Targeted Inactivation of the Gene \( psaK \) Encoding a Subunit of Photosystem I from the Cyanobacterium \( Synechocystis \) sp. PCC 6803.

Hitoshi Nakamoto\(^1 \) and Miki Hasegawa

Department of Biochemistry and Molecular Biology, Saitama University, Urawa, 338-8570 Japan

Mutant strains of the unicellular cyanobacterium \( Synechocystis \) sp. PCC 6803, in which the \( psaK \) gene was insertionally inactivated by targeted mutagenesis, were constructed. The gene is one of the two potential \( PsaK \)-coding genes which have been found as a result of the genome project with this cyanobacterium. One of the mutants was characterized in detail. A monocistronic, 480-nucleotide mRNA of \( psaK \) was absent in total RNA from the mutant cells. Inactivation of \( psaK \) had little effect on the accumulation of polypeptides in the isolated PSI complexes except for a polypeptide with an apparent molecular mass of 4.6 kDa which was absent in the mutant. The amino-terminal amino acid sequence of the 4.6-kDa polypeptide confirmed that it was the translation product of \( psaK \) and further revealed a presequence of \( PsaK \).

Characteristics of photosynthetic autotrophic growth at different temperatures, the amount of chlorophyll per cell, photosynthetic electron transport rates with various electron acceptors, the kinetics of charge recombination between P700\(^+\) and reduced \( F_A/F_B \), and the molar ratio of chlorophyll to P700, of the mutant were not significantly different from those of the wild type. Furthermore, the trimer to monomer ratio of the PSI complexes isolated from the mutant was similar to that isolated from the wild type.

Key words: Cyanobacterium — P700 — Photosystem — \( PsaK \) — \( Synechocystis \) sp. PCC 6803 — Targeted mutagenesis.

\( PSI \) is a multisubunit pigment-protein complex that consists of more than 10 different polypeptides and catalyzes light-driven electron transfer from \( Cyt c_6/plasto-cyanin \) to ferredoxin in thylakoid membranes of photoautotrophs (Golbeck 1994, Chitnis et al. 1995, Scheller et al. 1997, Hiyama 1997). The core of \( PSI \) is the heterodimer consisting of the \( PsaA \) and \( PsaB \) subunits to which most of the cofactors of \( PSI \) are bound (Golbeck 1994, Chitnis et al. 1995, Scheller et al. 1997, Hiyama 1997). The remaining cofactors, \( F_A \) and \( F_B \), are bound to \( PsaC \). Other subunits of \( PSI \) do not bind electron acceptors. Higher plants and algae contain additional subunits such as \( PsaG \), \( PsaH \), and \( PsaN \) which have not been identified in the PSI complexes from cyanobacteria (Golbeck 1994, Chitnis et al. 1995, Scheller et al. 1997, Hiyama 1997). All the subunits of \( PSI \) appear to be present in one copy per P700 (Golbeck 1994, Chitnis et al. 1995, Scheller et al. 1997, Hiyama 1997).

Trimeric \( PSI \) which may be the form in vivo in cyanobacteria, was isolated from the thermophilic cyanobacterium \( Synechococcus \) \( elongatus \) and crystallized (Röger et al. 1990, Witt et al. 1992). The structure of the trimeric \( PSI \) was analyzed at 4 Å resolution by an X-ray crystallographic method. It revealed the locations of \( \alpha \)-helices, the cofactors of \( PSI \), and Chl \( a \) molecules (Krauß et al. 1996). In the 4 Å X-ray structure model of the trimeric \( PSI \), six transmembrane \( \alpha \)-helices were located in the region facing adjacent monomers. Three of them defining the connection domain near the trimer axis were assigned to \( PsaL \) and \( Psal \) (Krauß et al. 1996) since deletion mutant studies indicated \( Psal \) to be directly engaged in trimerization of \( PSI \) and \( Psal \) to be involved in the proper organization of \( Psal \) (Chitnis and Chitnis 1993, Xu et al. 1995). The other two \( \alpha \)-helices were assigned to \( PsaK \) (Krauß et al. 1996) which contains two transmembrane, \( \alpha \)-helical regions (Golbeck 1994). The remaining one was assigned to \( Psam \) (Krauß et al. 1996). \( Psak \) and \( Psam \) are the two subunits which were removed together from the sulfobetain 12-extracted monomers of \( PSI \), which led to the suggestion that \( PsaK \) is located close to \( Psal \) (Jekow et al. 1995, Krauß et al. 1996). \( Psak \) from spinach may be tightly associated with the \( PsaA/PsAB \) heterodimer, since \( Psak \) was not removed along with other polypeptides of \( PSI \) from the heterodimer upon either heat treatment in the presence of ethylene glycol or treatments at high SDS concentrations (Hoshina et al. 1989, Wynn and Malkin 1990). However, \( Psak \) from spinach and pea was depleted from PSI when PSI was prepared by Triton X-100 treatment (Ikeuchi et al. 1990). Recently, \( Psak \) was shown to be closely located to light-harvesting complex I (Jansson et al. 1996).

Several cyanobacteria are naturally competent and incorporate foreign DNA into their genomes by homologous recombination (Golden et al. 1987, Thiel 1994), thus enabling mutation of a specific gene. Among these cyanobacteria, the unicellular cyanobacterium, \( Synechocystis \) sp. PCC 6803, has been used extensively for studies of photo-
synthetic reaction centers (Golbeck 1994, Chitnis et al. 1995), because it grows heterotrophically with glucose. All the genes encoding the subunits of PSI except psaK and psaM have been inactivated individually or together with another gene by targeted megagenesis with *Synechocystis* sp. PCC 6803 (Chitnis et al. 1989a, b, 1991, 1993, Smart et al. 1991, Rousseau et al. 1993, Shen et al. 1993, Smart and McIntosh 1993, Xu et al. 1994, 1995, Nakamoto 1995) to analyze the effect of a mutation of a specific gene of PSI in vivo.

Recently, psaK from the thermophilic cyanobacterium *Synechococcus elongatus* was inactivated and the gene was shown to be non-essential for phototrophic growth and for the trimerization of the PSI complex in this cyanobacterium (Mühlendorff and Chauvat 1996). However, it remains unknown whether Psak is dispensable for other structural and functional roles.

In the present work, we generated Psak-less mutants with *Synechocystis* sp. PCC 6803 through targeted-megagenesis and performed detailed characterization of the mutants.

**Materials and Methods**

Cyanobacterial strain and culture—A glucose-tolerant, wild type strain of *Synechocystis* sp. PCC 6803 was kindly provided by Dr. Masahiko Ikeuchi. The wild type and mutant cells were grown at 30°C under a light intensity of 30 μE m⁻² s⁻¹ in liquid BG-11 medium (Rippka et al. 1979) or on BG-11 plates containing 1.5% (w/v) agar and 0.3% (w/v) sodium thiosulfate. The BG-11 was modified to contain 5 mM TES-NaOH (pH 8.0) and 50 mg liter⁻¹ Na₂CO₃. The liquid culture was continuously aerated.

DNA manipulations, PCR, and DNA sequencing—Mini-preparation of plasmid DNA, restriction enzyme digestion, ligation, and agarose gel electrophoresis were performed according to Sambrook et al. (1989) and manufacturer's instructions. PCR was performed as described previously (Nakamoto 1995). DNA sequencing was carried out by using an AutoRead Sequencing Kit (Pharmacia, Uppsala, Sweden) and a DNA sequencer (DSQ-1, Shimadzu, Kyoto, Japan).

Probe preparation—A 955-bp DNA fragment containing ssr0390 was amplified by PCR and labeled with [α³²P]dCTP (3,000 Ci mmol⁻¹; Amersham International plc, Little Chalfont, U.K.) by the multiprime labeling method as directed by the manufacturer (Amersham), purified through a NICK column (Pharmacia), and then used as a probe for the Southern and Northern blot analyses.

Isolation of genomic DNA from *Synechocystis* sp. PCC 6803 and Southern blot analysis—Genomic DNA was isolated from the wild type and mutant cells by the 'total DNA miniprep' method of Golden et al. (1987). Southern blot analysis was performed using 1.5 μg of DNA completely digested with several restriction enzymes. Restriction fragments were separated on a 1.0% (w/v) agarose gel, transferred to a BA-S 83 nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) by a capillary transfer method (Sambrook et al. 1989), and then cross-linked to the membrane by ultraviolet illumination (FUNA-UV-Linker PS-800, Funakoshi, Tokyo, Japan). Prehybridization with the membrane was performed in a solution containing 6 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate), 5 × Denhardt's solution (Sambrook et al. 1989), 0.1% (w/v) SDS for 1 h at 65°C. Hybridization was done in a solution of the same constituents containing 100 μg of denatured salmon sperm DNA (Wako Pure Chemical, Osaka, Japan) per ml and the denatured DNA probe at 65°C overnight. After hybridization, the membrane was washed in 2 × SSC containing 0.5% (w/v) SDS at room temperature for 5 min, and then twice in 2 × SSC containing 0.1% (w/v) SDS at 42°C for 30 min. The hybridization signals were detected with a BAS1000 Mac bio-imaging analyzer (Fuji Film, Tokyo, Japan).

Preparation of total RNAs from *Synechocystis* sp. PCC 6803 and Northern blot analysis—Exponentially growing cells from a 70-ml culture were collected by centrifugation and resuspended with 3 ml of lysis buffer [30 mM Na-acetate (pH 5.5), 1% (w/v) Sarkosyl, and 1 mM EDTA]. After addition of 3 ml of phenol prewarmed at 60°C, the mixture was incubated at 60°C for 15 min with gentle shaking. After centrifugation, the aqeous phase was extracted with chloroform. After repeating the chloroform-extraction procedure several times, the total RNA was precipitated by adding one-tenth volume of 3 M Na-acetate (pH 5.5) and 2 volumes of ethanol to the aqueous phase, then incubated at −80°C for 30 min. After centrifugation, the pellet was dissolved in 10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA. 30 μg of the total RNA was electrophoresed on a denaturing 1.0% (w/v) agarose gel containing 6.6% (w/v) formaldehyde. Northern blotting and hybridization were performed as described above for Southern blot analysis. After hybridization, the membrane was washed in 6 × SSC at room temperature for 5 min, and then twice in 6 × SSC at 42°C for 30 min. The sizes of mRNA were determined by using an RNA ladder (Gibco-BRL, Tokyo, Japan). The hybridization signals were detected as mentioned above for Southern blot analysis.

Separation of monomeric and trimeric forms of PSI by sucrose-gradient centrifugation— Cultures (500 ml) of both the wild type and mutant strains were phototrophically grown in BG-11 medium. Cells in logarithmic phase growth were harvested from each culture and suspended in 5 ml of 50 mM Tris-HCl (pH 8.0) containing 0.4 M Sucrose, 10 mM NaCl, 100 μM phenylmethylsulfonyl fluoride, and 5 mM benzamidine. The cells were disrupted with a French pressure cell (American Instrument Company, Silver Spring, MD, U.S.A.) at 138 MPa for 2 times to isolate thylakoid membranes. After washed once with the above lysis buffer, the membranes containing 320 μg (the wild type) or 270 μg (the AKK6 mutant strain) Chl were suspended in 1 ml of 50 mM Tris-HCl (pH 8.0), 10 mM NaCl, and 0.4 M sucrose. The membrane suspension was incubated at 4°C for 15 min with dodecyl-beta-D-maltoside (1 : 15 Chl/detergent (w/v) ratio), centrifuged for 15 min at 20,000 × g at 4°C with a Hitachi S100AT5 fixed angle rotor and a Hitachi preparative ultracentrifuge (model CS120FX, Hitachi Koki, Tokyo, Japan). The supernatant was concentrated by ultrafiltration over a Molcut II membrane (Catalogue number UFP1 TGC 24, Millipore Co., Bedford, MA, U.S.A.) and then diluted to decrease the sucrose concentration. The solubilized membranes (300 μl) from the wild type or the mutant were layered onto a 3 ml of 10–30% sucrose gradient solution containing 20 mM MOPS-KOH (pH 7.0) and 0.01% (w/v) dodecyl-beta-D-maltoside and then centrifuged for 4 h at 200,000 × g at 4°C with the above rotor and ultracentrifuge.

**SDS-PAGE and amino-terminal amino acid sequence analysis**—Polypeptide composition of PSI was studied by SDS-PAGE (Ikeuchi and Inoue 1988), using samples treated with 2% (w/v) SDS, 50 mM Tris-HCl (pH 6.8), 0.3 M succrose, and 5% (v/v) 2-mercaptoethanol for 20 min at room temperature prior to appli-
cation to the gel. After electrophoresis, the gel was silver-stained (Oakley et al. 1980). Amino-terminal amino acid sequence analysis was performed as previously described (Nakamoto 1995).

Spectroscopy—Absorbance changes of P700 induced by red-light flash were measured at room temperature according to Hiyama (1985). The reaction mixture (1 ml) contained besides a sample: 100 mM Tris-HCl (pH 8.0), 0.05% (w/v) Triton X-100, 1 mM ascorbic acid, and 100 μM N,N,N,N-tetramethyl-p-phenylenediamine. The extinction coefficient of P700 determined by Hiyama and Ke (1972) was used to estimate the amount of P700.

Measurement of photosynthetic activities—Rates of oxygen evolution and uptake were determined with a DW1 oxygen electrode unit (Hansatech Ltd., Norfolk, U.K.) at 30°C at a photosynthetic photon flux density of 1.2 mmol m⁻² s⁻¹, using cells suspended in 20 mM Tricine-NaOH (pH 8.0). Oxygen evolution was measured in the presence of 10 mM NaHCO₃. Uptake of oxygen was measured either in the presence of 3 mM KCN and 1 mM methylviologen or in the presence of 1 mM 1,4-benzoquinone and 1 mM K₃Fe(CN)₆.

Chlorophyll determination—Chlorophyll was determined as described by MacKinney (1941).

Results and Discussion

Two orfs assigned as potential psaK genes in Synechocystis sp. PCC 6803—Recently, the entire genome of the unicellular cyanobacterium Synechocystis sp. PCC 6803 has been sequenced (Kaneko et al. 1996). In the sequence information on the cyanobacterial genome accessible through the database Cyanobase, there are two orfs, sll0629 and ssr0390, which are assigned as psaK.

Deduced primary sequences of the two orfs did not show a high degree of homology (42% overall identity) and were significantly different in size (128 amino acids for sll0629 and 86 amino acids for ssr0390) (Fig. 1). The sequence deduced from sll0629 contains an additional extension of 40-amino acid residues at the amino-terminus.

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<td>PDT—TWTPSGLVVLCNLFIALGRYAIQSRGKGPGPLIALPFE—G</td>
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<td>A. variabilis P—LEWSPTIIGMIANGVIAITFGRQTI</td>
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Fig. 1 Alignment of the sequences of Psak from Synechocystis sp. PCC 6803 (sll0629 and ssr0390), Synechococcus sp. (Mühlenhoff et al. 1993), Synechococcus vulcanus (Ikeuchi et al. 1991), and Anabaena variabilis ATCC 29413 (Ikeuchi et al. 1991). The first three sequences were deduced from nucleic acid sequences of the psaK genes and the sequences for S. vulcanus and A. variabilis were chemically determined. The amino-terminal sequence determined by protein sequencing of the Psak polypeptide encoded by ssr0390 is underlined. Residues that are conserved in all organisms are indicated by the asterisks below the last line. Hyphens indicate insertions/deletions created to optimize the sequence homology. The total number of amino acid residues for each polypeptide and the amino acid overall identity (%) are shown at the end of each sequence. The sequences were aligned and analyzed by using Genetyx Software, ver. 8.0 (Software Developing Co., Tokyo).
which is absent in amino acid sequences deduced from ssr0390 and psaK from other cyanobacteria (Fig. 1). Inspection of Fig. 1 indicates that all the PsAK polypeptides contain a highly conserved carboxyl-terminal region, two hydrophobic segments, and positively charged residues (i.e., arginine and lysine) between the two hydrophobic segments. Based upon the positive-inside rule (von Heijne and Gavel 1988) which predicts the abundance of arginine and lysine residues in the non-translocated segments of integral membrane proteins, the segment located between the two apolar segments of PsAK is suggested to be present in the cytoplasm. If this prediction is correct, the amino-terminus of PsAK should be directed into the lumenal side of the thylakoid membranes. The presence of an amino-terminal presequence in the translation product of ssr0390 as shown later supports this idea.

Targeted mutagenesis of ssr0390—The presence of two potential PsAK-coding genes in Synechocystis sp. PCC 6803 is peculiar since the PSI complexes prepared from Synechocystis sp. PCC 6803 contained only one kind of PsA in addition to all the other subunits of PSI (Ikeuchi et al. 1992). Only one kind of the PsA polypeptide has been identified in the PSI complexes isolated from various cyanobacteria such as Synechoccocus vulcanus, Synechoccoccus elongatus, and Synechococcus sp. PCC 7002 (Ikeuchi et al. 1992). The amino-terminal amino acid sequence of PsA from Synechocystis sp. PCC 6803 corresponded to that deduced from the nucleotide sequence of ssr0390 (Ikeuchi, M, personal communication). Thus, we decided to construct a ssr0390-disrupted mutant in order to study the role of PsA in PSI function. With two oligonucleotide primers, 5'-ACCGTATCATTGCTTTGCG-3' corresponding to the 'sense' strand of the Synechocystis sp. PCC 6803 genomic DNA and 5'-TAAGACCAA-CC-3' corresponding to the 'antisense' strand of the Synechocystis sp. PCC 6803 genomic DNA, a 956-bp DNA fragment starting from 380-bp upstream of the initiation codon of ssr0390 was amplified from the genomic DNA by PCR and then cloned in pGEM-T vector (Promega, Madison, WI, U.S.A.) to yield pGEMK. The accuracy of the polymerase reaction was confirmed by sequencing the PCR product. pGEMK was treated with MscI, which removes most of the structural gene (residues 6 to 70, see Fig. 1). A kanamycin-resistant gene cassette which was isolated as an EcoRI fragment from plasmid pUC4K (Pharmacia) and blunt-ended by using the Klenow fragment of DNA polymerase I was inserted into the restricted pGEMK to yield pGEMK. Recombination analysis revealed the generation of two kinds of constructs. In one construct, a kanamycin-resistant gene was inserted in the forward direction (the same gene-orientation as the disrupted ssr0390) and in the other one, the gene was inserted in the reverse direction. Both constructs were used to transform cells of Synechocystis sp. PCC 6803 through homologous recombination, and kanamycin-resistant transformants were selected and segregated for a few generations by a single colony selection on BG-11 agar plates containing kanamycin (25 μg ml⁻¹) to isolate mutant strains. Southern blot analysis of the genomic DNA from

![Fig. 2](https://academic.oup.com/pcp/article-abstract/40/1/9/1901204/104)
Gene-targeting in photosystem I

the wild type and mutant strains was carried out with the radio-labeled 956-bp PCR fragment as a probe. The size and the number of the restricted DNA fragments hybridized with the probe were as expected (Fig. 2) as the kanamycin-resistant gene cassette was introduced into the ssr0390 locus of all the copies of the mutant chromosome. In the following, characteristics of one (AKK6) of the clones in which kanamycin-resistant genes were inserted in the forward direction are presented.

To examine the expression of ssr0390 in the wild type and to confirm the inactivation of ssr0390 in the AKK6 mutant strain, Northern blot analysis with total RNA from the wild type and mutant strains was performed. The transcript of \textit{psaK} from a cyanobacterium has never been analyzed. A single signal, corresponding to a 480-nucleotide RNA, was detected in the wild type strain (Fig. 3, Lane 1). The uppermost band designated by G was due to contaminating genomic DNA (see Fig. 3 legend). Therefore, we could prove that ssr0390 is indeed expressed in \textit{Synechocystis} sp. PCC 6803. Considering the sizes of ssr0390 (261 bp) and the neighboring open reading frames analyzed by Cyanobase, it can be concluded that the mRNA for the \textit{psaK} gene (ssr0390) is monocistronic. The mRNA was not detected in the AKK6 mutant strain (Fig. 3, Lane 2), indicating that the expression of \textit{psaK} is completely inhibited in the mutant strain.

\textit{Characterization of the PsaK-less mutant}—Based on its proximity to the 3-fold axis in the trimeric PSI, PsaK was previously proposed to be responsible for the organization of monomers of PSI into the trimeric form (Krauss et al. 1993). Thus, we investigated the effect of the absence of PsaK on the trimeric structure of PSI with the AKK6 mutant strain. After sucrose-gradient ultracentrifugation of the thylakoid membranes from the wild type solubilized with dodecyl-\(\beta\)-d-maltoside, the pigmented complexes of the photosynthetic membranes were resolved into three distinct bands (Chitnis and Chitnis 1993), the upper orange band containing carotenoid-proteins and the middle and lower green bands containing Chl. The middle green band contained PSII and monomers of PSI and the lower one contained trimers of PSI. Chitnis and Chitnis (1993) showed that the thylakoid membranes from the mutant strains lacking PsaL did not produce the lower green band and thus concluded that PsaL is required for the formation of PSI trimers. We employed their method to resolve the monomeric and trimeric forms of PSI from the wild type and AKK6 mutant strains. Samples from both strains produced three distinct bands as they reported (Fig. 4). There was no apparent difference in the trimer to monomer ratio between the wild type and mutant strains. The lower green band did not contain the PsaK polypeptide encoded by ssr0390 as shown in Fig. 5, suggesting that PsaK is not necessary for trimerization of PSI. Recently, Mühlénhoff and Chauvat (1996) reported that PsaK is not essential for the trimerization of PSI in the thermophilic cyanobacterium \textit{Synechococcus elongatus}. They separated trimers of PSI from its monomers by gel filtration chromatography. Therefore, two independent results which were obtained with different cyanobacterial strains by different methods indicate that PsaK is dispensable for trimerization of PSI.

Polypeptides in the middle and lower green bands
separated by the sucrose-gradient centrifugation described above were analyzed by SDS-PAGE. The polypeptide profiles of the middle and lower bands of the wild type were essentially the same as those of the AKK6 strain except for a polypeptide with an apparent molecular mass of 4.6 kDa (Fig. 5, Lanes 1 and 2). The 4.6-kDa polypeptide was absent in the lower band from the mutant strain. The amino-terminal amino acid sequence of the 4.6-kDa polypeptide was chemically determined as TAV-PATLSWSPKVAGVMIA, confirming that it was indeed the translation product of ssr0390. Therefore, PsaK was absent in the trimeric PSI from the mutant strain. Fig. 5 also showed that the absence of PsaK did not affect the assembly of other proteins into thylakoid membranes. Comparison of the amino-terminal amino acid sequence of the 4.6-kDa polypeptide with the sequence deduced from ssr0390 revealed that the amino-terminal amino acid residues from 1 to 7, MHSFLLA, were absent in the mature polypeptide. Thus, the translation product of ssr0390 contained a cleavable presequence. The presequence is hydrophobic and its carboxy-terminal end has the sequence, Leu-Leu-Ala, which resembles the carboxy-terminal end of the eleven amino acids long presequence of the Psal protein from Anabaena variabilis ATCC 29413, Ile-Leu-Ala (Sonoike et al. 1992). Sonoike et al. (1992) suggested that the presequence in Anabaena Psal might be involved in targeting its amino-terminus to the thylakoid lumen. This could also be true for the PsaK polypeptide encoded by ssr0390. A predicted molecular mass of the mature PsaK polypeptide (79 amino acid residues) is 7,844 Da which deviated strongly from the apparent molecular mass determined by SDS-PAGE, 4.6 kDa. The migration of the PsaK polypeptides from other organisms in SDS-PAGE system was also anomalous. For example, Franzen et al. (1989) reported a molecular mass of PsaK from Chlamydomonas reinhardtii based on SDS-PAGE of approximately 3 kDa, although the deduced amino acid sequence of the mature protein indicates a molecular mass of 8.4 kDa.

When the PSI complexes in the lower band prepared from the wild type and AKK6 mutant strains by the sucrose-gradient centrifugation were excited by a flash of red light, absorption decrease due to the photo-oxidation of P700 was observed at 430 nm. The electron from P700 is transferred to F$_{A}$/F$_{B}$ through A$_{0}$, A$_{1}$, and F$_{X}$. The reduced F$_{A}$/F$_{B}$ re-reduces P700$^{+}$ with a half time of about 50 ms (Hiyama 1985). The kinetics and half time (see also Table 1) for the P700 re-reduction after the flash-photolysis were the same in the wild type and mutant PSI (Fig. 6). Therefore, the PSI complexes from the mutant and wild type cells did not differ in the characteristics of electron transfer from P700 to F$_{A}$/F$_{B}$.

Other aspects of the mutant phenotype are summarized in Table 1. The phenotype of the AKK6 mutant strain was similar to that of the wild type (Table 1). However, the following minor differences were observed. The mutant contained a slightly reduced amount of Chl per cell as compared with the wild type. The 4 Å X-ray structure model suggested the association of a few Chl molecules with PsaK (Kraufi et al. 1996). Thus, the loss of PsaK from PSI complexes may lead to a small reduction of Chl per PSI. The total Chl content per cell would be also reduced if there is no change in the number of the PSI complexes per cell. However, the Chl to P700 ratio in PSI from the mutant was not lower than that from the wild type (Table 1), suggesting that the decrease in Chl content is not due to the reduction of Chl per PSI. We speculate that PsaK may stabilize PSI in a cell and the absence of PsaK may cause a slight decrease in the PSI content in a cell. It is peculiar that the electron transport activity of PSII in the mutant cells as measured in the presence of 1,4-benzoquinone and K$_{3}$Fe(CN)$_{6}$ slightly decreased as compared to that in the

![Fig. 5 Polypeptide compositions in the lower (lanes 1 and 2) and middle (lanes 3 and 4) green bands separated by sucrose-gradient centrifugation after detergent-solubilization of the wild type (lanes 1 and 3) and the AKK6 mutant (lanes 2 and 4) thylakoids. A sample containing 5 µg of protein was loaded in each lane and fractionated on a 16-22% polyacrylamide gel containing SDS and urea. After electrophoresis, the gel was silver-stained. The arrow indicates the 4.6-kDa polypeptide which was identified as PsaK by amino-terminal amino acid sequence analysis. The subunits labeled in the figure have been identified by amino-terminal amino acid sequence analysis.](https://academic.oup.com/pcp/article-abstract/40/1/9/1901204)

![Fig. 6 Absorption change at 430 nm of the isolated PSI trimer (the lower green band shown in Fig. 4) from the wild type (WT) and the mutant (AKK6) cells. The reaction mixture (1 ml) contained the isolated PSI trimer from either the wild type (4.6 µg Chl) or mutant (4.3 µg Chl).](https://academic.oup.com/pcp/article-abstract/40/1/9/1901204)
Table 1 Characteristics of the wild type and the mutant strain AKK6 of *Synechocystis* sp. PCC 6803

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<th>Characteristics</th>
<th>Wild type</th>
<th>AKK6</th>
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<tr>
<td>Chlorophyll content of a cell (µg ml(^{-1}) A(_{730}))</td>
<td>4.49 ± 0.27 (n = 6)(^{a})</td>
<td>4.18 ± 0.34 (n = 6)(^{a})</td>
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<td>Oxygen evolution in the presence of 10 mM NaHCO(_3) (µmol O(<em>2) ml(^{-1}) A(</em>{730}) h(^{-1}))</td>
<td>1.60 ± 0.09 (n = 8)(^{b})</td>
<td>1.63 ± 0.17 (n = 8)(^{b})</td>
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<td>Oxygen uptake in the presence of 3 mM methylviologen and 1 mM KCN (µmol O(<em>2) ml(^{-1}) A(</em>{730}) h(^{-1}))</td>
<td>1.34 ± 0.09 (n = 6)(^{b})</td>
<td>1.21 ± 0.27 (n = 6)(^{b})</td>
</tr>
<tr>
<td>Oxygen evolution in the presence of 1 mM 1,4-benzoquinone and 1 mM K(_2)Fe(CN)(_6) (µmol O(<em>2) ml(^{-1}) A(</em>{730}) h(^{-1}))</td>
<td>1.70 ± 0.07 (n = 5)(^{c})</td>
<td>1.51 ± 0.11 (n = 5)(^{c})</td>
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<td>Chl (mol) per P700 (mol) of isolated trimeric PSI preparation(^{d})</td>
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<td>experiment 2</td>
<td>124</td>
</tr>
<tr>
<td>Half time (ms) for the reduction of the photooxidized P700 of isolated trimeric PSI preparation(^{d})</td>
<td>60.6 ± 5.1 (n = 5)(^{c})</td>
<td>61.5 ± 4.4 (n = 5)(^{c})</td>
</tr>
</tbody>
</table>

Data, except those for Chl per P700, are shown as the mean values with standard deviations. In a parenthesis, the number of total replicates of at least two separate experiments which were performed on different days using different cultures in logarithmic growth phase is shown.

\(^{a}\) The mean value obtained from 6 separate experiments using different cultures.

\(^{b}\) The mean value obtained from 3 separate experiments using different cultures.

\(^{c}\) The mean value obtained from 2 separate experiments using different cultures.

\(^{d}\) The lower green band shown in Fig. 4.

... wild type (Table 1). We cannot expect a physical interaction of PsaK with PSII since the structural model of PSI indicates that PsaK is located in the region facing adjacent monomers of PSI. Growth of the mutant and wild type strains was monitored by measuring apparent absorbance of cultures at 730 nm. The photoautotrophic growth rates at 22 and 30°C of the mutant in BG-11 were not significantly different from those of the wild type (Fig. 7).

The present findings suggest that the function of PsaK is dispensable for growth, photosynthesis, and the formation of PSI trimers in *Synechocystis* sp. PCC 6803, although it is still possible for PsaK to play a dispensable unknown role.

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**References**


Gene-targeting in photosystem I


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