Crystal Structure Analysis at Atomic Resolution of Photosystem II Complex

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Photosystem II (PSII) is a membrane protein complex consisting of 14 membrane-spanning proteins and 3 hydrophilic, peripheral proteins with a total molecular mass of 320,000 Da (including 40-45 chlorophylls). We have crystallized the PSII complex purified from a thermophilic cyanobacterium, *Synechococcus vulcanus*. The crystals contained PSII dimers which retained a high activity of oxygen evolution (1). In the previous studies, we have used BL41XU to collect diffraction data sets from both native crystals and multiple heavy atom derivatives. Diffraction data sets from PSII native crystals were processed with DPS/MOSFLM to 4.0 Å resolution, and the electron density map was obtained by SIRAS with a Ta_6Br_{14} derivative. In the present study, we aimed at analyzing the PSII structure at higher resolution. This depends critically on the quality of the crystal. Since PSII is a multi-subunit membrane protein-complex located on the thylakoid membrane in which, many other membrane-spanning and peripheral proteins co-exist, purity of the final preparation used for crystallization is one of the major factors influencing the crystal quality. Another important factor affecting the crystal quality is the type, purity and concentrations of detergent used for crystallization. By controlling these conditions, we obtained crystals which showed higher resolution than the previous ones. These crystals generated diffraction data set useful for structure analysis at 3.7 Å resolution; furthermore, diffraction spots beyond 3.5 Å resolution were observed, suggesting that higher resolution might be possible by further improving these factors along with the lines we are exploring (Table 1).

Table 1. Part of a data set from a PSII native crystal processed with DPS/MOSFLM.

Res. Range (Å)	Rfac	I/Sigma	Compl. (%)
4.18-3.91	0.198	3.9	89.5
3.91-3.69	0.290	2.7	85.0
3.69-3.50	0.448	1.8	83.4

Reference

(1) Shen J.-R. and Kamiya N. (2000) Biochemistry 39, 14739-14744.

Phase Extension for Photosystem II Complex by Multiple Isomorphous Replacement with Optimized Anomalous Scattering on Manganese

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Photosystem II (PSII) of *Synechococcus vulcanus* is a membrane protein complex consisting of 14 transmembrane subunits and 3 hydrophilic, peripheral subunits with a total molecular mass of 320 kDa (including 40-45 chlorophylls). The PSII complex contains intrinsically a 4-Mn cluster and 3 atoms of Fe for a monomer. We have succeeded in crystallization of the PSII complex in a dimeric form which retains full oxygen-evolving activity (*Biochemistry* **39** (2000) 14739).

We collected previously diffraction data sets at BL41XU with a native crystal and a heavy atom derivative of Ta6Br14 up to resolutions of 3.7 A and 4.0A, respectively. It was very difficult to maintain the PSII crystals isomorphous in soaking of various heavy atom compounds, and the cluster structure of Ta6Br12 cation was advantageous for interpretation of difference Patterson map. Unfortunately, however, phasing power of the Ta derivative, calculated by SIRAS technique, decreased drastically over 6.0 A because of scattering factor of Ta6Br12 cluster.

In order to obtain phase information at much higher resolution, we tried to perform MAD experiments using the intrinsic Mn cluster and Fe atoms of PSII. After XAFS spectroscopy at the Mn absorption edge, three data sets were collected with a MAR-CCD165 at 1.8892 A (peak), 1.8929 A (edge) and 1.0A (remote). For the Fe atoms, 1.7390 A (peak), 1.7409 A (edge) and 1.0 A (remote) were selected. All data reductions were carried out with DPS-MOSFLM and SIRAS-MAD combined phases were calculated with SHARP. In comparison of electron density maps with the Ta6Br14 SIRAS phase and the SIRAS-MAD combined phases, we confirmed significant improvements on structural detail in the latter map, for example, wavy feature of electron density distributions for transmembrane helices of PSII was observed. The model building at 4.0 A resolution is in progress.