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Phycobilisome model with novel skeleton-like structures in a glaucocystophyte *Cyanophora paradoxa*☆

Mai Watanabe [a,1], Momoko Sato [a,1], Kumiko Kondo [a,b], Rei Narikawa [a], Masahiko Ikeuchi [a,*]

[a] *Department of Life Sciences (Biology), Graduate School of Arts and Science, University of Tokyo, Komaba, Meguro, Tokyo 153-8902, Japan*
[b] *RIKEN Plant Science Center, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan*

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ABSTRACT

Phycobilisome (PBS) is a photosynthetic antenna supercomplex consisting of a central core subcomplex with several peripheral rods radiating from the core. Subunit structure of PBS was studied in a glaucocystophyte *Cyanophora paradoxa* strain NIES 547. Subunit composition of PBS was identified by N-terminal sequencing and genes for the subunits were determined by homology search of databases. They included rod linker proteins CpcK1 and CpcK2, rod-core linker proteins CpcG1 and CpcG2, and core linker proteins ApcC1 and ApcC2. Subfractionation by native polyacrylamide gel electrophoresis provided evidence for novel subcomplexes (ApcE/CpcK1/CpcG2/ApcA/ApcB/CpcD and ApcE/CpcK2/CpcG1/ApcA/ApcB), which connect rod and core subcomplexes. These skeleton-like structures may serve as a scaffold of the whole PBS assembly. Different roles of ApcC1 and ApcC2 were also suggested. Based on these findings, structural models for PBS were proposed. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Artificial.

1. Introduction

Light-harvesting antenna is universally functioning in support of chlorophyll-based photosynthesis but is highly diversified in evolution, reflecting phylogeny and different habitats. Phycobilisome (PBS) is a phycobilin-based light-harvesting antenna complex in cyanobacteria, red algae and glaucocystophytes, while light-harvesting chlorophyll complexes (LHC) is a chlorophyll-based antenna that is widely conserved; chlorophyll *a*-type in red algae [1,2], chlorophyll *a*/*c* fucoxanthin-type in brown algae [3,4], and chlorophyll *a*/*b*-type in green plants [5]. Pcb/Isi is another line of the chlorophyll-based antenna, which is related to a photosystem (PS) II internal antenna CP43 but is totally unrelated to LHC [6,7]. Pcb/Isi has been found only within cyanobacteria. Generally, the photosynthetic antenna is rather tightly associated with either PSII or PSI to efficiently transfer light energy to the PS. However, the linear electron transfer from water to $NADP^+$ must be driven with two coordinated PS-antenna systems.

A glaucocystophyte *Cyanophora paradoxa* is the ancient eukaryotic alga that harbors unique chloroplasts called cyanelles. Cyanelles possess a thin peptidoglycan wall between outer and inner envelope membranes and carboxysome-like structure in stroma [8,9]. The major photosynthetic antenna of *C. paradoxa* is the water-soluble PBS, although it has not been extensively studied so far [10]. An LHC-like polypeptide was suggested immunologically [11], while no such genes have been detected in the complete cyanelle genome [12] or the cDNA database [13–15].

The typical PBS is composed of a core and several rods subcomplexes, which radiate from the central core subcomplex. Both core and rod subcomplexes consist of various phycobilin-binding proteins (PBPs) connected with several classes of colorless linker proteins. Phycocyanin (PC) is the major PBP of the rod and allophycocyanin (APC) is the major PBP of the core cylinders. The linker proteins are critical for organization of the PBS supercomplex. They are classified into at least five distinct groups; (i) a rod linker (L_R, CpcC in case of the PC rod) locating in the rod structures to connect each rod disks, (ii) a rod-core linker (L_{RC}, CpcG) locating at the rod-core connection site to connect the rods with the core, (iii) a core linker (L_C, ApcC) locating at the end of core cylinders, (iv) a rod-capping linker (CpcD) locating at the end of the rods, (v) a core-membrane linker (L_{CM}, ApcE) locating in the core to connect the core with thylakoid membrane. ApcE having two to four linker domains also help assembling the bundle of core cylinders in the core subcomplex [16]. Several structural models for the PBS assembly have been proposed [16,17]. However, the precise assembly mechanism remains elusive, since the PBS structure including those linker proteins cannot be determined.

Previously, we suggested that two distinct types of PBS exist in a unicellular cyanobacterium *Synechocystis* sp. PCC 6803. One is the

Abbreviations: APC, allophycocyanin; LHC, light-harvesting chlorophyll complexes; PAGE, polyacrylamide gel electrophoresis; PBPs, phycobilin-binding proteins; PBS, phycobilisome; PC, phycocyanin.

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* Corresponding author. Tel.: +81 3 5454 4375; fax: +81 3 5454 4337.
E-mail address: mikeuchi@bio.c.u-tokyo.ac.jp (M. Ikeuchi).

[1] These authors contributed equally to this work.

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conventional PBS, which consists of the core and rods. Another is a unique rod-type complex, which lacks the central core [18]. A variant of the "rod-core" linker protein (CpcG2) having C-terminal hydrophobic tail may play a key role for the association of this rod-type complex (CpcG2-PBS) with the membrane or the PSI complex [19,20]. Such specific binding seems to reflect unusual hydrophobic nature of the linker protein CpcG2. However, these hydrophobic CpcG proteins are highly diverged and it remains still unclear whether the heterogeneity of PBS is universal or not. Here, we focused on the major antenna PBS in the ancient alga *C. paradoxa* and studied components and substructures of the PBS complex biochemically. We found novel linker polypeptides and unexpected skeleton-like structures, and suggest a new organization model.

2. Materials and methods

2.1. Cell growth and isolation of cyanelles

Cyanophora paradoxa strain NIES 547 was obtained from National Institute for Environmental Studies, Tsukuba, Japan. The cells were grown with aeration in the C medium at 25 °C [2].

2.2. Isolation of intact PBS and subcomplexes

Cells were harvested by centrifugation at 2000×*g* for 10 min and washed twice with HEMS buffer (50 mM HEPES–NaOH, pH 7.5, 2 mM EGTA, 1 mM $MgCl_2$ and 0.5 M sucrose) and were suspended in the same buffer. Cells were disrupted osmotically by 10 times dilution with HEM buffer (50 mM HEPES–NaOH, pH 7.5, 2 mM EGTA and 1 mM $MgCl_2$), and cyanelles were collected by centrifugation at 2500×*g* for 10 min according to [21].

Intact PBS was isolated by the standard protocol [18], with a slight modification. Isolated cyanelles were suspended in 0.8 M potassium phosphate (pH 7.0) and treated with lysozyme at 1 mg/ml for 15 min at 15 °C. Unbroken cyanelles were removed by centrifugation at 2500×*g* for 5 min. The supernatant was treated with Triton X-100 (final 2%) for 30 min and centrifuged for 20 min at 20,000×*g*. The lower blue phase containing PBS was collected, layered onto 10–50% linear sucrose gradient in 0.8 M phosphate and centrifuged at 130,000×*g* for 14 h at 18 °C.

The intact PBS complex in 0.8 M phosphate was partially disassembled by dilution to 0.3 M phosphate (pH 7.0). After dilution and concentration by ultramembrane centrifugation (Centriprep, Millipore), PBS subcomplexes were fractionated by linear sucrose gradient centrifugation (10–20% sucrose, 0.3 M phosphate, pH 7.0) at 130,000×*g* for 14 h at 18 °C.

2.3. Native-PAGE and SDS-PAGE

Native-PAGE was performed as described previously [22] followed by 2D SDS-PAGE [23]. SDS-PAGE was done using a 16% (w/v) polyacrylamide or a 16 – 22% (w/v) linear gradient of polyacrylamide gel containing 7.5 M urea [24] and proteins were stained with Coomassie brilliant blue R-250 or silver [25]. Samples of the same volume were loaded on the gel. Zinc-induced fluorescence was monitored by FMBIO II (Takara, Japan) after incubation with 10 mM zinc acetate for 30 min. Alternatively, SDS-gel was blotted to Immobilon-PSQ membrane (Millipore, USA) and subjected to N-terminal sequencing by the Edman degradation method (model PPSQ-21, Shimadzu, Japan).

2.4. Absorption and fluorescence spectra

Absorption spectra of PBPs were measured at room temperature using a spectrophotometer (model U3500, Hitachi, Japan) with an end-on photomultiplier. Fluorescence emission spectra of PBPs at 77 K were recorded upon excitation at 600 nm using a spectrofluorometer (model RF-5300PC, Shimadzu, Japan).

3. Results

3.1. Isolation of intact PBS

PBS was fractionated by a conventional extraction method of 0.8 M phosphate buffer with 2% Triton X-100 and the linear sucrose gradient centrifugation (Fig. 1). The intact PBS was obtained at fraction 4, while unassembled components were recovered at fractions 1 to 3. The fraction 1 peaking at 650 nm was enriched in APC (Fig. 1B). The fractions 2 and 3 contained mostly PC. The major fluorescence at 678 nm of fraction 4 is indicative of the intact PBS complex. Two fluorescence peaks at 678 and 655–660 nm in fractions 1, 2 and 3 suggested that not only PC and typical APC subunits but also long wavelength emitters such as ApcE and APC-B were fractionated together (Fig. 1C). Protein composition of the intact PBS was studied by two systems of SDS-PAGE (Fig. 2). A unique band at 53 kDa was observed in addition to the normal profile of linker proteins and PBPs.

3.2. Subunits of PBS

We determined N-terminal amino acid sequences by the Edman sequencing (Table 1). These sequences were then assigned to the

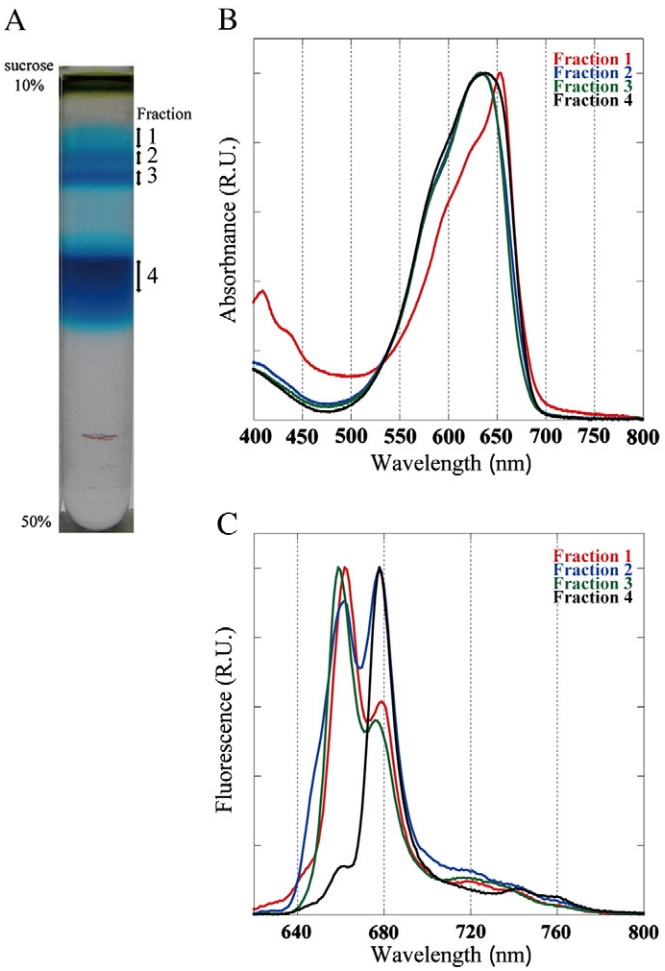

Fig. 1. Fractionation of intact PBS. A, separation profile; B, room temperature absorption spectra; C, 77 K fluorescence spectra with excitation at 600 nm.

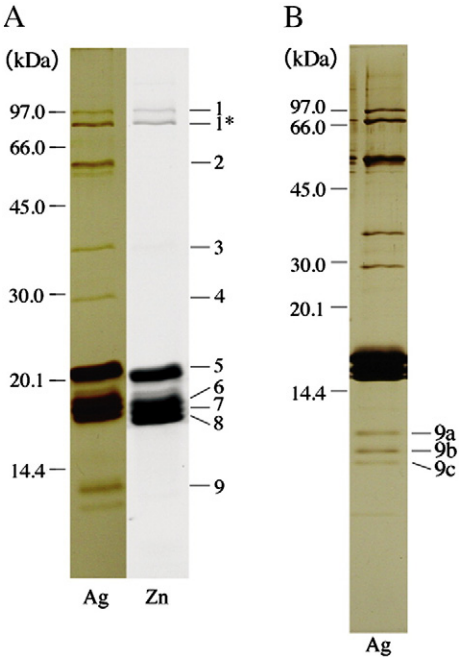

Fig. 2. SDS-PAGE of the intact PBS. A, 16% gel; B, 16–22% gel with 7.5 M urea. Proteins were stained with silver (Ag) or detected by zinc-induced fluorescence (Zn).

nucleotide sequences in the databases or in our on-going genome project. In summary, linker polypeptides at 53 kDa consisted of two proteins (CpcK1 and CpcK2), 37 kDa and 29 kDa bands were designated CpcG2 and CpcG1, respectively. APC linker proteins of 8 kDa and 10 kDa were designated ApcC1 and ApcC2, respectively. CpcD and several major phycobiliproteins (CpcA, CpcB, ApcA, ApcB and ApcE) were also detected but other minor PBPs (APC-B and ApcF) were not detected due to low abundance.

Protein sequences deduced from cDNA and genomic DNA are shown in Fig. 3. The complete sequences show that CpcK1 possesses a tandem repeat of the PC linker domain but no CpcD-like end-capping linker domain. Since the partial sequence of CpcK2 is homologous to CpcK1, a similar tandem repeat may be presumed for CpcK2. CpcG1 has domain architecture of a typical rod-linker polypeptide. Although the C-terminus of CpcG2 is slightly truncated, the overall sequence of CpcG2 is very similar to CpcG1 except for additional 63 amino acids in the N-terminal extension in the mature CpcG2.

3.3. Subfractionation by sucrose gradient centrifugation

To gain insights into structural organization of PBS, the intact PBS complex was partially disassembled by dilution to 0.3 M phosphate and fractionated by sucrose gradient centrifugation (Fig. 4). The fraction 1 at the top contained APC and two linker polypeptides ApcC1/C2, The fractions 2 to 4 contained most subunits including ApcE but not ApcC1/C2. Consistently, the absorption spectra showed more or less depletion of APC in the fractions 2–4 compared with the fraction 1. This means that major APC subunits (ApcA/B) and core linker polypeptides (ApcC1/C2) were dissociated concomitantly, while the core-membrane linker ApcE was still associated with some ApcA/B and other components. The fraction 5 containing PC subunits (CpcA/B), CpcD and two closely migrated bands of CpcK1 and CpcK2 was recovered at the bottom, indicative of a peripheral PC rod equipped with end capping linker CpcD and rod linker CpcK1/2. This fact strongly suggests that CpcK1 and CpcK2 are not the rod-core linker but the rod linker, although CpcK1 and CpcK2 are more homologous to the rod-core linker CpcG than the rod linker CpcC protein family (see Discussion).

3.4. Subfractionation by native PAGE

Subcomplex organization was further studied by native PAGE. Native PAGE and 2D SDS-PAGE of the intact PBS revealed unusual large subcomplexes (bands 1 and 2 in Fig. 5) in addition to typical subcomplexes of APC and core linkers (bands 3 and 4) and PBP subcomplexes without linkers (bands 5 and 6). 2D PAGE showed that the band 1 consisted of ApcE, CpcK1, CpcG2, ApcA, ApcB and CpcD. The band 2 consisted of ApcE, CpcK2, CpcG1, ApcA and ApcB. These subcomplexes demonstrate that the linker proteins of rod and core are interconnected to each other to form a skeleton-like structure. It is also evident that there are two distinct subpopulations of rod-core linkers and rod linkers, which are connected to ApcE in the core and also to CpcD at the end of the rod at least in the band 1. Although these bands 1 and 2 still retained some APC subunits (ApcA and ApcB), the bulk ApcA and ApcB were recovered in bands 3 to 5: the band 3 consisted of ApcA, ApcB and ApcC2, the band 4 consisted of ApcA, ApcB and ApcC1, and the band 5 consisted of only ApcA and ApcB

Table 1
Identified N-terminal sequences and the corresponding genes or EST cluster. Since EST data from Bhattacharya laboratory were not clustered, presence of corresponding EST clones are shown as +. –, not detected.

Band in Fig. 2	Apparent (kDa)	Predicted (kDa)	N-terminal sequences	Protein	Locus	Draft genome (our Lab)	Cyanelle genome or EST (Loffelhardt Lab)	EST (Durnford Lab)	EST (Bhattacharya Lab)
1	100	100.3	–	ApcE	cyanelle	contig00002, contig00501	NP_043160	–	–
1*	88	88.1	FNTGGADITPAGFKPINVSR						
2	53	53.2	SIPIIEFAPKTTNTRVAGFG	CpcK1	nucleus	contig03769, contig06606	CPL00000389	–	–
2	53		AVPLLAYKPKTTNTRVAGIG	CpcK2	nucleus	contig12959	CPL00000346	CDL00001908	+
3	37		KLKFSKTTGSAKDIKATGRA	CpcG2	nucleus	contig04538	CPL00000370	–	–
4	29	28.6	ALPLIAYKPNTQNNRVAAIG	CpcG1	nucleus	contig03519	CPL00000099	CDL00000055	+
5	19.7	17.6	MLDAFGKVVAQADARGEFIS	CpcB	cyanelle	contig00054	NP_043279	–	–
6	18	17.1	SIVTKSIVNADAEARYLSP	ApcA	cyanelle	contig00501	NP_043159	–	–
7	17.7	17.4	MKTPITEAIATADNQGRFLS	CpcA	cyanelle	contig00054	NP_043158	–	–
8	17.4	17.1	MQDAITAVINAADVQGKYLD	ApcB	cyanelle	contig00501	NP_043280	–	–
9a	10	10	DTSGSAIKRITNPSSSPIAG	ApcC2	nucleus	–	CPL00000360	CDL00000472	+
9b	9	9	EKEEAPSKSLVSPVVFAGKL	CpcD	nucleus	contig03571	CPE00000489	CDL00001225	+
9c	8	8	SARKFKVTVV	ApcC1	nucleus	contig09076	CPL00000292	CDL00001972	+

*proteolytic product.

A

CpcK1 CPL00000389

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  1 MAAFATPIAVAASRVAPVATATKLSSSFAGARLRSAPAAQKATTVFEVSASIPTIKFAPKTTNTRVAGFGTSTTDSQSEGYTVDQAFRTPSEIQEAIVAC 100
101 YRQIYGEHNIIAASKQATLESQLAQGTINVREFIKGLVASDFFLDSYFYTNSNYRFVEIVVERVLGRAVYNQAEKIAWSIVIANKGVEGFVDALTSSEEY 200
201 IENFGDYRVPYQRSRRLAGRAIGETPFNLKNPRYSYYYRSKYTNYTNPLARPANYRKKTSGTGADFYDTPFERTWATPVTQKSSRDFEVVAKTVEGEGAT 300
301 AIGLPTNGIPMINFAPSTNNWRVKTIGERITTDSPKTANTADQPTAADLDELIYQAYNQIFNVHHLKYNRNKQLETRVRDGRISVKQFIRGLITSPAFLE 400
401 STFETNNNYRFVQIVVQRLLGRDTYNQAEQIAWSIVIANKGVEGFIDAVLDSDEYNTNFGDCTVPYQRARVLAGRATGDRPFLLDTPRYSDWYRQRLGFS 500
501 MFNNVTPRRASPPSFRPKNGDPAQWVESAKRLSIEYQKAPLFLVDSGFLAKVPRRN* 556
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CpcK2 CDL00001908

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  1 EMAFVTPVPAVAGRALASSFAGEKCSTFKGTAVAAKAAAPAAPSTTFEVKAAVPLLAYKPKTTNTRVASIGENLSIDKPARYNIDYDYDNGEKEEVIAAA 100
101 YRQIFGEHQTIAYSRQVTLDSQLRNGQISVRQFIRGLVLSEQFLNQNFETATNYRFVEKLVQRVLGRDVYSNLEKIAWSIVIANKGVEGFVDELLASEEY 200
201 LSNFGENTVPFQRNRVLAGRATGDKPFNIKNARV 234
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CpcG1 CDL00000055, contig03519

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  1 MAFVTAAPVLASAGVVAPTTKVLKSDFVSTGIRKELSGAAVAQPAASFQAARTFEVKAALPLIAYKPNCQNNRVAAIGQAISDEKAKDFSIEKTTNPDDL 100
101 DEIXYAAYRQIFSEHQSIKYTKQPALESQFRNGEITVKDFIFGLVTSNAFLELNFQPNNNYRFAKIVIQRVLGRDVYSELECIAWSIVIVNEGVAGFVQA 200
201 LQDSQEYMENFGDFVVPFQRDRVLSGRAIGQTPFRLATPRYGTDWARKLGFPRAAETAIAKRAASTRVAAEEGSPFKWITVAKSLGYSIKQLRYVLPEID 300
301 GDYLKKVPSKKN* 312
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CpcG2 contig04538

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  1 SPAFHRVHLFALAVFLTIGRRSEHLGKSVQAAPAAQQASFEISAKLKFSKTTGSAKDIKATGRAGKSTSVVRRFVSVAQKNSIDFELASLTTEGKPAKVI 100
101 GVATTGASIPLLKYAPTCQNSRVAAGFERITTDAPTRFNTAEQPTVAERDAMIWAAYRQIMNVHHLAADRQVELETRVRDGRITVKEFVKGLATSPAFLA 200
201 TNFDTNSNYRSFVEIAVQRILGRDVYSQAEKIAWSIVIANKGVHGFIDALVESDEYKANFGEHTVPFQRQRVLAGRATGETPFLMKTPRYGADYRAKQPF 300
301 SVFSSVAPRRAFSASGSQSAGNPLAWLNVAKSVAVQPNKQPSWAVSQGWESKVPKRA 357
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B

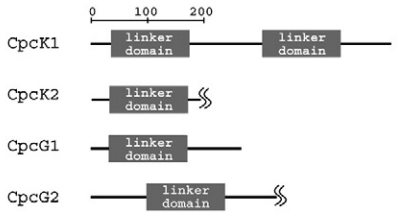

Fig. 3. Amino acid sequences (A) and domain architecture (B) of CpcK1, CpcK2, CpcG1 and CpcG2. N-terminal sequences are underlined. The PC linker domains are shaded. Note that *cpcK1* and *cpcG1* genes are complete but the others are deduced from partial nucleotide sequences (Table 1). Asterisks denote the stop codon. Domain architecture is shown for mature proteins.

but no core linkers. Since the core linker ApcC1 or ApcC2 was not recovered in the band 1 or band 2, we can conclude that the core subcomplex was separated into peripheral part carrying the core linkers and the central part carrying ApcE.

To get better resolution of the peripheral parts of the core subcomplex, the APC fraction of the sucrose gradient (fraction 1 in Fig. 4) was subjected to the native PAGE. Since this APC fraction was largely depleted of the PC, three APC subfractions were clearly resolved by native PAGE (Fig. 6). These three bands from 1 to 3 corresponded to the bands 3, 4 and 5 in Fig. 5, respectively. The distribution of the core linker polypeptides in these bands was identical to that in Fig. 5. Each blue band from the native PAGE in Fig. 6 was excised and the 77 K fluorescence spectra were measured. The band 1 that consisted of ApcA, ApcB and ApcC2 emitted at 680 nm, indicative of the presence of the long wavelength-emitting minor APC component, APC-B [26,27]. The band 2 that consisted of ApcA, ApcB and ApcC1 emitted at 661 nm. These results indicate that APC-B is specifically associated with the core linker ApcC2.

4. Discussion

In this study, we found novel rod linker proteins, CpcK1 and CpcK2, rod-core linker proteins, CpcG1 and CpcG2, and core linker proteins, ApcC1 and ApcC2, in PBS of the glaucocystophyte *Cyanophora paradoxa* NIES 547. Native PAGE of the intact PBS provided firm evidence for unique skeleton-like substructure of PBS such as ApcE/CpcK1/CpcG2/ApcA/ApcB/CpcD and ApcE/CpcK2/CpcG1/ApcA/ApcB, which connect rod and core subcomplexes.

4.1. Novel rod linker CpcK1 and CpcK2

Rod linker proteins, CpcK1 and CpcK2, possess two tandemly repeated linker domains but no CpcD-like rod cap linker domain. Generally, the typical rod linker protein CpcC having the PC linker domain and the CpcD-like domain connects the PC disks to form the PC rod [16]. However, such CpcC is missing in PBS or the database of *C. paradoxa.* In literature, unusual rod linker proteins having tandem repeats of the linker domain have been reported in several cyanobacteria. The unusual PC rod linker CpcJ having three tandem repeats of the PC linker domains was suggested to connect three PC rods to make a bundle, which is peculiar to the bundle-like shape PBS with six rods in a primitive cyanobacterium *Gloeobacter violaceus* [28]. On the other hand, a PE rod linker protein (MpeD) having two tandem repeats of phycoerythrin (PE) linker domains was identified in a marine cyanobacterium *Synechococcus* sp. WH 8102 [29]. This protein was proposed to connect two different PE rods in sequence. In *C. paradoxa*, there is only one type of PC disk (this study) and morphology of PBS is a typical hemidiscoidal shape [10]. Thus, we presume that the two linker domains of CpcK1/K2 connect two PC disks to elongate the PC rod, which may become somehow similar to the typical PC rod without the typical linker protein CpcC.

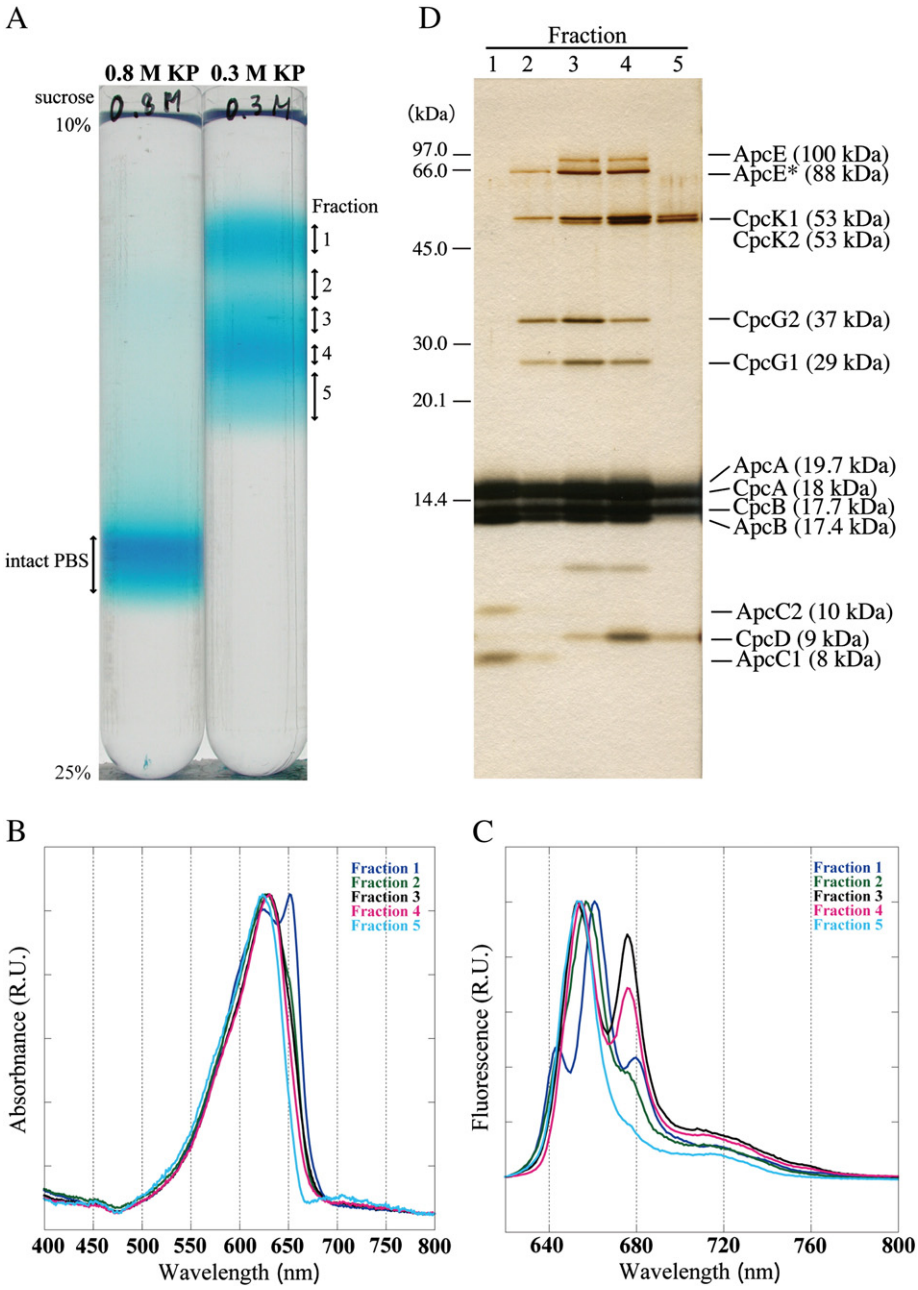

Fig. 4. Subfractionation of PBS subcomplexes. A, separation profile of the intact and disassembled PBS. Disassembled PBS in 0.3 M phosphate buffer was fractionated by 10–25% linear sucrose gradient containing 0.3 M phosphate. The intact PBS before dilution was fractionated by 10–25% sucrose containing 0.8 M phosphate. B, room temperature absorption spectra; C, 77 K fluorescence spectra with excitation at 600 nm; D, SDS-PAGE of 16–22% gel with 7.5 M urea. Proteins were stained with silver.

4.2. PBS model and novel skeleton-like structure

Native PAGE is powerful to separate subcomplexes as shown in Figs. 5 and 6. There are some reports to describe native PAGE of PBS [22]. However, to our knowledge, this is the first report to provide firm evidence for the unique skeleton-like subcomplexes. In *C. paradoxa*, the PC rods are assembled by the tandem linker proteins (CpcK1 and CpcK2), which are larger than the typical rod linker protein CpcC having a PC linker domain and a smaller CpcD-like end capping linker domain. This may be the reason why the skeleton-like structure of *C. paradoxa* was maintained after fractionation by native PAGE.

Here, we propose a model of PBS for *C. paradoxa* (Fig. 7). Morphology of PBS in *C. paradoxa* is just a typical hemidiscoidal shape [10], although a normal rod linker CpcC is absent (Figs. 2 and 3). Instead of CpcC, we propose that CpcK1 and CpcK2 having tandemly repeated PC linker domains interconnect two PC hexamer disks in the rod. Since two distinct subcomplexes (ApcE/CpcK1/CpcG2/ApcA/ApcB/CpcD and ApcE/CpcK2/CpcG1/ApcA/ApcB) were separately isolated by native PAGE, we assume two similar PBS models with distinct linker subunits (Fig. 7).

Here, we propose that PBS supercomplex may be assembled based on the skeleton-like structure (Fig. 7). It has been believed that the rod linker protein CpcC interconnects PC hexameric disks to form the PC rod (interlocking model) [16,30], while crystallographic study of the PC rod carrying CpcC did not confirm the model [17]. Recently the crystal structure of the N-terminal linker domain of CpcC supported the interlocking model [31]. In the classic work of [32], the core-membrane linker ApcE having multiple PC-linker domains connects several core cylinders. However, any further assembly

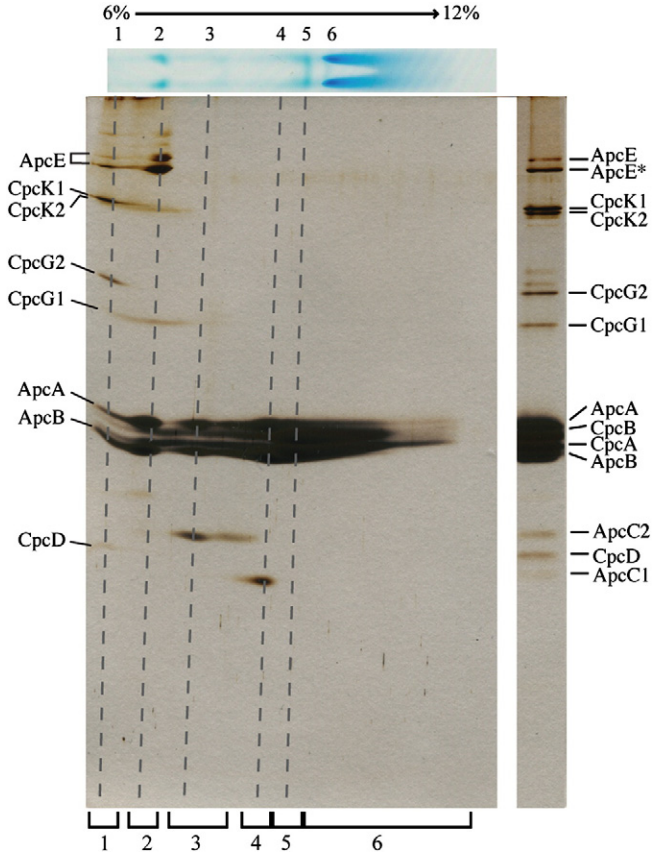

Fig. 5. Native PAGE and two-dimensional SDS-PAGE of the intact PBS. The reference profile of one-dimensional SDS-PAGE (16–22% gel with 7.5 M urea) is shown on the right. Proteins were stained with silver.

mechanism has not been proposed or suggested by experimental data. The skeleton-like structure isolated from *C. paradox* now allowed us to extend the model to the skeleton-like scaffold for assembly of the whole PBS supercomplex. It should be noted that the skeleton-like structure still retained some APC proteins. In Fig. 7, we included some APC for docking of CpcG-PC rods.

4.3. Antenna of *C. paradoxa*

According to the cDNA databases, LHC-like chlorophyll-based antenna may not be present in *C. paradoxa* like in cyanobacteria. On the other hand, LHC associated with PSI has been found in several red algae such as *Porphyridium cruentum* [33] and *Cyanidioschyzon merolae* [2], even though the abundant PBS serves as an antenna mainly for PSII. Similarly, some cyanobacteria such as *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120 possess a PBS subpopulation that possesses a variant of the "rod-core" linker protein having a C-terminal hydrophobic tail [18]. It is expected that the hydrophobic tail may directly bind to the stromal surface of the PSI complex. In *C. paradoxa*, two distinct CpcG copies were detected, but the C-terminal part of CpcG2 was not completely determined. Further study of PBS is needed for thorough understanding of the antenna system in *C. paradoxa*.

4.4. Phylogeny

In general, PC linker domains, which fill the central cavity of the hexameric disks of PBPs, are separated into two groups: the rod-core linker CpcG group and the rod linker CpcC group. The cluster analysis of these linker domains revealed that the linker domains of CpcG1/G2 and CpcK1/K2 of *C. paradoxa* are closely related to each other and belong to the CpcG group (Fig. 8). It is, thus, intriguing

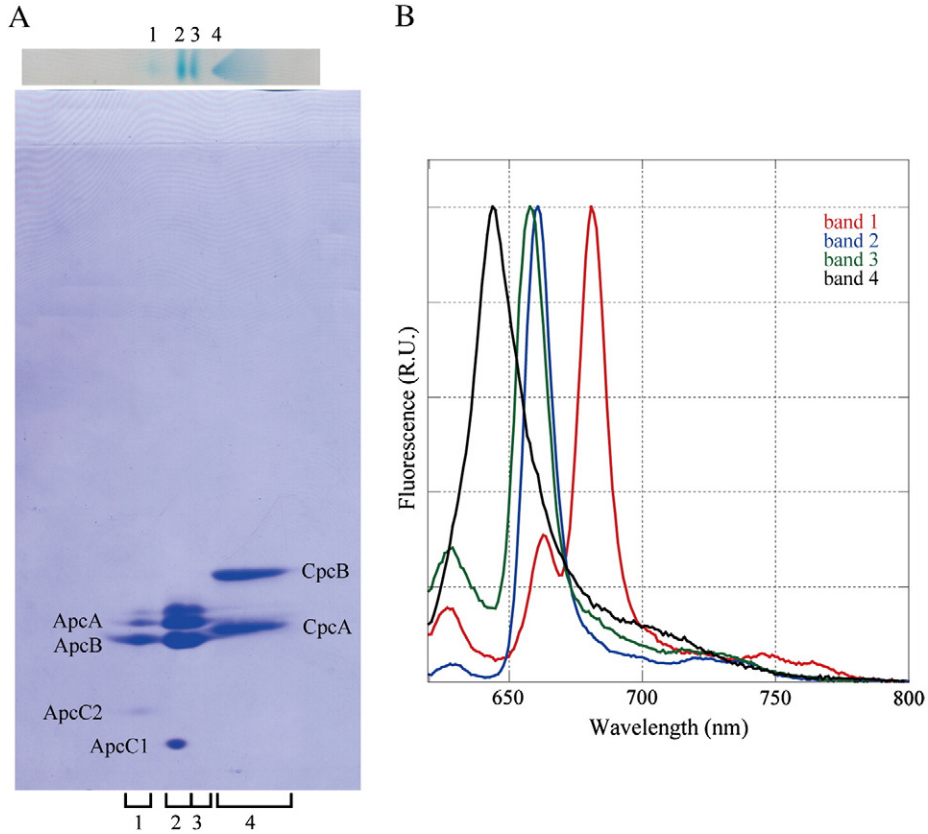

Fig. 6. Native PAGE fractionation of APC. A, native PAGE and two-dimensional SDS-PAGE (16% gel), stained with Coomassie brilliant blue; B, 77 K fluorescence spectra of blue bands in the native PAGE. Excitation at 600 nm. The APC fraction (Fig. 4, fraction 1) was analyzed.

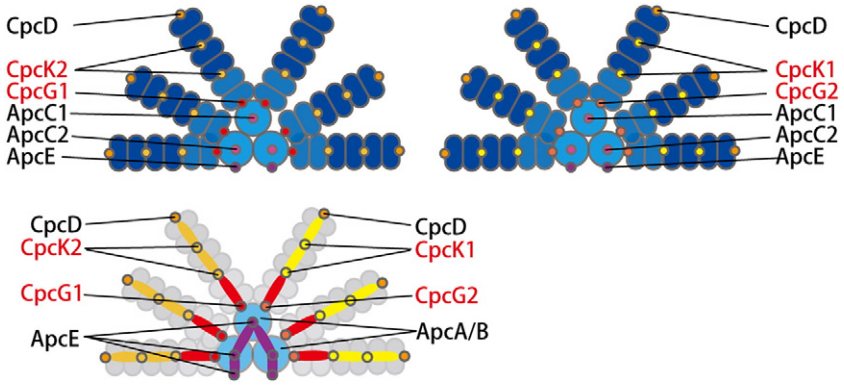

Fig. 7. Structural model of PBS and the skeleton-like structure. Note that two ApcE copies are shown in the skeleton-like structure. Subunits with gray color were not recovered in this structure.

that CpcK1 (and possibly CpcK2, too) is a unique rod linker protein having two linker domains of the CpcG group. Two PC linker domains of CpcK1 are deeply diverged from each other, suggestive of their different roles such as directional connection of the PC disks to the core subcomplex via the rod-core linker CpcG2 (Fig. 7).

PBS is conserved in glaucocystophyte and red algae. According to the phylogenetic tree of the linker domains, CpcG of red algae was closely branched from the hydrophobic variant of CpcG of certain cyanobacteria such as *Synechocystis*. Red algae also harbor the typical rod linker CpcC (e.g. CMP166C of *Cyanidioschyzon* in Fig. 8). These facts

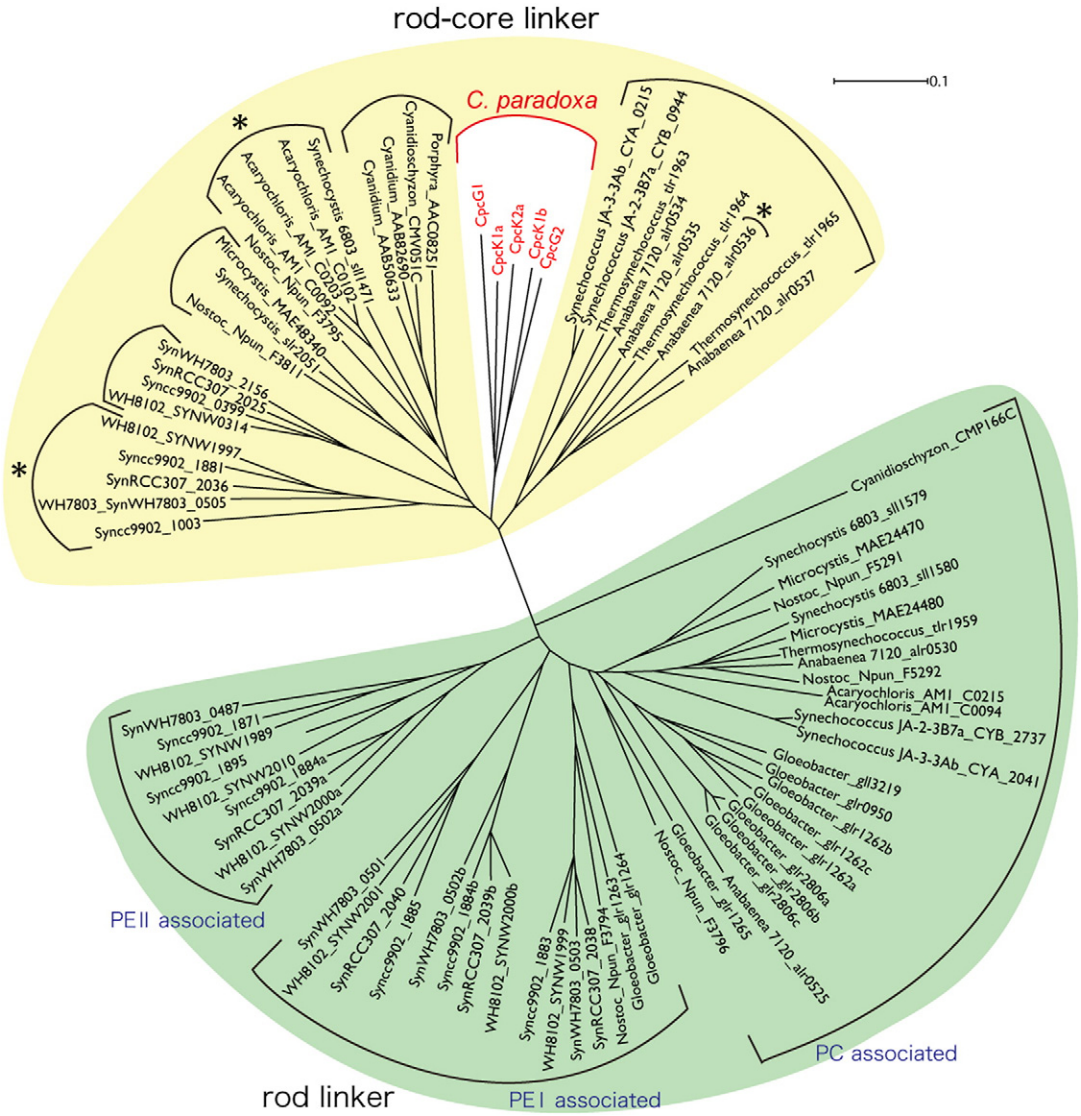

Fig. 8. Phylogenetic analysis of linker domains of rod linker and "rod-core" linker proteins. Gene ID and their groups with asterisk in the "rod-core" linker proteins possess a hydrophobic domain at the C-terminus.

suggest that the red algal PBS is more related to the cyanobacterial one than that of *C. paradoxa*.

4.5. Strains of *C. paradoxa*

There are three public cDNA Databases of *C. paradoxa* from Durnford laboratory (strain unspecified) [13], Loffelhardt laboratory (strain LB555 UTEX) [14] and a genome project of Bhattacharya laboratory (strain CCMP 329) [15]. Genome project of *C. paradoxa* strain NIES 547 is also in progress in our laboratory. Although origin of these strains must be the same Pringsheim strain, sequences from the Loffelhardt laboratory are slightly divergent from the other three including NIES 547. Since any database does not cover all the PBS genes at the moment, we listed genes and cDNA clones from them, which correspond to the protein sequences (Table 1).

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