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Purification and characterization of photosystem I complex from *Synechocystis* sp. PCC 6803 by expressing histidine-tagged subunits

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ABSTRACT

We generated *Synechocystis* sp. PCC 6803 strains, designated F-His and J-His, which express histidine-tagged PsaF and PsaJ subunits, respectively, for simple purification of the photosystem I (PSI) complex. Six histidine residues were genetically added to the C-terminus of the PsaF subunit in F-His cells and the N-terminus of the PsaJ subunit in J-His cells. The histidine residues introduced had no apparent effect on photoautotrophic growth of the cells or the activity of PSI and PSII in thylakoid membranes. PSI complexes could be simply purified from the F-His and J-His cells by Ni^{2+}-affinity column chromatography. When thylakoid membranes corresponding to 20 mg chlorophyll were used, PSI complexes corresponding to about 7 mg chlorophyll could be purified in both strains. The purified PSI complexes could be separated into monomers and trimers by ultracentrifugation in glycerol density gradient and high activity was recorded for trimers isolated from the F-His and J-His strains. Blue-Native PAGE and SDS-PAGE analysis of monomers and trimers indicated the existence of two distinct monomers with different subunit compositions and no contamination of PSI with other complexes, such as PSII and Cyt b_6f. Further analysis of proteins and lipids in the purified PSI indicated the presence of novel proteins in the monomers and about six lipid molecules per monomer unit in the trimers. These results demonstrate that active PSI complexes can be simply purified from the constructed strains and the strains are very useful tools for analysis of PSI.

1. Introduction

Oxygenic photosynthesis performed by cyanobacteria, algae, and higher plants provides oxygen and carbon sources for all organisms on earth. The initial step of this process is light-induced electron transfer, which is driven by photosystem I (PSI) and photosystem II (PSII). The electron transport between PSI and PSII is functionally coupled with a plastoquinone pool, Cyt b_6f complex and plastocyanin. PSI mediates the light-driven electron transfer from the plastocyanin at the lumenal side of the thylakoid membrane to ferredoxin at the stromal side.

In higher plants, PSI exists as a monomeric form, but in cyanobacteria, most PSI exists as a trimeric form [1]. The crystal structure of PSI trimers of *Thermosynechococcus elongatus* reveals that one monomer unit of PSI contains 12 different protein subunits, 128 cofactors including 96 chlorophylls (Chls), two phylloquinones, three iron–sulfur clusters, 22 carotenoids, four lipids, a putative Ca^{2+} as well as 201 water molecules [2]. In addition to the PSI trimer, also the PSI monomer and dimer have been characterized in a cyanobacterium *Synechococcus* sp. [3].

PSI complex is usually purified by a combination of ultracentrifugation with sucrose-density gradient and multiple ion exchange column chromatography steps [4,5]. These purification processes are time consuming, and the PSI yield is not high. Ni^{2+}-affinity column chromatography is a powerful tool for simple and rapid purification of protein complexes with a high yield. PSII has been purified from *Synechocystis* sp. PCC 6803 by expressing a histidine-tagged CP47 [6] and from *T. elongatus* by expressing a histidine-tagged CP43 [7]. Recently, purification of PSII with histidine-tagged PsbQ from *Synechocystis* sp. PCC 6803 [8] and with histidine-tagged Cyt b_{559} from tobacco [9] has also been reported. PSI has been purified with Ni^{2+}-affinity column chromatography from a green alga, *Chlamydomonas reinhardtii*, and from cyanobacteria, *Synechocystis* sp. PCC 6803 and *T. elongatus* [10–12]. Gulis et al. [10] expressed a histidine-tagged PsaA in *C. reinhardtii* and purified active PSI complexes. In *T. elongatus*, Prodöhl et al. [11] expressed histidine-tagged PsaF to purify PSI complexes, and the purified PSI complex was used for a semiartificial

Abbreviations: BN, blue native; Chl, chlorophyll; Cm, chloramphenicol; Cm^R, chloramphenicol-resistant gene; DGDG, digalactosyldiacylglycerol; DM, *n*-dodecyl β-D-maltoside; MGDG, monogalactosyldiacylglycerol; NDH, NADH dehydrogenase; PG, phosphatidylglycerol; PSI, photosystem I; PSII, photosystem II; SQDG, sulfoquinovosyldiacylglycerol

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device for hydrogen production. In *Synechocystis* sp. PCC 6803, Tang and Chitnis [12] constructed strains that express histidine-tagged PsaK1 and PsaL subunits of PSI and attempted to purify PSI. *Synechocystis* sp. PCC 6803 is indeed a useful strain for molecular-genetic approach to study PSI since it is easily transformable [13], can grow without PSI in the presence of glucose [14] and its genomic sequence has been completely determined [15]. However, in the previous study [12], the inserted histidine tag to the C-terminus of PsaK1 and PsaL subunits in *Synechocystis* sp. PCC 6803 had adverse effects on PSI, and the active PSI complex could not be purified.

In this study, we inserted a histidine tag into the C-terminus and N-terminus of the PsaF and PsaJ subunits of PSI, respectively, in *Synechocystis* sp. PCC 6803 (Fig. 1A). Because both the C-terminus of PsaF and the N-terminus of PsaJ, into which the histidine tag was inserted, are exposed to the cytoplasmic side of PSI according to the crystal structure of PSI of *T. elongatus* (Fig. 1B), we expected that the insertion of the histidine tag in both sites might be effective for purifying PSI with Ni^{2+}-affinity column chromatography and might not have adverse effects on the assembly and interaction of the PSI subunits. We were able to purify active PSI with a high yield from the constructed strains of *Synechocystis* sp. PCC 6803 and analyze the purified monomers and trimers of PSI. The findings demonstrate that the strains constructed in this study provide very useful tools for the study of PSI.

2. Materials and methods

2.1. Culture conditions and construction of F-His and J-His strains

Wild-type, F-His, and J-His strains of *Synechocystis* sp. PCC 6803 were grown photoautotrophically at 30 °C under continuous illumination at 30 μmol photons m^{-2} s^{-1} in BG11 medium [16]. Cultures were aerated on a rotary shaker (NR-3; TAITEC) at 120 strokes min^{-1}. Cells of F-His and J-His were grown in the presence of 30 μg mL^{-1} chloramphenicol (Cm). For purification of the PSI complex, F-His and J-His cells grown in 200 mL BG11 medium were transferred to 5 L of BG11 medium and cultivated under continuous aeration with 2% (v/v) CO_2 in air. Cells at the logarithmic growth phase were used for preparation of thylakoid membranes and purification of PSI complexes.

The F-His and J-His strains were generated by transforming wild-type cells of *Synechocystis* sp. PCC 6803 with *psaF*-His and *psaJ*-His plasmids. These plasmids were obtained by attaching the hexahistidine coding sequence to the 3' coding region of the *psaF* gene and the 5' coding region of the *psaJ* gene as described below. The chloramphenicol-resistant gene (Cm^R), which was obtained by digesting a plasmid, pCCm, with *Bgl*II, was inserted downstream of the *psaJ* gene as a selectable marker. The coding regions of *psaF* and *psaJ* were amplified by PCR with the following two sets of primers: 5'-CG(GAATTC)TTTCGCTCCCTCAGC-3' (F1) and 5'-ACTAATTAGTGGTGGTGGTGGTGGTGGCGGGGGGAAGTGGG-3' (R1), and 5'-CCCGCCACCACCACCACCACCACTAATTAGTTGGGCAT-3' (F2) and 5'-GA(AGATCT)CTAGGGGTGGAAAAG-3' (R2). The underlined sequence in R1 complements the underlined sequence in F2 and encodes six histidine residues. An *Eco*RI site and a *Bgl*II site, in parentheses, were added to the 5' region of the F1 and R2 primers, respectively. The DNA amplified with the two primer sets was annealed, extended, and used for the next PCR with primer set F1 and R2. The amplified DNA by the second PCR, which included the *psaF* gene encoding a histidine-tagged PsaF and the *psaJ* gene, was digested with *Eco*RI and *Bgl*II. The downstream region of the *psaJ* gene was also amplified by PCR with the following primer set: 5'-GA(AGATCT)TTTTGTTTGGCGAATAAATTC-3' (F3) and 5'-CG(GAATTC)GGCACCAACGGCATT-3' (R3). A *Bgl*II and an *Eco*RI site, in parentheses, were added to the 5' regions of the F3 and R3 primers, respectively. The amplified DNA was digested with *Bgl*II and *Eco*RI, mixed with the DNA amplified with the F1 and R2 primers, digested with *Eco*RI and *Bgl*II, and ligated into the *Eco*RI site of pBluescript II together with the Cm^R gene obtained by digestion of pCCm with *Bgl*II. The obtained plasmid was designated *psaF*-His and used for transformation of wild-type cells of *Synechocystis* sp. PCC 6803.

The *psaJ*-His plasmid was similarly constructed. The coding regions of *psaF* and *psaJ* were amplified by PCR with the following two sets of primers: 5'-CG(GAATTC)TTTCGCTCCCTCAGC-3' (F1) and 5'-CCGTC-

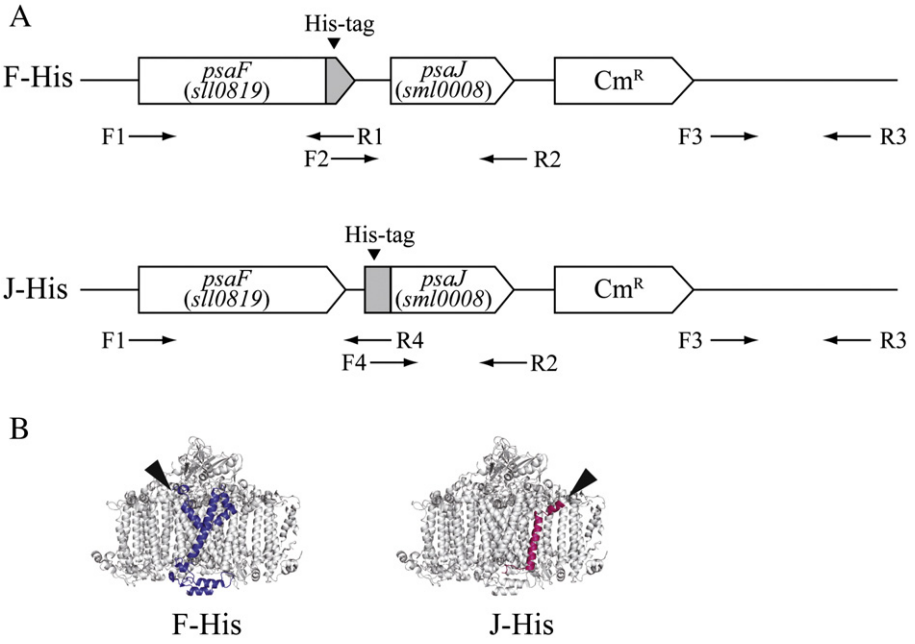

Fig. 1. The locus of *psaF* and *psaJ* genes and location of histidine-tags in the constructed F-His and J-His strains. (A) The locus of *psaF* and *psaJ* genes. The sequence encoding a hexahistidine tag (gray) was inserted to the 3' end of the coding region of *psaF* gene in F-His cells and the 5' end of the coding region of *psaJ* gene in J-His cells. Cm^R was inserted to the 3' downstream region of *psaJ* gene as a maker. (B) Structure of PSI of *T. elongatus* determined by X-ray crystal structural analysis [2]. As shown by arrow heads, the N terminus of the PsaJ subunit and the C terminus of the PsaF subunit, where histidine residues were inserted, were located on the cytoplasmic side of PSI complex. Arrows indicate locations of primers that were used for construction of plasmids, *psaF*-His and *psaJ*-His.

GTGGTGGTGGTGGTGGTGCATAAATTGAGTCTCCAA-3' (R4), and 5'-AATTTATGCACCACCACCACCACCACGACGGTTTGAAATCC-3' (F4) and 5'-GA(AGATCT)CTAGGGGTGGAAAAG-3' (R2). The underlined sequence in R4 complements the underlined sequence in F4 and encodes six histidine residues. The DNA amplified with the two primer sets was annealed, extended, and used for the next PCR with primer set F1 and R2. The amplified DNA by the second PCR, which included the *psaF* and *psaJ* genes encoding a histidine-tagged PsaJ, was digested with *Eco*RI and *Bgl*II. The downstream region of the *psaJ* gene was amplified by PCR and digested with *Bgl*II and *Eco*RI, as described above. The DNA obtained was mixed with the DNA amplified with primer set F1 and R2, digested with *Eco*RI and *Bgl*II, and ligated into the *Eco*RI site of pBluescript II together with the Cm^R gene obtained by digestion of pCCm with *Bgl*II. The obtained plasmid was designated *psaJ*-His and used for transformation of wild-type cells of *Synechocystis* sp. PCC 6803.

The transformants were selected for growth on BG11 agar plates containing 3 μg ml^{-1} Cm. Complete replacement of native genes with *psaF* and *psaJ* genes encoding histidine-tagged proteins was checked by PCR with a primer set, F1 and R3 (Fig. 1A), and by sequencing of the amplified DNA fragments.

2.2. Preparation of thylakoid membranes and purification of PSI complexes

Thylakoid membranes were prepared from 5 L cell culture with 6 mg Chl L^{-1} according to Kashino et al. [17]. PSI was purified from the thylakoid membranes according to the method for PSII purification [17] with minor modifications. Protein complexes in thylakoid membranes from each strain were solubilized with 1.0% *n*-dodecyl β-D-maltoside (DM) at a concentration of 1 mg Chl mL^{-1} in buffer A (50 mM HEPES-NaOH [pH 7.8], 10 mM $MgCl_2$, 5 mM $CaCl_2$, 25% [w/v] glycerol) at 4 °C for 30 min. The solubilized protein complexes were mixed with nickel nitrilotriacetic acid agarose (Qiagen) equilibrated with buffer A containing 0.04% DM and 5 mM histidine. After 1 h of continuous mixing, the mixture was applied to an open column. The column was subsequently washed with one volume of the same buffer containing 0.04% DM and 5 mM histidine, and then with nine volumes of buffer A containing 0.04% DM. PSI bound to the column was eluted with four volumes of buffer A containing 0.04% DM and 100 mM histidine. The obtained PSI complexes were further separated into monomers and trimers by ultracentrifugation at 180,000*g* for 14 h at 4°C with a 5% to 30% linear glycerol density gradient made with a Gradient Master (model 107ip; Biocomp) [18].

2.3. Measurement of photosynthetic activity

Photosynthetic activities were measured at 30 °C by oxygen exchange with a Clark-type oxygen electrode under illumination at 500 μmol photons m^{-2} s^{-1} as described by Gombos et al. [19] with minor modifications. Photosynthetic oxygen-evolving activity (net activity of photosynthesis) was monitored with intact cells washed with BG11 medium and suspended in the same medium. PSII activity of intact cells and thylakoid membranes was measured in the presence of 0.5 mM 2, 6-dichloro-*p*-benzoquinone and 1 mM $K_3Fe(CN)_6$. When PSII activity of thylakoid membranes was measured, thylakoid membranes were suspended in 50 mM MES-NaOH (pH 6.5) including 1 M sucrose and 5 mM $CaCl_2$. PSI activity of the thylakoid membranes and PSI complexes was measured as described by Pakrasi et al. [20] and Hagio et al. [21]. For measurement of PSI activity, thylakoid membranes and PSI complexes were suspended in 50 mM HEPES-NaOH (pH 8.0) including 1 M sucrose, 5 mM $CaCl_2$, 2.0 mM diaminodurene, 1.5 mM methylviologen, 1.0 mM Na-ascorbate, 10 μM 3-(3, 4-dichlorophenyl)-1,1-dimethylurea, and 2 mM KCN.

2.4. Protein analysis

Subunits of protein complexes were analyzed by SDS-PAGE as described by Kashino et al. [22] with minor modifications. Monomers and trimers of PSI corresponding to 2 μg Chl each were solubilized with 2% lithium dodecyl sulfate at 80 °C for 1 min. Then the solubilized proteins were applied to SDS-PAGE with an 18% to 24% polyacrylamide gradient gel (acrylamide/bisacrylamide = 99/1) containing 6 M urea. After electrophoresis for 20 h at 9 mA, proteins were visualized by silver staining [23].

Blue native PAGE (BN-PAGE) was performed according to Herranen et al. [24] with minor modifications. Protein complexes solubilized from thylakoid membranes and purified PSI complexes were analyzed by BN-PAGE. Thylakoid membranes were washed with a buffer (330 mM sorbitol, 50 mM BisTris [pH 7.0], 250 mg mL^{-1} Pefabloc [Roche Diagnostics, Indianapolis, IN, USA]) and resuspended in a resuspension buffer (20% [w/v] glycerol, 25 mM BisTris [pH 7.0], 10 mM $MgCl_2$, 0.1 units mL^{-1} RNase-Free DNase [Promega, Southampton, UK], and 250 mg mL^{-1} Pefabloc) at a Chl *a* concentration of 0.5 mg mL^{-1}. An equal volume of resuspension buffer containing 3% (w/v) DM was added, and the protein complexes in thylakoid membranes were solubilized for 30 min on ice. After incubation, insoluble materials were removed by centrifugation at 18,000*g* for 20 min. The obtained supernatant was mixed with 0.1 volumes of Coomassie Brilliant Blue solution (5% [w/v] Serva blue G [SERVA], 100 mM BisTris [pH 7.0], 30% [w/v] sucrose, 500 mM ε-amino-*n*-caproic acid, 10 mM EDTA) and loaded onto a 5% to 12.5% (w/v) polyacrylamide gradient gel (acrylamide/bisacrylamide = 48/1.5, 0.75 mm thick). Electrophoresis was performed according to Herranen et al. [24]. Protein subunits of separated protein complexes from BN-PAGE were analyzed by SDS-PAGE. Bands of protein complexes in lanes of the BN gel were excised and incubated in a sample buffer containing 5% (v/v) β-mercaptoethanol and 6 M urea for 90 min at room temperature. The bands were then layered onto a 15% (w/v) polyacrylamide gel (acrylamide/bisacrylamide = 50/1.3, 1.0 mm thick) containing 6 M urea. Electrophoresis was performed according to Laemmli [25]. Proteins were visualized by silver staining [23].

Identification of protein subunits was performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry as described previously [26].

2.5. Lipid analysis

Lipids were extracted from PSI using the method of Bligh and Dyer [27]. Lipid classes were separated by thin-layer chromatography and quantified by gas chromatography as described previously [28].

2.6. Pigment analysis

The concentration of Chl was determined using the method of Arnon et al. [29]. The amount of PSI was estimated from the molar ratio of Chl *a* to Chl *a*', which was determined by HPLC as described previously [30].

3. Results and discussion

3.1. Characterization of F-His and J-His strains

We constructed F-His and J-His strains of *Synechocystis* sp. PCC 6803, which express histidine-tagged PsaF and PsaJ subunits, respectively. Six histidine residues were genetically inserted into the C-terminus of PsaF (Sll0819) and the N-terminus of PsaJ (Sml0008; Fig. 1). In both constructs, the upstream and downstream parts of these genes remained intact, and Cm^R was inserted into the downstream part of *psaJ* as a marker (Fig. 1A). The native genes were

Table 1
Photosynthetic activity of whole transport of electrons (net), PSII, and PSI of intact cells and thylakoid membranes of wild-type, F-His and J-His cells.

		Activity (μmol O_2 mg Chl^{-1} h^{-1})		
Sample	Strain	Net ($H_2O \rightarrow CO_2$)	PSII	PSI
Cell	WT	340 ± 40	410 ± 10	–
	F-His	340 ± 20	410 ± 20	–
	J-His	310 ± 30	410 ± 20	–
Thylakoid	WT	–	270 ± 20	−930 ± 60
	F-His	–	240 ± 10	−970 ± 30
	J-His	–	270 ± 10	−903 ± 20

replaced with the genes encoding histidine-tagged proteins. Complete segregation of the replaced gene in the constructed strains was verified by PCR and sequence analysis (data not shown).

To determine the effect of histidine tags on PSI, we checked the photoautotrophic growth and photosynthetic activity of F-His and J-His cells and compared these with those of wild-type cells. The F-His and J-His cells grew as well as the wild-type cells (data not shown). There was no significant difference among the strains, demonstrating that the addition of histidine residues to PsaF and PsaJ does not affect the photoautotrophic growth of *Synechocystis* sp. PCC 6803. We also checked the photosynthetic activity of intact cells and thylakoid membranes of wild-type, F-His, and J-His cells (Table 1). The total photosynthetic electron transport (net) and PSII activity of F-His and J-His cells were similar to those of wild-type cells. No significant difference in activity was observed among the strains. PSII activity of thylakoid membranes isolated from the F-His and J-His cells was also similar to that of wild-type cells, although it was lower than that of intact cells in all strains. Oxygen uptake activity (PSI activity) of thylakoid membranes from F-His and J-His cells was similar to that of thylakoid membranes from wild-type cells. Again, no significant differences were observed among the strains. These results demonstrate that histidine residues added to the C-terminus of PsaF and the N-terminus of PsaJ had no adverse effect on photosynthetic activity.

3.2. Purification of histidine-tagged PSI

We purified the histidine-tagged PSI from F-His and J-His cells with Ni^{2+}-affinity column chromatography. Thylakoid membranes prepared from F-His and J-His cells were solubilized with 1.0% DM, and the solubilized protein complexes were applied to Ni^{2+}-affinity column chromatography to purify PSI complexes. We were able to purify PSI complexes (mixture of monomers and trimers), corresponding to about 7 mg Chl, from thylakoid membranes corresponding to 20 mg Chl. To separate the PSI monomers and trimers, the PSI complexes eluted from the Ni^{2+}-affinity column were subjected to glycerol density gradient centrifugation. As shown in Fig. 2 (lanes b and c), two clear bands corresponding to monomers and trimers of PSI were observed on the gradient after ultracentrifugation. Based on the Chl content of purified monomers and trimers, we calculated the ratio of trimers to monomers. This ratio was 10, indicating that trimers were much more abundant than monomers. In addition, we found a faint band between the monomers and trimers. To identify the band, we collected and concentrated the middle fraction between the monomers and trimers and applied it to BN-PAGE following SDS-PAGE. This fraction contained a small amount of PSI proteins (data not shown) and apparently represents a PSI dimer, which might be an intermediate in the assembly or disassembly processes of PSI.

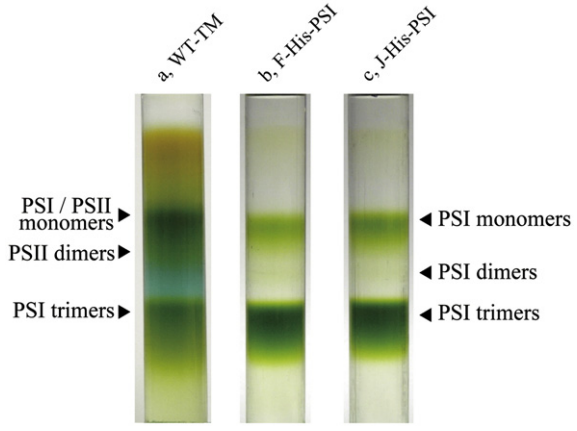

Fig. 2. Separation of protein complexes by ultracentrifugation with a 5% to 30% glycerol density gradient. (a) Protein complexes solubilized from thylakoid membranes of wild-type cells. Thylakoid membranes corresponding to 100 μg Chl were solubilized and loaded onto the glycerol density gradient. (b) PSI complexes purified from F-His cells. (c) PSI complexes purified from J-His cells. PSI complexes corresponding to 150 μg Chl were loaded onto the glycerol density gradient (b and c).

Protein complexes from thylakoid membranes of wild-type cells were also separated by ultracentrifugation with a glycerol density gradient. In this case, solubilized protein complexes were directly ultracentrifuged without Ni^{2+}-affinity column chromatography because there was no histidine tag in the PSI subunits. After ultracentrifugation, three major bands corresponding to monomers of PSI and PSII, dimers of PSII, and trimers of PSI were observed (Fig. 2, lane a). Monomers of PSI and PSII were not separated by ultracentrifugation with a glycerol density gradient.

3.3. Activity of PSI complexes

To check whether the purified PSI complexes were active, we measured the activity of PSI monomers and trimers from F-His and J-His cells (Table 2). PSI trimers from wild-type cells, purified as described above by ultracentrifugation with a glycerol density gradient, were used as a control. Because monomers of PSI from wild-type cells were contaminated with PSII monomers, only the trimers were used for measurement of PSI activity. The activity of PSI trimers purified from both F-His and J-His cells was around 1100 to 1200 μmol O_2 mg Chl^{-1} h^{-1} and that of PSI trimers from F-His cells was slightly higher. PSI trimers from both F-His and J-His cells were more active than PSI trimers from wild-type cells. Since wild-type trimers were isolated only by ultracentrifugation with glycerol density gradient, they might contain some components (e.g. PSII aggregates), which are not present in trimers from F-His and J-His cells, thereby lowering the activity (expressed on Chl basis) as compared to the trimers from F-His and J-His cells. The activity of PSI monomers purified from F-His and J-His cells was about 700 μmol O_2 mg Chl^{-1} h^{-1}, which was significantly lower than that of trimers. Although the reason for lower activity of the monomers than that of the trimers has not been clarified, we found many novel proteins in monomers but not in trimers, as described below. The interaction of monomers with such proteins might affect the activity of monomers. It is also possible that monomers lack some components, which are required for maximal activity of PSI. These findings demonstrate that PSI complexes purified from F-His and J-His cells are very active and that trimers are more active than monomers.

Table 2
Photosynthetic activity of PSI monomers and trimers purified from F-His and J-His cells.

	Activity (μmol O_2 mg Chl^{-1} h^{-1})		
Sample	WT	F-His	J-His
PSI monomer	–	−730 ± 30	−700 ± 100
PSI trimer	−830 ± 10[a]	−1240 ± 20	−1110 ± 30

[a] Purified by ultracentrifugation with a glycerol density gradient without Ni^{2+}-affinity chromatography.

3.4. Subunits of PSI complexes

To analyze the protein subunits of PSI, we applied the trimers and monomers, isolated by Ni^{2+}-affinity column chromatography and subsequently separated by ultracentrifugation with a glycerol density gradient, to SDS-PAGE. As shown in Fig. 3, the trimers (lanes a and b) from both the F-His and J-His cells contained PsaA, PsaB, PsaD, PsaF, PsaL, PsaE, PsaC, PsaK2, PsaK1, PsaI, PsaJ, and PsaM subunits. They contained only those proteins that already have been identified as subunits of PSI [31,32]. PsaX that was previously found in *T. elongatus* [32,33] was not detected, consistent with the lack of PsaX homolog in the genome of *Synechocystis* sp. PCC 6803 [15]. The molecular masses of PsaF and PsaJ in trimers from F-His and J-His cells, respectively, were shifted slightly higher than native proteins because of the addition of the histidine tag to the subunits. These results suggest that the trimers are pure and not contaminated with other protein complexes. In addition, PsaK2 was detected in trimers but not in monomers, suggesting that the attachment of PsaK2 to PSI occurs at a trimer stage. Because PSI monomer is considered to have only one binding site for the PsaK protein (either PsbK1 or PsbK2), the trimers might be a mixture of heterotrimers composed of PsaK1-binding and PsaK2-binding monomers. Our results also support the suggestion of Duhring et al. [34,35] reporting that PsaK is attached to PSI at the last stage of the assembly process.

The monomers from both the F-His and J-His cells contained PsaA, PsaB, PsaD, PsaF, PsaL, PsaE, PsaC, PsaK1, PsaI, PsaJ, and PsaM subunits (Fig. 3, lanes c and d). In addition to these protein subunits, 14 proteins

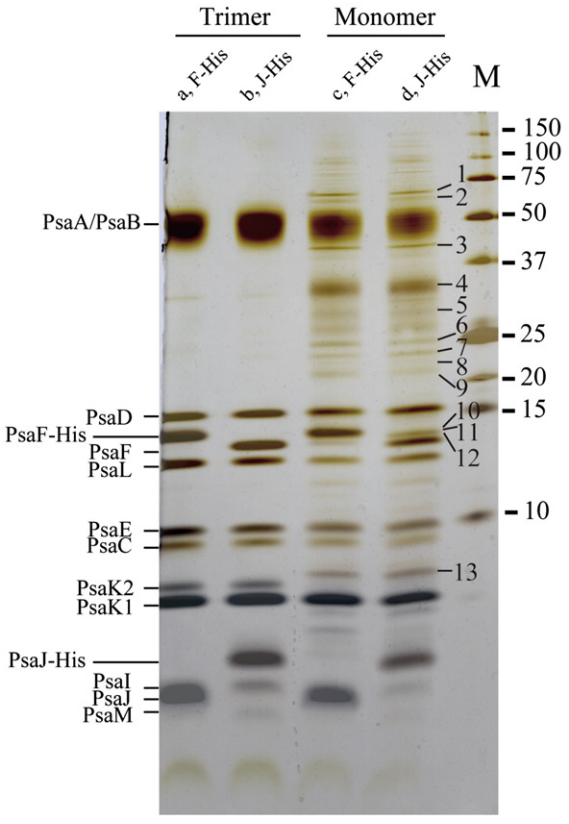

Fig. 3. Protein subunits of purified PSI. PSI complexes purified from F-His and J-His cells by Ni^{2+}-affinity column chromatography were separated to monomers and trimers by ultracentrifugation with glycerol density gradient and their protein subunits were analyzed by SDS-PAGE. Lanes a and b, trimers from F-His and J-His cells. Lanes c and d, monomers from F-His and J-His cells. Lane M, molecular weight markers. PSI monomers and trimers corresponding to 2 μg of Chl were loaded in each lane. Proteins were visualized by silver staining. Numbers in lane d indicate locations of proteins identified as novel proteins in monomers.

Table 3
Novel proteins identified by mass spectrometry in PSI monomers from F-His and J-His cells.

Band number	Protein assignment	Matched peptides	Cover (%)	Mascot score
1	Isoamylase (Slr1857)	9	16	46
2	Long-chain-fatty-acid CoA ligase (Slr1609)	20	41	97
3	NdhH (Slr0261)	20	53	141
4	Unknown protein (Slr0151)	14	60	111
5	Light-dependent NADPH-POR (Slr0506)	6	19	49
6	NdhK (Slr1280)	9	31	86
7	LexA repressor (Sll1626)	12	72	103
8	NdhI (Sll0520)	9	46	109
9	Hypothetical protein (Slr0147)	5	27	60
	NdhJ (Slr1281)	5	17	33
10	Hypothetical protein (Slr0146)	7	43	61
11	Hypothetical protein (Slr0149)	9	65	73
12	NdhN (Sll1262)	7	49	71
13	NdhO (Ssl1690)	6	68	88

(13 bands including 14 proteins) that have not previously been identified in PSI were detected in monomers from both the F-His and J-His cells (Table 3). If the latter proteins are only contaminants due to non-specific interaction with PSI complexes, they would be expected to be present not only in momomers but also in trimers. Thus, it is likely that at least part of them are specifically associated with PSI monomers. Six of these PSI monomer-associated proteins, NdhH, NdhI, NdhJ, NdhK, NdhN and NdhO, are subunits of the peripheral part of the NADH dehydrogenase 1 complex (NDH-1 complex) [36]. Intriguingly, recent studies with plant thylakoid membranes have identified a PSI-NDH supercomplex, which is possibly responsible for cyclic electron transfer around PSI [37,38]. It is likely that similar supercomplexes exist also in cyanobacterial membranes and are specifically interacting with PSI monomer complexes as in higher plants.

Four other proteins, Slr0146, Slr0147, Slr0149, and Slr0151, are encoded in a gene cluster composed of nine genes (*slr0144* to *slr0152*) in the genome of *Synechocystis* sp. PCC 6803 [39]. Recently, Wegener et al. [40] reported that this gene cluster is expressed as an operon and is involved in PSII assembly; thus, it was designated Pap (*P*hotosystem II *A*ssembly *P*roteins) operon. The expression of this operon is up-regulated in mutants that lack extrinsic proteins of PSII, such as PsbV, PsbQ, and PsbP [40], and down-regulated under oxidative stress and in mutants lacking PSI [39]. The proteins encoded in this operon contain domains for binding the cofactors, Chl, iron sulfur centers, and bilin, which are components of the electron transport chain [40]. These findings, together with identification of proteins encoded in the Pap operon in PSI monomers, suggest that this operon plays an important role in the assembly not only of PSII but also of PSI. As other novel proteins, the LexA repressor (Sll1626), light-dependent NADPH-protochlorophyllide oxidoreductase (Slr0506), isoamylase (Slr1857), and long-chain-fatty-acid CoA ligase (Slr1609) were identified in PSI monomers. Although further analyses are required to investigate the functions of these proteins, it is assumed that they have important roles in PSI.

3.5. Subcomplexes of PSI

Blue Native-PAGE is a powerful tool for separating protein complexes according to size without denaturing them [24]. In addition to ultracentrifugation with a glycerol density gradient, we also employed BN-PAGE to separate subcomplexes of PSI at higher

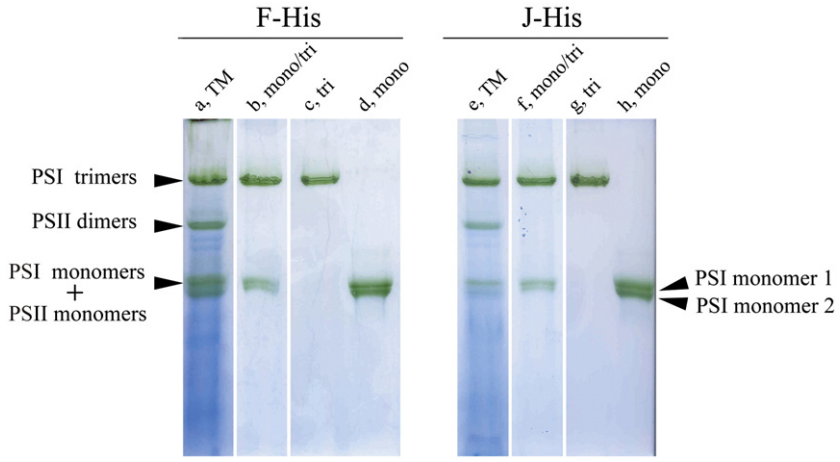

Fig. 4. Separation of protein complexes by BN-PAGE. Protein complexes solubilized from thylakoid membranes (a, e), a mixture of monomers and trimers of PSI (b, f), PSI trimers (c, g), and PSI monomers (d, h) purified from F-His (a to d) and J-His (e to h) cells were analyzed by BN-PAGE. Samples corresponding to 5 μg of Chl were loaded in each lane.

resolution. When protein complexes solubilized from thylakoid membranes of wild-type cells were analyzed by BN-PAGE, four major green bands and minor blue bands were detected (Fig. 4, lanes a and e). The two upper green bands corresponded to PSI trimers and PSII dimers, whereas the third and fourth green bands were close to each other and corresponded to a mixture of PSI monomers and PSII monomers. By contrast, PSI complexes purified from F-His (lane b) and J-His (lane f) cells had two green bands that corresponded to trimers and monomers of PSI, but no green bands of PSII dimers or minor blue bands. PSI trimers and monomers from F-His and J-His cells that were purified with Ni^{2+}-affinity column chromatography and separated by ultracentrifugation with a glycerol density gradient were also subjected to BN-PAGE. PSI trimers from both F-His (lane c) and J-His (lane g) cells showed only a single green band of PSI trimers and no other bands. PSI monomers from both F-His (lane d) and J-His (lane h) cells had two green bands, which were close to each other, and no other bands. The green bands of PSI monomers were designated monomer 1 and monomer 2. These results clearly demonstrate that the purified PSI complexes, monomers and trimers, are very pure and not contaminated with other protein complexes.

To check the difference between monomer 1 and monomer 2, we carefully excised the bands of PSI monomers (monomer 1 and monomer 2) detected in BN-PAGE and subjected them separately to SDS-PAGE. As shown in Fig. 5, monomer 1 and monomer 2 from both F-His and J-His cells contained most of the protein subunits identified in the analysis of monomers separated by ultracentrifugation with a glycerol density gradient. There was no significant difference in protein subunits between monomer 1 and monomer 2 from F-His and J-His cells except that the PsaL subunit was present in monomer 1 (panels a and b) but not in monomer 2 (panels c and d). Although the reason for the lack of PsaL subunit in monomer 2 has not been clarified, it can be assumed that monomer 2 is an assembly or disassembly intermediate of the PSI complex. The PsaL subunit is required for trimer formation in *Synechocystis* sp. PCC 6803 [41] and also in *Synechococcus* sp. PCC 7002 [42]. The C-terminal helix of PsaL is embedded inside the monomeric PSI and is involved in trimer formation [43]. Therefore, our data suggest that monomer 2 might be converted to monomer 1 and then to trimers. The other possibility is that PsaL subunit was partially released from monomers during purification of PSI complexes. In addition, the amounts of novel proteins in the monomer 1 and monomer 2 were lower than those in monomers separated by ultracentrifugation with a glycerol density gradient, presumably because of dissociation of the proteins during BN-PAGE.

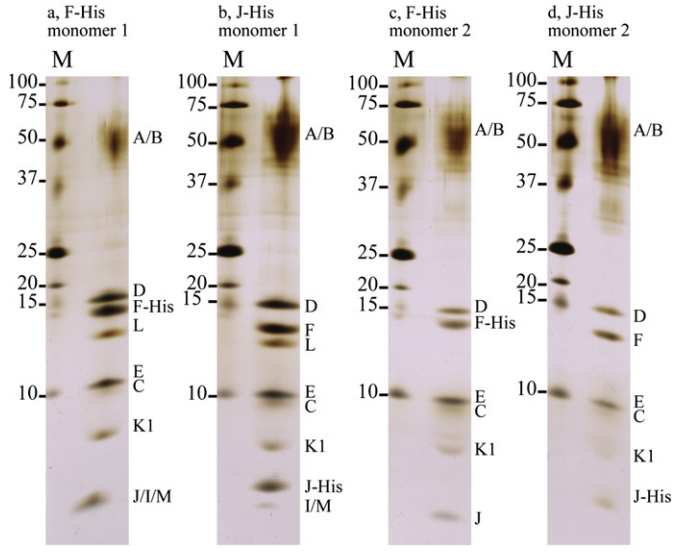

Fig. 5. Protein subunits of PSI complexes separated by BN-PAGE. PSI monomers separated as green bands by BN-PAGEs that correspond to 5 μg of Chl were excised from the BN gel, solubilized and subjected to SDS-PAGE with a 18–24% polyacrylamide gel. Panels a and b, monomer 1 from F-His and J-His cells. Panels c and d, monomer 2 from F-His and J-His cells. Lane M, molecular weight markers. Proteins were visualized with silver staining.

3.6. Lipids in PSI complexes

The lipid composition of PSI trimers purified from F-His and J-His cells is shown in Table 4. Lipid composition of thylakoid membranes from wild-type cells, which we determined previously [26], is also shown in the table as a control. There was a remarkable difference

Table 4
Lipid compositions of thylakoid membranes from wild-type cells and of PSI trimers purified from F-His and J-His cells.

Sample	Lipid class			
	MGDG	DGDG	SQDG	PG
	(mol%)			
TM (Wild type)	37 ± 2[a]	20 ± 2[a]	29 ± 1[a]	14 ± 1[a]
PSI (F-His)	32 ± 4	12 ± 3	19 ± 6	37 ± 7
PSI (J-His)	34 ± 4	9 ± 4	21 ± 7	36 ± 10

[a] These values are adapted from our previous data [26].

Table 5
Numbers of Chl and lipid molecules in PSI trimers purified from F-His and J-His cells.

Sample	Chl *a*/Chl *a*'	Lipid			
		MGDG	DGDG	SQDG	PG
		Number/monomer			
PSI (F-His)	72.3 ± 5.7	1.8 ± 0.6	0.7 ± 0.3	1.0 ± 0.3	2.0 ± 0.2
PSI (J-His)	67.6 ± 2.5	1.9 ± 0.3	0.6 ± 0.3	1.1 ± 0.2	2.1 ± 0.8

between the lipid composition of thylakoid membranes and that of PSI. In PSI from both F-His and J-His cells, the content on Chl basis of MGDG, DGDG and SQDG was lower than in thylakoid membranes, whereas the content of PG in PSI was about 2.5 times higher than in thylakoid membranes. These results demonstrate that PG is enriched in PSI complexes, as has been reported for PSII complexes [26]. The number of lipid molecules bound to PSI complexes was estimated based on the content of Chl *a* and Chl *a*' in PSI. As shown in Table 5, it was estimated that six lipid molecules (two MGDG, one DGDG, one SQDG, and two PG) per monomer were present in the PSI trimers.

Jordan et al. [2] analyzed the structure of the trimer complex of PSI from *T. elongatus* by X-ray crystallography at 2.5 Å resolution and identified one molecule of MGDG and three molecules of PG per monomer of the complex. In this study, we identified six lipid molecules per monomer in the PSI complex (Table 5). Although the number of lipid molecules was close to that found in the crystal structure of PSI from *T. elongatus*, the lipid composition of PSI from *Synechocystis* sp. PCC 6803 was different. Specifically, two MGDG, one DGDG, one SQDG, and two PG per monomer were identified in the PSI trimers from *Synechocystis* sp. PCC 6803. These observations suggest that the lipid composition of PSI depends on the cyanobacterial species and that some lipid molecules are interchangeable with other classes of lipids.

Our group [44] and Awai et al. [45] recently identified a *dgdA* gene presumably encoding a DGDG synthase of *Synechocystis* sp. PCC 6803 and made a mutant defective in the biosynthesis of DGDG by disrupting the *dgdA* gene. Because the mutant of *dgdA* contained no detectable DGDG, it was a good tool for elucidating the role of DGDG in photosynthesis. Analyses of the mutant in our previous studies demonstrated an important role of DGDG on the donor side of PSII in binding of extrinsic proteins required to stabilize the oxygen-evolving complex [44]. In this study, we identified one DGDG molecule per monomer unit in purified PSI trimers. The presence of DGDG in PSI prompted us to check the PSI activity of the *dgdA* mutant cells to investigate whether DGDG plays an important role in PSI. We measured PSI activity of thylakoid membranes from the *dgdA* mutant cells and found that the PSI activity was significantly lower than that of thylakoid membranes from wild-type cells (data not shown). This finding suggests that DGDG plays an important role not only in PSII but also in PSI, and it is consistent with the finding that DGDG is present in both PSI and PSII in *Synechocystis* sp. PCC 6803.

We also checked fatty acid composition of lipid classes in PSI (data not shown). The fatty acid composition of all lipid classes, MGDG, DGDG, SQDG, and PG, was similar to that of lipid classes in thylakoid membranes that we previously reported [26]. Because specific molecular species were not enriched in PSI, it is likely that lipid molecular species synthesized in thylakoid membranes are incorporated into PSI complexes in a non-specific manner during the assembly processes of PSI.

In conclusion, we generated F-His and J-His strains of *Synechocystis* sp. PCC 6803, which express histidine-tagged PsaF and PsaJ, respectively, for simple purification of active PSI complexes. We were able to purify PSI complexes from the constructed strains simply by using the Ni^{2+}-affinity column chromatography, and found that the purified complexes were very active and were not contaminated with other protein complexes. The strains constructed in this study are very useful tools that will facilitate further studies of PSI.

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