Crystallization and the Crystal Properties of the Oxygen-Evolving Photosystem II from *Synechococcus vulcanus*†

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ABSTRACT: A photosystem II (PSII) complex highly active in oxygen evolution was purified and crystallized from a thermophilic cyanobacterium, *Synechococcus vulcanus*. The PSII complex in the crystals contained the D1/D2 reaction center subunits, CP47 and CP43 (two chlorophyll-binding core antenna proteins of photosystem II), cytochrome *b*-559 α- and *β*-subunits, several low molecular weight subunits, and three extrinsic proteins, that is, 33 and 12 kDa proteins and cytochrome *c*-550. The PSII complex also retained a high rate of oxygen evolution. The apparent molecular mass of the PSII in the crystals was determined to be 580 kDa by gel filtration chromatography, indicating that the PSII crystallized is a dimer. The crystals diffracted to a maximum resolution of 3.5 Å at a cryogenic temperature using X-rays from a synchrotron radiation source, SPring-8. The crystals belonged to an orthorhombic system, and the space group was $P2_12_12_1$ with unit cell dimensions of $a = 129.7$ Å, $b = 226.5$ Å, and $c = 307.8$ Å. Each asymmetric unit contained one PSII dimer, which gave rise to a specific volume (V_M) of 3.6 Å^3/Da based on the calculated molecular mass of 310 kDa for a PSII monomer and an estimated solvent content of 66%. Multiple data sets of native crystals have been collected and processed to 4.0 Å, indicating that our crystals are suitable for structure analysis at this resolution.

Photosystem II (PSII)[1] is a large, multisubunit membrane−protein complex consisting of more than 10 membrane-spanning proteins and three peripheral, hydrophilic proteins with a total molecular mass of more than 250 kDa in its monomeric form (for review, see ref *1*). The function of PSII is to absorb and convert light energy into biologically useful chemical energy through a series of light-induced electron-transfer reactions, coupled with this is the splitting of water into molecular oxygen. Both the energy conversion and oxygen-evolving reactions catalyzed by PSII are of extreme importance to life on the earth. To understand the mechanisms of electron transfer and oxygen evolution in detail within PSII, it is essential to solve the structure of PSII. One of the significant features of PSII, however, is its highly fragile oxygen-evolving complex. This has largely hampered the structural studies on PSII, since a stable protein complex is a prerequisite for most of the structural studies. Low-resolution images of the isolated PSII were first obtained with single-particle analysis of isolated PSII (*2*−*4*) and cryoelectron microscopy of two-dimensional crystals (*5*−*7*). Recently, the structure of PSII was analyzed at 8 Å resolution by cryoelectron microscopy of two-dimensional crystals of a higher plant RC (reaction center)−CP47 complex that lacked the CP43 subunit and three extrinsic proteins involved in the oxygen-evolving reaction (*8*, *9*). This analysis revealed 23 transmembrane helices, of which 10 were assigned to the D1/D2 reaction center subunits and six to CP47 by comparison with the bacterial reaction center and PSI. A notable feature of the PSII structure revealed is its high similarity in the overall structure of the D1/D2 reaction center with that of both the bacterial reaction center and PSI, suggesting that all the reaction centers have a common evolutionary origin (*10*).

In addition to the limited resolution, the two-dimensional crystals of PSII so far utilized were obtained from higher plants, the PSII of which, in most cases, has lost the oxygen-evolving activity (*8*, *9*). Thus, the structural information concerning the oxygen-evolving complex is not available from analysis of the two-dimensional crystals. Three-dimensional crystallization of oxygen-evolving PSII from higher plants has also been reported (*11*−*13*). The crystals obtained, however, only yielded a low resolution (*13*) (Shen et al., unpublished results), which is not suitable for structural analysis in its current form. Very recently, two groups reported the success of three-dimensional crystallization of a PSII complex capable of oxygen evolution from the same thermophilic cyanobacterium, *Synechococcus elongatus* (*14*−*16*). The crystals reported by Kuhl et al. diffracted maximally to a 4.3 Å resolution (*16*), and Zouni et al. reported data collection to a 5.0 Å resolution from their PSII crystals (*14*, *15*). The crystals obtained by the two groups had the same space group and similar unit cell constants. However, while

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[1] Abbreviations: chl, chlorophyll; CP47 and CP43, two chlorophyll-binding core antenna proteins of photosystem II; D1 and D2, the reaction center proteins of photosystem II; PS, photosystem; SDS−PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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Zouni et al. (*14*) reported that the PSII in their crystals is in a monomeric form, Kuhl et al. (*16*) reported that the PSII in their crystals is in a dimeric form; this would result in a significant difference in the packing density of the crystals reported by the two groups. We have been crystallizing the PSII complex independently and have obtained PSII crystals from another thermophilic cyanobacterium, *Synechococcus vulcanus*. The crystals we obtained diffracted to a maximum resolution of 3.5 Å, which is the highest one so far reported. We determined that the PSII in our crystals is in the dimeric form, which retained a high rate of oxygen evolution. Multiple sets of X-ray diffraction data have been collected and processed to a 4.0 Å resolution from the native crystals, which indicated that our crystals are suitable for structural analysis at this resolution by the multiple isomorphous replacement method. We report here the crystallization and crystal properties of our PSII complex.

MATERIALS AND METHODS

Cells of the thermophilic cyanobacterium, *Synechococcus vulcanus*, were grown in four 10 L bottles at 55 °C. The oxygen-evolving PSII complex was isolated from the cyanobacterium according to the procedures described previously (*17*−*19*), with the only modification being that the crude PSII was solubilized with 1.2%, instead of 2.0%, *n*-dodecyl *β*-D-maltoside prior to Mono Q column chromatography. The isolated PSII was finally concentrated and suspended in a buffer containing 20 mM 2-(*N*-morpholino)-ethanesulfonic acid (pH 6.5), 20 mM NaCl, and 3 mM $CaCl_2$ and stored in liquid nitrogen until use. Crystallization was performed with a handmade microdialysis setup in which 10−20 *μ*L samples were placed in a capillary tube and dialyzed against a 1 mL reservoir buffer. The samples contained 4−5 mg chl/mL, 20 mM $MgSO_4$, and 8 mM $CaCl_2$, and the reservoir contained 6−7% poly(ethylene glycol) 1450, 40 mM $MgSO_4$, 20 mM $CaCl_2$, 20 mM Mes (pH 6.5), 20 mM NaCl, and 0.02% *n*-dodecyl *β*-D-maltoside. No additional detergents were added to the PSII samples. The dialysis was performed at 20 °C. Microcrystals appeared within 3−4 days and continued to grow until 10 days.

Chlorophyll concentration was determined according to ref *20*. Protein composition was analyzed by SDS-PAGE with a 16−22% acrylamide gradient gel containing 7.5 M urea as in ref *21*. Protein samples were solubilized with 2% (w/v) lithium dodecyl sulfate, 60 mM dithiothreitol, and 60 mM Tris-HCl (pH 8.5) at 0 °C immediately before electrophoresis. Oxygen evolution was measured with a Clark-type oxygen electrode at 30 °C under continuous, saturating light in a buffer containing 20 mM 2-(*N*-morpholino)ethanesulfonic acid (pH 6.5), 20 mM NaCl, and 10 mM $CaCl_2$. The chl concentration was 10 *μ*g/mL, and the electron acceptors used were 0.4 mM phenyl *p*-benzoquinone and 1 mM potassium ferricyanide. The molecular mass of the PSII complex was determined by gel filtration chromatography with a column (Superdex 200, HR30/10, Pharmacia) equipped to an HPLC setup (SMART system, Amersham Pharmacia Biotech Co.). The column was equilibrated with 30 mM 2-(*N*-morpholino)ethanesulfonic acid (pH 6.5), 20 mM NaCl, and 0.03% *n*-dodecyl *β*-D-maltoside and run in the same medium. The molecular weight standards used were thyroglobulin (669 000 Da), ferritin (440 000 Da), catalase (232 000 Da), and aldolase (158 000 Da). For comparison, a PSII core complex prepared from rice leaves according to ref *22*, which is a mixture of dimer and monomer, was run with the same column.

For X-ray diffraction measurements, PSII crystals were dialyzed against a solution containing 25% glycerol and 20% poly(ethylene glycol) 1450 as cryoprotectants and flash-frozen in a nitrogen gas stream at 100 K. The frozen PSII crystals were stored in liquid nitrogen until use. X-ray diffraction experiments were performed at beamlines BL41XU (*23*), BL44B2 (*24*), and BL45PX (*25*) of a synchrotron radiation facility, SPring-8, Japan. The diffraction patterns were recorded with an imaging plate detector, RAXIS-IV (RIGUKU), at an X-ray wavelength of 1.0 Å. The sample-to-detector distance was adjusted to 470 mm in order to give a maximum separation between adjacent diffraction spots, which yields enough resolution in the edge of the detector. Diffraction images obtained were processed with the softwares AUTO, newly developed by Dr. Higashi (RIGAKU, unpublished), and MOSFLM (*26*).

RESULTS

Highly purified and active oxygen-evolving PSII was prepared from the thermophilic cyanobacterium, *Synechococcus vulcanus*, according to the previously published procedures (*17*−*19*). Using this PSII preparation, crystals with different shape and size were obtained with the microdialysis method and the conditions as described in the Materials and Methods. First, rectangular rod-type crystals (Figure 1A) were obtained within 3−4 days; they grew to a maximum size of 0.5 mm × 0.03 mm × 0.01 mm. When the dialysis rate was slowed by decreasing the precipitant concentration, crystals with a rhombic shape and a typical size of 0.4 mm × 0.3 mm × 0.05 mm were obtained in 8−10 days (Figure 1B). These crystals may have grown larger, to a maximum size of 2 mm × 1 mm × 0.2 mm (Figure 1C), if the dialysis rate had been slowed further. Among these crystals, the needle-type crystals yielded a maximum resolution of 6 Å, whereas crystals with the rhombic shape yielded a higher resolution (see below). The crystals shown in Figure 1B,C gave rise to a same diffraction pattern, indicating that they belong to the same crystal system and space group (see below). The larger crystals, however, had a larger mosaicity than the small crystals, probably due to difficulties in flash-freezing the larger crystals; thus, the second type of crystals was used in the subsequent X-ray diffraction experiments.

Subunit composition of the PSII crystals was analyzed by SDS-PAGE after redissolving the crystal and then compared with the PSII preparation before crystallization. As shown in Figure 2A, the main subunits of PSII, namely, CP47, CP43, the D1/D2 reaction center proteins, the cytochrome *b*-559 α- and *β*-subunits, several low molecular mass subunits, together with three extrinsic proteins (33 and 12 kDa proteins and cytochrome *c*-550) required for oxygen evolution, were retained in the crystal. This composition is essentially the same as that of the PSII preparation before crystallization. Although the low molecular weight subunits cannot be unambiguously identified from the SDS-PAGE analysis, we may assume that they include at least the gene products *psb*I, *psb*K, *psb*L, and *psb*T, since these gene products have been shown to be required for the full activity

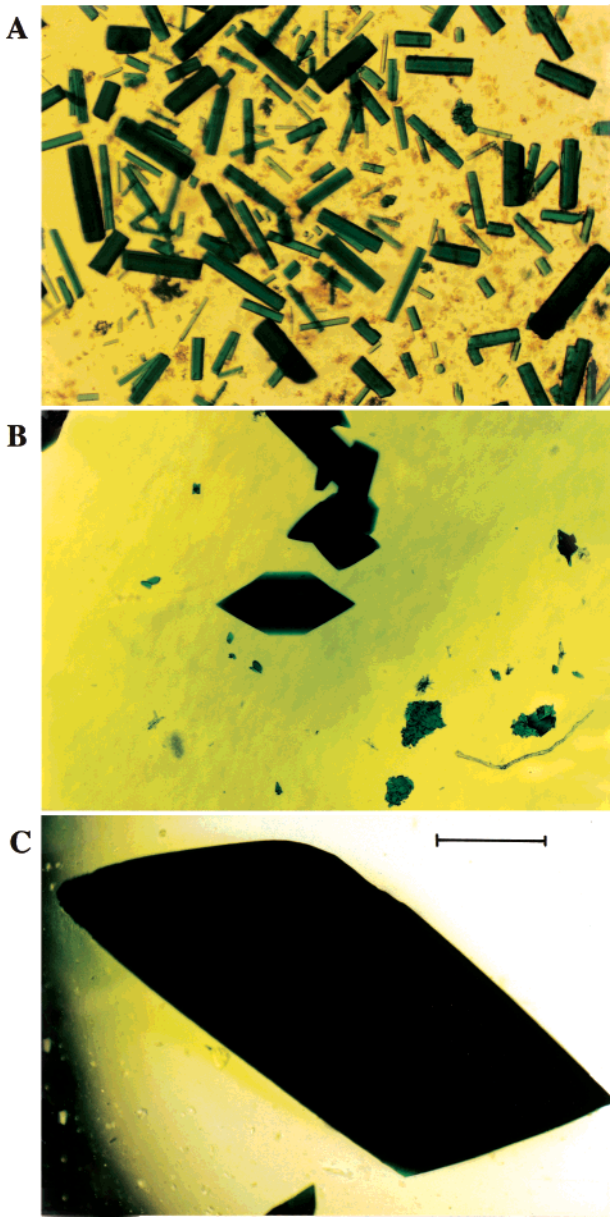

FIGURE 1: Crystals of different shapes and sizes of the oxygen-evolving PSII complex from *Synechococcus vulcanus*. The bar represents 0.5 mm in all the three panels.

of oxygen evolution (*1*, *27*−*30*) or are present in a purified PSII subcore complex from higher plants (*31*). With the presence of these low molecular weight subunits and using known sequences of the PSII subunits from another cyanobacterium, *Synechocystis* sp. PCC 6803 whose complete genome sequence has been determined (*32*), the molecular mass of PSII protein components was calculated to be 270 kDa. Since a PSII core complex contains about 40−45 chl *a* molecules (*27*, *31*), which amounts to a molecular mass of about 40 kDa, the total molecular mass of a PSII monomer is estimated to be 310 kDa.

In the PSII preparation before crystallization, there were four additional faint bands (indicated by arrowheads in Figure 2A); two are in the molecular mass region of 14−17 kDa, and two are in the region of 50−55 kDa. The staining intensity of these bands are very weak even though most of the PSII bands were heavily overloaded in the gel, which apparently indicates that these four bands are contaminating components in the PSII preparation. The two bands in the region of 14−17 kDa are phycobiliproteins (*17*), whereas the origins of the two bands in the region of 50−55 kDa are unknown at present but are probably residual components of F1 ATPase. These components were difficult to remove by the purification procedure, since extensive efforts employed to remove these contaminating components resulted in a partial loss of the PSII extrinsic components, especially cytochrome *c*-550 and the 12 kDa protein, which gave rise to a partially inactivated, heterogeneous PSII preparation not suitable for crystallization. These contaminating components, however, were successfully removed by the crystallization process, resulting in a purer PSII complex in the crystals (Figure 2A, lane 2).

Oxygen-evolving activity of PSII after crystallization was measured under continuous light after redissolving the crystals. Figure 2B shows the oxygen evolution traces of such measurement. PSII after crystallization had an oxygen-evolving activity of 2932 μmol O_2/mg chl/hr. Although this activity is slightly lower than that of PSII before crystallization (82%), the decrease caused by the crystallization procedure is low considering that the crystals were formed after dialysis for 8−10 days at 20 °C. Thus, the PSII complex in the crystals remains largely active in oxygen evolution.

The apparent molecular mass of PSII in the crystals was determined by gel filtration chromatography after redissolving the crystals. Figure 3 shows that the PSII preparation used for crystallization in the present study had a single peak in the gel filtration chromatography, the apparent molecular mass of which was determined to be 580 kDa. This molecular mass is close to the estimated molecular mass of 620 kDa for a PSII dimer. Indeed, the elution peak of the cyanobacterial PSII is exactly the same as that of the PSII dimer from a higher plant, rice, where both PSII dimer and monomer were present and resolved by the gel filtration chromatography (the PSII monomer eluted at an apparent molecular mass of 380 kDa). Thus, the cyanobacterial PSII complex used for crystallization in the present study is a dimer. After crystallization, the PSII complex eluted at exactly the same position as that before crystallization, and as that of the rice dimer, which unambiguously indicates that the crystallization process did not change the dimeric conformation of PSII.

For X-ray diffraction experiments, the PSII crystals were brought into a cryoprotectant solution containing concentrated glycerol and poly(ethylene glycol) by dialysis and then flash-frozen in a nitrogen gas stream at 100 K. Figure 4 shows an X-ray diffraction pattern of the PSII crystal. Diffraction spots with a maximum resolution of 3.5 Å were observed. We have collected several data sets from the PSII crystals at beamlines BL41XU as well as at BL44B2 and BL45PX of SPring-8; Table 1 presents an example of analysis of one such data set up to a 4.0 Å resolution. The crystal belonged to the orthorhombic system with the space group of $P2_12_12_1$ and unit cell dimensions of $a = 129.7$ Å, $b = 226.5$ Å, and $c = 307.8$ Å. Since PSII exists as a dimer in the crystal, each of the four asymmetric units in the $P2_12_12_1$ space group contains one PSII dimer; thus, each unit cell contains eight PSII monomers. This gave rise to a specific volume, V_M value, of 3.6 Å^3/Da, based on the calculated molecular mass of 310 kDa for the PSII monomer. On the basis of this value, the approximate solvent content was estimated to be 66% with the formula $V_{sc}(\%) = 100(1$

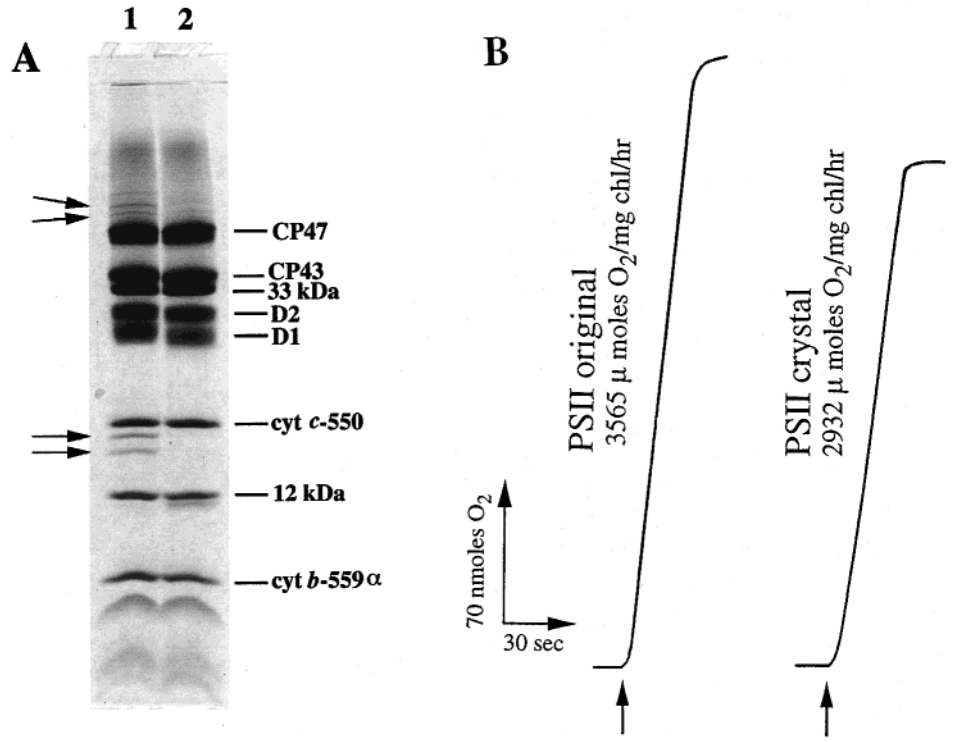

FIGURE 2: (A) Protein composition of the PSII complex before and after crystallization: lane 1, PSII before crystallization; lane 2, PSII after crystallization. The arrowheads indicate small amounts of contaminating components in PSII before crystallization which were removed in PSII after crystallization. (B) Oxygen evolution traces measured with a Clark-type oxygen electrode under continuous illumination. The left panel is PSII before crystallization, and the right panel is PSII after crystallization. The upward arrowheads indicate the initiation of saturating light.

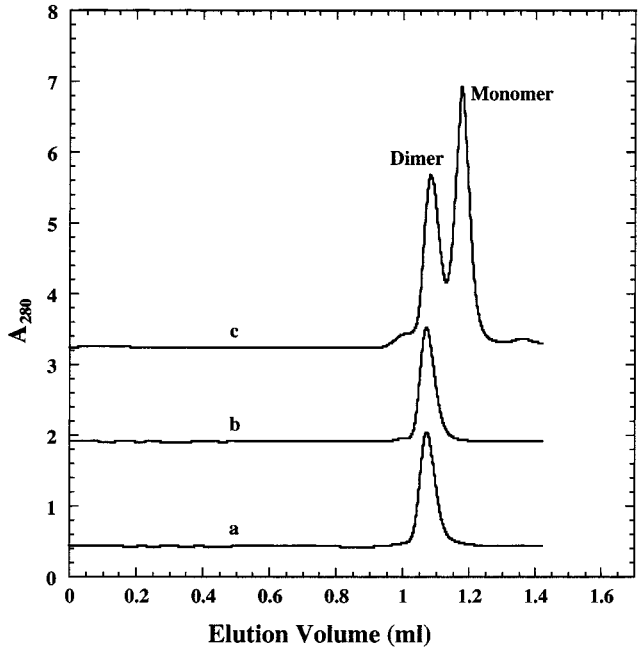

FIGURE 3: Elution pattern of various PSII from a gel filtration column, Superdex 200 (Amersham Pharmacia Biotech Co.): (a) *Synechococcus* PSII before crystallization; (b) *Synechococcus* PSII after crystallization; (c) rice PSII core complex.

$- 1.23V_M$) (*33*). From analysis of the full data sets with the software AUTO, the typical R_{merge} values lay between 6 and 10%, and those for the highest resolution shell (4.1−4.0 Å) were between 17 and 25%. The completeness of collected reflections was calculated to be 75−90%, and the mosaicities of the crystals were estimated to be 0.6−1.0°. Data reductions with the software MOSFLM were also carried out, and the results obtained were similar with those with AUTO (data not shown). Depending on the crystallization and flash-cooling conditions, the unit cell dimensions, R_{merge} value, completeness, and mosaicity may vary slightly among different crystals; however, we have found that under strictly controlled conditions, these variations are small enough to allow us to collect data sets from heavy-atom derivatives with which the PSII structure can be analyzed by the multiple isomorphous replacement method.

DISCUSSION

An oxygen-evolving PSII complex was purified and crystallized successfully from the thermophilic cyanobacterium, *Synechococcus vulcanus*. The crystals we obtained retained the full set of protein subunits for a native PSII complex and were capable of oxygen evolution at a high rate which is largely comparable to that before crystallization, even though the whole crystallization process took 8−10 days at 20 °C. Very recently, crystals of oxygen-evolving PSII have been reported from another thermophilic cyanobacterium, *Synechococcus elongatus*, by two groups (*14*−*16*). The subunit composition, space groups, and unit cell dimensions of our crystal are more or less the same as those reported by the two groups. In these two reports, Zouni et al. reported data collection and processing to 5.0 Å with their crystals (*14*−*15*), whereas Kuhl et al. reported a maximum resolution of 4.3 Å with their crystals (*16*). Our crystals showed a maximum resolution of 3.5 Å, and we have been able to collect and process data sets to 4.0 Å resolution from our crystals. Data analysis of our crystals showed reasonable values of R_{merge} and mosaic spread (although the mosaic spread is larger than those usually observed for water-soluble

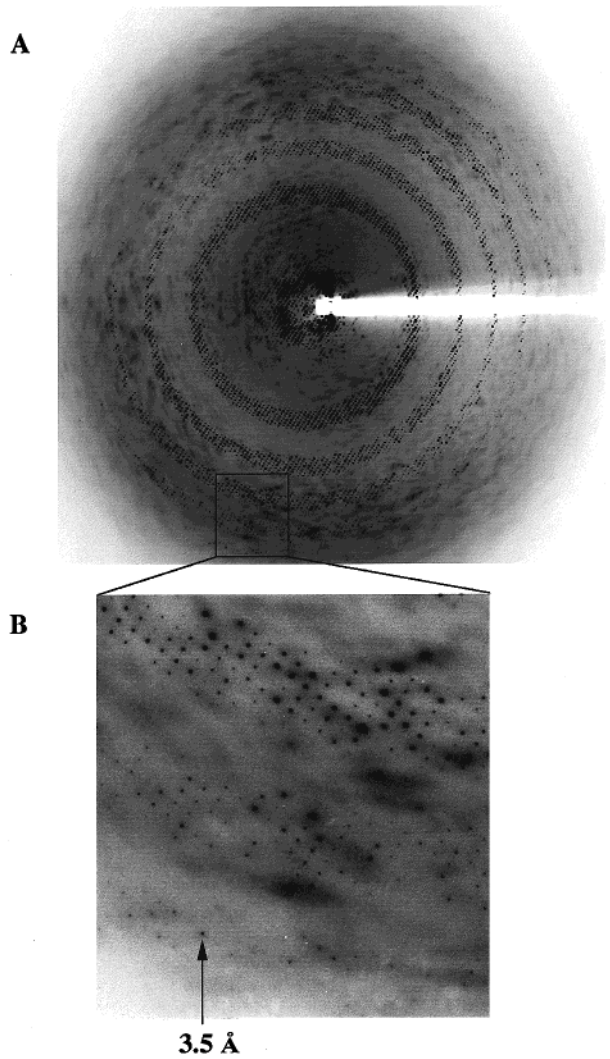

FIGURE 4: X-ray diffraction pattern of a PSII crystal. The diffraction image was recorded on an imaging plate detector, RAXIS-IV (Rigaku), at BL41XU of SPring-8, Japan. The PSII crystal with a size of 0.2 mm × 0.3 mm × 0.05 mm was frozen under a cooled nitrogen gas stream (100 K). The oscillation angle was 1.0° with an exposure time of 50 s. The X-ray wavelength and the crystal-to-detector distance were 1.0 Å and 470 mm, respectively. (A) The whole image of the diffraction pattern; (B) an enlarged section of the boxed area in panel A showing the resolution limit of the crystal.

proteins), which indicates that our crystals are suitable for structure analysis at 4.0 Å.

The apparent molecular mass of the PSII in our crystals was determined to be 580 kDa, indicating that PSII exists as a dimer in our crystals, since a monomeric PSII is estimated to have a molecular mass of 310 kDa (including chlorophylls). This is consistent with the report of Kuhl et al. (*16*), where they also showed that the PSII in their crystals is a dimer. On the basis of the calculated molecular weight, the specific volume (V_M) of our crystal was determined to be 3.6 $Å^3$/Da, which is slightly higher than those normally found for crystals of water-soluble proteins (most commonly observed value of 2.15 A^3/Da (*34*)) but comparable to some of the membrane proteins such as the photosynthetic reaction center of purple bacteria (for example, 3.4 A^3/Da (*35*) and 4.2 A^3/Da (*36*)) and matrix porin (2.9 A^3/Da, (*37*)). Zouni et al., however, reported that the PSII in their crystals is a monomer (*14*). Judging from the same space group and very similar unit cell dimensions between the crystals reported

Table 1: Crystallographic Data of a PSII Native Crystal[a]

crystal system	orthorhombic
space group	$P2_12_12_1$
unit cell dimensions	$a = 129.7$ Å, $b = 226.5$ Å, $c = 307.8$ Å
Z	8
MW of a monomer	310 kDa
V_M	3.6 $Å^3$/Da
resolution	4.0 Å
no. of measured reflections	189 881
no. of independent reflections ($I/\sigma > 0.0$)	67 854
no. of zero reflections ($I/\sigma \leq 0.0$)	4381
$\langle I \rangle$[b]	54.4 (11.0)[e]
$\langle \sigma \rangle$[c]	4.0 (3.1)[e]
R_{merge}[d]	7.3% (24.1%)[e]
completeness	0.87 (0.82)[e]

[a] The data set was collected at BL41XU of SPring-8, Japan, using an imaging plate detector, RAXIS-IV (RIGAKU). The X-ray wavelength and the sample-to-detector distance were 1.0 Å and 470 mm, respectively. The sample crystal was frozen at 100 K under a cooled nitrogen gas stream. An oscillation angle of 1.0° and an exposure time of 10 s were applied for each of 90 frames. The data set was reduced and analyzed with the software AUTO. [b] $\langle I \rangle$ = averaged reflection intensity. [c] $\langle \sigma \rangle$ = averaged standard deviation of the reflection intensities. [d] $R_{merge} = \sum hkl \sum i |Ii(hkl) - \langle I(hkl) \rangle| / \sum hkl \sum i Ii(hkl)$. [e] Values for the highest resolution shell (4.1−4.0 Å).

by the three groups, the possibility exists that the PSII in crystals obtained by Zouni et al. is also a dimer; this is indeed the case from the most recent report of the group.

PSII from thermophilic cyanobacteria has an advantage in that it is more stable than PSII from mesophilic cyanobacteria and higher plants. PSII crystals of higher plants have been obtained previously (*11*−*13*) (Shen et al., unpublished results). However, in the PSII crystals of higher plants, some of the PSII components were found to be degraded, and the resolution was limited to lower than 5.0 Å (*13*) (Shen et al., unpublished), which is not suitable for the structural analysis in its present form. The crystal packing of higher plant PSII crystal is also very different from the crystals obtained from thermophilic cyanobacteria. This may be caused by, in addition to the difference in thermostability, the difference of three extrinsic proteins between higher plants and cyanobacteria: Higher plant PSII has 33, 23, and 17 kDa proteins as its extrinsic proteins, of which the 23 and 17 kDa proteins were lost in the PSII core complex used for crystallization (*11*−*13*) (Shen et al., unpublished). This may suggest that the higher plant PSII core complex used for crystallization does not exist in its native form, although the oxygen-evolving activity is reasonably high in the absence of the 23 and 17 kDa proteins, as far as enough Ca^{2+} and Cl^- were supplemented. On the other hand, cyanobacterial PSII has 33 and 12 kDa proteins and cytochrome *c*-550 as extrinsic proteins (*17*, *18*, *38*, *39*), all of which remain bound to the PSII complex used for crystallization and also remain bound after crystallization ((*16*), present study). Since PSII is a large membrane−protein complex which has a limited area of hydrophilic surface exposed to the aqueous solution, binding of the three extrinsic proteins would largely contribute to the increase of hydrophilic surface area, which would be important for crystal packing. In this sense, increase of hydrophilic surface of higher plant PSII by binding of the three extrinsic proteins or other means might provide an effective way to improve the crystal quality, leading to formation of crystals suitable for structure analysis of higher plant PSII.

The central and major parts of PSII, however, are highly conserved from cyanobacteria to higher plants. Structural analysis of the thermophilic cyanobacterial PSII will thus provide a long awaited PSII model needed for further biophysical, biochemical, and molecular biological studies into the mechanism of electron transfer and oxygen evolution within this important complex. Such analysis is now underway using the crystals reported here.

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REFERENCES

1. Hankamer, B., Barber, J., and Boekema, E. J. (1997) *Annual Rev. Plant Physiol. Plant Mol. Biol. 48*, 641−671.
2. Rögner, M., Dekker, J. P., Boekema, E. J., and Witt, H. T. (1987) *FEBS Lett. 219*, 207−211.
3. Dekker, J. P., Boekema, E. J., Witt, H. T., and Rögner, M. (1988) *Biochim. Biophys. Acta 936*, 307−318.
4. Boekema, E. J., Hankamer, B., Bald, D., Kruip, J., Nield, J., Boonstra, A. F., Barber, J., and Rögner, M. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 175−179.
5. Lyon, M. K., Marr, K. M., and Furcinitti, P. S. (1993) *J. Struct. Biol. 110*, 133−140.
6. Nakazato, K., Toyoshima, C., Enami, I., and Inoue, Y. (1996) *J. Mol. Biol. 257*, 225−232.
7. Morris, E. P., Hankamer, B., Zheleva, D., Friso, G., and Barber, J. (1997) *Structure 5*, 837−849.
8. Rhee, K.-H., Morris, E.-P., Zheleva, D., Hankamer, B., Kühlbrandt, W., and Barber, J. (1997) *Nature 389*, 522−526.
9. Rhee, K.-H., Morris, E.-P., Barber, J., and Kühlbrandt, W. (1998) *Nature 396*, 283−286.
10. Hankamer, B., Morris, E. P., and Barber, J. (1999) *Nat. Struct. Biol. 6*, 560−564.
11. Adir, N., Okamura, M. Y., and Feher, G. (1992) in *Research in Photosynthesis* (Murata, N., Ed.), Vol. 2, pp 195−198, Kluwer Academic Publishers, Dordrecht, The Netherlands.
12. Fontinou, C., Kokkinidis, M., Fritzsch, G., Haase, W., Michel, H., and Ghanotakis, D. (1993) *Photosynth. Res. 37*, 41−48.
13. Adir, N. (1999) *Acta Crystallogr. D55*, 891−894.
14. Zouni, A., Lüneberg, C., Fromme, P., Schubert, W. D., Saenger, W., and Witt, H. T. (1999) in *Photosynthesis: Mechanisms and Effects* (Garab, G., Ed.), Vol. 2, pp 925−928, Kluwer Academic Publishers, Dordrecht, The Netherlands.
15. Zouni, A., Jordan, R., Schlodder, E., Fromme, P., and Witt, H. T. (2000) *Biochim. Biophys. Acta 1457*, 103−105.
16. Kuhl, H., Kruip, J., Seidler, A., Krieger-Liszkay, A., Bünker, M., Bald, D., Scheidiğ, A. J., and Rögner, M. (2000) *J. Biol. Chem. 275*, 20652−20659.
17. Shen, J.-R., Ikeuchi, M., and Inoue, Y. (1992) *FEBS Lett. 301*, 145−149.
18. Shen, J.-R., and Inoue, Y. (1993) *Biochemistry 32*, 1825−1832.
19. Shen, J.-R., and Inoue, Y. (1993) *J. Biol. Chem. 268*, 20408−20413.
20. Porra, R. J., Thompson, W. A., and Kriedemann, P. E. (1989) *Biochim. Biophys. Acta 975*, 384−394.
21. Ikeuchi, M., and Inoue, Y. (1988) *Plant Cell Physiol. 29*, 1233−1239.
22. Shen, J.-R. (1999) in *Photosynthesis: Mechanisms and Effects* (Garab, G., Ed.), Vol. 2, pp 941−944, Kluwer Academic Publishers, Dordrecht, The Netherlands.
23. Kamiya, N., Uruga, T., Kimura, H., Yamaoka, H., Yamamoto, M., Kawano, Y., Ishikawa, T., Kitamura, H., Ueki, T., Iwasaki, H., Kashihara, Y., Tanaka, N., Moriyama, H., Hamada, K., Miki, K., and Tanaka, I. (1995) *Rev. Sci. Instrum. 66*, 1703−1705.
24. Adachi, S., Oguchi, T., and Ueki, T. (1996) *SPring-8 Annual Report 1*, 239, 240.
25. Ueki, T., and Yamamoto, M. (1999) *Structure, 7*, R183−R187.
26. Leslie, A. (1993) in *Proceedings of the CCP4 Study Weekend*, 29−30 January 1993 (Sawyer, L., Isaac, N., and Bailey, S., Eds.), pp 44−51.
27. Bricker, T. M., and Ghanotakis, D. F. (1996) in *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R., and Yocum, C. F., Eds.) pp 113−136, Kluwer Academic Publishers, Dordrecht, The Netherlands.
28. Ikeuchi, M., Eggers, B., Shen, G., Webber, A., Yu, J., Hirano, A., Inoue, Y., and Vermaas, W. (1991) *J. Biol. Chem. 266*, 11111−11115.
29. Ikeuchi, M., Shukla, V. K., Pakrasi, H. B., and Inoue, Y. (1995) *Mol. Gen. Genet. 249*, 622−628.
30. Kitamura, K., Ozawa, S., Shiina, T., and Toyoshima, Y. (1994) *FEBS Lett. 354*, 113−116.
31. Zheleva, D., Sharma, J., Panico, M., Morris, H. R., and Barber, J. (1998*) J. Biol. Chem. 273*, 16122−16127.
32. Kaneko, T., Sato, S., and et al. (1996) *DNA Res. 3*, 109−136.
33. Sawyer, L., and Turner, M. A. (1992) in *Crystallization of Nucleic Acids and Proteins, A Practical Approach* (Ducruix, A. and Giegë, R. Eds.), pp 255−289, Oxford University Press, New York.
34. Matthews, B. W. (1968) *J. Mol. Biol. 33*, 491−497.
35. Allen, J. P., and Feher, G. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4795−4799.
36. Allen, J. P., Feher, G., Yeates, T. O., Rees, D. C., Deisenhofer, J., Michel, H., and Huber, R. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 8589−8593.
37. Garavito, R. M., Jenkins, J. A., Jansonius, J. N., Karlsson, R., and Rosenbush, J. P. (1983) *J. Mol. Biol. 164*, 313−325.
38. Shen, J.-R., Vermaas, W., and Inoue, Y. (1995) *J. Biol. Chem. 270*, 6901−6907.
39. Shen, J.-R., Ikeuchi, M., and Inoue, Y. (1997) *J. Biol. Chem. 272*, 17821−17826.

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