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A Comparison of Experimental and Broken Symmetry Density Functional Theory (BS-DFT) Calculated Electron Paramagnetic Resonance (EPR) Parameters for Intermediates Involved in the S_2 to S_3 State Transition of Nature's Oxygen Evolving Complex

Nathan J. Beal, Thomas A. Corry and Patrick J. O'Malley*

School of Chemistry, The University of Manchester, Manchester M13 9PL, UK.

Abstract

A broken symmetry density functional theory (BS-DFT) magnetic analysis of the S_2 , $S_2Y_Z^\bullet$ and S_3 states of Nature's oxygen evolving complex is performed for both the native Ca and Sr substituted forms. Good agreement with experiment is observed between the tyrosyl calculated g-tensor and ^1H hyperfine couplings for the native Ca form. Changes in the hydrogen bonding environment of the tyrosyl radical in $S_2Y_Z^\bullet$ caused by Sr substitution leads to notable changes in the calculated g-tensor of the tyrosyl radical. Comparison of calculated and experimental ^{55}Mn hyperfine couplings for the S_3 state presently favours an open cubane form of the complex with an additional OH ligand coordinating to Mn_D . In Ca models this additional ligation can arise by closed-cubane form deprotonation of the Ca ligand W3 in the $S_2Y_Z^\bullet$ state accompanied by spontaneous movement to the vacant Mn coordination site or by addition of an external OH group. For the Sr form, no spontaneous movement of W3 to the vacant Mn coordination site is observed in contrast to the native Ca form; a difference which may lead to the reduced catalytic activity of the Sr substituted form. BS-DFT studies on peroxo models of S_3 as indicated by a recent X-ray free electron

laser (XFEL) crystallography study give rise to a structural model compatible with experimental data and an $S=3$ ground state compatible with EPR studies.

Introduction

During oxygenic photosynthesis, for each visible light excitation of Photosystem II, the primary donor chlorophyll complex, P_{680} , forms a strongly oxidising radical cation ($P_{680}^{\bullet+}$).¹ This radical cation oxidises a nearby tyrosine residue (Y_Z , D1-Tyr-161) to form a tyrosyl radical ($Y_Z^\bullet$) which under physiological conditions can subsequently one-electron oxidise the oxygen evolving complex (OEC) and advance this Mn_4CaO_5 cluster to the next S_n state of the Kok cycle, Figure 1.^{2,3} Cryogenic temperatures (typically below 20 K) significantly perturb this single electron transfer between Y_Z and the OEC and may subsequently block the Kok cycle advancement from $S_n Y_Z^\bullet$ to $S_{n+1} Y_Z$.⁴⁻⁹ In a similar fashion these trapped intermediates can be obtained with various chemical modifications of the sample such as Ca depletion, acetate treatment and high pH.^{7,8} When this blockage occurs on the second photon absorption a characteristic $S_2 Y_Z^\bullet$ EPR split signal arises from the interaction of the $S = 1/2$ Y_Z radical with the $S = 1/2$ spin state S_2 . Another closed cubane form, $S = 5/2$ of the OEC has been associated with near infra-red induced $S_2 Y_Z^\bullet$ split signals.^{5,10} EPR studies of the split signal states have shown that the tyrosyl radical and the OEC weakly interact and that each $S_n Y_Z^\bullet$ state has a characteristic lineshape in the EPR spectra.^{9,11} These metallo-radical split signal states offer insights into the concerted changes that occur during S_n state transitions.¹² This is particularly important for the crucial S_2 to S_3 step where the catalyst is prepared for the O-O bond formation reaction. This transition is believed to involve a number of intermediate stages involving water binding and deprotonation.^{13,14}

Figure 1 The S-state cycle of the OEC illustrating the sequence of electron and proton releases required to oxidise two water molecules to molecular oxygen and four protons.

While there have been extensive efforts to provide a robust understanding of the S_2 state, the S_3 state is one of the least well understood. Recent attempts to rectify this situation have led to the investigation of the S_3 state using high resolution EPR techniques, which was interpreted as evidence for a $(\text{Mn}^{\text{IV}})_4$ oxidation state accompanied by hydroxide binding to Mn_D .¹⁵ In addition X-ray free electron laser (XFEL) studies have been used to directly study the atom-level structure of the S_3 state. One study supports the addition of an extra oxygen atom ligand to Mn_D in S_3 ,¹⁶ while the other study did not find any evidence for this.¹⁷ A recent assessment of these structures indicates that further refinements may be required before a definitive S_3 state atomic-level structure can be assigned.¹⁸ The origin of the second substrate water which may bind during the S_2 to S_3 transition is uncertain, with recent FTIR studies proposing a Ca bound water (W3 or W4).¹⁹ However debate still

exists in the literature regarding the identity of this substrate water and there are a number of alternative mechanisms based on so-called carousel and pivot mechanisms.^{20,21} In this report the transition from the S_2 state to the S_3 state is examined using BS-DFT calculations for both Ca and Sr cluster model forms of the OEC. The $S_2Y_Z^\bullet$ intermediate is initially investigated and the EPR parameters of the trapped tyrosyl radical calculated and compared with experimental determinations and other computational reports. The subsequent formation of the S_3 state is then investigated and its calculated EPR parameters compared with experimental determinations. A peroxo model based on the XFEL structure by Suga *et al*¹⁶ for S_3 is also investigated. Sr substitution is shown to introduce small but significant changes in hydrogen bonding at the $S_2Y_Z^\bullet$ state leading to changes in the tyrosyl radical EPR properties. Sr substitution is also shown to inhibit the movement of a proposed second water substrate (W3) in the S_2 to S_3 transition providing a possible explanation for the reduced catalytic activity of the Sr substituted catalyst.

Computational Details

Calculations were performed using ORCA 3.0.3 using procedures described previously.²² The geometries of all the models studied in this chapter were optimised in their respective high spin states using the BP86 functional,^{23,24} utilising the zeroth-order regular approximation (ZORA) Hamiltonian to include scalar relativistic effects.^{25–27} ZORA adapted segmented all-electron relativistically contracted (SARC) basis sets were employed for all atoms,²⁸ ZORA versions of the def2-SVP basis sets were used for C and H atoms with ZORA versions of the def2-TZVP basis set used for all other atoms with f functions removed.²⁹ The computational time of the calculations was decreased by invoking the resolution of identity approximation (RI) along with decontracted auxiliary def2-TZVP/J coulomb fitting basis sets.^{30–32} The optimizations also included the third

generation (D3) semi-empirical van der waals corrections proposed by Grimme.^{33,34} Increased integration grids (grid 4 and grid x4 in orca convention) and tight SCF convergence criteria were used throughout the calculations. The Heisenberg exchange coupling constants, hyperfine and nuclear quadrupole coupling values were calculated for all atoms of interest using the broken symmetry DFT methodology using the hybrid meta-GGA TPSSh functional with the chain of spheres (RIJCOSX) approximation to exact exchange using the same decontracted auxiliary basis sets that were used in the geometry optimization steps.^{35,36} Initial broken symmetry guesses were constructed using the ‘flipspin’ feature of ORCA.³⁷ Calculation of the hyperfine and quadrupole tensors used basis sets developed by Neese *et al.* based on the SARC def2-TZVP for the Mn, N and O atoms which contain fully decontracted s-shells with three additional steep primitives added to the core, in addition to this def2-TZVP(-f) was used for all other atoms.^{32,38} The integration grids were increased to an integration accuracy of 11 and 9 for Mn, N and O respectively. Picture change effects were applied for the calculation of hyperfine and nuclear quadrupole tensors. Heisenberg exchange coupling constants and spin projected hyperfine couplings were calculated using the methodology proposed by Pantazis *et al.*³⁹ The calculated ⁵⁵Mn isotropic hyperfine couplings were scaled with an empirically derived factor of 1.47 to account for well known deficiencies in the DFT description of the Fermi contact term.^{40,41} This factor has been validated for 6 models of mononuclear, dinuclear and tetranuclear manganese complexes (see Table S1 in Supporting Information; structures are shown in Figure S2). The BS-DFT approach and the use of the TPSSh functional in the calculations of Heisenberg exchange coupling constants in manganese systems has been discussed and compared to the performance of other functionals in earlier studies of manganese dimers, trimers and tetrameric systems.^{38–40,42,43} For the calculation of the **g**-tensor of the S₂Y_Z[•] tyrosyl radical, the approach used by Retegan *et al.* was followed

where the open shell manganese ions were replaced with diamagnetic ions of the same formal charge (Ga^{3+} and Ge^{4+}).⁴⁴ Convergence to the correct BS and HS states in all calculations was confirmed by examination of the calculated Mulliken spin populations.

Model Systems

Most of the starting models for the calculations (Figure S1) were first geometry optimised in the S_2 and $S_2Y_Z^\bullet$ states. The terminal carbon atom on each chain was constrained at the starting coordinates. The starting models were generated from the crystal structure determinations, PDB ID: 4UB6 (Ca), PDB ID: 4IL6 (Sr). The exception to this was the S_3 -peroxo model where the starting coordinates prior to geometry optimisation were taken from the S_3 XFEL coordinates, 5WS6. The high valent oxidation state scheme (Mn^{III}) (Mn^{IV})₃ was adopted for all S_2 models. All the OEC cluster models studied contain the seven directly coordinated amino acid residues (all found in the D1 protein chain unless otherwise indicated): Asp-170, Glu-189, His-332, Glu-333, Asp-342, Ala-344 and CP43-Glu-354. Two fully protonated water molecules W3 and W4 coordinated to the calcium/strontium ion were also included as well as a water molecule W1 and hydroxide group W2 coordinated to Mn_A . As well as this the models include the important second sphere residues: Asp-61, Tyr-161, Gln-165, His-190, Asn-298, His-337 and CP43-Arg-357. In addition to this twelve closely associated crystallographic water molecules as well as partial backbone of Glu-329 was involved, having previously been found to hydrogen bond with His-332. Inclusion of Tyr-161(Y_Z) and its hydrogen bonding partner His-190 is essential for all S-state models. All bridging oxygens were in the oxo form and the terminal W2 ligand on Mn_A was an hydroxo ligand with W1, W3 and W4 being aquo ligands. An additional label is used to distinguish between the open cubane (**O**) and closed

cubane (**C**) forms of the OEC. The numbering scheme for the tyrosyl radicals investigated in the $S_2Y_Z^\bullet$ state is shown below in Figure 2.

Figure 2. Numbering scheme for the atoms of interest in the $Y_Z^\bullet$ tyrosyl radical.

The numbering scheme for the OEC is shown in Figure 3. When discussing some S_3 models a third label is used to distinguish the identity of the extra water, W_x . This labelling scheme will be discussed further in the relevant results section.

Figure 3. Numbering scheme for the constructed cluster models of the OEC. Colour coding: manganese (purple), oxygen (red), nitrogen (blue), carbon (grey). Hydrogen atoms omitted for clarity

Results and Discussion

Results will first be presented for the S_2 and $S_2Y_Z^\bullet$ models arising from open cubane and closed cubane Ca and Sr models. Discussion will first focus on the structural changes that occur in the cluster models upon oxidation of the tyrosine residue. In addition the Heisenberg exchange coupling constants for these models will also be discussed before turning to the calculated EPR parameters. Following on from this discussion will then move to the S_3 state beginning firstly with a presentation of the structures before considering the exchange coupling constants and ^{55}Mn hyperfine couplings calculated for all the models studied.

The S_2 and $S_2Y_Z^\bullet$ States

Geometry

Analysis of the Mulliken spin populations (values close to 4.0 and 3.0 for Mn(III) and Mn(IV) respectively) for all the calculated models studied in this section show that the oxidation states of the manganese ions in $S_2Y_Z^\bullet$ remain essentially unchanged from S_2 . As a result of this, the changes in the interatomic distances of the OEC upon oxidation of the Y_Z residue are minor. Figure 4 shows the local environment of the Y_Z residue both before and after the oxidation of the Y_Z residue. The area surrounding the Y_Z residue changes upon formation of the tyrosyl radical. In all models it was found that the tyrosine residue is deprotonated with the proton moving spontaneously to the N_τ atom of the imidazole ring of the His-190 residue in line with the fast proton coupled electron transfer (PCET) mechanism suggested for Y_Z oxidation.^{45–47} The observed proton shift indicates that the tyrosyl residue in the $S_2Y_Z^\bullet$ form can be described as a neutral radical which is coupled to an imidazolium cation. From Figure 4 it can be seen that a significant difference between the calcium and strontium models is the Ca/Sr–W3 and Ca/Sr–W4 distances which were found to be 0.2 Å longer in the strontium models. As a result of the W3 and W4 bond length differences the hydrogen-bonding environment of the tyrosine residue is also altered (ca. 0.03 Å difference in water-tyrosine hydrogen bonds) following strontium substitution. The hydrogen bonding network of the Y_Z residue is thought to be optimised to ensure proper function and even relatively minor perturbations in the network have been thought to significantly impact its operation.^{48,49} It has been suggested that one of the potential roles for the calcium ion in the OEC is regulate the electronic structure and the redox potential of the Y_Z residue.⁵⁰ However recent work by Krewald *et al.* investigating the redox potential of the Y_Z residue in a number of calcium substituted S_n state transitions has

shown that the cofactors on the donor side of PSII are only sensitive to the charge of the cation and as such they ruled out this potential role.⁵¹ It should be noted however that Krewald *et al.* found that the OEC can respond to calcium substitution with a rearrangement of the hydrogen bonding waters (comparable with the perturbations observed here) suggesting that calcium may play a role in ordering the hydrogen bond network of the Y_Z residue and the OEC.⁵²⁻⁵⁴ QM/MM calculations by Nakamura *et al.* found a substantial rearrangement of the hydrogen bond network following tyrosine oxidation and observed a novel proton transfer mechanism where the histidine proton is transferred to a nearby water.⁵⁵ In all of the models studied here no rearrangement of the hydrogen bond network similar to that seen by Nakamura *et al.* was observed and the histidine was not deprotonated by a water molecule.

Figure 4. The hydrogen bonding environment of the Yz residue in the S_2 state (left) and $S_2Y_z^\bullet$ state (right) for all models studied, bond lengths shown in Å. Colour coding: calcium (yellow), strontium (brown), oxygen (red), carbon (grey), nitrogen (blue) and hydrogen (white).

Heisenberg Exchange Coupling Constants

Insight into the magnetic behaviour of S_2 and $S_2Y_Z^\bullet$ can be provided by the exchange coupling constants between the manganese ions.³⁹ Diagonalising the Heisenberg Hamiltonian leads to the complete spectrum of spin energy levels. Figure 5 shows the calculated Heisenberg exchange coupling constants as well as the first two spin states and the energy difference between them. In the open cubane $S_2Y_Z^\bullet$ models studied there are two antiferromagnetic interactions between Mn_A-Mn_B and Mn_C-Mn_D with a ferromagnetic interaction found between Mn_B-Mn_C . In the closed cubane $S_2Y_Z^\bullet$ cluster models all the exchange interactions between the three Mn^{IV} ions which make up the closed cubane unit are ferromagnetic, with the interaction between Mn_A-Mn_B antiferromagnetic. As a result of the minor structural perturbations found in the OEC structure following Y_Z oxidation, the Heisenberg exchange coupling constants and coupling topology for all models are essentially similar to those calculated for the corresponding S_2 models. Additionally Figure 5 shows that the $S_2Y_Z^\bullet$ models have the same $S=1/2$ (open) and $S=5/2$ (closed) OEC ground state spin found in the corresponding S_2 state form with a similar energy gap to the first excited state.

Figure 5. Calculated Heisenberg exchange coupling constants J (cm^{-1}), total spin of ground state (GS) and first excited state (ES) as well as the energetic separation between the two lowest energy levels (cm^{-1}) of the Mn_4CaO_5 cluster in the open cubane and closed cubane forms in the calcium/strontium $\text{S}_2\text{Y}_Z^\bullet$ state. Values in brackets refer to the S_2 state.

^{55}Mn Hyperfine Couplings

Table 1 shows that there is little change in the calculated ^{55}Mn hyperfine couplings of the OEC between the $\text{S}_2\text{Y}_Z^\bullet$ and S_2 states. These results, in conjunction with those discussed previously would indicate that the electronic structure of the Mn OEC in the S_2 state is unchanged on Y_Z oxidation.

Table 1. Calculated Spin Projected ^{55}Mn hyperfine couplings (in MHz) for the $\text{S}_2\text{Y}_Z^\bullet$ models in both the open and closed forms.

Model		A_{iso}	T_1	T_2	T_3
Ca-O	Mn _A	-293 (-287)	-28 (-30)	5 (7)	23 (23)
	Mn _B	204 (198)	-5 (-8)	-1 (-1)	6 (10)
	Mn _C	224 (224)	-7 (-5)	-1 (-1)	8 (6)
	Mn _D	-258 (-253)	-51 (-51)	-42 (-45)	93 (96)
Ca-C	Mn _A	119 (113)	-21 (-27)	0 (0)	21 (27)
	Mn _B	-76 (-71)	-6 (-6)	2 (3)	4 (3)
	Mn _C	-91 (-90)	-3 (-2)	-2 (-2)	5 (4)
	Mn _D	-90 (-87)	-2 (-2)	-1 (1)	3 (3)
Sr-O	Mn _A	-297 (-289)	-13 (-10)	1 (0)	12 (10)
	Mn _B	199 (195)	-6 (-6)	-3 (-2)	9 (8)
	Mn _C	235 (234)	-7 (-5)	-2 (-2)	9 (8)
	Mn _D	-257 (-253)	-57 (-58)	-39 (-36)	96 (93)
Sr-C	Mn _A	117 (112)	-25 (-31)	0 (0)	25 (31)
	Mn _B	-78 (-74)	-5 (-6)	2 (3)	3 (3)
	Mn _C	-89 (-87)	-3 (-2)	-1 (-2)	4 (4)
	Mn _D	-84 (-80)	-4 (-5)	1 (1)	4 (4)

T_i represents the traceless form of the full tensor. Values in brackets are for the corresponding S_2 state models.

$\text{Y}_Z^\bullet$ g-Tensor

We now investigate in detail the calculated EPR parameters of the tyrosyl radical formed in the $\text{S}_2\text{Y}_Z^\bullet$ state. Initial studies of tyrosyl radicals employed simplified models such as the phenoxyl-water or phenoxyl-imidazolium pairs to investigate the structural and spectroscopic properties of these organic radicals.⁵⁶⁻⁵⁹ In recent years more elaborate models have been constructed which also contain residues that surround the tyrosyl radical in order to study the histidine-tyrosine pair in both the Y_Z and Y_D residues.^{46,49,60,61} As well as considering the effects of the protein on the structure and spectroscopic properties, the larger models have also allowed the protein environment of the two redox active tyrosine residues found in PSII to be distinguished. Table 2 shows calculated g-tensors for all the $\text{S}_2\text{Y}_Z^\bullet$ models studied with Figure 6 showing the orientations of the calculated g-tensor.

Table 2. Calculated effective **g**-tensors for the tyrosyl radical found in all the investigated models of the $S_2Y_Z^\bullet$ state

	Ca-O	Ca-C	Sr-O	Sr-C	Exp. ⁶²
g_{iso}	2.00420	2.00420	2.00462	2.00462	2.00443
g_x	2.00622	2.00621	2.00749	2.00748	2.00689
g_y	2.00421	2.00420	2.00420	2.00420	2.00418
g_z	2.00218	2.00218	2.00219	2.00219	2.00221

The experimental results of Ioannidis *et al.* are for the Y_Z radical in the native OEC.

From Figure 6 it can be seen that the orientations of the calculated **g**-tensors is in agreement with earlier model systems.⁶³ In all models the g_x axis is found to be oriented along the C–O bond with g_z perpendicular to the plane of the ring and g_y in the tyrosine ring plane. Commonly EPR studies of the tyrosyl radical have been performed by selectively replacing the Y_D residue with phenylalanine and removing the tetranuclear Mn

Figure 6. The orientation of the calculated **g**-tensors for the tyrosyl radical in all the investigated models of the $S_2Y_Z^\bullet$ state

cluster.^{64,65} Ioannidis *et al.* have however studied the $Y_Z^\bullet$ radical in the presence of the Mn OEC.⁶² Table 2 shows a difference in the calculated g_x values between the **Ca** and **Sr** models. For the g_y and g_z components no such differences can be observed. Previous studies of the tyrosyl radical have shown that the g_x component is strongly dependent on the hydrogen bond length between the tyrosyl radical and the imidazolium cation.^{44,59} In this work a similar dependence is observed with the longer hydrogen bond (1.64 Å) for **Sr-O** and **Sr-C** yielding the larger calculated g_x value, while the shorter hydrogen bond length for the Ca models (1.59 Å) leads to a reduced g_x component. The **g**-tensor components in the absence of the Mn OEC have been measured by Matsuoka *et al.* who reported $g_x = 2.00714$ in frozen solution and 2.00705 in single crystals.⁵⁹ These values of g_x were found to be comparable to values reported for the more widely studied $Y_D^\bullet$ of PSII, where a range of g_x values for $Y_D^\bullet$ in various organisms from 2.0074 to 2.0078 have been reported.⁶⁶ The g_x component is known to strongly depend on the hydrogen bonding and the electrostatic environment surrounding the tyrosyl radical. An example of this effect is the tyrosyl radical $Y_{122}^\bullet$ of *Escherichia coli* ribonucleotide reductase where the g_x value increases significantly with removal of hydrogen bonding.^{57,67,68} The better agreement for the native **Ca** models with the experimental results of Ioannidis *et al.* for samples retaining the OEC compared with the Mn depleted OEC studies by Matsuoka *et al.*, is encouraging. These comparisons also suggest that the removal of the Mn OEC alters the waters surrounding the tyrosine residue, reducing their hydrogen bonding capability.^{59,62}

The Mulliken spin populations of the tyrosyl fragments are shown in Figure 7. As has previously been reported for a series of phenoxyl radical model systems,⁶⁹ the trend seen in the g_x components shown in Table 2 follows closely the changes observed in the unpaired spin population of the tyrosyl oxygen atom. Previous studies of the $Y_Z^\bullet$ system have found

a linear correlation between the oxygen unpaired spin population and the g_x component.^{44,59} As seen in Figure 4, on going from the **Ca** $S_2Y_Z^\bullet$ models to the **Sr** models, the hydrogen bond length between the neutral tyrosine and the imidazolium cation increases from 1.59 Å to 1.64 Å, this creates a weaker hydrogen bond to the $Y_Z^\bullet$ radical in the strontium models leading to an increased spin population at the carbonyl oxygen. Similar behaviour has been observed in the hydrogen bonding environment of semiquinone radicals and for the primary quinone ($Q_A/Q_A^{\bullet-}$) found in bacterial reaction centres.^{70,71}

Figure 7. Mulliken spin populations of the tyrosyl radical fragment in the open and closed forms of native and strontium substituted $S_2Y_Z^\bullet$ state.

$Y_Z^\bullet$, ^1H hyperfine couplings.

Insight into the electronic structure of the tyrosine radical may also be gained by studying the hyperfine couplings of the spin active nuclei which comprise the radical. Table 3 shows the calculated ^1H hyperfine couplings (HFCs) for the tyrosyl radical protons in all the investigated $S_2Y_Z^\bullet$ models. In addition Table 3 shows experimental values and assignments obtained from 2D-HYSCORE experiments performed by Nakazawa *et al.* on the green alga *Chlamydomonas reinhardtii*.⁷²

Table 3. Calculated ^1H hyperfine couplings (in MHz) for $S_2Y_Z^\bullet$ in both the open and closed forms and comparison with available experimental data.

	Label	A_{iso}	T_1	T_2	T_3
Ca-O	H2	3.9	-3.6	0.6	3.0
	H3	-17.3	-6.4	-2.3	8.7
	H5	-17.5	-6.8	-2.5	9.2
	H6	4.1	-3.6	0.5	3.1
	H7a	31.6	-2.6	-2.2	4.8
	H7b	4.4	-1.1	-1.0	2.1
	H8	0.2	-4.1	-3.9	8.0
Ca-C	H2	3.9	-3.4	0.4	3.1
	H3	-17.4	-6.1	-2.7	8.8
	H5	-17.9	-6.6	-2.5	9.1
	H6	4.0	-3.5	0.3	3.1
	H7a	30.5	-2.3	-2.1	4.3
	H7b	5.9	-1.1	-0.9	2.0
	H8	0.2	-4.1	-4.1	8.1
Sr-O	H2	4.2	-3.6	0.5	3.1
	H3	-17.2	-6.4	-2.4	8.8
	H5	-17.7	-6.4	-2.5	8.9
	H6	4.6	-3.6	0.4	4.0
	H7a	31.4	-2.6	-1.1	3.7
	H7b	4.9	-1.2	-0.9	2.1
	H8	0.1	-4.3	-4.0	8.3
Sr-C	H2	4.1	-3.4	0.4	3.1
	H3	-17.1	-6.2	-2.4	8.6
	H5	-17.6	-6.1	-2.4	8.5
	H6	4.4	-3.4	0.3	3.2
	H7a	31.1	-2.5	-1.2	3.6
	H7b	5.2	-1.1	-0.8	2.0
	H8	0.1	-4.2	-3.9	8.2

Exp. ⁷²	H2/H6	4.8	-0.9	-0.9	1.8
	H3/H5	-18.7	-6.1	-4.1	10.2
	H7a	30.2	-2.2	-2.2	4.3
	H7b	4.8	-0.9	-0.9	1.8
	H8	—	—	—	—

Similar experimental values have also been reported by Tommos *et al.* although there is disagreement for the reported anisotropic hyperfine couplings of the H2 and H6 protons (see main text).⁷³

The data contained in Table 3 shows that the calculated isotropic HFCs for all the tyrosyl radical split signal models agree very well with the associated experimental values. Moving on to consider the anisotropic HFCs, it can be seen that those calculated for the H3 and H5 protons are found in very good agreement with those determined from experiment. Table 3 shows disagreement between the calculated and experimental results for the H2 and H6 protons, where Nakazawa *et al.* found the same anisotropic HFCs for the 2/6 protons as the H7b proton. Tommos *et al.* found similar experimental results, when studying the $Y_Z^\bullet$ radical of *Synechocystis 6803*, to those shown in Table 3 apart from the anisotropic HFCs of the H2 and H6 protons which were found to be $(-3.5, 0.2 \text{ and } 2.7 (T_1, T_2 \text{ and } T_3 \text{ respectively}))$.⁷³ The experimental anisotropic HFCs reported by Tommos *et al.* are in better agreement with the calculated tyrosyl radical models. Of particular note in Table 3 is the good agreement observed for the H7a and H7b protons of the tyrosyl methylene group. As has been substantially documented in the literature, these methylene protons receive spin density via hyperconjugation with the π -spin density of the ring system and their isotropic HFCs vary depending on the level of the overlap with the spin density.⁷⁴ The extent of this overlap and therefore the resulting calculated hyperfine coupling is dependent on the C8-C7-C1-C2 dihedral angle.⁶⁰ In the literature discussions on this topic the “dihedral angles” typically discussed are the angles between the H7 protons and a hypothetical p_z orbital of C1 assuming ideal tetrahedral geometry. This is shown in Figure 8 where the calculated values of this study are compared with the experimentally determined values, from this it can be seen that the calculated values are

found to be within the experimental error in determining these angles. It should be noted that similar results for the experimental angles have been found by Tommos *et al.* for the $Y_Z^\bullet$ found in *Synechocystis 6803*,⁷³ similar EPR studies performed on the $Y_D^\bullet$ residue of PSII have found there to be a difference in the reported HFCs and angles.^{75,76} This has been interpreted to indicate that the protein environments of the Y_Z residue remain relatively similar between species but there is a larger difference in the environments of the Y_D residue.^{46,61,72} The reasoning behind this has been linked to the roles of the Y_Z and Y_D residues in photosynthesis; the Y_Z residue is responsible for electron transfer between the OEC and the P_{680} reaction centre and therefore central to water oxidation.⁷⁷ Whereas the Y_D residue is not required for correct PSII function and debate still rages as to its role in PSII,^{45,76,78} as a result of this the local protein environment of Y_D may be more amenable to changes. It is hoped that these calculations, supported by further studies of the $Y_Z^\bullet$ as well as the $Y_D^\bullet$ of PSII from a number of different species may provide more light to this important topic.

Figure 8. Comparison between calculated and experimentally derived orientations for the $Y_Z^\bullet$ methylene hydrogens, H7a and H7b with respect to C1'' p_z'' orbital (see text for details). The experimentally derived values from Nakazawa *et al* are shown in brackets for the native OEC.⁷² Note that Tommos *et al.* have found similar experimental values of 44° and 76°.⁷³

The S_3 State.

Geometry.

It is currently believed that on progressing from the S_2 to the S_3 state an additional water derived ligand coordinates to the OEC resulting in a conformational change in the structure.⁷⁹ This open coordination position along the Jahn-Teller axis of Mn^{III} is present

on either Mn_A (closed cubane S₂) or Mn_D (open cubane S₂). Deprotonation also occurs before progression to S₃. The combination of deprotonation and hexa coordination leads to oxidation of the remaining Mn^{III} ion to Mn^{IV} with reduction of Y_Z[•] to Y_Z. In one scenario, this series of events was modelled by inserting an additional hydroxide ligand in the open coordination site of the Mn^{III} ion of the S₂Y_Z[•] state. (These models are indicated by the W_x label). In addition, the water ligands (W1, W3 and W4) were selectively deprotonated in the S₂Y_Z[•] state to see if any ligand rearrangements occurred during subsequent geometry optimisation. The only instance where this did occur was in the closed form of the **Ca** model (**Ca-C**) where W3 in its hydroxide form ligates to Mn_A which was oxidised to Mn^{IV} with reduction of Y_Z[•] (this model is described by the W₃ label). A similar shift of deprotonated W3 on geometry optimisation has been reported by Ugur *et al.* who observed W3 hydroxide movement in both open and closed forms of the native OEC.⁸⁰ The observation concerning W3 movement in the open form was not observed in this study. Of particular note also is that a similar W3 shift in ligation could not be observed in either the **Sr-C** or **Sr-O** models. Analysis of the Mulliken spin populations for the open and closed **Ca** and **Sr** models studied found that the additional coordination of the hydroxide ligand to the vacant Mn^{III} coordination site resulted, on subsequent geometry optimisation, in an oxidation change from Mn^{III} to Mn^{IV} with reduction of Y_Z[•]. This resulted in all the manganese ions of the OEC being hexa coordinated and in the Mn^{IV} state. Key interatomic distances for the models studied can be seen in Table 4. Considering firstly the **Ca** results, it can be seen that the **Ca-O W_x** model provides Mn–Mn distances which are in very good agreement with the experimental EXAFS data.^{81,82} Looking at the closed form **Ca** model with either W_x or W₃, it can be seen from Table 4 that the calculated interatomic distances are broadly consistent with the EXAFS data. Table 4 also includes interatomic distances for the optimised **Sr** models, although the changes to the S₃ Mn–Mn values following

strontium substitution have not been experimentally determined. From Table 4 it can be seen that there are only small changes to the OEC core structure as a result of strontium substitution.

Table 4. Selected interatomic distances ($\text{\AA}$) of the S_3 models and comparison with available native S_3 state experimental data.

Interatomic Distance	Ca-O W_X	Sr-O W_X	Ca-C W_X	Ca-C W_3	Sr-C W_X	Exp.^{a)}
$Mn_A - Mn_B$	2.80	2.82	3.27	3.25	3.29	
$Mn_B - Mn_C$	2.82	2.85	2.80	2.81	2.82	2.80, 3.3
$Mn_C - Mn_D$	2.81	2.83	2.75	2.76	2.78	(3:1)
$Mn_B - Mn_D$	3.51	3.52	2.91	2.92	2.93	
$Mn_{A,B,C,D} - Ca/Sr$	3.99, 3.39, 3.35, 3.50	4.02, 3.45, 3.42, 3.54	3.99, 3.32, 3.41, 3.51	3.98, 3.34, 3.41, 3.52	4.04, 3.37, 3.44, 3.54	3.4, 4.0 (2:2 or 3:1)
$Mn_A - W1$	2.00	2.04	2.05	2.06	2.05	–
$Mn_A - W2$	1.79	1.81	1.80	1.81	1.80	–
$Ca/Sr - W3$	2.35	2.56	2.35	2.50 ^{b)}	2.57	–
$Ca/Sr - W4$	2.37	2.58	2.36	2.57	2.59	–

^{a)} Distances obtained from EXAFS studies of the native OEC in the S_3 state.^{79,81,82} ^{b)} note that in this model $W5$ binds to the calcium ion instead as $W3$ moves to coordinate to Mn_A

Heisenberg Exchange Coupling Constants.

Figure 9 illustrates the calculated Heisenberg exchange coupling constants (J_{ij}) for the S_3 models studied as well as the ground and first excited spin states and the energy difference between them. All S_3 models produce an effective total spin $S_t = 3$ ground state and an $S_t = 4$ first excited state in line with observations from experimental EPR and other computational studies.¹⁵

Examining the J coupling topology of the different S_3 models, shows that the open and closed cubane forms display different magnetic connectivity. For the open form models, **Ca-O W_X** and **Sr-O W_X** , three main exchange pathways are found: i) a ferromagnetic

coupling between Mn_C and Mn_D (J_CD); ii) a ferromagnetic coupling between the Mn_B and Mn_C (J_BC); and iii) an antiferromagnetic coupling between Mn_A and Mn_B (J_AB). The remaining exchange couplings (J_AC , J_AD and J_BD) are considerably smaller reflecting the larger distances between the ions. The coupling topology can be described by a dimer of dimers model⁸³ made up of two interacting dimer units, S_A comprising Mn_A and Mn_B and S_B (Mn_C and Mn_D). Antiferromagnetic coupling observed between Mn_A and Mn_B (J_AB) results in a low spin dimer subunit of total spin $S_\text{X} = 0$. Ferromagnetic coupling between Mn_C and Mn_D (J_CD) leads to a high spin dimer of total spin $S_\text{Y} = 3$. The interaction between these two dimer subunits then results in the overall $S_\text{t} = 3$ observed. The closed form magnetic connectivity can best be explained using a monomer-trimer model. The trimer comprising Mn_B – Mn_C – Mn_D coupled ferromagnetically producing a high spin fragment ($S_\text{X} = 9/2$). This spin state is a characteristic feature of biomimetic models which feature a $\text{Mn}^\text{IV}_3 \text{O}_4 \text{Ca}$ unit with the internal geometry of the cubane dictating the ferromagnetic character of the coupling within the cubane.^{86,87} The antiferromagnetic coupling between this trimer in the OEC and the $S=3/2$ Mn_A , described by the J_AB coupling then produces the ground state spin of $S = 3$. The effects of strontium substitution on the S_3 state, Figure 9, shows there to be only minor changes to the calculated exchange coupling values indicating that the substitution has little or no effect on the underlying magnetic structure of the models studied.

Figure 9. Calculated Heisenberg exchange coupling constants, J (cm^{-1}), total spin of ground state (GS) and first excited state (ES) as well as the energetic separation between the two lowest energy levels (cm^{-1}) for the investigated S_3 models.

Spin Projection Coefficients.

All of the S_3 state cluster models produce a coupling topology of ‘ $\beta a a a$ ’ with a negative spin projection coefficient being found for Mn_A and positive spin projection coefficients being found for all other manganese ions of the OEC. Table 5 shows that the manganese ions of the **Ca-O W_X** model couple in such a manner so that the on-site spin projection coefficients can be categorised into two unique classes: Mn_C and Mn_D are found to possess a spin projection coefficient of roughly 0.5, with the Mn_A and Mn_B possessing significantly smaller on-site spin projection coefficients in the range of 0.1 to 0.2 approximately. For **Ca-C** models (in either the W_X attack or W_3 attack scenario) the on-site spin projection coefficients are of a similar magnitude. The **Sr** models show identical patterns to their Ca analogues.

Table 5. Calculated on-site spin projection coefficients, c_i , for the investigated OEC S_3 models.

	Mn_A	Mn_B	Mn_C	Mn_D	Coupling
Ca-O W_X	-0.121	0.171	0.459	0.490	$\beta a a a$
Sr-O W_X	-0.106	0.164	0.456	0.487	$\beta a a a$
Ca-C W_X	-0.362	0.416	0.473	0.473	$\beta a a a$
Ca-C W_3	-0.366	0.424	0.474	0.467	$\beta a a a$
Sr-C W_X	-0.362	0.415	0.474	0.473	$\beta a a a$

^{55}Mn Hyperfine Couplings.

Table 6 shows the isotropic and anisotropic hyperfine couplings (HFCs) calculated for all the S_3 state models using the on-site spin projection coefficients shown in Table 5. In addition to this, Table 6 also shows experimental W-band ^{55}Mn -EDNMR data by Cox *et al.* on the native OEC in the S_3 state.¹⁵ Cox *et al.* found the HFCs for the four manganese ions could be separated into two classes. The strongly coupled HFC class

which was found to have an A_{iso} around 100 MHz and the more weakly coupled class having an isotropic couplings of a few MHz.¹⁵ In addition to the two HFC classes, Cox *et al.* also reported small hyperfine anisotropy for all the manganese ions which was interpreted as evidence of the presence of Mn^{IV} ions with octahedral coordination geometry in the S_3 state of the OEC. Experimental and computational studies of biomimetic complexes which contain Mn^{IV} ions with trigonal bipyramidal geometry as well as all Mn^{III} complexes have found larger hyperfine anisotropies.^{85–88} All of the S_3 models studied in this section find the four manganese ions to have the same oxidation state (Mn^{IV}) and also possess similar octahedral coordination environments. The similar environments should lead to similar on-site HFC values for all Mn ions and the differences in the experimentally measured coupled values lie in different spin projection coefficients for the four Mn ions. From Table 6 it can be seen that the calculated isotropic HFCs for the **Ca-O W_X** model are in reasonable agreement with the experimental values. In particular the two large HFCs calculated at –87 MHz and –88 MHz are in good agreement with the larger set of experimental HFCs in terms of magnitude and sign. The calculated smaller set of couplings for Mn_C and Mn_D are significantly larger than the experimental values however. Similar findings have been found by Cox *et al*¹⁵ and Krewald *et al*⁸⁹. It should be noted that the two smaller experimental HFCs, by virtue of their smaller magnitude, could not be completely characterised in the experimental study.¹⁵ In particular the anisotropy was not specifically determined but assumed to be small. The calculated HFCs for the closed form of the native OEC in either the **Ca-C W_X** or **Ca-C W_3** models, Table 6, are in poorer agreement with the experimental data as the magnitudes of the calculated isotropic HFC in both models are broadly similar across all four manganese ions and do not separate into two distinctive levels of calculated isotropic HFC. This can be rationalised by the broadly similar spin projection coefficients reported in Table 5 for the closed forms. Our BS-DFT

analysis therefore broadly supports an open cubane form of the S_3 state with hydroxo – oxo hydrogen bonding between the nearby oxygens which then presumably undergo O-O bond formation in the S_4 state.

Table 6 also shows calculated HFC data for the Sr substituted form where identical trends to the native Ca form are observed between the open and closed forms of the OEC. As already noted above geometry optimisation on W3 deprotonation leads to a minimum energy structure with the W3 (OH) ligated to Sr. This contrasts with the Ca form where this movement was observed to spontaneously occur on W3 deprotonation leading to a minimum energy structure with the W3 (OH) ligated to $Mn_D(IV)$. Currently we do not have an obvious reason for this different behaviour between Ca and Sr models, and possible sources are being investigated. Absence of movement of W3 hydroxide for the Sr form results in Mn_D remaining unoxidised as Mn^{III} in the S_3 state. It would be expected therefore that the EPR properties of the S_3 state for the Sr substituted samples would be quite different to the native Ca form as the presence a Mn^{III} ion would introduce higher anisotropy. Clearly inhibited movement of the second substrate water on Sr substitution would provide an explanation for the lower activity observed for this form of the catalyst.

Table 6. Calculated ^{55}Mn hyperfine couplings (in MHz) for the S_3 models and comparison with experimental data.

Model		A_{iso}	T_1	T_2	T_3
Ca-O W_X	Mn_A	28	-2	1	1
	Mn_B	-42	-4	1	3
	Mn_C	-87	-3	1	2
	Mn_D	-88	-4	1	3
Sr-O W_X	Mn_A	25	-1	0	1
	Mn_B	-40	-3	1	2
	Mn_C	-87	-4	1	3
	Mn_D	-88	-4	2	3

Ca-C W_X	Mn _A	72	-4	1	3
	Mn _B	-67	-4	2	2
	Mn _C	-84	-1	0	1
	Mn _D	-80	-2	1	1
Ca-C W₃	Mn _A	75	-4	2	2
	Mn _B	-59	-4	1	3
	Mn _C	-86	-3	1	2
	Mn _D	-81	-2	1	1
Sr-C W_X	Mn _A	74	-4	1	3
	Mn _B	-64	-3	1	2
	Mn _C	-81	-3	1	2
	Mn _D	-77	-4	2	2
Exp. ¹⁵	1	-99.0	-1.2	-1.2	2.4
	2	-95.6	-3.8	1.8	1.8
	3 ^{a),b)}	-25.9 or + 7.0	—	—	—
	4 ^{b)}	0.5 (≤ 5)	—	—	—

T_i represents the traceless parts of the anisotropic tensor.^{a)} The experimental value of A₃ is dependent upon the assumed sign of the hyperfine coupling, It could potentially take either one of the two values shown in Table .6. ^{b)} The A₃ and A₄ hyperfine couplings are experimentally assumed to be isotropic.

S₃-peroxo model

As mentioned in the introduction atomic level structures for the S₃ state have very recently been reported using XFEL crystallography.^{17,16} In one case, Suga *et al*¹⁶, an extra oxygen atom has been located compared with the S₁ state which is similar to the additional OH ligand of our open form models. The crystal structure O-O bond distance of 1.5 Å is however much shorter than the average 2.4 Å value found in our oxo-hydroxo S₃ models. The short O-O bond distance reported in the XFEL structure of Suga *et al*¹⁶ indicates peroxo ligand formation has occurred rather than an oxo-hydroxo interaction that we locate in our models and similar ones reported by Cox *et al.*¹⁵ and Krewald *et al.*⁸⁹ To test the feasibility of peroxo formation in S₃, an OEC model, using as starting structure the XFEL coordinates has been geometry optimised assuming peroxo formation. The minimum energy structure, Figure 10, shows minimal differences to the XFEL determination and agrees with S₃ EXAFS structural data, thereby indicating that this is, structurally, a highly viable model for the S₃ state. Based on Mulliken population analysis,

both Mn_A and Mn_D are in oxidation state III with Mn_B and Mn_C present in oxidation state IV. The Jahn-Teller axis for both Mn_A and Mn_D lies along their respective bonds to the peroxo oxygen atoms, see Figure 10. Similar to the XFEL starting coordinates, distinctive ligation of the peroxo oxygen atoms is found, where one of the peroxo oxygens is ligated to Mn_B (1.9 Å), Mn_A (2.2 Å) and Ca (2.4 Å) with the other peroxo oxygen ligated solely to Mn_D (2.1 Å). A DFT exchange coupling analysis gives rise to an $S = 3$ ground state in agreement with S_3 EPR determinations, Sanakis *et al*⁹⁰, Boussac *et al*⁹¹. Intriguingly as well, the $S = 3$ ground state can be rationalised within a dimer of dimers model with an $S_X = 0$ dimer fragment (antiferromagnetic coupling, accommodated by superexchange via the peroxo ligand, between Mn(III)_A and Mn(III)_D) coupled with an $S_Y = 3$ (ferromagnetic coupling between Mn(IV)_B and Mn(IV)_C). The low spin $S_X = 0$ dimer would render it EPR silent. This coupling scheme would give rise to Mn_A and Mn_D spin projections close to zero and Mn_B and Mn_C spin projections close to 0.5, leading to two large ⁵⁵Mn isotropic hfcs from Mn_B and Mn_C and close to zero small magnitude values for Mn_D and Mn_A. This is similar to the pattern of ⁵⁵Mn hfcs obtained experimentally by Cox *et al*.¹⁵ Because the Mn(III)_A/Mn(III)_D dimer is EPR silent, an overall low anisotropy would be expected. It is therefore possible to explain the S_3 EPR characteristics based on the peroxo model revealed in the XFEL study of Suga *et al*.¹⁶ Another possibility is that an equilibrium exists between the peroxo and oxo-hydroxo species as proposed some years ago by Renger⁹² and references therein and more recently by Isobe *et al*⁹³. ¹⁸O/¹⁶O isotope water exchange rates⁹⁴ which show two different rates for substrate waters in the S_3 state, have been argued to rule out peroxo formation in this state. The distinctly different binding modes of the peroxo oxygens described above, Figure 10, could explain this however. The surprising similarity in exchange rates between S_2 and S_3 together with drastic reduction for the S_3Y_Z state are also difficult to incorporate in any current mechanism. X-ray absorption near

edge structure (XANES) studies ⁸¹ do not support Mn reduction in the S₂ to S₃ transition but here again the significant structural change to peroxo bridging ligation may complicate the edge shape and shift ⁸². Clearly further clarification of the conflicting S₃ structures and associated modelling studies needs to be performed to provide us with a definitive picture of the S₃ state, an essential step in determining any overall mechanism for water oxidation by the OEC.

Figure 10. (a) Geometry optimised S₃-peroxo model. Mn_A and Mn_D are oxidation state III (green) and Mn_C and Mn_B are in oxidation state IV (purple). The peroxo bond of length 1.4 Å is highlighted. (b) Geometry optimised S₃ (oxo-hydroxo) model in its high spin state Mn_A, Mn_D, Mn_C and Mn_B are in oxidation state IV (purple). The O-O bond distance of 2.4 Å is highlighted. See text for details.

Conclusions

In this study a BS-DFT magnetic analysis of the S_2 , $S_2Y_Z^\bullet$ and S_3 states of Nature's oxygen evolving complex was performed for both the native Ca and Sr substituted forms. Excellent agreement with experimental data is observed between the tyrosyl calculated g-tensor and ^1H hyperfine couplings for the native Ca form. Changes in the hydrogen bonding environment of the tyrosyl radical in $S_2Y_Z^\bullet$ caused by Sr substitution leads to notable changes in the calculated g-tensor of the tyrosyl radical. Comparison of calculated and experimental ^{55}Mn hyperfine couplings for the S_3 state favours an open cubane form of the complex with an additional OH ligand coordinating to Mn_D . In Ca models this additional ligation can arise by closed-cubane form deprotonation of the Ca ligand W3 in the $S_2Y_Z^\bullet$ state accompanied by movement to the vacant Mn coordination site or by addition of an external OH group. For the Sr form, no spontaneous movement of W3 to the vacant Mn coordination site is observed in contrast to the native Ca form. This difference may lead to the reduced catalytic activity of the Sr substituted form. The oxo-hydroxo bond distance for such S_3 models is much larger than that found in the XFEL study of the S_3 state by Suga *et al*¹⁶ where the O-O bond distance of 1.5Å indicates peroxo ligation. BS-DFT studies on such a peroxo model is shown to be consistent with the experimental structural data. In addition an $S = 3$ ground state is calculated in agreement with EPR studies indicating that this scenario needs further investigation.

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ASSOCIATED CONTENT

Supporting Information

Additional analysis of models, derivation of empirical scaling factor for ^{55}Mn isotropic hyperfine couplings plus representative model coordinates. Available free of charge at <http://pubs.acs.org>

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