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Crystal structure analysis of photosystem II from *Thermosynechococcus vulcanus*

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Introduction

Photosystem II (PSII) complex is the site of oxygen evolution and electron transfer leading to conversion of light energy into chemical energy which is continuously supporting our life on the earth. PSII is a multi-subunit membrane protein complex consisting of 13-14 transmembrane subunits and 3 peripheral subunits with a total molecular mass of 310 kDa. In order to understand the mechanism of electron transfer and oxygen evolution reactions within PSII, significant efforts have been made in the recent years to analyze the 3-dimensional structure of PSII by means of both cryo-electron microscopy and X-ray crystallography. In particular, Zouni et al. [1] succeeded in obtaining 3-D crystals of PSII dimer from a thermophilic cyanobacterium, *Thermosynechococcus elongatus* (formerly *Synechococcus elongatus*) and reported its 3-dimensional structure at 3.8 Å resolution analyzed by X-rays of synchrotron radiation. In their structural model, 36 transmembrane helices were found, of which, 22 were assigned to D1, D2, CP47, CP43, and the rest were assigned to low molecular mass subunits based on biochemical results and cryo-electron microscopy of 2-dimensional crystals so far obtained (some of the helices have not been assigned). In the lumenal side, the structure of two out of three extrinsic proteins, cytochrome (cyt) *c*-550 and the 33 kDa protein, were determined. Most of the electron transfer cofactors including 32 chlorophyll (chl) *a* molecules, cyt *b*-559, Q_A, 2 pheophytins and the non-heme iron, were identified. In addition, a model of the Mn-cluster was proposed based on the electron density map obtained.

We have succeeded in crystallizing PSII from another thermophilic cyanobacterium, *Thermosynechococcus vulcanus* (formerly *Synechococcus vulcanus*). Our PSII crystals contained PSII dimer, retained a high rate of oxygen evolution, and diffracted to a maximum resolution of 3.5 Å with X-rays of synchrotron radiation [2]. We determined phases by a combination of single isomorphous replacement with anomalous scattering (SIRAS) and multiwavelength anomalous dispersion (MAD), and obtained the PSII structure from our crystals. In the present report, we present the structural model of PSII analyzed to a resolution of 3.7 Å with our crystals, and compare it with the model reported for *T. elongatus*.

Materials and Methods

PSII complex highly active in oxygen evolution was purified and crystallized *from T. vulcanus* as described previously [2]. X-ray diffraction data were collected at beamlines BL41XU [3] of SPring-8, a third generation synchrotron radiation facility in Japan. The crystals were flash-cooled with a nitrogen gas stream to 100 K, and a wavelength of 1.0 Å of X-rays was used to collect native and heavy atom derivative data. The detector used was a CCD detector, MarCCD-165. Diffraction data were processed with DPS-MOSFLM [4]. The space group was determined to be $P2_12_12_1$, and the unit cell parameters are a=129.5 Å, b=225.6 Å, c=307.6 Å. The phases were determined by a combination of SIRAS and MAD. The SIRAS data were collected with a Ta_6B_{14}-derivative which gave rise to an experimental data set to 4.0 Å resolution, but the phasing power decreased quickly beyond 6.0 Å resolution due to the large cluster of $Ta_6Br_{12}^{2+}$ cation used (data to be published elsewhere). In order to improve the phasing statistics, MAD data were collected with native crystals at BL41XU around K-absorption edges of the Mn-cluster and Fe, respectively. The SIRAS/MAD combined phases were calculated with SHARP [5] to 4.0 Å resolution and extended to 3.7 Å resolution with DM of the CCP4 program suite [6]. The resulted electron density map was visualized and interpreted with the program TURBO-FRODO [7], and some of the transmembrane helices in the model of PSII from *T. elongatus* (PDB No. 1FE1) were used to help building our model. Figures were drawn with TURBO-FRODO and Molscript [8].

Results and Discussion

Previous N-terminal sequencing analysis has shown that oxygen-evolving PSII from *T. vulcanus* contained low-molecular mass subunits of PsbE, PsbF, PsbH, PsbK [9], PsbI, PsbL [10], PsbN, PsbT (previously called PsbM) [11], and PsbX (H, Koike, personal communication). These are very similar to the composition reported for PSII of *T. elongatus*, except for PsbJ, which was present in *T. elongatus* but has not been identified in our PSII from *T. vulcanus*. In addition to these low-molecular mass subunits, there are four large transmembrane subunits, D1, D2, CP47, CP43, and three extrinsic subunits, PsbO, PsbU and PsbV (cyt *c*-550) [2]. We have determined that each PSII monomer contained 33-36 chl *a* molecules (data not shown). In total, there are at least 13 transmembrane subunits and 3 peripheral proteins with a calculated molecular mass of 310 kDa.

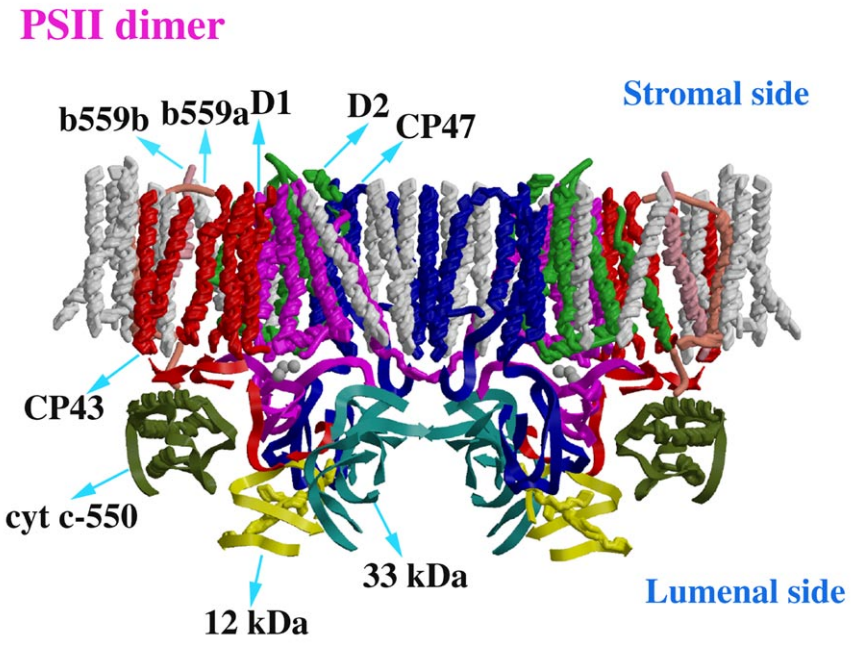

Fig. 1. Structure of PSII dimer determined for *T. vulcanus*. Assignments of the transmembrane helices, PsbO and PsbV were according to Zouni et al. The PsbU subunit (12 kDa protein) were newly determined and shown. Large loops of CP47, CP43 protruding into the lumenal side were also shown.

Fig. 1 shows the preliminary structural model of PSII dimer determined for *T. vulcanus*. Our structural modelling is still in progress and the final one will be published elsewhere. The arrangement of transmembrane helices is largely the same as that reported for *T. elongatus* [1]. Each of the 5 helices of D1 and D2 could be assigned based on their homology to the L, M subunits of photosynthetic bacterial reaction center [12]. At the present level of resolution, the assignment of other helices to other subunits is tentative; we follow the assignment of Zouni et al. [1] based on biochemical results as well as cryo-electron microscopy of two-dimensional crystals so far obtained. We also identified most of the cofactors including 32 chl *a* molecules; the position of them are more or less similar to those reported by Zouni et al. (a detailed comparison of our structure with the *T. elongatus* one will be published elsewhere). Large differences were found in the lumenal side of PSII. We extended preliminally some of the helices of CP43 and CP47 into the lumenal side, which have been proposed to have large loops protruding into the lumen. This results in a close contact of the loops of CP47 with PsbO, which has been reported to be cross-linked. A loop extended from CP43 was also close to PsbO, suggesting that these two components are also close to each other in the lumen. In addition, an isolated part of electron density was found between PsbO and PsbV (cyt *c*-550); this was assigned to PsbU (12 kDa protein) based on previous results that this subunit can bind and function in PSII only in the presence of PsbO and PsbV [13]. This subunit was not found in the structure

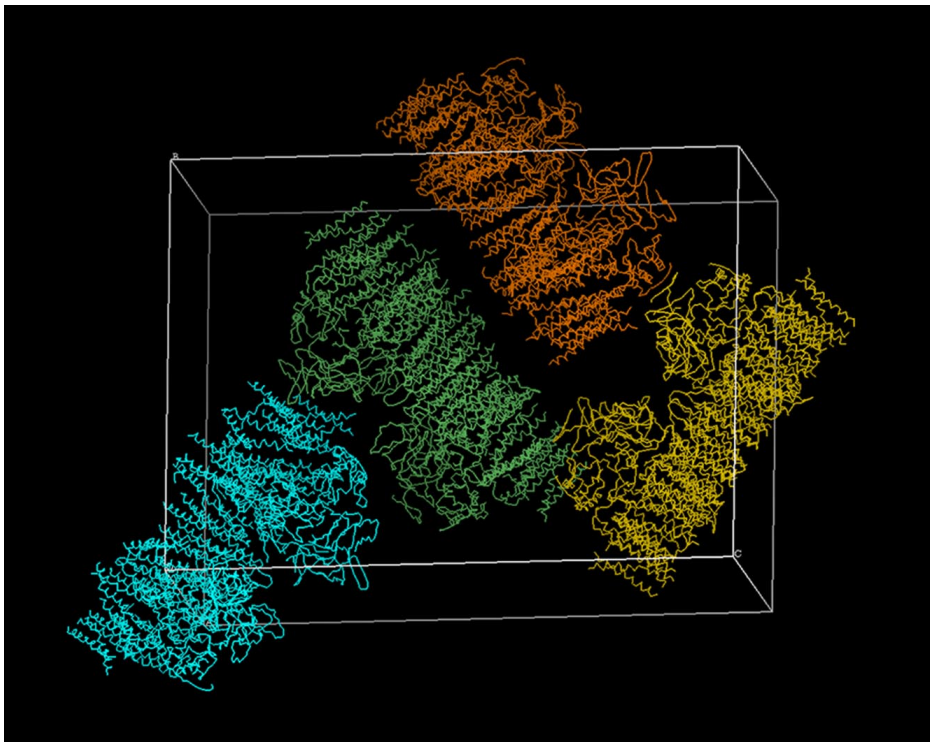

Fig. 2. Crystal packing of the PSII dimers. Four PSII dimers were shown in the unit cell of $P2_12_12_1$.

of *T. elongatus*. In addition, we confirmed the global position of the Mn-cluster from its anomalous scattering data. Since MAD phasing disturbed the electron density around the region of the Mn-cluster, we used the SIRAS phase with the Ta_6Br_{14} derivative to calculate the electron density around the Mn-cluster, which was extended by DM from 6 Å to 3.7 Å resolution. The result showed that the electron density map contoured at a 5 sigma level is slightly different in its fine structure from that reported for *T. elongatus*. At present, our map for the Mn-cluster can be fitted with the structural model proposed for *T. elongatus*; however, a different model may also be accommodated (data to be published elsewhere).

Fig. 2 shows the molecular contacts between adjacent PSII dimers in a unit cell of our PSII crystals. The predominant contact was found to be between the large hydrophilic region in the lumenal side of one PSII dimer and the hydrophobic, transmembrane helices in the edge of adjacent dimer. This hydrophilic-hydrophobic interaction provides an example for crystal packing of membrane proteins, which may be important for formation of crystals of other membrane proteins or their complexes.

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