**Toward understanding the S$_2$-S$_3$ transition in the Kok Cycle of Photosystem II: Lessons from Sr-Substituted Structure**

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**ABSTRACT:** Understanding the water oxidation mechanism in Photosystem II (PSII) stimulates the design of biomimetic artificial systems that can convert solar energy into hydrogen fuel efficiently. The Sr$^{2+}$ substituted PSII is active but slower than with the native Ca$^{2+}$ as an oxygen evolving catalytic structure. Here, we use Density Functional Theory (DFT) to compare the energetics of the S$_2$ to S$_1$ transition in the Mn$_4$O$_5$:Ca$^{2+}$ and Mn$_4$O$_5$:Sr$^{2+}$ clusters. The calculations show that deprotonation of the water bound to Ca$^{2+}$ (W3), required for the S$_2$ to S$_1$ transition, is energetically more favorable in Mn$_4$O$_5$:Ca$^{2+}$ than Mn$_4$O$_5$:Sr$^{2+}$. In addition, we have calculated the pK$_a$ of the water that bridges Mn4 and the Ca$^{2+}$/Sr$^{2+}$ in the S$_2$ using continuum electrostatics. The calculations show that the pK$_a$ is higher by 4 pH units in the Mn$_4$O$_5$:Sr$^{2+}$.

The oxygen evolving complex (OEC) is a unique natural bioinorganic cluster that catalyzes the water oxidation reaction in the 5-steps ($S_0$, $S_1$, $S_2$, $S_3$, $S_4$) Kok cycle.$^{1,2}$ The core of the OEC contains a metal cluster of four Mn and one Ca$^{2+}$ connected through bridging oxygens.$^{2-4}$ Ca$^{2+}$ depletion$^{5,6}$ blocks the S$_2$-S$_3$ transition, while replacing Ca$^{2+}$ with Sr$^{2+}$ reduces the catalytic activity.$^{7-10}$

Calcium and strontium belong to group 2 alkaline earth metals in the periodic table. Thus, they are chemically similar and have a stable oxidation state of +2. However, Ca$^{2+}$ is a stronger Lewis acid, which indicates that aqua-Ca$^{2+}$ compounds have a lower pK$_a$ than aqua-Sr$^{2+}$ (measured pK$_a$ is 2 pH unit lower). This difference in proton affinity of the bound waters may be the reason for the difference in the catalytic activity in the Sr-substituted PSII.$^{10-12}$ Here, we use Density Functional Theory (DFT) to compare the energetics of the S$_2$-S$_3$ transition in the native and Sr-substituted PSII.

Experimental$^{13}$ and theoretical studies$^{14-16}$ have proposed that the S$_2$-S$_3$ transition passes through an intermediate step in which the S$_2$ EPR signal changes from the multiline g=2 signal to the g=4.1. In the g=4.1 EPR state Mn1, Mn2, Mn3 are in the IV oxidation state, while Mn4 is in the III state (Fig 1).$^{17}$ In the g=2 redox intermediate M1 is Mn$^{3+}$ while M4 is Mn$^{4+}$. In addition, time-resolved photothermal beam deflection measurements suggest that a proton is released from the OEC or surroundings when the nearby Tyr, Y$_z$, is oxidized before Mn oxidation in the S$_2$-S$_3$ transition.$^{18,19}$

Based on classical electrostatic calculations and DFT study,$^{14}$ we previously proposed that the S$_2$-S$_3$ transition starts by the transition from g=2 to g=4.1 structure followed by deprotonation of the W3 Ca$^{2+}$ ligand.$^{20}$ This is coupled to the protonation of HIS190 upon the oxidation of the secondary donor Yz$^*$. The deprotonated W3 moves toward Mn4 adding the sixth ligand to its coordination shell to facilitate its oxidation to IV state. Similar mechanisms have been proposed by previous theoretical$^{21-24}$ and experimental$^{10}$ studies.

Here, we compare the energies of two structures of the S$_2$ g=4.1 state, A in which HIS190 and W3 are neutral (Figure 1A) and B with protonated HIS190$^+$ and W3 is a OH$^-$ bridge between Mn4 and Ca$^{2+}$ (Figure 1B) in both Mn$_4$O$_5$:Ca$^{2+}$ and Mn$_4$O$_5$:Sr$^{2+}$ clusters. The structures were optimized at the DFT level using the B3LYP
functional and 6-31G(d) basis sets for N, O, C and H atoms, while SDD are used for Mn, Ca and Sr. All the Mn ions are in the high spin state. Furthermore, the energies are compared using different levels of theory; B3LYP/6-31G(d) and B97D/6-31G+(d).25,26

The energy differences between the A and B states (ΔG_{B-A}) at different level of theory are shown in (Table 1). In general, the B state (protonated HIS190 and hydroxyl on W3) is always more favorable for the Mn₄Ο₂Ca²⁺ than the Mn₄Ο₂Sr²⁺ cluster. The large energy difference obtained for the Mn₄Ο₂Sr²⁺ cluster using B3LYP/6-31G(d) level of theory indicates the importance of including diffuse functions in the basis sets when modeling large ions. These diffuse functions provide flexible representation to the tail part of the atomic orbitals further from the nucleus. 25,26

Sr²⁺ is larger than Ca²⁺ by 0.1Å, which elongates the interatomic distances between the Sr²⁺ and the rest of atoms in the Mn cluster. This is seen in the optimized structures of the A and B states with Ca²⁺ and Sr²⁺ clusters (Table 2). In addition, the dispersion interaction between the metal and the water ligand is expected to push the water away in case of Sr²⁺, which will result in smaller electrostatic interactions and a higher pKₐ. This is found for aqua-Ca²⁺ and aqua-Sr²⁺ compounds, where the water bound to Sr²⁺ have a higher pKₐ than those bound to Ca²⁺. Thus, the Sr²⁺ structure is more stable with neutral W3 (Figure 1A). However, with Ca²⁺, W3 deprotonates forming a hydroxide that moves to bridge Mn4 and Ca²⁺ (Figure 1B).

![Figure 1](image.png)

**Figure 1.** A represents the S₂ state with HIS190 neutral. B represents the S₂ state with HIS190⁺ protonated. M is Ca²⁺ or Sr²⁺. Mn1, Mn2, Mn3 are in the IV oxidation state. Mn4 is III. The rest of atoms in the model were removed for clarity.

The optimized DFT structures show that Mn-Sr²⁺ distances are in general longer than Mn-Ca²⁺. In the A state the Sr²⁺-W₃(HOH) distance is longer by 0.1Å than Ca²⁺-W₃(HOH), while in the B state the Sr²⁺-W₃(OH⁻) distance is 0.2Å longer. The Mn1 to Ca²⁺, is longer because Ca²⁺ moves significantly toward Mn4 after the deprotonation of W3.

| Compound       | B3LYP/ 6-31G(d) | B3LYP/6-31G+(d) | B97D/6-31G+(d) |
|----------------|----------------|----------------|----------------|
| Mn₄Ο₂Ca²⁺      | -2.3           | -8.7           | -6.8           |
| Mn₄Ο₂Sr²⁺      | 13.0           | -8.0           | -6.4           |

Energy differences are expressed in Kcal/mol. The transition from A to B state is more favorable in the Mn₄Ο₂Ca²⁺ cluster than the Mn₄Ο₂Sr²⁺.

To further compare the Mn₄Ο₂Ca²⁺ the Mn₄Ο₂Sr²⁺, we calculated the pKₐ of W3 in the A state for both clusters using continuum electrostatics.27,28 W3 has a pKₐ of 6.5 for the Mn₄Ο₂Ca²⁺ and 10.3 in the Mn₄Ο₂Sr²⁺. The lower pK of W3 in Ca²⁺ structure is expected as W3 is significantly closer to the positively charged ions (Ca²⁺, Mn4(III) and Mn1(IV)) (Table 2). This conclusion is supported by the DFT calculations, which show that B is lower energy than A indicating the easier deprotonation in case of the Mn₄Ο₂Ca²⁺.

An open question is what the source of the proton which is released after Y₁ is oxidized but before the OEC
advances to the $S_3$ state.\textsuperscript{3,29} As there are no protons bound to the bridging oxygens in the $S_2$ state, the donors are likely to be terminal water ligands bound to Mn4 water\textsuperscript{30,31} or to Ca.\textsuperscript{32} Previous studies have shown the Mn4-bound water W1 to be upon the formation of tyrosyl radical, however the proton is trapped by the nearby acceptor D61.\textsuperscript{33,34}

The present study utilizes the $S_2$ g=4.1 models for Ca\textsuperscript{2+} and Sr\textsuperscript{2+} to understand the nature of deprotonation event. Our DFT calculations support the deprotonation of W3 in the $S_2$ to $S_3$ transition, which is also supported by the XFEL structures comparing $S_1$, $S_2$ and $S_3$ states.\textsuperscript{28}

Table 2. Interatomic distances in A and B states.

| A   | Ca\textsuperscript{2+} | Sr\textsuperscript{2+} | Ca\textsuperscript{2+} | Sr\textsuperscript{2+} |
|-----|------------------------|------------------------|------------------------|------------------------|
| Mn1 | 2.50                   | 3.58                   | 3.49                   | 3.67                   |
| Mn4 | 4.35                   | 4.51                   | 3.58                   | 2.64                   |
| W3  | 2.55                   | 2.66                   | 2.39                   | 2.58                   |
| W4  | 2.33                   | 2.50                   | 2.35                   | 2.53                   |
| O5  | 2.66                   | 2.78                   | 2.66                   | 2.77                   |

All distances are reported in Å. In general, the interatomic distances are longer for Sr\textsuperscript{2+}.

Supporting Information

The Supporting Information includes details of the continuum electrostatic calculations and the optimized atomic coordinates of the optimize A and B structures.

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