Water oxidation chemistry of photosystem II

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Photosystem II (PSII) uses light energy to split water into protons, electrons and O₂. In this reaction, nature has solved the difficult chemical problem of efficient four-electron oxidation of water to yield O₂ without significant amounts of reactive intermediate species such as superoxide, hydrogen peroxide and hydroxyl radicals. In order to use nature's solution for the design of artificial catalysts that split water, it is important to understand the mechanism of the reaction. The recently published X-ray crystal structures of cyanobacterial PSII complexes provide information on the structure of the Mn and Ca ions, the redox-active tyrosine called Y_Z and the surrounding amino acids that comprise the O₂-evolving complex (OEC). The emerging structure of the OEC provides constraints on the different hypothesized mechanisms for O₂ evolution. The water oxidation mechanism of PSII is discussed in the light of biophysical and computational studies, inorganic chemistry and X-ray crystallographic information.

Keywords: calcium; manganese; oxygen-evolving complex; photosystem II; water oxidation

1. PHOTOSYSTEM II AND THE OXYGEN-EVOLVING COMPLEX

The photosynthetic protein complex photosystem II (PSII) is found in the thylakoid membranes of green plants, algae and oxyphotobacteria, this last group being dominated by the cyanobacteria. PSII is a multi-subunit membrane protein that acts as the first light-transducing complex in the redox pathway of oxygenic photosynthesis. It uses the energy of sunlight to oxidize water, producing dioxygen and protons, and reduces lipid-soluble plastoquinone. The water-derived electrons pass to the cytochrome *b₆f* complex and photosystem I and are eventually used to reduce CO₂ in the Calvin cycle. PSII is the source of nearly all of the O₂ in the Earth's atmosphere and is, therefore, of considerable importance and practical interest.

Water is oxidized at the metal-containing oxygen-evolving complex (OEC) of PSII, which catalyses the four-electron reaction described in equation (1.1) as follows:

where PQ is plastoquinone and PQH₂ is plastoquinol.

Protons are liberated from H₂O and bound by PQ on opposite sides of the thylakoid membrane; the pH gradient thereby established contributes to the transmembrane free energy gradient used for ATP synthesis. The standard reduction potential of this reaction at pH 5.0, the pH at which the OEC typically operates, is +0.93 V. Because four electrons are transferred, this potential equates to a reaction free energy of 0.93 V × 4 = 3.72 eV, or 359 kJ mol⁻¹. The stringent energetic and catalytic requirements of this reaction are

indicated by the fact that the OEC appears to be identical in every type of PSII, while nature is able to use a variety of metal catalysts for many other bioenergetic catalytic reactions (e.g. those performed by hydrogenases, nitrogenases and terminal oxidases).

Kok *et al.* (1970) established that the OEC accumulates oxidizing equivalents by proceeding through five redox states, commonly called 'S states', of which the most reduced during the catalytic cycle is S₀ and the most oxidized is the transiently stable S₄ (figure 1*a*). The S₄ state oxidizes water and is itself reduced to the S₀ state, almost certainly via one or more intermediates (Clausen & Junge 2004; Haumann *et al.* 2005).

In the past 6 years, three groups have published X-ray crystal structures of PSII at 3.8 Å (Zouni *et al.* 2001), 3.7 Å (Kamiya & Shen 2003), 3.5 Å (Ferreira *et al.* 2004) and 3.0 Å (Loll *et al.* 2005) resolution. The recent 3.5 Å crystal structure of Barber, Iwata and co-workers (Ferreira *et al.* 2004) is the first crystal structure of PSII that was refined to atomic resolution. The OEC is modelled as an Mn₃CaO₄ cuboid, with the fourth manganese ion attached to the exterior of the cuboid via a μ₄ oxide ion at one of the cuboid corners (figure 1*a*). This so-called 'dangler' Mn ion (Mn(4)) is positioned so that its coordination sphere is close to that of calcium. It seems probable that the Mn(4)–Ca surface of the metal ion cluster is the area of catalytic activity. The modelled structure of the OEC is generally compatible with a large body of X-ray absorption fine structure (EXAFS) and electron paramagnetic resonance (EPR) data, computational models (Sproviero *et al.* 2005), and with site-directed mutagenesis studies (Debus 2001; Diner 2001), although there remain many questions of the exact structure of the manganese-oxo cluster and its ligation (McEvoy & Brudvig 2006).

The crystallographic model suggests a well-defined proton exit channel of hydrophilic residues leading

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Figure 1. Structure-based mechanisms proposed for the oxidation of water by (a) PSII (McEvoy & Brudvig 2004) and by (b) a functional mixed-valent Mn^{III/IV}-terpy model complex (complex 1; Limburg *et al.* 2001).

from near Mn(4) to the luminal exterior of PSII. Importantly, the channel is on the opposite side of the Mn₄ cluster from Y_Z (D1-Tyr161) and leads directly away from Y_Z, which is shown as being hydrogen bonded to D1-His190 alone. This finding casts doubt on Y_Z's function as the catalytic base in water splitting (Hoganson & Babcock 1997) because the crystallographic evidence suggests that protons would be unable to escape from the Y_Z/H190 residue pair. Studies of the kinetics of proton release and electron transfer by Junge *et al.* (2002) also indicate that protons are not released from Y_Z into the lumen.

We have recently suggested that the arginine residue CP43-Arg357, found to be very close to the OEC, plays the role of the thermodynamically indispensable redox-coupled base (McEvoy & Brudvig 2004). The recent computational study of Knapp and co-workers (Ishikita *et al.* 2006) has concluded that the pK_a of CP43-Arg357 is dramatically lowered by oxidation of the Mn cluster from the S₀ state to the higher S states and also that a series of acid/base residues form a pathway for proton transfer leading away from CP43-Arg357 to the luminal protein surface, in agreement with our proposal that CP43-Arg357 functions as the catalytic base in the OEC.

2. PROPOSED MECHANISMS FOR PHOTOSYNTHETIC WATER OXIDATION

The mechanism of water oxidation by PSII remains unclear. Mass spectrometry measurements show that none of the lower S states (S₀ through S₃) contain a non-exchangeable form of water, a result that seems to rule out O–O bond formation before the S₄ state is produced (Hillier *et al.* 1998; Hendry & Wydrzynski 2002; Hillier & Wydrzynski 2004).

There have been a large number of proposals for the mechanism of O–O bond formation since the first modern 'molecular' mechanism was proposed by Brudvig & Crabtree (1986); most of the earlier proposals lack molecular detail and are reviewed by Volkov (1989). Proposals for the mechanism of O–O bond formation can be grouped into three major categories based on the nature of the bound substrate water molecules (Hillier & Messinger 2005; McEvoy & Brudvig 2006). The earliest proposals for the mechanism of O–O bond formation involved the coupling of Mn-bridging oxo (μ-oxo) ligands (Brudvig & Crabtree 1986; Vincent & Christou 1987) and some current proposals fall in this category (Ruettinger & Dismukes 2000). Following from evidence that Mn-centred oxidation may not occur in the S₂→S₃-state transition, mechanisms have been proposed involving coupling reactions of an oxyl radical (Yachandra *et al.* 1996; Siegbahn 2000; Dau *et al.* 2001; Messinger 2004). The third category of mechanisms for water oxidation involves nucleophilic attack of a calcium-bound water (or hydroxide) ligand on the electrophilic oxygen atom of an Mn^V=O intermediate in the O–O bond-forming step (Pecoraro *et al.* 1998; Szalai *et al.* 1998). Related proposals involve coupling of an Mn-bound hydroxide and an Mn=O species (Hoganson & Babcock 1997; Hillier & Wydrzynski 2000).

Based on a consideration of both the available biophysical data and the inorganic chemistry of Mn, we proposed a detailed mechanism for the S-state cycle in which O–O bond formation occurs in the S₄ state through nucleophilic attack of a calcium-bound water on the electrophilic oxygen atom of an Mn^V=O species (Limburg *et al.* 1999a; Vrettos *et al.* 2001a). Mass

spectrometry measurements using H_2^{18}O corroborate calcium's role in substrate water binding (Hendry & Wydrzynski 2003). The structural model of Ferreira *et al.* (2004) also fits well with our proposed mechanism. We have extended our mechanistic proposal based on the modelled structure of the OEC; the O–O bond-forming step is shown in figure 1 (McEvoy & Brudvig 2004). In order to test this and other proposed mechanisms, we have investigated the kinetics of ligand exchange in a series of inorganic Mn model complexes, the properties of calcium-substituted PSII, and the mechanism of water oxidation by the mixed-valent $\text{Mn}_2^{\text{III/IV}}$ -terpy complex (complex 1 shown in figure 1; terpy = 2,2': 6,2''-terpyridine). These studies provide insight into the water oxidation chemistry of PSII and are described in §§3–5.

3. SUBSTRATE BINDING TO THE OXYGEN-EVOLVING COMPLEX

Proposed molecular mechanisms of water oxidation by the OEC must specify the timing and nature of substrate (water) binding to the catalytic centre. The timing of water binding is specified by the S states (S_0 through S_4) of the OEC at which substrate water binds. The nature of binding is determined by the site and mode of binding; for example, terminal aqua or hydroxo ligands bound to a single Mn or Ca atom (Hoganson & Babcock 1997; Pecoraro *et al.* 1998; Szalai *et al.* 1998; Hillier & Wydrzynski 2001; McEvoy & Brudvig 2004), or μ -oxo or μ -hydroxo bridges between metal centres (Brudvig & Crabtree 1986; Vincent & Christou 1987; Pecoraro *et al.* 1994; Yachandra *et al.* 1996; Nugent *et al.* 2001; Dasgupta *et al.* 2004; Messinger 2004; Isobe *et al.* 2005; Siegbahn & Lundberg 2005). Important constraints have been provided on the substrate binding to the OEC by isotope exchange experiments between bulk water and evolved oxygen in PSII preparations (Messinger *et al.* 1995; Hillier *et al.* 1998; Hillier & Wydrzynski 2000, 2004; Hendry & Wydrzynski 2002, 2003), as well as from studies of proton release from the OEC during the S-state cycle (Junge *et al.* 2002). However, even mechanisms proposed after the availability of the recent PSII crystal structures disagree on the mode of water binding to the OEC; some authors favour terminal water binding (McEvoy & Brudvig 2004; Isobe *et al.* 2005), and others invoke conversion of the substrate water into a μ -O bridge (Dasgupta *et al.* 2004; Messinger 2004; Siegbahn & Lundberg 2005). In order for the μ -O bridges in the OEC to function as binding sites for substrate waters, the substrate water must be able to form a μ -O bridge and the μ -O moiety must exchange with bulk water on a time scale faster than or equal to the time scale of OEC turnover. Measurement of the latter time scale has been provided by the ^{18}O isotope exchange experiments on the OEC referred to above.

Unfortunately, there has been a dearth of information on the rates of ligand exchange in oxo-Mn model complexes to aid in the interpretation of data on substrate exchange in the OEC. In particular, it has not been clear whether or not μ -oxo ligands could exchange with bulk water on a fast enough time scale in the OEC. Using time-resolved electrospray mass spectrometry,

we recently determined the rates of isotope exchange between μ -O bridges and ^{18}O -labelled water in a series of di- μ -O dimanganese complexes that are structural models for the OEC (Tagore *et al.* 2006, 2007).

A comparison of the ligand exchange rates for different Mn model complexes showed that: (i) all Mn^{IV} complexes exchange ligands much more slowly than mixed-valence complexes in which the Mn ions can switch between slow-exchanging Mn^{IV} and fast-exchanging Mn^{III} states, (ii) terminal water ligands exchange very much faster than μ -O ligands, irrespective of the Mn oxidation states, (iii) the availability of a terminal water-binding site on manganese greatly enhances the μ -O exchange rate, and (iv) the structure, nuclearity and ancillary ligands (other than water) of the Mn complexes do not significantly affect the μ -O exchange rates. These factors place significant constraints on the interpretation of the substrate exchange rates in the OEC in terms of specific modes of water binding to the Mn and Ca ions.

The absolute rates measured in Mn model complexes (Tagore *et al.* 2006) can be compared with the fast and slow isotope exchange rates measured in PSII samples (Messinger *et al.* 1995; Hillier *et al.* 1998; Hillier & Wydrzynski 2000, 2004; Hendry & Wydrzynski 2002, 2003). The S_1 and S_2 oxidation states of the OEC are widely accepted to be $\text{Mn}_2^{\text{III}}\text{Mn}_2^{\text{IV}}$ and $\text{Mn}^{\text{III}}\text{Mn}_3^{\text{IV}}$, respectively (McEvoy & Brudvig 2006). The exchange rates in mixed-valent $\text{Mn}_2^{\text{III/IV}}$ complexes are, therefore, considered to be reasonable estimates for the exchange rates in the S_1 and S_2 states of the OEC. The fast exchange rates in the S_1 and S_2 states are approximately 10^5 times greater than the μ -O exchange rate in the mixed-valent $\text{Mn}_2^{\text{III/IV}}$ -terpy complex 1 (figure 1*b*). The rate of fast exchange in the OEC is smallest in the S_3 state, but is still approximately 10^3 times greater than in complex 1. Thus, it is very unlikely that the fast isotope exchange rates measured in the OEC are μ -O exchange rates.

The slow isotope exchange rates measured in the S_0 , S_2 and S_3 states are approximately 800–4000 times greater than in complex 1 and it is, therefore, also very unlikely that the slow isotope exchange rates measured in the S_0 , S_2 and S_3 states of the OEC are μ -O exchange rates. The slow exchange rate in the S_1 state of the OEC, however, approaches the μ -O exchange rate for complex 1, being approximately eight times greater. The activation energy for the slow exchange measured in the S_1 state is approximately 83 kJ mol^{-1} (Hillier & Wydrzynski 2004), which is close to the activation energy for μ -O exchange observed for complex 1 (approx. 84 kJ mol^{-1} ; Tagore *et al.* 2007). Thus, the slow-exchanging substrate in the S_1 state could be bound as a bridging oxo.

From the perspective of metal oxidation states, the direct comparison of μ -O exchange rates between complex 1 and the OEC is reasonable. However, differences in other factors need to be considered to make a rigorous comparison between the μ -O exchange rates in the OEC and complex 1, such as the dielectric surrounding the OEC, the accessibility of water to the OEC and the functional groups from the protein surrounding the active site, which may be difficult to investigate by a study of model complexes. There is one other system where a comparison can be made between

Table 1. Ionic radii and pK_a s of the aqua ions of metal cations.

metal ion	ionic radius ($\text{\AA}$) ^a	pK_a of aqua ion ^b
<i>monovalent cations</i>		
Na ⁺	0.97	14.77
K ⁺	1.33	16
Cs ⁺	1.67	> 17
<i>divalent cations</i>		
Mg ²⁺	0.66	11.41
Ni ²⁺	0.69	9.86
Cu ²⁺	0.72	8.00
Co ²⁺	0.72	9.85
Cd²⁺	0.97	9.00
Ca²⁺	0.99	12.80
Sr²⁺	1.12	13.18
Ba ²⁺	1.34	13.36
<i>trivalent cations</i>		
Lu ³⁺	0.85	7.94
Dy ³⁺	0.91	8.10
Gd ³⁺	0.92	9.78
Pr ³⁺	1.01	8.91
La ³⁺	1.02	8.82

^a Data from Weast (1978).^b Data from Dean (1985).

the rates of μ -O ligand exchange in a protein active site with model complexes in solution. The μ -O exchange rate measured for the Fe-(μ -O)-Fe centre of ribonucleotide reductase (Sjöberg *et al.* 1982) is approximately $8 \times 10^{-4} \text{ s}^{-1}$. This value is approximately 10^4 times smaller than the rates of μ -O exchange measured in solution (approx. $1\text{--}40 \text{ s}^{-1}$) for inorganic Fe-(μ -O)-Fe porphyrin complexes (Fleischer *et al.* 1971) and μ -O-bridged Fe(III) dimers coordinated to EDTA and related chelators that are structurally similar to the active site in ribonucleotide reductase (Wilkins & Yelin 1969). The possibility of a similar disparity in the comparison of complex 1 and the OEC should be kept in mind, considering the restricted and rigidly structured nature of the OEC active site, which could inhibit exchange. This would make it even less probable that the substrates are bound to the OEC as μ -O ligands.

The mechanism of μ -O exchange has been studied for di- μ -O dimanganese complexes with and without terminal water-binding sites in order to determine how the rate of μ -O exchange is enhanced in Mn complexes that have terminal water ligands. It was found that the acidic terminal water ligand serves as the proton donor to the μ -O species in the first step of the exchange mechanism (Tagore *et al.* 2007). The absence of terminal water-binding sites on manganese inhibits μ -O exchange because, in this case, one of the ancillary ligands must dissociate to open up a site for a terminal water ligand to bind (Tagore *et al.* 2007). Dissociation of ancillary Mn ligands from protein residues seems unlikely in the OEC and, therefore, μ -O exchange would not readily occur in the OEC unless terminally bound waters are present. Thus, if the slow-exchanging substrate in the S_1 state of the OEC were to be bound as a μ -O ligand, the OEC would also need to contain terminal water ligands in order to account for the rapid substrate exchange rates.

On the basis of the mechanistic insight gained from studies of Mn model complexes, it can be concluded

that the fast-exchanging substrate in the OEC and the slow-exchanging substrate in the S_0 , S_2 and S_3 states are terminally bound water or hydroxide ligands. A μ -O-binding mode remains a possibility for the slow-exchanging substrate in the S_1 state, with the constraint that the exchange of such a μ -O substrate would need to be facilitated by an adjacent fully protonated water terminally bound to one of the Mn ions in the OEC. Overall, the recent studies of ligand exchange rates in Mn model complexes argue against mechanisms of O_2 evolution by the OEC in which the substrate waters are bound as μ -O bridges between Mn atoms.

4. ROLE OF CALCIUM IN THE OXYGEN-EVOLVING COMPLEX

One prediction of the model shown in figure 1 is that Ca^{2+} functions as a Lewis acid in the OEC. In order to test this idea, we have studied the binding of a series of cations to the Ca^{2+} site in PSII (Vrettos *et al.* 2001b).

Considering the large number of metal ions that will compete for the Ca^{2+} -binding site in PSII, it is surprising that only Ca^{2+} and Sr^{2+} support O_2 evolution. It would be expected that if the role of Ca^{2+} in PSII is purely structural, then metal ions of the same size and charge should be functional replacements. For example, Cd^{2+} (0.97 $\text{\AA}$) is almost the same size as Ca^{2+} (0.99 $\text{\AA}$), carries the same charge, is a closed-shell ion and binds to PSII with an affinity that is comparable to that of Ca^{2+} . Moreover, Cd^{2+} replaces Ca^{2+} in other proteins without large structural perturbations and even preserves hydrogen-bonding networks (McPhalen *et al.* 1991; Bouckaert *et al.* 2000). If the role of Ca^{2+} in the OEC is purely structural, then Cd^{2+} should function too. However, Cd^{2+} -substituted PSII is inactive. What is the distinguishing factor among the metal ions that determines the functional competence of Ca^{2+} and Sr^{2+} over other cations that can bind in the Ca^{2+} -binding site in PSII? Values of ionic radii and pK_a s of the aqua ions are tabulated in table 1; the ions discussed in this section (Ca^{2+} , Sr^{2+} and Cd^{2+}) are highlighted in bold. Owing to its larger size, Sr^{2+} is not a particularly good match to the size of the Ca^{2+} -binding site in PSII and does not bind to PSII as tightly as Ca^{2+} (Vrettos *et al.* 2001b). However, among the cations that can bind with reasonable affinity to the Ca^{2+} -binding site in PSII, only Sr^{2+} has a pK_a of its aqua ion close to that of Ca^{2+} . We postulate that only Sr^{2+} can functionally substitute for Ca^{2+} because it is the only cation that can bind with reasonable affinity and whose Lewis acidity is well matched to that of Ca^{2+} . Other metal ions fail to support O_2 evolution because either their ionic radius is too large (or too small) to allow good binding, or their Lewis acidity is out of the range required for activity, which makes the coordinated water too strong (or weak) a Brønsted acid. The result of a mismatch in the Lewis acidity is that the H-bonding network of the OEC is disturbed, and the coordinated water either deprotonates to form an unreactive metal cation-bound OH^- (too strong a Brønsted acid) or cannot be readily deprotonated in the O–O bond-forming reaction (too weak a Brønsted acid).

There are two possibilities for the protonation state of the Ca^{2+} -bound water, either OH^- or H_2O . When buried in the hydrophobic interior of a protein, the pK_a of water bound to a metal ion is usually lowered several units; nonetheless, the relative ordering of the Lewis acidity of the metal ions in table 1 will remain the same. Taking this into consideration, the Ca^{2+} aqua ion is still a weak Lewis acid ($\text{pK}_a = 12.8$) compared with the other metal ions in table 1, and so we expect that H_2O , and not OH^- , is the form of the Ca^{2+} -bound substrate molecule. Sr^{2+} is the only other catalytically competent metal ion because its pK_a is sufficiently high that it provides H_2O and not OH^- as a ligand. The conclusion that a Ca^{2+} - OH_2 species is present in the OEC, rather than a Ca^{2+} -hydroxide species, fits with the dependence of cation binding on ionic radius (Vrettos *et al.* 2001b). It was found that both the divalent and trivalent cations bind equivalently for a given ionic radius, which was interpreted to mean that they have the same charge. This can be explained if the trivalent metal ions, which are strong Lewis acids, bind as a metal ion–hydroxide species and that the divalent metal ions bind as a metal ion–water species, both of which have a +2 charge. This has also been proposed to account for structural effects on the tetramanganese cluster induced by substituting Dy^{3+} for Ca^{2+} in the OEC (Riggs-Gelasco *et al.* 1996).

If the role of Ca^{2+} as a Lewis acid is only required in the O–O bond-forming reaction that occurs during the $\text{S}_3 \rightarrow \text{S}_0$ transition, then it may be expected that other metal cations could functionally substitute for Ca^{2+} in the earlier S-state transitions. Indeed, in recent work (Lee & Brudvig 2007), it was found that the S_2 -state multiline EPR signal is formed in Dy^{3+} - and Cd^{2+} -inhibited PSII by 200 K illumination of dark-adapted (S_1 state) samples (Brudvig *et al.* 1983). This observation provides direct support for the proposal that Ca^{2+} plays a structural role in the early S-state transitions, which can be fulfilled by other cations of similar ionic radius, and that the functional role of Ca^{2+} to activate water in the O–O bond-forming reaction that occurs in the final step of the S-state cycle can only be fulfilled by Ca^{2+} and Sr^{2+} , which have similar Lewis acidities.

These results suggest that Ca^{2+} is not only a structural cofactor in the OEC, but also directly involved in the chemistry of water oxidation as a Lewis acid. They provide good support for the proposed functional role of Ca^{2+} as a Lewis acid to bind a substrate water molecule and tune its nucleophilic reactivity.

5. ELECTRON TRANSFER BETWEEN MANGANESE IONS IN THE OXYGEN-EVOLVING COMPLEX

Complex 1 is a water oxidation catalyst in aqueous solution when O-atom transfer reagents such as potassium hydroperoxymonosulphate (KHSO_5) or sodium hypochlorite (NaOCl) are used as the primary oxidant (figure 1b) and, thus, provides a functional model system for the oxygen-evolving complex of PSII (Limburg *et al.* 1999b, 2001). Based on steady-state kinetics and ^{18}O isotope labelling studies, the reaction was shown to

exhibit saturation (Michaelis–Menten-like) kinetics and to involve a high-valent Mn species that could exchange with solvent $^{18}\text{OH}_2$ (Limburg *et al.* 2001). A mechanism was proposed to explain these observations in which an $\text{Mn}^{\text{V}}=\text{O}$ intermediate reacts with water to form the O_2 product, as shown in figure 1b.

In order to investigate the mechanism of the water oxidation reaction and to characterize the intermediates, we have studied the reaction of complex 1 with HSO_5^- by using EPR, UV–visible and rapid-mix X-ray spectroscopy (Chen *et al.* 2007) and by stopped-flow UV–visible spectroscopy (Tagore *et al.* in press). Conversion of the mixed-valence $[\text{Mn}^{\text{III}}(\mu\text{-O})_2\text{Mn}^{\text{IV}}]^{3+}$ complex (1) to its one-electron oxidized product, $[\text{Mn}^{\text{IV}}(\mu\text{-O})_2\text{Mn}^{\text{IV}}]^{4+}$ (2) occurs within seconds and in high yield.

With excess HSO_5^- , the conversion of 1 into 2 is monophasic. The rate of the reaction is first order in [1] and nearly zero order in $[\text{HSO}_5^-]$. These observations are consistent with a reaction that involves the two-electron oxidation of 1 by HSO_5^- to form a $[\text{Mn}^{\text{IV}}(\mu\text{-O})_2\text{Mn}^{\text{V}}=\text{O}]^{3+}$ intermediate, followed by rapid reaction of the two-electron oxidized intermediate with another molecule of 1 to give two molecules of 2 (equations (5.1) and (5.2)).

However, in the stopped-flow UV–visible spectroscopic measurements, it was found that the reaction is distinctly biphasic for low concentrations of HSO_5^- and becomes monophasic at higher concentrations of HSO_5^- . The data are well fit by a model in which redox chemistry proceeds only when HSO_5^- is bound to Mn^{III} and that interconversion of HSO_5^- bound to the Mn^{IV} and Mn^{III} sites is slow. The kinetic distinctness of the Mn^{III} and Mn^{IV} sites allows estimates to be made on the upper limits for the rates of intramolecular electron transfer between Mn^{IV} and Mn^{III} sites of an oxo-manganese complex.

It has generally been assumed that the rate of electron transfer between Mn ions in the OEC is much faster than the chemical steps in the water oxidation reaction. However, the water oxidation site in the OEC is probably a manganese ion that is more oxidized than the neighbouring manganese sites. In order to prevent the dissipation of oxidizing power, electron transfer between the water-oxidizing manganese and its neighbours may be required to be slow on the time scale of the water oxidation step. The water oxidation site has been variously proposed to be an $\text{Mn}^{\text{IV}}=\text{O}$ adjacent to an Mn^{III} site in the S_4 state (Hillier & Wydrzynski 2001), an $\text{Mn}^{\text{IV}}-\text{O}\cdot$ adjacent to an Mn^{III} site in the S_3 state (Siegbahn & Crabtree 1999), and an $\text{Mn}^{\text{V}}=\text{O}$ moiety adjacent to Mn^{IV} sites in the S_4 state (Pecoraro *et al.* 1998; McEvoy & Brudvig 2004; Isobe *et al.* 2005; Sproviero *et al.* 2005). In the former cases, a high reorganization barrier is expected for electron transfer

between the water-oxidizing Mn^{IV} site and the Jahn–Teller distorted Mn^{III} site. In the latter case, an octahedral Mn^{V} water-oxidizing site would lead to a lower reorganization barrier due to a lower degree of Jahn–Teller distortion in Mn^{V} as compared with Mn^{III} , but electron exchange between a square pyramidal Mn^{V} (a probable geometry for an $\text{Mn}^{\text{V}}=\text{O}$ moiety; Collins & Gordon-Wylie 1989; Collins *et al.* 1990; MacDonnell *et al.* 1994) and an adjacent octahedral Mn^{IV} would again lead to high reorganization barriers. In all cases, the manganese with the oxo or oxyl radical bound to it would be expected to have a preference for the higher-oxidation state, leading to slow electron exchange. The effects of Jahn–Teller distortion and non-identical ligands on the exchanging partners are, therefore, expected to be operative for electron exchange in the OEC.

Intramolecular electron transfer between Mn^{III} and Mn^{IV} is required to be slow in order to account for biphasic kinetics of the reaction of **1** with HSO_5^- . The maximum electron-transfer rate constant for the interconversion of HSO_5^- bound to the Mn^{IV} and Mn^{III} sites on **1** is 5 s^{-1} ($t_{1/2} \geq 140\text{ ms}$; Tagore *et al.* *in press*). This can be compared with the measured lifetimes of approximately 200 μs and approximately 1 ms for the S_3 and S_4 states, respectively, of the OEC (Haumann *et al.* 2005). Rates for intramolecular electron transfer in oxo-bridged manganese complexes are not available, but an upper limit of $10^6\text{--}10^8\text{ s}^{-1}$ has been proposed for the mixed-valent $[\text{Mn}_2^{\text{III/IV}}(\text{O})_2(\text{bpy})_4]^{3+}$ complex ($\text{bpy}=2,2'$ -bipyridine) on the basis of its EPR and near-IR absorption spectra (Cooper & Calvin 1977; Cooper *et al.* 1978). Barriers ranging from approximately 13 to approximately 19 kcal mol^{-1} have been calculated by density functional theory for the same process for $\text{Mn}^{\text{III}}(\mu\text{-O})_2\text{Mn}^{\text{IV}}$ complexes with water, hydroxo and formate ligands (Lundberg & Siegbahn 2005). Assuming a pre-exponential factor of $k_{\text{B}}T/h$, these barriers translate to rates of $2400\text{--}0.1\text{ s}^{-1}$. From these observations, it is apparent that slow electron transfer between Mn ions in the OEC could lead to the existence of a localized water oxidation site on a specific manganese in the OEC and this may be important for the mechanism of water oxidation catalysis.

6. SUMMARY AND CONCLUSIONS

As discussed in this article, studies of inorganic Mn model complexes provide important information on the structure and properties of high-valent oxo-manganese complexes, the rates of ligand exchange and the rates of intramolecular electron transfer. This information helps to define the reactions of the OEC leading to water oxidation. There remain many unanswered questions about the structure and function of the Mn_4Ca cluster in the OEC. However, a body of evidence provides strong support for binding of the substrate water molecules as terminal ligands to manganese and/or calcium and for a direct role of Ca^{2+} in the water-oxidation chemistry as a Lewis acid to activate a substrate water molecule as a nucleophile. Mn model chemistry also supports the possibility that water is activated for O–O bond formation in the OEC by binding to a high-valent manganese ion that is more

oxidized than its neighbouring manganese sites and that this high-valent Mn does not dissipate its oxidizing power by electron transfer to its neighbouring Mn ions because the inter-Mn electron-transfer reactions are slow on the time scale of the water-oxidation step. Overall, these results are consistent with a mechanism for photosynthetic water oxidation that involves nucleophilic attack of a calcium-bound water ligand on the electrophilic oxygen atom of an $\text{Mn}^{\text{V}}=\text{O}$ intermediate in the O–O bond-forming step, as shown in figure 1.

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Discussion

V. Pecoraro (*University of Michigan, USA*). Firstly, I would like to comment that the rate of oxo exchange for di- μ -oxo- $Mn^{III,IV}$ complexes is facilitated by disproportionation. This is unavailable in a protein; therefore, oxo transfer will be even slower. My question is, under what condition were pK_a values determined?

G. Brudvig. All of the pK_a values I discussed in my talk were determined by pH-dependent measurements in aqueous solutions.

H. Dau (*Freie University, Berlin, Germany*). You suggested that in an Mn^{III} – Mn^{IV} complex the inner-molecule electron transfer may be slow. Why is it so slow? Is the rate limited by ligand movements (or proton movements)?

G. Brudvig. The reaction involves slow conversion of the unreactive H_2O – $Mn^{III}(O)_2Mn^{IV}$ – SO_5H -terpy complex into the reactive HSO_5 – $Mn^{III}(O)_2Mn^{IV}$ – OH_2 complex. Although this reaction could occur by electron transfer from Mn^{III} to Mn^{IV} , the electron-transfer reaction will be significantly uphill owing to the asymmetric ligation. I expect that the reaction is limited by ligand movements.

A. Aukauloo (*University of Paris-Sud, France*). Comparing the mechanisms of formation of the oxygen–oxygen bond, in the natural system you have an Mn-oxyl (terminal oxo) radical that gets attacked by an activated water molecule having some spin density through hydrogen bonding to a μ -oxo fragment. In the synthetic model, you have an Mn(V) oxo low spin or an Mn-oxyl (terminal oxo) type fragment. If in the first case, an activated water molecule comes and attacks the electrophilic oxo fragment, then how do you get triplet dioxygen out? In the second case, the activated water molecule must be a more radical type to do the O–O bond. Can you comment on this?

G. Brudvig. We still do not have any direct information on the electronic state of the Mn-oxo species involved in the O–O bond-forming step for either the OEC or synthetic models. Whether or not it is an Mn-oxyl radical species remains to be determined. Perhaps DFT calculations will clarify this question once a complete atomic-resolution structure of the OEC has been determined. As for formation of triplet oxygen, this is an important question, but I expect that intersystem crossing will be very fast for oxygen bound to paramagnetic manganese complexes.

D. Nocera (*MIT, Boston, USA*). Do you have an estimate for the reorganization energy of $Mn^{III} \rightarrow Mn^{IV}$ transfers versus electron exchange for a possible $Mn^{IV} \rightarrow Mn^V$ transfer? How does this play into your ideas of electron localization within the OEC cluster, as it pertains to your model?

G. Brudvig. This will depend greatly on the coordination environments of the Mn ions. For asymmetric ligation,

as is probably to be the case in the OEC, the reactions probably will be limited by ligand movements.

P. Siegbalm (*Stockholm University, Sweden*). I have tested your mechanism with an attack on a terminal Mn=O by a water bound to calcium (Siegbahn 2006). Among the ones I tried, this mechanism has one of the highest barriers (32 kcal mol⁻¹). Actually, an attack by a second-sphere water has a much lower barrier (approx. 20 kcal mol⁻¹). However, by far the lowest barrier is found for an attack by an oxyl radical bound to the dangling manganese on an oxo ligand in the cube. The barrier for this mechanism is as low as 5 kcal mol⁻¹ for the latest Berlin X-ray structure.

J. Messinger (*Muelheim, Germany*). Your mass spec data on water exchange in model systems are done in acetonitrile with only a few per cent of water. Can you give us an estimate what the actual water exchange rates would be in pure water?

G. Brudvig. There is a first-order dependence of the rate of μ -oxo exchange on the water concentration in acetonitrile solution for concentrations of water up to 0.5 M. We have not measured the rate of exchange in pure water because a rapid mixing experiment cannot be done in this case. However, I do not think an extrapolation of the μ -oxo exchange rates for Mn complexes measured with low water concentrations in acetonitrile solution to pure water will give a valid comparison to the substrate exchange data for PSII because it is very likely that the substrate exchange rates for the protein become saturated with fairly low concentrations of water.

Additional reference

Siegbahn, P. E. M. 2006 O–O bond formation in the S4 state of the oxygen-evolving complex in photosystem II. *Chem. Eur. J.* 12, 9217–9227.