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

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Supporting Information

ABSTRACT: The oxygen-evolving complex (OEC) of photosystem II has been studied in the S₃ state by electron paramagnetic resonance, extended X-ray absorption fine structure (EXAFS), and femtosecond X-ray diffraction (XRD). However, the actual structure of the OEC in the S₃ state has yet to be established. Here, we apply hybrid quantum mechanics/molecular mechanics methods and propose a structural model that is consistent with EXAFS and XRD. The model supports binding of water ligands to the cluster in the S₂ → S₃ transition through a carousel rearrangement around Mn4, inspired by studies of ammonia binding.

Photosystem II (PSII) is a protein–pigment complex found in the thylakoid membranes of the higher plants and internal membranes of cyanobacteria. During the light period of photosynthesis, PSII performs water oxidation with oxygen evolution.^{1–3} The reaction is triggered by the photochemical oxidation of the chlorophyll *a* species P₆₈₀ that induces electron–hole separation at the reaction center. The electron hole is transferred from P₆₈₀ to the redox-active tyrosine, Y_Z, which in turn oxidizes the CaMn₄O₅ cluster of the oxygen-evolving complex (OEC). The high efficiency and low overpotentials^{4,5} make the OEC a prototype for development of artificial water-oxidation catalysts. However, the structural changes of the OEC responsible for efficiency and functionality, along the catalytic cycle, have yet to be established at the molecular level.

In each turn of the cycle, the OEC evolves through five “storage” states, S_{*n*} (*n* = 0–4), with S₀ and S₄ being the most reduced and oxidized forms, respectively.^{6,7} After the S₄ state is formed, O₂ is spontaneously released and the S₀ state is restored, completing the catalytic cycle. Structural models for the S₀–S₂ states have been found to be consistent with femtosecond X-ray diffraction (XRD) and high-resolution extended X-ray absorption fine structure (EXAFS) data.^{8–11} However, the structure of the OEC cluster in the S₃ state remains controversial.

W-Band electron paramagnetic resonance (EPR) spectroscopy has determined that the OEC in the S₃ state has Mn(IV) centers with octahedral environments.¹² It is established that the S₂ → S₃ conversion involves binding of a water molecule (W_x) to the OEC,^{13–15} although the coordination site remains uncertain. Ammonia binding to the S₂ state^{16–20} provided valuable insights because water and ammonia are electronic and

structural analogues. On the basis of EPR^{18,21} and FTIR²² spectroscopy and measurements of reduction potentials,²³ we have recently proposed that ammonia binds in a terminal motif as the sixth ligand of the dangling Mn4.¹¹ In the work presented here, we examine analogous water binding. We introduce an S₃ model based on our studies of ammonia binding,¹¹ using quantum mechanics/molecular mechanics (QM/MM) hybrid methods and calculations of EXAFS spectra that allow for direct comparisons to EXAFS data, and difference electron-density maps from XRD.

We find that a structure in which Mn4 is linked to Mn3 with two μ-oxo bridges and Mn1 has O5 coordinated as an OH ligand (Figure 1) is most consistent with EXAFS spectroscopy.

Figure 1. QM/MM-optimized structure of the OEC in the S₃ state (Mn oxidation states: IV, 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–Mn3 distance, from 2.83 to 2.78 Å, forming three equivalent Mn–Mn distances in the range of 2.75–2.78 Å, and stretches the long Mn1–Mn3 and Mn1–Mn4 distances by ~0.15 Å, leading to an overall expansion of the cluster when compared to the OEC in the S₁ state. These structural changes are supported by experimental data of observed S₃–minus–S₁ electron-density differences,²⁵ and direct comparisons to differences of simulated electron-density maps based on the S₁ and S₃ QM/MM models.

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

Our QM/MM model of the S_3 state is consistent with the so-called “open” structure of the OEC,^{33,34} which can be formed from the “closed” cubane structure upon proton transfer from a water ligand of Mn4 (W2) to O5, forming the HOS ligand of Mn1 and an additional μ -oxo bridge linking Mn4 and Mn3. Our “open” structure is more stable than the “closed” form by 35 kcal mol^{−1} and shows excellent agreement with EXAFS data (Figure 2).

Figure 2. Comparison of calculated (red) and experimental²⁴ (black) Mn EXAFS spectra of the S_3 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 S_3 EXAFS sample.²⁴

Figure 2 shows that there is very good agreement between the experimental EXAFS spectrum of the OEC in the S_3 state and the calculated spectrum based on our S_3 QM/MM model. The structure is stabilized by a very strong hydrogen bond between the OH ligand of Mn1 and the additional μ -oxo bridge linking Mn4 and Mn3. Refinement of the QM/MM structure by simulated annealing Monte Carlo (MC) leads to no significant changes in the Mn–Mn distances (Table 1), with three short Mn–Mn distances [Mn1–Mn2 (2.78 Å), Mn2–Mn3 (2.78 Å), and Mn3–Mn4 (2.76 Å)] and one short intermetallic distance corresponding to the Mn1–Mn3 distance (3.50 Å). The resulting spectrum is thus similar to the EXAFS spectrum of the S_2 $g = 4.1$ state, as previously reported.^{35,36}

We note that while the theoretical and experimental spectra show very good agreement, the sample used to collect the EXAFS data was not 100% S_3 .²⁴ In fact, misses and double hits resulting from the Xe lamp flashes led to a final composition of 60% S_3 , 25% S_2 , 12% S_0 , and 3% S_1 .²⁴ Therefore, for the sake of completeness, we compared the EXAFS spectrum based on the 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 ($S = 1/2$ spin isomer),¹¹ S_1 ¹⁰ and S_0 ¹⁰ states (Figure 2C,D). To assess our results, we calculated the root-mean-square deviation (rmsd) between the calculated and experimental EXAFS spectra in k-space. We found that including the fractions of other S states leads to a slight improvement of the comparison with the experiment in k-space (Supporting Information) without significantly affecting the results in the reduced distance space.

Mn–Mn distances of >5 Å, such as Mn1–Mn4 and Mn2–Mn4 distances, can be probed by XRD, although they are perhaps too long to be reliably determined by EXAFS spectroscopy. XRD has been essential in determining the structure and ligation scheme of the OEC in the dark-stable S_1 state.^{37–41} In addition, recent advances in XRD techniques based on high-intensity femtosecond X-ray pulses have permitted collection of PSII data in intermediate states of the catalytic cycle, although at low resolution.^{25,42,43} Here, we analyze the data from ref 25, at 5.5 Å resolution, where the dark-stable S_1 state was advanced by two flashes to achieve some population of the S_3 state. Therefore, changes in the electron-density maps before and after the flashes should report on the underlying changes due to S_3 formation.

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

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 electron-density 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 were obtained from ref 25, as follows. First, the two observed data sets were scaled to each other to remove any Wilson B factor differences. Second, a $2F_{\text{obs}}(S_3) - F_{\text{obs}}(S_1)$ map was calculated in the S_3 unit cell, using the S_3 model phases, and the $2F_{\text{obs}}(S_1) - F_{\text{obs}}(S_3)$ map was calculated in the S_1 unit cell,

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

The most striking common feature in the OEC of both monomers is a negative peak in the S_3 -minus- S_1 electron-density difference maps inside the OEC (red in Figure 3), corresponding to an overall expansion of the OEC when evolving from the S_1 state to the S_3 state. As we mentioned before, such an expansion is due to elongation of the Mn1–Mn4 and Mn2–Mn4 distances (Table 1) upon formation of the “open” S_3 structural model. In addition, there are several peaks outside the OEC due to protein rearrangements. Some of these differences are consistent with those previously reported,²⁵ although they are beyond the scope of our study.

In summary, our proposed model of the S_3 state is consistent with XRD and EXAFS data. The model is also mechanistically consistent with water binding to the OEC during the $S_2 \rightarrow S_3$ transition, as previously suggested for ammonia binding at the primary ammonia binding site of the OEC in the S_2 state (Figure 4). The additional water molecule (Wx) most likely

Figure 4. Proposed mechanism for S_3 -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.

enters from the “narrow”⁴⁶ water channel, when hydrogen bonded to O4 in the second coordination sphere of the five-coordinate Mn4(III) in the $g = 4.1$ S_2 state (Figure 4A). Upon III $\rightarrow$ IV oxidation of Mn4, during the $S_2 \rightarrow S_3$ transition, Wx must bind as the sixth ligand to Mn4, completing its octahedral environment (Figure 4B) and leading to rearrangement of the water ligands in a carousel around Mn4, as first proposed in our ammonia binding studies,¹¹ and the subsequently reported “pivot” mechanism.⁴⁷

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

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

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.6b00041.

Description and analysis of EXAFS and QM/MM simulations (PDF)

PDB file with the proposed model (PDB)

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Notes

The authors declare no competing financial interest.

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