Targeted Inactivation of the Gene psaL Encoding a Subunit of Photosystem I of the Cyanobacterium Synechocystis sp. PCC 6803*

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Photosystem I is a multisubunit pigment-protein complex that functions as a light-driven plastocyanin-ferredoxin oxidoreductase in thylakoid membranes of cyanobacteria and higher plants. A 16-kDa protein subunit of photosystem I complex was isolated from the cyanobacterium Synechocystis sp. PCC 6803. The sequence of its N-terminal residues was determined and a corresponding oligonucleotide probe was used to isolate the gene encoding this subunit. The gene, designated as psaL, codes for a protein of 16,605 Da. The deduced amino acid sequence is homologous to the subunit Psal of barley photosystem I. There are two conserved hydrophobic regions in the subunit Psal that may cross or interact with thylakoid membranes. The gene psaL exists as a single copy in the genome and is expressed as a monocistronic RNA. Stable mutant strains in which the gene psaL was interrupted by a gene conferring resistance to chloramphenicol, were generated by targeted mutagenesis. Growth and photosynthetic characteristics of a selected mutant strain under photautotrophic conditions were similar to those of the wild type, suggesting that the function of Psal is dispensable for photosynthesis in Synechocystis sp. PCC 6803. Western analysis and subunit composition of purified photosystem I revealed that the mutant strain contained other subunits of photosystem I in thylakoid membranes and in the assembled complex. When photosystem II activity was inhibited and glucose was supplied in the medium, mutant strains grew faster than the wild type. Under these conditions of growth, re-reduction of P700 in the mutant cells, but not in the wild type cells, showed a component with an uncharacteristically rapid half-time.

Photocystem I (PS I) is one of two pigment-protein complexes of the photosynthetic apparatus of cyanobacterial and plant thylakoid membranes. It accepts electrons on the luminal side of the membranes from plastocyanin or cytochrome c553 and donates them to ferredoxin present in the cytoplasm of cyanobacteria and stroma of chloroplasts. The PS I reaction center has been isolated from higher plants, algae, and cyanobacteria (1). Its function, structure, and subunit composition are all remarkably conserved during evolution (2). PS I complex typically contains at least 13 polypeptide subunits, approximately 100 chlorophyll a molecules, a pair of phylloquinones, and three Fe-S clusters (1). The PsA and PsB subunits are two homologous chlorophyll-binding polypeptides that carry the electron acceptors P700, A6, and the Fe-S center Fx1 (1, 3). The PsA protein contains secondary electron acceptors, Fa and Fb (4). The remaining subunits of PS I do not contain any electron transfer centers and are accessory in their function. The PsAD subunit has been proposed to function in the docking of ferredoxin to PS I reaction centers (5, 6). It is required for correct orientation of PsAC so that Fa is preferentially reduced (2). The PsD subunit is also essential for stable assembly of other peripheral subunits on the n-side of the photosynthetic membranes (8, 9). The polypeptide designated as PsAF is thought to regulate the interaction of plastocyanin or cytochrome c553 with P700 (10–12). The PsAE subunit is apparently not required for the linear flow of electrons through photosystem I. Cyanobacterial mutants lacking PsAE, however, show impaired cyclic electron flow (13).

The cyanobacterium Synechocystis sp. PCC 6803 provides an attractive system for studying photosynthesis. The structure of its PS I is similar to that from higher plants, but its genetic organization is simpler and can be more easily manipulated. This organism is able to take up extraneous DNA and incorporate it into the genome by homologous recombination (14), thus enabling one to mutate specific genes encoding proteins of the photosynthetic apparatus. We have embarked on a program to decipher functions of the accessory subunits of PS I through targeted mutagenesis of the genes encoding these subunits in Synechocystis sp. PCC 6803. Previously we have cloned the genes encoding PsAD, PsAE, and PsAF subunits from Synechocystis sp. PCC 6803 and subsequently generated mutants in which these genes have been mutated (15–18). This approach has allowed us to demonstrate the functions of these subunits in vivo. In the present work, we have cloned and sequenced the gene encoding subunit Psal of PS I and then generated a mutant strain in which the psal gene has been inactivated by insertion of a gene conferring resistance to chloramphenicol.
Inactivation of Cyanobacterial psaL Gene

EXPERIMENTAL PROCEDURES

Materials—Radioactive chemicals were purchased from Du Pont-New England Nuclear. All enzymes and reagents used for molecular cloning were obtained from New England Biolabs (Beverly, MA), Bethesda Research Laboratories, or Promega Biotech. Reagents for oligonucleotide synthesis were from Applied Biosystems. The majority of other chemicals and antibiotics were purchased from Sigma or Fisher Biotech. The antibody specific to the carboxyl-terminal sequence of PsaB was kindly provided by Dr. James Guikema (19). The antibodies against PsaC, PsaD, and PsaE were produced at the University of Nebraska, Lincoln Antibody Production Facility.

Isolation and Characterization of the 16-kDa Subunit from PS I—The PS I reaction center was isolated by previously described methods from the wild type strain of Synechocystis sp. PCC 6803 (15). Subunits of PS I were separated by electrophoresis on a 15% polyacrylamide gel using a mini-gel. The subunit and presequence were used as a probe for diulfide membranes (Immobilon) for determination of NH2-terminal sequences (20). The NH2 terminus of the 16-kDa subunit showed some homology to the product of the psaL gene of barley. Therefore, the 16-kDa subunit of Synechocystis sp. PS I is designated as Psal (21).

Screening and Nucleotide Sequencing—Oligonucleotide probes corresponding to the amino-terminal residues of Psal were used to screen a genomic library of Synechocystis DNA in phBlueScript-KS. The oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer (Model 381A), and phosphorylated with [32P]TP by T4 polynucleotide kinase. Plasmid DNA was isolated from positive colonies by alkaline lysis method and screened further by dot blots and Southern blots (22).

Both strands of a 700-bp Sau3A fragment containing the psaL gene were sequenced completely. Overlapping deletions of this fragment were obtained by exonuclease III digestion (23) and their nucleotide sequences determined by the dideoxy termination method (24). Nucleotide sequences were aligned and analyzed using DNAstar software.

Southern and Northern Analysis—Southern blotting was performed using 1 µg of Synechocystis genomic DNA for each restriction digestion. An 470-bp fragment containing the complete psaL gene was labeled with [32P]dCTP by random primer labeling (Promega) and the same fragment was used as a probe for Northern analysis. Total RNA was isolated from cells of Synechocystis sp. PCC 6803 (25) and 5 µg were subjected to electrophoresis on a denaturing 1.2% agarose gel containing formaldehyde and then transferred to Magnachrome (Micron Separations, Inc.) nylon membranes.

Targeted Mutagenesis of psaL—A mutant strain lacking the gene psaL was generated by transforming the wild type strain of Synechocystis sp. PCC 6803 with DNA of a clone (pK18) in which the gene psaL was interrupted by a gene conferring resistance to chloramphenicol (see “Results” for more details). Transformation was carried out according to previously described methods (14, 18). The transformants resistant to chloramphenicol were segregated for a few generations. A colony was resuspended in single culture and growth in a liquid BG11 medium. The strains with interrupted psaL were identified by PCR and confirmed by Southern analysis.

Characterization of the Strain with Inactive psaL Gene—The wild type and mutant strains were grown at 34 °C with constant shaking at 200 rpm and their growth was monitored by measuring absorbance of the cultures at 730 nm. Rates of oxygen evolution or uptake were determined with a DWI oxygen electrode unit (Hansatech Ltd., United Kingdom) at 25 °C at a light intensity of 1600 µmol m⁻² s⁻¹ photosynthetically active radiation, using cells suspended in 20 mM Tricine-NaOH (pH 8.0) or 40 mM Hepes (pH 7.0). Total photosynthetic activity was measured in the presence of 10 mM NaHCO3 and 20 mM Tricine-NaOH (pH 8.0). PS II activity was determined in the presence of 1 mM 2,6-dichloro-β-d-phenoxazone and 20 mM Tricine-NaOH (pH 8.0). PS I activity was measured as the rate of oxygen uptake via Mehler reaction in the presence of 100 µM HEPEs (pH 7.0), 50 µM DCMU, 1 mM ascorbic acid, 2 mM methyl viologen, and 1 µM DCMU. In this reaction, photosystem I activity was inhibited by DCMU while assimilated electron flow to PS I via 3,6-diaminodurene. Methyl viologen accepted electrons from PS I and donated them in turn to oxygen, thereby reducing oxygen concentration in the reaction mixture. Photosynthetic membranes from the cells of wild type and mutant strains were isolated and purified and analyzed according to previously published methods (15). Polypeptide composition of photosystem I was studied by SDS-polyacrylamide gel electrophoresis (26). Western blotting using an hanced chemiluminescence was performed using an ECL kit (Amerham).

P700 Turnover Kinetics—Oxidation and reduction of P700 was measured in whole cells of Synechocystis sp. PCC 6803 with modulated light and phase-sensitive detection (13), similar in principle to that described in Ref. 27. Modulated light was generated by mechanically chopping an 820-nm laser diode (Spindler & Hoyer DC25F) at frequency 1.7 kHz. The 50-milliwatt collimated beam was enabled to a height of 10 mm and a width of 5 mm with a laser beam expander and passed through a 5-mm wide sample cuvette with a path length of 10 mm. A colored glass band-pass filter (λ<sub>cut-off</sub> > 800 nm) was inserted between the cuvette and the detector to minimize fluorescence artifacts. The signal was detected with a photodiode (RCA 30810) operated in the photoconductive mode and demodulated with a lock-in amplifier (EG & G, Model 5101). The instrument operated with a time constant of 1 ms. The sample was excited with white light generated from a 150-watt quartz-tungsten lamp and was passed through a 700-nm cutoff filter to eliminate the near-infrared. An illumination period of 1.5 s following a dark period of 6 s permitted full activation and recovery of electron transport components. The signal-to-noise ratio was improved by averaging 64 light-dark cycles with a Nicolet 4095A Digital Oscilloscope (12-bit resolution, 500 µs/pt). The average data were transferred to a Macintosh Iic computer for curve fitting using a commercial software package ( Igor).

RESULTS

Characterization of psaL—PS I was isolated from the wild type strain of Synechocystis sp. PCC 6803. The 16-kDa polypeptide was isolated from PS I as described and an NH2-terminal sequence of AENQVQYAGNYDPVFV was determined. A genomic library was screened using a 32P-labeled oligonucleotide probe corresponding to sequence in the NH2 terminus. Positive clones were isolated, and Southern analysis indicated that the psaL gene resided in an 0.7-kilobase Sau3A fragment. Overlapping deletions in this fragment were generated by digestion with exonuclease III for sequencing by the dideoxy termination method. The nucleotide sequence of the region between the 0.7-kilobase fragment containing the psaL gene revealed an open reading frame comprising psaL gene. It is 471 bp long and encodes a protein of 157 residues. The amino acid sequence determined by chemical protein sequencing is found beginning at residue 2 of the Psal protein. Therefore, the initiator methionine encoded in the gene psaL is post-translationally removed. Lack of a lumen-targeting presequence in the protein encoded by the gene psaL indicates that the subunit Psal is not a luminal protein.

Overall, the Psal subunit of Synechocystis shows considerable homology to Psal proteins from barley (45% identity) and Synechococcus elongatus (67% identity) (Fig. 1). Furthermore, the protein from Synechocystis shows homology to mino-terminal sequences of subunits of PS I of Synechococcus sp. PCC 7002 (28) and of Synechococcus vulcanus (29) (results not shown). These results confirm the identity of the 16-kDa polypeptide from Synechocystis as the Psal subunit. Hydrophathy analysis indicated the presence of a hydrophilic NH2-terminal region followed by three potential transmembrane regions (Fig. 2). The third putative transmembrane region is highly conserved between cyanobacteria and higher plants but the first two share less homology. The length of the middle hydrophobic region in cyanobacteria is smaller than in the Psal of barley.

Southern and Northern Analysis of the psaL Gene—For Southern blot analysis, a 470-bp DNA fragment containing psaL was labeled with [32P]dCTP by random priming. This probe hybridized predominately to a single band in all restriction digests (Fig. 3, panel A). The probe hybridized with two fragments when the DNA was digested with Sty I, this enzyme recognizes a site in psaL gene. Therefore, our results indicate that psaL is present as a single gene in the genome of Synechocystis sp. PCC 6803. Hybridization of a psaL probe to total
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Synecochystis
MAE---
MAE---

Synecochoccus
MAE---
MAE---

Barley
AVSKDKPTQ
AVSKDKPTQ

FIG. 1. Comparison of the deduced amino acid sequences of the PsaL subunit of PS I from Synecochystis sp. PCC 6803 and barley. The sequences were aligned using GeneWorks, the computer program from Intelligenetics. Conservative amino acid replacements are shaded while the identical amino acids are boxed. The protein sequence determined by chemical sequencing of the 16-kDa subunit of PS I starts at amino acid 2 and is shown in italic letters. The transit sequence of the polypeptide encoded by barley psaL is not shown. Sources for sequences: Synecochystis sp. PCC 6803 (this work), barley (21) and Synecochoccus elongatus (GenBank accession No. X63763).

FIG. 2. Hydropathy profile of the deduced amino acid sequence for subunit Psal. Analysis was performed by a GeneWorks analysis program using Eisenberg algorithms (7) with a window size of 21 amino acids. The positions of the charged amino acids in Psal are indicated on the plot.

FIG. 3. Southern and Northern blot analysis of the gene psal from Synecochystis sp. PCC 6803. A, genomic Southern. One µg of DNA, isolated from the wild type strain of Synecochystis sp. PCC 6803, was digested with: EcoRI (lane 1), BamHI (lane 2), HindIII (lane 3), EcoRI + BamHI (lane 4), EcoRI + HindIII (lane 5), and BamHI + HindII (lane 6), and Styl (lane 7). The digested DNA was electrophoresed on a 0.75% agarose gel, transferred to Magnacharge nylon membrane, and probed with a DNA fragment containing psaL that had been labeled by random primer labeling. B, 5 µg of total RNA was subjected to electrophoresis in a 1.2% agarose gel containing formaldehyde, transferred to a Magnacharge nylon membrane, and hybridized with a probe specific to psaL.

FIG. 4. Construction of a clone for insertional inactivation of the psaL gene. Restriction map of cloned Synecochystis sp. PCC 6803 DNA containing the gene psaL is shown on the upper line. A DNA fragment containing the coding region of the gene psaL (middle line) was amplified by PCR and cloned in the EcoRI and XhoI sites of the polylinker of pBluescript-SK. The chloramphenicol resistance cassette with Smal ends (lower line) was isolated from pUC4C and cloned in the Stul site in psaL. The resultant clone was used for transforming the wild type cells of Synecochystis sp. PCC 6803. Numbers indicate size of the DNA fragments. The chloramphenicol resistance cassette is not drawn to scale.

RNA from Synecochystis sp. PCC 6803 revealed a single RNA species of approximately 500 nucleotides in size (Fig. 3, panel B). Considering the sizes of psaL genes (468 bp), it can be concluded that the predominant mRNA species for psaL in the total RNA in the cells is monocistronic.

Targeted Mutagenesis of psaL—Our goal was to generate a subunit-specific mutant using homologous recombination in Synecochystis sp. PCC 6803 to study the role of subunit Psal in photosystem I function. A 470-bp fragment containing Psal-coding regions was amplified by PCR. EcoRI and XhoI restriction sites were added to the end of this fragment during PCR (Fig. 4). This fragment was cloned in pBluescript-SK to yield the plasmid pK17 and was sequenced completely to ensure fidelity of PCR amplification. The plasmid pK17 was digested with: EcoRI + HindIII (lane 5), and BamHI + HindII (lane 6) and Styl (lane 7). The digested DNA was electrophoresed on a 0.75% agarose gel, transferred to Magnacharge nylon membrane, and probed with a DNA fragment containing psaL that had been labeled by random primer labeling.

3 V. P. Chitnis and P. R. Chitnis, unpublished results.
digestion with the restriction endonuclease Smal.

The resultant plasmid, pK18, was used to transform cells of Synechocystis sp. PCC 6803, and chloramphenicol-resistant transformants were selected and segregated to isolate mutant strains. Six mutant strains were characterized for most of the properties investigated in these studies. All of them were indistinguishable from each other and therefore characteristics of only one (strain ALC7–3) are presented in this paper. Fig. 5 shows the Southern blot analysis of genomic DNA from wild type and ALC7–3 strains. The genomic DNAs were digested completely with EcoRI and HindIII simultaneously, transferred to nylon membrane, and hybridized with the [32P]dCTP-labeled DNA fragments containing the psaL genes. The probe for the Psal-coding region hybridized only with a 2.7-kilobase fragment in wild type DNA but two fragments of 2.1 and 1.7 kilobases were seen in the mutant DNA (Fig. 5). Introduction of an additional EcoRI site in the mutant chromosome was expected since the gene for chloramphenicol acetyltransferase contains a site for EcoRI. Therefore the strain ALC7–3 contains only the interrupted psaL gene.

Growth of the mutant and wild type strains was monitored by measuring absorbance of cultures at 730 nm (Fig. 6). The photoautotrophic growth rate of ALC7–3 in BG11 was not significantly different from that of wild type cells (Fig. 6, triangles). Both strains grew five times faster when the medium was supplemented with 5 mM glucose; however, their growth rates did not differ from each other in that medium (Fig. 6, circles). When 10 μM DCMU, which inhibits electron transfer through PS II, and 5 mM glucose were added to the growth medium, the mutant strain grew more rapidly than the wild type (Fig. 6, diamonds). When glucose was omitted from the medium, DCMU prevented growth of wild type and mutant cells (data not shown). Table I compares the chlorophyll contents and photosynthetic characteristics of the wild type and ALC7–3 cells. The mutant cells contained about the same amount of chlorophyll (approximately 4 μg/A730) as the wild type irrespective of their conditions or stage of growth. The light-dependent oxygen evolution or uptake measurements of the cells were used as indication of the rates of photosynthetic electron transfer. Both wild type and ALC7–3 cells showed about the same rate of overall photosynthetic activity (Table I). Similarly, the activity of PS II remained unchanged in the mutant. To measure PS I activity (as the rate of oxygen uptake) in the intact cells, PS I alone (as the intact complexes isolated from the mutant and wild type cells did not differ in amount or characteristics of reaction center P700 and electron transfer centers Fx, Fa, and Fp (data not shown). PS I preparation from photosynthetic membranes of wild type and mutant strains by the Triton X-100 solubilization method (30). SDS-polyacrylamide gel electrophoresis was used to compare the polypeptide composition of PS I preparations from wild type and mutant cells (Fig. 8). The PS I preparation from ALC7–3 specifically lacked the 16-kDa Psal subunit. Both preparations contained approximately the same amounts of other subunits. Therefore PS I complex was assembled in thylakoids.

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**Table I**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Wild type</th>
<th>ALC7–3</th>
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<tbody>
<tr>
<td>Chlorophyll content (μg·A−1·mg−1)</td>
<td>4.1 ± 0.2</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>Oxygen evolution (μmol O2·mg chl−1·h−1)</td>
<td></td>
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<tr>
<td>Whole chain</td>
<td>151 ± 7</td>
<td>147 ± 6</td>
</tr>
<tr>
<td>PS II alone</td>
<td>304 ± 18</td>
<td>323 ± 26</td>
</tr>
<tr>
<td>PS I alone*</td>
<td>−116 ± 6</td>
<td>−118 ± 12</td>
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* Negative values represent oxygen uptake via Mehler reaction.

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**Fig. 5. Southern blot of genomic DNA from wild type (wt) and mutant strains (ALC7–3) of Synechocystis sp. PCC 6803.**

Genomic DNA was completely digested with two restriction endonucleases, EcoRI and HindIII, and then electrophoresed on 0.75% agarose gel, transferred to nylon membrane, and probed with [32P]CTP-labeled probes specific for the genes psaL.
of the mutant strain without subunit PsaL. Silver staining and Coomassie Blue staining of polyacrylamide gels showed PsaL as a lightly stained band in the wild type PS I. The decreased staining of PsaL compared to other subunits may be due to the low proportion of basic residues (4%) in PsaL (31) or may reflect a lower stoichiometry. However, it is less likely that PsaL is lost during isolation of PS I, because the homologous protein in barley is resistant to removal from PsaA-B by chaotropic agents (21). There were polypeptides of 40 and 45 kDa in PSI preparations from wild type and mutant thylakoids. These polypeptides reacted with antibodies against the subunits PsaA and PsaB and were probably the degradation products. Their presence in different preparations varied and did not depend on the presence or absence of the PsaL subunit.

**P700 Turnover**—In the wild type and ALC7–3 mutant *Synechocystis* sp. PCC 6803 were grown photoautotrophically, the 1/e time for P700* reduction is 23 ms (Table II). Although this corresponds to an electron transfer rate of only 40 s⁻¹, the cells are grown under conditions that favor the presence of 3–5 times as much PS I as PS II. Hence, many PS I reaction centers are being supplied by a few PS II reaction centers, and the apparent P700* reduction kinetics will appear correspondingly slower. In the presence of 10 μM DCMU, electrons are supplied only by the respiratory NAD(P)H dehydrogenase complex and by cyclic electron flow around PS I, and the 1/e time for P700* reduction increases to about 165 ms in both the wild-type and mutant strains. The addition of glucose accelerates this rate slightly to 145 ms in the wild type strain but not in the mutant; this difference is not considered meaningful. The addition of 1 mM cyanide to the DCMU-poisoned sample accelerates the 1/e time for P700* reduction in wild type cells to 121 ms due in large part to inhibition of the competing oxidase. The addition of 5 mM glucose to these samples slightly affected the rate. The same trend is followed in the ALC7–3 mutant, except for a slower rate of P700* reduction of 143 ms. The further addition of 100 μM methyl viologen removes electrons from the acceptor side of PS I, thereby inhibiting cyclic electron flow. The decrease in the 1/e time to about 300 ms in both wild type and mutant strains shows that the cyclic pathway is unaffected by the absence of PsaL (13). Dibromomethylisopropyl benzoquinone (DBMIB) at 5 μM concentration inhibits electron donation to the cytochrome b₆/f complex from PS II, the respiratory NAD(P)H dehydrogenase complex, and the cyclic pathway, and the rate of P700* reduction is slowed to a basal level of 640–680 ms in the wild type and ALC7–3 mutant strains. This rate probably represents a “leak” due to the reduction of P700* from non-physiologically significant reductants. We conclude that the absence of PsaL has no effect on cyclic electron flow around PS I, or on the linear electron flow from PS II or the NAD(P)H dehydrogenase complex.

The only difference between the wild type and ALC7–3 mutant strains was found when the cells were grown photoheterotrophically in medium supplemented with glucose and DCMU. Without added DCMU, the rate of P700* reduction in wild type cells is equal to that of photoautotrophically grown cells in the presence of added 10 μM DCMU. Although the cells were centrifuged prior to analysis to remove DCMU, it is likely that DCMU remains tightly bound to the Q₀ site during measurement. As expected, the addition of cyanide caused the reduction rate to increase and the further addition of methyl viologen caused it to decrease. However, unlike the wild type cells, the ALC7–3 mutant cells showed biphasic P700* reduction kinetics in which one-third of the P700* reduction occurred in 40 ms and the remaining two-thirds in 815 ms (Fig. 9). The addition of cyanide caused the slower reduction rate to increase to 634 ms and the addition of methyl viologen caused it to increase to 750 ms. These additions had the expected effect on the slower kinetic component, but the ratio of the slow to fast kinetic phases did not change. Both wild type and the ALC7–3 mutant strains showed identical monophasic P700* re-reduction kinetics in the presence of DBMIB. We have no adequate explanation for the biphasic kinetics in the mutant strain, nor do we understand the reason for the fast kinetic component in the absence of DBMIB.

**DISCUSSION**

The composition of the PS I reaction center and primary sequences of its subunits are remarkably conserved in cyanobacteria and higher plants. Genes *psaD* (15), *psaE* (17), *psaF* (18), *psaA-B* (32, 33), and *psaC* (34) encoding subunits of PS I of *Synechocystis* sp. PCC 6803 have been isolated and characterized. Like these genes, the gene *psaL* encodes a polypeptide that is similar to the homologous counterpart from a higher plant (Fig. 1). As expected, the PsaL of the *Synechocystis* sp. PCC 6803 does not contain obvious consensus sequences for binding of prosthetic groups. Interestingly, its hydropathy profile was similar to that of the PsaL subunit.
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Table II

<table>
<thead>
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<th>Inhibitors</th>
<th>Wild type</th>
<th>ALC7-3</th>
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<tr>
<td></td>
<td>PA1</td>
<td>PA2</td>
<td>PH</td>
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<tr>
<td>None</td>
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<tr>
<td>DCMU</td>
<td>162</td>
<td>145</td>
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<td>298</td>
<td>286</td>
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</tr>
<tr>
<td>DBMIB</td>
<td>640</td>
<td>644</td>
<td>660</td>
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</table>

<sup>a</sup>PA1, photoautotrophic growth in BG11, assay in BG11; PA2, photoautotrophic growth in BG11 supplemented with glucose (5 mM) and DCMU (10 μM).

<sup>b</sup>Sample showed biphasic kinetics; two-thirds of the P700<sup>+</sup> rereduction is represented by the slower component and one-third by the faster component.

<sup>c</sup>CN, cyanide (sodium salt); MV, methyl viologen.

Fig. 9. P700 turnover in wild type and ALC7-3 mutant strains of Synechocystis sp. PCC 6803. The cyanobacterial strains (wild type, panels A and B; mutant, panels C and D) were grown in BG11 medium (panels A and C) or in BG11 medium supplemented with 10 μM DCMU and 5 mM glucose (panels B and D). The cells were then pelleted, resuspended in BG11 medium, and used to study P700 turnover kinetics without any additions. The arrows show start of light (upward arrow) or darkness (downward arrow).

from barley (21); there are three domains that potentially could span the membrane. It is unclear whether all of these hydrophobic sequences indeed serve as transmembrane helices to anchor the protein to the membrane or, alternatively, are involved in interaction with other subunits of PS I. The PsAL subunit of barley is resistant to removal by chaotropic agents (21). The PsAL protein from intact spinach thylakoids is only partially cleaved by Pronase and partitions into the hydrophobic phase in Triton X-114 extraction experiments (35, 36). Therefore, PsAL seems to be an integral membrane protein containing as many as three transmembrane helices. The amino-terminal hydrophilic region of PsAL contains several positively charged residues. If the positive inside rule for predicting topology of membrane proteins applies to PsAL, the amino terminus of this protein may face the cytoplasmic side of the thylakoid membranes (35). Thus, the distribution of charged amino acids, the presence of hydrophobic regions, and location of the protease-susceptible site indicates that PsAL contains three transmembrane helices with the amino terminus exposed to the n-side (stromal or cytoplasmic side) and the carboxyl terminus on the p-side (luminal side) of thylakoids. Homology between subunit PsAL from cyanobacteria and a higher plant reveals some interesting features. Like other subunits of PS I, the cyanobacterial proteins lack several amino acids from the amino terminus of the mature polypeptide. These amino-terminal extensions may be required for correct and efficient proteolytic cleavage of the precursors of plant proteins by the leader peptidease. The third hydrophobic region in PsAL is highly conserved between the cyanobacterial and plant proteins while the middle region has variation in sequence and length (Fig. 1). The significance of these differences is not understood.

The conservation of the primary structure of PsAL implies similarity in its function in cyanobacterial and higher plant photosynthesis. Biochemical methods have been used to identify functions of some of the subunits of PS I (10, 12, 38). Incubation of purified PS I from barley with an antibody raised against barley PsAL does not affect NADP photoreduction (39). Generation of cyanobacterial mutant strains lacking specific proteins has been successfully used to study functions of some proteins of PS I (16–18, 33). We used a similar approach to generate mutant strains lacking the subunit PsAL of PS I. The mutant strain ALC7-3 has an interrupted psaL gene (Fig. 5) and lacks the PsAL in its PS I (Fig. 8). The lack of this subunit, however, does not affect photoautotrophic growth (Fig. 6), as well as assembly of other subunits into the thylakoid membranes (Fig. 7) or into PS I (Fig. 8). The P700 turnover kinetics in cells grown under photoautotrophic growth was also similar in wild type and mutant strains (Table II). Therefore, the absence of PsAL in photosystem I does not affect linear or cyclic electron flow during photoautotrophic growth of ALC7-3 mutant strain.

There were some differences between the wild type and mutant strains. When the cyanobacterial strains were grown under photoheterotrophic conditions in the presence of DCMU and glucose, the mutant grew faster than the wild type strain (Fig. 6). Under the same conditions the mutant cells showed differences in P700 re-reduction kinetics (Table II). The turnover of P700 in the mutant was biphasic; the faster phase was considerably more rapid than the rates of turnover in the wild type (Fig. 9). This may be responsible for the faster growth rates of mutant strains under these conditions. These results may indicate existence of an unidentified mechanism that is induced under photoheterotrophic growth conditions and can donate electrons to P700 in the absence of PsAL. This hypothesis still remains to be tested. We also observed another difference in the PsAL-less

<sup>4</sup>Y. Cohen, V. P. Chitnis, R. Nechushtai, and P. R. Chitnis, unpublished results.
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mutant. Our method of PS I isolation involves sucrose gradient centrifugation that results in isolation of PS I in two fractions; the heavier fraction presumably represents the trimeric form of PS I. During the isolation of PS I from mutant photosynthetic membranes, we did not observe the trimeric form of PS I under the conditions that resulted in resolution of trimeric and monomeric forms of PS I from the wild type membrane. This observation suggests that PsaL may play a role in trimerization of PS I. We are currently investigating this possibility.

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