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Evidence of O-O Bond Formation in the Final Metastable S_3 State of Nature's Water Oxidising Complex Implies a Novel Mechanism of Water Oxidation

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ABSTRACT: A novel mechanism for the final stages of Nature's photosynthetic water oxidation to molecular oxygen is proposed. This is based on a comparison of experimental and broken symmetry density functional theory (BS-DFT) calculated geometries and magnetic resonance properties of water oxidising complex models in the final metastable oxidation state, S_3 . We show that peroxo models of the S_3 state are in vastly superior agreement with the current experimental structural determinations compared with oxo-hydroxo models. Comparison of experimental and BS-DFT calculated ^{55}Mn hyperfine couplings for the EPR visible form shows better agreement for the oxo-hydroxo model. An equilibrium between oxo-hydroxo and peroxo models is proposed for the S_3 state and the major implications for the final steps in the water oxidation mechanism are analysed and discussed.

TOC Graphic

Nature's invention of a molecular device to dissociate water into molecular oxygen and metabolically bound hydrogen by using visible light as the source of Gibbs energy was the key stage in the development of all oxygenic photosynthetic organisms in the early biosphere.¹ The catalytically active water oxidising complex (WOC) located in Photosystem II, is a $\text{Mn}_4\text{O}_5\text{CaW}_4$ cluster shown in Figure 1. To produce molecular oxygen from two water molecules, Kok proposed that the WOC active site undergoes four one-electron oxidation steps, giving rise to five distinct progressively oxidizing storage states designated S_n , where $n = 0-4$, represents the number of one-electron oxidations stored, before releasing O_2 during the rapid decay of $S_4 \rightarrow S_0$.^{2,3} The final metastable state that can be detected and characterised experimentally is the S_3 state. The exact structural and electronic characterisation of this state is crucial to a full understanding of the water oxidation reaction. This goal was greatly enhanced by the recent report of an atomic-level X-ray crystal structure of the WOC using X-ray free electron lasers (XFEL), after two light flashes (2F) and poised predominantly in the S_3 state.^{4,5} Previously high resolution electron paramagnetic resonance (EPR) spectroscopy of an EPR active, $S=3$ state population, had been used to propose that all four Mn ions are present in the IV oxidation state and octahedrally coordinated.⁶ This was facilitated by the addition of an extra hydroxo ligand O6 to the Mn_1 ion, on S_3 formation, leading to an oxo (O5) - hydroxo (O6) interaction apparently poised for O-O bond formation in the S_4 state.⁷ While the 2F X-ray crystal structure revealed by Suga et al⁴ does indicate an additional oxygen atom (O6) coordinating to the Mn_1 ion, the O5-O6, Mn_1 -O6 and Mn_4 -

O5 bond lengths were recently shown to be best modelled by a bridging peroxo with Mn₁ and Mn₄ present as Mn (III) ions.⁸ This additional O6 was not resolved in the 2F crystal structure presented earlier by Young *et al*⁵ most likely due to the poorer resolution in that determination as explained previously by Suga *et al*⁴, Kato *et al*⁹ and Wang *et al.*¹⁰ In this communication we use broken symmetry density functional theory (BS-DFT) calculations on large models of the WOC to further investigate these possibilities. We calculate the structural and magnetic properties for both oxo-hydroxo and peroxo models generated directly from the S₃ state crystal structure coordinates and compare these with the experimental determinations. We show that the peroxo models are in vastly superior agreement with the experimental structural determinations compared with oxo-hydroxo models. Comparison of experimental and BS-DFT calculated ⁵⁵Mn hyperfine couplings (hfc) shows somewhat better agreement for the oxo-hydroxo model. Both the likelihood of the S₃ state corresponding to an equilibrium between oxo-hydroxo and peroxo forms and novel implications for the final steps in water oxidation are discussed.

Figure 1: Double flashed (2F) structure of the WOC core (PDB file 5WS6), with numbering scheme used throughout this report for the cluster models. The colour code used is: Mn (pink), calcium (white) and oxygen (red).

As starting coordinates, large models of the Suga *et al.*⁴ crystal structure determination (PDB: 5WS6 Chain A) were optimised either as oxo-hydroxo or peroxo forms and all models located are shown in Figure 2. For oxo-hydroxo models only O6 protonated forms

were found to represent stationary points on the potential energy surface and these differed by the presence (**oxo-hydroxo-A**) or absence (**oxo-hydroxo-B**) of hydrogen bond donation to O5. In both oxo-hydroxo models, W2 is represented as an hydroxo ligand, as strong evidence has been presented for this protonation state in the prior S_2 state.^{11,12} Three peroxo models were examined depending on the protonation and hydrogen bond interaction of W2. In **peroxo-A**, W2 is a fully protonated water with hydrogen bond donation to O5 and O6 while in **peroxo-B**, fully protonated W2 does not hydrogen bond to either O5 or O6. As stated both have fully protonated W2 in their optimised forms as starting initially with either O5 or O6 protonated gave rise on optimisation to transfer of the proton to the W2 hydroxo, generating **peroxo-A**. In addition, for comparison, we used another model **peroxo-C** in which W2 is an hydroxo ligand and O5 and O6 are both deprotonated.

Figure 2: Peroxo and oxo-hydroxo models geometry optimised from X-ray crystal structure coordinates of PDB:5WS6 (Chain A) with relevant optimised bond lengths shown for comparison. The colour code used for models is: Mn(IV) [pink], Mn(III) [green], Mn(unassigned oxidation state) [silver], calcium [white], hydrogen [blue] and oxygen [red]. Oxygen bond forming O5 and O6 highlighted in red.

The structure and some key optimised interatomic distances calculated for all models are shown in Figure 2 and compared with the X-ray determined structure. Overlays of the crystal structure coordinates and the optimised models are also shown in Figure S3 along with a more extensive comparison, Table S2, of interatomic distances. It is clear that all peroxo models are in vastly superior agreement with the experimentally determined structure compared with the two oxo-hydroxo models and indeed it can be stated categorically that only a peroxo model is compatible with the current X-Ray structure determination of Suga et

al.⁴ Comparison with extended X-ray absorption fine structure (EXAFS) data in Table S2, shows similar agreement for both types of model. For the peroxo models, Mulliken spin population analysis, Table S4 shows that Mn₁ and Mn₄ are in oxidation state III (spin populations near 4.0 respectively) while Mn₃ and Mn₂ are present in the IV oxidation state (spin populations near 3.0). This explains the relatively long Mn₁-O6 and Mn₄-O5 bond lengths which lie along the Jahn-Teller axes of these ions. The Mn₃-O5 bond length is somewhat shorter for all peroxo models due to the absence of Jahn-Teller distortion and again agrees with the X-ray structure determination. It would appear that Mn₃ is the ion which anchors the peroxo in the WOC structure. The peroxo ligand is therefore bound asymmetrically in the complex with its O5 terminus bonded to both Mn₄ and Mn₃ and its O6 terminus bonded to Mn₁. O5 and O6 to a lesser extent are additionally ligated to Ca. **Peroxo-A**, where W2 acts as a donor to both O5 and O6, has a noticeably narrower W4Mn₄O5 bond angle of 77.6° compared with 89.9° for **peroxo-B**. **Peroxo-A** may represent an intermediate between oxo-hydroxo and **peroxo-B** where the O6 proton is transferred from O6 to the W2 hydroxo in the process of O-O bond formation between O5 and O6, see below.

EPR studies have identified two populations of S₃. The first is an EPR detectable form identified as arising predominantly from an S=3 ground state.^{13,14} The second has been shown, on near infrared (NIR) illumination at cryogenic temperatures, to give rise to a distinct EPR resonance, a so-called split signal assumed to arise due to induced oxidation of Y_Z by the photoactivated WOC.^{15,16} The first S=3 population has been further studied by Eldor Detected Nuclear Magnetic Resonance (EDNMR) which measured the ⁵⁵Mn hfcs for the four Mn ions present. These have been interpreted as indicating that all Mn ions are present in the IV oxidation, octahedrally coordinated and composed of a pair of two large and two small magnitude hfcs.⁶

It has been demonstrated that exchange coupling constants J_{ij} , the ground spin state and the projected ^{55}Mn hfcs can be reliably calculated by using BS-DFT calculated exchange couplings constants in the diagonalization of the isotropic Heisenberg Van Vleck Hamiltonian.^{7,17} The results of such an analysis is given in Table 1 which shows the calculated ground state spin and the projected isotropic and anisotropic ^{55}Mn hfcs for all models studied. The calculated values for the oxo-hydroxo models are similar to those calculated previously for “open-cubane” models generated from the dark adapted crystal structure.^{6,8,7} As all ions are Mn(IV), low anisotropy is exhibited throughout and grouping of the couplings into a high magnitude and low magnitude pairs, as found experimentally, is apparent especially for **oxo-hydroxo-B**. We note however that the magnitudes of the smaller pair are significantly larger than the experimentally reported values. These smaller hfcs are probably the least accurately determined in the experimental report and have also been found to be highly sensitive to small variations in the J_{34} exchange coupling value.^{7,8} **Peroxo-A**, and **peroxo-B** have calculated hfcs which are similar in magnitude for all four Mn ions in disagreement with the experimental determination. **Peroxo-C** does display calculated values which fall into two sets of large and small magnitude values but a ground state of $S_{\text{GS}}=4$ is calculated for this model using the exchange coupling analysis. In general it would appear that the oxo-hydroxo models better reproduce some of the experimentally determined values while the peroxo models show poorer agreement. So, based on the comparison with the structure determination of Suga et al.⁴, the S_3 state is best described as a peroxo model. On the other hand, comparison of calculated and experimental ^{55}Mn hfcs would, with certain caveats, favour an oxo-hydroxo model for the $S=3$ species detected by EPR. As mentioned in the introduction, another separate population, 30-40%, of S_3 exists^{15,16} which is only EPR detectable on NIR illumination at cryogenic temperatures. This has been found to be in

Table 1: BS-DFT calculated ground spin state and projected ^{55}Mn isotropic (A_{iso}) and T_i hfcs constants (in MHz) for all models studied compared to experimental determinations.

Model		A_{iso}	T_1	T_2	T_3
Peroxo-A $S_{\text{GS}}=3$	Mn_4	-130	-26	-8	34
	Mn_3	-72	-3	1	3
	Mn_2	-68	-3	1	3
	Mn_1	83	-24	10	14
Peroxo-B $S_{\text{GS}}=4$	Mn_4	-128	-28	-6	34
	Mn_3	-109	-10	-1	12
	Mn_2	-58	2	-1	-1
	Mn_1	85	14	8	-22
Peroxo-C $S_{\text{GS}}=4$	Mn_4	-103	-24	1	23
	Mn_3	-83	-7	-1	7
	Mn_2	22	-1	0	1
	Mn_1	-45	-7	-5	12
Oxo-hydroxo-A $S_{\text{GS}}=3$	Mn_4	40	-1	0	1
	Mn_3	-39	-3	0	3
	Mn_2	-76	-5	1	4
	Mn_1	-84	-5	-1	6
Oxo-hydroxo-B $S_{\text{GS}}=3$	Mn_4	18	-1	0	1
	Mn_3	-19	-1	0	1
	Mn_2	-80	-5	1	4
	Mn_1	-83	-5	1	4
Exp⁶ $S_{\text{GS}}=3$	A_1	-99.0	-1.2	-1.2	2.4
	A_2	-95.6	-3.8	1.8	1.8
	A_3	-25.9 or + 7.0	—	—	—
	A_4	0.5 (≤ 5)	—	—	—

equilibrium with the S=3 form¹⁵. Importantly this is distinct from the S=3 form¹⁵ and not its precursor as has been suggested by Retegan et al.¹⁸

It is generally believed that photoactivation of the WOC by NIR requires the presence of Mn (III)^{19,20} so a potential candidate for this is the peroxo form. The combination of integer spin (S=3 or 4) and large zero field splitting magnitude (due to Mn(III)) for this component would also explain its lack of detection by EPR.²¹ NIR activation with expected reduction would lead to a half-integer spin (Kramers doublet) system more readily detected by EPR.²¹ Supported by the presence of two distinct populations for S₃ measured by EPR, we propose that in S₃ an equilibrium exists between oxo-hydroxo and peroxo with the experimental conditions detecting/favouring one

Scheme 1. Schematic representation of oxo-hydroxo/peroxo equilibrium described in text, highlighting the electron movements involved.

form over the other, Scheme 1 and Figure 3. As demonstrated in Scheme 1 this equilibrium can be envisioned as a redox coupling mechanism involving concerted one-electron movements. This is similar to the equilibrium known for peroxo/oxo conversion in high-capacity layered-oxide electrodes²² and indeed has been previously speculated as arising in

the S_3 state by Renger.^{23,1} based on kinetic analysis of the S state transitions. Isobe et al²⁴ have also suggested that S_3 exists as an equilibrium of structures including oxo-hydroxo, peroxo, superoxo and oxyl radical species based on broken symmetry density functional states calculated using models based on the S_1 crystal structure coordinates. Pushkar et al²⁵ have recently combined crystallographic, spectroscopic and DFT results to illustrate the possibility of low barrier O-O bond formation prior to S_4 . For the equilibrium model proposed here the relative populations are likely to be dependent on sample preparation and temperature. Based on the 1.5 Å bond length observed in the crystal structure reported by Suga et al⁴, the predominant species present in that study would appear to be the peroxo form.⁸ For a more mixed state an intermediate O5-O6 bond length between oxo-hydroxo and peroxo might be expected. In this respect the accuracy limitations of the current crystal structures must be borne in mind and further more highly resolved crystal structure determinations are desirable.²⁶ As has been previously found by Isobe et al²⁴ energetic comparisons between the different oxo-hydroxo and peroxo models are notoriously dependent on the percentage of Hartree-Fock (HF) exchange used with low HF exchange levels favouring the oxo-hydroxo form and high HF exchange levels favouring the peroxo forms. Our own energetic comparisons are given in Table S5 which clearly demonstrates this effect as well. A calculation beyond density functional theory methods may clarify this but are for the foreseeable future infeasible for models of the size used here. A similar equilibrium between oxo and bridged peroxo has been shown to exist for bridged copper oxygen complexes.^{27,28} The experimentally verified copper oxo and peroxo equilibrium importantly demonstrates the feasibility of O-O bond formation arising solely through such an equilibrium without the need for generation of Mn(V) or oxyl radicals. In this respect we find that starting with a protonated O5 or O6 leads to transfer of this proton to the W2 OH group on optimisation of the peroxo model. **Peroxo-A** could be interpreted as an intermediate in

this proton transfer process which accompanies oxo-hydroxo conversion to peroxo, Scheme 1. X-ray absorption and emission spectroscopy have been interpreted to show a significantly smaller average Mn oxidation change on S_2 to S_3 advancement^{29,30} compared with the previous S-state transitions. This has been attributed to absence of Mn oxidation in the S_2 to S_3 transition.^{31,32} As discussed in Yano and Yachandra³⁰ interpretations of such changes in terms of formal oxidation changes on the Mn ions may be too simplified for a covalent complex like the WOC. By contrast, the changes occurring on the S_2 to S_3 transition have been attributed to Mn oxidation accompanied by a coordination change.^{33,34,35} The smaller average oxidation change between S_2 and S_3 is also fully compatible with the equilibrium model proposed here assuming the oxo-hydroxo form is in excess. $^{18}\text{O}/^{16}\text{O}$ exchange rates for the different S-states which show both oxygens exchanging in the S_3 state have been interpreted as indicating that O-O bond formation can be ruled out in this state.³⁶ However, the actual mechanism via which oxygens exchange in the WOC is unknown and may even involve multiple S-states or be diffusion limited. The equilibrium suggested here could contribute to the fast exchange rate observed.

The mechanism of O-O bond formation proposed here arising from an equilibrium established in the S_3 state, is quite distinct from the current two leading proposals in the literature i.e. nucleophilic attack^{37,38} and oxo-oxyl radical coupling^{39,40} which are proposed to both occur in the S_4 state. The formation of the O-O bond in the S_3 state is quite appealing from a mechanistic and kinetics point of view as it establishes O-O bond formation before the final electron transfer out of the WOC to oxidised Y_Z . Babcock *et al* have shown that there is virtual kinetic coincidence between the rate of O_2 evolution and Y_Z^{OX} reduction in the final $\text{S}_3\text{Y}_Z^{\text{OX}}$ to S_4 step ($t_{1/2} \sim 1\text{ms}$).⁴¹ This instantaneous formation of oxygen on Y_Z reduction is difficult to rationalise if the O-O bond formation reaction is still required to take place. In addition oxidation of the oxo-hydroxo form would require oxidation of Mn(IV) to Mn(V) or

Mn(IV) oxyl. The electrode potential for this couple in acidic aqueous solution ranges from +2.7 to +4.7 V⁴² which is well above the electrode potential of Y_Z^{ox}/Y_Z of +1.1 V.³⁵ A drastic site-induced reduction in the Mn(V)/Mn(IV) electrode potential would be required to facilitate Mn(IV) oxidation by Y_Z^{ox} . This conclusion is supported by a recent x-ray emission spectroscopy study by Jensen *et al* who found no evidence of Mn(IV) oxidation in this final step.⁴³ By contrast if peroxo formation has already occurred, this final electron transfer to Y_Z^{ox} is likely to result in exclusive oxidation of peroxo to superoxo, $[E^0(O_2^{-1}/H_2O_2) = +890 \text{ mV}]^{42,44}$ shifting the S_3 equilibrium towards the peroxo. The resultant superoxo would be expected to be rapidly oxidised to triplet oxygen $[E^0(O_2/O_2^{-1}) = -180 \text{ mV}]^{44}$ by $Mn_3(IV)$, see Figure 3. This would explain the instantaneous appearance of oxygen on Y_Z reduction leaving the active site vacant for occupation of another water molecule (new O5) to restart the cycle.

Figure 3: Proposed final intermediates in water oxidation cycle where oxidation only of the peroxo form of S_3 leads to superoxo formation in S_4 with eventual release of O_2 . Colouring as in Figure 2. The S_4 superoxo shown is generated by one-electron oxidation and deprotonation of the S_3 peroxo form followed by geometry optimisation. Formation of triplet oxygen leads to movement of the formed $O_6=O_5$ oxygen molecule from the active site. Arrows indicate Jahn-Teller axes for Mn(III).

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Supporting Information Available: Details of calculations performed and models used, calculated exchange couplings for all models, additional data tables plus comparison of model geometries with additional structural studies. XYZ coordinates of models used.

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