactivities follow broadly the trends seen for Ru

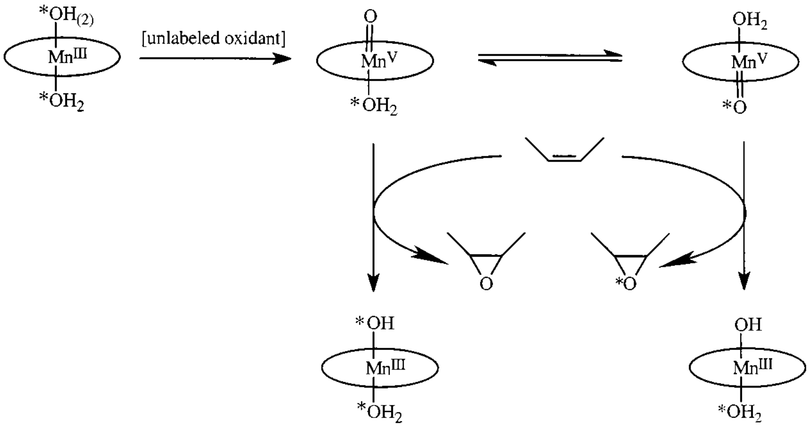


Fig. 6 Mechanism of incorporation of ^{18}O from solvent water into epoxides formed from the reaction of Mn^V=O(porphyrin) species with olefins. Based on refs. 105 and 107.

catalysts, that is to say, less stabilizing ligands lead to more reactive intermediates. Chellamani *et al.*[110,112] looked at the electronic effects on the oxidation of thioanisoles by $[(salen)Mn^{III}]^+$, analogous to that published for $(O)(P(p\text{-}C_6H_5R)_3)Ru^{IV}$ complexes.[95] As with Ru, it was found that the less basic the ligand, the more reactive the catalyst. Further, Hammett plots similar to those obtained for $(O)(P(p\text{-}C_6H_5R)_3)Ru^{IV}$ complexes were reported in which both the salen ligand and substrate basicities were varied, suggesting that $[(salen)Mn^{III}]^+$ and $(O)(P(p\text{-}C_6H_5R)_3)Ru^{IV}$ both oxidize sulfides by analogous mechanisms, *i.e.* single electron transfer from a M=O intermediate. Unfortunately, the manganese system did not oxidize the sulfoxides to sulfones under the reported conditions so no information is available on whether a two-electron reaction would predominate here. These electronic effects manifest themselves dramatically in Jacobsen's epoxidation catalyst (an enantioselective version of the $[(salen)Mn^{III}]^+$ complex) which shows a direct correlation between electronic effects and selectivity, *i.e.* the more reactive the catalyst, the less selective the oxidation.[113] In both of these cases, an analogy to the Ru chemistry can be drawn and the oxo on manganese can be thought of as becoming increasingly electrophilic as the other ligands to manganese become less basic.

A further, interesting, aspect of Mn-oxidation chemistry is the role of the ligand *trans* to the active site on the complex. It was found early on that adding a base such as pyridine or imidazole as a co-catalyst increases greatly the reactivity of Mn porphyrins.[108,114] Subsequent proposals suggest that these bases act as π-donors to the Mn=O LUMO, an antibonding orbital (Fig. 7).[86,115] This has the effect of weakening the Mn=O bond and so increasing the reactivity of the oxo group by, again, making it more electrophilic.[115] As a result, a basic group *cis* to the active site stabilizes a terminal oxo, whereas a π-donor *trans* to the active site increases its reactivity.

2.2.1 O–O bond formation: O_2 evolution.

There have been only a few reports of manganese complexes able to catalyse homogeneous O–O bond-forming reactions that lead to dioxygen evolution (reviewed in references 5 and 37). Naruta *et al.* have reported homogeneous catalytic water oxidation from a face-to-face Mn(porphyrin) dimer (Fig. 8).[116] The active species was suggested to be a Mn^V=O dimer, and this would be consistent with the other Mn(porphyrin) catalysts. This mechanism has since been supported by studies involving olefin epoxidation.[117]

We have developed a Mn di-μ-oxo dimer capable of catalysing O_2 evolution from oxone[5,118] or sodium hypochlorite.[119] The catalyst is ultimately lost by formation of permanganate which is unreactive under these conditions. This is the first example of a di-μ-oxo Mn dimer, which is a structural element present in the OEC, that has the relevant functional chemistry. When the reaction with hypochlorite is run in $H_2{}^{18}O$ the label is incorporated into the dioxygen.[119] The ratio $^{36}O_2:{}^{34}O_2$ is consistent with water being the source of the oxygen in the O_2 evolved, and we interpret the mechanism to involve exchange of oxygen from water with a Mn=O species. In analogy to the porphyrin dimer,

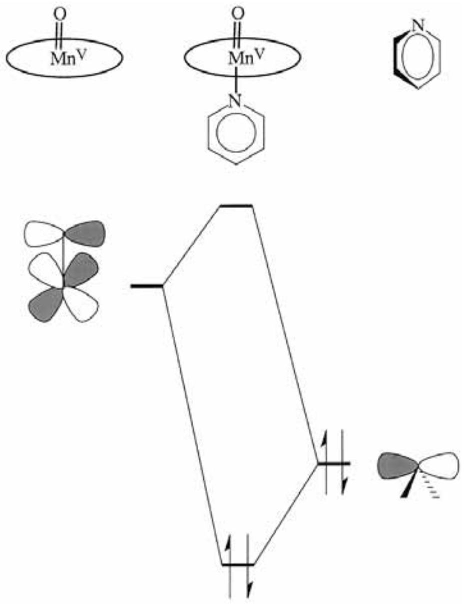


Fig. 7 Molecular orbitals of a M=O species showing π-donation of bases into the antibonding orbital. Adapted from Jørgensen and Swanstrøm.[115]

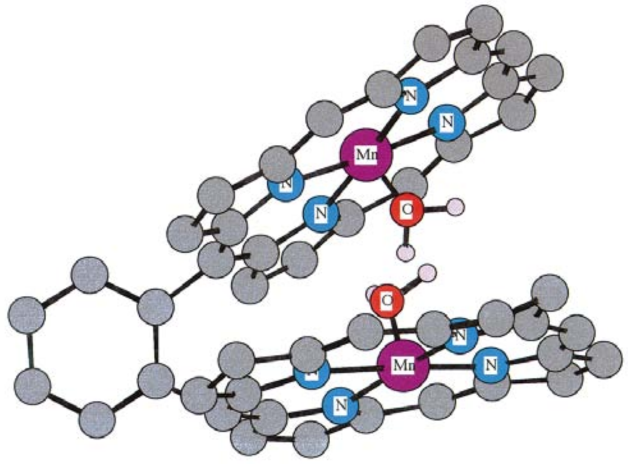


Fig. 8 Structure of the face-to-face $[Mn(porphyrin)]_2$ complex reported by Naruta *et al.*[116]

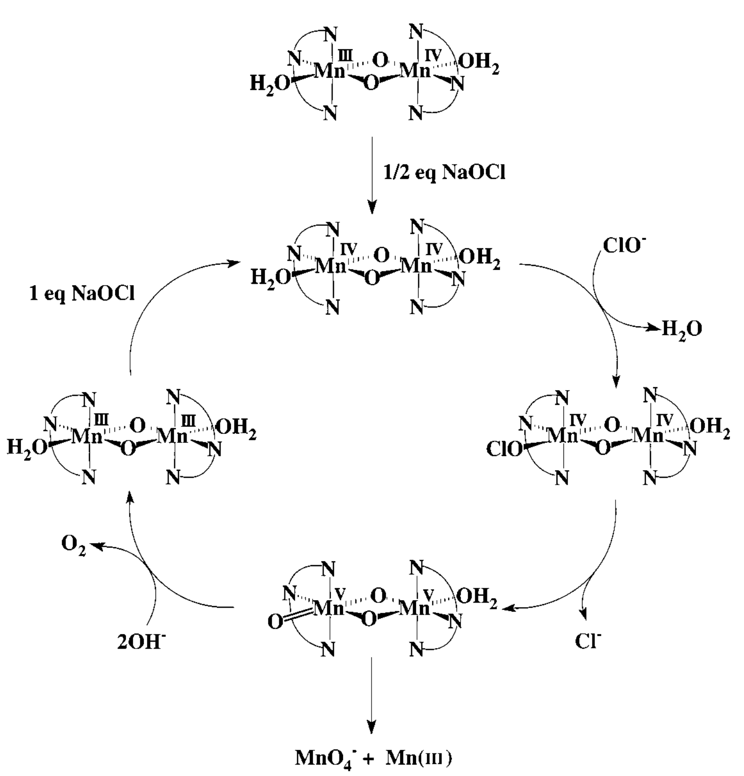


Fig. 9 Possible mechanism of O–O bond formation from the reaction between $[(Terpy)(H_2O)Mn(O)_2Mn(OH_2)(Terpy)](NO_3)_3$ (Terpy = 2,2′ : 6′,2″-terpyridine) and sodium hypochlorite.[118]

we have proposed a mechanism with a Mn^V=O intermediate (Fig. 9); one possibility for O–O bond formation is attack of a hydroxide on the oxo ligand.

3.0 A mechanism for photosynthetic water oxidation

We have presented a basis for an O–O bond-forming reaction that proceeds *via* nucleophilic attack on a Mn^V=O. In this section, we consider how such a reaction could be promoted by the OEC and consider the implications that the chemistry of M=O species has on the suggestion that a Mn=O species is formed during photosynthetic water oxidation. In addition, we tie together observations about Ca^{2+} and Cl^- and propose that their role is to align and tune the reactivity of hydroxide as a nucleophile. This is incorporated into a new structural and mechanistic model that extends the H-atom abstraction model presented by Hoganson *et al.*[32,33] Assembling all of this information, we present a detailed picture of the O–O bond-forming reaction which occurs between the S_3 and S_0 states of the S-state cycle of the OEC.

Fig. 10 shows our model of the intermediates in the S-state cycle of photosynthetic water oxidation focusing on the Mn_4 cluster. The structure of the manganese cluster shown is based on the structure proposed by Yachandra *et al.* from X-ray absorption data.[9] However, in Fig. 10, a single terminal Mn ion is proposed to be directly involved in the O–O bond-forming step, with the other three Mn ions serving as a source of oxidizing equivalents and/or as structural elements. Therefore, other structures for the Mn_4 cluster are readily accommodated by this model. As suggested by recent EPR results,[17–19,120] the oxidation states of the manganese atoms in the S_0 state are assigned as $Mn^{II}Mn^{III}Mn^{IV}_2$ in which one dimer consists of Mn^{IV} atoms and the other dimer is a $Mn^{II}Mn^{III}$ pair. As there are no examples of di-μ-oxo $Mn^{II}Mn^{III}$ complexes, the $Mn^{II}Mn^{III}$ dimer in the cluster is suggested to be bridged by one μ-hydroxo and one μ-oxo ligand. Advance from the S_0 to the S_1 state would be followed by deprotonation of the $Mn^{II}Mn^{III}$ μ-hydroxo to form a di-μ-oxo $Mn^{III}Mn^{III}$ dimer; there are three such dimers known from model chemistry.[121,122] The role of such proton-coupled steps involving interconversion of μ-hydoxo and μ-oxo bridges in mediating oxidation potentials have been studied in Mn model complexes,[123,124] and analogous reactions involving the OEC may allow for approximately equipotential sequential oxidations of the Mn_4 cluster in the early S states. Oxidation of the Mn_4 cluster from S_1 to the S_2 state would involve electron transfer from the manganese cluster to $Y_Z^•$ together with protonation of $Y_Z^•$ by a non-substrate species. In these steps, the role of BDE's in providing sufficient driving force for the reactions may be unimportant because a variety of inhibited states of the OEC, including calcium- and chloride-depleted samples, can advance to the S_2 state.

Advancement from the S_2 to S_3 and S_3 to S_4 states would involve oxidation of the Mn_4 cluster together with a proton abstraction from a water ligand, ultimately converting the water ligand to a terminal oxo in the S_4 state. Importantly, in this proposal these steps cannot proceed if the proton transfer from the substrate water to $Y_Z^•$ is inhibited. Fig. 11 shows a scheme of OEC oxidation from the S_3 to the S_4 state which incorporates interpretations of current biophysical data on Ca^{2+}, Cl^-, the Mn_4 cluster and Y_Z. In the final step, the S_4 state would collapse to the S_0 state through nucleophilic attack of hydroxide on a Mn^V=O group with the concomitant release of O_2. Because advance to S states higher than S_2 has been proposed to proceed through proton-coupled electron transfer and because Ca^{2+} and Cl^- have been found to be required for the S_2-to-S_3-state transition,[69,74] we propose detailed structural models of only the higher S-state transitions.

Fig. 12 shows a structural model for the OEC incorporating the proposed roles of Ca^{2+} and Cl^-. The geometric center-to-center distance between $Y_Z^•$ and the Mn_4 cluster is ≈9 Å in this model, and the point-dipole distance[125] is within the currently accepted 7–10 Å distance estimate limits.[42–45,125] Another feature of this model is that it includes a well-ordered hydrogen-bonded network between Ca^{2+}, Y_Z, the Mn_4 cluster, and a histidine residue (D1-His 190, the proton acceptor for Y_Z upon its oxidation). In this hydrogen-bonded network, calcium serves as an anchor between a hydroxide ligated to the manganese cluster and Y_Z. Part of the chloride's role would be to position calcium

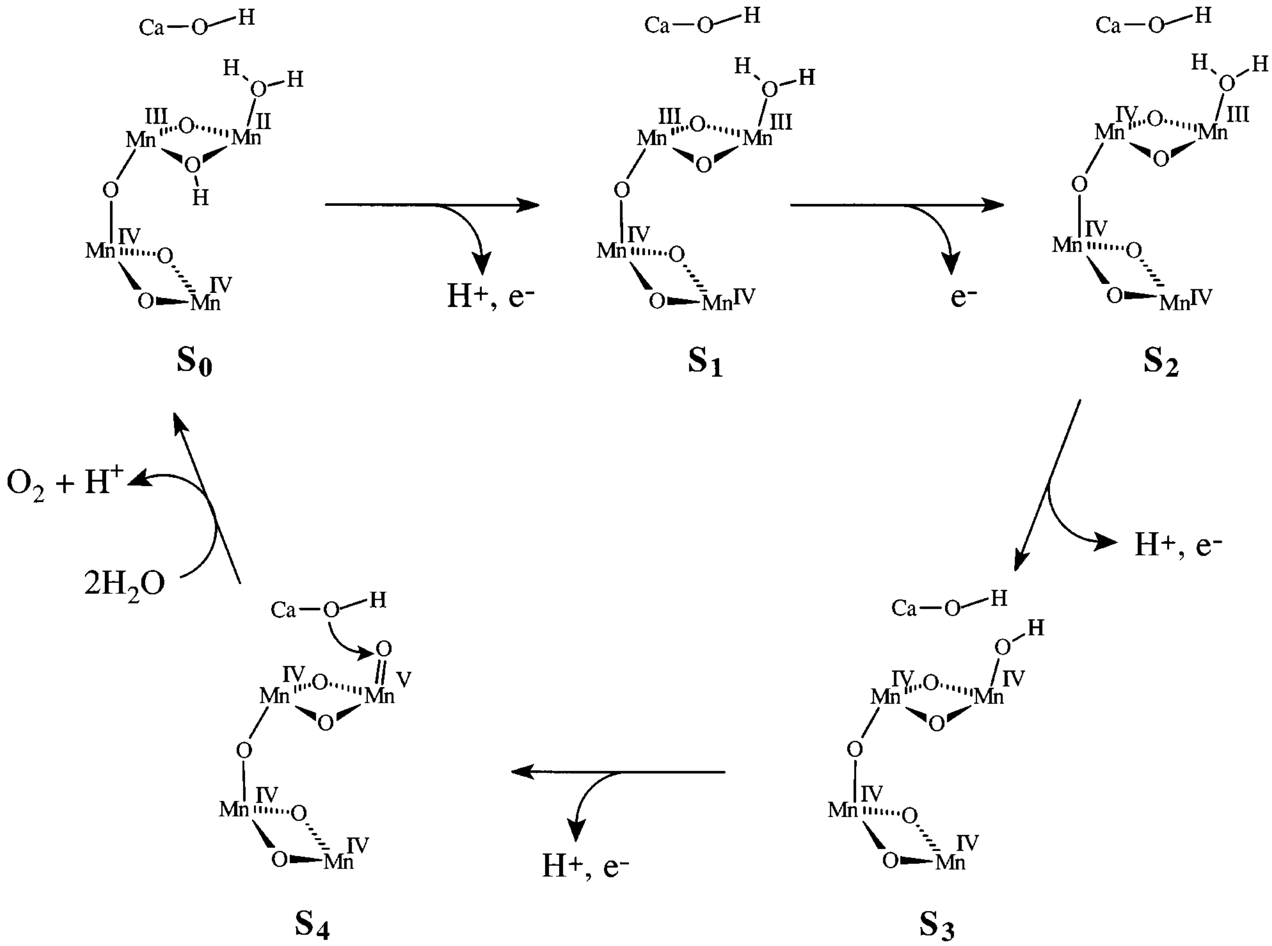


Fig. 10 Proposed S-state cycle of the OEC in PSII. The Mn_4 cluster structure shown is the structure proposed by Yachandra *et al.*[9]

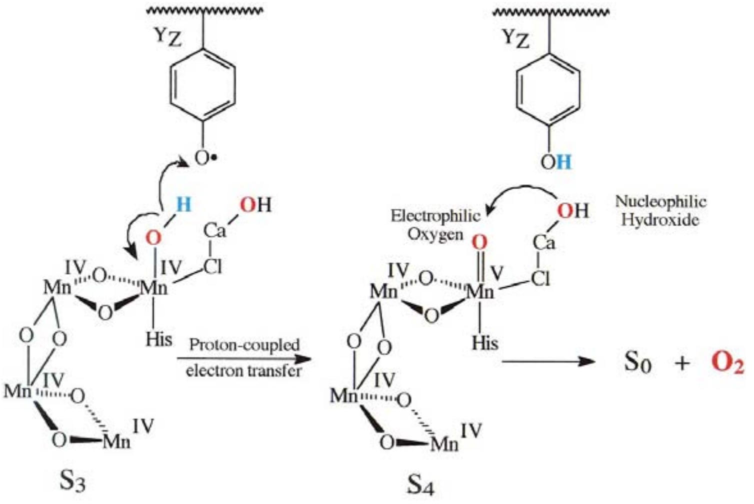


Fig. 11 Hypothetical O–O bond-forming steps (S_3 to S_0 states) of the OEC in PSII showing the positions of Ca^{2+}, Cl^-, Y_Z and the Mn_4 cluster. The Mn_4 cluster is based on the model complex of Blondin *et al.*[25]

between Y_Z and the Mn_4 cluster. Because $Y_Z^{\bullet}$ abstracts a hydrogen atom from the S_2 state to convert it to the S_3 state, Ca^{2+} and Cl^- together could create a local structure uniquely designed to make advance to the S_3 state energetically and kinetically accessible. Removal of Ca^{2+} or Cl^- would disrupt the H-bonded structure between the Mn_4 cluster and Y_Z, thereby preventing the S_2 to S_3-state oxidation step. Fig. 12 also shows a histidine ligated to the manganese *trans* to where the oxo group forms. This is consistent with ESEEM studies which have shown that there is at least one histidine ligated to the Mn_4 cluster.[126] An aromatic amine *trans* to the oxo group could play an activating role as shown in Fig. 7.

The final step, advance to the S_4 state, involves H-atom abstraction to form a Mn^V=O. We support the hypothesis that O–O bond formation is achieved by nucleophilic attack on the Mn^V=O by a hydroxide ligated to calcium.[26,41] This type of mechanism is consistent with the calculations of Siegbahn and Crabtree which suggest that Mn=O groups are electron deficient.[127] It is also consistent with the idea that M=O species react with substrates that are difficult to oxidize by one

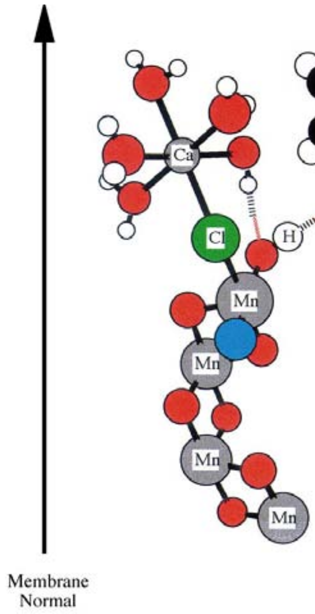


Fig. 12 A structural model of the OEC showing the proposed hydrogen-bonded network between Ca^{2+}, Cl^{-}, the Mn_4 cluster and Y_Z. The model was generated by using Chem 3D and metrical parameters from the Cambridge Structural Database, as described in ref. 125. The blue atom *trans* to OH on the terminal Mn represents a nitrogen atom of a histidine ligand.

electron, such as hydroxide, by a two-electron S_N2-like process (see preceding sections).

In order for the O–O bond-forming reaction to occur, the nucleophile must be delivered at the same step in which the Mn^V=O is created. The proposed mechanism also includes a new functional role for chloride as a bridge that communicates the formation of the Mn^V=O to Ca^{2+} in order to activate the hydroxide for nucleophilic attack. We envision that by binding between the Mn_4 cluster and Ca, chloride tunes the nucleophilicity of the calcium-hydroxide by responding to changes in the oxidation state of the Mn_4 cluster. As the oxidation level of the Mn_4 cluster is increased, chloride binds more tightly to it. As a result of this, the chloride is pulled away from calcium, thereby causing calcium to become a stronger Lewis acid. In effect, advance to the S_4 state would change the interaction between calcium and chloride so that a nucleophilic hydroxide would be created in concert with an electrophilic Mn^V=O species, to facilitate formation of the O–O bond.

4.0 Summary

We have presented a new model for photosynthetic water oxidation that combines inorganic and biophysical studies to support the idea that the O–O bond-forming step involves nucleophilic attack of a calcium-bound hydroxide on an electrophilic Mn^V=O species. In our proposal, the reactivity of the terminal oxo is enhanced by a histidine ligated *trans* to it. Another feature of the model assigns roles for calcium and chloride as mediating proton-coupled electron transfer between the Mn_4 cluster and $Y_Z^{\bullet}$ by way of a hydrogen-bonded network. Further, having chloride as a bridging ligand between calcium and the Mn_4 cluster allows it to control the Lewis acidity of calcium, thereby tuning the reactivity of the substrate hydroxide.

Acknowledgements

We would like to thank Drs. G. T. Babcock, R. H. Crabtree and P. E. M. Siegbahn for copies of submitted manuscripts. This work was supported by grants from the National Institutes of Health.

References

1 B. A. Diner and G. T. Babcock, in *Oxygenic Photosynthesis: The Light Reactions*, eds. D. R. Ort and C. F. Yocum, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1996, p. 213.
2 R. J. Debus, *Biochim. Biophys. Acta*, 1992, **1102**, 269.
3 V. K. Yachandra, K. Sauer and M. P. Klein, *Chem. Rev.*, 1996, **96**, 2927.
4 R. D. Britt, in *Advances in Photosynthesis, Oxygenic Photosynthesis: The Light Reactions*, eds. D. R. Ort and C. F. Yocum, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1996, p. 137.
5 J. Limburg, G. W. Brudvig and R. H. Crabtree, in *Biomimetic Oxidation Chemistry*, ed. B. Meunier, Imperial College Press, Singapore, 1999, in press.
6 K. Wieghardt, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 725.
7 R. Manchanda, G. W. Brudvig and R. H. Crabtree, *Coord. Chem. Rev.*, 1995, **144**, 1.
8 G. W. Brudvig, *Adv. Chem. Ser.*, 1995, **246**, 249.
9 V. K. Yachandra, V. J. DeRose, M. J. Latimer, I. Mukerji, K. Sauer and M. P. Klein, *Science*, 1993, **260**, 675.
10 B. Kok, B. Forbush and M. McGloin, *Photochem. Photobiol.*, 1970, **11**, 457.
11 J. P. Dekker and H. J. van Gorkom, *J. Bioenerg. Biomembr.*, 1987, **19**, 125.
12 L. Iuzzolini, J. Dittmer, W. Doerner, W. Meyer-Klauke and H. Dau, *Proceedings of the XIth International Congress on Photosynthesis*, ed. G. Garab, Kluwer Academic Publishers, Dordrecht, The Netherlands, in press.
13 T. Ono, T. Noguchi, Y. Inoue, M. Kusunoki, T. Matsushita and H. Oyanagi, *Science*, 1992, **258**, 1335.
14 T. A. Ono, T. Noguchi, Y. Inoue, M. Kusunoki, T. Matsushita and H. Oyanagi, *Jpn. J. Appl. Phys. Part 1*, 1993, **32**, 530.
15 T. A. Roelofs, W. Liang, M. J. Latimer, R. M. Cinco, A. Rompel, J. C. Andrews, K. Sauer, V. K. Yachandra and M. P. Klein, *Proc. Natl. Acad. Sci. U.S.A.*, 1996, **93**, 3335.
16 G. C. Dismukes and Y. Siderer, *FEBS Lett.*, 1980, **121**, 78.
17 K. A. Åhrling, S. Peterson and S. Styring, *Biochemistry*, 1997, **36**, 13148.
18 J. Messinger, J. H. A. Nugent and M. C. W. Evans, *Biochemistry*, 1997, **36**, 11055.
19 C. Goussias, N. Ioannidis and V. Petrouleas, *Biochemistry*, 1997, **36**, 9261.
20 J. Bonvoisin, G. Blondin, J.-J. Girerd and J.-L. Zimmermann, *Biophys. J.*, 1992, **61**, 1076.
21 J. E. Penner-Hahn, P. J. Riggs-Gelasco, E. Yu, P. DeMarois and C. F. Yocum, in *Photosynthesis: From Light to Biosphere*, ed. P. Mathis, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1995, p. 241.
22 J. E. Penner-Hahn, *Structure and Bonding*, 1998, **90**, 1.
23 M. Zheng and G. C. Dismukes, *Inorg. Chem.*, 1996, **35**, 3307.
24 M. Zheng and G. C. Dismukes, in *Research in Photosynthesis*, ed. N. Murata, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1992, vol. 2, p. 305.
25 G. Blondin, R. Davydov, C. Philouze, M.-F. Charlot, S. Styring, B. Åkermark, J.-J. Girerd and A. Boussac, *J. Chem. Soc., Dalton Trans.*, 1997, 4069.
26 V. L. Pecoraro, M. J. Baldwin, M. T. Caudle, W. Hsieh and N. A. Law, *Pure Appl. Chem.*, 1998, **70**, 925.
27 M. R. A. Blomberg, P. E. M. Siegbahn, S. Styring, G. T. Babcock, B. Åkermark and P. Korall, *J. Am. Chem. Soc.*, 1997, **119**, 8285.
28 G. W. Brudvig and R. H. Crabtree, *Proc. Natl. Acad. Sci. U.S.A.*, 1986, **83**, 4586.
29 G. W. Brudvig and R. H. Crabtree, *Prog. Inorg. Chem.*, 1989, **37**, 99.
30 M. L. Gilchrist, G. A. Lorigan and R. D. Britt, *Proc. Natl. Acad. Sci. U.S.A.*, 1995, **92**, 9545.
31 J. A. Halfen, S. Mahapatra, E. C. Wilkinson, S. Kaderli, V. G. Young, L. Que, A. D. Zuberbuhler and W. B. Tolman, *Science*, 1996, **271**, 1397.
32 C. W. Hoganson, N. Lydakis-Simantiris, X.-S. Tang, C. Tommos, K. Warncke, G. T. Babcock, B. A. Diner, J. McCracken and S. Styring, *Photosynth. Res.*, 1995, **46**, 177.
33 C. W. Hoganson and G. T. Babcock, *Science*, 1997, **277**, 1953.
34 J. Messinger, M. Badger and T. Wydrzynski, *Proc. Natl. Acad. Sci. U.S.A.*, 1995, **92**, 3209.
35 D. M. Proserpio, R. Hoffmann and G. C. Dismukes, *J. Am. Chem. Soc.*, 1992, **114**, 4374.
36 D. M. Proserpio, A. K. Rappe and S. M. Gorun, *Inorg. Chim. Acta*, 1993, **213**, 319.
37 W. Rüttinger and G. C. Dismukes, *Chem. Rev.*, 1997, **97**, 1.
38 C. Tommos and G. T. Babcock, *Acc. Chem. Res.*, 1998, **31**, 18.
39 J. B. Vincent and G. Christou, *Inorg. Chim. Acta*, 1987, **136**, L41.

40 T. Wydrzynski, W. Hillier and J. Messinger, *Physiol. Plant.*, 1996, **96**, 342.

41 V. A. Szalai and G. W. Brudvig, *Am. Sci.*, 1998, **86**, 542.

42 P. Dorlet, M. Di Valentin, G. T. Babcock and J. L. McCracken, *J. Phys. Chem. B*, 1998, **102**, 8239.

43 K. V. Lakshmi, S. S. Eaton, G. R. Eaton, H. A. Frank and G. W. Brudvig, *J. Phys. Chem. B*, 1998, 8327.

44 J. M. Peloquin, K. A. Campbell and R. D. Britt, *J. Am. Chem. Soc.*, 1998, **120**, 6840.

45 V. A. Szalai, H. Kühne, K. V. Lakshmi, G. R. Eaton, S. S. Eaton and G. W. Brudvig, *Biophys. J.*, 1998, **74**, A75.

46 A.-M. A. Hays, I. R. Vassiliev, J. H. Golbeck and R. J. Debus, *Biochemistry*, 1998, **37**, 11352.

47 B. A. Diner, P. J. Nixon and J. W. Farchaus, *Curr. Opin. Struct. Biol.*, 1991, **1**, 546.

48 P. J. Nixon and B. A. Diner, *Biochem. Soc. Trans.*, 1994, **22**, 338.

49 R. A. Roffey, D. M. Kramer, Govindjee and R. T. Sayre, *Biochim. Biophys. Acta*, 1994, **1185**, 257.

50 C. Tommos, X.-S. Tang, K. Warncke, C. W. Hoganson, S. Styring, J. McCracken, B. A. Diner and G. T. Babcock, *J. Am. Chem. Soc.*, 1995, **117**, 10325.

51 M. J. Baldwin and V. L. Pecoraro, *J. Am. Chem. Soc.*, 1996, **118**, 11325.

52 M. T. Caudle and V. L. Pecoraro, *J. Am. Chem. Soc.*, 1997, **119**, 3415.

53 J. Lind, X. Shen, T. E. Eriksen and G. Merényi, *J. Am. Chem. Soc.*, 1990, **112**, 479.

54 F. G. Bordwell and J.-P. Cheng, *J. Am. Chem. Soc.*, 1991, **113**, 1736.

55 V. A. Szalai and G. W. Brudvig, *Biochemistry*, 1996, **35**, 15080.

56 C. F. Yocum, *Biochim. Biophys. Acta*, 1991, **1059**, 1.

57 P. H. Homann, *Biochim. Biophys. Acta*, 1988, **934**, 1.

58 F. Rappaport, M. Blanchard-Desce and J. Lavergne, *Biochim. Biophys. Acta*, 1994, **1184**, 178.

59 P. O. Sandusky and C. F. Yocum, *Biochim. Biophys. Acta*, 1986, **849**, 85.

60 A. W. Rutherford, *Trends Biochem. Sci.*, 1989, **14**, 227.

61 J. Tso, M. Sivaraja and G. C. Dismukes, *Biochemistry*, 1991, **30**, 4734.

62 A. Boussac and A. W. Rutherford, *Biochemistry*, 1988, **27**, 3476.

63 V. A. Szalai, H. Kühne, K. V. Lakshmi and G. W. Brudvig, *Biochemistry*, 1998, **37**, 13549.

64 V. A. Szalai and G. W. Brudvig, *Biochemistry*, 1996, **35**, 1946.

65 D. J. MacLachlan and J. H. A. Nugent, *Biochemistry*, 1993, **32**, 9772.

66 X.-S. Tang, D. W. Randall, D. A. Force, B. A. Diner and R. D. Britt, *J. Am. Chem. Soc.*, 1996, **118**, 7638.

67 B. J. Hallahan, J. H. A. Nugent, J. T. Warden and M. C. W. Evans, *Biochemistry*, 1992, **31**, 4562.

68 H. Kühne, V. A. Szalai and G. W. Brudvig, *Biochemistry*, submitted for publication.

69 H. Wincencjusz, H. J. van Gorkom and C. F. Yocum, *Biochemistry*, 1997, **36**, 3663.

70 K. Lindberg and L.-E. Andréasson, *Biochemistry*, 1996, **35**, 14259.

71 D. A. Force, D. W. Randall and R. D. Britt, *Biochemistry*, 1997, **36**, 12062.

72 J. E. Penner-Hahn, R. M. Fronko, V. L. Pecoraro, C. F. Yocum, S. D. Betts and N. R. Bowlby, *J. Am. Chem. Soc.*, 1990, **112**, 2549.

73 M. Baumgarten, J. S. Philo and G. C. Dismukes, *Biochemistry*, 1990, **29**, 10814.

74 A. Boussac, J.-L. Zimmermann and A. W. Rutherford, *FEBS Lett.*, 1990, **277**, 69.

75 A. Boussac and A. W. Rutherford, *J. Biol. Chem.*, 1994, **269**, 12462.

76 R. Damoder, V. V. Klimov and G. C. Dismukes, *Biochim. Biophys. Acta*, 1986, **848**, 378.

77 K. Kalosaka, W. F. Beck, G. W. Brudvig and G. Cheniae, in *Current Research in Photosynthesis*, ed. M. Baltscheffsky, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1990, vol. 1, p. 721.

78 M. Sivaraja, J. Tso and G. C. Dismukes, *Biochemistry*, 1989, **28**, 9459.

79 P. van Vliet, A. Boussac and A. W. Rutherford, *Biochemistry*, 1994, **33**, 12998.

80 P. van Vliet and A. W. Rutherford, *Biochemistry*, 1996, **35**, 1829.

81 S. Ferguson-Miller and G. T. Babcock, *Chem. Rev.*, 1996, **96**, 2889.

82 M. Sono, M. P. Roach, E. D. Coulter and J. H. Dawson, *Chem. Rev.*, 1996, **96**, 2841.

83 *Cytochrome P450. Structure, Mechanism and Biochemistry*, ed. P. R. Ortiz de Montellano, Plenum, New York, 1996.

84 R. H. Holm, *Coord. Chem. Rev.*, 1990, **100**, 183.

85 R. S. Drago, *Coord. Chem. Rev.*, 1992, **117**, 185.

86 K. A. Jørgensen, *Chem. Rev.*, 1989, **89**, 431.

87 F. Montanari and L. Casella, in *Metalloporphyrins Catalyzed Oxidations*, eds. R. Ugo and B. R. James, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1994.

88 W. P. Griffith, *Chem. Soc. Rev.*, 1992, **21**, 179.

89 *Selective Hydrocarbon Activation: Principle and Progress*, ed. J. A. Davies, VCH, New York, 1994.

90 R. A. Sheldon and J. K. Kochi, *Metal Catalyzed Oxidation of Organic Compounds*, Academic Press, New York, 1991.

91 *Metalloporphyrins in Catalytic Oxidations*, ed. R. A. Sheldon, Marcel Dekker, New York, 1994.

92 C.-M. Che, *Pure Appl. Chem.*, 1995, **67**, 225.

93 J. K. Hurst, J. Zhou and Y. Lei, *Inorg. Chem.*, 1992, **31**, 1010.

94 J. G. Muller, J. H. Acquaye and K. J. Takeuchi, *Inorg. Chem.*, 1992, **31**, 4552.

95 J. H. Acquaye, J. G. Muller and K. J. Takeuchi, *Inorg. Chem.*, 1993, **32**, 160.

96 M. Chanon and S. Andre, in *Chemistry of Sulfones and Sulfoxides*, eds. S. Patai, Z. Rappoport and C. J. M. Stirling, John Wiley and Sons Ltd, 1988, p. 1048.

97 S. W. Gersten, G. J. Samuels and T. J. Meyer, *J. Am. Chem. Soc.*, 1982, **104**, 4029.

98 D. Geselowitz and T. J. Meyer, *Inorg. Chem.*, 1990, **29**, 3894.

99 T. J. Collins and S. W. Gordon-Wylie, *J. Am. Chem. Soc.*, 1989, **111**, 4511.

100 T. J. Collins, R. D. Powell, C. Slebodnick and E. S. Uffelman, *J. Am. Chem. Soc.*, 1990, **112**, 899.

101 F. M. MacDonnell, N. L. P. Fackler, C. Stern and T. V. O'Halloran, *J. Am. Chem. Soc.*, 1994, **116**, 7431.

102 C. G. Miller, S. W. Gordon-Wylie, C. P. Horwitz, S. A. Strazisar, D. K. Peraino, G. R. Clark, S. T. Weintraub and T. J. Collins, *J. Am. Chem. Soc.*, 1998, **120**, 11540.

103 J. T. Groves and M. K. Stern, *J. Am. Chem. Soc*, 1987, **109**, 3812.

104 J. T. Groves and M. K. Stern, *J. Am. Chem. Soc.*, 1988, **110**, 8628.

105 J. T. Groves, J. Lee and S. S. Marla, *J. Am. Chem. Soc.*, 1997, **119**, 6269.

106 R. Song, A. Sorokin, J. Bernadou and B. Meunier, *J. Org. Chem.*, 1997, **62**, 673.

107 J. Bernadou, A.-S. Fabiano, A. Robert and B. Meunier, *J. Am. Chem Soc.*, 1994, **116**, 9375.

108 B. Meunier, E. Guilmet, M.-E. De Carvahlo and R. Poilblanc, *J. Am. Chem. Soc.*, 1984, **106**, 6668.

109 D. L. Hughes, G. B. Smith, J. Liu, G. C. Dezeny, C. H. Senanayake, R. D. Larsen, T. R. Verhoeven and P. J. Reider, *J. Org. Chem.*, 1997, **62**, 2222.

110 A. Chellamani, N. M. I. Alhaji and S. Rajagopal, *J. Chem. Soc., Perkin Trans. 2*, 1997, 299.

111 D. Feichtinger and D. A. Plattner, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1718.

112 A. Chellamani, N. I. Alhaji, S. Rajagopal, R. Sevvel and C. Srinivasan, *Tetrahedron*, 1995, **51**, 12677.

113 M. Paluki, N. S. Finney, P. J. Pospisil, M. L. Gueler, T. Ishida and E. N. Jacobsen, *J. Am. Chem. Soc.*, 1998, **120**, 948.

114 O. Bortolini and B. Meunier, *J. Chem. Soc., Perkin Trans. 2*, 1984, 1967.

115 K. A. Jørgensen and P. Swanstrøm, *Acta Chem. Scand.*, 1988, **43**, 822.

116 Y. Naruta, M. Sasayama and T. Sasaki, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1839.

117 K. Ichihara and Y. Naruta, *Chem. Lett.*, 1998, 185.

118 J. Limburg, G. W. Brudvig and R. H. Crabtree, *J. Am. Chem. Soc.*, 1997, **119**, 2761.

119 J. Limburg, J. S. Vrettos, L. M. Liable-Sands, A. L. Rheingold, R. H. Crabtree and G. W. Brudvig, *Science*, 1999, **283**, 1524.

120 J. Sarrou, N. Ioannidis, Y. Deligiannakis and V. Petrouleas, *Biochemistry*, 1998, **37**, 3581.

121 P. A. Goodson, A. R. Oki, J. Glerup and D. J. Hodgson, *J. Am. Chem. Soc.*, 1990, **112**, 6248.

122 N. Kitajima, U. P. Singh, H. Amagai, M. Osawa and Y. Moro-oka, *J. Am. Chem. Soc.*, 1991, **113**, 7757.

123 H. H. Thorp, J. E. Sarneski, G. W. Brudvig and R. H. Crabtree, *J. Am. Chem. Soc.*, 1989, **111**, 9249.

124 R. Manchanda, H. H. Thorp, G. W. Brudvig and R. H. Crabtree, *Inorg. Chem.*, 1992, **31**, 4040.

125 V. A. Szalai, D. A. Stone and G. W. Brudvig, *Proceedings of the XIth International Congress on Photosynthesis*, ed. G. Garab, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1998, in press.

126 X.-S. Tang, B. A. Diner, B. S. Larsen, M. L. Gilchrist, G. A. Lorigan and R. D. Britt, *Proc. Natl. Acad. Sci. U.S.A.*, 1994, **91**, 704.

127 P. E. M. Siegbahn and R. H. Crabtree, *J. Am. Chem. Soc.*, 1999, **121**, 117.

Paper 8/07583B