The Membrane-Associated CpcG2-Phycobilisome in *Synechocystis*: A New Photosystem I Antenna[1][C][OA]

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The phycobilisome (PBS) is a supramolecular antenna complex required for photosynthesis in cyanobacteria and bilin-containing red algae. While the basic architecture of PBS is widely conserved, the phycobiliproteins, core structure and linker polypeptides, show significant diversity across different species. By contrast, we recently reported that the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 possesses two types of PBSs that differ in their interconnecting ''rod-core linker'' proteins (CpcG1 and CpcG2). CpcG1-PBS was found to be equivalent to conventional PBS, whereas CpcG2-PBS retains phycocyanin rods but is devoid of the central core. This study describes the functional analysis of CpcG1-PBS and CpcG2-PBS. Specific energy transfer from PBS to photosystems that was estimated for cells and thylakoid membranes based on low-temperature fluorescence showed that CpcG2-PBS transfers light energy preferentially to photosystem I (PSI) compared to CpcG1-PBS, although they are able to transfer to both photosystems. The preferential energy transfer was also supported by the increased photosystem stoichiometry (PSI/PSII) in the *cpcG2* disruptant. The *cpcG2* disruptant consistently showed retarded growth under weak PSII light, in which excitation of PSI is limited. Isolation of thylakoid membranes with high salt showed that CpcG2-PBS is tightly associated with the membrane, while CpcG1-PBS is partly released. CpcG2 is characterized by its C-terminal hydrophobic segment, which may anchor CpcG2-PBS to the thylakoid membrane or PSI complex. Further sequence analysis revealed that CpcG2-like proteins containing a C-terminal hydrophobic segment are widely distributed in many cyanobacteria.

Oxygenic phototrophic organisms possess two photosystems (PSI and PSII), which coordinate two independent photochemical reactions catalyzing the transfer of electrons from water to $NADP^+$. To collect light energy efficiently, a number of sophisticated antenna systems have evolved for each photosystem. Cyanobacteria, rhodophytes, and glaucocystophytes are unique in that they contain the phycobilisome (PBS), an extrinsic antenna protein supercomplex that harbors bilin chromophores and is positioned on the stromal surface of thylakoids, where it traps light in the blue to red region, filling the gap in chlorophyll absorption (Sidler, 1994; MacColl, 1998; Adir, 2005). Although the macromolecular structure and energy transfer properties of PBS have been extensively studied, a number of controversial areas remain, in particular, how the PBS interacts with photosystems and how energy transfer to photosystems is regulated.

PBS is a supercomplex that is composed of a core complex and multiple peripheral rod complexes. Typically, the core consists of two to five cylinders lying on the membrane with, in most cases, multiple rods radiating from the core to form a hemidiscoidal structure. The building units of the core cylinders and the peripheral rods are trimeric and hexameric discs, in which a monomer consists of a pair of related phycobiliproteins, such as phycoerythrins, phycoerythrocyanins, phycocyanins, and allophycocyanins. The discs are connected to each other via specific linker polypeptides to form peripheral rods or core cylinders. The basic architecture of PBS is widely conserved in cyanobacteria and bilin-containing algae, except prochlorophytes and cryptophytes. However, great diversity can be seen in the phycobiliproteins, core structure and linker polypeptides. (1) Various phycobiliproteins that absorb shorter wavelengths than allophycocyanin are found in the peripheral rods. Their chromophores (phycoerythrobilin, phycoviolobilin, phycourobilin, and phycocyanobilin) are covalently ligated to apobiliproteins and further assembled into a hexameric disc at the core-distal part of the peripheral rods. They efficiently transfer light energy to phycocyanins in the core-proximal part of the rods and then to allophycocyanins in the core of PBS. In many cyanobacterial species such as *Calothrix* and marine *Synechococcus*, the peripheral rods can be rearranged to cope with changes in the light environment, a process called chromatic acclimation (Kehoe and Gutu, 2006). (2) With regard to the core structure, bicylindrical, tricylindrical, and pentacylindrical types have been identified to date in

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cyanobacteria, while the tricylindrical type is predominantly found in red algae. These core structures have been shown to correspond to the number (two to four) of linker domains in the core anchor polypeptide ApcE (Capuano et al., 1991; Ducret et al., 1998). The linker domains of ApcE connect allophycocyanin discs of core cylinders and support assembly of the core structure, eventually controlling the entire PBS complex and physiological antenna size. (3) The diversity of linker polypeptides is important for the structural diversity of peripheral rods. The linker polypeptides, which carry the above-mentioned linker domain in the N-terminal part, are rod linkers and rod-core linkers. The linker domain of each rod linker specifically fills a central channel of hexameric discs of phycoerythrin, phycoerythrocyanin, and phycocyanin (Yu and Glazer, 1982; Liu et al., 2005). Likewise, a small additional domain in the C-terminal part, which resembles the rod-terminating linker, determines which partner is connected (de Lorimier et al., 1990). As a result, various hexameric discs are correctly connected to form the peripheral rod, which allows the directional energy transfer from a core-distal disc of higher energy to a core-proximal disc of lower energy. More important for diversity are the so called rod-core linker proteins, which consist of an N-terminal conserved linker domain and a small C-terminal nonconserved domain (Liu et al., 2005). This protein is essential for reconstitution of rods and allophycocyanins (Glick and Zilinskas, 1982). Disruption of the *cpcG* gene results in the disconnection of the peripheral rods from the central core in *Synechococcus* sp. PCC 7002 (Bryant, 1991). On the other hand, three or four distinct CpcG proteins have been detected in a pentacylindrical-type PBS in filamentous cyanobacteria *Anabaena* sp. PCC 7120 and *Mastigocladus laminosus* (Bryant et al., 1991; Glauser et al., 1992a, 1992b). Multiple CpcG proteins in a pentacylindrical core complex point to the existence of distinct docking sites on the complexed core (Ducret et al., 1996). A unique variant of the rod-core linker polypeptide (CpcJ) was detected in *Gloeobacter violaceus* sp. PCC 7421, which has a bundle-shaped PBS (Guglielmi et al., 1981; Koyama et al., 2006); it harbors three linker domains and is predicted to fix a bundle of three peripheral rods on to a core complex.

The diversity in PBSs described above has been found within specific organisms and mainly in cyanobacteria. It is very likely that such diversity has been selected in evolution to adapt to the various light environments these organisms inhabit. By contrast, we previously reported that two distinct forms of PBS are assembled via different CpcG proteins (CpcG1 and CpcG2) in the cyanobacterium *Synechocystis* sp. PCC 6803 (Kondo et al., 2005). Gene disruption showed that CpcG1-PBS is equivalent to the conventional PBS supercomplex having peripheral rods and central tricylindrical core, and retarded growth of the *cpcG1* disruptant under white light of medium intensity supported that CpcG1-PBS plays a major role in light harvesting. On the other hand, CpcG2-PBS retained phycocyanin rods but was devoid of a typical central core consisting of allophycocyanins. But at that time, we could not elucidate the physiological role of CpcG2-PBS, as the *cpcG2* disruptant grew as fast as the wild type under the experimental conditions. It was tentatively proposed that CpcG2-PBS transfers light energy via a yet-unidentified minor allophycocyanin to photosystems. To further elucidate the functional role of CpcG2-PBS, we analyzed the protein composition of CpcG2-PBS by high-resolution two-dimensional gel electrophoresis and immunodetection with newly prepared anti-ApcD and anti-ApcF. However, results suggested that CpcG2-PBS does not carry a minor allophycocyanin component for energy transfer (data not shown).

We also reported that CpcG2 is unique in that its mRNA (formerly *sll1471*) is preferentially expressed under PSII light conditions (Hihara et al., 2001a), which usually up-regulates accumulation of the PSI complex (Fujita, 1997). By contrast, the conventional *cpcG* copy (*cpcG1*, *slr2051*) or any other PBS component is not induced under PSII light. This led to the further exploration of the regulatory mechanism of *cpcG2* induction; a phytochrome-like photoreceptor gene (*sll1473-5*, *scaS*) and an OmpR-type transcriptional regulator gene (*slr1584*, *scaR*) are responsible for induction of *cpcG2* specific for PSII light (Katayama and Ikeuchi, 2006; M. Katayama, X.X. Geng, M. Kobayashi, F. Yano, M. Kanehisa, and M. Ikeuchi, unpublished data). This demonstrates a new type of chromatic acclimation in *Synechocystis* sp. PCC 6803.

In this communication, we present analysis that aims to elucidate the physiological role of CpcG2-PBS by measurement of fluorescence energy transfer to photosystems in cells and isolated thylakoids. The results show that the efficiency from CpcG2-PBS to PSI is approximately 3-fold higher than from CpcG1-PBS, although they are able to transfer to both photosystems. Immunoblot analysis further reveals that CpcG2-PBS is tightly associated with the thylakoid membrane, while CpcG1-PBS is loosely associated. These observations suggest that two types of PBSs with distinct properties function for optimal light harvesting in *Synechocystis*.

RESULTS

Energy Transfer from CpcG2-PBS to PSI in Cells

Apparent energy transfer efficiency from PBS to PSI can be estimated from 77 K fluorescence spectra of whole cells (Fig. 1A). The emission spectra obtained through excitation of phycocyanin show three major peaks: a broad peak at around 650 to 660 nm corresponding to emission of phycocyanin at 650 nm and allophycocyanin at 665 nm, a broad peak at around 690 nm corresponding to emission of ApcE (680 nm) and PSII (approximately 685 nm and 690–695 nm), and a peak at approximately 720 nm from PSI (Su et al., 1992; Shen et al., 1993). Clearly, CpcG2-PBS as well as

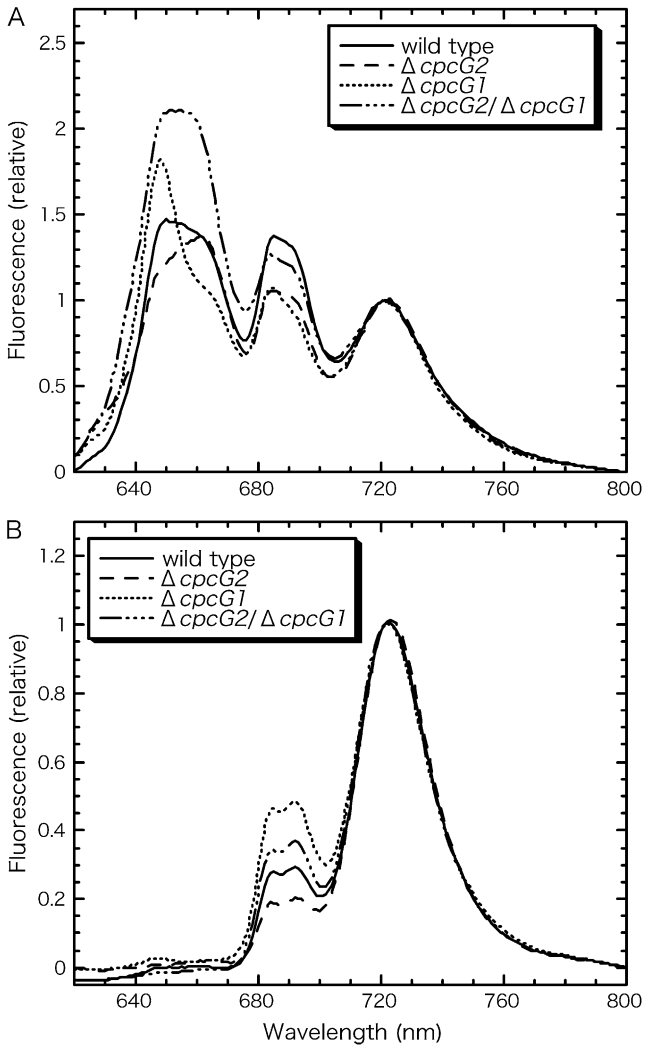

Figure 1. 77 K fluorescence emission spectra of whole cells. Excitation is at 600 nm (A) or 435 nm (B). Cells were resuspended in growth medium at a chlorophyll concentration of 5 μg mL^{-1} and dark adapted for 10 min prior to measurements. Each spectrum was normalized to the PSI fluorescence peak at 721 nm.

CpcG1-PBS in the mutants transfer light energy more or less to both photosystems. The apparent energy transfer from PBS to PSI relative to the energy transfer from PBS to PSII was deduced as the peak ratio of fluorescence emission at 721 nm (F_{PSI}) to fluorescence emission at 692 nm (F_{PSII}), designated F_{PSI}/F_{PSII} (Ex λ = 600 nm; Table I, row A). This value also reflects the photosystem stoichiometry, which was found to be conversely affected by disruption of *cpcG1* or *cpcG2* (Fig. 1B). Therefore, the values of F_{PSI}/F_{PSII} (Ex λ = 600 nm) should be corrected for photosystem stoichiometry (Table I, row B; F_{PSI}/F_{PSII} [Ex λ = 435 nm]). The corrected values thus obtained were high in the *cpcG1* disruptant but low in the *cpcG2* disruptant (Table I, row C). As seen in four independent experiments, the results were reproducible. The results showed that CpcG2-PBS in the *cpcG1* disruptant transfers energy to PSI 3.1-fold (= 0.630/0.202) more efficiently than CpcG1-PBS in the *cpcG2* disruptant, although they are able to transfer to both photosystems. In other words, the conventional CpcG1-PBS transfers energy to PSII more efficiently than CpcG2-PBS. It is also of note that the corrected value of the wild type is slightly lower than the *cpcG2* disruptant, suggesting that both CpcG1-PBS and CpcG2-PBS are active in the wild-type cells. Also shown is the value of the *cpcG2/cpcG1* double mutant, which was found to occur between that of the two single mutants. This again supports our conclusion, although the double mutant may contain some free phycocyanins and allophycocyanins (Kondo et al., 2005).

Generally, photosystem stoichiometry PSI/PSII is redox regulated, and prolonged excess excitation of PSII versus PSI induces a higher photosystem stoichiometry PSI/PSII and vice versa (Fujita, 1997). So, the higher photosystem stoichiometry PSI/PSII in the *cpcG2* disruptant (Fig. 1B) is in itself quite a good indication that the CpcG2-PBS is predominantly a PSI antenna. It is very reasonable that the opposite effect was seen in the *cpcG1* disruptant (Fig. 1B).

Association to the Thylakoid Membrane of CpcG2-PBS

To obtain insights into the mechanism of photosystem-specific energy transfer, we isolated the thylakoid-associated PBS. Cells were broken in the presence of 0.8 M potassium phosphate buffer (pH 7.0) at room temperature, allowing the structural integrity of the PBS supercomplex to be maintained (Gantt et al., 1979). The cell extracts were fractionated into the low-speed (20,000*g*) precipitate (P1) containing the thylakoid membranes, the high-speed (100,000*g*) precipitate (P2) containing small vesicles and high-molecular-mass particles, and the high-speed supernatant (S2) containing soluble proteins. Fluorescence detection showed similar recovery of phycobiliproteins in fraction P2 between the wild type and the *cpcG2* disruptant (Fig. 2A). Consistently, Coomassie blue staining confirmed the presence of all components of the conventional PBS but near absence of thylakoid proteins or soluble proteins in fraction P2 of the wild type and *cpcG2* disruptant (data not shown). This means that the conventional PBS supercomplex was detached from the membranes. By contrast, almost no phycobiliproteins of the *cpcG1* disruptant were recovered in fraction P2 (Fig. 2A) but were predominantly recovered in fraction P1. Immunoblot analysis further confirmed that CpcG2 was also predominantly recovered from fraction P1 in the *cpcG1* disruptant (Fig. 2B). These results strongly suggest that CpcG2-PBS is tightly associated with the thylakoid membrane. The S2 fraction from the wild type and the *cpcG1* disruptant contained phycocyanins but very little CpcG2 (Fig. 2, A and B). This may suggest that CpcG2 itself has an intrinsic affinity with the membranes. It is assumed that, as discussed later, CpcG2-PBS may bind specifically to the membrane, possibly with the aid of the C-terminal hydrophobic region.

Table I. *Energy transfer from phycocyanin to PSI/PSII in cells*

Row A, Apparent energy transfer from phycocyanin to PSI relative to PSII is estimated as the fluorescence intensity ratio as F_{PSI}/F_{PSII} excited at 600 nm. Row B, Photosystem stoichiometry is represented as the fluorescence ratio of PSI (F_{PSI}) to PSII (F_{PSII}) upon excitation of chlorophyll at 435 nm. A fluorescence emission at 721 nm is designated as F_{PSI}, and an emission at 692 nm is designated as F_{PSII}. Four independent experiments were performed and averages along with SDS are shown.

	Wild Type	Δ*cpcG2*	Δ*cpcG1*	Δ*cpcG2*/Δ*cpcG1*
(A) F_{PSI}/F_{PSII} excitation at 600 nm	0.72 ± 0.041	0.92 ± 0.063	0.86 ± 0.16	0.81 ± 0.072
(B) F_{PSI}/F_{PSII} excitation at 435 nm	3.00 ± 0.37	4.54 ± 0.40	1.40 ± 0.45	2.49 ± 0.30
(C) = A/B	0.243 ± 0.023	0.202 ± 0.012[a]	0.630 ± 0.069[a]	0.325 ± 0.018

[a]The difference is significant (*t* test, $P = 0.0009$).

CpcG1 was recovered in equal amounts from both the membrane fraction P1 and the particulate fraction P2 in the *cpcG2* disruptant as well as the wild type (Fig. 2B). This is in agreement with the notion that the conventional CpcG1-PBS is not associated with the membrane as tightly as CpcG2-PBS. It is also of note that ApcE was retained in fraction P1 from the *cpcG2*/*cpcG1* double mutant. This is consistent with the view that ApcE itself has an affinity with the membrane to dock CpcG1-PBS, although the docking domain has not yet been specified (Capuano et al., 1991; Ajlani and Vernotte, 1998). Finally, it is clear that no phycocyanins (CpcA and CpcB) were associated with thylakoids in the absence of both CpcG1 and CpcG2.

Energy Transfer from Membrane-Associated CpcG2-PBS to PSI

We estimated apparent energy transfer efficiency from the membrane-associated PBS to PSI from 77 K fluorescence spectra of fraction P1. The energy transfer from PBS to PSI relative to that from PBS to PSII could not be determined as in cells because the isolated thylakoids showed prominent fluorescence of PBS at 680 nm, which masked the PSII fluorescence at 692 nm (data not shown). Instead, the energy transfer from PBS to PSI was represented as excitation peak of phycocyanin at 618 nm when PSI fluorescence was monitored at 718 nm and the excitation peak of phycocyanin was normalized to the excitation peak of chlorophyll at 673 nm (Fig. 3A; Table II, row A). These values were then corrected for phycocyanin content relative to chlorophyll (Table II, row B), which was monitored by absorption spectra (Fig. 3B), and corrected for F_{PSI}/F_{PSII} (Ex λ = 435 nm; Fig. 3C), representing photosystem stoichiometry (Table II, row C). The values thus obtained were high in the *cpcG1* disruptant but low in the *cpcG2* disruptant (Table II, row D). This suggests that CpcG2-PBS transfers energy to PSI 2.7-fold more efficiently than CpcG1-PBS, which is consistent with the value estimated from cell-fluorescence spectra (Table I).

Comparison of CpcG1 and CpcG2 Amino Acid Sequences

Both CpcG1 and CpcG2 possess a conserved "linker" domain in their N-terminal 180 residues, while the remaining C-terminal part of CpcG2 shows little homology to that of CpcG1 (Fig. 4A). The hydropathy plot clearly shows that CpcG2 has a hydrophobic segment of at least 25 amino acid residues within the C-terminal region, which is absent in CpcG1 (Fig. 4B). It has been suggested that the N-terminal region of CpcG1 is buried within the hexameric phycocyanin disc of the rod and the C-terminal region protrudes to connect the allophycocyanin core (Liu et al., 2005). By contrast, we reported that CpcG2-PBS consists of phycocyanin rods but no detectable core polypeptides (Kondo et al., 2005). In light of this data, we propose that the C-terminal hydrophobic segment of CpcG2 interacts directly with the thylakoid membrane or the PSI complex.

In agreement with this, we found that CpcG2 was preferentially recovered in the hydrophobic phase when PBS proteins were extracted by the conventional protocol using 2% Triton X-100 and 0.8 M potassium phosphate buffer (Yamanaka et al., 1978; Gantt et al., 1979). Since these two solutions are not freely miscible, the extracts were separated into two liquid phases: the upper green Triton X-100 layer containing chlorophyll and hydrophobic proteins, and the lower blue aqueous layer containing most of the phycobiliproteins. By western-blotting analysis, we found that more CpcG2 was recovered in the upper layer than the lower layer,

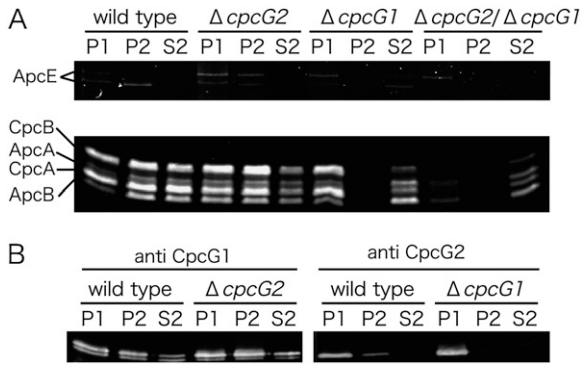

Figure 2. Localization of PBS proteins in the wild type and *cpcG* disruptants. Cell extracts were fractionated into low-speed precipitate (P1), high-speed precipitate (P2), and high-speed supernatant (S2). Phycobiliproteins were detected by zinc-induced fluorescence after SDS-PAGE (A), whereas CpcG1 and CpcG2 were detected by immunoblotting (B).

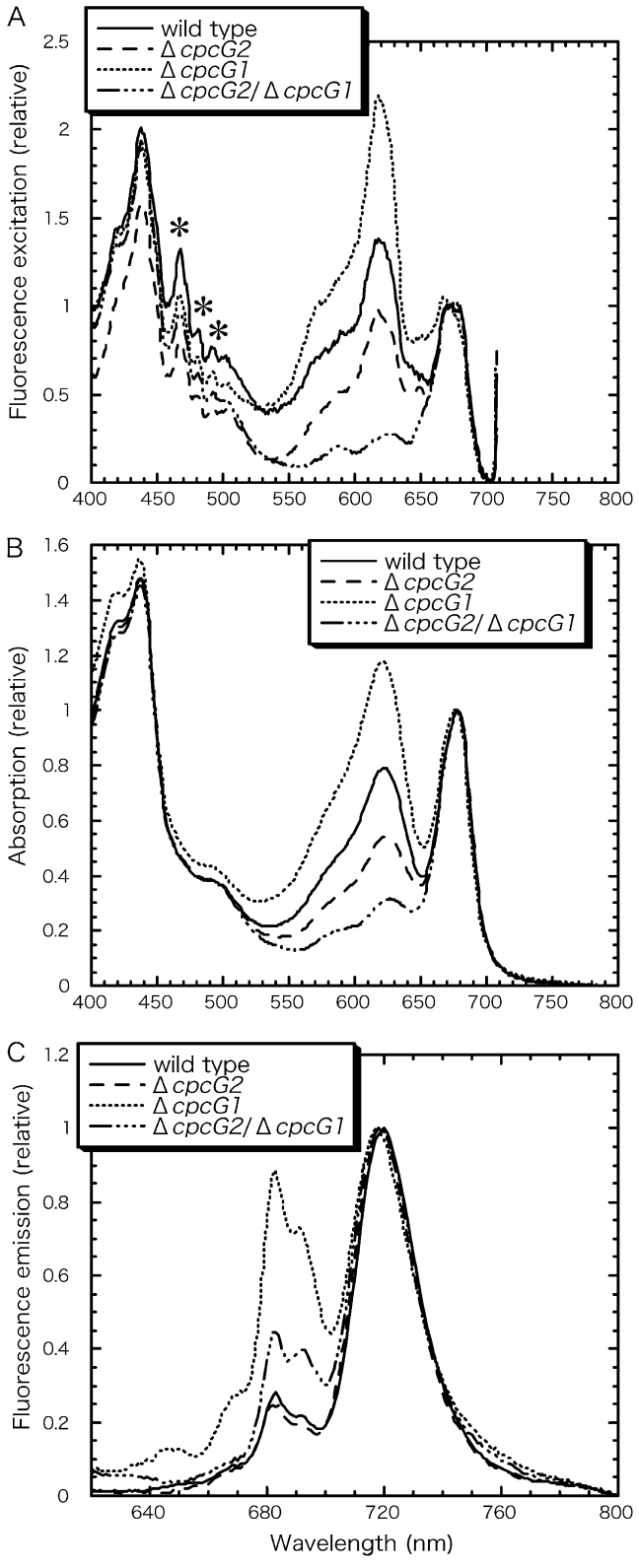

Figure 3. 77 K fluorescence spectra (A and C) and absorption spectra (B) of thylakoid fractions P1. A, The excitation spectra were normalized to the chlorophyll peak at 673 nm. PSI fluorescence was measured at 718 nm. Asterisks indicate artificial peaks derived from a Xenon light source. B, The absorption spectra were normalized to chlorophyll peak at 678 nm. C, Emission spectra excited upon 435 nm were normalized to PSI fluorescence peak at 721 nm.

while CpcG1 was recovered in the lower layer (Fig. 5). In addition, significant amount of phycocyanin proteins was also recovered in the upper layer (data not shown). This means that a large part of CpcG2-PBS was recovered into the hydrophobic layer, while CpcG1-PBS was recovered into the aqueous layer.

Cell Growth under Weak PSII Light

The effects of *cpcG* disruption on photoautotrophic growth under white light of medium intensity (40 μE m^{-2} s^{-1}) were already reported (Kondo et al., 2005). However, the *cpcG2* disruptant showed virtually no difference in growth compared to the wild type under the experimental conditions, while the *cpcG1* disruptant and the double mutant grew remarkably slower than the wild type. Here, photoautotrophic growth under weak PSII light ($\lambda = 610$ nm $\pm$ 20 nm, 10 μE m^{-2} s^{-1}) was carefully compared (Fig. 6). The results showed that the *cpcG2* disruptant reproducibly showed slightly slower growth than the wild type. The phycocyanin content per cell was much reduced in both the *cpcG1* and the *cpcG2* disruptant (data not shown). But, the slower growth of the *cpcG2* disruptant than the wild type cannot be explained by reduced phycocyanin content, since the same *cpcG2* disruption on the *cpcG1* mutant background slightly recovered the retarded growth. The photosystem stoichiometry PSI/PSII of the *cpcG2* disruptant was higher than that of the wild type under weak PSII light (data not shown). Therefore, the changed stoichiometry cannot explain the slower growth of the *cpcG2* disruptant, either. It is very probable that CpcG2-PBS transfers light energy preferentially to PSI in contrast with CpcG1-PBS.

DISCUSSION

In this study, we estimated that energy transfer efficiency to PSI is approximately 3-fold higher from CpcG2-PBS than from CpcG1-PBS in both cells and thylakoids, although they are able to transfer to both photosystems. This was also qualitatively supported by photosystem stoichiometry, which was conversely affected in the *cpcG1* and *cpcG2* disruptants. When thylakoid membranes were isolated under high-salt conditions, CpcG2-PBS was found to be tightly associated with the thylakoid membranes, while CpcG1-PBS was partly released. These results suggest that wild-type cells have two distinct types of PBSs (CpcG1-PBS and CpcG2-PBS) and that CpcG2-PBS preferentially transfers energy to PSI. Consistent with this data, the *cpcG2* disruptant showed slightly retarded growth under PSII light conditions. It is suggested that the unique behavior of CpcG2 is derived from its C-terminal hydrophobic segment.

Table II. *Energy transfer from phycocyanin to PSI in thylakoids*

Row A, Energy transfer from phycocyanin to PSI is represented as the ratio of phycocyanin excitation peak at 618 nm to the chlorophyll excitation peak at 673 nm in excitation spectra monitored with fluorescence emission at 718 nm. Row B, Values in row A were corrected based on phycocyanin content relative to chlorophyll, which is represented as absorption peak at 622 nm. Row C, Photosystem stoichiometry is represented as the fluorescence ratio of PSI (F_{PSI}) to PSII (F_{PSII}) upon excitation of chlorophyll at 435 nm.

	Wild Type	Δ*cpcG2*	Δ*cpcG1*	Δ*cpcG2*/Δ*cpcG1*
(A)[a] Excitation peak ratio	1.13	0.72	1.95	0
(B)[a] Phycocyanin content	0.48	0.23	0.87	0
(C) F_{PSI}/F_{PSII} (excitation at 435 nm)	4.57	5.12	1.37	2.52
(D) = A/BC	0.51	0.61	1.64	–

[a]Values in A and B were corrected for contribution of chlorophyll in Δ*cpcG2*/Δ*cpcG1*.

Clustering analysis of whole CpcG proteins and hydrophobicity analysis of the C-terminal domain (Fig. 7) showed that CpcG proteins can be divided into two distinct categories characterized by a hydrophobic or hydrophilic C terminus. The hydrophobic ones are clustered into three distinct groups (Fig. 7, red circles). The *Synechocystis* group covers many cyanobacteria, including non-N_2-fixing and N_2-fixing species, and the marine group is found in all the marine *Synechococcus* species but not in the closely related protochlorophytes. With the exception of some marine *Synechococcus* spp. that have two hydrophobic copies, these two groups have one copy each of the hydrophilic CpcG and the hydrophobic CpcG. The third group is found only in N_2-fixing *Anabaena* sp. PCC 7120; this organism harbors one hydrophobic copy (Alr0536) that shows some homology to the three hydrophilic CpcG copies. The three hydrophilic copies were detected in the conventional PBS, but detection of the hydrophobic copy remains unsettled (Bryant et al., 1991; Ducret et al., 1996; Cai et al., 1997). Interestingly, N_2-fixing heterocyst cells of *Anabaena* contain an unusual PBS that is devoid of allophycocyanins (Yamanaka and Glazer, 1983); it resembles the CpcG2-PBS in *Synechocystis*. This is in agreement with the finding that heterocyst cells are devoid of O_2-evolving PSII (Wolk et al., 1994). On the other hand, freshwater *Synechococcus elongatus* contains only one copy of the hydrophilic CpcG (Sugita et al., 2007), and *Thermosynechococcus* has three copies of the hydrophilic one (Nakamura et al., 2002). Similarly, rhodophytes, including *Cyanidioschyzon merolae*, possess only one hydrophilic copy of *cpcG* on the plastid genome (Matsuzaki et al., 2004). The primitive cyanobacterium *G. violaceus* appears to be unique in that it does not have any copies of CpcG (Nakamura et al., 2003), though a potential ortholog may be a unique rod-core linker protein CpcJ containing three linker domains (Koyama et al., 2006). Thus, we can conclude that the hydrophilic CpcG protein is almost universally required for the assembly of the peripheral rods and the central core into the conventional PBS, whereas the hydrophobic CpcG protein may have evolved at least three times from the hydrophilic CpcG in cyanobacteria alone. Judging from the domain architecture, the N-terminal linker domain of the authentic rod-core linker proteins packs the central channel of the hexameric phycocyanin disc, whereas the C-terminal

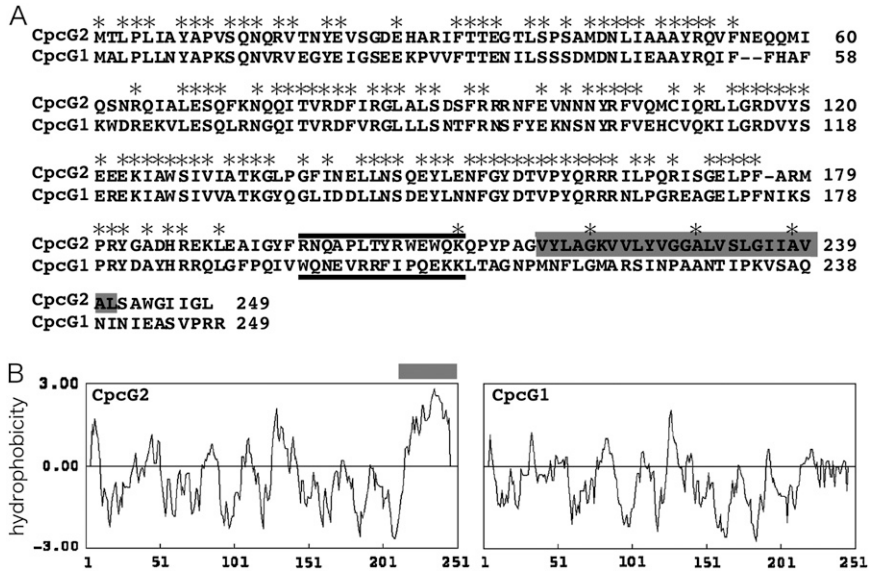

Figure 4. Sequence alignment (A) and hydropathy plots (B) of CpcG2 and CpcG1. A hydrophobic segment specific to CpcG2 is highlighted in gray. Black bars shown in A indicate sequences of synthetic peptides used for anti-peptide antibodies.

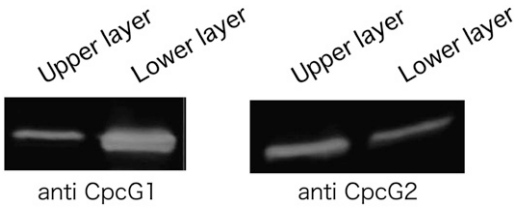

Figure 5. Phase partitioning of wild-type cell extracts using Triton X-100 and high-salt buffer. CpcG1 (left) and CpcG2 (right) were detected by immunoblotting after SDS-PAGE.

hydrophilic domains have differentiated to adapt to the docking sites on the core complex (Glick and Zilinskas, 1982; Liu et al., 2005). However, the absence of core cylinder proteins in *Synechocystis* CpcG2-PBS suggests an additional role of the C-terminal hydrophobic domain other than that of binding to the core. Similarly, N_2-fixing heterocysts of *Anabaena* accumulated PBS without allophycocyanins (Yamanaka and Glazer, 1983). This observation seems to support our conclusions, although CpcG copies of this preparation were not experimentally specified. It is of note that the position and length of the hydrophobic segment are somewhat conserved in CpcG among the three groups, although little sequence homology was detected between them.

The C-terminal segment of CpcG2 may interact directly with the PSI complex or the thylakoid membrane to support selective energy transfer. Previous biochemical analysis showed that CpcG2-PBS consists of the phycocyanin rods and CpcG2, but no central core proteins, such as ApcA, ApcB, ApcE, etc. (Kondo et al., 2005). This observation led us to interpret the isolated large CpcG2-PBS supercomplex as an artificial aggregation likely due to the hydrophobic tail of CpcG2. In situ docking sites of CpcG2-PBS may be located on the photosystem complexes. Alternatively, the hydrophobic tail of CpcG2 may be responsible for anchoring it to the membrane. A study of CpcG2-binding site(s) by fractionation of thylakoid membranes is currently under way. To date, a PSI trimer complex that retains CpcG2 weakly but specifically has been isolated (data not shown). This finding may support the former idea of a direct hydrophobic interaction between PSI and CpcG2-PBS.

Energy transfer from PBS to PSI has been observed as a state transition for nearly 40 years (Murata, 1969; Fork and Satoh, 1983). The state transition is a dynamic process that regulates the way absorbed light energy is distributed between PSI and PSII, although its molecular mechanism has not yet been elucidated (van Thor et al., 1998; Mullineaux and Emlyn-Jones, 2005). This is in contrast with the state transition in green plants, in which the light-harvesting chlorophyll complexes migrate in the membrane due to reversible phosphorylation and dephosphorylation in a redox-dependent manner (Haldrup et al., 2001; Wollman, 2001; Depege et al., 2003; Allen and Mullineaux, 2004; Takahashi et al., 2006). Random mutagenesis and phenotypic screening in cyanobacteria revealed that a hypothetical protein RpaC with transmembrane helices maintains the correct interaction between PSII and PBS and is essential for the state transition, although the actual function of this protein remains to be elucidated biochemically (Emlyn-Jones et al., 1999; Joshua and Mullineaux, 2005). PsaK2, an alternative of PSI peripheral subunit, was proposed on the photosystem side in the state transition in *Synechocystis*. It was expressed and incorporated into the PSI complex instead of PsaK1 during acclimation to high light (Hihara et al., 2001b; Fujimori et al., 2005). Furthermore, the state transition induced under high-light conditions was found to be impaired in the *psaK2* disruptant. This suggests that PsaK2 is involved in the energy transfer from PBS to PSI under high-light conditions. CpcG2 may interact with PsaK2 for energy transfer. Components of the central core of the conventional PBS are ApcD, ApcE, and ApcF; these have been implicated in energy transfer to both PSI and PSII and in the state transition (Gindt et al., 1994; Ashby and Mullineaux, 1999). ApcD and ApcF are variants of major allophycocyanin subunits, while, possessing multiple linker domains, ApcE connects the core cylinders and also anchors PBS to the membrane (Redlinger and Gantt, 1982; Capuano et al., 1991). Specific antibodies were raised against ApcD and ApcF and used to analyze various fractions of PBS and phycobiliproteins in *Synechocystis*. However, these proteins were not detected in the CpcG2-PBS fraction, suggesting that they are involved in docking or energy transfer of CpcG1-PBS alone (data not shown). On the other hand, Mullineaux and co-workers showed that PBS diffusion is required for state transition (Joshua and Mullineaux, 2004). By using fluorescence recovery after photobleaching, they showed that PBS is a mobile complex diffusing rapidly on the surface of the thylakoids in *S. elongatus* (Mullineaux et al., 1997). Since this organism has only one copy of hydrophilic CpcG, this PBS

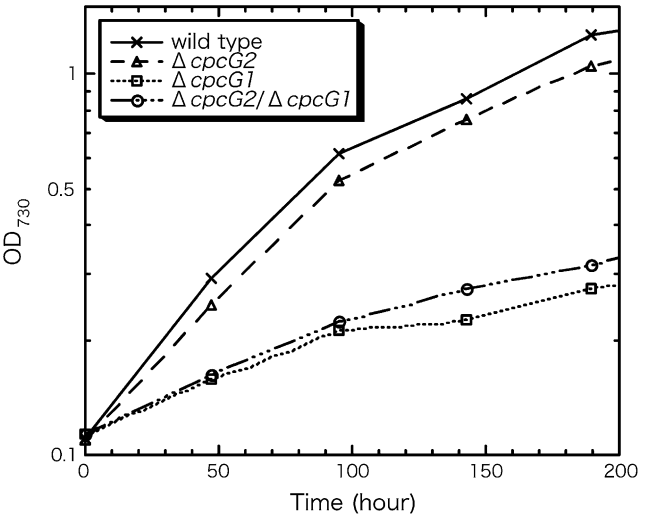

Figure 6. Growth curves under 10 μE m^{-2} s^{-1} PSII light.

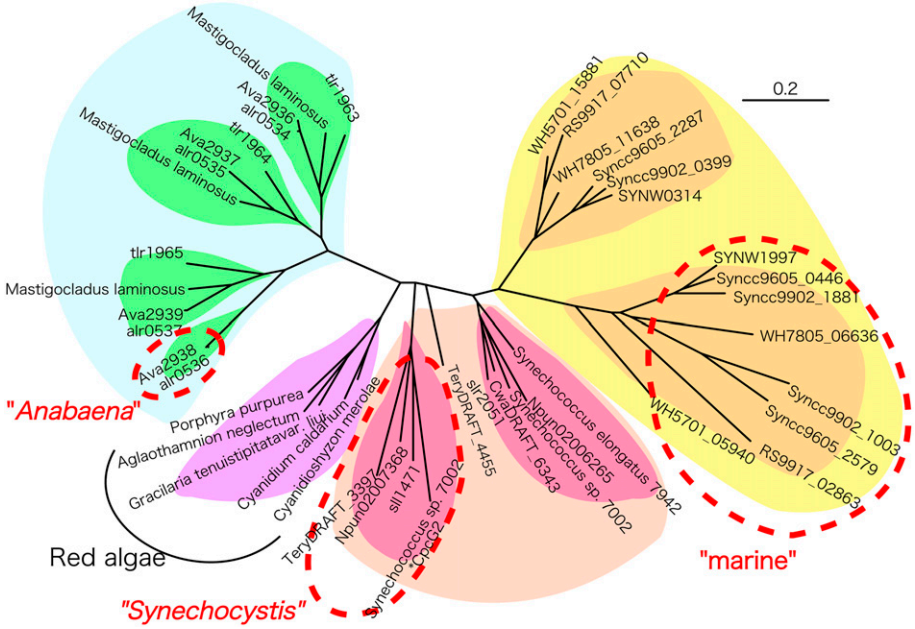

Figure 7. Clustering of whole amino acid sequences of CpcGs with the neighbor-joining method. CpcG proteins encircled by a red dotted line possess a C-terminal hydrophobic segment, whereas those that are not possess a hydrophilic segment. Gene names belong to the following organisms; Sll1471 and Slr2051, *Synechocystis* sp. PCC 6803; Npun02007368 and Npun02006265, *Nostoc punctiforme*; CwatDRAFT_6343, *Crocosphaera watsonii* WH8501; TeryDRAFT_3397 and TeryDRAFT_4455, *Trichodesmium erythraeum* IMS101; SYNW0314 and SYNW1997, *Synechococcus* sp. WH8102; WH5701_15881 and WH5701_05940, *Synechococcus* sp. WH5701; RS9917_02863 and RS9917_07710, *Synechococcus* sp. RS9917; WH7805_06636 and WH7805_11638, *Synechococcus* sp. WH7805; Syncc9605_0446, Syncc9605_2579, and Syncc9605_2287, *Synechococcus* sp. CC9605; Syncc9902_0399, Syncc9902_1881, and Syncc9902_1003, *Synechococcus* sp. CC9902; Alr0534, Alr0535, Alr0536, and Alr0537, *Anabaena* sp. PCC 7120; Ava2936, Ava2937, Ava2938, and Ava2939, *Anabaena variabilis* ATCC29413; Tlr1963, Tlr1964, and Tlr1965, *Thermosynechococcus elongatus* BP-1. *CpcG2 of *Synechococcus* sp. PCC 7002 was retrieved from the draft genome sequence. [See online article for color version of this figure.]

must be CpcG1-PBS (Fig. 7). In the monomeric PSI mutant, PBS diffuses nearly three times as fast as in the wild type. This suggests that the normal trimeric PSI is a platform for docking of CpcG1-PBS (Aspinwall et al., 2004). By way of a similar method, CpcG2-PBS was observed to be somewhat static, while CpcG1-PBS was observed to be mobile (W. Ma, K. Kondo, T. Ogawa, and M. Ikeuchi, unpublished data). This is consistent with our results suggesting that CpcG2-PBS is more tightly associated with the membrane than CpcG1-PBS. Analysis of the contribution of CpcG1-PBS and CpcG2-PBS to the state transition is currently being undertaken.

Another candidate for the PSI antenna is IsiA in PBS-containing cyanobacteria. It forms a ring-shaped antenna complex of 18-mers that surrounds the trimeric PSI and transfers light energy to PSI (Bibby et al., 2001a; Boekema et al., 2001; Nield et al., 2003). Curiously, IsiA is not expressed under normal physiological conditions but is induced under iron-starvation conditions (Laudenbach and Straus, 1988) and other stress conditions, such as salt stress, oxidative stress (Jeanjean et al., 2003; Li et al., 2004), and high-light stress (Havaux et al., 2005). IsiA may contribute to energy dissipation rather than light harvesting under stress conditions (Sandström et al., 2001). In prochlorophytes, where the PBS supercomplex and CpcG are absent, chlorophyll *a*/*b*-binding Pcb proteins serve as an antenna for both PSI and PSII (Bibby et al., 2001b; Bumba et al., 2005). In red algae, the conventional PBS containing the hydrophilic CpcG has been studied extensively, whereas the hydrophobic CpcG has never been detected (to our knowledge). Instead, red algae have developed chlorophyll *a*-containing antenna proteins that are specifically associated with PSI (red algal light-harvesting complex I [LHCI]; Wolfe et al., 1994a; Wolfe et al., 1994b). Many other algae of non-green lineage do not have phycobiliproteins but FCP (fucoxanthin chlorophyll *a*/*c* complex). The functional differentiation of FCP as antenna has been studied for decades, but no clear evidence for a PSI-specific FCP has been obtained. However, according to recent genomic data of a diatom (*Thalassiosira pseudonana*; Armbrust et al., 2004), we found that one FCP (JGI | thaps1 | 119497) is clustered into the clade of red algal LHCI. Thus, it can be assumed that PSI antennae such as CpcG2-PBS, Pcb, and LHCI are universally essential for coordinated excitation of the two photosystems.

Chromatic acclimation of photosystem stoichiometry has been widely observed in cyanobacteria, red algae, green algae, and higher plants (Myers et al., 1978, 1980; Manodori and Melis, 1986; Kim et al., 1993; Fujita, 1997). Namely, cells accumulate more PSI under

PSII light than PSI light to drive linear electron transport from PSII to PSI more efficiently. A similar mode of chromatic regulation of PSI accumulation in *Synechocystis* and PSII light-induced expression of *cpcG2* was recently reported (Hihara et al., 2001a; Katayama and Ikeuchi, 2006; M. Katayama, X.X. Geng, M. Kobayashi, F. Yano, M. Kanehisa, and M. Ikeuchi, unpublished data). Taken together, it is concluded that CpcG2-PBS accumulates in parallel with PSI to compensate for the reduced excitation of PSI under PSII light. To our knowledge, this is the first report of a PSI antenna that is regulated by light quality. Since the chromatic regulation of PSI has been reported in eukaryotic algae and green plants (Chow et al., 1990; Melis et al., 1996), we may also expect similar regulation of the PSI antenna in cyanobacteria.

CONCLUSION

Energy transfer efficiency from CpcG2-PBS to PSI was found to be approximately 3-fold higher than that from CpcG1-PBS in both cells and thylakoids, although they are able to transfer to both photosystems. The preferential energy transfer to PSI was also supported by the increased photosystem stoichiometry PSI/PSII in the *cpcG2* disruptant. When thylakoid membranes were isolated under high-salt conditions to stabilize PBS structure, CpcG2-PBS was found to be tightly associated with the thylakoid membranes, while CpcG1-PBS became partially unbound. The results suggest that wild-type cells have two distinct types of PBSs: the conventional CpcG1-PBS and the unusual CpcG2-PBS, which lacks the central core. The *cpcG2* disruptant showed slightly retarded growth under PSII light conditions. The unique behavior of CpcG2 was discussed in terms of its C-terminal hydrophobicity.

MATERIALS AND METHODS

Strain and Media

The original motile strain of *Synechocystis* sp. PCC 6803 showing positive phototaxis was used as the wild type. Previously, *cpcG1* and *cpcG2* were disrupted by insertion of spectinomycin-resistant and kanamycin-resistant genes, respectively (Kondo et al., 2005). The wild type and mutants were grown at 30°C in BG11 medium supplemented with 20 mM TES-KOH (pH 7.8; Rippka, 1988) and bubbling with 1% (v/v) CO_2 under continuous illumination with white fluorescent lamps (30–50 μE m^{-2} s^{-1}). Alternatively, cells were cultured under weak orange LED light with a λ_{max} of 610 nm and a 20 nm half-bandwidth (TLOH180P; TOSHIBA) at 10 μE m^{-2} s^{-1}. Cell density was monitored as optical density at 730 nm with a spectrophotometer (model UV-2400PC; Shimadzu).

77 K Fluorescence Spectrometry

Cells at log phase were harvested and resuspended at 5 μg chlorophyll mL^{-1} with BG11 medium. After dark adaptation for 10 min, cells were frozen in liquid N_2. Fluorescence was measured with a spectrofluorometer (model RF-5300PC; Shimadzu). Emission spectra were recorded by excitation at 435 nm (chlorophyll) or 600 nm (phycocyanin). The bandwidth of the excitation light was 10 nm for cells or 5 nm for thylakoids. The PSI/PSII fluorescence ratio was evaluated from a peak at 692 nm divided by a peak at 721 nm. For measurement of membranes, PSI fluorescence excitation spectra were recorded with a fixed emission at 718 nm. PSI excitation by PBS was estimated from a peak at 618 nm from the excitation spectra normalized to the chlorophyll peak at 673 nm. Relative phycocyanin content was estimated from the absorption peak at 622 nm normalized to the chlorophyll peak at 678 nm, measured with a spectrophotometer (model UV-2400PC; Shimadzu).

Isolation of Thylakoid-Associated PBS

To preserve PBS structure, procedures were carried out at room temperature unless otherwise specified. Cells were harvested by centrifugation, washed twice with 0.8 M potassium phosphate buffer (pH 7.0), and resuspended in the same buffer. The cells were then broken by vortexing with zircon beads, and the homogenate was centrifuged for 10 min at 4,000*g* at 18°C to remove cell debris. The supernatant (cell extract) was centrifuged for 30 min at 20,000*g* at 18°C to yield low-speed precipitate (P1). The supernatant was then centrifuged at 100,000*g* for 60 min at 18°C to yield high-speed precipitate (P2) and supernatant (S2).

Phase Partitioning

The classic protocol for isolation of PBS developed by Gray and Gantt (Gray and Gantt, 1975) and modified by others (Yamanaka et al., 1978; Gantt et al., 1979) was adopted for hydrophobicity-based phase partitioning. The cell extract was treated with 2% Triton X-100 in 0.8 M potassium phosphate buffer for 30 min and then centrifuged at 20,000*g* for 20 min at 18°C to separate into the upper green Triton X-100 layer and the lower blue aqueous layer.

SDS-PAGE and Zinc-Induced Fluorescence

Proteins were resolved by SDS-PAGE using 15% acrylamide gel (Laemmli, 1970), followed by staining with Coomassie Brilliant Blue R-250. For the zinc-induced phycobiliprotein fluorescence assay, the SDS-gel was soaked in 20 mM zinc acetate for 30 min and fluorescence was visualized through a 605-nm filter upon excitation at 532 nm (FMBIO II; Takara).

Immunoblotting

Proteins resolved in the SDS gel were blotted onto a polyvinylidene difluoride membrane (Immobilon; Millipore). After blocking with 5% skim milk (Wako), the membrane was probed with rabbit anti-peptide antibodies in an incubation solution (20 mM Tris-HCl [pH 7.5], 0.5 M NaCl, 0.05% [v/v] Tween 20), followed by a goat anti-rabbit IgG-alkaline phosphatase conjugate (Jackson Immunoresearch). Immunoreaction was detected by 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.

The anti-peptide antibodies were produced by Takara Bio. Synthetic peptides (for CpcG1, WQNEVRRFIPQEKKLC; for CpcG2, RNQAPLTYRWEWQKC; C-terminal Cys was added for conjugation) were conjugated to keyhole limpet hemocyanin and injected into rabbits.

Clustering Analysis of CpcG

CpcG sequences were obtained from the database. Clustering analysis was performed by automatic sequence alignment and classification with the neighbor-joining algorithm using the ClustalX program (Thompson et al., 1997). The cluster was visualized as a nonrooted tree. Hydropathy plots were obtained based on the method of Kyte and Doolittle (Kyte and Doolittle, 1982). The window size was 9 amino acid residues. In the clustering analysis of CpcG proteins, hydrophobicity of the C-terminal region was estimated by the hydropathy plot of Kyte and Doolittle and the SOSUI program (Hirokawa et al., 1998).

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