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Photosystem II

Location of Potential Substrate Water Binding Sites in the Water Oxidizing Complex of Photosystem II**

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Water is the substrate for production of molecular oxygen in photosystem II (PSII). In a cyclic four-electron process, the Mn_4Ca cluster in the water-oxidizing complex (WOC) of PSII advances through four metastable (S_0 to S_3) and one unstable state (S_4) known as the S states.[1] However, despite intense scrutiny, the geometry and mechanism of the Mn_4Ca site and the metal oxidation states at which water oxidation occurs, remain contentious.

Clues to unraveling the mechanism of water oxidation lie principally in the active-site geometry, specifically, the location of the substrate water binding sites. Several medium-resolution (3.8 to 2.9 Å) PSII crystal structures have been reported,[2–5] but while these structures bear common features, considerable disparity exists between the assigned positions of some of the Mn atoms and ligating protein residues. Further structural information has been obtained from EXAFS studies (extended X-ray absorption fine structure)[6] but there is still no universally accepted geometry for the PSII active site.

Clearly, complementary approaches to PSII structure characterization are needed. Computational chemistry is one such approach adopted by several groups including ourselves.[7–13] We have explored the widest class of possible Mn_4Ca geometries indicated by the 2.9 Å "Berlin" (**I**), 3.7 Å "Hyogo" (**II**), and 3.5 Å "London" (**III**) crystal structures,[2–5] across several charge states and magnetic coupling configurations of the Mn. Surprisingly, this work[12,13] suggested that the diverse crystal structure geometries might be simply related as calculations on a series of $[CaMn_4C_9H_{10}N_2O_{16}]^{q+}\cdot 3H_2O$ clusters ($q = -1$ to $+3$) showed facile interconversion between forms strikingly similar to the Mn_4 geometries of the "Hyogo", "London", and "Berlin" structures.

In the density functional calculations[14,15] reported here, we have explored the effect of sequential hydration (up to 7 water molecules) on these Mn_4Ca structure types across charge states from $q = -1$ to $q = +3$ and all feasible magnetic coupling modes (see Supporting Information). In addition, we have performed calculations on relevant charge compensated structures in order to maintain the overall charge at zero or $+1$, consistent with the experimental data on sequential proton loss in the real system. The relevance of these structure types as models of the WOC active site, and importantly, the location of substrate water binding sites in catalytically active PSII, may be assessed against recent experimental data on substrate water interaction with the WOC.

The locations of the preferred water binding sites in the optimized geometries of the heptahydrate $\mathbf{I}^{2+}\cdot(H_2O)_7$, $\mathbf{II}^{2+}\cdot(H_2O)_7$, and $\mathbf{III}^{2+}\cdot(H_2O)_7$ structures are shown in Figure 1. In all structure types, Mn(2) is coordinatively saturated and cannot bind water. In **III**, an additional tri-μ-oxo bridge which links Mn(1), Mn(3) and Mn(4), renders Mn(1) coordinatively saturated in the absence of hydration, while in **I** and **II** there is a vacant hydration site on Mn(1). This site is unoccupied in all charge states for **I** as the "pocket" between Mn(1) and the μ-oxo bridge linking Mn(3) to Mn(4) is too small. In **II**, the distance between Mn(1) and the Mn(3)/Mn(4) μ-oxo bridge is larger and so admits water ligation.

Structure type **II**, in the neutral charge state, is unique in possessing two distinct hydration patterns with similar energy ($\mathbf{II}^0$, $\mathbf{IIa}^0$) (Figure 2). Addition of the seventh water provokes an unexpected structural change—spontaneous proton transfer from the water strongly bound to Ca/Mn(3) converts the μ-O bridge between Mn(3) and Mn(4) to a μ-OH bridge. The resulting hydroxide ligand is H-bonded both to the nascent μ-OH bridge and to a water molecule now bound to Mn(1). The μ-OH-bridged structure is ca. 10 kJ mol^{-1} lower in energy than its μ-O-bridged tautomer.

The above proton transfer effect is highly significant in the context of the experimental water exchange kinetics.[16] To address this and the known S state transition proton loss sequence,[17,18] we performed calculations on structures $\mathbf{II}^{0}*$ and $\mathbf{II}^{+}*$, versions of $\mathbf{II}^{-}$ and $\mathbf{II}^{2+}$ with appropriate charge compensation. $\mathbf{II}^{0}*$ contains a protonated μ-O bridge between Mn(3) and Mn(4), while $\mathbf{II}^{+}*$ is a deprotonated version of $\mathbf{II}^{2+}$ (Figure 3). The proton is lost from the water strongly bound to Mn(4). As shown in Figure 3, S states from S_0 to S_3 correspond to structures $\mathbf{II}^{0}*$, $\mathbf{IIa}^{0}$, $\mathbf{II}^{+}$, and $\mathbf{II}^{+}*$, respectively. The latest 2.9 Å crystal structure[5] locates a possible water site near the cluster, below Mn(4), on the opposite side of the cluster from Ca and the redox-active tyrosine, Y_z. This water site is adjacent to a newly identified position for Cl^-, a known cofactor of the water oxidizing reaction.[1] A water molecule bound to Mn(4) is consistently located near this putative water site in all S states for structure **II**, with a binding energy of 40–90 kJ mol^{-1}. Consideration of the likely roles of Ca and Y_z in O–O bond formation[1] argues against this water

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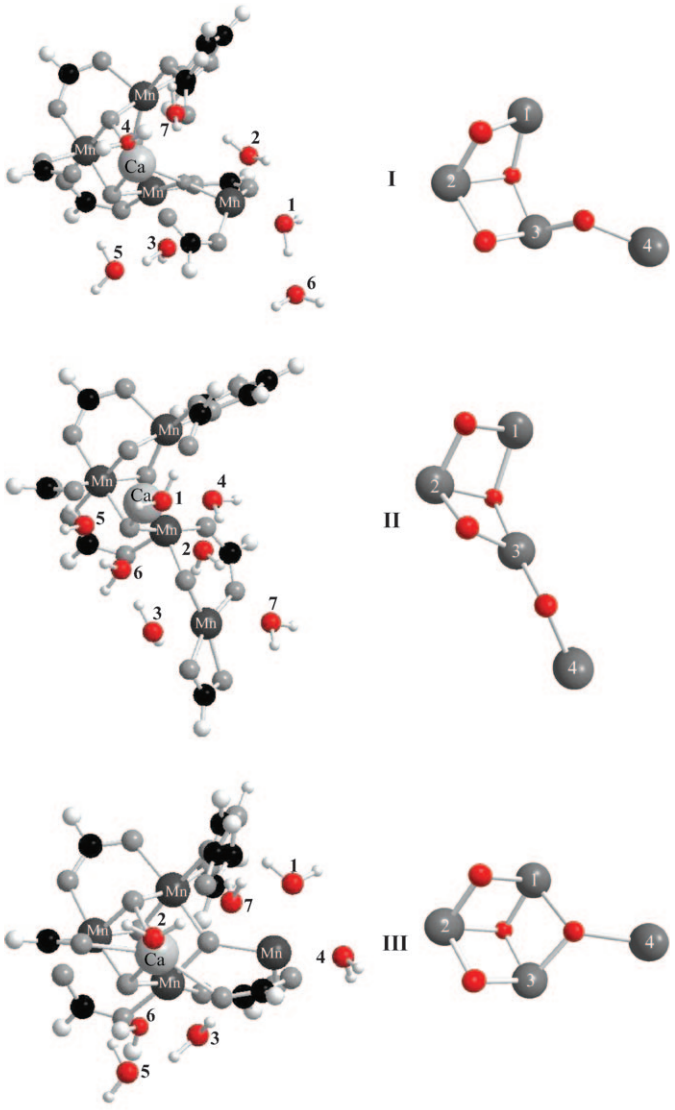

Figure 1. The left column shows the lowest-energy heptahydrated complexes of clusters **I**, **II**, and **III**, each in the dicationic charge state, with water ligands highlighted in color. Structures are viewed along a line-of-sight nearly coincident with the axis passing down through Ca to the tri-μ-oxo bridge linking Mn(1), Mn(2) and Mn(3), with Mn(1) oriented approximately north, and Mn(2) due west, of the projected Ca atom. Annotations refer to the hierarchies of water ligand attachment, with "1" indicating the strongest bound water ligand. The right column displays the Mn–oxo connectivity within each cluster, with Mn atoms enumerated.

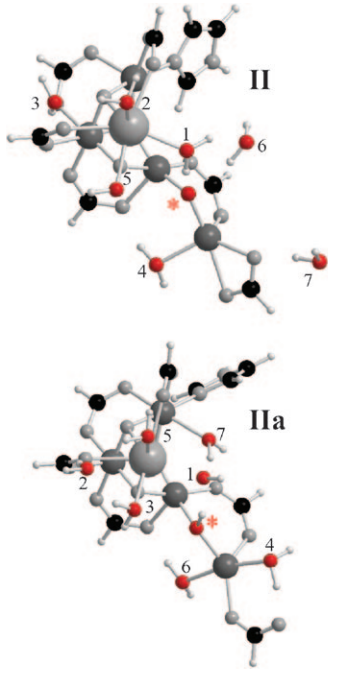

Figure 2. Lowest-energy heptahydrated complexes of clusters **II** and **IIa**, each in the neutral charge state, with water ligands highlighted in color. Cluster **II** is the "conventional" "Hyogo-like" cluster, analogous to that obtained at the other charge states surveyed, while **IIa** is a tautomer of **II** which is marginally lower in energy. The transferred proton in **IIa** converts the μ-O bridge between Mn(3) and Mn(4) to μ-OH (highlighted by a red asterisk). Cluster orientation is analogous to that already described for the anionic complexes in Figure 1. Annotations refer to the hierarchies of water ligand attachment, with "1" indicating the strongest bound water ligand (or, for **IIa**, the hydroxide/proton pair resulting from tautomerization).

molecule being a substrate species. However, its deprotonation in S_3 to maintain charge balance is mechanistically attractive as the adjacent Cl^- is thought to communicate with a proton-only channel.[5]

Substrate water exchange kinetics are known for all stable S states, from which two exchanging sites, one "fast" and one "slow", were identified.[16] For S_0 through to S_3, the slow exchange kinetics reveal simple, unimolecular cleavage from the catalytic site. The fast exchange process indicates "single file" diffusion through a proteinaceous pore and carries no "memory" of the binding site.[18] The latter must have a substantially lower water binding energy than the "slow" site.

The slow rate is nearly constant across S_0, S_2, and S_3, but markedly lower in S_1. This is counterintuitive as one expects the rates to systematically decline as the Mn cluster is progressively oxidized and protons lost through the S state cycle. The experimental H_2O binding energy is ca. 75 kJ mol^{-1} for S_2 and S_3 but increases by ca. 10 kJ mol^{-1} in S_1. This suggests that the water binding site is not progressively altered throughout the S cycle, but reversibly modifies in S_1. Our results provide a natural candidate for this site which not only rationalizes the anomalous exchange kinetics, but is also consistent with the substrate water interactions inferred from recent ESEEM (electron spin echo envelope modulation) and FTIR studies.[20–22]

Alone of the three structure types, **II** possesses a water accessible "cleft" region between Mn(1), Ca, and Mn(3) in which two water/hydroxide ligands may be accommodated (Figure 3). One water molecule, generally the strongest or second strongest ligated water, is predominantly bound to Ca but distantly bridges to Mn(3). This water molecule also forms H bonds with the μ-oxo bridge between Mn(3) and Mn(4). Based on our calculations, proton transfer from this water to the oxo bridge occurs in S_1 (**IIa**^{0}) triggered by Mn(2) oxidation, which reverses in S_2 (**II**$^+$) when Mn(4) is oxidized.

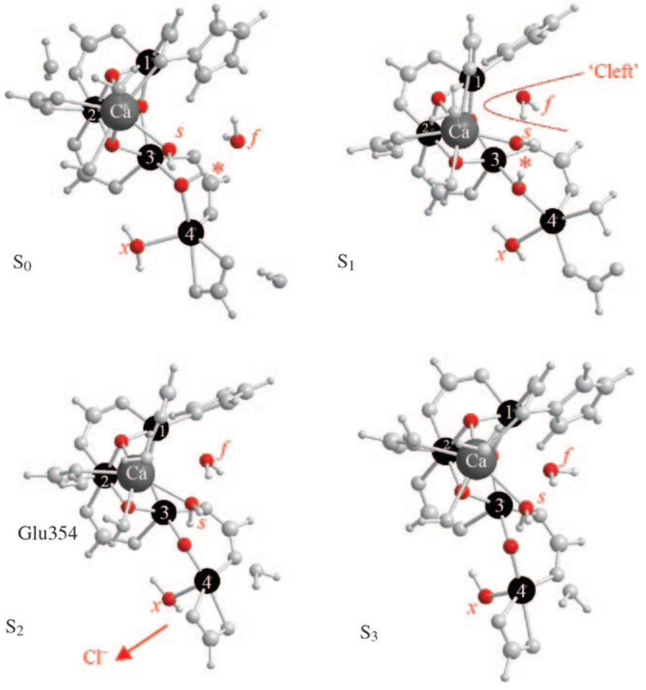

Figure 3. Heptahydrated II^{0*}, IIa^0, II^+, and II^{+*} species corresponding to calculated WOC structures in S_0 to S_3 with metal atoms, oxo bridges, and "important" water ligands highlighted. Mn atoms are labeled 1 to 4. Water ligands labeled "*f*" and "*s*" correspond to "fast" and "slow" exchanging substrate waters. "*x*" denotes the water ligand tentatively identified in the latest 2.9 Å XRD structure. Protonated oxo bridge denoted by asterisk. "Cleft" region, Glu354 equivalent residue, and approximate location of XRD-identified chloride are also shown.

Consistent with the experimental data, the above water is bound with similar energy (93 ± 12 kJ mol^{-1}) in S_0, S_2, and S_3 based on our calculated hydration energies (see Table S1 in Supporting Information). For the proton-transferred form of S_1 (**IIa**^{0}), this water is now formally OH^- so its calculated binding energy is significantly above that in the other S states, in agreement with the observed low water exchange rate, where it binds and displaces as the neutral species. Thus, we suggest that this water (or OH^-) ligand is the slow exchanging substrate species in the protein, in all S states. Further, the near-constancy of the S_0, S_2, and S_3 "slow" ligand binding energies is enhanced with inclusion of solvent corrections (Table S2) across a range of dielectric constant values (64 ± 4 kJ mol^{-1} for $\varepsilon = 5.0$, 61 ± 4 kJ mol^{-1} for $\varepsilon = 10.0$, 58 ± 5 kJ mol^{-1} for $\varepsilon = 78.4$). While the absolute H_2O binding values from these solvent-corrected calculations are uniformly lower than expected for the true protein, these results are highly encouraging for the interpretation of structure type **II** as a realistic model of the true PSII active site.

Neither structure **I** or **III** offers an obvious candidate for the slow exchanging substrate water, leading to the wrong S state binding trend in **I** and too weak binding overall in **III**. The situation is not resolved by shifting the oxidation pattern for the cluster, that is, taking $q = 1$ to be S_0, as the binding energies just monotonically increase with S state.

The most recent ESEEM study[20] of substrate water interaction with the WOC in the S_0 and S_2 states, involving "physiological" flash advanced functional turnover, identified a minimum set of three deuteron/Mn coupling classes, very similar in all S_0 and S_2 samples, from which effective Mn–D distances were estimated. The protons from the "slow" water are unlikely to be strongly coupled magnetically to the cluster in S_2 or S_0 as one proton is 2.7 Å from Mn(3) while the other is over 4 Å away from any Mn.

The other more weakly bound water molecule in the cleft is associated with both Mn(1) and Mn(3). Its computed binding energy is generally < 30 kJ mol^{-1} and it has protons sufficiently close to Mn(1) for strong magnetic coupling in S_2 or S_0. In fact, the S_2 distances are within 0.1 Å of those inferred experimentally.[19] This is a strongly suggestive result and therefore we assign the strong substrate water proton couplings to the likely "fast" exchanging water bound between Mn(1) and Mn(3).

FTIR difference spectroscopy has identified one substrate water molecule binding to the WOC in the S_1 and S_2 states with considerably different H bonding interactions for the two protons.[21] One water proton is strongly H bonded and the other only weakly, or not at all. This inequivalence alters substantially on the S_1 to S_2 transition. The above "fast" water is most likely the species identified by the FTIR measurements. In **IIa**^{0} (S_1), this water makes one strong H bond to the adjacent OH^- ligand, inferred to be the deprotonated "slow" exchanging substrate water, while the "fast" water's other H atom has no intermolecular connections. In **II**$^+$ (S_2), the OH^- group becomes H_2O and the H bond between this newly protonated species and the above "fast" water ligand weakens. This is qualitatively consistent with the pattern seen in FTIR for the OH stretch of the presumed substrate water species. Further, the recent mutation studies[22] on Glu354 suggest that this water is associated with either Mn(2) or Mn(3) as this ligand bridges both metals, again consistent with structure **II**.

In summary, several key features of structure **II** provide a natural "fit" to the functional water exchange, deuteron coupling and FTIR data for PSII, namely its "openness", the near constancy of the "slow" water's chemical environment, and its propensity for H bonding to the Mn(3)–Mn(4) μ-oxo bridge. All of these features are contingent on structure **II**'s metal–oxo connectivity in that the Mn(3)–Mn(4) oxo bridge connects only these metals, and does not bridge to the Ca or Mn(1) as occurs in structures **I** and **III**. Furthermore, because the water exchange and ESEEM studies involved fully functional turnover states, we suggest that structure **II** is actually closest to the active enzyme site of those possibilities examined here.

Overall, six waters satisfy the cluster of which only three water molecules actually bind at or near Mn throughout the whole cycle. These are the two substrate waters bound in the cleft formed between Mn(1), Ca and Mn(3), and the water on Mn(4) which probably corresponds to the putative water resolved in the latest XRD structure. A second water molecule sometimes associated with Mn(4) for structure **II** (Figure 1) is actually very weakly bound and can be removed with no significant change to the results. The remaining three water molecules are bound solely to Ca.

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