Time-resolved protein crystallography – Structural changes of the catalyst for water oxidation captured by XFEL

Photosynthetic water oxidation is catalyzed by a Mn_4CaO_5 cluster, which is bound to photosystem II (PSII), a huge membrane protein complex (Figs. 1(a,b)) [1]. Through this reaction, two molecules of water are split into four electrons, four protons, with the concomitant generation of one dioxygen, using the light energy from the sun. This reaction is important because it converts light energy to biologically useful chemical energy and generates molecular oxygen, both of which are indispensable for sustaining almost all life forms on the earth. This reaction proceeds through the so-called S-state cycle (Fig. 1(c)), in which the Mn_4CaO_5 cluster undergoes state transitions upon absorption of photons in the sequence of $S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow (S_4) \rightarrow S_0$. The structure of the dark-stable S_1 state, as well as those of the meta-stable S_2, S_3 states, have been solved [2,3], and some time-resolved studies have also been conducted [4]. However, the full time-resolved structural changes of the S_2 and S_3 states have not yet been elucidated.

Using the X-ray free electron laser (XFEL) at SACLA **BL2**, we conducted pump-probe serial femtosecond X-ray crystallography (SFX) experiments of PSII microcrystals at room temperature at delay times of 20 ns, 200 ns, 1 μs, 30 μs, 200 μs, and 5 ms after either one (1F) or two (2F) flashes, corresponding to the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions, respectively [5]. We calculated difference *Fourier* maps between those after light illumination and before illumination ($F_{o\,(after\ pump)} - F_{o\,(before\ pump)}$), which sensitively detect maps of areas where structural changes have occurred. In this way, we are able to detect transient structural changes of the catalyst during the formation of the S_2 and S_3 states.

While we observed many structural changes following either 1F or 2F illumination, including both the electron acceptor and donor sides of PSII [5], we will focus these changes on the electron donor side and around the Mn_4CaO_5 cluster here. At 200 ns following 1F, P680, a chlorophyll *a* molecule of the reaction center of PSII, showed some positive density at its Mg ion, reflecting the charge separation event after light illumination (Fig. 2(a)). Accompanying this, an electron is donated to P680 through Y_Z, a Try residue of D1-Y161, from the Mn_4CaO_5 cluster. D1-F186, a residue lying between Y_Z and P680, is shifted toward P680 together with Y_Z and D1-Q165 (Fig. 2(a)), reflecting the electron donation from Y_Z to P680 at this time range. D1-H190, which forms a short, low-barrier H-bond with Y_Z in the S_1 state, moved away from Y_Z,

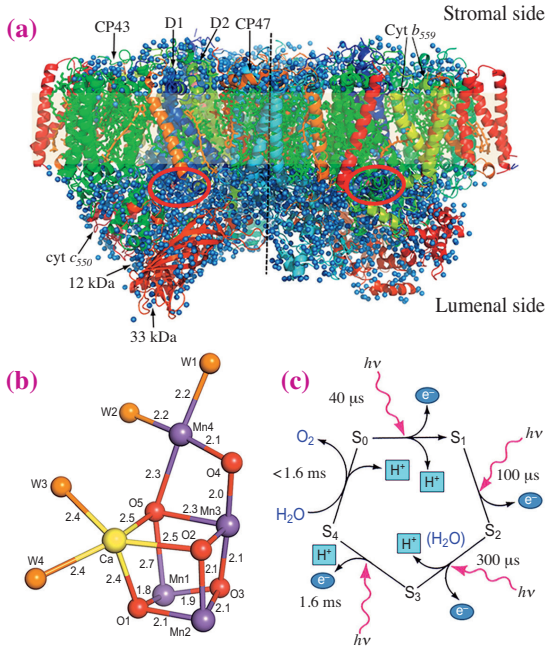

Fig. 1. Structure of PSII and the S-state model of water oxidation. **(a)** Structure of a PSII dimer with a side view of the membrane. The area highlighted in weak yellow in the middle is the trans-membrane area, and a dashed line divides two monomers. The areas encircled with red lines are the site where the Mn_4CaO_5 cluster is bound. **(b)** Structure of the Mn_4CaO_5 cluster, with distances indicated in Å. **(c)** Kok cycle (S-state model) for water oxidation.

making the H-bond between Y_Z 'normal' (Figs. 2(b,c)). These structural changes disappeared gradually following time progression, indicating that the structure returned to its original state after electron donation (Fig. 2). The structural changes following 2F are more or less the same as those following 1F in this area, but they appeared later and smaller than those following 1F, indicating some loss of efficiency as well as the time required to donate the second electron than donating one electron.

In the Mn_4CaO_5 cluster and its immediate environment, a number of difference densities appeared after either 1F or 2F (Fig. 3), indicating large structural changes during the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions. At 200 ns following 1F, Mn1–Mn3 were found to be unstable (Fig. 3(a)), which corresponds to the electron donation from the Mn_4CaO_5 cluster to P680. After the meta-stable S_2 is formed (5 ms after 1F), these Mn ions mostly become stable again; instead, Mn4 slightly shifted its position, corresponding to the stable donation of an electron from Mn4 to P680. At this time, W16, a water molecule that participates

in the H-bond network in the O4-channel, has disappeared, and W10 is largely displaced. In addition, D1-E189, a unique carboxylate ligand that is bound to Mn1 in a monodentate way and thus has the freedom to rotate, rotated its side chain slightly (Fig. 3(a)).

Following 2F, there are also a number of structural changes observed at various time points. Among them, the most remarkable one is that a new water molecule named O6*, appeared near Ca and the side chain of D1-E189 at 1 μs following 2F (Fig. 3(b)). The density for O6* increased at 30–200 μs following 2F, but disappeared completely by 5 ms. Instead, the density for O6 (or Ox), a water molecule previously reported to be inserted into a position close to O5 [2,3], appeared at 200 μs after 2F, and became maximum at 5 ms after 2F (Fig. 3(b)). This indicates that O6* is the precursor for O6, and is translocated to the O6 position during formation of the meta-stable S_3 state. Accompanying these structural changes, Mn1 and Mn4 shifted toward the opposite side, resulting in the elongation of the distance between them which is required for the insertion of O6. D1-E189 also shifted its side chain largely in order for the insertion of O6. Finally, O6 is ligated to the Ca ion, making it from 7-coordinated in the S_1 state to 8-coordinated in the S_3 state.

In addition to the above structural changes, we also found a number of structural changes at the channels and H-bond networks in PSII. First, new water molecules are found in the O1-channel following 2F, suggesting that this channel may function to pump water into the reaction site. Second, different movements are found in the Cl-1 channel between 1F and 2F, suggesting that this channel may play different roles in the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions. Details can be found in the original publication [5].

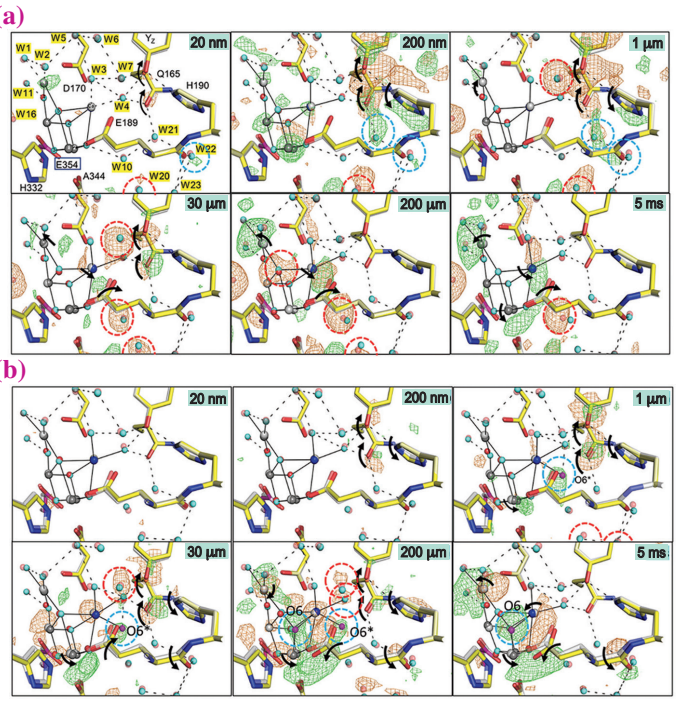

Fig. 3. Structural changes of the Mn_4CaO_5 cluster and its immediate environment following **(a)** 1F or **(b)** 2F. Residue names not encircled in a rectangle are of D1 subunit, whereas that encircled in a rectangle is of the CP43 subunit. Residues or water molecules encircled with red dashed lines indicate their disappearance, and those encircled with cyan dashed lines indicate their appearance.

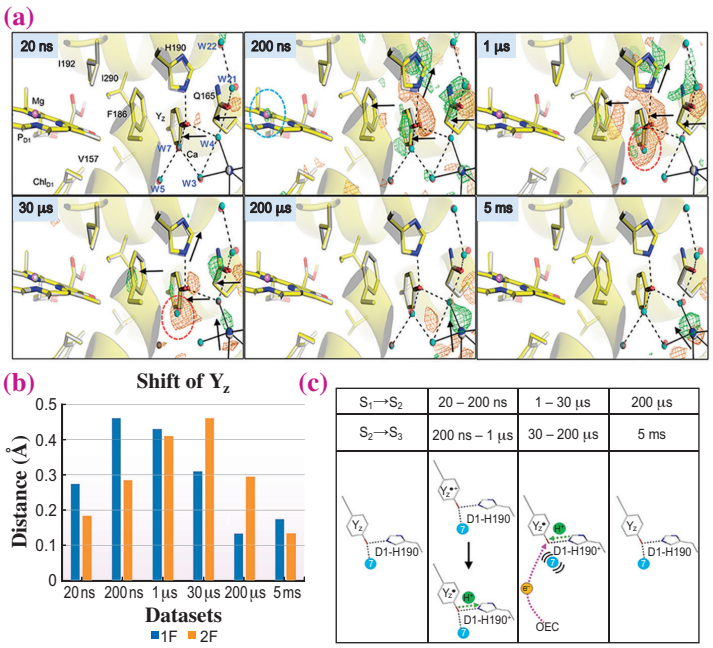

Fig. 2. Structural changes in the Y_Z-P680 area after light excitation. **(a)** Structural changes at various time points after 1F. Green and red meshes represent positive and negative difference densities between 1F-0F, and arrows indicate movements of residues and water molecules. **(b)** Distance changes in the H-bond between D1-H190 and Y_Z after 1F or 2F. **(c)** Schematic of the relationship between D1-H190 and Y_Z after 1F or 2F.

Jian-Ren Shen* and Michihiro Suga

Research Institute for Interdisciplinary Science, Okayama University

*Email: shen@cc.okayama-u.ac.jp

References

[1] Y. Umena *et al.*: Nature **473** (2011) 55.
[2] M. Suga *et al.*: Nature **543** (2017) 131.
[3] M. Suga *et al.*: Science **366** (2019) 334.
[4] A. Bhowmick *et al.*: Nature **617** (2023) 629.
[5] *H. Li, Y. Nakajima, E. Nango, S. Owada, D. Yamada, K. Hashimoto, F. Luo, R. Tanaka, F. Akita, K. Kato, J. Kang, Y. Saitoh, S. Kishi, H. Yu, N. Matsubara, H. Fujii, M. Sugahara, M. Suzuki, T. Masuda, T. Kimura, T. N. Thao, S. Yonekura, L.-J. Yu, T. Tosha, K. Tono, Y. Joti, T. Hatsui, M. Yabashi, M. Kubo, S. Iwata, H. Isobe, K. Yamaguchi, M. Suga and J.-R. Shen: Nature* **626** *(2024) 670.*