

Using small molecule complexes to elucidate features of photosynthetic water oxidation

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The molecular oxygen produced in photosynthesis is generated via water oxidation at a manganese–calcium cluster called the oxygen-evolving complex (OEC). While studies in biophysics, biochemistry, and structural and molecular biology are well known to provide deeper insight into the structure and workings of this system, it is often less appreciated that biomimetic modelling provides the foundation for interpreting photosynthetic reactions. The synthesis and characterization of small model complexes, which either mimic structural features of the OEC or are capable of providing insight into the mechanism of O₂ evolution, have become a vital contributor to this scientific field. Our group has contributed to these findings in recent years through synthesis of model complexes, spectroscopic characterization of these systems and probing the reactivity in the context of water oxidation. In this article we describe how models have made significant contributions ranging from understanding the structure of the water-oxidation centre (e.g. contributions to defining a tetrameric Mn₃Ca-cluster with a dangler Mn) to the ability to discriminate between different mechanistic proposals (e.g. showing that the Babcock scheme for water oxidation is unlikely).

Keywords: oxygen-evolving complex; water oxidation; manganese; photosynthesis

1. INTRODUCTION

Molecular oxygen, which is crucial to life on earth, is produced in cyanobacteria, algae and green plants (Wydrzynski 2005). The enzyme responsible for the conversion of water into dioxygen is the oxygen-evolving complex (OEC), which is located within the larger framework known as photosystem II (PSII). The OEC contains a cluster of manganese (Cheniae & Martin 1970; Sauer 1980; Yocum *et al.* 1981) and calcium (Ghanotakis *et al.* 1984; Han & Katoh 1993; Adelroth *et al.* 1995; van Gorkom & Yocum 2005) ions. The detailed structural aspects of this centre are still to be resolved; however, it is widely accepted that this unit contains four manganese ions, which can change oxidation states, as well as one calcium(II) ion. A chloride ion is known to be necessary for oxygen evolution (Yocum 1992; Lindberg & Andreasson 1996; Homann 2002; van Gorkom & Yocum 2005), but neither its structural location nor its function are fully understood yet. The kinetic profile of the enzyme follows the S-clock, a cycle that is composed of five states given designations S_{*n*}, where *n* = 0–4 representing the most reduced to the most oxidized enzyme forms, respectively. Several key issues need to be resolved in order to assess the chemical mechanism of water oxidation. Probably most important are the oxidation states of the Mn ions in each of the S-states, especially S₄ and S₀. Additionally, the roles of Ca^{II} and

chloride need to be established. In this article we will address some of these issues using small molecule models as a guide.

2. STRUCTURE

The stoichiometric composition of the OEC is widely accepted to be Mn₄Ca, whereas the question whether chloride is attached to the cluster, or if it is even a crucial part of photosynthesis, is under debate (Wincencjusz *et al.* 1999; Yocum & Pecoraro 1999; Popelkova *et al.* 2006). Researchers agree that the metal ions of the cluster are linked by μ-oxo bridges. Additionally, they are coordinated to amino acids, namely to carboxylate oxygen atoms and a histidine nitrogen. A number of structure suggestions have been put forth by various research groups, mainly derived from electron paramagnetic resonance (EPR), X-ray absorption (XAS) and X-ray diffraction spectroscopies. Early models suggested a Mn cubane structure (Brudvig & Crabtree 1986) which was ruled out by extended X-ray absorption fine structure (EXAFS) data that were inconsistent with such a symmetric structure (Kim *et al.* 1990; Penner-Hahn *et al.* 1990). An alternative proposal called the ‘dimer of dimers’ proved very popular for a number of years (Yachandra *et al.* 1993); however, analysis (Pecoraro & Hsieh 2000; Peloquin *et al.* 2000) of the EPR spectral features in the S₂ state (a *g* = 2 multiline (Dismukes & Siderer 1981) and a broad low-field signal at *g* ~ 4 (Casey & Sauer 1984)) suggested that the dimer-of-dimers model was inappropriate, and that a 3+1 formulation was probably more accurate (Pecoraro & Hsieh 2000;

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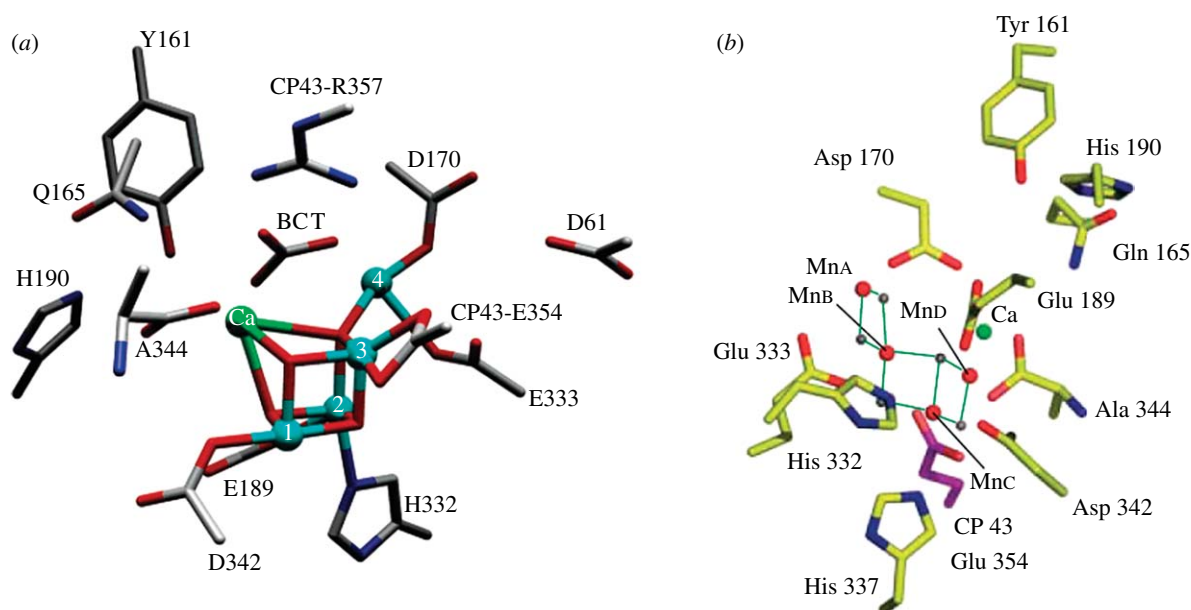


Figure 1. (a) Structure of the OEC based on the X-ray diffraction measurements done by Barber and co-workers (Ferreira *et al.* 2004) and (b) a recent model (Yano *et al.* 2006) of the Mn₄Ca cluster derived from polarized Mn-EXAFS and the 3.0 Å resolution X-ray structure (Loll *et al.* 2005). (Reproduced with permission from McEvoy & Brudvig (2006) and Yano *et al.* (2006).)

Peloquin *et al.* 2000). Pulsed EPR studies of the enzyme (Peloquin *et al.* 2000; Britt *et al.* 2004) and small molecule models suggested an alternative ‘dangler’ model, which was reported in a linear topology but could easily accommodate a more three-dimensional structure. In 2000 the first low-resolution structure appeared which suggested that the Mn formed a 3+1 cluster (Zouni *et al.* 2000, 2001). Subsequent crystallographic refinement at 3.5 Å resolution done by Barber and co-workers (Ferreira *et al.* 2004) shows a Mn₃Ca-oxo cluster with a dangler Mn ion attached. The manganese and calcium ions are coordinated by glutamic and aspartic acids, a terminal alanine and a histidine. Furthermore, a tyrosine (Y_z), which is not part of the metals’ first coordination sphere but within a 5 Å distance, is poised to play a vital role in the oxidation mechanism. Most importantly, this structure demonstrated for the first time that calcium was an integral part of the Mn cluster, a point which had been debated for many years based on EXAFS studies (Riggs-Gelasco *et al.* 1996; Cinco *et al.* 1998). More recently, a structure from Loll and co-workers (Loll *et al.* 2005) was obtained at higher resolution (3.0 Å), but unfortunately it fails to locate critical bridging oxygen atoms in the cluster. Also, it differs from the Barber structure (Ferreira *et al.* 2004) showing a modified spatial distribution of the metal ions, as well as comprising a different ligation of the nearby amino acids. However, available crystal structures of the OEC still lack atomic resolution. More importantly, recent XAS experiments have shown that X-ray structures could be distorted by X-ray-induced photo degradation (Yano *et al.* 2006). A combination of XAS and crystallography has led to the best available models (Loll *et al.* 2005; Yano *et al.* 2006) shown in figure 1. Thus, at present it appears that the best description for the OEC cluster, in the lower oxidation levels, is a tetrameric assembly of Mn

(best thought of as a 3+1 Mn-oxo cluster) with a calcium ion integral to the structure.

Unfortunately, models for such complex structures are difficult to prepare, and the interested reader is referred to numerous reviews on synthetic models for the OEC (Wiegardt 1989; Pecoraro *et al.* 1994; Law *et al.* 1998; Mukhopadhyay *et al.* 2004). Among the best are the various Mn-oxo structures reported by Christou and co-workers (Bashkin *et al.* 1987; Vincent *et al.* 1989; Libby *et al.* 1990, 1991; Bouwman *et al.* 1992). While most of these structures form butterfly or pseudocubane assemblies that do not match the precise stoichiometry or include calcium, they do provide deeper insight into the system. An alternative formulation has been reported from our group using a class of molecules known as metallacrowns (Gibney *et al.* 1996; Pecoraro *et al.* 1997). The 12-MC-4 structure type shown in figure 2 contains four Mn^{III} ions in a ring. A fifth ion is linked to these ring metals through carboxylate bridges and Mn^{III}-O-M linkages (Lah & Pecoraro 1989). Stoichiometrically, these compounds are interesting links to the OEC with a Mn₄M composition (Gibney *et al.* 1996). The structure in figure 2a contains a capping Mn^{II} as the fifth ion, whereas figure 2b contains two Na^I ions. Of particular interest, the Na^I is bridged to the Mn^{III} ions with halides, mimicking a possible role for chloride in the OEC. Na^I is a competitive inhibitor (Waggoner *et al.* 1989) versus calcium of the OEC. Another class of metal inhibitors are lanthanide ions (Bakou *et al.* 1992). Figure 2c shows the Mn₄Dy^{III} structure with additional carboxylate bridges. The propensity of lanthanides for their higher charge and higher coordination number suggests a mechanism for lanthanide inhibition of the OEC. While we do not yet have crystals of the Mn₄Ca or Mn₄Sr analogues, we do have mass spectral evidence that such compounds exist. Thus, while these compounds are topologically rather

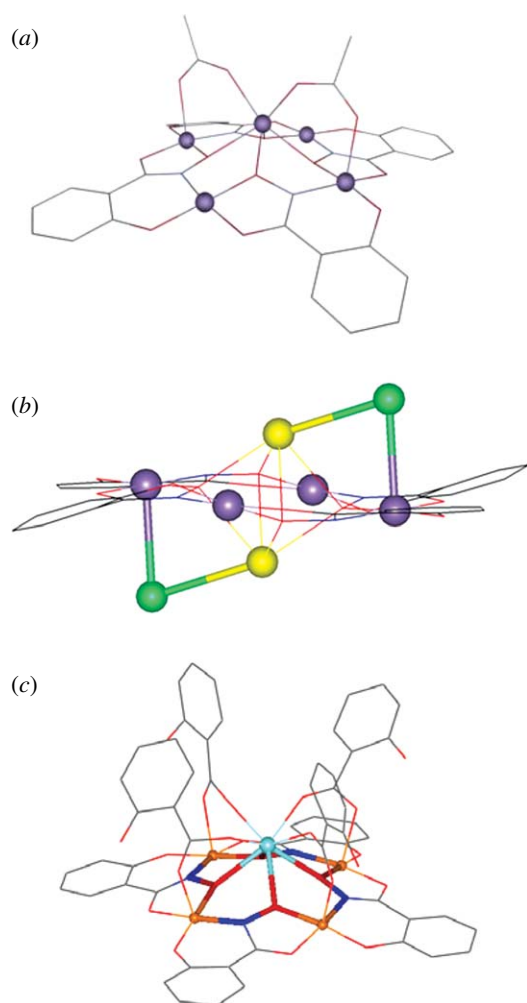


Figure 2. Representation of three 12-MC-4 type manganese(III) metallacrowns with: (a) Mn^{II} and acetates as bridges (Lah & Pecoraro 1989), (b) two Na^{I} and two bromide bridges (Gibney *et al.* 1996) and (c) Dy^{III} and four salicylate bridges. We expect the Ca 12-MC-4 to be isostructural with the structure with Mn^{II} (a).

different from the Mn_4Ca cluster of the OEC, they are the structures that as a group have most closely modelled the stoichiometric basis for active and related inhibited forms of the enzyme.

Thinking about the OEC as a 3+1 formulation of Mn ions, it is worth considering trinuclear Mn compounds as models for this centre. A plethora of structure classes is possible in clusters comprising three manganese centres (Mukhopadhyay *et al.* 2004). May be the simplest structure type is the Mn_3O core, in which all three metals are linked by oxygen and further ligated by carboxylates. This core type is usually referred to as the basic carboxylate type. A noteworthy complex was synthesized by Weatherburn and co-workers, in which a peroxo moiety and two carboxylates bridge the Mn centres (Bhula *et al.* 1988). Despite the poorly resolved crystal structure, this complex provided the first indication of potential dioxygen coordination to a trinuclear manganese cluster. Another structure class of interest is the open structure type, especially, the mixed-valent $\text{Mn}^{\text{III}}\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ clusters. The first compound of this class was published by Pecoraro and co-workers, a tridentate Schiff base complex with acetate and methanol ligands (Kessissoglou *et al.* 1989). Similar complexes have been synthesized with other

Schiff base ligands, all resulting in weakly antiferromagnetically coupled complexes with an EPR signal at approximately $g=4$, indicating a $S=3/2$ spin groundstate (Kessissoglou *et al.* 1992; Tangoulis *et al.* 1996). Kitajima *et al.* (1994) have used trispyrazolylborate ligands to synthesize a $\text{Mn}^{\text{III}}\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}$ complex, in which manganese ions are linked by one μ -hydroxo and two acetato groups. A reversed order of manganese oxidation states, namely $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}\text{Mn}^{\text{II}}$ was achieved by Tanase and co-workers, who used tris[(*N*-aldosyl)-aminoethyl]amine as a ligand (Yano *et al.* 1997). Most trinuclear complexes of this type are low valent. Therefore, higher valent complexes such as $\text{Mn}^{\text{II}}\text{Mn}^{\text{IV}}\text{Mn}^{\text{II}}$ are very interesting and will be discussed in greater detail in §3.

Trinuclear structures that model the S_2 and possibly S_3 states include an open trinuclear Mn_3^{IV} complex that has been recently reported by Christou and co-workers, comprising a nonlinear Mn_3O_4 unit (Bhaduri *et al.* 2002), which can be found in many proposed structures for the OEC. The ligands are bipyridines and acetate. The central metal is solely coordinated by oxygen ions, which seems to increase the stability of this high-valent cluster. In addition, adamantane-like Mn_3^{IV} arrangements have been published by Wieghardt and co-workers, who synthesized a series of oxo-bridged complexes using triazacyclononane (Wieghardt *et al.* 1988). Despite all these advances, true corroborative models of the OEC appear to be far in the future.

3. OXIDATION STATES

While there are five S-states, the two most important are S_0 , the product state, and S_4 , the catalytic state that forms and releases dioxygen. In most cases, it is believed that S-state advancement corresponds to manganese oxidation, although there is some controversy as to the $S_2 \rightarrow S_3$ (Yachandra *et al.* 1996; Robblee *et al.* 2001; Yachandra 2005) transition. But even this ambiguity has as its primary consequence the assignment of the proper manganese oxidation levels in S_4 . The EPR-active S_2 state often serves as the calibration point for the S-clock, being assigned by most workers as the mixed valent $\text{Mn}^{\text{III}}\text{Mn}_2^{\text{IV}}\text{Ca}^{\text{II}}$ oxidation level. There is no disagreement that the $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ transitions correspond to manganese-centred oxidation. This leads to S_1 being assigned as $\text{Mn}_2^{\text{III}}\text{Mn}_2^{\text{IV}}\text{Ca}^{\text{II}}$ and S_0 as $\text{Mn}_4\text{Ca}^{+15}$. We have chosen this latter formulation because there are two different possible formulations, namely $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}\text{Mn}_2^{\text{IV}}\text{Ca}^{\text{II}}$ (supported by X-ray absorption near-edge structure spectroscopy—XANES— and EPR; Ahrling *et al.* 1997; Messinger *et al.* 1997) and $\text{Mn}_3^{\text{III}}\text{Mn}^{\text{IV}}\text{Ca}^{\text{II}}$ (supported by recent EPR studies). Also, recent ^{55}Mn electron-nuclear double resonance (ENDOR) spectroscopy argues against the presence of Mn^{II} in S_0 (Kulik Leonid *et al.* 2005). The $\text{Mn}^{\text{II}}\text{Mn}^{\text{III}}\text{Mn}_2^{\text{IV}}\text{Ca}^{\text{II}}$ arrangement is particularly interesting, as it would be a rare example where manganese ions are found in three different oxidation states.

Model compounds can provide insight into this controversy. There are only two small molecule compounds that have Mn in three different oxidation states (Chan & Armstrong 1990; Mukherjee *et al.* 2006), while a handful of compounds contain at least

two manganese ions separated by two charge units. In the latter case, there are two interesting pairs of compounds that are redox isomers, which serve to evaluate the ability of XANES to discriminate between the two S_0 -oxidation state formulations (Alexiou *et al.* 2003; Zaleski *et al.* submitted).

Comparative spectroscopic and magnetic studies between $Mn^{II}Mn^{IV}Mn^{II}$ and $Mn^{III}Mn^{II}Mn^{III}$ compounds suggested that one can properly assign oxidation states to these Mn_3^{8+} systems using XANES spectroscopy (Alexiou *et al.* 2003). The oxidation states in these compounds could be clearly distinguished from each other due to the fact that they display different shapes in the XANES spectra, with Mn^{II} -rich compounds exhibiting a more intense X-ray edge absorption. Since XAS spectroscopy is an averaging technique, the ability to resolve oxidation states diminishes as the number of manganese ions increases. Therefore, we have recently investigated a more rigorous test of the XANES technique using tetrameric systems. In particular, we have examined two Mn_4^{10+} systems ($Mn_3^{II}Mn^{IV}$ (Afrati *et al.* 2002) versus $Mn_2^{II}Mn_2^{III}$ (Zaleski *et al.* submitted)). While not as clear cut as with the trinuclear system, we can still resolve the appropriate oxidation states for these compounds. These data suggest that XANES can, in principle, distinguish properly between the $Mn^{II}Mn^{III}Mn_2^{IV}Ca^{II}$ and the $Mn_3^{III}Mn^{IV}Ca^{II}$ formulations; however, in practice, this may be very difficult.

The S_1 state is almost certainly $Mn_2^{III}Mn_2^{IV}$. Despite being the dark stable state, little attention was focused on this oxidation level, because it was spectroscopically less interesting than the one-electron-oxidized S_2 level. Britt's discovery of a parallel mode EPR signal confirmed the non-Kramer's electronic state and strongly supported the accepted manganese oxidation assignment (Campbell *et al.* 1998). Subsequently, we demonstrated that a tetranuclear manganese complex displays a very similar parallel-mode signal to that of the enzyme (Hsieh *et al.* 2004). Because this model complex has a Mn_4^{IV} oxidation level, the hyperfine shifts are slightly reduced from that of S_1 . Nonetheless, this is still the only compound known to exhibit a parallel mode multiline signal reminiscent of S_1 .

The number of model compounds prepared to mimic S_2 are too numerous to mention. The interested reader should look at the many reviews on this topic (Mukhopadhyay *et al.* 2004; Wu *et al.* 2004). In contrast, models for S_3 and S_4 are very rare. The highest oxidized S-state is S_4 , which has a very short lifetime and its individual manganese oxidation states are unclear. The best descriptions are either a $Mn_3^{IV}Mn^V=O$ or Mn_4^{IV} -oxy radical (to some extent differentiating these species may be equivalent to asking whether the metal or ligand molecular orbitals have been more depopulated in electron density). Alternatively, one could envision a lower valent S_4 state if partial substrate oxidation occurs in S_3 , prior to the formation of S_4 . Certainly there are neither reported examples of crystallographically characterized clusters containing Mn^V nor an equivalent Mn^{IV} -oxy radical. There are numerous examples of Mn_4^{IV} species (Wiegardt 1989; Pecoraro *et al.* 1994; Law *et al.* 1998; Mukhopadhyay *et al.* 2004), but these complexes are

generally inert (non-reactive). The reason for the ambiguity in the S_4 -oxidation level arises from the conversion of $S_2 \rightarrow S_3$. Initially, XANES data were interpreted as corresponding to a non-metal-centred oxidation. Several accounts have suggested that the Mn are oxidized in this transition. Simply put, there are significant amounts of data supporting either metal- or non-metal-centred oxidation, making this point very difficult to resolve at the present time.

4. MECHANISMS

Given our present understanding of structure (a Mn_4Ca core) and kinetics (five states with S_4 either $Mn_3^{IV}Mn^V=O$ or Mn_4^{IV} -oxy radical, and S_0 either $Mn^{II}Mn^{III}Mn_2^{IV}Ca^{II}$ or $Mn_3^{III}Mn^{IV}Ca^{II}$), one can begin to evaluate the mechanism for water oxidation (McEvoy & Brudvig 2006). There are several ways to categorize water-oxidation proposals. The most useful starting point is to differentiate models based on whether O–O bond formation begins in S_3 or S_4 . One can then assess whether bridging oxo groups, a combination of bridging oxo and terminal water/hydroxide/oxo functionalities or solely terminal moieties are responsible for the formation of an O–O bond. From there, more subtle points such as the appropriate oxidation states of the manganese ions and the origin of oxo atoms (e.g. bound to Mn versus Ca) can be assessed.

5. O–O BOND FORMATION BEGINNING IN S_3

Proposed as an S_3 -initiated reaction by Yachandra and co-workers (Yachandra *et al.* 1996; Robblee *et al.* 2001; Yachandra 2005), the formation of a Mn-oxyl-radical occurs in the $S_2 \rightarrow S_3$ transition, followed by the formation of a second oxygen radical during $S_3 \rightarrow S_4$. Both oxo radicals then combine yielding a peroxo species. The μ -oxo bridges of the cluster would be the origin of the oxygen atoms in this model. While this mechanism was originally proposed for a dimer-of-dimers structure, which was ruled out by ^{55}Mn ENDOR spectroscopy (Peloquin *et al.* 2000) and X-ray crystallography (Zouni *et al.* 2001; Ferreira *et al.* 2004; Loll *et al.* 2005), it can certainly be incorporated into the present 3+1 structural motif. This model would never require the manganese ions of the cluster to exceed a $Mn^{III}Mn_3^{IV}Ca$ oxidation level. As an advantage, this model accounts for an invariant metal oxidation state from $S_2 \rightarrow S_3$ and would be consistent with a structural change in the cluster at higher S-states. One potential drawback of this system is that water exchange between bridging μ -oxo groups would be expected to be much slower than has been reported. Wydrzynski and co-workers have measured the exchange rates of substrate water (Hillier *et al.* 1998, 2001). They determined that at least two water molecules are bound to the OEC throughout the cycle, one of them by S_2 . Although μ -oxo exchange could not be measured, the comparison with other metal-oxo complexes leads to the conclusion that the observed rates were too fast for an exchange of a bridging oxygen atom.

Similar to the Yachandra proposal, Messinger and co-workers have invoked oxygen radicals in the S_3 and S_4 states (Messinger 2004). A major difference

to the previous mechanisms, Messinger incorporated a deprotonation step in the $S_0 \rightarrow S_1$ transition (Haumann *et al.* 2005), which rationally explains the Mn–Mn bond-length shortening from 2.85 to 2.70 Å. Such a deprotonation is probably essential in order to maintain a relatively constant potential for the water-oxidation catalyst. This model again suffers from the water-exchange-rate problem described above.

Siegbahn and co-workers proposed an alternative strategy based on density functional theory (Lundberg & Siegbahn 2004; Siegbahn & Blomberg 2005). In their model, oxygen radicals are involved in S_3 and S_4 and deprotonation leads to a bridging hydroxide. One major difference from the Messinger or Yachandra models is that the oxygen atoms are terminally bound to the Mn ions. These authors propose that terminal Mn^{V} -oxo species may be insufficiently reactive for water oxidation, requiring a spin-state alteration to a Mn^{IV} -oxyl radical for reactivity. One can think of this model as a homolytic coupling of two Mn^{IV} -oxyl radicals.

One of the most influential early proposals for water oxidation was presented by Babcock (Tommos *et al.* 1995; Hoganson & Babcock 1997). Proton-coupled electron transfer (PCET) and, more specifically, H-atom abstraction significantly influenced the thinking behind this proposal. The mechanism involves the tyrosyl radical Y_z not only as an oxidizing agent but also as an H-atom acceptor (the Y_z simultaneously accepts protons and electrons). The first critical catalytic step takes place in the transition $S_2 \rightarrow S_3$, where a Mn^{IV} terminal oxo species in proximity to a hydroxyl group (attached to Mn^{IV}) is formed. After a final oxidation and proton abstraction in S_4 , a peroxo bridge linking the two dangler manganese centres is formed. Eventually O_2 is released, substrate water is attached and S_0 is re-established. While a very appealing process when first published, recent structural and spectroscopic data appear to make this mechanism non-viable. The most recent structures place Y_z too far away from the cluster to be involved in direct H-atom abstraction.

Model compounds have served to both support the basic premise of this proposal and refute it. For example, a key requisite for this chemistry (and that proposed by Siegbahn) is the ability to abstract hydrogen atoms from either terminal or bridging oxygen atoms. Several reports on model compounds demonstrated that H-atom abstraction, with homolytic bond dissociation energies (HBDEs) of the order of 87 kcal mol^{-1} , could occur with either terminal or bridging water or hydroxides with biologically relevant manganese oxidation levels. This HBDE is precisely the thermodynamic value necessary for Babcock's H-atom abstraction model (Gardner & Mayer 1995; Caudle & Pecoraro 1997). More recent work on a single set of compounds suggests that going higher than Mn^{IV} leads to a large increase in the HBDE (Gupta *et al.* 2002). Nonetheless, these studies demonstrated that PCET was thermodynamically important in the water-oxidation process.

Model compounds have also served to exclude the Babcock proposal, and any other mechanisms requiring a $Mn=O$ formulation in S_3 . An important class of mononuclear manganese complexes incorporates Mn

terminal oxygen moieties, since many mechanistic proposals are based on manganyl species in the higher S-states. Early synthetic work in this field was done by Collins and co-workers, who published the first structurally characterized Mn^{V} -oxo compound, a 1,2-bis(2-hydroxy-2-methylpropanamido)benzene Mn^{V} complex, in 1989 (Collins & Gordon-Wylie 1989). Later on, two further Mn^{V} complexes, also comprising oxidatively stable and highly negatively charged amidate ligands, were published by the same group (Collins *et al.* 1990; Miller *et al.* 1998). XANES spectra of Collins' first (alcoholato) complex, contributed strongly to the understanding of the $S_3 \rightarrow S_4$ transition (Weng *et al.* 2004). An intense XANES pre-edge signal was found in this Mn^{V} -oxo compound. The origin of the feature is a consequence of the mixing of metal d- and oxygen p-orbitals, which removes the symmetry forbidden nature of the $1s \rightarrow 3d$ transition. Furthermore, it was shown that $Mn^{IV}=O$ (also having significant metal d- and oxygen p-orbital mixing) should give rise to a similar intense pre-edge transition. In contrast, $Mn^{IV}OH$ or other related species do not have such strong pre-edge absorptions. Analysis of the XANES spectra of the OEC demonstrated that a strong pre-edge transition was not present in the native S_3 . Therefore, this work demonstrated that manganyl species do not exist in the OEC prior to the S_4 state (Weng *et al.* 2004). Dau and co-workers later extended this analysis demonstrating that $Mn=O$ species also do not exist in the region until S_4 (Haumann *et al.* 2005); however, there is no experimental evidence to assess whether such species are viable in the actual water-oxidation step. It should be noted that even though the Babcock model has now been shown not to be the proper description for water oxidation, the components of these model were tremendously inspirational and significantly moved the mechanistic thinking of the field forward.

Meyer and co-workers (Meyer *et al.* 2007) have proposed a 14-step mechanism that scrupulously accounts for proton movement through the S-cycle. These authors propose the formation of an O–O bond during S_3 . One oxygen atom of the product is bound to manganese while the second originates on calcium. Subsequent photo-oxidation and proton transfer lead to the release of dioxygen. This is the only model with chemistry beginning in S_3 that uses calcium explicitly in the reaction process. In summary, there are several viable water-oxidation mechanisms that require substrate oxidation to begin in S_3 . The most prominent (Hoganson & Babcock 1997) of these proposals has recently been eliminated. Whether any of these proposals remain viable is dependent on the resolution of the controversy for Mn oxidation in the $S_2 \rightarrow S_3$ transition.

6. O–O BOND FORMATION BEGINNING IN S_4

The second major class of mechanisms for water oxidation relies on the entire oxidation process occurring during the $S_3 \rightarrow S_4 \rightarrow S_0$ transition. An example that invokes oxidation of bridging oxygen atoms was proposed by Dismukes based on a Mn_4O_4 cuboidal model compound (Ruettinger *et al.* 2000).

Two μ -oxo bridging oxygen atoms combine generating a peroxo bridge. After the liberation of dioxygen, the cluster displays a Mn_4O_2 butterfly structure. The observation of dioxygen in the gas phase, using mass spectroscopy, has been taken as support of this model. Hückel calculations, which had been carried out for the S_4 state of cuboidal Mn–oxo clusters comprising peroxo oxygen atoms coordinated by three Mn ions, have predicted an energetic advantage for this process (Proserpio *et al.* 1992). It is unclear how this model can be rationalized with the water exchange results of Wydrzynski.

Dau and co-workers suggested that oxygen is formed during a reaction of a terminal oxyl and an exogenous hydroxyl radical in S_4 (Dau *et al.* 2001). Furthermore, they use the concept of proton-assisted oxidation, where the μ -oxide bridges act as bases and abstract H atoms from non-coordinated water molecules. A highly reactive Mn^{V} -oxo species that switches to a Mn^{IV} -oxyl-radical is proposed in S_4 . A H atom is abstracted from a coordinated water by an adjacent μ -oxide, providing a poised hydroxyl radical. This OH moiety immediately forms an O–O–H bond, with the hydroperoxo H atom abstracted by another μ -oxide. Model chemistry has been particularly important to assess this mechanistic aspect. It has been shown that μ -OH bridges are generally acidic and this acidity increases as the oxidation level of the cluster increases (Larson *et al.* 1992; Baldwin *et al.* 1993; Baldwin *et al.* 1994). Thus, one would not expect μ -oxo bridges to be sufficiently basic as to act as proton acceptors, especially as the cluster is oxidized.

Pecoraro *et al.* (1998) proposed a reaction process in 1997 that explicitly accounted for the role of Ca. It was suggested that an electrophilic Mn^{V} -oxo is formed in the S_4 state. Hydroxide coordinated to a nearby calcium ion could then serve as a nucleophile, attacking this terminal oxo group to directly form dioxygen. In addition to accounting for the need of calcium for the first time, this proposal is consistent with Wydrzynski's experiment (Hillier *et al.* 1998, 2001), which suggests two water molecules displaying different exchange rates. Most likely, the slower exchanging substrate is bound to manganese and the faster one only to calcium. Subsequently, Brudvig and co-workers expanded on these ideas to provide a detailed step-by-step mechanism that accounts for the chemistry through each S-transition (McEvoy & Brudvig 2006).

Model chemistry has been generally supportive of this proposal although direct proof for the presence of a $\text{Mn}^{\text{V}}=\text{O}$ has not been presented. Brudvig and co-workers have produced a $\text{Mn}^{\text{III}}\text{Mn}^{\text{IV}}$ terpyridine complex, which served as the first model for catalytic O–O bond formation when sodium hypochlorite or KHSO_5 was used as an oxidant (Tagore *et al.* 2006, 2007). Some of the early experiments have been reinterpreted to release dioxygen through a disproportionation reaction; however, Yagi has indicated that these water-oxidation catalysts are efficient when placed on a clay surface. More recently, McKenzie and co-workers have used a similar complex to probe reactivity towards a mechanistic model, using t-butyl hydroperoxide as an oxidant (Poulsen *et al.* 2005). It is well known that singlet dioxygen can be liberated from

the reaction of Mn dimers and t-butyl hydroxide (Caudle *et al.* 1996); however, it appears that this may be a legitimate water-oxidation process as ^{18}O -labelled water is found in the product dioxygen. A highly reactive Mn_2^{IV} species was suggested to play a crucial role in this process. Further definition of this system is required, with particular attention being focused on the origin of the second oxygen atom.

In summary, the field of photosynthetic oxygen evolution has made tremendous advances in understanding the structure of the cluster responsible for water oxidation. Furthermore, definition of cluster oxidation states has been obtained in several, although not all, of the S-states. Due in part to oxidation state ambiguity, the number of proposed water-oxidation mechanisms seems to be increasing, rather than narrowing. One of the main objectives of the next decade will be to bring a similar level of clarity to the chemical mechanism of this extraordinary process, as is now being revealed for the lower S-state catalyst structure.

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Discussion

J. Barber (*Imperial College London, UK*). In your scheme of O–O bond formation involving a nucleophilic attack of a hydroxyl oxygen on a highly electrophilic oxygen, how important is the directionality of this attack?

V. Pecoraro. The directionality of the attack is important as one would want one of the p orbitals on the hydroxide to be positioned in such a way as to be able to shift electron density into the LUMO of the electron-deficient oxo atom bound to the manganese. This should not be a significant issue, however, because there should be high symmetry for the orbitals on this oxo atom so that the only restriction would really come from the hydroxide group. Again, this is unlikely to be a problem as the only orbitals that are restricted from participating in this attack are the ones engaged in bonds to the Ca and to the proton. Of course, the proton, unless restricted by a strong H-bond, should have rapid movement to all three potential orbitals of the hydroxide oxygen atom.

G. Brudvig (*Yale University, USA*). What are your thoughts about Ca-hydroxide versus Ca-water as the nucleophile in the O–O bond formation?

V. Pecoraro. I believe that the more proton deficient the oxygen atom becomes, the better base it should be. Therefore, my preference would be to use a hydroxide that had the proton involved in an H bond to another acceptor (making it more oxide-like than even

hydroxide-like). I think water is not as good a nucleophile (e.g. it has lower charge) so my preference would be for hydroxide rather than water.

A. Aukauloo (*University of Paris-Sud, France*). What was the rationale for making your tetranuclear Mn-Ca system and were the Mn atoms of this cluster redox active?

V. Pecoraro. I'll answer the second question first. We have not examined the electrochemistry of this particular compound so I can't give a definitive answer. The corresponding compound with Mn(II) has a one-electron oxidative wave which probably corresponds to the oxidation of Mn(II) to Mn(III). If this new compound parallels the previous molecule, then it is unlikely that the Mn(III) atoms are redox active within a reasonable electrochemical window. As for the rationale, we have long known that the class of molecules we described as metallacrowns leads to controllable assemblies of metal atoms. Given our observations with other related metallacrown systems that bind manganese, lanthanides or calcium, it was rather straightforward to design a synthesis that led to the structures that were described in my lecture. I would refer you to our *Progress in Inorganic Chemistry* review in 1996 for a general overview of this strategy and some subsequent papers in *Inorganic Chemistry* and *Angewandte Chemie* if you'd like more synthetic details.

J. Messinger (*Muelheim, Germany*). I have a comment I wish to make. On the basis of my first mass spectrometric data on the substrate water exchange in PSII (Messinger *et al.* 1995) I also proposed a nucleophilic attack mechanism which involves a transient Mn(IV)- $\equiv\text{O}^+$ species in the S_4 state (this is isoelectronic with Mn(V)=O or Mn(IV)-O \cdot), and a water molecule that was said to be either free, bound to a protein residue or to Ca (symbolized in the corresponding scheme II as X-OH $_2$). At that point in time the exchange rate for the fast exchanging substrate molecule was unresolved due to a mixing time of 30 ms, and therefore appeared to be much faster than the slow exchange. After improving the time-resolution to 8 ms it turned out that the fast exchange rate is only approximately 20 times faster than that of the slowly exchanging substrate molecule (Hillier *et al.* 1998). On that basis it appears unlikely that the two substrate molecules are bound in completely different environments (i.e. one to Mn, the other being free or ligated only to Ca) and hence I now consider the nucleophilic attack mechanism as not very likely. Furthermore, substrate water exchange measurements on the S_2 state show that the slow exchange occurs essentially with the same rate in the S_2 and S_3 states (Hillier & Wydrzynski 2000). Nucleophilic attack mechanisms during this step often involve the oxidation of the Mn ion to which the slowly exchanging substrate molecule (at this stage usually shown as Mn=O) is bound, which appears to be inconsistent with the above result. Other findings add to this argument: several different experiments and theoretical calculations (summarized in (Messinger 2004; Hillier & Messinger 2005)) indicate the formation of a radical in the S_3 state, and consequently argue against a Mn(IV) $\equiv\text{O}^+$ (or Mn(V)=O) formation and, thereby, also against a nucleophilic attack mechanism for O-O bond formation in PSII.

V. Pecoraro. Johannes, you raise a number of interesting points which I will attempt to address in order. With respect to your proposed nucleophilic attack mechanism, there are points about it that never made sense. First, as you point out, Mn(V)=O and Mn(IV)-O \cdot are isoelectronic; however, that does not mean that they react identically or even similarly. In your proposal, an oxygen atom behaves as a non-innocent ligand which is formally oxidized to the radical form by a Mn(V). Such a system would be quite similar to that of Fe(III)catecholate versus Fe(II)(semiquinone) in which there is a non-innocent ligand (catechol) bound to an oxidizing metal. The two compounds do not react the same with the Fe(II)(semiquinone) being air sensitive whereas the Fe(III)catecholate is stable. As Dan Nocera also has pointed out in this symposium session, a reactivity difference is also expected for Mn(V)=O and Mn(IV)-O \cdot . The oxygen on a Mn(V)=O is electrophilic, meaning that it is susceptible to attack by a nucleophile. The oxygen of Mn(IV)-O \cdot is non-nucleophilic and reacts as a radical. This is the basis for Per Siegbahn's model from his quantum calculations. Thus, to have invoked a nucleophilic attack on Mn(IV)-O \cdot was simply wrong. Next, I find little insight in describing the nucleophile water as 'either free, bound to a protein residue or to Ca (symbolized in the corresponding scheme II as X-OH $_2$)'. One could even suggest that X could have been manganese. In this case, you would have invoked your non-electrophilic Mn(IV)-O \cdot being attacked by bulk water, water trapped in a protein or water bound to a metal. Said differently, no source of water is excluded. More important, though, is that your proposed nucleophile was water. The reason that we have suggested that water is likely bound to calcium, with its non-redox active Lewis acid properties, is that it makes the water more acidic. Thus, calcium helps activate the water to form hydroxide (a much better nucleophile than water) without itself being prone to oxidation. The premise of the idea is that the oxygen atom on manganese must be highly activated (as achieved with an Mn(V)=O) and the nucleophile also must be highly activated (as occurs with a hydroxide bound to calcium). You are correct to question your original proposition as one would never expect a non-electrophilic oxygen (as represented by Mn(IV)-O \cdot) to be attacked by a really poor nucleophile (from bulk water onward to water on X). Thus, I think we are both in agreement that your original proposal is wrong and needs no more discussion. Now let's discuss the water exchange rates. Here one needs to be careful discriminating between experimental results and experimental interpretation. The observation is that the exchange rates for water are within 20 times that of one another and that they do not change going from S_2 to S_3 . Unfortunately, we do not know what process is rate limiting for water exchange in this case. You are assuming that the rate limitation that you and others measure is a consequence of the dissociation rate of water from a metal ion. Gary Brudvig demonstrated in his talk here that the rates of exchange on Mn are kinetically competent with these exchange rates so there would be no problem with our model. In fact, your arguments are of more concern for an oxo bridge (especially for high valent manganese) as water

exchange should be very different if one oxygen was in the bridge and the other not. Furthermore, if both were in bridges, one might expect truly slow exchange rates. None-the-less, the main problem with this discussion is that you do not know why you observe the rates that you do. With respect to no change in rate going from S_2 to S_3 , this is also completely consistent with a non-metal dependent rate limitation. While some mechanisms from other groups may invoke a $Mn=O$ in S_3 , we have said that this is highly unlikely based on our collaborative work with Jim Penner-Hahn (Weng *et al.* 2004). Thus, there is no inconsistency in our proposal based on the oxidation level of S_3 . Finally, you are concerned with the appropriate oxidation state description for the Mn ions in higher S states. This is, of course, the controversy to which I referred in my talk. It is wrong to imply that the S_3 state is a radical; this has not been proven in my opinion. There are some people, such as yourself, that feel that the manganese ions are not oxidized going from S_2 to S_3 . Others, such as myself and Gary Brudvig, feel the data presented so far is most consistent with a manganese-centered oxidation. There are still others that think that one can not reach Mn(V) because their formulation of the oxidation levels for S_2 are $Mn(III)_3 Mn(IV)$, not the $Mn(III)Mn(IV)_3$ assignment which I think both you and I prefer. Clearly, reasonable scientists have a disagreement on this point

which I do not think has been clarified further at today's meeting. Thus, my conclusion is as I stated in my talk, if one has sequential oxidation of the manganese ions through the entire S cycle, the most reasonable mechanism for water oxidation is by a hydroxide ion bound to calcium attacking an electrophilic oxygen of a manganyl ($Mn(V)=O$) species.

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