Structural evidence for intermediates during O_2 formation in Photosystem II

The splitting of water into electrons, protons and molecular oxygen driven by sunlight is the first step in the solar-to-chemical energy conversion in natural photosynthesis. This reaction is catalyzed by a tetra-manganese cluster (Mn_4CaO_5 cluster; called oxygen evolving complex or OEC) located in a negatively charged pocket of the enzyme Photosystem II (PS II, Fig. 1), coordinated by several water molecules and amino acids such as Aspartate, Glutamate and Histidine. The enzyme, located in the thylakoid membrane, is connected to the lumen via several water channels that are likely responsible for water and proton transfer in PS II. Understanding the enzymatic process in PS II is important for developing efficient artificial photosynthetic devices, preferably from earth-abundant elements like Mn and Ca.

In order to catalyze the reaction, the OEC progressively accumulates four oxidizing equivalents from the charge separation that happens at the reaction center upon absorption of each photon. A redox active tyrosine residue, Y_Z, connects the reaction center with the OEC. With the generation of each oxidizing equivalent, the enzyme forms an intermediate (called S-states, S_i, $i = 0$ to 4) in the Kok cycle formalism, (Fig. 1(c)) with structural and electronic changes previously tracked by our group using XFELs for the meta-stable S-states as well as during the $S_2 \rightarrow S_3$ transition [1,2]. Structural studies of various S-state intermediates of PS II have also been reported by others [3]. However, the structural details of the final $S_3 \rightarrow [S_4] \rightarrow S_0$ steps are unclear. This transition, which is the slowest (taking several milliseconds to complete) and perhaps the most difficult transition during the catalytic reaction, is where the O–O bond is formed along with the release of two protons, insertion of a water molecule and the resetting of the enzyme to the most reduced S_0 state. By performing time-resolved X-ray crystallography at SACLA **BL2** and at the MFX beamline at LCLS, our group has now determined the structure of PS II at various timepoints along the $S_3 \rightarrow S_0$ transition [4]. We used our drop-on-tape (DOT) sample delivery setup to perform all the experiments that allows for precise and efficient illumination of the PS II samples, with the required delay times between the S-state transitions necessary for opening of the acceptor quinone sites. Further details of the experimental and analysis methods can be found in the following Ref. 4. In total, seven timepoint structures as well as the structures of the meta-stable S_3 and the most reduced S_0 states were determined. For clarity, the timepoints discussed are the time-delays between the 3rd visible laser flash and the XFEL X-ray probe.

We tracked the electron density changes of the oxygen O5, the newly inserted oxygen O_X (also called O6, inserted in the $S_2 \rightarrow S_3$ transition) and the terminal waters (W1–W4) by constructing omit maps of the individual oxygen atoms (Fig. 2(a)). The electron density of the O_X atom becomes asymmetric between 250–500 μs with a reduction in intensity after 500 μs, dropping to the noise level between 1200–2000 μs. For O5, the density remained approximately constant in all the timepoints except for a decrease in the 1200 μs dataset. At 730 μs, the omit densities of O5, W2 and W3 become anisotropic and suggest a high mobility of these ligands. We also tracked the Mn1–Mn4 distance and the Y_Z-H190 distance over the timepoints. The Y_Z re-reduction (based on Y_Z-H190 environment changes) occurs after 500 μs and is mostly complete

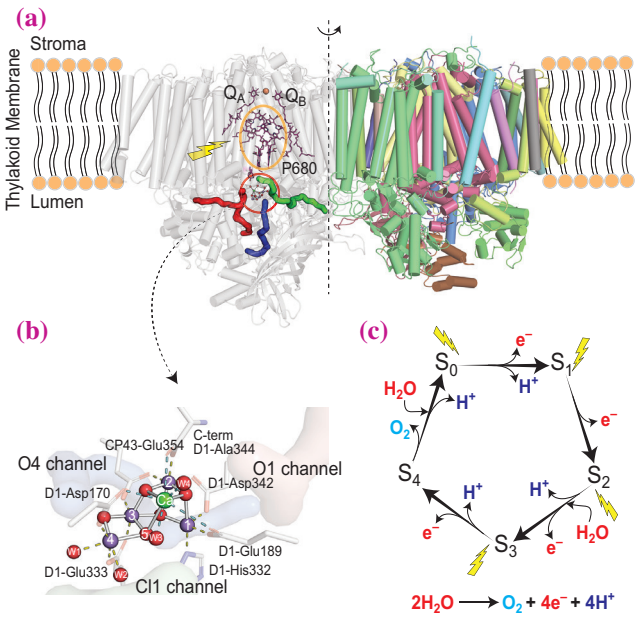

Fig. 1. Overview of Photosystem II (PS II) and the water oxidation reaction **(a)** The enzyme PS II is embedded in the thylakoid membrane of cyanobacteria, algae, and higher plants. The highlighted regions include the reaction center (orange circle) where charge separation occurs and the oxygen evolving complex (OEC; red circle) where the water oxidation reaction takes place. **(b)** Detailed view of the OEC in the dark-stable S_1 state and the surrounding amino acids and water channels. **(c)** The Kok cycle for the water oxidation reaction. Starting from the S_0 state, absorption of a photon at the reaction center leads to extraction of an electron from the OEC and oxidation of the Mn-cluster to the next S-state. Water oxidation occurs after the formation of the transient S_4 state.

by 1200 μs. The Mn1–Mn4 distance, which is an indicator for the presence of O_X contracted after 1200 μs, suggesting a delay between Y_Z re-reduction and O_X disappearance. This indicates the possibility of a reduced intermediate (such as a peroxide-like species) prior to O_2 formation and release. Among the O–O bond formation candidates (Fig. 2(b)), O5–O_X appears to best fit the current data based on their proximity and lower occupancy of O5 at 1200 μs. However, bond formation involving O5–W2 or O5–W3 cannot be excluded at this time.

We also observed several changes in the protein environment, including regions 10–15 Å away from the OEC like the proton gate region (Glu65–Glu312–Arg334) that are possible structural signatures of a proton release. For example, a clear rotation of Glu65 by about 60° is seen (Fig. 2) at two different time-points, similar to the change seen in the $S_2 \rightarrow S_3$ transition, that can be attributed to the release of two protons during this transition. The events leading to the resetting of the enzyme also appears to be quite slow as seen in the reappearance of W20, a water molecule that is part of a key hydrogen bond network near the OEC, in the 4000 μs timepoint, which is later than the disappearance of O_X.

Overall, the results show the well-orchestrated time-resolved changes between the Mn-cluster and the water and protein environment which is necessary to catalyze the water splitting reaction under ambient conditions. These findings bring us closer to understanding one of the most consequential chemical reactions in nature and using the knowledge for designing sustainable energy technologies from earth-abundant materials.

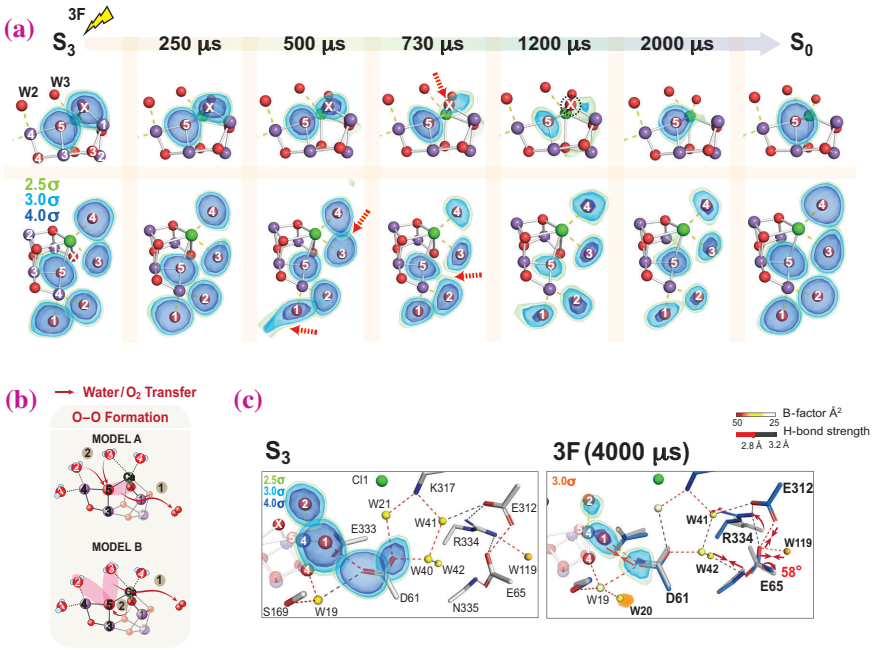

Fig. 2. Changes in the OEC and the broader enzyme environment in the $S_3 \rightarrow S_0$ transition **(a)** Electron density changes of O5, O_X and terminal waters (W1–W4) over the course of the transition. **(b)** The two possible O–O bond formation models based on the results of the study. **(c)** Changes in the proton gate region of the Cl1 channel. A rotation of the Glu65 is seen which is indicative of proton transfer to the bulk. W20, a key water molecule near the OEC, also appears to return very late in the transition suggesting a slow resetting of the enzyme.

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