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NH_3 Binding to the S_2 State of the O_2-Evolving Complex of Photosystem II: Analogue to H_2O Binding during the $S_2 \rightarrow S_3$ Transition

Mikhail Askerka, David J. Vinyard, Gary W. Brudvig, and Victor S. Batista*

Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107, United States

Supporting Information

ABSTRACT: Ammonia binds directly to the oxygen-evolving complex of photosystem II (PSII) upon formation of the S_2 intermediate, as evidenced by electron paramagnetic resonance spectroscopy. We explore the binding mode by using quantum mechanics/molecular mechanics methods and simulations of extended X-ray absorption fine structure spectra. We find that NH_3 binds as an additional terminal ligand to the dangling Mn4, instead of exchanging with terminal water. Because water and ammonia are electronic and structural analogues, these findings suggest that water binds analogously during the $S_2 \rightarrow S_3$ transition, leading to rearrangement of ligands in a carrousel around Mn4.

Photosystem II (PSII) is a protein−pigment complex responsible for production of molecular oxygen (O_2) in higher plants, algae, and cyanobacteria during the light reactions of photosynthesis.[1−4] O_2 evolves from catalytic water oxidation,[5,6] driven by light absorption and charge separation across the membrane bilayer. The primary chlorophyll *a* donor P_{680}, formed in the charge-separated state $P_{680}^{+\bullet}$, oxidizes the redox-active tyrosine Y_Z to form $Y_Z^{\bullet}$, which in turn oxidizes the oxygen-evolving complex (OEC) where substrate water molecules bind. The OEC is a Mn_4CaO_5 cluster in which metal centers are linked by μ-oxo bridges forming a cuboidal $CaMn_3O_4$ core with a dangling Mn. As the catalytic cycle proceeds, the OEC accumulates oxidizing equivalents, evolving through so-called "storage states" S_n (n = 0−4), with S_0 being the most reduced and S_4 the most oxidized state.[5,6] The S_0 state is regenerated from the S_4 state when the substrate water molecules transform into O_2, reducing the OEC and releasing protons to the thylakoid lumen. The OEC is able to oxidize water efficiently at low overpotentials,[7,8] which makes it a prototype for artificial homogeneous and heterogeneous water oxidation catalysts. However, despite the tremendous effort put into elucidating the mechanism of water oxidation by the OEC, one of the main questions, assigning substrate water molecules, remains a matter of debate.

Studies of ammonia binding provide valuable insights into the active site for water coordination because water and ammonia are electronic and structural analogues. Ammonia binds to two sites in PSII. Secondary binding site is in the outer shell of amino acids around the OEC and is competitive with chloride.[9−12] In the primary binding site, which is the focus of this work, ammonia coordinates to Mn in the OEC upon formation of the S_2 state.[13,14] The binding of ammonia, however, does not completely block oxygen evolution,[14,15] indicating that substrate waters can still bind and react at the OEC in the presence of ammonia.[14−17]

Earlier studies of ammonia binding have proposed two possibilities: the terminal binding model in which ammonia replaces a terminal water ligand on the dangling Mn (Mn4) and the bridging model in which ammonia replaces the O5 μ-oxo bridge (see Figure 1 for the labeling of atoms in the OEC). The bridging model has been supported by early experiments of electron spin echo envelope modulation (ESEEM).[13] $^{14}NH_4Cl$- and $^{15}NH_4Cl$-treated PSII samples exhibit nuclear quadrupole couplings that could be interpreted in terms of an NH_2 bridge between two Mn centers by comparison with model compounds.[13] The bridging model is also in line with the Fourier transform infrared spectroscopy (FTIR) experiments,[18] showing that the 601 cm^{-1} mode (assigned to a Mn−O−Mn vibration) is lost upon ammonia binding. In addition, the kinetic analysis of flash-induced oxygen evolution experiments also favored a bridging model that could stabilize the S_2 state because there is a significant (>120 mV) decrease in the S_2 reduction potential upon ammonia binding.[19] Another important observation is that the binding site of ammonia is accessible only to NH_3, while substituted amines with similar ligating properties do not bind to the OEC.[12]

The terminal binding model has been supported by various electron paramagnetic resonance (EPR) measurements.[16] Changes in the spectral envelope widths and hyperfine couplings in the ^{17}O-EDNMR W-band spectra of $^{14}NH_3$-treated PSII were interpreted in terms of exchange of water (W1) with ammonia, while the perturbation of the O5 μ-oxo bridge signal was assigned to a *trans* effect. Such an interpretation was supported by density functional theory (DFT) calculations, showing that terminal binding can be energetically favored.[20] Very recently, a study based on pulsed EPR experiments[21] found a significant change in the ammonia signal upon comparison of wild-type PSII with D1-D61A mutated PSII. That observation offered compelling evidence of direct hydrogen bonding of ammonia to D61, thus supporting the terminal binding model.

In this work, we explore various motifs of ammonia binding by using quantum mechanics/molecular mechanics (QM/MM)

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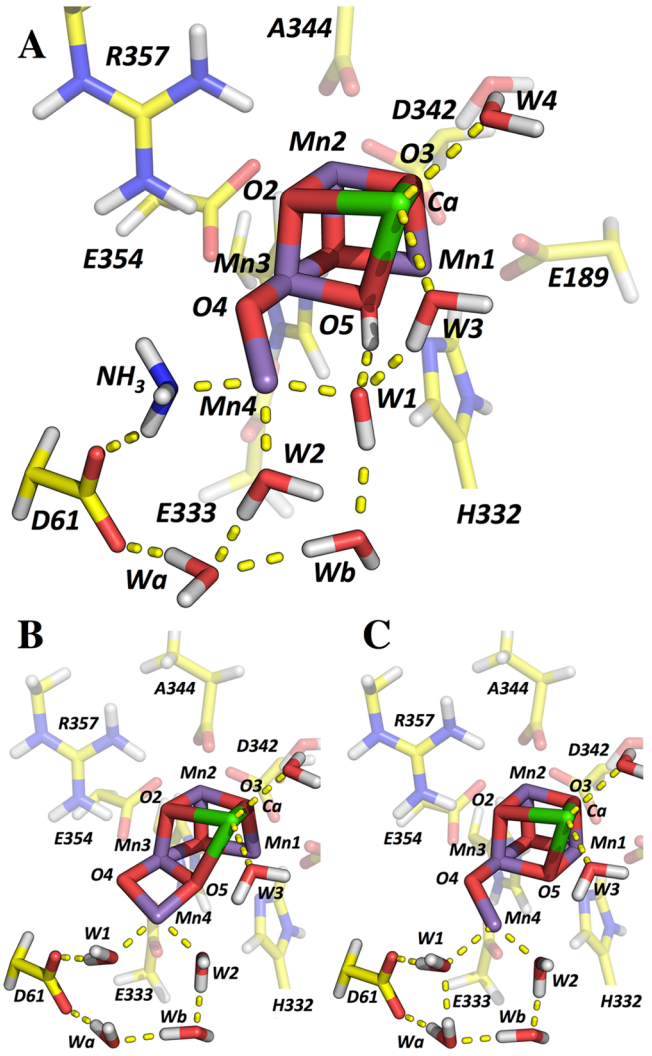

Figure 1. (A) Quantum mechanics/molecular mechanics optimized structure of the NH_3-bound OEC S_2 state (III, IV, IV, IV). (B) Native S_2 state in the g = 2.0 form (III, IV, IV, IV). (C) Native S_2 state in the g = 4.1 form (IV, IV, IV, III), including coordination of water ligands as well as D1-D61, D1-His337, and CP43-R357. For the sake of clarity, D1-D170 is not shown.

hybrid methods and calculations of extended X-ray absorption fine structure (EXAFS) spectra that allow for direct comparisons to experimental data (methods described in the Supporting Information). We find that ammonia binds as an additional terminal ligand to the dangler manganese of the OEC (Mn4) in the S_2 state as shown in Figure 1A, completing the octahedral coordination sphere and stabilizing oxidation states Mn1(III), Mn2(IV), Mn3(IV), and Mn4(IV) by triggering the protonation of O5 via deprotonation of W2. As we discuss below, such a binding motif is most consistent with experimental data, including EXAFS, EPR, and FTIR spectroscopy.

The g = 4.1 EPR signal of the S_2 state has been attributed to an OEC spin isomer with oxidation states Mn1(IV), Mn2(IV), Mn3(IV), and Mn4(III), where Mn4 is pentacoordinate and linked to Mn3 by a single μ-oxo bridge established by O4, while O5 links Mn1 and Mn3 (Figure 1C).[22] Such a model shares common structural features with the model shown in Figure 1A. This S_2 isomer becomes the predominant component when ammonia binds competitively with Cl^- to a secondary site.[12] It is, therefore, plausible that ammonia binds first to the secondary site (likely in the second coordination sphere of Mn4), stabilizing the OEC structure of the g = 4.1 isomer before binding to Mn4 by displacing W1 and W2 in a carrousel around Mn4.

According to the proposed scenario, ammonia binds as the sixth ligand of the dangling Mn4, inducing W2 to protonate O5 and become a stronger ligand of Mn4, while making the protonated O5 a weaker ligand of Mn1 (Table S1 of the Supporting Information). As a result, ammonia binding as the sixth terminal ligand to Mn4 triggers a redox-switch mechanism that changes the oxidation states of the OEC from Mn1(IV), Mn2(IV), Mn3(IV), and Mn4(III) to Mn1(III), Mn2(IV), Mn3(IV), and Mn4(IV), respectively. The resulting oxidation state pattern (III, IV, IV, IV) is consistent with the g = 2.0 EPR signal of ammonia-bound PSII in the S_2 state.[14]

We note that protonation of O5 is consistent with the loss of the 601 cm^{-1} Mn−O−Mn mode reported by FTIR experiments.[18] In addition, the resulting structure of hydrogen bonds and completion of the Mn4 octahedral coordination sphere provide significant stability to the ammonia-bound OEC, consistent with the decrease in the S_2 reduction potential upon ammonia binding.[19] This increased stability as well as the fast rate of binding of ammonia to PSII (0.5 s at 240 K)[15] suggests that ammonia makes the OEC less susceptible to X-ray radiation damage.[20] Therefore, EXAFS measurements are expected to be particularly informative.

Figure 2 shows the comparison of calculated and experimental EXAFS spectra, validating the QM/MM model of the NH_3-bound S_2 state shown in Figure 1A. QM/MM optimizations were performed using the B3LYP[23,24] functional with the LANL2DZ[25,26] pseudopotential for Ca and Mn and the 6-31G*[27] basis set for all other atoms as in our previous models.[28−30] The AMBER force field[31] was used for all MM

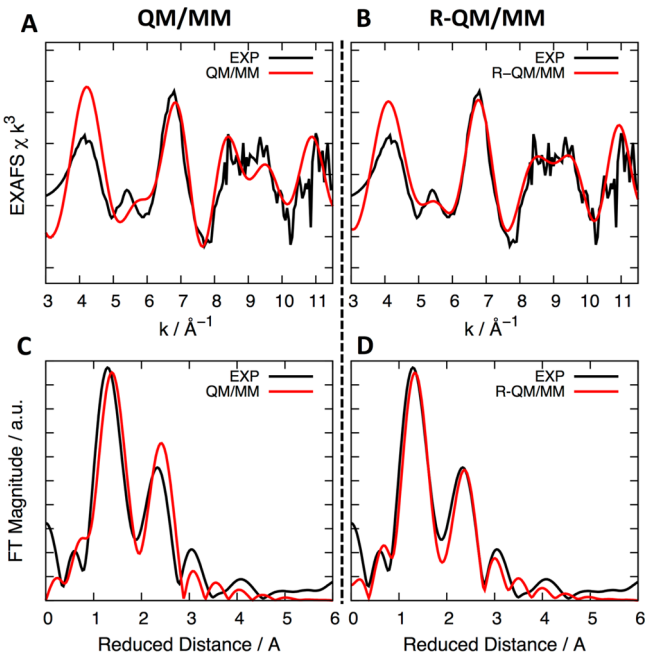

Figure 2. Comparison of calculated (red) and experimental[35] (black) EXAFS Mn spectra of ammonia-bound PSII in the S_2 state in k-space (A and B) and reduced distance (C and D) for the QM/MM-optimized (I_t, A and C) and QM/MM-refined (I_t^{ref}, B and D) structures.

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layer atoms. The EXAFS spectrum was calculated using the *ab initio* real space Green's function approach as implemented in FEFF (version 8.30).[32] The comparison of Figure 2 shows very good agreement between calculated and experimental spectra, validating the model in which binding of ammonia to Mn4 stabilizes two long Mn−Mn distances, including Mn3−Mn4 (3.21 Å) and Mn1−Mn3 (3.10 Å) distances, and two short intermetallic distances corresponding to Mn1−Mn2 (2.75 Å) and Mn2−Mn3 (2.73 Å) distances. The resulting spectrum is thus similar to the EXAFS spectrum of the S_2 g = 4.1 state, as previously reported.[33,34] Refinement of the QM/MM-optimized structure (I_t) by simulated annealing Monte Carlo (described in the Supporting Information) generates structure I_t^{ref} in excellent agreement (Table S1 of the Supporting Information) with experimental data, exhibiting only a slight elongation (0.04 Å) of the Mn3−Mn4 distance, within the range of error for DFT geometry optimization.

The EXAFS spectrum of the ammonia-bound S_2 state is not only similar to the spectrum of the native S_2 g = 4.1 state but also similar to the spectrum of the native S_3 state.[33] Therefore, the QM/MM model of the ammonia-bound S_2 structure is expected to provide valuable insights into the structure of the OEC in the S_3 state. We propose that ammonia binds to Mn4(III) in the S_2 state and that under normal conditions water binds to the same site in the S_3 state [now Mn4(IV)]. Therefore, the binding of ammonia, a Lewis base that is "harder" than water, in S_2 is an analogue of the binding of water in S_3, as previously suggested.[36] This model differs from previous DFT studies that suggest water binds to Mn1 during the $S_2 \rightarrow S_3$ transition.[37,38] Here, we find that water binding as a sixth terminal ligand of Mn4 would be more consistent with the analysis of ammonia binding, leading to rearrangement of the W1 and W2 ligands in a carrousel around Mn4, deprotonation of W2 by protonation of O5, and redox-switch transition to oxidation states III, IV, IV, and IV before advancing to the S_3 state. A similar binding mode for an additional water molecule, completing the coordination sphere of Mn4 in the S_2 state, has been proposed by previous DFT calculations,[39] although the redox-switch mechanism was not suggested so the proposed oxidation states were different [i.e., Mn4(III) and Mn1(IV)] and, therefore, inconsistent with the recent pulsed EPR/mutagenesis study suggesting that D1-H332 is bound to Mn1(III) while the new ligand binds to Mn4(IV), as shown by the asymmetric spectroscopic feature that becomes symmetric in the D1-D61A mutant.[21]

Further work is required to analyze the implications of the proposed S_2 model with Mn4(IV) stabilized by an additional terminal water ligand for the $S_2 \rightarrow S_3$ transition and beyond. However, the considered scenario of ammonia binding unambiguously suggests W2 to be important for the catalytic mechanism. Because of the proximity between W3 and W2 and interaction through a H-bond, W3 might also be mechanistically important. Finally, we note that O5 plays an important role as a base for W2 proton abstraction resulting in O5 being protonated as suggested by previous computational models.[30]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.5b00974.

Description and analysis of EXAFS and QM/MM simulations and exploration of various sites of ammonia binding (PDF)

PDB file with the proposed model (PDB)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: victor.batista@yale.edu. Phone: (203) 432-6672. Fax: (203) 432-6144.

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Notes

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