

# A mechanistic and structural model for the formation and reactivity of a $\text{Mn}^{\text{V}}=\text{O}$ species in photosynthetic water oxidation

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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  $\text{Mn}=\text{O}$  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,  $\text{Y}_Z$ , abstracts hydrogen atoms directly from substrate water molecules bound to the  $\text{Mn}_4$  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*  $\text{M}=\text{O}$  intermediates. We also interpret biophysical data concerning the roles of  $\text{Ca}^{2+}$  and  $\text{Cl}^-$  in photosynthetic water oxidation, proposing that they are involved in a hydrogen-bonded network between the  $\text{Mn}_4$  cluster and  $\text{Y}_Z$ . Connecting the observed reactivities of model complexes containing  $\text{M}=\text{O}$  groups to spectroscopic information on the environment of the  $\text{Mn}_4$  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  $\text{Mn}=\text{O}$  intermediate. In addition, a new role for  $\text{Cl}^-$  is proposed in which  $\text{Cl}^-$  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  $\text{O}_2$  evolution. The model extends previous proposals and includes a new mechanistic role for  $\text{Ca}^{2+}$  and  $\text{Cl}^-$  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,  $\text{Y}_Z$ .<sup>1–4</sup> While the precise structure of the  $\text{Mn}_4$  cluster is unknown, interpretations of EPR and X-ray absorption data have allowed for some fairly detailed proposals,<sup>3,5–9</sup> some of which are shown in Fig. 2. A key structural element indicated from EXAFS studies is the di- $\mu$ -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  $\text{S}_0$  to  $\text{S}_4$  (Fig. 3).<sup>10</sup> Each S-state advance is associated with

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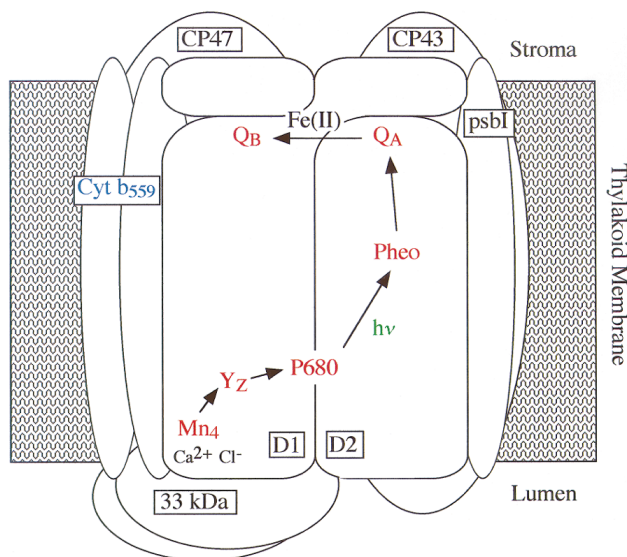


Julian Limburg

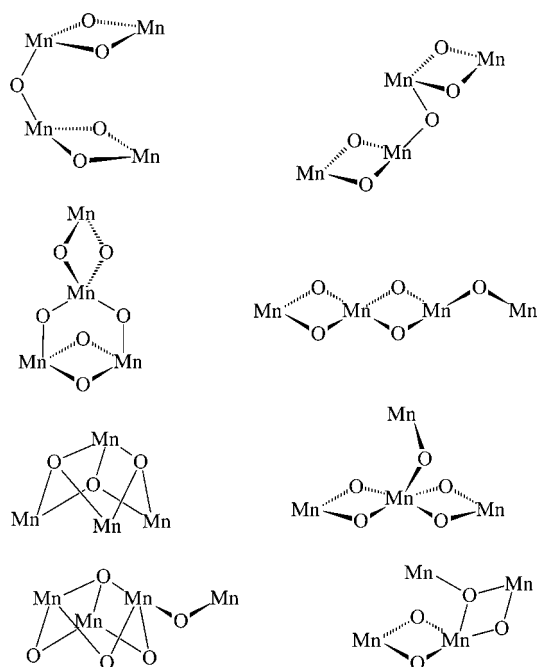


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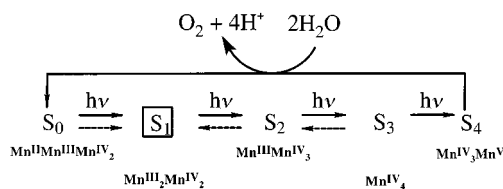
**Fig. 1** Model of photosystem II in the thylakoid membrane. The arrows show the direction of electron-transfer reactions. After initial photoexcitation, the special chlorophyll called P680 transfers an electron to a pheophytin (Pheo) molecule. The electron is rapidly passed to  $Q_A$ , a tightly-bound 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.



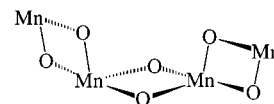
**Fig. 2** Structural models of the OEC based on X-ray absorption spectroscopic data of PSII. Adapted from ref. 3.

light-induced charge separation at the chlorophyll-containing pigment P680 to form the strong oxidant  $P680^+$ . The tyrosyl radical,  $Y_Z^{\cdot}$ , formed upon reduction of  $P680^+$  oxidises the manganese cluster which, in turn, is reduced by electrons stripped from water. Flash-induced UV<sup>11</sup> and XANES<sup>12–14</sup> 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.<sup>15</sup>

The manganese tetramer in PSII exhibits EPR signals from the odd-electron  $S_0$  and  $S_2$  states.<sup>16–19</sup> A combination of EPR<sup>8,20</sup>



**Fig. 3** The S-state cycle proposed by Kok *et al.*<sup>10</sup> 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.*<sup>25</sup>

and XANES<sup>3,14,21,22</sup> data has led to the assignment of  $S_2$  as  $Mn^{III}Mn^{IV}_3$  although  $Mn^{III}_3Mn^{IV}$  cannot be ruled out.<sup>23,24</sup> 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.*<sup>25</sup> 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.<sup>3,5,26–41</sup> 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  $Y_Z^{\cdot}$  and the  $Mn_4$  cluster, estimated to be 7–10 Å.<sup>42–45</sup> Spectroscopic studies of  $Y_Z$  indicate that its proton is released to a nearby histidine residue<sup>46–49</sup> upon oxidation to generate the neutral tyrosyl radical,  $Y_Z^{\cdot}$ ,<sup>50</sup> 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<sup>-1</sup>.<sup>27,51,52</sup> This is comparable to the O–H bond-dissociation energy of tyrosine, estimated at 86.5 kcal mol<sup>-1</sup>,<sup>53,54</sup> 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  $Y_Z^{\cdot}$ . It has been argued on the basis of studies of inhibited PSII that  $Y_Z^{\cdot}$  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  $Y_Z^{\cdot}$  is invoked.<sup>55</sup> As a result, an attractive possibility is that the  $S_4$  state contains a  $Mn=O$  species, formed by  $Y_Z^{\cdot}$  abstracting hydrogen atoms from a water bound to manganese.<sup>26,27,30,32–34,41</sup> 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<sup>2,56,57</sup> and, primarily, these cofactors have been assigned structural or electrostatic charge-balance roles.<sup>12,26,40,57–61</sup> 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<sup>62</sup> 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  $S_2Y_Z^{\cdot}$ .<sup>43,45,55,63–67</sup> Chloride can be competitively replaced by acetate,<sup>63,68</sup> to produce an  $S_2Y_Z^{\cdot}$  state, although the effects of acetate inhibition do not mimic all the effects of chloride depletion.<sup>63,69,70</sup> Importantly, acetate-inhibited samples display characteristics of both chloride-depleted and calcium-depleted PSII

preparations,<sup>63</sup> 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,<sup>68,71</sup> and could be bridging between them in a similar manner to that observed in Concanavalin A.<sup>63,72</sup>

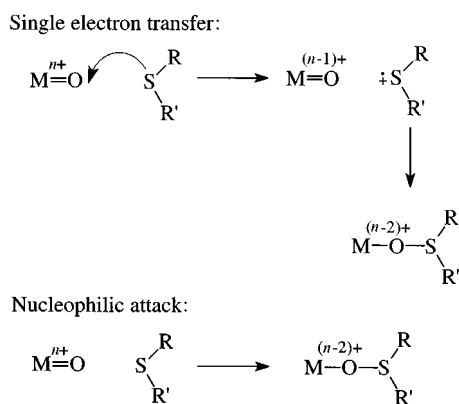
The body of biophysical data on the OEC provides evidence for a mechanism in which  $Y_Z$  is involved in H-atom abstraction from Mn-bound substrate water molecules ultimately leading to the formation of a Mn=O species. In section 3, we propose a model that explains these effects by assigning chloride 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.<sup>1,70,73-80</sup> 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  $Mn^V=O$ .<sup>26,41</sup>

## 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.<sup>84-91</sup>

Scheme 1 shows a simplified view of how a metal-oxo species



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

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.

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.<sup>92</sup> Ru=O complexes (Ru = +4 to +7) can oxidize organic substrates<sup>87,88,92</sup> and, most importantly for our interests, have been identified as key intermediates in homogeneous catalysis of water oxidation.<sup>93</sup>

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.<sup>94,95</sup> 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.<sup>92</sup>

Acquaye *et al.*<sup>95</sup> studied the oxidation of thioanisoles and methyl phenyl sulfoxides by  $(O)(P(p-C_6H_5R)_3)_2Ru^{IV}$  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  $E_3$  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:  $E^0_{thioanisole} = 1.45 V$ ,<sup>95</sup>  $E^0_{methylphenylsulfoxide} = 2.30 V$ <sup>96</sup>). 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.*<sup>97</sup> in 1982, and which

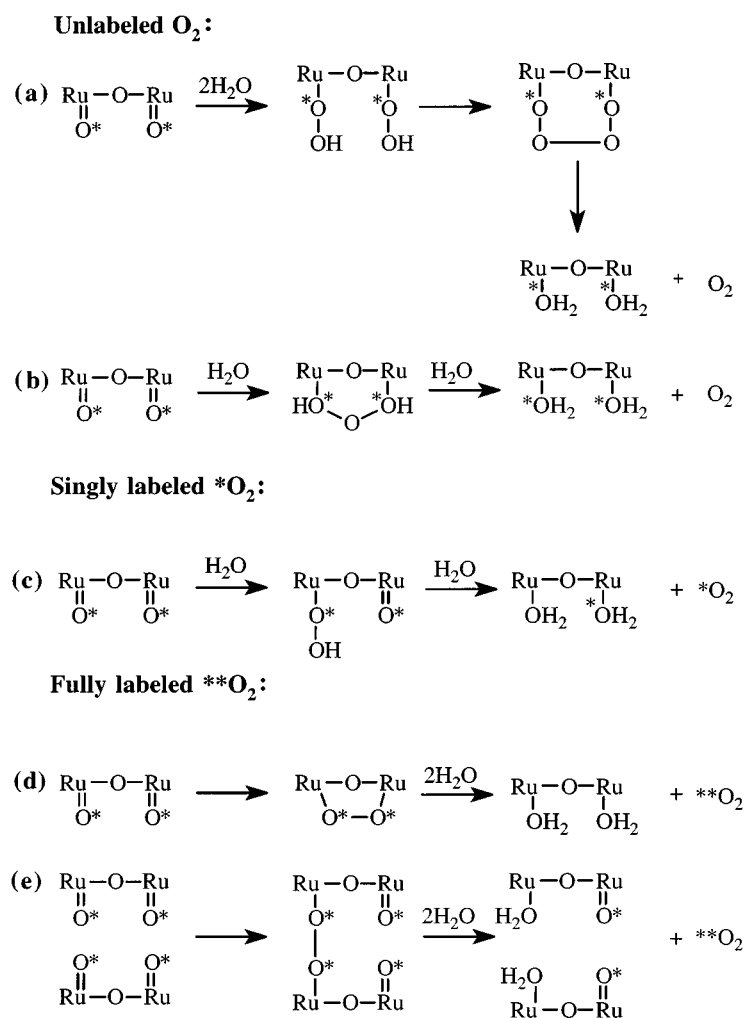


Fig. 5 Mechanisms to explain <sup>18</sup>O incorporation into [(bpy)<sub>2</sub>(H<sub>2</sub>O)RuORu(H<sub>2</sub>O)(bpy)<sub>2</sub>]<sup>4+</sup>. Adapted from ref. 5.

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

Single-turnover labeling studies using H<sub>2</sub><sup>18</sup>O–Ru and unlabeled solvent water, where the O<sub>2</sub> formed was analysed using mass spectrometry, were inconclusive as to the precise mechanism of O–O bond formation. Geselowitz and Meyer<sup>98</sup> reported a <sup>36</sup>O<sub>2</sub>:<sup>34</sup>O<sub>2</sub>:<sup>32</sup>O<sub>2</sub> ratio of 13:64:23, whereas a similar study by Hurst *et al.*<sup>93</sup> gave a <sup>34</sup>O<sub>2</sub>:<sup>32</sup>O<sub>2</sub> ratio of approximately 50:50, and no <sup>36</sup>O<sub>2</sub>, 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,<sup>5,37,93,98</sup> and multiple pathways must be invoked to account for all the products. Importantly, <sup>34</sup>O<sub>2</sub> 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

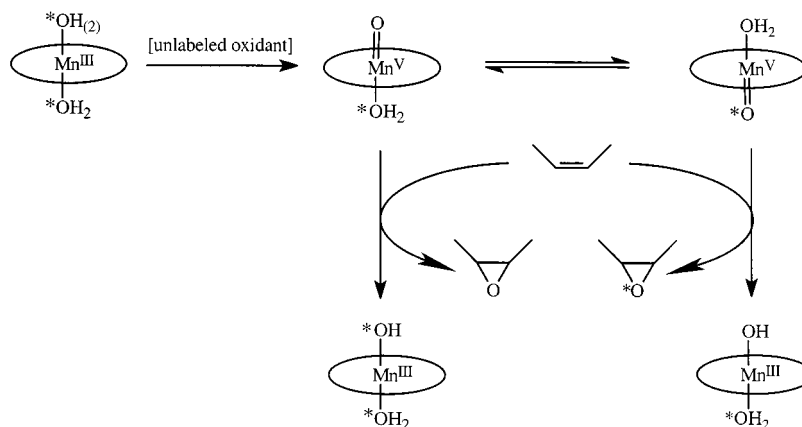
In contrast to the Ru chemistry, the detection of Mn=O catalytic intermediates has been a challenge. Manganates excepted, there are only four structurally characterized Mn=O complexes,<sup>99–102</sup> only one of which has shown reactivity.<sup>102</sup> The reactive system was developed by engineering a ligand that could coordinate cations close to the Mn(v)=O, thereby tuning the electrophilicity of the terminal oxo.<sup>102</sup> For instance, adding 5 equivalents of Sc<sup>3+</sup> to the complex increased the rate of Ph<sub>3</sub>P

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

[(salen)Mn<sup>III</sup>]<sup>+</sup> (salen = *N,N'*-bis(salicylidene)ethylenediamine di-anion) Catalysed epoxidations are thought to require a Mn<sup>V</sup>=O species analogous to that seen in the porphyrin systems.<sup>109,110</sup> Recently, Feichtinger and Plattner<sup>111</sup> used ES–MS to study the reaction between [(salen)Mn<sup>III</sup>]<sup>+</sup> and iodosobenzene, and saw a *m/z* peak that was attributable to a Mn<sup>V</sup>=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



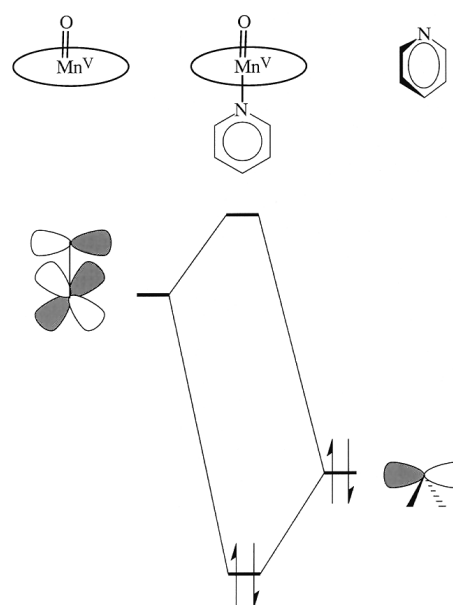
**Fig. 6** Mechanism of incorporation of  $^{18}\text{O}$  from solvent water into epoxides formed from the reaction of  $\text{Mn}^{\text{V}}=\text{O}(\text{porphyrin})$  species with olefins. Based on refs. 105 and 107.

catalysts, that is to say, less stabilizing ligands lead to more reactive intermediates. Chellamani *et al.*<sup>110,112</sup> looked at the electronic effects on the oxidation of thioanisoles by  $[(\text{salen})\text{Mn}^{\text{III}}]^+$ , analogous to that published for  $(\text{O})(\text{P}(p\text{-C}_6\text{H}_5\text{R})_3)\text{Ru}^{\text{IV}}$  complexes.<sup>95</sup> 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  $(\text{O})(\text{P}(p\text{-C}_6\text{H}_5\text{R})_3)\text{Ru}^{\text{IV}}$  complexes were reported in which both the salen ligand and substrate basicities were varied, suggesting that  $[(\text{salen})\text{Mn}^{\text{III}}]^+$  and  $(\text{O})(\text{P}(p\text{-C}_6\text{H}_5\text{R})_3)\text{Ru}^{\text{IV}}$  both oxidize sulfides by analogous mechanisms, *i.e.* single electron transfer from a  $\text{M}=\text{O}$  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  $[(\text{salen})\text{Mn}^{\text{III}}]^+$  complex) which shows a direct correlation between electronic effects and selectivity, *i.e.* the more reactive the catalyst, the less selective the oxidation.<sup>113</sup> 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.

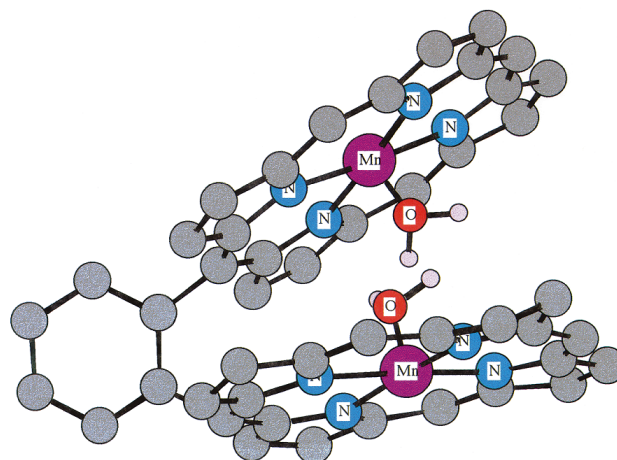
A further, interesting, aspect of Mn-oxidation chemistry is the role of the ligand *trans* to the active site on the complex. It was found early on that adding a base such as pyridine or imidazole as a co-catalyst increases greatly the reactivity of Mn porphyrins.<sup>108,114</sup> Subsequent proposals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

**2.2.1 O–O bond formation:  $\text{O}_2$  evolution.** There have been only a few reports of manganese complexes able to catalyze 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).<sup>116</sup> The active species was suggested to be a  $\text{Mn}^{\text{V}}=\text{O}$  dimer, and this would be consistent with the other Mn(porphyrin) catalysts. This mechanism has since been supported by studies involving olefin epoxidation.<sup>117</sup>

We have developed a Mn di- $\mu$ -oxo dimer capable of catalyzing  $\text{O}_2$  evolution from oxone<sup>5,118</sup> or sodium hypochlorite.<sup>119</sup> The catalyst is ultimately lost by formation of permanganate which is unreactive under these conditions. This is the first example of a di- $\mu$ -oxo Mn dimer, which is a structural element present in the OEC, that has the relevant functional chemistry. When the

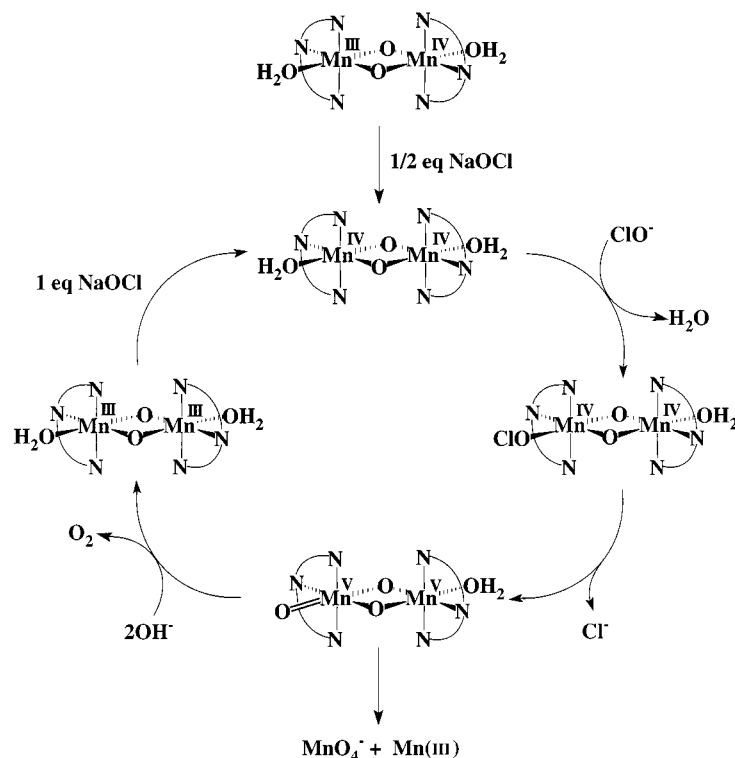


**Fig. 7** Molecular orbitals of a  $\text{M}=\text{O}$  species showing  $\pi$ -donation of bases into the antibonding orbital. Adapted from Jørgensen and Swanstrøm.<sup>115</sup>



**Fig. 8** Structure of the face-to-face  $[\text{Mn}(\text{porphyrin})]_2$  complex reported by Naruta *et al.*<sup>116</sup>

reaction with hypochlorite is run in  $\text{H}_2^{18}\text{O}$  the label is incorporated into the dioxygen.<sup>119</sup> The ratio  $^{36}\text{O}_2 : ^{34}\text{O}_2$  is consistent with water being the source of the oxygen in the  $\text{O}_2$  evolved, and we interpret the mechanism to involve exchange of oxygen from water with a  $\text{Mn}=\text{O}$  species. In analogy to the porphyrin dimer,



**Fig. 9** Possible mechanism of O–O bond formation from the reaction between  $[(\text{Terpy})(\text{H}_2\text{O})\text{Mn}(\text{O})_2\text{Mn}(\text{OH}_2)(\text{Terpy})](\text{NO}_3)_3$  (Terpy = 2,2':6',2''-terpyridine) and sodium hypochlorite.<sup>118</sup>

we have proposed a mechanism with a  $\text{Mn}^{\text{V}}=\text{O}$  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  $\text{Mn}^{\text{V}}=\text{O}$ . In this section, we consider how such a reaction could be promoted by the OEC and consider the implications that the chemistry of  $\text{M}=\text{O}$  species has on the suggestion that a  $\text{Mn}=\text{O}$  species is formed during photosynthetic water oxidation. In addition, we tie together observations about  $\text{Ca}^{2+}$  and  $\text{Cl}^-$  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.*<sup>32,33</sup> Assembling all of this information, we present a detailed picture of the O–O bond-forming reaction which occurs between the  $\text{S}_3$  and  $\text{S}_0$  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  $\text{Mn}_4$  cluster. The structure of the manganese cluster shown is based on the structure proposed by Yachandra *et al.* from X-ray absorption data.<sup>9</sup> 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  $\text{Mn}_4$  cluster are readily accommodated by this model. As suggested by recent EPR results,<sup>17–19,120</sup> the oxidation states of the manganese atoms in the  $\text{S}_0$  state are assigned as  $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}_2$  in which one dimer consists of  $\text{Mn}^{\text{IV}}$  atoms and the other dimer is a  $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$  pair. As there are no examples of di- $\mu$ -oxo  $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$  complexes, the  $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$  dimer in the cluster is suggested to be bridged by one  $\mu$ -hydroxo and one  $\mu$ -oxo ligand. Advance from the  $\text{S}_0$  to the  $\text{S}_1$  state would be followed by deprotonation of the  $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$   $\mu$ -hydroxo to form a di- $\mu$ -oxo  $\text{Mn}^{\text{III}}\text{Mn}^{\text{III}}$  dimer; there are three such dimers known from model chemistry.<sup>121,122</sup> The role of such proton-

coupled steps involving interconversion of  $\mu$ -hydroxo and  $\mu$ -oxo bridges in mediating oxidation potentials have been studied in Mn model complexes,<sup>123,124</sup> and analogous reactions involving the OEC may allow for approximately equipotential sequential oxidations of the  $\text{Mn}_4$  cluster in the early S states. Oxidation of the  $\text{Mn}_4$  cluster from the  $\text{S}_1$  to the  $\text{S}_2$  state would involve electron transfer from the manganese cluster to  $\text{Y}_Z^*$  together with protonation of  $\text{Y}_Z^*$  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  $\text{S}_2$  state.

Advancement from the  $\text{S}_2$  to  $\text{S}_3$  and  $\text{S}_3$  to  $\text{S}_4$  states would involve oxidation of the  $\text{Mn}_4$  cluster together with a proton abstraction from a water ligand, ultimately converting the water ligand to a terminal oxo in the  $\text{S}_4$  state. Importantly, in this proposal these steps cannot proceed if the proton transfer from the substrate water to  $\text{Y}_Z^*$  is inhibited. Fig. 11 shows a scheme of OEC oxidation from the  $\text{S}_3$  to the  $\text{S}_4$  state which incorporates interpretations of current biophysical data on  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ , the  $\text{Mn}_4$  cluster and  $\text{Y}_Z$ . In the final step, the  $\text{S}_4$  state would collapse to the  $\text{S}_0$  state through nucleophilic attack of hydroxide on a  $\text{Mn}^{\text{V}}=\text{O}$  group with the concomitant release of  $\text{O}_2$ . Because advance to S states higher than  $\text{S}_2$  has been proposed to proceed through proton-coupled electron transfer and because  $\text{Ca}^{2+}$  and  $\text{Cl}^-$  have been found to be required for the  $\text{S}_2$ -to- $\text{S}_3$ -state transition,<sup>69,74</sup> 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  $\text{Ca}^{2+}$  and  $\text{Cl}^-$ . The geometric center-to-center distance between  $\text{Y}_Z^*$  and the  $\text{Mn}_4$  cluster is  $\approx 9$  Å in this model, and the point-dipole distance<sup>125</sup> is within the currently accepted 7–10 Å distance estimate limits.<sup>42–45,125</sup> Another feature of this model is that it includes a well-ordered hydrogen-bonded network between  $\text{Ca}^{2+}$ ,  $\text{Y}_Z$ , the  $\text{Mn}_4$  cluster, and a histidine residue (D1-His 190, the proton acceptor for  $\text{Y}_Z$  upon its oxidation). In this hydrogen-bonded network, calcium serves as an anchor between a hydroxide ligated to the manganese cluster and  $\text{Y}_Z$ . Part of the chloride's role would be to position calcium

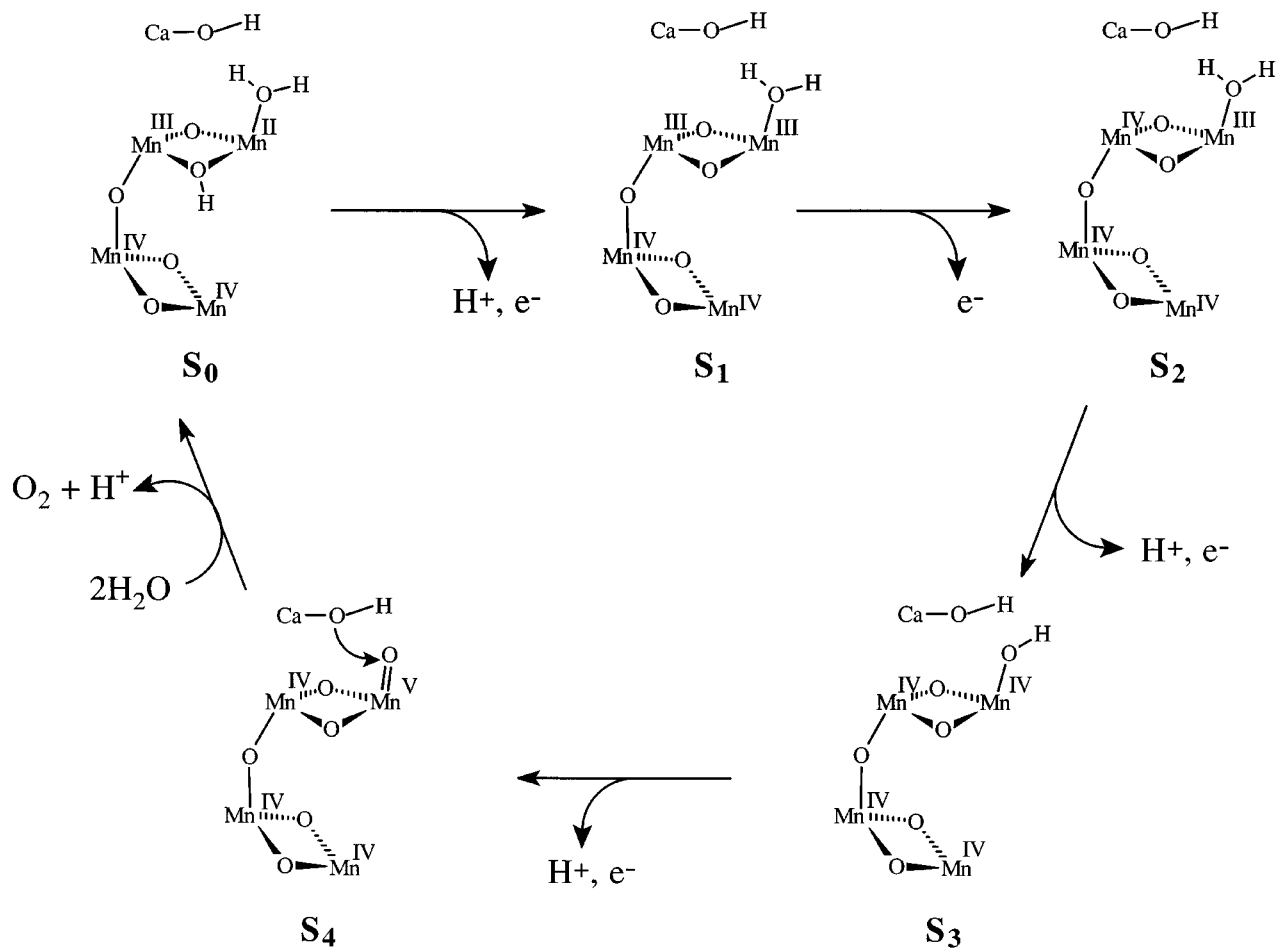


Fig. 10 Proposed S-state cycle of the OEC in PSII. The  $Mn_4$  cluster structure shown is the structure proposed by Yachandra *et al.*<sup>9</sup>

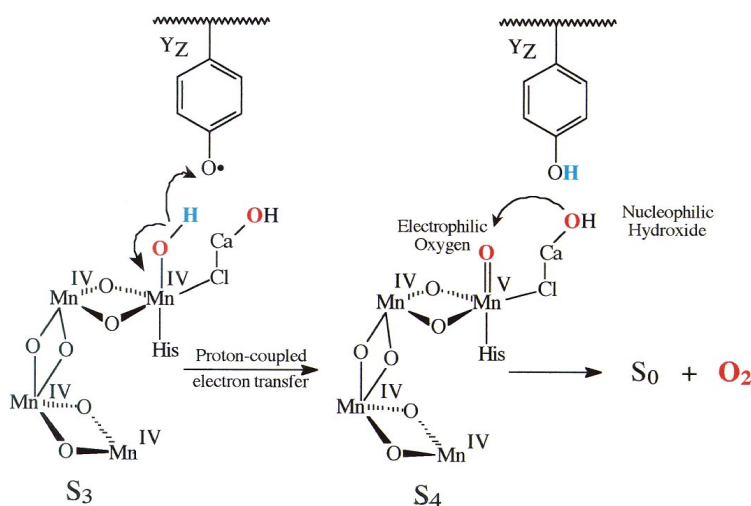
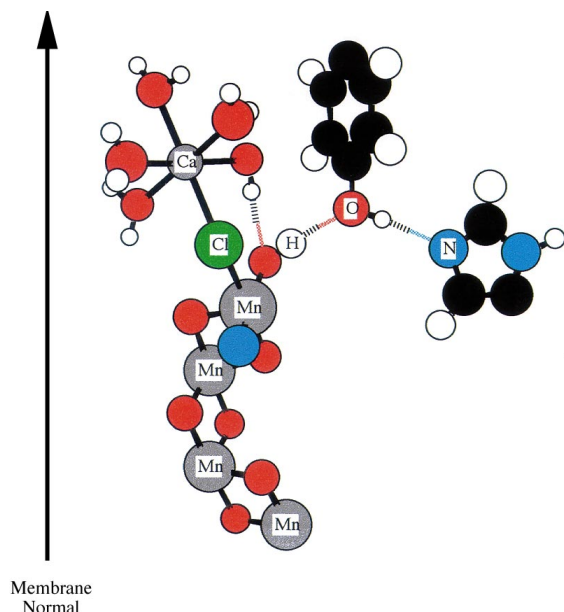


Fig. 11 Hypothetical O–O bond-forming steps ( $S_3$  to  $S_0$  states) of the OEC in PSII showing the positions of  $Ca^{2+}$ ,  $Cl^-$ ,  $Y_Z$  and the  $Mn_4$  cluster. The  $Mn_4$  cluster is based on the model complex of Blondin *et al.*<sup>25</sup>

between  $Y_Z$  and the  $Mn_4$  cluster. Because  $Y_Z^{\cdot}$  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.<sup>126</sup> 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.<sup>26,41</sup> This type of mechanism is consistent with the calculations of Siegbahn and Crabtree which suggest that  $Mn=O$  groups are electron deficient.<sup>127</sup> 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  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ , the  $\text{Mn}_4$  cluster and  $\text{Y}_Z$ . 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  $\text{S}_{\text{N}}2$ -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  $\text{Mn}^{\text{V}}=\text{O}$  is created. The proposed mechanism also includes a new functional role for chloride as a bridge that communicates the formation of the  $\text{Mn}^{\text{V}}=\text{O}$  to  $\text{Ca}^{2+}$  in order to activate the hydroxide for nucleophilic attack. We envision that by binding between the  $\text{Mn}_4$  cluster and Ca, chloride tunes the nucleophilicity of the calcium-hydroxide by responding to changes in the oxidation state of the  $\text{Mn}_4$  cluster. As the oxidation level of the  $\text{Mn}_4$  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  $\text{S}_4$  state would change the interaction between calcium and chloride so that a nucleophilic hydroxide would be created in concert with an electrophilic  $\text{Mn}^{\text{V}}=\text{O}$  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  $\text{Mn}^{\text{V}}=\text{O}$  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  $\text{Mn}_4$  cluster and  $\text{Y}_Z$  by way of a hydrogen-bonded network. Further, having chloride as a bridging ligand between calcium and the  $\text{Mn}_4$  cluster allows it to control the Lewis acidity of calcium, thereby tuning the reactivity of the substrate hydroxide.

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