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Psb28 is involved in recovery of photosystem II at high temperature in *Synechocystis* sp. PCC 6803

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ABSTRACT

Psb28 is an extrinsic protein of photosystem II (PSII), which is conserved among photosynthetic organisms from cyanobacteria to higher plants. A unicellular cyanobacterium, *Synechocystis* sp. PCC 6803, has two homologs of Psb28, Psb28-1 and Psb28-2. However, the role of these proteins remains poorly understood. In this study, we disrupted the *psb28-1* (*sll1398*) and *psb28-2* (*slr1739*) genes in wild-type *Synechocystis* sp. PCC 6803 and examined their photosynthetic properties to elucidate the physiological role of Psb28 in photosynthesis. We also disrupted the *psb28-1* gene in a *dgdA* mutant defective in the biosynthesis of digalactosyldiacylglycerol, in which Psb28-1 significantly accumulates in PSII. The disruption of the *psb28-1* gene in the wild-type resulted in growth retardation under high-light conditions at high temperatures with a low rate of restoration of photodamaged photosynthetic machinery. Similar phenomena were observed at normal growth temperatures in the *psb28-1*/*dgdA* double mutant. In contrast, disruption of *psb28-2* in the wild-type and *dgdA* mutant did not affect host strain phenotype, suggesting that Psb28-2 does not contribute to the recovery of PSII. In addition, protein analysis using strains expressing His-tagged Psb28-1 revealed that Psb28-1 is mainly associated with the CP43-less PSII monomer. In the *dgdA* mutant, the CP43-less PSII monomer accumulated to a greater extent than in the wild-type, and its accumulation caused greater accumulation of Psb28-1 in PSII. These results demonstrate that Psb28-1 plays an important role in PSII repair through association with the CP43-less monomer, particularly at high temperatures.

1. Introduction

In the initial step of oxygenic photosynthesis, the photosystem II (PSII) of plants, algae, and cyanobacteria catalyzes light-driven water splitting, one of the most important biochemical reactions responsible for the production of oxygen in the atmosphere [1,2]. PSII is a large cofactor–protein supercomplex embedded in thylakoid membranes of chloroplasts and cyanobacteria, which contains approximately 20 protein subunits and many cofactors, such as pigments, metals, and lipids. PSII normally exists as a dimer when it is active in oxygen evolution. Recently, the dimeric structure of the PSII complex was resolved by X-ray crystallographic analysis using the thermophilic cyanobacteria, *Thermosynechococcus elongatus* [3] and *Thermosynechococcus vulcanus* [4]. The PSII core complex is composed of a D1/D2 heterodimer, CP47, CP43, and a number of small polypeptides including cytochrome b_{559}. The D1/D2 heterodimer harbors most of the cofactors necessary for PSII photochemistry, including the primary electron donor P680. CP47 and CP43 function as inner antenna proteins and are located on the periphery of the D1/D2 heterodimer. These four large proteins are surrounded by small membrane-spanning polypeptides. An Mn_4Ca cluster, which is responsible for the water-splitting reaction, is located on the luminal side of the D1/D2 heterodimer and stabilized by several extrinsic proteins (PsbO, PsbV, PsbU in cyanobacteria) attached to the luminal surface of the PSII core complex [5].

Although the static structure of the PSII dimer has been resolved in detail by structural analysis, questions regarding the dynamic structure of PSII remain, such as assembly of PSII and repair of photodamaged PSII. Within the D1/D2 reaction center of PSII, oxidative damage inevitably occurs due to the excess energy generated by light absorption, leading to loss of PSII activity known as photoinhibition. Photoinhibition limits plant growth and lowers productivity, especially when combined with other abiotic stresses [6,7]. Photosynthetic organisms have evolved a highly specialized mechanism to restore the function of photodamaged PSII. According to recent genetic and biochemical studies, several steps are involved in the repair of PSII as follows [8–10]. The D1 protein in active PSII dimers is photodamaged under strong illumination. Extrinsic proteins and manganese ions are released from the luminal side of photodamaged PSII, leading to monomerization of the complex. Then

Abbreviations: BN-PAGE, blue native-polyacrylamide gel electrophoresis; BQ, *p*-benzoquinone; Chl, chlorophyll; Cm^R, chloramphenicol-resistant gene cassette; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DGDG, digalactosyldiacylglycerol; Fecy, potassium ferricyanide; HL, high light; Km^R, kanamycin-resistant gene cassette; LL, low light; ML, moderate light; PCR, polymerase chain reaction; PG, phosphatidylglycerol; PSII, photosystem II; SDS–PAGE, SDS–polyacrylamide gel electrophoresis

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CP43 is dissociated from the monomer complex, allowing efficient replacement of damaged D1 with a newly synthesized D1 precursor (preD1). The damaged D1 is subjected to degradation, and Psb27 binds to the luminal side of the complex. After reassociation, CP43, the C-terminal extension of preD1, is processed into the mature form by a processing enzyme, CtpA. Then Psb27 is released from the complex and the Mn_4Ca cluster is formed, followed by binding of extrinsic proteins. Finally, the active PSII monomer is dimerized to the active dimer. The *de novo* assembly of PSII also proceeds through a multi-step pathway, which partly overlaps with the repair process [10].

Due to the structural complexity of PSII, it is thought that a number of proteins participate in its assembly and repair. In recent studies, several proteins involved in PSII assembly have been identified and characterized (for review, see [9,11]). Because these proteins transiently associate with assembly intermediate complexes, they are often found in association with purified PSII complex in a nonstoichiometric manner. One of these, Sll1398, was originally identified as a nonstoichiometric component of PSII prepared from *Synechocystis* sp. PCC 6803 [12]. Although this protein has been called Psb28, Psb13, and Ycf79, it is referred to as Psb28-1 in this study because *Synechocystis* sp. PCC 6803 has another protein, Psb28-2 (Slr1739), which is homologous to Psb28-1. Psb28-1 is a soluble protein with a molecular mass of ~13.5 kD and is conserved from cyanobacteria to higher plants. Recently, Dobáková et al, showed that Psb28-1 is peripherally bound to the cytoplasmic side of thylakoid membranes and involved in the biogenesis of chlorophyll (Chl) and/or CP47 [13]. NMR structural analysis showed that Psb28-1 consists of two antiparallel β-sheets, one long α-helix, two short helices, and nine loop regions, and possibly interacts with other proteins via a conserved cavity [14]. In *T. elongatus*, deletion of *psbJ* led to accumulation of Psb27–Psb28 PSII complexes [15]. In higher plants, Psb28 is sometimes annotated as PsbW or PsbW-like protein because it has a 16% amino acid sequence identity to PsbW, a 6 kD membrane subunit of PSII. *Arabidopsis thaliana* contains a single copy of the *Psb28* gene (*At4g28660*). DNA microarray analysis has demonstrated that this gene is highly expressed in leaves, especially in cotyledons (Arabidopsis eFP browser: Winterhttp://bbc.botany.utoronto.ca/efp, [16]), and its expression is controlled by a phytochrome signal via the transcription factor phytochrome-interacting factor 3 [17,18]. In *Oryza sativa*, transcription of *Psb28* (*Os01g71190*) is induced by high illumination levels, and inactivation of this gene by T-DNA insertion results in a pale-green phenotype [19].

In spite of recent intense research, the physiological role of Psb28 remains poorly understood. A previous study by Dobáková et al. [13] indicated that Psb28-1 is dispensable for cell viability and maintenance of photosynthetic activities in *Synechocystis* sp. PCC 6803 under normal growth conditions. However, in previous studies [20,21], we found that Psb28-1 significantly accumulates in PSII monomer prepared from *pgsA* and *dgdA* mutants of *Synechocystis* sp. PCC 6803, which are defective in the biosynthesis of phosphatidylglycerol (PG) and digalactosyldiacylglycerol (DGDG), respectively. These findings suggest that Psb28-1 plays an important role in the PSII assembly and repair processes. In addition, proteomic analysis of *Synechocystis* sp. PCC 6803 demonstrated that Psb28-1 levels increase after heat shock treatment [22]. Based on these observations, the *pgsA* and *dgdA* mutants may be useful tools for investigating the function of Psb28 in PSII. In this study, we disrupted *psb28-1* and *psb28-2* in wild-type and *dgdA* mutant *Synechocystis* sp. PCC 6803 strains and examined their photosynthetic properties, to clarify the physiological role of Psb28. Analyses of photoautotrophic growth and photoinhibition of photosynthesis using intact cells revealed that Psb28-1, but not Psb28-2, is necessary for efficient PSII repair, especially under high-temperature stress conditions. Furthermore, we constructed wild-type and *dgdA* mutant strains expressing His-tagged Psb28-1 to purify the protein complexes associated with Psb28-1. Psb28-1 was mainly associated with the CP43-less PSII monomer. In the *dgdA* mutant, CP43-less monomer level markedly increased with a concomitant decrease in PSII dimer, and so Psb28-1 accumulates in this mutant. These findings suggest that Psb28-1 is involved in PSII repair through binding to assembly intermediates that require Psb28-1 for stabilization.

2. Materials and methods

2.1. Organisms and growth conditions

The following strains, which were previously constructed in *Synechocystis* sp. PCC 6803, were used: (1) the *dgdA* disruption mutant (*slr1508*) [21]; (2) the CP47-His strain expressing a CP47 subunit of the PSII complex with six His residues at the C terminus [23]; (3) the CP47-His/*dgdA* strain generated from the *dgdA* mutant [21]. In addition to these strains, a *psb28-1* mutant, *psb28-2* mutant, *psb28-1*/*psb28-1* double mutant, and a Psb28-1-His strain expressing a Psb28-1 protein with a C terminus histidine tag were newly constructed in this study, as described below. The *psb28-1* disruption mutant was also constructed using the CP47-His and *dgdA* mutants as background strains.

Cells were grown photoautotrophically at 30 °C on BG11 agar plates or in liquid BG11 media under continuous fluorescent white light at an intensity of 20 μmol photons m^{-2} s^{-1} [low light (LL) conditions] unless otherwise mentioned. Liquid cultures were aerated on a rotational shaker (NR-3; TAITEC) at 120 rpm. For high light (HL) conditions, liquid cultures were illuminated at 200 μmol photons m^{-2} s^{-1}. Growth was monitored by determining the optical density at 730 nm (OD_{730}). To assay the growth of mutant cells on agar BG-11 plates, cells that had been cultured at 30 °C under LL conditions were washed twice with fresh BG11 medium, and 10 μL washed cell suspensions adjusted to OD_{730} of 0.5, 0.25, and 0.05 were spotted onto BG11 agar plates and incubated at 30 °C or 38 °C for 3 days under continuous fluorescent white light at an intensity of 10 (LL), 30 [moderate light (ML)], or 100 (HL) μmol photons m^{-2} s^{-1}. For purification of PSII complexes, cells grown in 200 mL BG11 medium were transferred to 6 L BG11 medium and cultivated at 30 °C under continuous aeration with 2% (v/v) CO_2 in air. Cells in the logarithmic growth phase were used to prepare thylakoid membranes, which were used to purify PSII complexes.

2.2. Construction of psb28-1 and psb28-2 disruption mutants

The primers used in the study are listed in Table 1. The *psb28-1* disruption mutant (*sll1398*) was constructed by transforming wild-type cells with a *psb28-1* plasmid (see Fig. 1A). The *psb28-1* plasmid was obtained by replacing a section of *psb28-1* with chloramphenicol resistant gene cassette (Cm^R), as follows. The regions upstream and downstream of *psb28-1* were amplified by polymerase chain reaction (PCR) using two sets of primers: 1398-F1 and 1398-R1, and 1398-F2 and 1398-R2, respectively. As shown in parentheses in Table 1, these primers included *Sal*I, *Hin*dIII, *Hin*dIII, and *Pst*I sites at the 5′ end. Amplified upstream and downstream fragments were digested with *Sal*I and *Hin*dIII, and *Hin*dIII and *Pst*I, respectively, and ligated together into the *Sal*I and *Pst*I sites of pBluescript II (Stratagene). The obtained plasmid was digested with *Hin*dIII and ligated with Cm^R obtained by digestion of plasmid pCCm with *Hin*dIII to construct the *psb28-1* plasmid. The disruption *psb28-2* mutant (*slr1739*) was similarly constructed by transforming wild-type cells with a *psb28-2* plasmid in which most of the *psb28-2* gene was replaced with kanamycin resistant gene cassette (Km^R, see Fig. 1A). To construct the *psb28-2* plasmid, the upstream and downstream fragments of *psb28-2* were amplified by PCR using the following two sets of primers: 1739-F1 and 1739-R1, and 1739-F2 and 1739-R2, respectively. These primers included *Sal*I, *Hin*dIII, *Hin*dIII, and *Spe*I sites at the 5′ end. Amplified upstream and downstream fragments were digested with *Sal*I and *Hin*dIII, and *Hin*dIII and *Spe*I, respectively, and ligated together into the *Sal*I and *Spe*I site of pBluescript II. The obtained plasmid was digested with *Hin*dIII and ligated with Km^R, itself obtained by digestion of the plasmid pUC4KIXX (Amersham) with *Hin*dIII to construct the *psb28-2* plasmid. The obtained plasmids were used to transform the wild-type *Synechocystis* sp. PCC 6803. Transformants were

Table 1
List of primers used in this study.

Primer	Sequence
1398-F1	5′-ACGC(GTCGACG)TTCTACCTGCTCGATCGC-3′
1398-R1	5′-CCC(AAGCTT)TGCTGAGGCGCACTTCTG-3′
1398-F2	5′-CCC(AAGCTT)CTTGGAACCGACAATTCTAGCG-3′
1398-R2	5′-AA(CTGCAG)TGGGGGCAATGGGAGAGT-3′
1739-F1	5′-CG(GAATTC)ATGGGGTCTGTGAAAACTGC-3′
1739-R1	5′-CCC(AAGCTT)AATTCGATGGTGGGAGTGAG-3′
1739-F2	5′-CCC(AAGCTT)TCATTGGGAACGCTTTATCC-3′
1739-R2	5′-GG(ACTAGT)ACACCACATCCCCTGGTTTA-3′
1398His-R1	5′-CCC(AAGCTTA)ATGATGATGATGATGATGTTCAGATTTGGAAAAAC C-3′
1398His-F2	5′-CCC(AAGCTT)ATCAATGGCCGTCCTACG-3′
1398His-R2	5′-GG(ACTAGT)CCACCGAAATGGGTACAAAC-3′
1398-R3	5′-GGACTAGTCCCGAGGTAAGCGTAATCAA-3′

The sequences in parentheses represent the restriction sites added to the primers, and the underlined sequence regions represent sequences coding hexahistidines.

selected for growth on agar BG11 plates containing 5 μg mL^{-1} chloramphenicol or kanamycin. Complete segregation was verified by PCR using the following primer sets: 1398-F1 and 1398-R3 for *psb28-1*, and 1739-F1 and 1739-R2 for *psb28-2*. A *psb28-1* disruption mutant was also constructed using the *psb28-2* mutant, *dgdA* mutant, and CP47-His strains as parent strains to obtain the *psb28-1*/*psb28-2* double mutant, *psb28-1*/*dgdA* double mutant, and CP47-His/*psb28-1* strain. These strains were obtained by transforming parent strains with chromosomal DNA from *psb28-1* mutant cells, and transformants were selected based on additional resistance to chloramphenicol.

2.3. Construction of Psb28-1-His strains

The Psb28-1-His strains expressing Psb28-1 protein with six His residues as a tag at the C terminus under control of the native promoter was constructed by transforming wild-type cells with a *psb28-1-His* plasmid to replace the native *psb28-1* gene with *psb28-1-His* in which the hexahistidine coding sequence was attached to the 3′ coding region (see Fig. 6A). Cm^R was inserted at the 5′ site of the stop codon of *psb28-1* gene as a selectable marker. To avoid the side effect of insertion of Cm^R into the downstream gene (*sll1399*), ~210 bp upstream of the adjacent gene (*sll1399*) including the 3′ coding region of *psb28-1* was inserted the downstream of Cm^R. The *psb28-1-His* plasmid was constructed using a procedure similar to that used to generate the *psb28-1* and *psb28-2* plasmids. The upstream and coding regions, and the region downstream of *psb28-1*, were amplified by PCR using the following two primer sets: 1398-F1 and 1398His-R1, and 1398His-F2 and 1398His-R2, respectively. The 1398His-R1 primer contains a hexahistidine coding sequence (underlined in Table 1). Amplified fragments were digested with the appropriate restriction enzymes, ligated together into pBluescript II, and a Cm^R cassette was inserted into the *Hind*III site added at the 5′ site of the stop codon of *psb28-1*. The obtained plasmid was used to transform the CP47-His and CP47-His/*dgdA* strains of *Synechocystis* sp. PCC 6803, generating the Psb28-1-His and Psb28-1-His/*dgdA* strains. Transformation and verification of complete segregation were performed as described above.

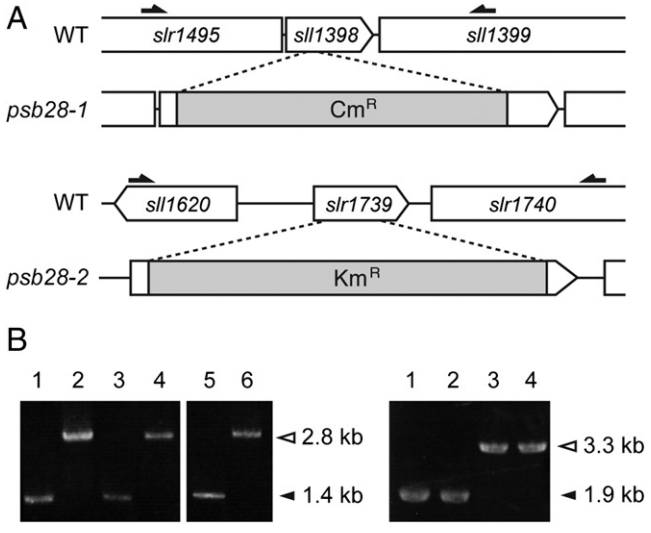

Fig. 1. Disruption of *psb28-1* and *psb28-2*. (A), Schematic design of disruption of the *psb28-1* (*sll1398*) and *psb28-2* (*slr1739*) genes. Arrows indicate PCR primers. (B), Insertion of Cm^R or Km^R confirmed by PCR analysis. PCR was performed using genomic DNA of wild-type (lane 1), *psb28-1* mutant (lane 2), *psb28-2* mutant (lane 3), *psb28-1*/*psb28-2* double mutant (lane 4), *dgdA* mutant (lane 5), and *dgdA*/*psb28-1* double mutant (lane 6) strains. Left panel, PCR was performed using the primers 1398-F1 and 1398-R3. Right panel, PCR was performed using the primers 1739-F1 and 1739-R2.

2.4. Analysis of photosynthetic activity

Photosynthetic oxygen-evolving activity of intact cells was measured using a Clark-type oxygen electrode according to Gombos et al. [24]. The samples were illuminated with white light filtered through thermo-cutting and red filters. Chl concentrations were determined as described by Arnon et al. [25]. To measure PSII activity, 0.5 mM *p*-benzoquinone (BQ) and 1 mM potassium ferricyanide (Fecy) were added as electron acceptors.

To assay the susceptibility of mutant cells to high-intensity light, cells suspended in BG-11 medium containing 10 μg Chl mL^{-1} were illuminated at 30 °C or 38 °C with white light at 2500 μmol photons m^{-2} s^{-1} in the presence or absence of 0.25 mg mL^{-1} lincomycin. Recovery of photosynthetic activity after photoinhibition at high temperature was measured in cells that had been subjected to photoinhibitory treatment at 40 °C in the presence of lincomycin, washed twice with distilled water and once with fresh BG-11 to remove lincomycin, and then their oxygen-evolving activity was allowed to recover under LL (20 μmol photons m^{-2} s^{-1}) at 30 °C or 40 °C. Duration of photoinhibitory treatment of the wild-type and *psb28-1* mutant strains, and the *dgdA* and *psb28-1*/*dgdA* mutant cells, was 90 and 30 min, respectively, because oxygen-evolution activity in DGDG-deficient mutants (*dgdA* and *psb28-1*/*dgdA* mutant) is more sensitive to HL than it is in wild-type cells, as reported previously [26].

2.5. Preparation of thylakoid membranes and PSII complexes

Thylakoid membranes were prepared from 6 L of cell culture grown to the late logarithmic phase, according to Kashino et al. [27]. PSII complexes binding CP47-His, and protein complexes binding Psb28-1-His, were purified by Ni-affinity column chromatography (Ni-NTA column; Qiagen) as described by Sakurai et al. [20]. PSII complexes binding CP47-His were further separated into monomeric and dimeric fractions by ultracentrifugation, as described by Sakurai et al. [20].

2.6. Protein analysis

Polypeptide compositions were analyzed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) as described by Kashino et al. [28] with the minor modifications of Kubota et al. [29] using a gradient gel of 18 to 24% polyacrylamide containing 6 M urea. Monomeric and dimeric fractions of PSII purified from the CP47-His and CP47-His/psb28-1 strains corresponding to 2 μg Chl each were subjected to SDS–PAGE.

Subunits of PSII monomer and dimer complexes were identified by matrix-assisted laser desorption ionization time of flight mass spectrometry as described previously [23].

Blue Native-PAGE (BN-PAGE) was performed using the NativePAGE ™ Novex® Bis-Tris Gel System (Invitrogen) with 4–16% polyacrylamide bis-tris precast gels according to the manufacturer's instructions, with

minor modifications to the run conditions. Purified PSII complexes corresponding to 3 μg Chl (CP47-His and CP47-His/*dgdA*) or 1.5 μg Chl (Psb28-1-His and Psb28-1-His/*dgdA*) were subjected to BN-PAGE, and electrophoresis was performed at 4 °C by increasing voltage gradually from 50 up to 200 V during the 4.5 h run. To separate polypeptides in the second dimension, a strip corresponding to each lane of the native gel was excised and incubated in SDS sample buffer containing 10% (*v*/*v*) β-mercaptoethanol and 6 M urea for 60 min at room temperature. The gel strips were then layered onto 1-mm-thick SDS–PAGE gels, and subjected to SDS–PAGE analyses as described above. After electrophoresis, polypeptides were visualized by silver staining [30].

3. Results

3.1. Construction of *psb28* mutant strains

Synechocystis sp. PCC 6803 has two homologous proteins of Psb28, Psb28-1 (Sll1398) and Psb28-2 (Slr1739), with 23.8% identity and 36.1% similarity in their amino acid sequences. To elucidate the physiological function of Psb28, we constructed *psb28-1* and *psb28-2* disruption mutants in wild-type and *dgdA* mutant *Synechocystis* sp. PCC 6803 strains. Regions of the *psb28-1* and *psb28-2* genes were replaced with Cm^R and Km^R, respectively (Fig. 1A). Because *psb28-1* and *psb28-2* might complement each other, we also constructed a *psb28-1*/*psb28-2* double mutant. As cyanobacterial cells normally contain many copies of chromosomal DNA [31], complete replacement of all copies in each mutant strain was verified by PCR (Fig. 1B). PCR using a primer set (1398-F1 and 1398-R3) for amplification of *psb28-1* resulted in amplification of DNA fragments of 1.4 kbp from the wild-type, *psb28-2* and *dgdA* mutants, and 2.8 kbp from the *psb28-1* mutant, *psb28-1*/*psb28-2* double mutant, and *psb28-1*/*dgdA* double mutant (Fig. 1B, left panel). On the other hand, PCR using a primer set (1739-F1 and 1739-R2) for amplification of *psb28-2* resulted in amplification of DNA fragments of 1.9 kbp from the wild-type and *psb28-1* mutant, and 3.3 kbp from the *psb28-2* mutant and *psb28-1*/*psb28-2* double mutant (Fig. 1B, right panel). These results clearly demonstrate that *psb28-1* and *psb28-2* were completely disrupted in the *psb28-1* mutant and *psb28-1*/*dgdA* double mutant, and in the *psb28-2* mutant, respectively, and both were completely disrupted in the *psb28-1*/*psb28-2* double mutant.

Initial characterization of mutant cells showed that the photoautotrophic growth and oxygen-evolving activities of *psb28-1* mutant cells were retarded under HL and high-temperature conditions, as described below. In contrast, the photosynthetic properties of *psb28-2* mutant cells were the same as those of wild-type cells in terms of growth rates, photosynthetic activities, and HL susceptibility (Table 2 and Supplemental Fig. 1). In addition, the *psb28-1*/*psb28-2* double mutant showed the same properties as the *psb28-1* mutant (Table 2 and Supplemental Fig. 1). These data suggest that Psb28-1, but not Psb28-2, plays an important role in growth and photosynthesis, and Psb28-2 cannot complement the function of Psb28-1. Therefore, we further analyzed only the *psb28-1* mutants in detail.

Table 2
Cellular Chl content and photosynthetic oxygen-evolving activities of intact cells of wild-type and mutant strains grown at 30 °C under low-light conditions.

Strain	Chl content (μg mL^{-1})	Activity (μmol O_2 mg Chl^{-1} h^{-1})	
		Net ($H_2O \rightarrow CO_2$)	PSII ($H_2O \rightarrow BQ$)
Wild-type	5.21 ± 0.16	330 ± 20	410 ± 10
psb28-1	4.73 ± 0.12	320 ± 10	400 ± 10
psb28-2	5.02 ± 0.09	310 ± 10	390 ± 40
psb28-1/*psb28-2*	4.79 ± 0.22	300 ± 40	390 ± 40
dgdA	3.91 ± 0.14	270 ± 20	260 ± 30
psb28-1/*dgdA*	3.47 ± 0.21	230 ± 10	220 ± 40

Values represent averages ± SD of independent preparations ($n > 3$).

In our previous studies [20,21], Psb28-1 accumulated predominantly in the monomeric PSII complex in the *pgsA* and *dgdA* mutants, which are defective in the biosynthesis of PG and DGDG, respectively. Thus, it was assumed that Psb28-1 plays an important role in the assembly and maintenance of PSII complex in these mutants. To analyze the function of Psb28-1 in these mutants, we constructed a *psb28-1*/*dgdA* double mutant. We expected that the effect of the lack of Psb28-1 on the photosynthetic machinery would be clearly observed by comparing the photosynthetic properties of the *psb28-1*/*dgdA* double mutant to those of the *dgdA* mutant.

3.2. Cellular Chl content and photosynthetic activities in the *psb28-1* mutant

Table 2 shows the Chl content and photosynthetic oxygen-evolving activities of wild-type and mutant strains grown at 30 °C under LL conditions. The *psb28-1* mutant cells had slightly lower Chl content than wild-type cells. However, on a Chl basis, there was no significant difference in the net oxygen-evolving or PSII activity between wild-type and *psb28-1* mutant cells. This result indicates that Psb28-1 is not indispensable for photosynthesis under normal growth conditions. In a previous study [13], the *psb28-1* mutant showed more PSII activity on a Chl basis than the wild-type due to the decreased PSI content. However, under our conditions, no significant change in PSI/PSII ratio was observed by low-temperature (77 K) Chl fluorescence measurement (Supplemental Fig. 2). Indeed, the PSII activity of our mutant

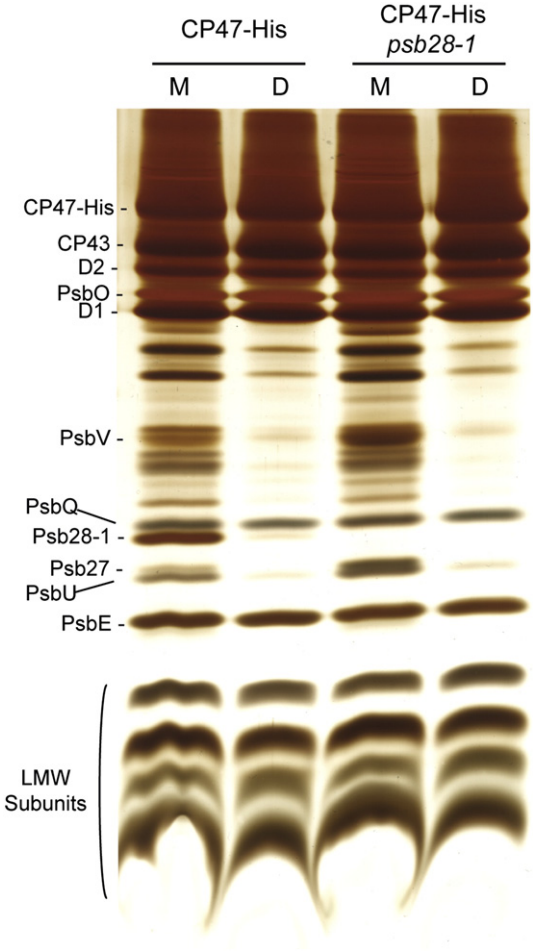

Fig. 2. Polypeptide compositions of monomeric (M) and dimeric (D) fractions of PSII prepared from CP47-His and CP47-His/*psb28-1* mutant cells. Purified PSII complexes corresponding to 2 μg Chl were loaded in each lane. Polypeptides were visualized by silver staining. LMW subunits: low-molecular- weight subunits of PSII.

was equivalent to that of wild-type cells on a Chl basis. On the other hand, the net oxygen-evolving and PSII activities of the *psb28-1/dgdA* double mutant were lower than those of the *dgdA* mutant, indicating that accumulated Psb28-1 in the *dgdA* mutant is important for maintenance of photosynthetic activities.

3.3. Polypeptide composition of PSII complex purified from the psb28-1 mutant

To analyze the effects of a lack of Psb28-1 on PSII, PSII complexes were purified by Ni-affinity column chromatography from thylakoid membranes of CP47-His [23] and CP47-His/*psb28-1* cells in which the His-tagged CP47 protein was expressed. The CP47-His/*psb28-1* strain was constructed by disrupting *psb28-1* in the CP47-His strain [23]. Monomeric and dimeric fractions of PSII complex were further separated by ultracentrifugation on a glycerol density gradient, subjected to SDS–PAGE analysis, and subunits of PSII complexes shown in Fig. 2 were identified by mass spectrometry. Psb28-1 was preferentially detected in the monomeric fraction of the wild-type, but was not detected in that of the mutant strain. This result demonstrates that *psb28-1* was completely inactivated in the mutant. Except for the absence of Psb28-1, there was no significant difference in the polypeptide composition of monomeric and dimeric fractions between PSII complexes of CP47-His and CP47-His/*psb28-1*.

3.4. Growth of the psb28-1 mutant under high-temperature conditions

In a proteomic analysis [22], Psb28-1 increased in abundance by more than two-fold when *Synechocystis* sp. PCC 6803 cells were subjected to heat shock. Therefore, we examined the effect of moderately high temperatures on photoautotrophic growth. Fig. 3A shows the growth of wild-type and various mutant cells on agar plates. Cells were incubated at 30 °C or 38 °C under LL, ML, and HL conditions. The *psb28-1* mutant cells grew as well as the wild-type cells at 30 °C, but showed slight growth retardation at 38 °C under ML and HL conditions. In contrast, when the cells were grown photoheterotrophically at 38 °C under ML conditions in the presence of 5 mM glucose and 10 μg mL^{-1} 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (DCMU), the mutant cells grew normally, suggesting that the growth retardation was related to photosynthesis. As reported previously [32], the growth of *dgdA* mutant cells was retarded at 30 °C especially under HL conditions compared to wild-type cells, and this effect was more pronounced at 38 °C. The growth of *psb28-1/dgdA* double mutant cells, compared to that of *dgdA* mutant cells, was significantly suppressed under HL conditions even at 30 °C. The growth retardation of the double mutant was also observed under ML conditions at 38 °C. Similar results were obtained with cells grown in liquid medium (Fig. 3B). At 30 °C and 38 °C, no difference in growth rate was observed between the wild-type and *psb28-1* mutant cells. However, when the temperature was increased to 40 °C, the growth of *psb28-1* mutant cells became slower than that of wild-type cells under HL conditions. The *psb28-1/dgdA* double mutant cells showed lower growth rates than the *dgdA* mutant cells under HL conditions, and this effect was more pronounced at high temperatures. These data indicate that Psb28-1 is important for maintenance of viability of *Synechocystis* sp. PCC 6803 when exposed to high-intensity light at moderately high temperatures. Furthermore, these results confirm that Psb28-1 accumulated in PSII of the *dgdA* mutant is important for survival under HL and high temperature conditions.

3.5. Effects of disruption of psb28-1 on photodamage and repair of photosynthetic machinery under high-temperature conditions

To clarify whether the suppression of the growth of *psb28-1* mutant under HL conditions at moderately high temperatures was caused by photoinhibition of photosynthesis, we examined HL susceptibility of the oxygen-evolving activities in wild-type and mutant cells at 30 °C and 40 °C. Photoinhibition occurs when the rate of photodamage to the photosynthetic machinery exceeds that of the repair processes. The photodamage and repair processes of photosynthesis can be measured separately by monitoring the time course of oxygen-evolving activity when cells are exposed to intense light in the presence and absence of lincomycin, which inhibits the protein

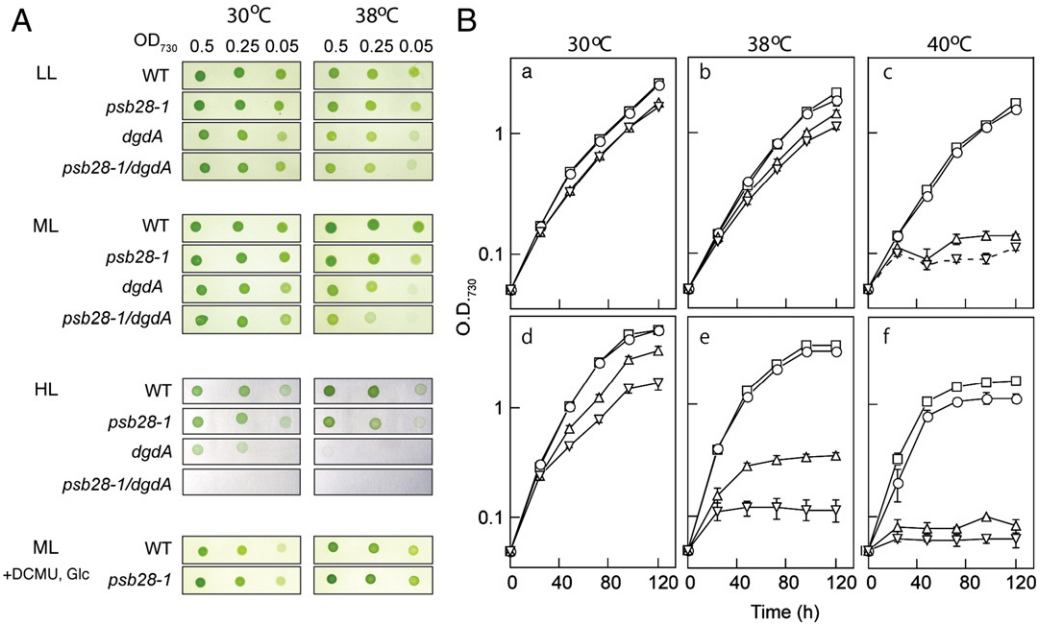

Fig. 3. Effects of high-temperature stress on growth in wild-type and mutant cells. Growth of the cells on agar plates (A) or in liquid media (B). (A) Cell suspensions adjusted to an optical density of 0.5, 0.25, and 0.05 at 730 nm were spotted onto agar plates, and then cultured for 3 days under LL (10 μmol photons m^{-2} s^{-1}), ML (30 μmol photons m^{-2} s^{-1}), or HL (100 μmol photons m^{-2} s^{-1}) conditions at 30 °C (left panels) or 38 °C (right panels). (B) The growth of wild-type (squares), *psb28-1* mutant (circles), *dgdA* mutant (triangles), and *psb28-1/dgdA* double mutant (inverted triangles) cells was determined by measuring optical density at 730 nm. The cells were grown at 30 °C (a and d), 38 °C (b and e), or 40 °C (c and f) under LL (a, b, and c, 20 μmol photons m^{-2} s^{-1}) or HL (d, e, and f, 200 μmol photons m^{-2} s^{-1}) conditions. Error bars represent SD of three independent cultures, even though they are not clearly visible in the figure because of low variation.

synthesis required for repair. At 30 °C, both wild-type and *psb28-1* mutant cells showed an HL-induced decline in oxygen-evolving activity (Fig. 4). However, the decline observed in the presence of lincomycin (Fig. 4A) was much higher than that in its absence (Fig. 4B), suggesting that inactivated photosynthetic activity is readily recovered during HL treatment. At 40 °C, in the absence of lincomycin, the rate of decline was higher in mutant than in wild-type cells (Fig. 4D), although the decline was at the same rate as wild-type cells in the presence of lincomycin (Fig. 4C). In agreement with the above results, oxygen-evolving activity declined faster in the *psb28-1*/*dgdA* mutant cells than in the *dgdA* mutant cells, even at 30 °C in the absence of lincomycin (Fig. 4B). At 40 °C, in the absence of lincomycin, *dgdA* mutant cells lost oxygen-evolving activity rapidly by HL treatment, as reported previously [26], whereas the activity loss in double mutant cells was even faster (Fig. 4D). The decline occurred at the same rate in the *dgdA* and *psb28-1*/*dgdA* mutants at both temperatures in the presence of lincomycin, as was the case in wild-type and *psb28-1* mutant cells (Fig. 4A, C). These data suggest that the photosynthesis repair process is specifically retarded by disruption of *psb28-1* without any change in the rate of photodamage.

To further focus on the photosynthesis repair process, we monitored restoration of oxygen-evolving activity under LL conditions after HL treatment (Fig. 5). The wild-type and mutant cells were pre-incubated with intense light at 30 °C in the presence of lincomycin until the oxygen-evolving activity declined to less than 20% of the original level. The activity of cells was restored upon transfer to LL conditions after washing to remove lincomycin. The restoration of activity in mutant cells was slower than in wild-type cells at 30 °C (Fig. 5A); this difference was more pronounced at 40 °C (Fig. 5B). The rate of restoration declined in the following order: wild-type, *psb28-1* mutant, *dgdA* mutant, and *psb28-1*/*dgdA* double mutant. These results clearly demonstrate that Psb28-1 is necessary for efficient repair of photosynthesis after photodamage under high-temperature conditions.

3.6. Characterization of protein complexes associated with Psb28-1

Psb28-1 was shown to be involved in the photosynthesis repair process, as described above. To obtain further information on its function, we purified protein complexes associated with Psb28-1. For purification, Psb28-1-His and Psb28-1-His/*dgdA* strains, which express Psb28-1 with a hexahistidine tag at the C terminus under the control of the native promoter, were constructed by replacing the original *psb28-1* gene with that encoding a His-tagged version (Fig. 6A). Complete segregation of chromosomal DNA was confirmed by PCR analysis (Fig. 6B) and by sequencing of the amplified DNA fragments. The protein complexes binding Psb28-1 were purified from thylakoid membranes of Psb28-1-His strains by Ni-affinity column chromatography using the same method as for purification of PSII complexes binding CP47-His [23]. The yield of the protein complexes associated with Psb28-1-His was extremely low compared to that of PSII complexes binding CP47 purified from CP47-His cells. The yield on a Chl basis of the purified protein complexes from thylakoid membranes was approximately ≤0.05% and 0.1–0.2% in the Psb28-1-His and Psb28-1-His/*dgdA* strains, respectively, but 3–4% in the case of PSII-binding CP47 from the CP47-His strain.

Purified protein complexes were analyzed by BN-PAGE, followed by separation of protein subunits of individual complexes in the second dimension by SDS–PAGE. In protein complexes purified from the Psb28-1-His strain, the most abundant was a putative assembly intermediate of PSII lacking the CP43 subunit (CP43-less monomer) (Fig. 7A). In the Psb28-1-His/*dgdA* strain, the CP43-less monomer was also the most abundant (Fig. 7B). In addition, a CP43-less dimer-like complex was observed (Fig. 7B, indicated by an asterisk). These results suggest that Psb28-1 is mainly associated with CP43-less PSII monomers. This conclusion is consistent with a previous study [13], although that report used a

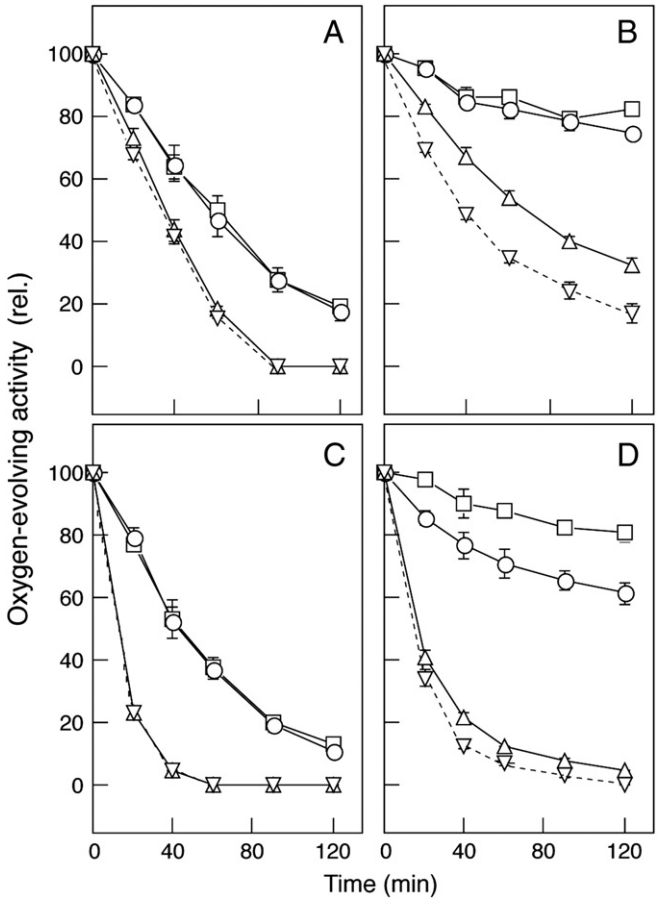

Fig. 4. Effects of high-temperature treatment under HL conditions on photosynthetic oxygen-evolving activity of wild-type and mutant cells. Wild-type (squares), *psb28-1* mutant (circles), *dgdA* mutant (triangles), and *psb28-1*/*dgdA* double mutant (inverted triangles) strains cultured under LL conditions at 30 °C were suspended in culture medium at a concentration of 10 μg Chl mL^{-1} and incubated under intense light in the presence (A and C) or absence (B and D) of lincomycin at 30 °C (A and B) or 40 °C (C and D). One-hundred percent activities representing the activities before HL treatment were 340 ± 20, 330 ± 10, 310 ± 20, and 280 ± 20 μmol O_2 $mgChl^{-1}$ h^{-1} for the wild-type, *psb28-1*, *dgdA*, and *dgdA*/*psb28-1* strains, respectively. Error bars represent SD of three independent cultures.

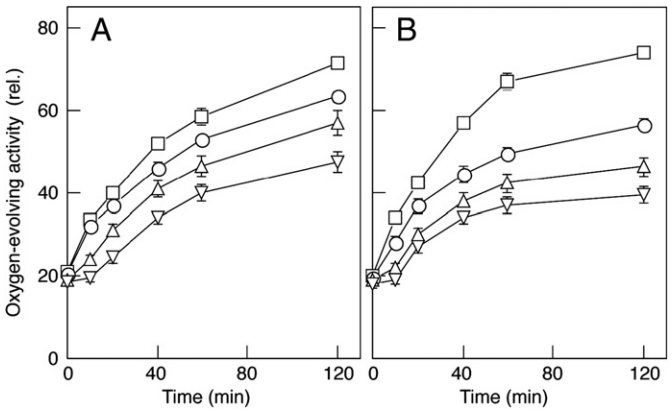

Fig. 5. Recovery of photodamaged-photosynthetic activity under low light conditions. Wild-type (squares), *psb28-1* mutant (circles), *dgdA* mutant (triangles), and *psb28-1*/*dgdA* double mutant (inverted triangles) cells that had been subjected to photoinhibitory treatment for 90 min (wild-type and *psb28-1* mutant) or 30 min (*dgdA* and *psb28-1*/*dgdA* mutants) in the presence of lincomycin were washed twice with distilled water and once with fresh BG-11, and then their oxygen-evolving activity was allowed to recover under LL (20 μmol photons m^{-2} s^{-1}) conditions. The photoinhibitory treatment and recovery were performed at 30 °C (A) or 40 °C (B). Error bars represent SD of three independent cultures.

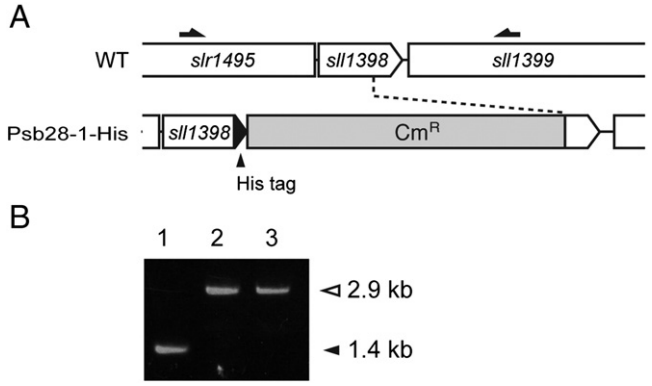

Fig. 6. Construction of Psb28-1-His strains. (A) Schematic of the addition of an His tag to the C terminus of Psb28-1. Cm^R was inserted at the 5′ site of the stop codon of the *psb28-1* gene as a selectable marker. To avoid the side effect of insertion of Cm^R into the downstream gene, a ~210 bp region of the adjacent gene (*sll1399*) including the 3′ coding region of *psb28-1* gene was inserted downstream of Cm^R. Arrows indicate PCR primers. (B) Insertion of a Cm^R confirmed by PCR analysis. PCR was performed using genomic DNA of the wild-type (lane 1), Psb28-1-His (lane 2), and Psb28-1-His/*dgdA* (lane 3) strains.

Synechocystis sp. PCC 6803 strain overexpressing Psb28-1 with the Myc-His tag at the N terminus under the control of *psbA2* promoter.

Furthermore, we performed a similar analysis using PSII complexes of the *dgdA* mutant to elucidate why Psb28-1 accumulates in this mutant. In a control sample (PSII complexes purified from CP47-His cells), most PSII complexes were dimers, and only a small quantity of monomers and CP43-less monomers were detected (Fig. 7C). On the other hand, in PSII complexes purified from CP47-His/*dgdA* cells, the ratio of monomers and CP43-less monomers to dimers was significantly increased. In addition, two smaller subcomplexes (Fig. 7D, indicated by arrows) and a CP43-less dimer-like complex (Fig. 7D, indicated by an asterisk) were also detected. These results suggest that the assembly of the PSII complex is impaired and/or its stability is reduced by depletion of DGDG in the *dgdA* mutant. The accumulation of Psb28-1 in the *dgdA* mutant was presumably due to the increased CP43-less monomer level in this mutant.

4. Discussion

To elucidate the function of Psb28 in photosynthesis, we constructed *psb28-1* and *psb28-2* deletion mutants of *Synechocystis* sp. PCC 6803 using the wild-type and *dgdA* mutant as background strains and examined the effects of the lack of Psb28 on the photosynthetic properties. Photoautotrophic growth of the *psb28-1* mutant was comparable to that of wild-type cells under normal growth conditions (Fig. 3). The oxygen-evolving activities of the mutant cells were equivalent to those of wild-type cells on a Chl basis and the Chl content of the *psb28-1* mutant cells was only slightly lower than that of wild-type cells (Table 2). The polypeptide composition of PSII complex purified from cells grown under normal growth conditions was not affected by the absence of Psb28-1 in neither the monomeric nor dimeric fractions (Fig. 2). These results clearly indicate that Psb28-1 is not essential for photosynthesis or the function of the PSII complex in *Synechocystis* sp. PCC 6803, at least under normal growth conditions. The small impact of Psb28-1 absence on PSII function is supported by the fact that Psb28-1 was absent from the crystal structure of the PSII dimer complex purified from the thermophilic cyanobacteria, *T. elongatus* [3] and *T. vulcanus* [4]. However, we found that the growth retardation occurred in the *psb28-1* mutant when cells grown in liquid medium under LL conditions at 30 °C were transferred to HL conditions at 40 °C (Fig. 3B, panel f). The HL-induced growth retardation at 40 °C observed in the *psb28-1* mutant was well correlated with the suppression of repair of photodamaged photosynthetic machinery without affecting the photodamage process (Figs. 4 and 5). In addition, Psb28-1 was associated only with intermediate PSII assembly complexes, not with functional PSII dimers (Figs. 2, 7A). These results suggest that Psb28-1 is involved in PSII repair, especially under high-temperature conditions.

Dobáková et al. [13] performed the initial characterization of *psb28-1* mutant cells, which they independently generated. They demonstrated the dispensability of Psb28-1 for photosynthesis, but their mutant seemed to have several characteristics different from ours. Their *psb28-1* mutant exhibited slower autotrophic growth and lower Chl content per cell than the wild-type grown under both LL and HL conditions, although the absence of Psb28-1 did not affect the functional properties of PSII. Irrespective of low growth rates, their *psb28-1* mutant cells showed that the oxygen-evolving activity of PSII on a Chl basis was considerably higher than that of the wild-type. This likely resulted at least partly from the decreased cellular PSI content in their mutant. In contrast, in our *psb28-1* mutant, the cellular PSI content estimated from the measurement of Chl fluorescence at 77 K, was unaffected (Supplemental Fig. 2). Therefore, we think that our mutant retains PSII activity similar to that of the wild-type. However, why the decreased PSI content does not occur in our mutant cells is not known. The growth properties also differed between the two *psb28-1* strains. In liquid culture, the wild-type and our *psb28-1* mutant cells showed similar growth rates under LL and HL conditions at 30 °C and 38 °C (Fig. 3B). To detect growth retardation in our mutant, it was necessary to increase the growth temperature to 40 °C.

Dobáková et al. [13] also showed that the photosynthetic machinery repair rate of *psb28-1* mutant cells at 30 °C was higher than that of the wild-type, with a faster D1 turnover. Conversely, in our mutant, the repair rates were slightly lower than the wild-type, even at 30 °C (Fig. 5). The reason for this difference remains unclear, but it may be due to different photoinhibitory treatments. We used light at an intensity of 2000 μmol photons m^{-2} s^{-1} for HL treatment, while Dobáková et al. used a lower intensity (500 μmol photons m^{-2} s^{-1}). Thus, it is possible that the effect of the absence of Psb28-1 on photosynthesis repair is limited until the light intensity reaches a threshold level.

In previous studies [20,21], we found that a relatively large amount of Psb28-1 accumulated in the PSII monomeric fraction from the *pgsA* and *dgdA* mutants, which are defective in the biosynthesis of PG and DGDG, respectively. Meanwhile, accumulation of Psb28 was not observed in the PSII dimeric fraction. These results suggest that Psb28-1 plays an important role in the assembly and maintenance of PSII in these lipid-deficient mutants. Therefore, in this study, we generated a *psb28-1*/*dgdA* double mutant and analyzed its photosynthetic properties. As expected, the *psb28-1*/*dgdA* double mutant exhibited a more severe phenotype than the single mutants. Even at 30 °C, the double mutant cells showed the growth retardation observed in the *psb28-1* single mutant at high temperatures. The double mutant cells showed lower photosynthetic activities (Table 2), growth retardation (Figs. 3 and 4), and increased sensitivity to HL because of inefficient photosynthesis repair (Figs. 5 and 6).

Furthermore, we demonstrated that protein complexes purified from a His-tagged Psb28-1-expressing strain mainly contained an intermediate PSII assembly complex that lacks CP43 (Fig. 7A). Dobáková et al. [13] detected a similar assembly intermediate using a strain overexpressing Myc-His-tagged Psb28-1 under the control of the *psbA2* promoter. Because in our Psb28-1-His strain the expression of Psb28-1-His was driven by the native promoter, the CP43-less complex binding Psb28-His was considered to be generated under physiological, but not artificial, conditions. The protein complexes purified from the Psb28-1-His/*dgdA* strain also mainly contained the CP43-less monomer (Fig. 7B). The yield on a Chl basis of protein complexes binding Psb28-1-His was very low compared to PSII complexes binding CP47-His purified from CP47-His strain, indicating that the PSII complex contained only a small proportion of the CP43-less monomer (Fig. 7C). In contrast, in the *dgdA* background, the yield was about four times higher than in the wild-type background. Concomitant with this increased yield, a greater accumulation of CP43-less monomer was observed in the PSII complex of the *dgdA* mutant (Fig. 7D). The correlation between CP43-less monomer complex in the

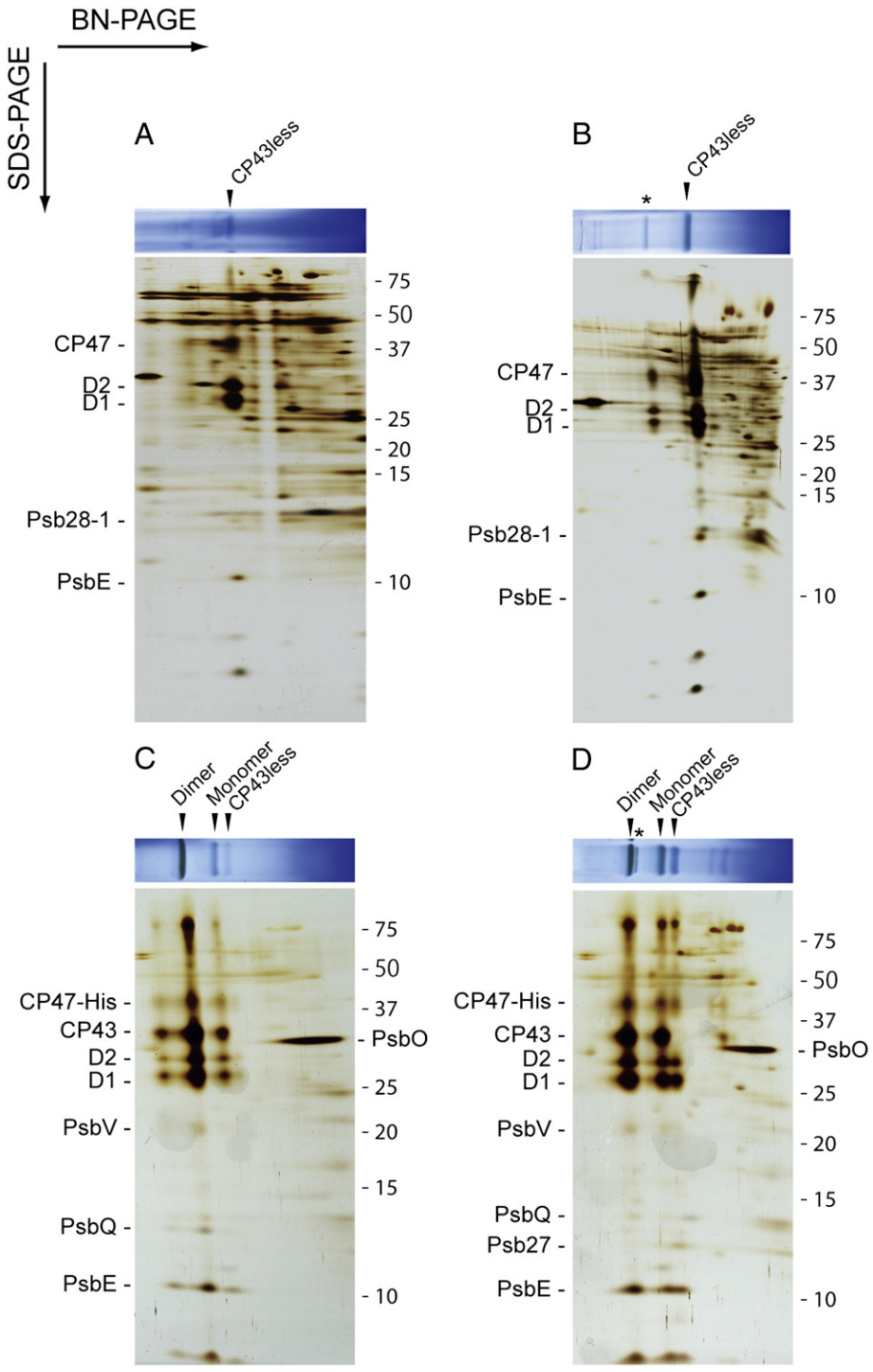

Fig. 7. BN-PAGE/SDS–PAGE analysis of purified protein complexes binding Psb28-1-His or CP47-His. Protein complexes purified from the thylakoid membranes of Psb28-1-His (A), Psb28-1-His/*dgdA* (B), CP47-His (C), and CP47-His/*dgdA* (D) cells were subjected to BN-PAGE/SDS–PAGE analysis. Protein complexes corresponding to 1.5 μg Chl (A and B) or 3 μg Chl (C and D) were loaded in each lane of BN gels. Proteins were visualized with silver staining. Asterisk and arrows indicate CP43-less dimer-like complexes and unidentified small PSII subcomplexes, respectively.

CP47-His and Psb28-1-His strains suggests a close association of Psb28-1 with the CP43-less PSII monomer.

Interestingly, the protein complexes purified from the Psb28-1/*dgdA* strain contained CP43-less dimer-like complexes (Fig. 7B, asterisk). This was also detected by BN-PAGE of the PSII complex purified from the CP47-His/*dgdA* strain (Fig. 7D, asterisk). Although this may be an aggregation of CP43-less monomers, it is also possible that the CP43-less dimer exists *in vivo* as an intermediate complex during dissociation of photodamaged PSII. Recently, a novel dimeric PSII complex containing Psb27 was found in *T. elongatus* by Grasse et al. [33]. Psb27 is thought to be associated with PSII monomer and to function in PSII complex assembly to support Mn_4Ca cluster formation in *T. elongatus* [34] and *Synechocystis* sp. PCC 6803 [35]. These findings led to an interesting hypothesis: that Psb27 might function not only in the assembly but

also in the disassociation of the PSII complex [33]. Similarly, it is possible that Psb28-1 associated with CP43-less dimers functions in the disassociation of photodamaged PSII complexes.

X-ray crystallographic analyses of dimeric PSII complexes of *T. elongatus* [3] and *T. vulcanus* [4] have identified four and three molecules of DGDG, respectively, in the region between CP43 and D1–D2 heterodimer. It can be expected that the lack of these DGDG molecules in the *dgdA* mutant impairs association of CP43 to PSII core, leading to accumulation of CP43-less monomer and inefficient PSII assembly. This assembly disorder in the *dgdA* mutant is thought to be one of the reasons for the accumulation of Psb28-1. However, whether the accumulation of Psb28-1 occurs whenever the CP43-less monomer is abundant, or if it is triggered by stresses such as high temperature, remains unknown. Because high-temperature stress is sensed by the altered fluidity of thylakoid membranes [36,37], it is possible that situations similar to high-temperature stress induce changes in membrane properties in these mutants as a result of lipid depletion. Further research is required to clarify the mechanism underlying Psb28-1 accumulation in lipid-deficient mutants.

Recently, Liu et al. [38] reported that Psb28-1 is associated with the intermediate PSII assembly complexes purified from a strain expressing His-tagged Psb27. They further showed that Psb28-1 and PsbO are not associated with assembly intermediates binding Psb27-His when the *ctpA* gene, which encodes the D1 processing enzyme, is inactivated. Consistent with this, we found that complexes binding Psb28-1-His included a small quantity of PSII monomer. Thus, although Psb27 and Psb28-1 are mainly associated with PSII monomer and CP43-less monomer, respectively, the complexes associated with these proteins partly overlap. Komenda et al. [39] have recently reported that Psb27 binds to and stabilizes unassembled CP43 and both proteins bind to CP43-less monomer during the PSII assembly pathway. It was also previously reported that Psb28-1 mainly binds to the CP43-less monomer [13]. Thus, PSII complex assembly is assumed to occur in the following order: the C-terminal extension of preD1 in CP43-less monomer is processed by CtpA, Psb28-1 and PsbO bind to the complex, CP43 together with Psb27 bind to the complex, Psb27 is released from the complex, manganese–calcium cluster and other extrinsic subunits bind to the complex, and then finally dimerization of the complex occurs to form dimer complex. Psb28-1 likely dissociates from the complex during the CP43-binding process.

The role of Psb28-1 in assembly of the PSII complex remains unclear, but one hypothesis is that Psb28-1 stabilizes CP43-less PSII monomers during assembly. It is possible that the absence of Psb28-1 has little effect under normal conditions because CP43-less monomers are quickly assembled, as suggested by the small proportion of CP43-less monomers in PSII complexes of the wild-type strain (Fig. 7C). In contrast, in the DGDG-deficient mutant, in which a larger amount of PSII exists as CP43-less monomer for longer periods due to the assembly disorder, PSII complex assembly might be impaired in the absence of Psb28-1.

5. Conclusion

In summary, we demonstrated that Psb28-1 is involved in PSII repair, particularly at high temperatures. The *psb28-1* mutant showed growth retardation under HL conditions at moderately high temperatures with a low rate of restoration of photosynthetic activities after photoinhibition. In the *psb28-1*/*dgdA* double mutant, similar phenomena were observed even at normal growth temperatures, suggesting that Psb28-1 plays an important role in photosynthesis in the *dgdA* mutant. Protein analysis using a strain expressing His-tagged Psb28-1 revealed that Psb28-1 is mainly associated with an intermediate PSII assembly complex that lacks CP43. In the *dgdA* mutant, CP43-less monomer and other intermediate PSII assembly complexes accumulated, suggesting that PSII assembly was impaired. This assembly disorder is one of the reasons for accumulation of Psb28-1. In contrast, the *psb28-2* mutant showed no significant phenotype, suggesting that Psb28-2 cannot complement the function of Psb28-1, at least under the conditions examined in this study.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbabio.2012.10.004.

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