NH₃ Binding to the S₂ State of the O₂-Evolving Complex of Photosystem II: Analogue to H₂O Binding during the S₂ → S₃ Transition

Mikhail Askerka  
_Yale University_

David J. Vinyard  
_Yale University_

Gary W. Brudvig  
_Yale University_

Victor S. Batista  
_Yale University_

Follow this and additional works at: https://repository.lsu.edu/biosci_pubs

**Recommended Citation**

This Article is brought to you for free and open access by the Department of Biological Sciences at LSU Scholarly Repository. It has been accepted for inclusion in Faculty Publications by an authorized administrator of LSU Scholarly Repository. For more information, please contact ir@lsu.edu.
NH₃ Binding to the S₂ State of the O₂-Evolving Complex of Photosystem II: Analogue to H₂O Binding during the S₂ → S₃ 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 S₂ 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 NH₃ 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 S₂ → S₃ transition, leading to rearrangement of ligands in a carousel around Mn₄.

Photosystem II (PSII) is a protein–pigment complex responsible for production of molecular oxygen (O₂) in higher plants, algae, and cyanobacteria during the light reactions of photosynthesis.¹⁻⁴ O₂ evolves from catalytic water oxidation,⁵,⁶ driven by light absorption and charge separation across the membrane bilayer. The primary chlorophyll a donor P₇₃₀⁺⁺⁺⁺⁺ oxidizes the redox-active tyrosine Y₇ to form Y₇⁺, which in turn oxidizes the oxygen-evolving complex (OEC) where substrate water molecules bind. The OEC is a Mn₄CaO₅ cluster in which metal centers are linked by µ-oxo bridges forming a cuboidal CaMn₃O₄ core with a dangling Mn. As the catalytic cycle proceeds, the OEC accumulates oxidizing equivalents, evolving through so-called "storage states" Sₙ (n = 0–4), with S₀ being the most reduced and S₄ the most oxidized state.⁵,⁶ The S₀ state is regenerated from the S₄ state when the substrate water molecules transform into O₂, 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.⁹⁻¹² 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 S₂ 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 OS 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).¹³,¹⁴NH₄Cl- and ¹⁵NH₄Cl-treated PSII samples exhibit nuclear quadrupole couplings that could be interpreted in terms of an NH₂ 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 S₂ state because there is a significant (>120 mV) decrease in the S₂ reduction potential upon ammonia binding.¹⁹ Another important observation is that the binding site of ammonia is accessible only to NH₃ 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 ¹⁷O-EDNMR W-band spectra of ¹⁴NH₃-treated PSII were interpreted in terms of exchange of water (W1) with ammonia, while the perturbation of the OS 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 experiments²¹ 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)
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 dangling manganese of the OEC (Mn4) in the S2 state as shown in Figure 1A, completing the octahedral coordination sphere and stabilizing oxidation states 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 S2 state.

We note that protonation of OS is consistent with the loss of the 601 cm\(^{-1}\) Mn\(\text{–O–Mn}\) mode reported by FTIR experiments. 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. This increased stability as well as the fast rate of binding of ammonia to PSII (0.5 s at 240 K) suggests that ammonia makes the OEC less susceptible to X-ray radiation damage. 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 S2 state shown in Figure 1A. QM/MM optimizations were performed using the B3LYP functional with the LANL2DZ pseudopotential for Ca and Mn and the 6-31G* basis set for all other atoms as in our previous models. The AMBER force field was used for all MM calculations.

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, 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.

Figure 2. Comparison of calculated (red) and experimental 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 (IT, A and C) and QM/MM-refined (IT\(_{\text{ref}}, B\) and D) structures.
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 S2 state, as previously reported.\(^{33,34}\) Refinement of the QM/MM-optimized structure (I) by simulated annealing Monte Carlo (described in the Supporting Information) generates structure I\(_{\text{ref}}\) 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 S2 state is not only similar to the spectrum of the native S1 state but also similar to the spectrum of the native S3 state.\(^{35,36}\) Therefore, the QM/MM model of the ammonia-bound S2 structure is expected to provide valuable insights into the structure of the OEC in the S1 state. We propose that ammonia binds to Mn4(III) in the S2 state and that under normal conditions water binds to the same site in the S3 state [now Mn4(IV)]. Therefore, the binding of ammonia, a Lewis base that is “harder” than water, in S2 is an analogue of the binding of water in S3, as previously suggested.\(^{30,36}\) This model differs from previous DFT studies that suggest water binds to Mn1 during the S2 → S3 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 carousel around Mn4, deprotonation of W2 by protonation of OS, and redox-switch transition to oxidation states III, IV, IV, and IV before advancing to the S3 state. A similar binding mode for an additional water molecule, completing the coordination sphere of Mn4 in the S2 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.\(^{41}\)

Further work is required to analyze the implications of the proposed S2 model with Mn4(IV) stabilized by an additional terminal water ligand for the S2 → S3 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 OS plays an important role as a base for W2 proton abstraction resulting in OS being protonated as suggested by previous computational models.\(^{30}\)

**ASSOCIATED CONTENT**

1. 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.*

**Funding**

We acknowledge support by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences, via Grants DE-FC0001423 (V.S.B.) and DE-FG0205ER15646 (G.W.B.) and computational resources from NERSC and Yale University.

**Notes**

The authors declare no competing financial interest.

**REFERENCES**


