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¹ S₃ State of the O₂-Evolving Complex of Photosystem II: Insights from ² QM/MM, EXAFS, and Femtosecond X-ray Diffraction

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6 **Supporting Information**

ABSTRACT: The oxygen-evolving complex (OEC) of 7 photosystem II has been studied in the S3 state by electron 8 paramagnetic resonance, extended X-ray absorption fine 9 structure (EXAFS), and femtosecond X-ray diffraction 10 11 (XRD). However, the actual structure of the OEC in the S_2 state has yet to be established. Here, we apply hybrid 12 quantum mechanics/molecular mechanics methods and 13 propose a structural model that is consistent with EXAFS 14 and XRD. The model supports binding of water ligands to 15 the cluster in the $S_2 \rightarrow S_3$ transition through a carousel 16 rearrangement around Mn4, inspired by studies of 17 ammonia binding. 18

hotosystem II (PSII) is a protein–pigment complex found 19 in the thylakoid membranes of the higher plants and 2.0 21 internal membranes of cyanobacteria. During the light period of 22 photosynthesis, PSII performs water oxidation with oxygen 23 evolution.¹⁻³ The reaction is triggered by the photochemical 24 oxidation of the chlorophyll a species P_{680} that induces 25 electron-hole separation at the reaction center. The electron $_{26}$ hole is transferred from P_{680} to the redox-active tyrosine, $Y_{\text{Z}\prime}$ 27 which in turn oxidizes the CaMn₄O₅ cluster of the oxygen-28 evolving complex (OEC). The high efficiency and low 29 overpotentials^{4,5} make the OEC a prototype for development 30 of artificial water-oxidation catalysts. However, the structural 31 changes of the OEC responsible for efficiency and functionality, 32 along the catalytic cycle, have yet to be established at the 33 molecular level.

In each turn of the cycle, the OEC evolves through five 35 "storage" states, S_n (n = 0-4), with S_0 and S_4 being the most 36 reduced and oxidized forms, respectively.^{6,7} After the S_4 state is 37 formed, O_2 is spontaneously released and the S_0 state is 38 restored, completing the catalytic cycle. Structural models for 39 the S_0-S_2 states have been found to be consistent with 40 femtosecond X-ray diffraction (XRD) and high-resolution 41 extended X-ray absorption fine structure (EXAFS) data.⁸⁻¹¹ 42 However, the structure of the OEC cluster in the S_3 state 43 remains controversial.

W-Band electron paramagnetic resonance (EPR) spectros-4s copy has determined that the OEC in the S₃ state has Mn(IV) 46 centers with octahedral environments.¹² It is established that 47 the S₂ \rightarrow S₃ conversion involves binding of a water molecule 48 (Wx) to the OEC,¹³⁻¹⁵ although the coordination site remains 49 uncertain. Ammonia binding to the S₂ state¹⁶⁻²⁰ provided 50 valuable insights because water and ammonia are electronic and structural analogues. On the basis of EPR^{18,21} and FTIR²² 51 spectroscopy and measurements of reduction potentials,²³ we 52 have recently proposed that ammonia binds in a terminal motif 53 as the sixth ligand of the dangling Mn4.¹¹ In the work 54 presented here, we examine analogous water binding. We 55 introduce an S₃ model based on our studies of ammonia 56 binding,¹¹ using quantum mechanics/molecular mechanics 57 (QM/MM) hybrid methods and calculations of EXAFS spectra 58 that allow for direct comparisons to EXAFS data, and Fourier 59 difference electron-density maps from XRD. 60

We find that a structure in which Mn4 is linked to Mn3 with $_{61}$ two μ -oxo bridges and Mn1 has O5 coordinated as an OH $_{62}$ ligand (Figure 1) is most consistent with EXAFS spectrosco- $_{63}$ fi



Figure 1. QM/MM-optimized structure of the OEC in the S_3 state (Mn oxidation states: IV, IV, IV), including coordination of water ligands as well as D1-D61, D1-His337, and CP43-R357.

py.²⁴ Such a rearrangement of ligands shrinks the short Mn2– 64 Mn3 distance, from 2.83 to2.78 Å, forming three equivalent 65 Mn–Mn distances in the range of 2.75–2.78 Å, and stretches 66 the long Mn1–Mn3 and Mn1–Mn4 distances by ~0.15 Å, 67 leading to an overall expansion of the cluster when compared to 68 the OEC in the S₁ state. These structural changes are supported 69 by experimental data of observed S₃-minus-S₁ electron-density 70 differences,²⁵ and direct comparisons to differences of 71 simulated electron-density maps based on the S₁ and S₃ QM/ 72 MM models. 73

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Our QM/MM models were optimized as in previous 75 studies^{26,27} by using the B3LYP functional with the 76 LANL2DZ^{28,29} basis set for calcium and manganese, and the 77 $6-31G^{*30}$ basis set for all other atoms.^{8–11} The AMBER force 78 field³¹ was used for the MM layer. EXAFS spectra were 79 calculated using the *ab initio* real space Green's function 80 approach as implemented in FEFF (version 8.30).³²

⁸¹ Our QM/MM model of the S₃ state is consistent with the so-⁸² called "open" structure of the OEC, ^{33,34} which can be formed ⁸³ from the "closed" cubane structure upon proton transfer from a ⁸⁴ water ligand of Mn4 (W2) to O5, forming the HO5 ligand of ⁸⁵ Mn1 and an additional μ -oxo bridge linking Mn4 and Mn3. ⁸⁶ Our "open" structure is more stable than the "closed" form by ⁸⁷ 35 kcal mol⁻¹ and shows excellent agreement with EXAFS data ⁸⁸ (Figure 2).



Figure 2. Comparison of calculated (red) and experimental²⁴ (black) Mn EXAFS spectra of the S₃ state in k-space (A and C) and reduced distance (B and D) for the QM/MM-optimized (A and B) and weighted average of spectra, based on the QM/MM models for states $S_0-S_3^{10,11}$ and the experimental composition of the S₃ EXAFS sample.²⁴

Figure 2 shows that there is very good agreement between 89 90 the experimental EXAFS spectrum of the OEC in the S₃ state 91 and the calculated spectrum based on our S₃ QM/MM model. 92 The structure is stabilized by a very strong hydrogen bond 93 between the OH ligand of Mn1 and the additional μ -oxo bridge 94 linking Mn4 and Mn3. Refinement of the QM/MM structure 95 by simulated annealing Monte Carlo (MC) leads to no 96 significant changes in the Mn-Mn distances (Table 1), with 97 three short Mn-Mn distances [Mn1-Mn2 (2.78 Å), Mn2-Mn3 (2.78 Å), and Mn3-Mn4 (2.76 Å)] and one short 98 99 intermetallic distance corresponding to the Mn1-Mn3 distance (3.50 Å). The resulting spectrum is thus similar to the EXAFS 100 spectrum of the S₂ g = 4.1 state, as previously reported.^{35,36} 101 102 We note that while the theoretical and experimental spectra

¹⁰³ show very good agreement, the sample used to collect the ¹⁰⁴ EXAFS data was not 100% S_3 .²⁴ In fact, misses and double hits ¹⁰⁵ resulting from the Xe lamp flashes led to a final composition of ¹⁰⁶ 60% S_3 , 25% S_2 , 12% S_0 , and 3% S_1 .²⁴ Therefore, for the sake of ¹⁰⁷ completeness, we compared the EXAFS spectrum based on the ¹⁰⁸ weighted average of the spectra produced with that S-state

Table 1. Comparison of Mn–Mn Distances (angstroms) in the QM/MM-Optimized Structures of the OEC in the S_1 and S_3 States and the MC-Refined Model

	S_1	S ₃	S ₃ ref	$S_3 - S_3$ ref	$S_3 - S_1$
Mn1-Mn2	2.79	2.78	2.78	0.00	-0.01
Mn1-Mn3	3.34	3.50	3.50	0.00	0.16
Mn1-Mn4	4.90	5.15	5.15	0.00	0.25
Mn2-Mn3	2.83	2.78	2.78	0.00	-0.05
Mn2-Mn4	5.15	5.19	5.19	0.00	0.05
Mn3-Mn4	2.72	2.76	2.75	-0.01	0.04

composition, using our previously reported models of S_2 ($S = 109^{1/2}$ spin isomer), 11 S_1^{10} and S_0^{10} states (Figure 2C,D). To 110 assess our results, we calculated the root-mean-square deviation 111 (rmsd) between the calculated and experimental EXAFS 112 spectra in k-space. We found that including the fractions of 113 other S states leads to a slight improvement of the comparison 114 with the experiment in k-space (Supporting Information) 115 without significantly affecting the results in the reduced 116 distance space.

Mn–Mn distances of >5 Å, such as Mn1–Mn4 and Mn2– 118 Mn4 distances, can be probed by XRD, although they are 119 perhaps too long to be reliably determined by EXAFS 120 spectroscopy. XRD has been essential in determining the 121 structure and ligation scheme of the OEC in the dark-stable S₁ 122 state.^{37–41} In addition, recent advances in XRD techniques 123 based on high-intensity femtosecond X-ray pulses have 124 permitted collection of PSII data in intermediate states of the 125 catalytic cycle, although at low resolution.^{25,42,43} Here, we 126 analyze the data from ref 25, at 5.5 Å resolution, where the 127 dark-stable S₁ state was advanced by two flashes to achieve 128 some population of the S₃ state. Therefore, changes in the 129 electron-density maps before and after the flashes should report 130 on the underlying changes due to S₃ formation. 131

Following the approach implemented in our analysis of 132 changes of the OEC structure due to the $S_1 \rightarrow S_2$ transition,⁸ 133 we calculated the S_3 -minus- S_1 Fourier difference electron- 134 density maps based on our QM/MM models and compared the 135 resulting electron-density difference to the observed S_3 -minus- 136 S_1 data (Figure 3). 137 fB



Figure 3. (A) Calculated S_3 -minus- S_1 difference Fourier maps based on the QM/MM S_3 and S_1 models. (B) Observed S_3 -minus- S_1 electrondensity difference maps for monomer "A" using data from ref 25. Negative difference densities are shown as red mesh nets and positive differences as green mesh nets.

The observed S_3 -minus- S_1 electron-density difference maps 138 were obtained from ref 25, as follows. First, the two observed 139 data sets were scaled to each other to remove any Wilson *B* 140 factor differences. Second, a $2F_{obs}(S_3) - F_{obs}(S_1)$ map was 141 calculated in the S_3 unit cell, using the S_3 model phases, and the 142 $2F_{obs}(S_1) - F_{obs}(S_3)$ map was calculated in the S_1 unit cell, 143

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144 using the S_1 model phases to ensure that exactly the same set of 145 Bragg reflections was used for calculation of both maps. Third, 146 the two maps were aligned by maximizing an electron-density 147 correlation function within the boundary of the dimer. Finally, 148 the differences between two aligned and scaled electron-density 149 maps were calculated. Our XRD calculations were conducted 150 using the Rave and CCP4 packages.^{44,45} We argue that our 151 approach is more reliable than a side-by-side visual comparison 152 of the two electron-density maps at a fixed σ level²⁵ because the 153 σ value varies with resolution. Given the resolution limit of 154 \sim 5.5 Å and the fact that difference features are typically 155 averaged within a sphere \sim 5.5 Å in diameter, the only visible 156 features would be overall changes in electron density within the 157 OEC.

The most striking common feature in the OEC of both 158 159 monomers is a negative peak in the S₃-minus-S₁ electron-160 density difference maps inside the OEC (red in Figure 3), 161 corresponding to an overall expansion of the OEC when 162 evolving from the S_1 state to the S_3 state. As we mentioned 163 before, such an expansion is due to elongation of the Mn1-164 Mn4 and Mn2-Mn4 distances (Table 1) upon formation of 165 the "open" S₃ structural model. In addition, there are several 166 peaks outside the OEC due to protein rearrangements. Some of 167 these differences are consistent with those previously ¹⁶⁸ reported,²⁵ although they are beyond the scope of our study. In summary, our proposed model of the S₃ state is consistent 169 170 with XRD and EXAFS data. The model is also mechanistically 171 consistent with water binding to the OEC during the $S_2 \rightarrow S_3$ 172 transition, as previously suggested for ammonia binding at the 173 primary ammonia binding site of the OEC in the S2 state 174 (Figure 4). The additional water molecule (Wx) most likely



Figure 4. Proposed mechanism for S₃-state formation. The key step of the mechanism is the addition of the new water molecule Wx and the carousel rearrangement of W1 and W2 around Mn4.

175 enters from the "narrow"⁴⁶ water channel, when hydrogen bonded to O4 in the second coordination sphere of the five-176 coordinate Mn4(III) in the $g = 4.1 \text{ S}_2$ state (Figure 4A). Upon 177 III \rightarrow IV oxidation of Mn4, during the S₂ \rightarrow S₃ transition, Wx 178 must bind as the sixth ligand to Mn4, completing its octahedral 179 environment (Figure 4B) and leading to rearrangement of the 180 water ligands in a carousel around Mn4, as first proposed in our 181 182 ammonia binding studies,¹¹ and the subsequently reported "pivot" mechanism." 183

Rearrangement of W2 and O5 (Figure 4C,D) forms the 184 185 proposed "open" S₃ model, found to be consistent with EXAFS 186 and XRD data, even when considering incomplete $S_1 \rightarrow S_3$

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conversion, as discussed above. Our findings thus suggest 187 formation of three short Mn-Mn distances with an overall 188 expansion of the OEC cluster, consistent with the analysis of 189 the S_1 and S_3 electron densities from the XFEL data. The 190 proposed S3 structure is also consistent with W2 being one of 191 the substrate water molecules. However, more work is required 192 to determine the second substrate water molecule and the 193 mechanism of O-O bond formation. 194

ASSOCIATED CONTENT	195

Supporting Information

The Supporting Information is available free of charge on the 197 ACS Publications website at DOI: 10.1021/acs.bio- 198 chem.6b00041. 199

Description	and	analysis	of	EXAFS	and	QM/MM	200
simulations	(PDF)					201
PDB file with the proposed model (PDB)							202

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