

Oxyl/oxo coupling mechanism for dioxygen formation in photosystem II

Photosynthesis in plants and algae is a process of synthesizing biomass in the form of sugars using light energy from the sun. The initial step of oxygenic photosynthesis is the light-driven water oxidation reaction of photosystem II (PSII), in which four electrons and protons are extracted from two water molecules, and a dioxygen molecule is released into the atmosphere. A manganese complex called the oxygen-evolving center (OEC) catalyzes this reaction through five linear oxidation intermediates of the S_i -state cycle ($i = 0-4$). Unveiling the mechanism of natural water oxidation is of considerable importance as it may have great potential in applications for developing a sustainable energy source, that is, a framework for the S_i -state cycle may provide structural insights into a rational design for catalysts capable of artificial water oxidation by which clean, renewable energy from the sun may be harvested.

The structure of PSII in the dark-adapted S_1 state was first analyzed at 1.9 Å resolution at SPring-8 **BL41XU** and **BL44XU**, and the detailed organization of the OEC was revealed (Fig. 1) [1]. The OEC is a Mn_4CaO_5 cluster with a shape resembling a distorted chair, located at the luminal surface of the thylakoid membrane and linked to four hydrogen-bonded water channels [1]. The S_1 -state structure was further analyzed using X-ray free electron lasers (XFELs) provided by the SACLA **BL2** and **BL3** to obtain its radiation-damage-free structure [2], which confirmed the presence of a unique, central oxo-bridged oxygen (O5) located between two manganese atoms, Mn1 and Mn4, with unusually long bond distances to the two Mn ions. This feature suggests weak binding of O5 with the nearby Mn ions and therefore possibly a crucial

role of O5 in dioxygen formation [2]. Furthermore, pump-probe, time-resolved serial femtosecond crystallography (SFX) at room temperature on the S_3 -state structure induced by two-flash illumination at 2.35 Å resolution revealed that the OEC becomes a Mn_4CaO_6 cluster in the S_3 state by incorporating an additional oxygen O6 in the vicinity of O5. This result supports a dioxygen formation mechanism between O5 and O6 [3]. While the insertion of O6 seems reasonable, the chemical structures of O5 and O6 remain controversial because of the uncertainty in the O5-O6 distance of 1.5 Å reported at the limited resolution. Therefore, several possible mechanisms for the O=O bond formation have been under debate.

To reveal detailed structural changes during the water oxidation reaction, we further analyzed the XFEL structures of PSII in the S_1 , S_2 , and S_3 states by using fixed-target serial femtosecond crystallography with SACLA-XFELs. The S_2 and S_3 states were generated by excitations with one or two flashes at room temperature with PSII microcrystals evenly sprayed on a mesh and trapped immediately at 77 K. Single-shot diffraction images were collected by fixed-target data collection at cryogenic temperature. Compared with the previous SFX approach, this method reduced sample consumption by one order of magnitude and ensured low-background images, allowing us to analyze the structure of multiple intermediate states at 2.15 Å resolution [4].

The results showed that no insertion of water occurs in the S_2 state, but upon transition to the S_3 state, the flipping of D1-Glu189, the only monodentate carboxyl ligand of the OEC, provides a space for incorporating the additional oxygen O6, and the

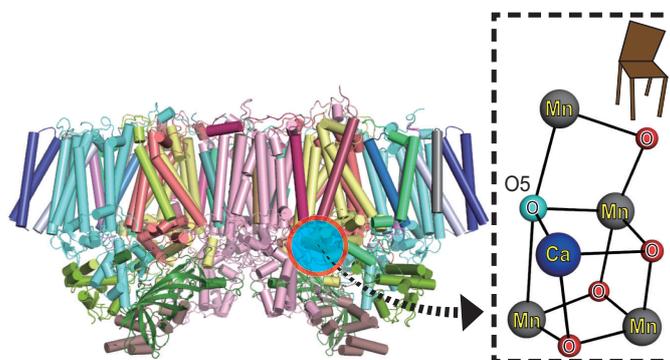


Fig. 1. Overall structure of PSII and an enlarged view of the OEC, the Mn_4CaO_5 cluster. PSII is a large membrane protein-pigment complex consisting of twenty protein subunits and exists in a dimeric form. Red circle indicates the location of the OEC, and its enlarged view on the right side shows the architecture of the OEC, which resembles the form of a distorted chair.

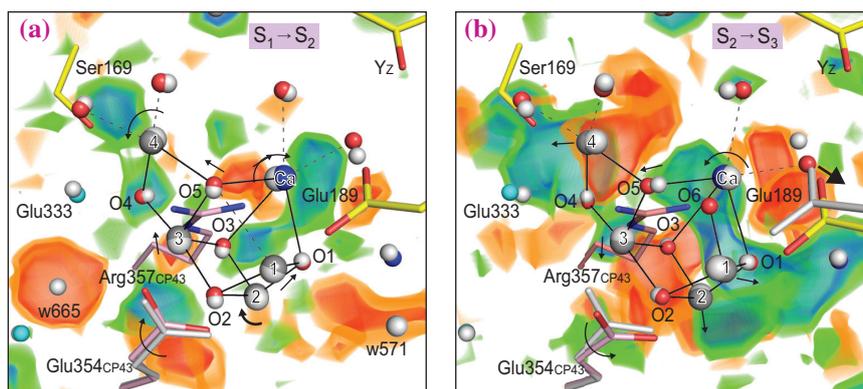


Fig. 2. Structural changes in the vicinity of the OEC during the S_i -state transitions. OEC structures were superposed with the difference Fourier maps of (a) S_2 minus S_1 and (b) S_3 minus S_2 datasets. Difference maps are contoured at -4σ (red), -3σ (orange), 3σ (green), and 4σ (blue).

Mn_4CaO_5 cluster remains in the open-cubane form (Fig. 2). To determine the exact chemical structure of O5-O6 we examined four possible chemical species, namely, superoxo, peroxy, oxyl/oxo, and oxyl/hydroxo. By altering the O5-O6 distance and examining the residual densities in the difference map, we found that a distance of 1.9 Å between O5 and O6 resulted in the lowest residual density. This finding suggests an oxyl/oxo coupling mechanism for dioxygen formation in the OEC. Moreover, the flipping of Glu-189 induces van der Waals repulsion between the carbonyl oxygen of D1-Glu189 and Ala-411_{CP43},

thereby moving a short loop of the CP43 subunit that restricts the size of the O1-water channel, leading to the widening of the O1-channel. This channel thus likely plays a role in delivering the substrate water to the Mn cluster during the S_2 to S_3 transition. Furthermore, O6 is coordinated to the Ca ion in the S_3 state, leading to the change in the coordination number of Ca from 7 in the S_1 state to 8 in the S_3 state. These structural changes revealed the mechanism of photosynthetic water oxidation via the cooperative action of substrate water access, proton release, and O=O bond formation (Fig. 3) [4].

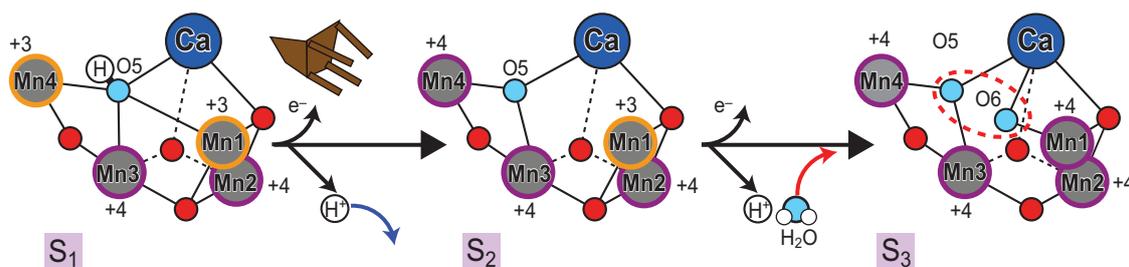


Fig. 3. Summary of structural changes of the OEC and the proposed mechanism of dioxygen formation. The protonated O5 atom in the S_1 state is located between Mn1 and Mn4 atoms and may be deprotonated in the S_2 state. Upon transition to the S_3 state, the OEC incorporates an additional water molecule as the O6 atom into the vicinity of O5 from the O1 channel. The species formed between O5 and O6 is determined to be oxyl/oxo in the S_3 state, which is considered to proceed to the O=O bond formation and dioxygen release upon further transition beyond the S_3 state.

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