

Structure of photosystem II oxygen-evolving complex at 1.9 Å resolution

The process of photosynthesis is composed of two reactions, the light and dark reactions. In the light reaction, solar energy is utilized to synthesize the cellular energy source, adenosine triphosphate (ATP), and the cellular reducing reagent, nicotinamide adenine dinucleotide phosphate (NADPH). In the dark reaction, carbon dioxide (CO₂) is fixed and converted to carbohydrates with the consumption of ATP and NADPH. The first light reaction is catalyzed by photosystem II (PSII) embedded in the thylakoid membrane. PSII catalyzes photoinduced water oxidation leading to the production of protons, electrons, and molecular oxygen. The former two products are utilized by ATPase and NADP reductase in the light reaction, respectively, and the third product is indispensable for sustaining oxygenic life on the earth. Cyanobacterial PSII contains 17 membrane-spanning subunits and 3 membrane-extrinsic subunits in each monomer. The catalytic center of the oxygen-evolving complex (OEC) is located in the luminal surface of PSII. Upon light-induced charge separation at the PSII reaction center, electrons are abstracted from the OEC via a redox-active tyrosine, D1-Y161 (Yz). The OEC is composed of 4 Mn atoms and 1 Ca atom forming a Mn₄Ca cluster. In the last decade, the structures of PSII have been reported at resolutions in the range of 3.8-2.9 Å [1-4], from two closely related thermophilic cyanobacteria, *Thermosynchococcus elongatus* and *T. vulcanus*. These structural studies provided the arrangement of the protein subunits, the location of the cofactor molecules; however, these resolutions were inadequate to solve the detailed structure of the Mn₄Ca cluster as well as the precise arrangement of the amino acid residues and cofactors.

In addition, no water molecules, which are important as substrates and ligands, have been found in these structures. In order to uncover the mechanism of light-induced water oxidation, it is essential to solve the detailed structure of the Mn₄Ca cluster as well as to locate the water molecules.

In this study, we prepared PSII dimers from *T. vulcanus*, and improved their purity and homogeneity by introducing a recrystallization step. PSII crystals were then obtained and their quality was improved by post-crystallization dehydration treatments, which yielded crystals diffracted to a resolution of 1.9 Å. Here, we report the PSII structure at 1.9 Å resolution (PDB code: 3ARC) [5] using diffraction data collected at beamline **BL44XU**.

The overall PSII dimer structure is shown in Fig. 1. Each PSII monomer contained 19 subunits, among which the small membrane-spanning subunit, psbY, was not found. The superposition of our PSII dimer with the dimeric structure composed of two monomers (PDB code: 3BZ1 and 3BZ2), which were reported at 2.9 Å resolution from *T. elongatus* [4], yielded a root mean square deviation of 0.78 Å, indicating that our structure from *T. vulcanus* is similar as the structure from *T. elongatus*. In addition to the protein subunits, there were 81 or 82 cofactors: 35 chlorophylls, two pheophytins, 11 β-carotenes, 20 lipids, two plastoquinones, two haems, one non-haem iron, one bicarbonate, one magnesium ion, three chloride ions, two or three calcium ions, and one Mn₄Ca cluster, in each PSII monomer. We found about 2,800 water molecules in PSII dimer, for the first time. The water molecules were organized into two layers located in the stromal and luminal regions, with the latter having

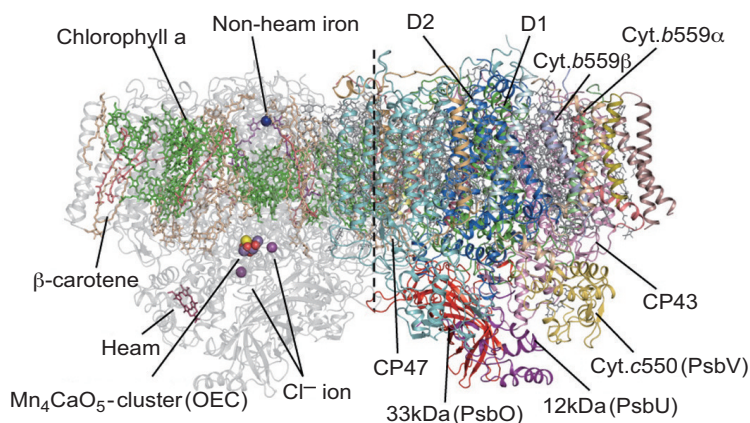


Fig. 1. Overall structure of PSII dimer from *T. vulcanus* and the location of the Mn₄CaO₅ cluster. The protein subunits on the right-hand side were colored individually and the locations of the cofactors are illustrated on the left-hand side. The central broken line is the non-crystallographic pseudo-C2 axis relating the two monomers.

four-folds more water molecules than the former. Some water molecules were found within the membrane region, most of which served as ligands to chlorophylls, and four water molecules were found to be associated with the Mn_4Ca cluster, two of which were associated with Mn_4 (W1 and W2) and the other two with Ca atoms (W3 and W4) (Fig. 2). Some water molecules participated in the hydrogen-bond network linking the OEC to bulk solvent region, and filled some channels from the bulk solvent to the OEC. They may therefore serve as channels for the transport of products (proton and dioxygen) and substrates (water) from and to the OEC.

We determined unambiguously the positions of the metal atoms in the Mn_4Ca cluster using the electron density map corresponding to each of the five metal atoms. Five oxygen atoms, which linked each metal atom with oxo-bridges, were also clearly observed with the omitted difference Fourier map (Fig. 2). We therefore determined the Mn_4Ca cluster to be a Mn_4CaO_5 cluster, for the first time.

There are six carboxylate ligands and one histidine ligand for the Mn_4CaO_5 cluster, among which, D1-D170, D1-E333, D1-D342, D1-A344, and CP43-E354 served as bidentate ligands and D1-189 and D1-H332 served as monodentate ligands (Fig. 3). Three oxygen atoms in the cluster, O2, O3, and O4, were hydrogen-bonded by CP43-R357, D1-H337, and CP43-R357, respectively, and two of the oxygen atoms, O1 and O4, were linked to water networks by the hydrogen-bond, but one of the oxygen, O5, was located in the hydrophobic environment surrounded by D1-V185. These hydrogen bonds to the oxygen atoms may

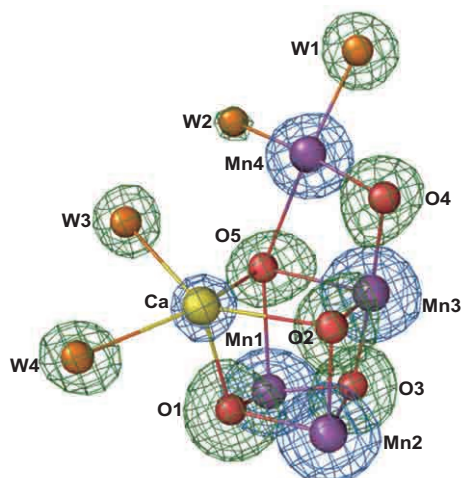


Fig. 2. Determination of individual atoms associated with the Mn_4CaO_5 cluster. The structure of the Mn_4CaO_5 cluster was superposed with the $2F_o-F_c$ map (blue) contoured at 5σ for manganese and calcium atoms, and the omitted F_o-F_c map contoured at 7σ for oxygen atoms and water molecules. The cluster formed resembled a distorted chair.

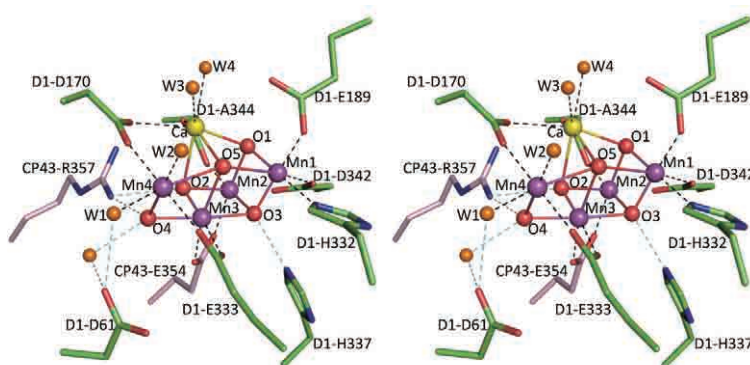


Fig. 3. Stereoview of the Mn_4CaO_5 cluster and its ligand environment. The hydrogen bonds are indicated by cyan broken lines, and the coordinating bonds are indicated by black broken lines. Water molecules are indicated by orange dots [5].

stabilize the structure of the cluster (Fig. 3). The cluster structure determined had a distorted chair form. The distortion was caused by the differences in the bond distances between Mn-O and Ca-O. While most of the Mn-O distances are within the range of 1.9-2.1 Å, the distances between one oxygen atom (O5) and 3 Mn atoms (Mn1, Mn3, and Mn4) are in the range of 2.4-2.6 Å, and Ca-O distances are in the range of 2.3-2.5 Å, which are longer than the normal Mn-O distances except for the Mn-O5 distance. Thus, the bond distances between O5 and metal atoms were considerably long. This is a significant feature of the cluster, and may suggest a higher reactivity of O5 compared with other oxo-atoms. Interestingly, two water ligands, W2 and W3, which are bonded to Mn4 and Ca atoms, respectively, are within the range of the hydrogen bond of O5. This suggests that W2, W3, and O5 constitute the site for the water oxidation that reaction leads to the generation of molecular oxygen.

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