Radiation damage-free structure of photosystem II at 1.95 Å resolution revealed by femtosecond X-ray pulses at SACLA

Oxygenic photosynthesis utilizes light energy from the sun to convert carbon dioxide and water into carbohydrates, thereby providing chemical energy required for sustaining life on the earth. During this process, molecular oxygen is evolved as a by-product by the splitting of water at the first step of a series of electron transfer reactions, which transformed our atmosphere into an oxygenic one that enabled aerobic life to evolve and flourish as we enjoy today. This water-splitting reaction is catalyzed by photosystem II (PSII), a membrane-protein complex consisting of 17 trans-membrane subunits and 3 peripheral, hydrophilic subunits with a total molecular mass of 350 kDa for a monomer.

In order to reveal the water-splitting mechanism, it is essential to solve the structure of PSII, especially the catalytic center for water-splitting. After decades of extensive studies, the structure of PSII dimer was solved at resolutions gradually increased up to 1.9 Å in 2011 with X-rays of SPring-8 [1-3], which made PSII the largest membrane protein complex with its structure solved beyond 2.0 Å resolution. In this high resolution structure, the detailed organization of the catalytic center for water-splitting was clearly elucidated, which showed that it is a Mn_4CaO_5-cluster organized in a distorted chair form (Fig. 1(a)). The distorted structure suggested that it is unstable in some extent, and this is considered to be one of the most important features required for the catalytic activity. Since the water-splitting reaction proceeds through an S-state cycle composed of four steps, and each step is driven by the absorption of one photon causing a slight structural re-arrangement of the catalyst, the distorted, unstable structure should allow such structural changes to occur easily.

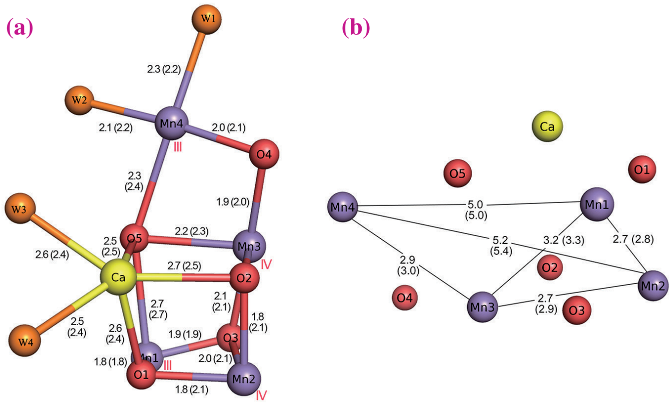

Fig. 1. **(a)** Structure of the Mn_4CaO_5-cluster. Interatomic distances of the XFEL-structure at 1.95 Å are shown in Å, with the corresponding distances obtained in the previous synchrotron radiation (SR) structure shown in parentheses. **(b)** Comparison of the Mn-Mn distances between those obtained by XFEL and SR (parentheses).

The reasons causing the distortion in the structure of the Mn_4CaO_5-cluster are two-folds: One is that the Ca-O distances are slightly longer (2.3–2.5 Å) than the typical Mn-O distances (1.8–2.1 Å), making the cubane core asymmetric; the other one is that, among the five O atoms bridging four Mn ions, four O atoms have normal Mn-O distances, whereas the 5th O (O5) atom has unusually longer distances to its nearby Mn ions in the range of 2.4–2.7 Å (Fig. 1(a)). This suggested that the O5 atom binds weakly to the Mn ions, and therefore may be cut out during the reaction, providing one of the substrates for O=O bond formation.

However, the high-resolution structure of PSII described above was solved with SPring-8 beamlines, and a possibility was raised that the structure has suffered some radiation damage, causing elongation of the Mn-Mn and Mn-O distances. Indeed, previous EXAFS as well as theoretical (QM/MM) studies based on the X-ray structure have suggested slightly shorter Mn-Mn distances, which hampered unambiguous elucidation of the structure and reaction mechanism.

In order to eliminate the radiation damage and solve the PSII structure at an atomic resolution, we utilized femtosecond XFEL pulses from SACLA to collect the X-ray diffraction data (**BL3**), which allowed collection of the diffraction data before radiation damage occurs (diffraction before destruction). Since PSII is an extremely large membrane protein complex, its crystal diffracts very weakly, and small crystals have been shown to have a very low resolution (4.5–5.5 Å) with the XFEL pulses [4]. Thus, we used large PSII crystals, and adopted an approach in which, the diffraction data was collected by irradiating one point of the crystal with one XFEL pulse, then translating the crystal by 50 μm to enable collection of the diffraction image from a fresh, undamaged volume of the crystal (Fig. 2) [5]. In this way, a full data set was collected by use of more than 100 large PSII crystals, and we collected two independent data sets from two different batches of crystals. Both data sets were analyzed at 1.95 Å resolution, and the atomic distances were taken from the average of the two structures [5].

Our results showed that most of the Mn-Mn distances were shortened by 0.1–0.2 Å (Fig. 1(b)),

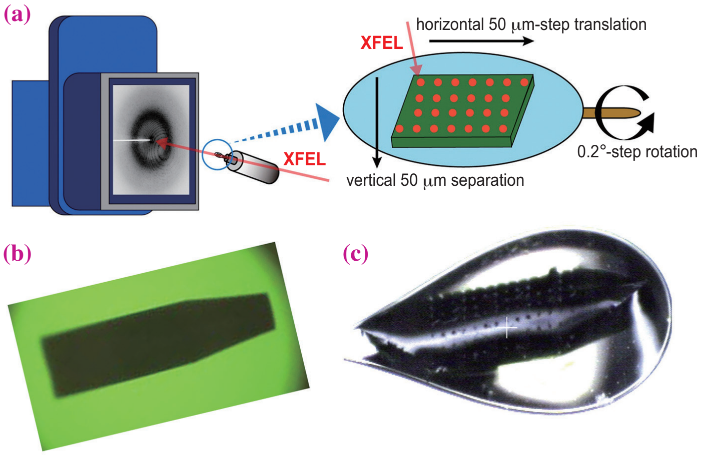

Fig. 2. **(a)** Schematic drawing of the XFEL-diffraction experiment. Still diffraction images were recorded from different spots of large PSII crystals, and the crystals were rotated by 0.2° between every two consecutive images over a range of 180°. Adjacent irradiation spots were separated by 50 μm in the horizontal direction for any rotational angle. Translation in the vertical direction was varied depending on the rotational angle so that the irradiation spots were separated by at least 50 μm in the vertical direction, too. **(b)** and **(c)** A picture of the PSII crystal before (b) and after the XFEL diffraction experiment (c). The path where the XFEL beam passed through became hollowed out, resulting in footprints of the irradiation spots by the XFEL beam which were well separated.

suggesting the elimination of the radiation damage. The O5-Mn1 and O5-Mn4 distances became 2.7 and 2.3 Å, respectively. Compared with the previous structure, these distances were slightly changed. However, they are still extremely longer than the typical Mn-O distances. Based on the Mn-O and Mn-ligand distances, we also assigned the valences of the four Mn ions to be (Mn1, Mn2, Mn3, Mn4)=(III, IV, IV, III) (Fig. 1(a)) [5].

The above results indicated that the unusual feature of the O5 atom is retained in the damage-free structure, suggesting that the O=O bond formation occurs in the area involving the O5 site. Based on this and other theoretical studies, a reaction mechanism was proposed in which, the O5 atom may move closer either to Mn4 or Mn1 upon one electron transfer from the S_1-state to S_2-state, resulting in two types of structures, namely, one with an open space between O5 and Mn1 (right-side open) (Fig. 3(a)) and the other one with an open space between O5 and Mn4 (left-side open) (Fig. 3(b)). These two types of structures have been shown to be in a quasi-equilibration in the S_2-state, which may enable an additional water (O atom) to come into either of the open spaces upon transition from S_2 to the higher S-state. Thus, the O=O bond formation may proceed either in the right-side or left-side between O5 and the newly inserted water (O atom) (Fig. 3) [5].

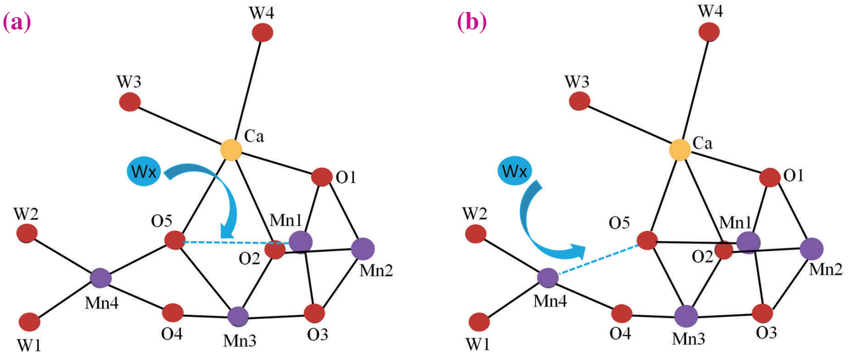

Fig. 3. Proposed mechanism for the O=O bond formation. **(a)** The O5 atom moved toward Mn4, resulting in an open space between O5 and Mn1 (right-side open), allowing a new water molecule (Wx) to come in and form the O=O bond between O5. **(b)** The O5 atom moved toward Mn1, resulting in the left-side open structure and allows a new water molecule to come in and form the O=O bond.

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