Roles of PsbI and PsbM in photosystem II dimer formation and stability studied by deletion mutagenesis and X-ray crystallography

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Abbreviations: Chl, chlorophyll; DDM, n-dodecyl-β-D-maltoside; hrCNE, high resolution clear native electrophoresis; LDAO, lauryldimethylamine N-oxide; Mes, 4-morpholineethanesulfonic acid; PSII, photosystem II; PSIIcc, photosystem II core complex; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Abstract

PsbM and PsbI are two low molecular weight subunits of photosystem II (PSII), with PsbM being located in the center, and PsbI in the periphery, of the PSII dimer. In order to study the functions of these two subunits from a structural point of view, we crystallized and analyzed the crystal structure of PSII dimers from two mutants lacking either PsbM or PsbI. Our results confirmed the location of these two subunits in the current crystal structure, as well as their absence in the respective mutants. The relative contents of PSII dimers were found to be decreased in both mutants, with a concomitant increase in the amount of PSII monomers, suggesting a destabilization of PSII dimers in both of the mutants. On the other hand, the accumulation level of the overall PSII complexes in the two mutants was similar to that in the wild type strain. Treatment of purified PSII dimers with lauryldimethylamine N-oxide at an elevated temperature preferentially disintegrated the dimers from the PsbM-deletion mutant into monomers and CP43-less monomers, whereas no significant degradation of the dimers was observed from the PsbI-deletion mutant. These results indicate that although both PsbM and PsbI are required for the efficient formation and stability of PSII dimers in vivo, they have different roles, namely, PsbM is required directly for the formation of dimers and its absence led to the instability of the dimers accumulated. On the other hand, PsbI is required in the assembly process of PSII dimers in vivo; once the dimers are formed, PsbI was no longer required for its stability.

1. Introduction

Photosystem II (PSII) is a multi-subunit, pigment protein complex located in the thylakoid membranes from cyanobacteria to higher plants. PSII core complex (PSIIcc) from thermophilic cyanobacteria contains 17 membrane-spanning subunits and 3 peripheral, hydrophilic subunits, with a total molecular mass of around 350 kDa [1]. Both dimeric and monomeric forms of PSIIcc have been purified from cyanobacteria and higher plants; however, the amount of PSIIcc dimers is much larger than that of monomers from thermophilic cyanobacteria [2, 3], suggesting that PSIIcc exists predominately as a dimer, at least in cyanobacteria, although recent reports have shown that the dimer/monomer ratio is subjected to changes depending on the detergent solubilization and purification conditions [4, 5]. The structure of dimeric PSIIcc has been solved from two thermophilic cyanobacteria, *Thermosynechococcus elongatus* and *T. vulcanus*, at resolutions of 3.8-2.9 Å (6-8). These structural studies revealed the location and organization of all 20 subunits, in which the D1 and D2 subunits constitute the reaction center core of PSII and are located in the center of PSII. Surrounding the reaction center core are two large trans-membrane subunits CP47 and CP43, which bind chlorophylls and serve as the core antenna for trapping and transferring the light energy into the reaction center. In addition to these four large trans-membrane subunits, there are 13 small subunits with molecular masses lower than 10 kDa; they are PsbE, PsbF, PsbH, PsbI, PsbJ, PsbK, PsbL, PsbM, PsbTc, PsbX, PsbY, PsbZ, Psb30 (Ycf12). Among these subunits, only PsbZ has two trans-membrane helices and the other 12 subunits have a single trans-membrane helix each. The roles of most of the low molecular mass subunits remain obscure.

Among the low molecular mass subunits of PSII, PsbM is located in the center of PSIIcc dimer. In the most recent crystal structure of PSIIcc dimer [8], PsbM from one monomer is closely associated with the same protein from another monomer, forming a heptad motif of aliphatic side chains as seen in a leucine zipper. This interaction is considered important for the formation and stabilization of PSIIcc dimer. However, deletion of the *psbM* gene from both tobacco [9] and *Synechocystis* sp. PCC 6803 [10] was shown to have no significant effect on the formation of PSII dimer, although both mutants showed a slightly lower activity of oxygen evolution. These results suggested

that PsbM is not the only factor that controls the formation and stability of PSII dimer. Thus, the exact roles of PsbM in the assembly and functioning of PSIIcc remain to be elucidated.

PsbI is another low molecular mass subunits, and is present in the periphery of PSIIcc monomer close to D1 and CP43 [6-8]. In fact, PsbI was first identified in the PSIIcc containing D1/D2 and PsbE, PsbF, in agreement with its location in the current PSII structure [11]. Deletion of PsbI from *Chlamydomonas reinhardtii* has been shown to decrease the oxygen evolution to 10-20% of the wild type level, and the resulted mutant strain has a high sensitivity to photoinhibition so that it could not grow under high light conditions [12]. A *psbI* gene knock-out mutant of tobacco also showed high sensitivity toward photoinhibition, and the levels of PSIIcc were reduced by 50% [13]. In contrast, a mutant of *Synechocystis* sp. PCC 6803 lacking the *psbI* gene was shown to be able to grow photoautotrophically nearly as well as the wild type strain, and the PSII core proteins are present at a similar level as that in the wild type [14]. The PsbI-deficient cyanobacterial cells were shown to be only slightly more sensitive to photoinhibition than the wild type cells. In addition, deletion of PsbI was shown to affect the stability of PSII in a thermophilic cyanobacterium *Thermosynechococcus elongatus* [15]. These results suggested that the PsbI protein may have multiple roles in the structural assembly and functioning of PSIIcc, although the precise functions of this protein in PSII is still unclear.

In order to elucidate the roles of PsbM and PsbI in PSIIcc from a structural point of view, we purified, crystallized PSIIcc from two mutants of a thermophilic cyanobacterium *Thermosynechococcus elongatus* lacking either PsbM or PsbI, and solved the crystal structure of the mutant PSIIccs. These results confirmed the position of these two subunits in the current structure, as well as the lack of these subunits in the mutant strains. Combined with functional analysis of purified PSIIcc, we showed that whereas PsbM is partly required for the stable formation of PSIIcc dimer, PsbI is required in the process of PSIIcc dimer assembly; once the PSIIcc dimer is formed, PsbI is no longer required for its stability.

2. Materials and methods

2.1. Construction of mutant strains and purification of PSIIcc

T. elongatus cells were grown at 45-49°C with bubbling of air containing 5% CO_2, as described previously [16]. DNA fragments containing the *psbM* and *psbI* genes were amplified by PCR. The *psbM* gene was deleted by replacing a Hinc II fragment at a position 7 bp upstream of the start codon of the gene with a chloramphenicol-resistant cassette. The *psbI*-deletion mutant was constructed as described previously [15]. The deletion of *psbM* and *psbI* genes were confirmed by PCR (not shown). Both mutants were maintained in the presence of 5 μg/ml chloramphenicol. For large scale purification of PSIIcc samples, the mutant cells were grown in a 50 L liquid culture for 10-15 days in the absence of chloramphenicol.

PSIIcc dimers were purified from *psbM*- and *psbI*-deletion mutants according to Shen and Inoue [17] with modifications as described in [18]. The crude-PSII obtained by solubilization of thylakoid membranes with lauryldimethylamine N-oxide (LDAO) was solubilized with n-dodecyl-β-D-maltoside (DDM) and purified by two subsequent ion-exchange columns of TOYOPEARL DEAE 650M (Tosoh Co.) and Q-sepharose High Performance (GE Healthcare UK Ltd.). The final preparation of PSIIcc dimers was suspended in a medium containing 20 mM Mes (pH 6.0), 10 mM NaCl and 3 mM $CaCl_2$, and either used immediately for analysis or stored in liquid nitrogen.

2.2. Electrophoresis, fluorescence emission spectra and oxygen-evolving activity measurements

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with a 16%-22% polyacrylamide gradient gel containing 7.5 M urea at room temperature [19]. Samples were solubilized with 2% lithium dodecyl sulfate, 60 mM dithiothreitol and 60 mM Tris-HCl (pH 8.5), and incubated for 10 min on ice.

For analysis of the amounts of PSII dimer and monomer in thylakoids, high resolution Clear Native Electrophoresis (hrCNE) was performed according to [20, 21], with modifications described in [22]. The gels of each lane in the native PAGE were cut out and subjected to a second dimensional electrophoresis with the SDS-PAGE described above. For disintegration of PSIIcc dimers into monomers, PSIIcc dimers purified from the wild type and mutant strains were treated with LDAO at designated concentrations at 40^{o}C, and the resulted samples were analyzed by hrCNE and second

dimensional SDS-PAGE.

Fluorescence emission spectra of thylakoid membranes from wild type and mutant strains were recorded at 77 K with a fluorescence spectrometer (F-4500, Hitachi) with an excitation wavelength of 435 nm at a Chl concentration of 5 μg/ml [22].

Oxygen evolution was measured with a Clark-type oxygen electrode under continuous, saturating illumination at 30°C with 0.5 mM phenyl-*p*-benzoquinone and 0.5 mM potassium ferricyanide as electron acceptors, at a Chl concentration of 10 μg Chl/ml. The medium used was 20 mM Mes-NaOH (pH 6.0), 20 mM NaCl, 3 mM $CaCl_2$.

2.3. Crystallization and crystal structure analysis

Crystallization was performed with the hanging drop vapor diffusion method as described in [2, 6, 18]. The crystals grew to a size of 1.0 x 0.5 x 0.2 mm^3 at 20^oC in 1 week. The crystals were cryoprotected in a solution containing 25% glycerol and 20% PEG1,450, and flash-cooled in a nitrogen gas stream at 100 K. X-ray diffraction data were collected at beamline BL41XU and BL44XU of a synchrotron radiation facility, Spring-8, Japan [23]. The diffraction patterns were recorded with a CCD detector Mar225 at an X-ray wavelength of 0.9 Å, with an oscillation angle of 0.6^o, and an exposure time of 1 sec. The data collected was processed with *HKL2000* [24]. Difference-Fourier maps were calculated with *FFT* in the CCP4 program suit [25].

2.4. Disintegration of PSIIcc dimers by detergent treatment

Purified PSIIcc dimers from the wild type and two mutant strains were treated with 0.1%-0.2%LDAO at a chlorophyll concentration of 0.5 mg/ml in the dark for various times, and then the treated samples were analyzed by clear native PAGE as well as 2-dimensional electrophoretic analysis.

Results

Purification and protein composition of PSIIcc dimers from PsbM- and PsbI-deletion mutants

PSIIcc dimers were purified from both PsbI and PsbM deletion mutants with a

procedure similar to that used for the wild type strain. During the purification procedure, we constantly found a higher yield of PSIIcc monomer in the final elution pattern of column chromatography (data not shown, see later), so that we have to use a larger amount of thylakoid membranes in order to obtain PSIIcc dimers sufficient for crystallization. Fig. 1 shows the protein composition of PSIIcc dimers from the wild type strain as well as the two mutants analyzed by SDS-PAGE. Apparently, the protein composition of PSIIcc dimers from the two mutants were the same as that from the wild type strain, except in the low molecular weight region where the bands labeled PsbI, PsbM appears to be weaker in their respective mutants. However, due to the overlap with other low molecular weight subunits, it is impossible to identify the lack of these subunits in the mutants unambiguously from the SDS-PAGE analysis alone.

Crystallization and crystal structure analysis of mutant PSIIcc

Since PSIIcc dimers have been successfully crystallized and its structure was analyzed by X-ray crystallography [6-8], we used dimers from both mutants for crystallization. Both mutant PSIIs gave rise to crystals with similar shapes as that of wild type PSIIcc (data not shown), under the conditions similar to that used for wild type PSIIcc (Materials and Methods). Table 1 shows the statistics of X-ray diffraction data collected from PSIIcc crystals of PsbM and PsbI deletion mutants, as well as those from the wild type that showed a high isomorphism with those of PsbM- and PsbI-deletion mutants. Crystals from the PsbM-deletion mutant diffracted to a 4.20 Å resolution, whereas that from the PsbI-deletion mutant diffracted to a 3.45 Å resolution. Difference-Fourier map of wild type-minus-PsbM mutant showed two dense, positive regions of electron density with a helix-like shape that are located near each other (Fig. 2). Superimposition of this difference-Fourier map with the PSIIcc dimer structure showed that these two regions corresponded exactly to the PsbM subunit located in the middle of the dimer, indicating that the PsbM subunit was indeed absent in the mutant PSIIcc. This also confirms the location of PsbM in the current crystal structure. No strong signals other than that arising from the PsbM subunit was observed in the difference-Fourier map, indicating that no remarkable structural changes have occurred due to the deletion of PsbM.

Difference-Fourier map of wild type-minus PsbI mutant also showed two positive

electron density regions which are separated distantly (Fig. 3). Superimposition of this difference-Fourier map with the PSIIcc dimer structure showed that these two regions corresponded exactly to the PsbI subunit in the periphery of PSIIcc dimer, thus confirming the absence of this subunit in the mutant as well as its location in the current crystal structure. Similar to the PsbM mutant, there were also no strong signals other than that arose from the PsbI subunit in the difference-Fourier map, indicating that there were no major structural changes occurred due to deletion of the PsbI subunit.

Functional analysis of mutant PSIIcc

Table 2 compares oxygen-evolving activity of thylakoid membranes and PSIIcc dimers purified from the wild type and mutant strains. The activity of thylakoid membranes of both mutants were slightly lower than that of wild type strain (the activity of two mutants ranged in 70-80% of that of wild type), and this decrease was similarly observed in purified PSIIcc dimers, although the activity of both mutant and wild type strains varied to some extent depending on the growth conditions, as we used a large scale culture of cells. As a result, both mutants were able to grow photoautotrophically at a rate slightly slower than that of the wild type (data not shown, see 10, 15). These results suggest that both subunits are not essential for the photoautotrophic growth of the cyanobacterium as well as the formation of functional PSIIcc.

In order to examine whether the content of PSII was affected in the two mutants, we measured the fluorescence yields of PSII and PSI from the cells of wild type, PsbM-deletion mutant, and PsbI-deletion mutant, at 77 K. As shown in Fig. 4, the fluorescence yields of PSII at 685 and 695 nm from both PsbI and PsbM deletion mutants were similar as that from the wild type strain when the spectra were normalized at 730 nm, the emission peak of PSI, although the emission peaks of PSII from the PsbI-deletion mutant seemed to be slightly lower than those of the wild type or PsbM-deletion mutant. This indicates that the ratio of PSI/PSII, and thereby the content of PSII, was essentially not affected by the deletion of PsbM as well as PsbI, although the latter may slightly affected the accumulation level of PSII.

Since both PsbI and PsbM subunits have been suggested to be involved in the formation or stabilization of PSII dimer, we analyzed the relative content of PSII

monomers and dimers in the thylakoid membranes from the wild type and mutant strains by clear native-PAGE. In the clear native-PAGE, three major green bands were resolved (Fig. 5A), they were identified to be PSI trimers, PSIIcc dimers and PSIIcc monomers, respectively, ranging from the higher molecular weight region to the lower molecular weight region, by two-dimensional gel analysis (Fig. 5B). The amount of PSIIcc monomers was very low compared with that of the dimers in the wild type strain. In contrast, both PsbI and PsbM deletion mutants showed a decreased amount of PSIIcc dimers and an increased amount of monomers. These results suggested that both PsbI and PsbM subunits are required for the stable formation of PSIIcc dimers in vivo. The total amounts of PSIIcc dimers and monomers in the two mutants were similar to that of the total amounts of PSII in the wild type strain, confirming the above results from the fluorescence measurements that the overall amount of PSII was not affected in the two mutants.

A destabilization of PSIIcc dimer in the absence of PsbM is consistent with the crystal structure of PSIIcc, in which, PsbM is located in the center of dimer joining two monomers and has been suggested to be important for the formation of the dimers. However, a decrease in the amount of dimer in the absence of PsbI is difficult to explain based on the current crystal structure of PSIIcc, as PsbI is located in the periphery of PSIIcc not directly involved in the interaction of the two monomers. In order to examine whether there is a difference in the stability of the dimers formed between the PsbM-deletion mutant and PsbI-deletion mutant, we attempted to find conditions which allows the decomposition of PSIIcc dimers into monomers and other low molecular weight forms. As a result, we found that treatment with a suitable concentration of LDAO at 40^{o}C induced degradation of PSIIcc dimers into monomers with no significant disintegration of the PSIIcc core complex in the absence of PsbM, whereas the same treatment did not destabilize PSIIcc dimers from both wild type and the PsbI-deletion mutant. As shown in Fig. 6, treatment of PSIIcc dimers purified from the PsbM-deletion mutant with 0.1% LDAO for 30-60 min induced a green band with a lower molecular weight than the original PSIIcc dimer, with a parallel decrease in the amount of dimers. This low molecular weight band was identified to be PSIIcc monomer by two-dimensional gel analysis. A longer treatment for 90-120 min further increased the amount of monomers and decreased the amount of dimers. Moreover, the longer

treatment induced the formation of a band with a molecular mass even lower than that of the monomer. This was confirmed to be the monomer without CP43 (CP43-less monomer) from the two-dimensional gel analysis. The same treatment up to 120 min yielded little degradation of PSIIcc dimers, and almost no monomer formation was observed in both the wild type and PsbI-deletion mutant after this treatment. The same results were observed following treatment with an increased concentration of LDAO from 0.1% to 0.2%, where PSIIcc dimers were degraded into monomers and CP43-less monomers in the PsbM-deletion mutant, whereas almost no degradation of the dimers were observed in the wild type as well as the PsbI-deletion strains (data not shown). These results clearly indicate that PSIIcc dimers from the PsbM-deletion mutant was unstable against the LDAO-treatment than that of the wild type strain, whereas the dimers from the PsbI-deletion mutant had essentially the same stability as that of the wild type strain.

Discussion

Both dimeric and monomeric PSII have been purified from cyanobacteria and higher plants, and the structures of both of them have been reported [6-8, 26]. The amount of PSIIcc dimers purified from thermophilic cyanobacteria is generally larger than that of monomers, suggesting that PSII exists predominately in a dimeric form in vivo [2, 3], and it is generally considered that PSII assembles first as a monomer, and subsequently from monomer to dimer [27-29]. However, the amount of purified PSII dimers/monomers may change depending on the solubilization and/or purification conditions, and sometimes the amount of monomers may exceeds that of dimers [4,5].

The present results showed that both PsbM and PsbI-deletion mutants had a decreased amount of PSIIcc dimers and an increased amount of monomers, indicating that both subunits are required for the formation and/or stability of PSIIcc dimers in vivo. The destabilization of PSIIcc dimers due to deletion of PsbI is consistent with the results reported previously [15], whereas deletion of PsbM has been reported to have no effect on the dimer formation in tobacco [9] or *Synechocystis* sp. PCC 6803 [10]. The PsbM-deletion mutant of *Synechocystis* sp. PCC 6803 showed a lowered level of total

PSII accumulated, as well as an increase in the amount of CP43-less PSII [10]. Presumably, PSII monomers generated due to the absence of PsbM in the *Synechocystis* PsbM-deletion mutant were unstable, some of which may be degraded to CP43-less PSII and some may be degraded more completely, leading to the apparently unchanged ratio of PSII dimer and monomer. On the other hand, PSII monomers in the absence of PsbM from the thermophilic cyanobacterium may be still stable to some extent, allowing the accumulation of the PSII monomers degraded from the dimers. The association of CP43 with PSII in the thermophilic cyanobacterial PSIIcc may also be more stable, leading to the absence of CP43-less PSII in the PsbM-deletion mutant of *T. elongatus*. However, the differences observed between the thermophilic cyanobacterial PsbM-deletion mutant and tobacco mutant regarding the stability of PSII dimers is not clear at present. In this respect, it should be pointed out here that a destabilization of PSII dimer due to the absence of PsbM is consistent with the crystal structure, where PsbM is located in the area joining two monomers.

Oxygen-evolving activities were found to be slightly decreased in both PsbM and PsbI mutants, and this can be ascribed to the increased instability of the PSIIcc dimer in vivo. Indeed, our results showed that the overall level of PSII accumulated in the two mutants are more or less similar as that found in the wild type strain, which is apparently different from PsbZ or Ycf12 (Psb30) deletion mutants, where the relative ratio of PSII dimers to PSII monomers was not changed but a decrease in the overall amount of PSII was found [22]. The accumulation level of PSII in the absence of PsbI has been reported to be almost unaffected in the mesophilic cyanobacterium *Synechocystis* sp. PCC 6803 [14], in agreement with the present results. On the other hand, a remarkable decrease in the level of PSII was observed with tobacco (a decrease of ca. 50%) [13] and *Chlamydomonas reinhardtii* (a decrease of ca. 80-90%) mutants lacking PsbI [12]. These suggest that the assembly process of PSII in eukaryotes may be more complicated than that in prokaryotes, and thus requires a more sophisticated collaboration among different subunits. A defect in one of such subunits then affects the whole assembling process more profoundly. In fact, such a difference has been reported for other small subunits such as PsbK, the lack of which induced a remarkable destabilization of PSII in *Chlamydomonas reinhardtii* [30] but not in cyanobacteria [31, 32].

Based on the crystal structure of PSIIcc, PsbM is located in the center of PSIIcc dimer joining two monomers; thus, it has been suggested that this subunit is required for the formation of dimer. Nevertheless, a significant amount of the PSIIcc dimer was still found in the PsbM-deletion mutant, suggesting that the deletion of PsbM alone was not adequate to completely inhibit the formation of dimer. This suggests that there may be factors other than PsbM that are required for the formation and stability of PSIIcc dimer. In fact, the crystal structure at 2.9 Å has shown that there are 7 lipids from each monomer that are located in the interface of the two monomers within the dimer, forming a layer of 14 lipids in the interface. In addition, 8 detergent (β-DDM) molecules were also found in the interface, some of which may be lipids in vivo which were replaced by the detergents during the purification procedure. Thus, lipid molecules may be important for the formation and stability of PSII dimer in vivo, and the presence of a significant amount of dimer in the absence of PsbM may suggest that some of the lipid molecules important for the stability of PSII dimer are still present in the absence of PsbM. Our structural analysis of PSIIcc dimer from the PsbM-deletion mutant, unfortunately, did not give rise to a resolution high enough to allow us to identify the lipid molecules or structural changes possibly induced by the deletion of the subunit.

In addition to PsbM, there are two other small subunits, PsbL and PsbT, that were found to be located in the interface between the two monomers within the dimer. Among the three small subunits, only PsbM of one monomer was found to be in a position directly interacting with its counterpart from the other monomer. PsbT of one monomer is located relatively close to PsbB from the other monomer, but distantly from its counterpart in the other monomer. On the other hand, PsbL is shielded by PsbM and PsbT, and is not possible to interact with any subunits from the other monomer. However, PsbT has been shown to destabilize PSII dimer significantly [33-35], leading to a decrease in the amount of dimers even larger than that found in the PsbM-deletion mutant. These results suggested that absence of PsbT may have induced some structural changes and/or loss of some of the lipid molecules in the interface of two monomers, leading to the instability of PSIIcc dimer. Furthermore, deletion of the *psbL* gene has been shown to inactivate the PSII activity completely in both *Synechocystis* sp. PCC 6803 [36] and tobacco [27], leading to an significant inhibition in the assembly of the PSII complexes.

In contrast to PsbM, PsbI is located in the periphery of PSIIcc dimer, which is distant from the interface of two monomers. From this location, PsbI would be considered not directly involved in maintaining the formation and/or stability of PSIIcc dimers. However, the present results showed that the amount of PSII dimer is also decreased in the PsbI-deletion mutant, in agreement with the previous reports [15]. This decrease is comparable, or even larger than that found in the PsbM-deletion mutant. In order to find the causes for this decrease, we examined the stability of PSIIcc dimers purified from the PsbI- and PsbM-deletion mutants using a detergent treatment at an elevated temperature, as the PSIIcc dimers from the thermophilic cyanobacterium was stable against the treatment at room temperature. Our results showed that, while PSIIcc dimers from the PsbM-deletion mutant was unstable and degraded to monomers rabidly by the LDAO treatment, dimers from the PsbI-deletion mutant was as stable as that from the wild-type strain against the detergent treatment. This indicates that PSIIcc dimers in the absence of PsbI had an essentially similar stability as that in the presence of PsbI. In other words, once the PSIIcc dimer was formed in the PsbI-deletion mutant, it no longer requires PsbI to maintain its stability. The decrease in the amount of dimers observed in the PsbI-deletion mutant can thus be ascribed to a requirement of this subunit for the assembly of dimers, in agreement with early suggestions that PsbI participates in the early stage of PSIIcc assembly from the PsbI-deletion mutant of *Synechocystis* sp. PCC 6803 [28], as well as a close association of this subunit with the PSII reaction center core, especially D1. In contrast, PSIIcc dimers in the absence of PsbM was remarkably unstable during the detergent treatment, which is in agreement with the role of this subunit directly participating in the formation of dimers. Like PsbM, however, deletion of PsbI did not completely inhibit the formation of PSIIcc dimers, indicating that there are factors other than PsbI that participates in the assembly and stability of the dimers in vivo.

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Table 1 X-ray diffraction data statistics of PSIIcc crystals from the wild type and mutant strains*.

Strains	Wild type	ΔPsbM	ΔPsbI
Resolution (Å)	25-3.30	50-4.20	50-3.45
Unique reflections	136179	63266	108504
Rmerge (%)	8.3 (41.6)	8.5 (43.6)	7.6 (60.8)
I/σ (I)	8.2 (2.10)	25.0 (2.59)	22.9 (2.00)
Completeness (%)	91.4 (77.7)	97.1 (82.6)	93.7 (75.8)
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions (Å)			
a	127.5	129.4	127.7
b	224.4	226.9	225.3
c	304.3	305.7	305.7
	□=β=λ=90°	□=β=λ=90°	□=β=λ=90°

*The X-ray data was collected at BL41XU of SPring-8. Values in parenthesis represent those of the highest resolution shell.

Table 2 Effects of deletion of PsbM or PsbI on the oxygen-evolving activities (μmoles O_2/mg chl/hr) of thylakoid membranes and purified PSIIcc dimers from the wild type and mutant strains. Each of the values was obtained from 4-6 independent samples.

Strains	Wild type	ΔPsbM	ΔPsbI
Thylakoid membranes	400-500	300-400	300-400
PSIIcc dimers	3000-3500	2400-2700	2500-2700

Figure legends

Fig. 1. SDS-PAGE analysis of PSIIcc dimers from the wild type, PsbM-deletion, and PsbI-deletion mutants. Lane 1, Wild type; lane 2, PsbM-deletion mutant; lane 3, PsbI-deletion mutant. Right panel is an enlarged view of the low molecular weight region boxed in the left panel.

Fig. 2. Difference-Fourier map of wild type-minus-PsbM deletion mutant contoured at 4.0 σ, superimposed with the PSIIcc dimer structure expressed in a ribbon model. Red, Positive electron densities; blue, negative electron densities. Right panel is an enlarged view of the region around two PsbM helices in the PSIIcc dimer, corresponding to the boxed area in the left panel.

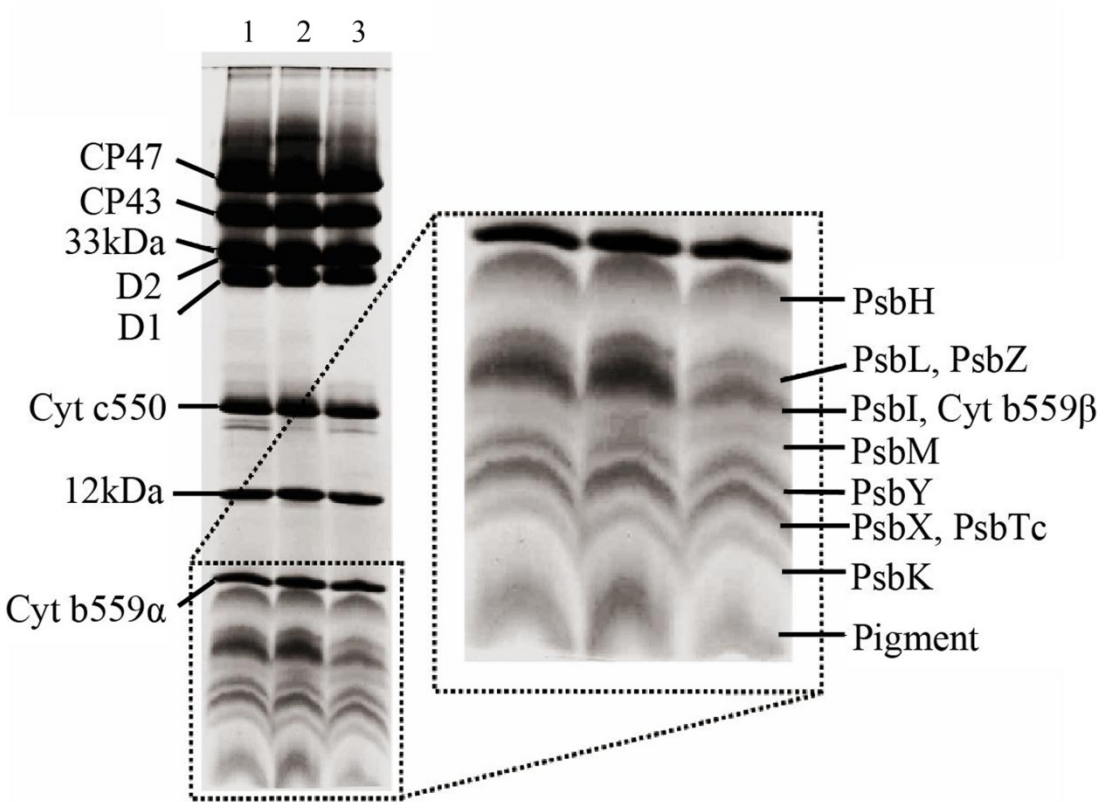
Fig. 3. Difference-Fourier map of wild type-minus-PsbI deletion mutant contoured at 4.0 σ, superimposed with the PSIIcc dimer structure expressed in a ribbon model. Red, Positive electron densities; blue, negative electron densities. Right panel is an enlarged view of the region around one of the two PsbI helices present in the PSIIcc dimer, corresponding to the boxed area in the left panel.

Fig. 4. Low temperature fluorescence emission spectra of cells of wild type and two mutants strains measured at 77 K, with an excitation wavelength of 430 nm. Black, lower black curve is the baseline, and upper black line is from wild type cells; blue, PsbM-deletion mutant cells; red, PsbI-deletion mutant cells.

Fig. 5. Analysis on relative contents of PSII dimers and monomers in the thylakoid membranes from wild type and two mutant strains. A, Clear native PAGE of thylakoid membranes. The amount of thylakoid membranes loaded onto each lane is equivalent to 3 μg chl. Lane 1, wild type; lane 2, PsbM-deletion mutant; lane 3, PsbI-deletion mutant. B, Two-dimensional SDS-PAGE of the clear native PAGE gels shown in panel A. The area shown in a red box corresponds to PSIIcc dimer, and that in a blue box corresponds to PSIIcc monomer.

Fig. 6. Clear native PAGE analysis of purified PSIIcc dimers treated with 0.1% LDAO at 0.5 mg chl/ml for various times in the dark at 40^{o}C. A, PSIIcc dimers of wild type; B, PSIIcc dimers of the PsbM-deletion mutant; C, PSIIcc dimers of the PsbI-deletion mutant. Lane 1, Control; lane 2, after 30 min LDAO-treatment; lane 3, after 60 min LDAO-treatment; lane 4, after 90 min LDAO-treatment; lane 5, after 120 min LDAO-treatment.

Fig. 1


1
2
3
CP47
CP43
33kDa
D2
D1
Cyt c550
12kDa
Cyt b559α
PsbH
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PsbI, Cyt b559β
PsbM
PsbY
PsbX, PsbTc
PsbK
Pigment

Fig. 2

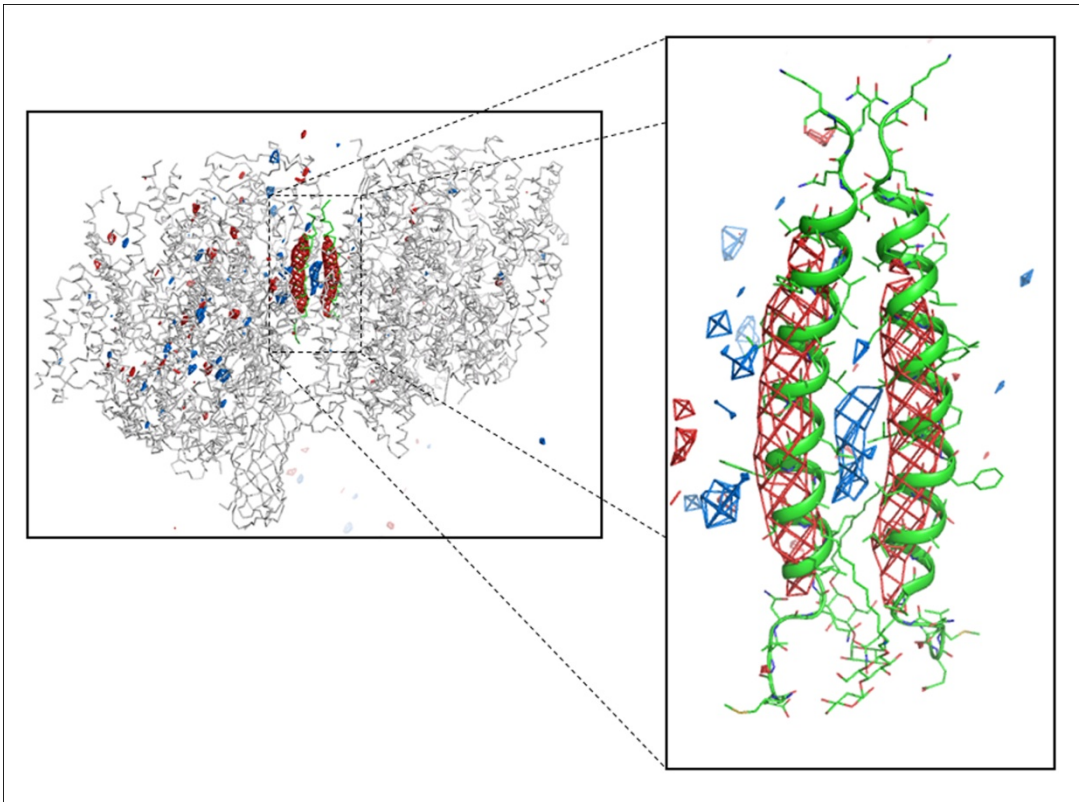


Fig. 3

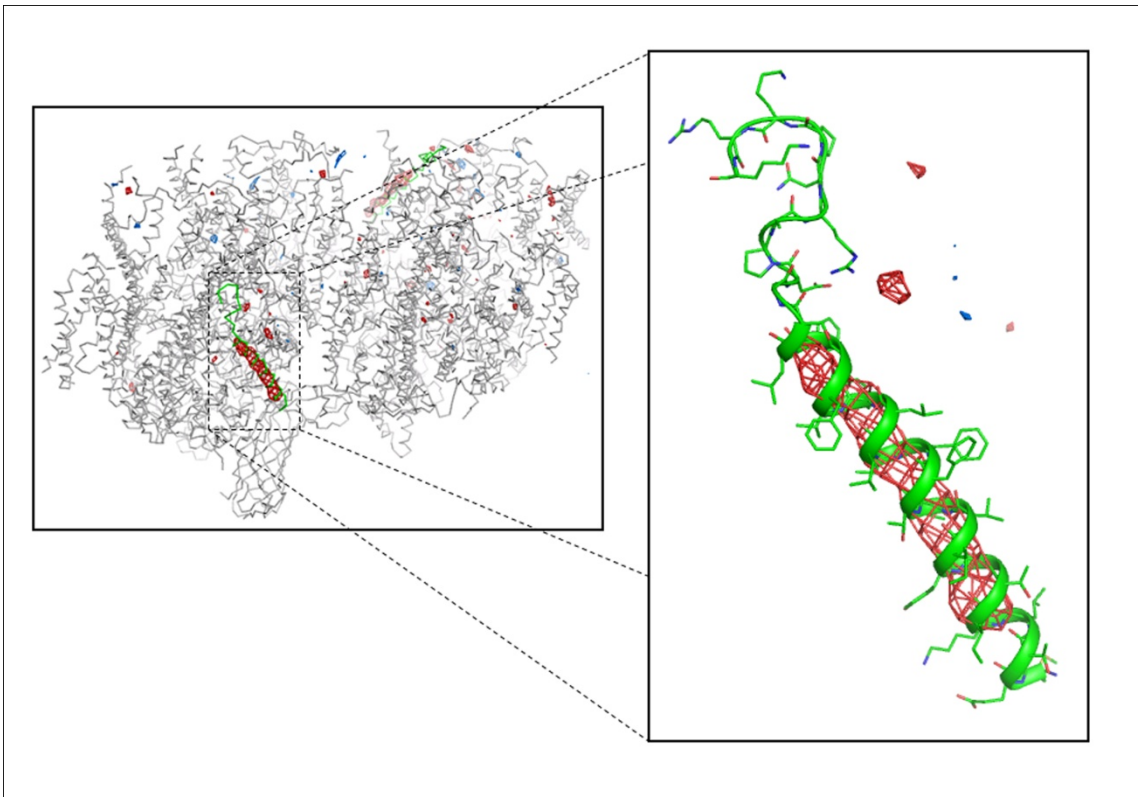


Fig. 4

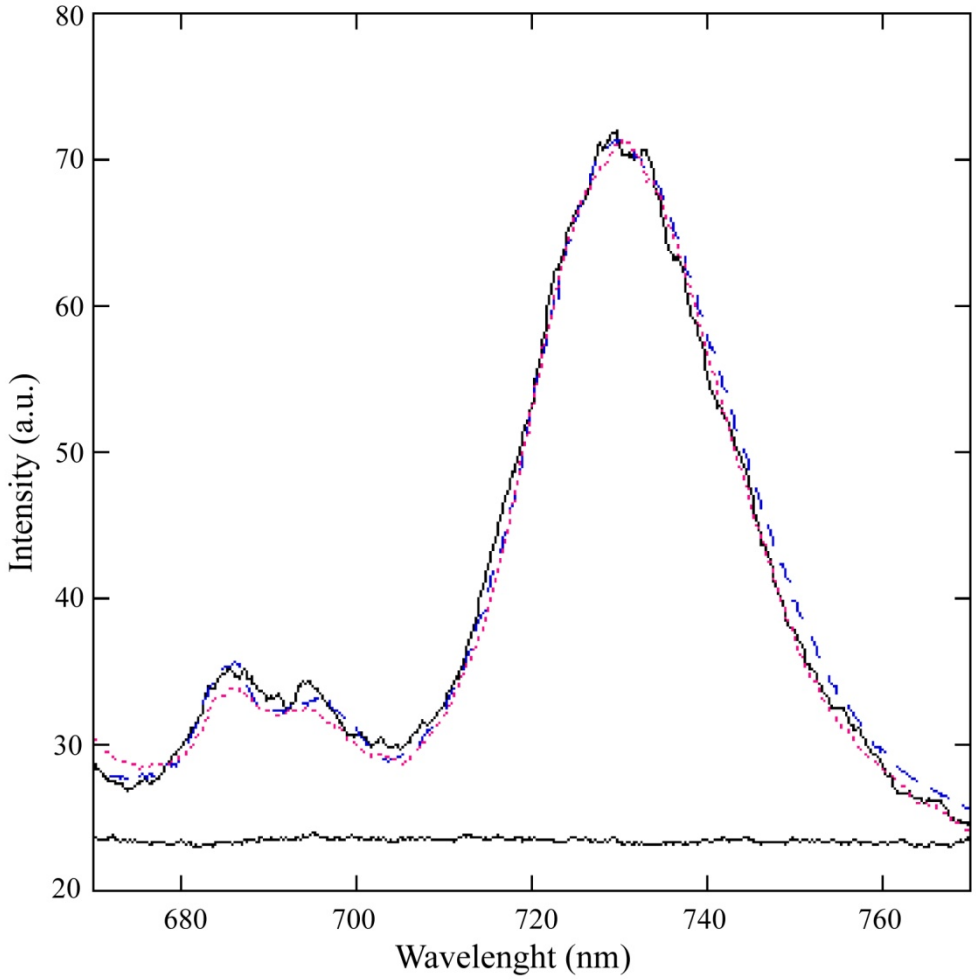

80
70
60
50
40
30
20
Intensity (a.u.)
680
700
720
740
760
Wavelenght (nm)

Fig. 5

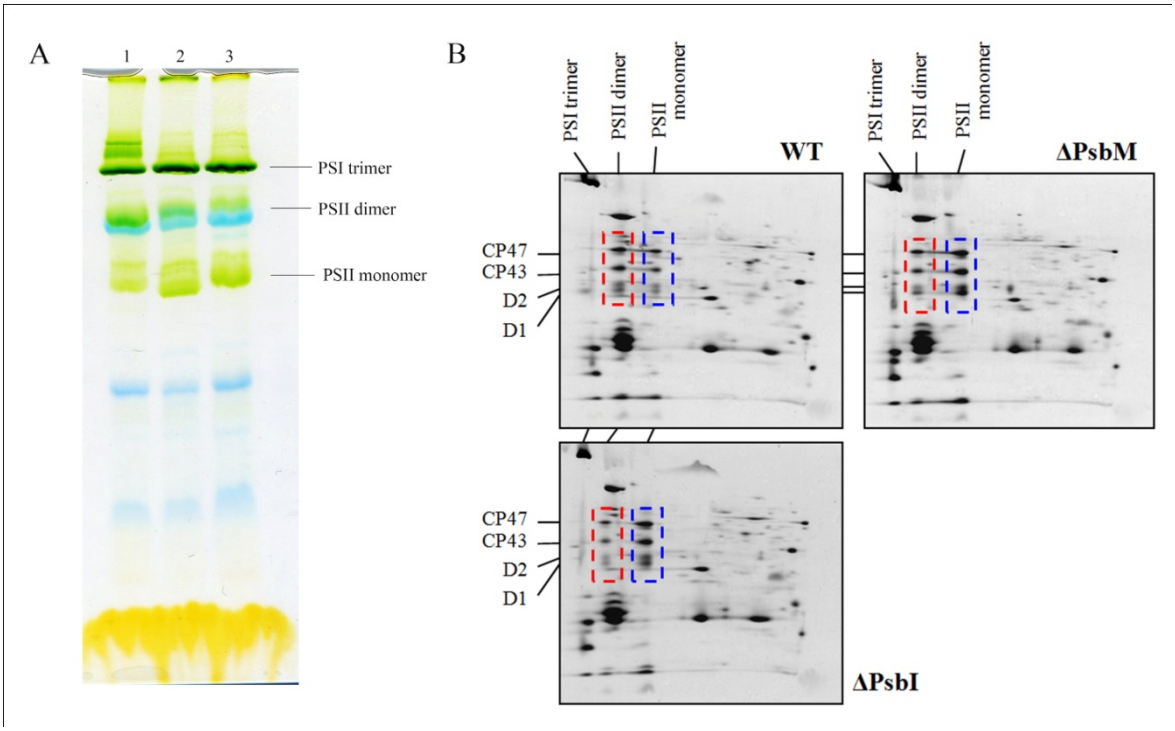
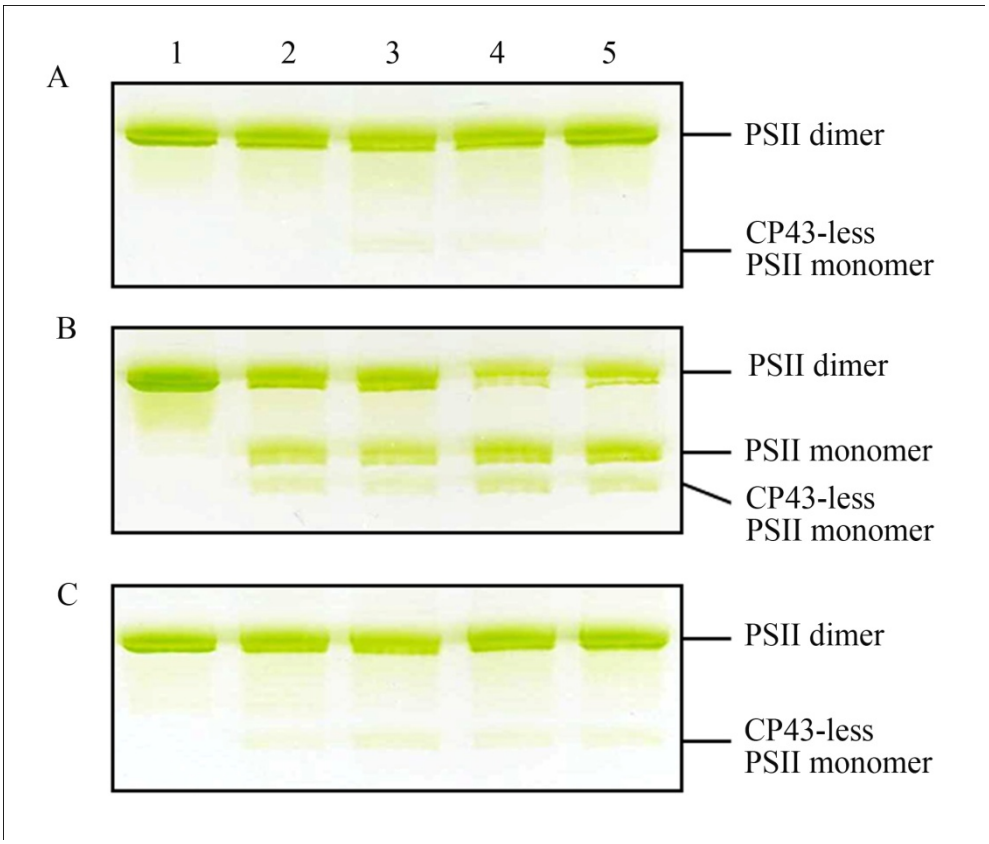

A
1
2
3
PSI trimer
PSII dimer
PSII monomer
B
PSI trimer
PSII dimer
PSII monomer
WT
ΔPsbM
CP47
CP43
D2
D1
CP47
CP43
D2
D1
ΔPsbI

Fig. 6


1
2
3
4
5
A
PSII dimer
CP43-less
PSII monomer
B
PSII dimer
PSII monomer
CP43-less
PSII monomer
C
PSII dimer
CP43-less
PSII monomer