Unique constitution of photosystem I with a novel subunit in the cyanobacterium *Gloeobacter violaceus* PCC 7421

Hidetoshi Inoue[a], Tohru Tsuchiya[a,b], Soichirou Satoh[c], Hideaki Miyashita[a,b], Takakazu Kaneko[d], Satoshi Tabata[d], Ayumi Tanaka[c], Mamoru Mimuro[a,b,*]

[a] *Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan*
[b] *Department of Technology and Ecology, Hall of Global Environmental Research, Kyoto University, Kyoto 606-8501, Japan*
[c] *Institute of Low Temperature Science, Hokkaido University, Sapporo 060-0819, Japan*
[d] *Kazusa DNA Research Institute, Kazusa-kamatari, Kisarazu, Chiba 292-0812, Japan*

Received 4 October 2004; revised 1 November 2004; accepted 8 November 2004

Available online 19 November 2004

Edited by Peter Brzezinski

Abstract Constitution of the photosystem I complex isolated from the cyanobacterium *Gloeobacter violaceus* PCC 7421 was investigated by tricine–urea–SDS–PAGE, followed by peptide mass fingerprinting or N-terminal sequencing. Eight subunits (PsaA, PsaB, PsaC, PsaD, PsaE, PsaF, PsaL and PsaM) were identified as predicted from the genome sequence. A novel subunit (PsaZ) was discovered, but PsaI, PsaJ, PsaK and PsaX were absent. PsaB has a C-terminal extension with 155 amino acids in addition to the conserved region and this domain is similar to the peptidoglycan-binding domain. These results suggest that PS I complexes of *G. violaceus* have unique structural properties.

Keywords: Cyanobacterium; Genome information; Peptidoglycan-binding domain; Photosynthesis; Photosystem I; *Gloeobacter violaceus*

1. Introduction

Gloeobacter violaceus PCC 7421 is a unicellular cyanobacterium assigned to a species that branched off at the earliest stage in the phylogenetic tree of cyanobacteria on the basis of 16S rRNA sequences [1]. This organism shows unique properties; it lacks intracellular thylakoid membranes [2], and this indicates that the lumenal side of the thylakoid membrane corresponds to the periplasmic side in *G. violaceus*. Since the water activity and ionic environment in the periplasm are different from those in the lumen, this localization of components might induce significant changes in amino acid sequences.

Recently, the complete genome sequence of *G. violaceus* was determined and many unique properties were demonstrated [3]. Usually, cyanobacterial photosystem (PS) I consists of 11 highly conserved subunits; PsaA, PsaB, PsaC, PsaD, PsaE, PsaF, PsaI, PsaJ, PsaK, PsaL and PsaM [4]. A small subunit called PsaX was reported only in *Synechococcus vulcanus* and *Anabaena variabilis* [5,6]. Four of the PS I genes, *psaI*, *psaJ*, *psaK* and *psaX*, are not found in *G. violaceus* [3]. On the basis of the PS I trimer structure of *Synechococcus elongatus* [7], these four subunits are localized in the periphery of the monomer; a few of them are localized in the attaching site of monomers. They might interact with other polypeptides and finally contribute to the stability of the whole structure. Absence of the four subunits in *G. violaceus* may thus induce a malfunction of PS I complexes or the missing subunits might be replaced with an additional component(s).

To investigate these possibilities, we purified the PS I complex and characterized its biochemical properties. We discovered a novel subunit specific to *G. violaceus* and also found a C-terminal extension of PsaB, which shows a significant homology to peptidoglycan-binding domains in several eubacteria. Based on these properties, we discussed the biological significance of this species in the evolution of cyanobacteria.

2. Materials and methods

Gloeobacter violaceus PCC 7421 was grown photoautotrophically in BG11 medium [8] at 25 °C under a fluorescent lamp with a light intensity of 5 μE/(m^2 s). Air was continuously supplied.

PS I complexes were purified by the procedures described by Sun et al. [9] with slight modification. All procedures were done under dim light conditions. Cells were suspended in SMN buffer (0.4 mM sucrose, 10 mM NaCl, and 50 mM MOPS, pH 7.0) containing 0.2 mM phenylmethylsulfonyl fluoride and 5 mM benzamidine, and broken with a bead beater. The membrane fraction was recovered by centrifugation (50000 × *g*, 30 min, 4 °C) after removal of beads and unbroken cells. This fraction was incubated for 30 min at room temperature with 1 mM $CaCl_2$ in SMN buffer and then PS I complexes were solubilized with dodecyl β-D-maltoside (final concentration of 0.75%) at 4 °C for 15 min in the dark. After centrifugation (18800 × *g*, 15 min, 4 °C), the supernatant was layered on a linear sucrose density-gradient (10–25%). The samples were centrifuged at 160000 × *g* for 16 h at 4 °C and the green band corresponding to the PS I trimer was collected. The samples were stored at −80 °C until use.

Tricine–urea–SDS–PAGE was performed according to the standard procedure [10] using a 14% acrylamide gel with 6 M urea. Subunits were separated by tricine–urea–SDS–PAGE and identified by peptide mass fingerprinting (PMF). The bands corresponding to the proteins stained with silver (SilverQuest, Invitrogen, CA, USA) were excised

*Corresponding author. Fax: +81 75 753 6855.
E-mail address: mamo-mi@mm1.mbox.media.kyoto-u.ac.jp (M. Mimuro).

Abbreviations: Chl, chlorophyll; LHC, light-harvesting complex; ORF, open reading frame; P700, the primary electron donor of photosystem I; PAGE, polyacrylamide gel electrophoresis; PMF, peptide mass fingerprinting; PS, photosystem; PVDF, polyvinylidene fluoride; RC, reaction center; SDS, sodium dodecyl sulfate

0014-5793/$22.00 © 2004 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/j.febslet.2004.11.013

from the gel and the silver was removed by chemical reducers [11]. After in-gel trypsin digestion, proteins were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The experimental mass values were compared with a database of peptide mass values and each subunit was then identified. Two low-molecular-mass subunits were transferred to a polyvinylidene fluoride (PVDF) membrane (Immobilon-P^{SQ}, Millipore, MA, USA) by semi-dry blotting after separation and their N-terminal sequences then analyzed.

P700 content was estimated by the oxidation–reduction difference spectrum with a Hitachi 557 spectrophotometer as reported previously [12]. Chl *a* content was measured in methanol extracts by using the extinction coefficient reported by Porra et al. [13].

3. Results

3.1. Identification of PS I subunits

The Chl *a*/P700 ratio in our preparations was estimated to be approximately 100. This value is consistent with that found in other cyanobacteria [12,14], and is close to the predicted value based on the crystal structure of *S. elongatus*, i.e., 96 Chl *a* in PS I [7], thus confirming the intactness of our preparations.

Fig. 1A shows the sedimentation pattern in the sucrose density-gradient centrifugation. The top colored band contained a small amount of free Chl *a* and carotenoids (mainly oscillol). Two green bands were detected below this band; the lower major band was ascribed to the PS I trimer, and the upper faint band to a mixture of PS I monomer and PS II. Therefore, we collected the lower green band. This pattern was consistent with that of *Synechocystis* sp. PCC 6803 (*Synechocystis*) [9].

We separated subunits of PS I complexes by tricine–urea–SDS–PAGE and then identified them by PMF. Seven subunits were unequivocally identified as PsaA, PsaB, PsaF, PsaD, PsaL, PsaE and PsaC (Table 1 and Fig. 1B). Two low-molecular-mass subunits (band-1 and band-2 in Fig. 1B) were not identified by PMF. Therefore, we transferred them to a PVDF membrane after separation and their N-terminal sequences were analyzed. Band-1 was assigned to PsaM. A partial and unknown N-terminal sequence was obtained for band-2 (MXSYNVFPALVIXT, X being an unidentified residue).

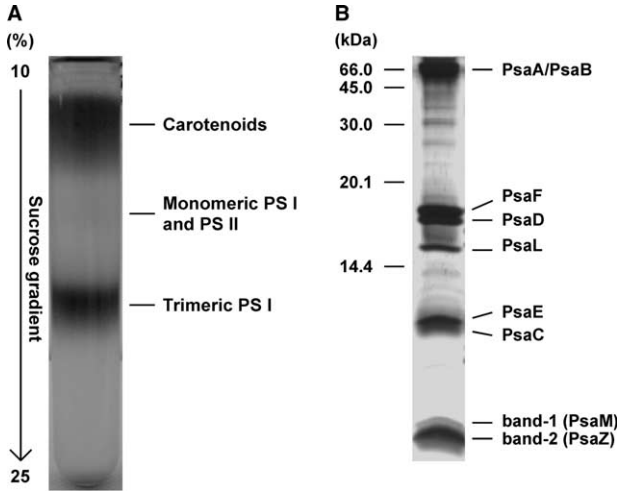

Fig. 1. Purification of *G. violaceus* PS I. (A) Sedimentation pattern in the sucrose density-gradient (10–25%) centrifugation. (B) Tricine–urea–SDS–PAGE pattern of the purified PS I. Subunits identified by PMF or N-terminal sequencing are shown.

Table 1
Identification of PS I subunits in *G. violaceus*

Subunit	Identifier	Method	PMF	
			Number of fragments	Coverage
PsaA	*glr3438*	PMF	15	164/783
PsaB	*glr3439*	PMF	18	188/872
PsaC	*gsl3287*	PMF	7	69/81
PsaD	*glr3701*	PMF	11	105/144
PsaE	*gsl3408*	PMF	6	49/65
PsaF	*glr2732*	PMF	6	57/181
PsaL	*glr2236*	PMF	5	46/147
PsaM	*gsl2401*	N-terminal		
PsaZ	*gsr5001*	N-terminal		

PMF and N-terminal mean peptide-mass-fingerprinting and N-terminal sequencing for identification of subunits, respectively. Coverage indicates the numbers of amino acid residues identified in fragments by PMF/numbers of total amino acid residues.

However, a gene corresponding to this sequence was not found among annotated genes [3]. TBLASTN [15] was then performed using the partial sequence as a query and a novel open reading frame (ORF) (3942197–3942304) encoding a polypeptide corresponding to MQSYNVFPALVIITTLVVPFMAA-AALLFIIERDPS was identified. We analyzed the secondary structure of the polypeptide by the SOSUI program (http://sosui.proteome.bio.tuat.ac.jp/sosuimenu0.html) [16], and it predicted that 23 residues (8–30) out of 35 total residues formed a trans-membrane α-helix. Based on these results, we assigned band-2 to a new subunit of *G. violaceus* PS I and named it PsaZ (Fig. 1B). When a novel ORF is annotated in *G. violaceus*, the gene number is given sequentially from 5001 as designated by the Kazusa DNA Research Institute. Accordingly, the gene corresponding to a new ORF was named *gsr5001* (CyanoBase: http://www.kazusa.or.jp/cyanobase/). No homolog of *psaZ* was found by our database search, indicating that PsaZ is unique in *G. violaceus*. PsaI, PsaJ, and PsaK, which did not have corresponding genes in *G. violaceus*, were not detected in our preparations. Therefore, we concluded that the PS I complexes of *G. violaceus* consist of nine subunits.

3.2. A C-terminal extension in PsaB

We found unique features in the amino acid sequence of *G.violaceus* PsaB. Fig. 2 shows the sequence alignment of the deduced amino acid sequences of *psaB* in three species (CyanoBase: http://www.kazusa.or.jp/cyanobase/). Eleven transmembrane regions were well conserved among three species, but minor differences were discernible. Two deletion sites were found in *G. violaceus* between transmembrane regions 3 and 4, and between transmembrane regions 7 and 8; both sites are to be localized in the periplasm.

A C-terminal extension was found in *G. violaceus psaB*; 155 amino acid residues (from residues 718 to 872) were attached to the C-terminus of the PsaB conserved region (Fig. 2). PMF confirmed the presence of this region in PsaB. A mixture of PsaA and PsaB separated by tricine–urea–SDS–PAGE (Fig. 1B) was subjected to PMF analysis; 15 and 18 peptide fragments were obtained for PsaA and PsaB, respectively (Table 1). Among the 18 PsaB fragments, five corresponded to the C-terminal extension and one of these fragments contained the C-terminus. This confirmed the existence of the C-terminal extension in PsaB that was predicted

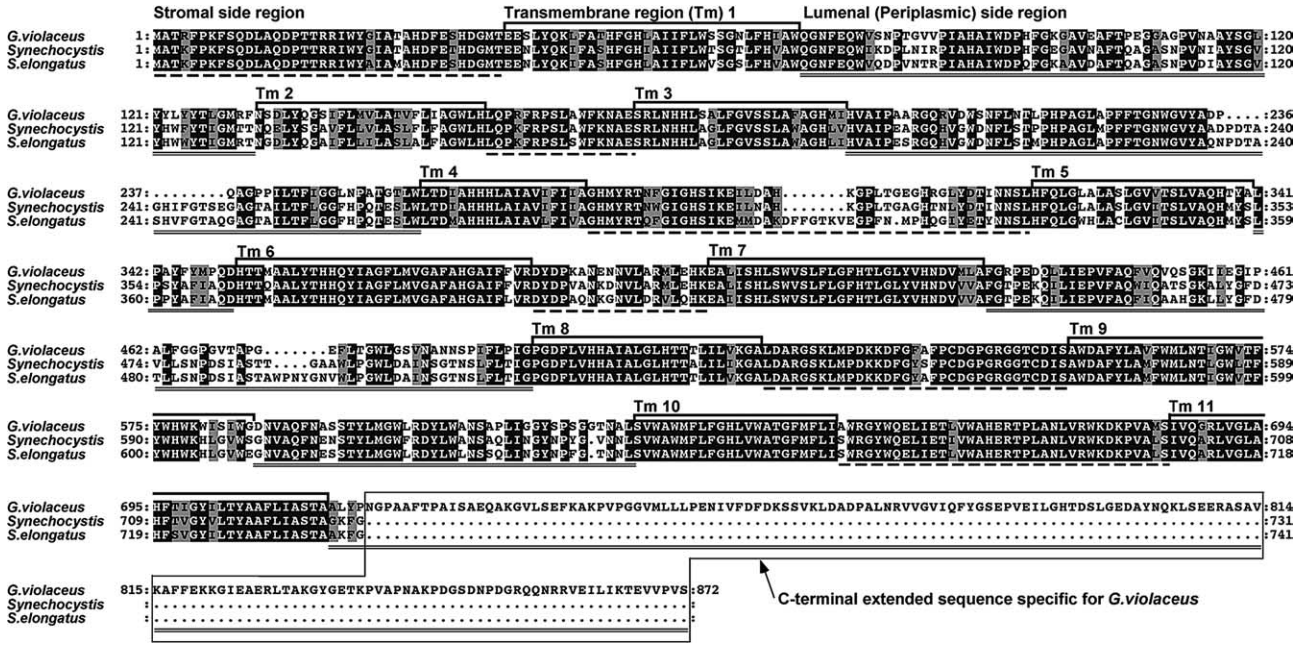

Fig. 2. Alignment of PsaB protein sequences. Amino acid residues that are conserved in the three species are highlighted and similar amino acid residues are shaded in gray. Transmembrane (Tm) regions are shown by upper brackets based on the crystal structure of *S. elongatus* PS I. The sidedness of the loop regions, the stroma and lumen (periplasm in *G. violaceus*), are shown by broken underlines and double underlines, respectively. The C-terminal extended sequence specific for *G. violaceus* is emphasized.

by the gene sequence. This extension showed similarity to the peptidoglycan-binding domain of eubacteria, as discussed below.

4. Discussion

4.1. Subunit composition

We identified nine subunits in the PS I complexes of *G. violaceus*. In our preparations, a trimer structure is stably formed (Fig. 1A) even if PsaI, PsaJ, and PsaK are absent in *G. violaceus*. This is consistent with a report that PsaL is essential for trimer formation in cyanobacteria [17] and which is supported by the crystal structure [7]. The crystal structure also showed that PsaJ and PsaK are localized in the periphery of the trimer structure, and that PsaI is localized in the attaching site of individual monomers [7]. It has been reported that PsaI and PsaM, which is adjacent to PsaI, maintain the structure of the PS I trimer in *Synechocystis* [18,19]. Therefore, we presumed that PsaM might replace the function of PsaI in *G. violaceus*. PsaZ, a novel, specific subunit in *G. violaceus*, might function to stabilize the whole structure when PsaI, PsaJ and PsaK are missing. To examine this possibility, further experiments will be necessary.

Mangels et al. [20] have analyzed the subunit composition of *G. violaceus* PS I. They detected a large number of unidentified polypeptides in their preparation, including the light-harvesting complex (LHC) identified by an antibody raised against the LHC of *Cyclotella cryptica*, which is a diatom. However, our study did not find a gene corresponding to the LHC in *G. violaceus* [3]; as well the LHC was not detected in the PS I complexes (Fig. 1B). These disagreements between studies may be caused by differences in the isolation and detection methods.

4.2. Possible function of the C-terminal extension in PsaB

We found that the PsaB extension shows a significant homology to the peptidoglycan-binding domains in several eubacteria. A database search showed that the C-terminal extended sequence was homologous to the C-terminal sequences of RmpM (reduction-modifiable protein M) in *Neisseria meningitidis* [21] (Fig. 3), OmpA (outer membrane protein A) in *Escherichia coli* [22] and OprF (*Pseudomonas aeruginosa* protein F) in *P. aeruginosa* [23]. Similar sequences were also found in other bacteria, including Gram-positive and Gram-negative bacteria [22].

A function of the C-terminal domain of RmpM is believed to be peptidoglycan binding by the crystal structure [21]. Gizot and Buchanan [21] reported the crystal structure of the C-terminal domain (from residues 68 to 207) of RmpM in 1.9 Å resolution, as well as the mechanism of binding to the peptidoglycan. Four residues were postulated to interact directly and six residues indirectly with peptidoglycan (Fig. 3). Computer simulation indicated that three out of the four residues (one tyrosine and two arginine residues) could form hydrogen bonds with the disaccharide, *N*-acetylglucosamine and *N*-acetylmuramic acid, which are essential elements of peptidoglycan [21]. We predicted the secondary structure of the C-terminal extension of *G. violaceus* PsaB by the PSIPRED method [24]. It was shown that the secondary structure was very similar to that of RmpM (Fig. 3), suggesting that the whole structure of the C-terminal extension is similar to that of RmpM. Furthermore, the above-noted ten residues are completely conserved in *G. violaceus* PsaB. Based on the PS I trimer structure of *S. elongatus*, the C-terminal of PsaB faced the lumenal side of thylakoid membranes [7], implying that the C-terminal extension of PsaB faces the periplasm in *G. violaceus*. These results suggest that the C-terminal extension in *G. violaceus* PsaB binds to the peptidoglycan layer.

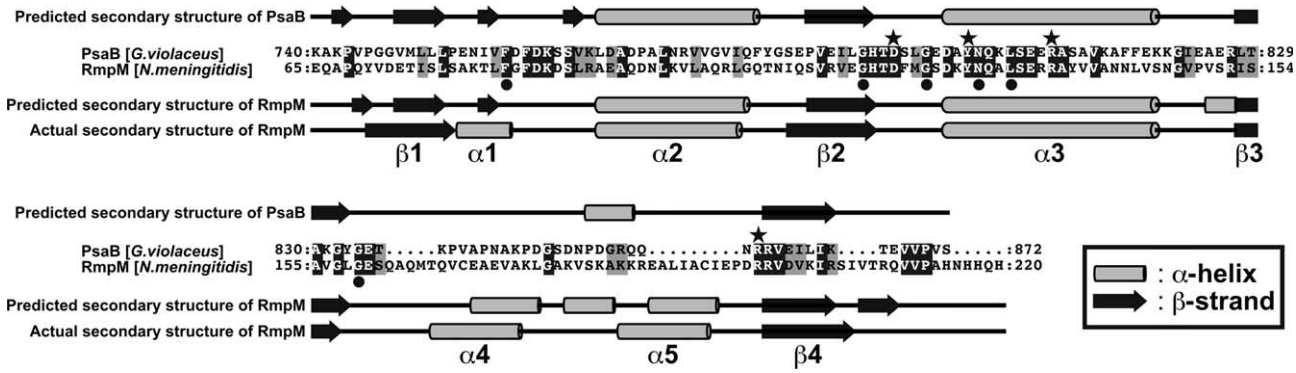

Fig. 3. Comparison of the C-terminal domains of *G. violaceus* PsaB and RmpM. The sequences for C-terminal domains of PsaB and RmpM are aligned. Amino acid residues that are conserved in the two species are highlighted and similar amino acid residues are shaded in gray. Asterisks and dots denote residues that may directly and indirectly interact with peptidoglycan, respectively. The secondary structures of PsaB and RmpM predicted by the PSIPRED method and actual secondary structure of RmpM are shown.

Reaction center subunits possessing the peptidoglycan-binding domain have not been reported in cyanobacteria or photosynthetic bacteria. In *G. violaceus*, PsaA has a high homology with other cyanobacteria without any extension (data not shown), therefore the presence of *G. violaceus* PsaB possessing this domain is interesting. The existence of the gene *glr1828* in *G. violaceus* is noteworthy with regard to the C-terminal extension of PsaB. This gene encodes a probable outer membrane protein comprising 227 amino acid residues. The C-terminal region of *glr1828* (approximately 150 residues) has high homology with the C-terminal region of *psaB* (155 residues) based on the amino acid sequence as well as nucleotide sequence. This suggests that both have the same origin and that the C-terminal region of *glr1828* might fuse with *psaB* after gene duplication. This is the next issue to be studied in our laboratory.

Gloeobacter violaceus might keep partly ancestral photosystems as indicated by the smaller number of subunits in the PS I complexes. Small subunits (PsaI, PsaJ, PsaK and PsaX) might not have been present in an earlier stage of evolution, but could have been acquired afterwards. The origin and biological significance of the peptidoglycan-binding domain are enigmatic but interesting issues. *G. violaceus* is thus suitable for analyses of the development of photosystems.

Acknowledgments: We thank Mr. T. Sakawa, Yamaguchi University, for help in the early stage of this work, Dr. T. Shimada, Shimadzu Biotech, for aid in analysis of amino acid sequences, and Mr. K. Araki, Kyoto University for aid in sequence alignment. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan to MM (No. 15370021).

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