Advancements in understanding oxygen-evolving complex through structural models in photosystem II

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Photosynthesis is a fundamental chemical process that enables cyanobacteria, algae, and plants to utilize solar energy to convert water and carbon dioxide into oxygen and carbohydrates. Developed over billions of years, this process has critical effects on ecosystems by providing energy and organic matter. In addition, photosynthesis helps regulate atmospheric oxygen levels by releasing oxygen.

Photosystem II (PSII), a protein-pigment complex in thylakoid membranes, is responsible for a series of light-dependent reactions in plants and algae. Assembled into a homodimer, PSII utilizes light energy to split water molecules, producing oxygen, electrons, and protons. In PSII, the oxygen-evolving complex (OEC) that catalyzes the water oxidation reaction comprises four manganese (Mn) ions, one calcium (Ca) ion, and several oxygen atoms. The water-splitting process involves a light-driven five-step cycling of S states (i = 0–4); S0 represents the initial OEC state, whereas S1 to S4 correspond to the intermediate states involving sequential oxidations under light.1 In S0, the oxidation states of four Mn ions are MnII, III, IV, and II. The subsequent S1-to-S2 and S2-to-S3 transitions involve changes in the Mn state from MnIII to MnIV and MnIV to MnIII. In S0, the reaction center chlorophylls P680 in PSII is in an unexcited reduced state. After absorbing photon energy, P680 transitions from its ground state to an excited state, followed by a rapid charge separation that produces the paired positive and negative charges of P680+/Pheophytin. The electron subsequently moves from PheoII to the plastoquinone Qa and Qb, while P680+ is reduced by a tyrosine residue from the D1 subunit (Yj) located between P680 and OEC. Next, Yj is further reduced by accepting electron from the OEC. Throughout the S0–S4 cycle, four electrons and four protons are extracted from two water molecules by the OEC, resulting in the formation of one dioxygen molecule. Subsequently, the OEC returns to its S0 state (Figure 1).

Investigations on how PSII utilizes solar energy to split water molecules, studies on the substrate water source, and research on the mechanism mediating the formation of the O=O bond are crucial for advancing our understanding of photosynthesis. Therefore, elucidating the atomic arrangement of the OEC and the structural modifications in the S0–S4 transitions under light are major research objectives. In 2011, Yasufumi et al. determined the crystal structure of PSII from Thermosynechococcus vulcanus, achieving an impressive resolution of 1.9 Å. The structure clearly indicated that the water-splitting OEC is a Mn5CaO5 cluster composed of four Mn ions (Mn1–Mn4), one Ca ion, and five oxygen atoms (O1–O5). The Mn5CaO5 complex in this structure has a ‘distorted chair’ shape, representing the stable S1 state. The five oxygen atoms serve as oxo bridges linking the five metal atoms. In addition, the four water molecules detected in the vicinity of the OEC are likely substrates for the dioxygen formation catalyzed by PSII. Numerous water molecules form hydrogen-bond networks, some of which connect the Mn5CaO5 cluster to the luminal surface of PSII. This connection is potentially indicative of channels for releasing protons and transporting the substrate water during the S-state cycle. The four channels detected in this structure were designated as O1, O4, Cl-1, and Cl-2 in the later resolved structures.2 The O1 and O4 channels were named after their proximity to O1 and O4 of the Mn5CaO5 cluster, respectively, while Cl-1 and Cl-2 include hydrogen-bond networks mediated by two chloride ions (designated as Cl−1 and Cl−2, respectively) near the Mn5CaO5 cluster. These Cl− ions play crucial roles in stabilizing the structure of the OEC and facilitating the water-splitting reaction. The O1 channel, which is broader than the other channels, likely facilitates the entry of substrate water molecules into the OEC.

An exposure to X-rays can reduce the Mn cations in the OEC, leading to slightly longer Mn–Mn distances in the X-ray crystal structure compared with the calculated distances in theoretical analyses. The serial femtosecond X-ray free electron laser (XFEL) technique generates ultra-short, high-intensity pulses to capture diffraction images before radiation-induced damage occurs to the crystal structure. In an earlier study by Michihiro et al., this technique was used to present the damage-free structure of T. vulcanus PSII in the S1 state, achieving a resolution of 1.95 Å.2 The Mn–Mn distances in this structure were 0.1–0.2 Å shorter than those in the X-ray diffraction structure. Furthermore, the Mn–O5 distance was notably longer than the other Mn–O distances, implying that O5 may be a hydroxide ion in S1 rather than a typical oxygen anion. Thus, O5 may represent a water molecule in S0, which transforms into the O− species in S1, releasing a proton during the S0–S1 transition. Hence, O5 likely provides one of the substrate oxygen atoms involved in the formation of the O=O bond.

The XFEL technique is ideal for investigating the intermediate S-states of the PSII OEC because it can synchronize XFEL pulses with short visible light laser pulses to excite the OEC into different S-states. To clarify the formation of the O=O bond, the structural characterization of the other intermediate S states is necessary. In 2017, Michihiro et al. utilized time-resolved serial femtosecond crystallography (TR-SFX) with XFEL to solve the structure of PSII at a resolution of 2.35 Å treated with a dual-flash exposure at room temperature. This approach helped to elucidate the structural details of the OEC in S1.3 Compared with the dark-adapted state, PSII under the two-flash state exhibits significant structural changes, particularly in regions surrounding the Qa/non-heme iron and Mn5CaO5 cluster due to the electron and proton transfers induced by the dual-flash treatment. A water molecule (W665) positioned 3.5 Å away from the Mn5CaO5 cluster in the S1 structure becomes undetectable after
exposure to two light flashes. Another water molecule (W567), which forms hydrogen bonds with both W665 and O4 in S1, moves toward O4 in the S3 structure, suggesting a proton transfer during the S1-to-S2-to-S3 transitions. Notably, a new unique oxo-bridge atom (O6) was detected close to O5 and Mn1. The movement of the Glu189 side chain in the D1 subunit creates enough space to accommodate O6, which binds to Mn1 and Ca, leading to the formation of a Mn$_2$CaO$_6$ cluster with a cubane shape. Moreover, O6 is positioned close to O5, with an average distance of 1.5 Å, enabling the formation of a peroxide in S3.

Later, Michihiro et al. successfully resolved the PSII structures from S1 to S3 at resolutions of 2.15 to 2.40 Å. These structures were used to more precisely calculate the distances between atoms in the OEC, while also providing insights into the chemical entities of the O5 and O6 species, as well as the corresponding changes in the proteins and ligands adjacent to the OEC. These changes contribute to the movement of protons and water during the S-state cycle. In this S1 structure, the Mn–Mn distances are similar to those determined previously, except for the Mn1–Mn2 distance (2.60 Å), which is slightly shorter than reported before. In S2, there are slight changes in Mn–Mn distances with no detectable insertion of water; however, an open cubane structure of the Mn$_2$CaO$_6$ cluster was observed. Additionally, Mn4 shifts towards Glu333, while O5 shifts towards Mn4. These changes are consistent with the Mn4 oxidation during the S2-to-S3 transition, resulting in Mn$^{IV}$ and Mn$^{II}$ in S2. Following the transition to S3, Mn1, Mn2, and Mn3 remain static, but Mn4 moves toward Ser169, resulting in an increase in the Mn1–Mn4 distance. The rotation of the D1 Glu189 side chain creates the space needed to incorporate O6, forming an open cubane Mn$_2$CaO$_6$ cluster. To precisely measure the distance between O5 and O6, difference Fourier maps of O5 and O6 were generated. The analysis revealed an approximate 1.9 Å distance separating O5 and O6, indicating the presence of oxyl/oxo species at these positions. Accordingly, an oxyl/oxo coupling mechanism likely mediates the formation of the O=O bond. This indicates that the O5–O6 site is where the O=O bond forms in S3.

Previous research has elucidated the structural changes in the Mn$_2$CaO$_6$S$_5$ cluster and its surroundings as it transitions through the different S-states within microseconds (μs). In a recent study, Li et al. utilized pump-probe TR-SFX with XFEL to comprehensively investigate the dynamic structural changes of PSII in nanosecond (ns) to millisecond (ms) timeframes after PSII crystals were exposed to one and two flashes. This method involves a rapid succession of light pulses followed by capturing the transient states of molecular structures, providing insights into the dynamic processes within the OEC. Using this method, dynamic structures were obtained during the S1-to-S2 and S2-to-S3 transitions within a timeframe ranging from 20 ns to 5 ms. Structural changes related to electron transfer, proton release, and water transport were detected in various areas, including Q$_{A_{0}}$–Q$_{B_{0}}$, Y$_Z$, OEC, and the O1, O4, and Cl-1 channels after one and two light pulses. Notably, the tyrosine residue Y$_Z$, which connects the Mn$_2$CaO$_6$S$_5$ cluster and P680, along with its surrounding amino acids and water molecules, were revealed to undergo structural changes at the nanosecond timescale. These changes reflect the rapid electron and proton-transfer-related processes involving Y$_Z$ after exposure to light. During the early stage of the S2-to-S3 transition, following two light pulses, a water molecule (O6$^*$) near Glu189 of the D1 subunit was observed to bind to the Ca atom in the OEC within 1 μs. While the electron densities of O6$^*$ and O6 co-existed at 200 μs, the O6$^*$ signal disappeared, and the O6 signal increased at 5 ms. This indicates that the water molecule located at O6$^*$ moves to O6 between 200 μs and 5 ms, serving as the source of O6. During the S2-to-S3 transition, Mn1 is oxidized from Mn1$^{III}$ to Mn1$^{IV}$, thereby attracting the negatively charged O6$,^*$ resulting in its shift to the O6 position. Furthermore, water molecules and amino acids in the three water channels near the OEC (i.e., O1, O4, and Cl-1 channels) also undergo dynamic changes, reflecting their sensitivity to electron transfer, proton release, and substrate water molecule transfer, or their involvement in these processes. During the S1-to-S2-to-S3 transitions, the synergetic effects of the first and second flashes result in the deprotonation of a water molecule close to the OEC. This leads to its binding to the OEC and subsequent movement to the O6 position.

With the availability of a series of high-resolution PSII structures, the structural details of the OEC in most stages of the S-state cycle as well as, the dynamic structural changes during each stage transition, are gradually being revealed. These studies represent significant milestones in understanding the mechanism behind the S-state transitions of the OEC and the production of oxygen during photosynthesis. Currently, characterizing the structure of OEC in S4 remains a challenge. This is primarily because of its highly transient nature and the technical limitations of current spectroscopy and crystallography methods. The available information is mostly based on theoretical computational models. Understanding the S2 state is crucial for completing the picture of the OEC’s mechanism. In future studies, researchers will need to combine experimental techniques and incorporate computational modeling methods to resolve the structures of each S$_i$ state. The comprehensive characterization of photosynthesis will have critical implications for various research fields, including biology, energy, and materials science. It will provide important mechanistic explanations for the PSII S-state cycle and the production of oxygen during photosynthesis. Additionally, it will form the theoretical basis for designing more efficient artificial systems for decomposing water and producing hydrogen and other renewable energy sources, utilizing solar energy as the driving force.

**REFERENCES**


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**DECLARATION OF INTERESTS**

The authors declare no competing interests.