Computational Insights on Crystal Structures of the Oxygen-Evolving Complex of Photosystem II with either Ca^{2+} or Ca^{2+} Substituted by Sr^{2+}

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KEYWORDS: *Photosystem II, Oxygen-Evolving Complex, QM/MM, Sr substitution*

ABSTRACT: The oxygen-evolving complex of photosystem II can function with either Ca^{2+} or Sr^{2+} as the heterocation, but the reason for differing turnover rates remains unresolved despite reported X-ray crystal structures for both forms. Using quantum mechanics/molecular mechanics (QM/MM) calculations, we optimize structures with each cation in both the resting state (S_1) and in a series of reduced states (S_0 , S_{-1} , and S_{-2}). Through comparison with experimental data, we determine that X-ray crystal structures with either Ca^{2+} or Sr^{2+} are most consistent with the S_{-2} state, $\text{Mn}_4[\text{III},\text{III},\text{III},\text{II}]$ with O4 and O5 protonated. As expected, the QM/MM models show that $\text{Ca}^{2+}/\text{Sr}^{2+}$ substitution results in elongation of the heterocation bonds and displaces terminal waters W3 and W4. The optimized structures also show that hydrogen-bonded W5 is displaced in all S states with Sr^{2+} as the heterocation, suggesting that this water may play a critical role during water oxidation.

Photosystem II (PSII) is a large, multi-subunit protein complex found in the thylakoid membranes of higher plants and algae, and in the internal membranes of cyanobacteria.¹⁻³ Using solar energy absorbed by the reaction center chlorophylls, PSII oxidizes two water molecules and generates molecular O_2 as a by-product. Water oxidation occurs at the oxygen-evolving complex (OEC), composed of a CaMn_4O_5 catalytic core.⁴ The catalytic mechanism requires four sequential oxidation and deprotonation steps to advance the OEC through the series of S states, with S_0 and S_4 the most reduced and oxidized states, respectively.^{5, 6} Intriguingly, Sr^{2+} is the only cation that can functionally replace Ca^{2+} in the OEC, although turnover rates are slower.⁷⁻¹⁰ Here, we address the structural changes that might modify the function upon substitution of Ca^{2+} by Sr^{2+} .

The X-ray diffraction (XRD) structure of PSII from a thermophilic cyanobacteria, reported at 1.9 Å resolution (PDB: 3ARC),¹¹ shows that the OEC core is a cuboidal CaMn_3 cluster with a dangling Mn (i.e., Mn4) held together by μ -oxo bridges (O1-O5) and protein side chains (Figure 1). Mn4 is linked to the cube by two μ -oxo bridges (O4 and O5) and the carboxylate group of D170 bridges it to Ca^{2+} . Two terminal water molecules (W1, W2) bind to Mn4 and another two terminal water molecules (W3, W4) bind to Ca^{2+} . A 2.1 Å resolution XRD model of the OEC with Ca^{2+} substituted by Sr^{2+} has also been reported with a similar cuboidal OEC structure (PDB: 4IL6), denoted here as Sr^{2+} -OEC.¹²

Figure 1. QM/MM optimized Ca^{2+} -OEC structure in the S_{-2} state with both O4 and O5 protonated. Atoms in light red show positions that are different when Sr^{2+} is the heterocation. Bonds to OEC ligands (from subunit D1 unless otherwise noted) and terminal waters (W1-W4) are shown as grey and black dashed lines, respectively. Hydrogen bonds are shown as red dashed lines. For clarity, His332, CP43-Arg357, and other waters are not shown.

In S_1 , the dark-adapted resting state, the oxidation state of the OEC is most likely $\text{Mn}_4[\text{III},\text{IV},\text{IV},\text{III}]$.^{13, 14} (Throughout this article, square brackets are used to indicate the oxidation state of each Mn in the order reported in the 1.9 Å XRD structure.) However, during crystallographic data collection, at least 20% of the Mn centers are fully reduced to $\text{Mn}(\text{II})$,³ according to the X-

ray dose dependent presence of Mn(II) in PSII samples.¹⁵ From this, we infer that each photoreduced OEC should contain at most one Mn(II) center. In fact, it has been proposed that the 1.9 Å Ca²⁺-OEC structure is reduced beyond the S₀ state,¹⁴ possibly by one¹⁶ or more^{17, 18} electrons. However, comparison of the photo-reduced structures of Ca²⁺-OEC and Sr²⁺-OEC has not yet been reported.

Building upon our previous work based on density functional theory quantum mechanics/molecular mechanics (DFT-QM/MM) models, calculations of extended X-ray absorption fine structure (EXAFS) spectra, and direct comparisons to experimental raw data (in *k*-space) for the S₁¹⁴ and S₀¹⁹ states of the Ca²⁺-OEC, we now explore the effects of Sr²⁺ substitution and X-ray reduction. We report models of the OEC structure in the S₁, S₀, S₋₁ and S₋₂ states with either Ca²⁺ or Sr²⁺ as the heterocation obtained at the DFT-QM/MM level. These models are larger than recent work concerning the protonation of the S₁ state²⁰ and do not consider the geometry of the OEC in the higher S states,^{21, 22} which are not relevant in the context of the crystal structures. Comparison of our models to XRD coordinates shows that both Ca²⁺ and Sr²⁺ XRD structures best fit the S₋₂ Mn₄[III,III,III,II] state with at least one protonated μ -oxo bridge. Furthermore, comparison of atomic locations between Ca²⁺-OEC and Sr²⁺-OEC for each S state reveals that the W5 water molecule bridging W2 and W3 is consistently displaced upon Sr²⁺ substitution.

COMPUTATIONAL METHODS

The DFT-QM/MM models were built and optimized using a two layer ONIOM H-atom link approach²³ implemented in Gaussian09 v.C01 or v.D01,²⁴ as recently reported for the S₀ state.¹⁹ The QM layer includes the side chains of D1 (D61, D170, E189, H332, E333, H337, D342, A344) and CP43 (E354, R357), and ten surrounding water molecules, including those bound to Mn4 and Ca²⁺/Sr²⁺. We use the B3LYP functional^{25, 26} with the LANL2DZ pseudo-potential^{27, 28} for Ca, Sr, and Mn, and the 6-31G* basis set²⁹ for all other atoms. Residues with C α atoms within 15 Å of the OEC are included in the MM layer and described using the AMBER force field.³⁰ In the present work, D1-His337 is assumed to be doubly protonated; although structures with neutral D1-His337 give very similar results, the agreement with the XRD coordinates is slightly better when this residue is positively charged (see SI, Section S6). The Sr²⁺-OEC structures are prepared analogously with Ca²⁺ substituted by Sr²⁺. Further details of the QM/MM method can be found in the Supporting Information, along with optimized coordinates, Mn spin densities, interatomic distances for each state, and calculations of EXAFS spectra. Thermal fluctuations are included in our EXAFS calculations as Debye-Waller factors accounting for fluctuations of distances due to structural and/or thermal disorder under the assumption of

small displacements and Gaussian distributions of distances. While the analysis is focused on direct comparisons with experimental data at low temperature (110 K), explicit treatment of thermal fluctuations could also be modeled at room temperature by molecular dynamics simulations, as previously reported.³¹ Nevertheless, the reported comparison of structures at low temperature is biologically meaningful since it has been shown that temperature effects are negligible when analyzing the structure, or protonation state of PSII intermediates at 20 K and room temperature.³²

The S₁ state is optimized with neutral terminal waters and all μ -oxo bridges deprotonated. Upon reduction, a proton is typically added along with each electron to maintain the net charge during oxidation-state transitions. For the S₀ state, results are reported for calculations in which the proton is placed on either O4 or O5. For the S₋₁ and S₋₂ states, both O4 and O5 are protonated.

RESULTS AND DISCUSSION

The QM/MM optimized structures show good agreement with Mn³² and Sr³³ K-edge EXAFS data for the S₁ state with Ca²⁺ and Sr²⁺, respectively (see SI Section S5). Furthermore, the QM/MM models of the Ca²⁺-OEC in the S₋₁ and S₋₂ states, each with one Mn(II), produce Mn EXAFS spectra similar to the spectrum of X-ray exposed PSII solution samples with ~25% Mn(II) content¹⁵ (SI Figure S5). Although neither S₋₁ nor S₋₂ give a complete match, the experimental data come from a fully hydrated sample likely to contain a mixture of states from exposure to higher X-ray doses than in the crystal structure. In general, the ability of QM/MM models to produce EXAFS spectra similar to the experimental results in several S states and with both Ca²⁺ and Sr²⁺ provides validation of our computational method.

Using the QM/MM models, we first look for trends that result from Ca²⁺/Sr²⁺ substitution in the OEC. Figure 1 shows the DFT-QM/MM optimized structures for the OEC in the S₋₂ state, including terminal water molecules bound to Ca²⁺ (red) and Sr²⁺ (light red) (analogous OEC models for the other S states are shown in SI, Figure S3). We find that there are no long-range effects induced by Sr²⁺ on the structure of the surrounding protein. As in the Ca²⁺ and Sr²⁺ XRD structures, where the root mean square deviation (RMSD) of all C α atoms for residues in the QM/MM selection is 0.09 Å,¹² the optimized QM/MM models also show good agreement in protein positions for each state. Furthermore, heterocation substitution does not significantly affect the cuboidal structure of the OEC (see, e.g., Mn-Mn distances in SI Tables S3 and S4). This is consistent with experimental and computational reports suggesting that the OEC structure does not undergo significant changes upon Ca²⁺ depletion^{34, 35} or Ca²⁺/Sr²⁺ substitution.³⁶ Therefore, the heterocation is not expected to play a dominant

role in the stabilization of the OEC core and is consistent with the low occupancy of Sr^{2+} (0.7) in the XRD structure.¹²

The QM/MM models indicate that Sr^{2+} is positioned slightly further away from the OEC when compared to Ca^{2+} , consistent with the larger ionic radius of Sr^{2+} vs. Ca^{2+} . The coordination bond lengths for W3 and W4 to the heterocation are also longer for Sr^{2+} (Table 1). Another change upon $\text{Ca}^{2+}/\text{Sr}^{2+}$ substitution is in the location of the water molecule (W5) that bridges W2 and W3 via hydrogen bonds (Figure 1).

Table 1. Bond lengths (in Å) between $\text{Ca}^{2+}/\text{Sr}^{2+}$ and μ -oxo bridges or terminal waters in XRD and QM/MM models.

$\text{Ca}^{2+}/\text{Sr}^{2+}$ to:		O1	O2	O5	W3	W4
XRD^a	A	2.33	2.49	2.49	2.39	2.49
	Ca^{2+} B	2.40	2.46	2.79	2.41	2.38
QM/MM	S_1	2.47	2.49	2.60	2.42	2.52
	S_0^{O4H}	2.45	2.87	2.46	2.45	2.53
	S_0^{O5H}	2.42	2.78	2.47	2.48	2.51
	S_{-1}	2.46	2.79	2.47	2.45	2.52
	S_{-2}	2.39	2.68	2.46	2.50	2.53
XRD^a	A	2.42	2.64	2.56	2.54	2.32
	Sr^{2+} B	2.46	2.67	2.62	2.74	2.27
QM/MM	S_1	2.57	2.86	2.70	2.57	2.70
	S_0^{O4H}	2.58	2.99	2.58	2.57	2.70
	S_0^{O5H}	2.54	2.89	2.61	2.61	2.69
	S_{-1}	2.58	2.89	2.60	2.58	2.70
	S_{-2}	2.53	2.81	2.58	2.63	2.71

^a Distances from XRD monomers A and B of Ca^{2+} (PDB: 3ARC¹¹) and Sr^{2+} (PDB: 4IL6¹²) structures.

W5 is located between the heterocation and the active Mn centers, Mn1 and Mn4. As a hydrogen-bonded link between W2 and W3, the position of W5 directly affects the hydrogen-bonding network and electrostatic interactions around the OEC. The altered location of W5 upon $\text{Ca}^{2+}/\text{Sr}^{2+}$ substitution might, therefore, be responsible for the 3–4 fold increase in the exchange rate of “slow” substrate water with bulk water when Sr^{2+} is used to re-constitute Ca^{2+} -depleted PSII,³⁷ and the reported differences in 2D hyperfine sub-level correlation (HYSCORE) spectra.^{38, 39} The new positions of W3 and W5 in Sr^{2+} -substituted PSII also affect the hydrogen-bond network connecting the OEC to Y_Z (reviewed in Ref. ⁴⁰), which may account for the slower reduction of Y_Z^* in all S states,⁴¹ and especially in S_3 .⁴²

The observed shift in the W3 position is also consistent with a recent DFT model of the Sr^{2+} -OEC structure, optimized in the $\text{Mn}_4[\text{III},\text{III},\text{III},\text{III}]$ state,⁴³ although our DFT-QM/MM analysis suggests that the OEC S_{-1} state

is $\text{Mn}_4[\text{III},\text{IV},\text{III},\text{II}]$ (see SI Tables S1 and S2). The Mn oxidation states assigned by DFT-QM/MM are also in agreement with Density Matrix Renormalization Group (DMRG) results for S_{-1} using the Ca^{2+} -OEC XRD coordinates, although the geometry of the OEC could not be optimized at the DMRG level and the S_{-2} state with $\text{Mn}[\text{III},\text{III},\text{III},\text{II}]$ was not considered.¹⁶

The position of W4 as a terminal ligand to the heterocation is also affected by $\text{Ca}^{2+}/\text{Sr}^{2+}$ substitution (Table 1). We note that QM/MM structures have W4 bound to Sr^{2+} as H_2O , although the Sr-W4 bond is 0.4 Å longer than in the XRD model. Nevertheless, the Sr K-edge EXAFS spectrum shows reasonable agreement with experimental data (SI Figure S4c). We argue that the XRD position of W4 is due to the only 70% occupancy of Sr^{2+} , combined with crystal dehydration that can remove the hydrogen-bond acceptor for W4 (*i.e.*, the neighboring water not resolved in monomer B¹²), although deprotonation of W4 has been suggested.⁴³

In addition to the analysis of bond lengths (Table 1), we compare the overall structure of the OEC in both XRD and QM/MM models. We find S states with minimum RMSDs of the OEC atoms, computed after aligning the QM/MM models to both XRD monomers with respect to the protein C α atoms. The resulting RMSDs are shown in Figure 2 (values in SI, Table S7). We find that QM/MM structures for the S_0 state with O5H exhibit smaller RMSDs than with O4H, in accordance with our previous work based on the analysis of the Mn EXAFS data.¹⁹ However, the S_{-1} and S_{-2} states with both O4 and O5 protonated fit the XRD model better than the S_1 or S_0 states with either O4H or O5H. Therefore, we propose that the OEC initially in the S_1 state might be quickly reduced by the X-ray radiation more than once, as in the presence of chemical reductants,⁴⁴ leaving little population in the S_0 form.

Figure 2. RMSDs for OEC atoms in QM/MM models relative to XRD coordinates when aligned using protein backbone atoms.

An optimized linear combination of QM/MM coordinates indicates that both Ca^{2+} and Sr^{2+} XRD structures are at least 40% in the S_{-2} state (see SI Section S6). For the better-resolved Ca^{2+} -OEC structure, the S_1 state also contributes around 40%, consistent with the dark-adapted crystals becoming photoreduced during data

collection. We note that the reduced states considered here contain at most one Mn(II) center, unlike the more reduced state previously suggested to account for the crystal structure of the Ca^{2+} -OEC.^{17, 18}

For comparison, we have also aligned the models based on the five OEC atoms with the lowest B-factors: O1, Mn1, Mn2, O3, and Mn3 (see SI, Figure S8 for XRD B-factors). The subsequent RMSDs for these atoms (< 0.14 Å for all cases) are close to the experimental error and show no major change upon reduction of the OEC. Figure 3 shows the RMSDs for atoms used in the alignment as open symbols; RMSDs for the remaining OEC atoms (Ca/Sr, O2, O4, Mn4, O5) are shown as shaded symbols. The second set of atoms includes those with larger B-factors that are more likely to move upon reduction during X-ray radiation. For these atoms, the RMSDs are consistently larger for all S states, but unlike the protein-aligned comparison, there is no clear improvement in XRD alignment as the OEC is reduced, indicating that no one state fits all of the XRD assigned coordinates.

Figure 3. RMSDs for OEC atoms in QM/MM models relative to XRD coordinates when aligned using well-resolved OEC atoms (O1, Mn1, Mn2, O3, Mn3).

To resolve which atoms are responsible for the improved structural alignment to XRD coordinates as the

OEC is reduced, we also determine the individual displacements of each atom. For the set of well-resolved OEC atoms, we again find little variation in the position relative to the XRD coordinates for either Ca^{2+} -OEC or Sr^{2+} -OEC (see SI Section S6). Only in the case of O1 in monomer A of the Sr^{2+} -OEC structure is the displacement for all S states larger than the experimental error, coinciding with O1 having a larger relative B-factor in this structure than in any other. The QM/MM position matches well with that of O1 in monomer B; this result suggests localized QM/MM optimization as an effective tool to refine XRD-assigned coordinate positions.

For the five OEC atoms with larger B-factors ($\text{Ca}^{2+}/\text{Sr}^{2+}$, O2, O4, Mn4, O5), analyzing individual displacements after partial OEC alignment proves to be instructive (Figure 4). For example, the positions of Ca^{2+} and Sr^{2+} in S_1 QM/MM models are in line with the XRD data. For more reduced states, Ca^{2+} is typically closer to the Sr^{2+} XRD position (open symbols in Figure 4). In general, however, no particular trend is apparent in the displacements of $\text{Ca}^{2+}/\text{Sr}^{2+}$ or O2 as the OEC is reduced. The larger B-factors of these atoms are likely due to difficulty resolving the electron density next to the Mn atoms rather than atomic movement. For O4 and Mn4, the RMSDs are not improved in the more reduced S states, which is unsurprising given their large B-factors and their role as atoms that move upon two-electron reduction. However, O5 behaves quite differently. In this case, there is a clear improvement in the location of the O5 atom upon protonation that is concurrent with OEC reduction in our models. Since O4 is already stabilized by a water hydrogen bond, it is likely that O5 is the μ -oxo bridge most accessible for protonation. We note that since the S_1 state that best fits the EXAFS data does not have O5 protonated,^{15,16} and both the Ca^{2+} and Sr^{2+} XRD structures have O5 in the same position, the protonation of O5 may occur during crystal preparation or early during the data collection procedure (probably as the OEC is reduced to the S_0 state).

Figure 4. Displacement of selected OEC atoms for each QM/MM model relative to X-ray coordinates when aligned using well-resolved OEC atoms.

CONCLUSIONS

Based on the comparison of bond lengths and atomic positions in QM/MM models to the reported XRD co-

ordinates for PSII, we determine the effect of X-ray reduction and protonation on the structure of the OEC. The QM/MM optimized coordinates with an S₂ oxidation state of Mn₄[III,III,III,II] and both O4 and O5 protonated fits the XRD data of both Ca²⁺ and Sr²⁺ PSII better than any other S state. The QM/MM models with either heterocation reproduce the XRD position of O5 only when this atom is protonated. Based on the optimized coordinates, we can also suggest improved positions for atoms that are difficult to resolve, such as the μ -oxo bridges that are next to the partially occupied Sr²⁺ atom. In addition, the displacement of W5 as a result of Sr²⁺ substitution in all of the S states studied here leads us to propose that this water may play an important role in the mechanism of water oxidation.

ASSOCIATED CONTENT

Supporting Information. QM/MM method details; optimized coordinates, Mn spin densities, interatomic distances for OEC atoms, and structures for each S state; EXAFS simulations; RMSDs after protein or OEC alignment. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Funding Sources

The authors acknowledge funding by the Division of Chemical Sciences, Geosciences & Biosciences, Office of Basic Energy Sciences, U.S. Department of Energy (DOE): V.S.B. Grant DE-SC0001423; M.Z.E. Contract DE-AC02-98CH10886, a Computational Materials and Chemical Sciences (CMCSN) project at Brookhaven National Laboratory; G.W.B. Grant DE-FG02-05ER15646 and super-computer time from NERSC and from the High Performance Computing facilities at Yale University.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

The authors acknowledge helpful discussions with Sahr Khan and Drs. Christian Negre, David Vinyard, and Ravi Pokhrel. Figures in this manuscript were prepared with VMD⁴⁵ using colors selected from ColorBrewer 2.0.⁴⁶

ABBREVIATIONS

DFT, Density Functional Theory; DMRG, Density Matrix Renormalization Group; EXAFS, Extended X-ray Absorption Fine Structure; OEC, Oxygen-Evolving Complex; ONIOM, "our own n-layered integrated molecular orbital and molecular mechanics"; PDB, Protein

Data Bank; PSII, Photosystem II; QM/MM, Quantum Mechanics/Molecular Mechanics; RMSD, Root Mean Square Deviation; VMD, Visual Molecular Dynamics; XRD, X-ray Diffraction

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Table of Contents Graphic

Photosystem II
Oxygen-Evolving
Complex
(with Ca^{2+} or Sr^{2+})

X-ray
reduction