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Directed mutagenesis of an iron-sulfur protein of the photosystem I complex in the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413

(photosynthesis/*psaC* gene/electron transport/gene replacement/EPR spectroscopy)

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ABSTRACT **In oxygenic photosynthetic organisms the PSI-C polypeptide, encoded by the *psaC* gene, provides the ligands for two [4Fe-4S] centers, F_A and F_B, the terminal electron acceptors in the photosystem I (PSI) complex. An insertion mutation introduced in the *psaC* locus of the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413 resulted in the creation of a mutant strain, T398-1, that lacks the PSI-C polypeptide. In medium supplemented with 5 mM fructose, the mutant cells grew well in the dark. However, when grown in the same medium under light, the doubling rate of T398-1 cells was significantly decreased. In intact cells of T398-1, bicarbonate-dependent whole-chain electron transport (PSII and PSI) could not be detected, although partial electron transport reactions involving either one of the two photosystems could be measured at significant rates. The low-temperature EPR signals attributed to the [4Fe-4S] centers F_A and F_B were absent in the mutant cells. Chemical titration measurements indicated that the ratios of chlorophyll to the primary donor P700 were virtually identical in membranes from the wild-type and mutant cells. Moreover, room-temperature optical spectroscopic analysis of the thylakoid membranes isolated from T398-1 showed flash-induced P700 oxidation followed by dark rereduction, indicating primary photochemistry in PSI. Thus stable assembly of the reaction center of PSI can occur in the absence of the Fe-S cluster cofactors F_A and F_B. These studies demonstrate that *Anabaena* 29413 offers a useful genetic system for targeted mutagenesis of the PSI complex.**

Photosystem I (PSI) is a membrane-bound pigment–protein complex that mediates electron transfer from reduced plastocyanin and cytochrome *c*553 to ferredoxin. Biochemical and biophysical analyses of thylakoid membranes and isolated PSI particles from cyanobacteria and higher plants have helped in formulating the functional organization of redox intermediates involved in the transfer of electrons from P700, the reaction-center chlorophyll(s) (Chl) of PSI, to ferredoxin. Five different electron-transfer intermediates—namely, A_0, A_1, F_X, F_A, and F_B—are known to be involved in electron transfer from P700 to ferredoxin (reviewed in ref. 1). A_0 and A_1 are believed to be a monomeric Chl and a phylloquinone, respectively, whereas F_X, F_A, and F_B are all iron-sulfur (4Fe-4S) clusters (1, 2). The apoproteins of these cofactors have been identified. According to the current model, P700, A_0, A_1, and F_X are coordinated in the heterodimer of PSI-A and PSI-B polypeptides, whereas the iron-sulfur centers F_A and F_B are located on a single 9-kDa polypeptide (PSI-C), the product of the *psaC* gene (1).

In recent years, site-directed mutagenesis has been used to create targeted mutations in various protein components of the photosystem II (PSII) complex of the unicellular cyanobacterium, *Synechocystis* sp. PCC 6803 (reviewed in ref. 3). These mutants are capable of photoheterotrophic growth in the absence of PSII-mediated electron transport activity. Similar molecular genetic analysis is expected to provide valuable information regarding the function of various proteins of PSI. However, employing the strategy used for the PSII complex to carry out site-specific mutagenesis of genes encoding PSI polypeptides has, so far, been unsuccessful in creating any mutant that completely lacks PSI activity. For example, in *Synechocystis* 6803, the *psaD* and *psaE* genes, encoding PSI-D and PSI-E, two peripheral proteins of PSI, have been insertionally inactivated (4, 5). The mutant strain lacking the PSI-E polypeptide shows only a minor difference in growth under photoautotrophic condition, and its PSI activity is comparable to that of wild type. The *psaD* deletion mutant also shows photoautotrophic growth, albeit at a reduced rate. However, attempts to mutagenize the genes encoding the two reaction-center polypeptides, PSI-A and PSI-B, the products of *psaA* and *psaB* genes in the unicellular cyanobacterium *Synechococcus* sp. PCC 7002, have been unsuccessful (6, 7). The cells carrying mutagenized *psaA* and *psaB* genes also maintain copies of the wild-type genes. Failure of these alleles to segregate has been interpreted as an indication that cells lacking functional PSI are not viable.

Our research objective is to develop an experimental system for "reverse genetic" analysis of various PSI proteins. Toward this goal, we have chosen *Anabaena variabilis* ATCC 29413 (henceforth called *Anabaena* 29413), a filamentous nitrogen-fixing cyanobacterium. The principal reasons behind this choice are as follows. (*i*) *Anabaena* 29413 can grow in the dark in the presence of 5 mM fructose (8), indicating that photosynthetic electron transfer is not an obligate requirement for growth. (*ii*) Exogenous DNA may be introduced into *Anabaena* 29413 cells via conjugation with appropriate *Escherichia coli* cells (9). Moreover, positive selection vectors have been constructed so that gene replacement mutations may be created via homologous double reciprocal recombination (10).

In this report, we describe the creation and characterization of a targeted PSI-deficient mutant of this organism, T398-1, in which the *psaC* gene, encoding the PSI-C protein, has been interrupted by the introduction of a neomycin-resistance (Nm^r) gene cartridge in the coding region. The T398-1 mutant has photoactive PSI reaction center, even though the Fe-S centers F_A and F_B are missing.

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Abbreviations: Asc, sodium ascorbate; Chl, chlorophyll; DAD, 3,6-diaminodurene; DCBQ, 2,6-dichloro-*p*-benzoquinone; MV, methyl viologen; Nm, neomycin; Sm, streptomycin; Sp, spectinomycin; PSI, photosystem I; PSII, photosystem II.

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MATERIALS AND METHODS

Materials. Enzymes for recombinant DNA work were from New England Biolabs, [α-^{32}P]dCTP (2000 Ci/mmol; 1 Ci = 37 GBq) was from Amersham, and most of the other chemicals were from Sigma.

Plasmids, Bacterial Strains, and Culture Conditions. *Anabaena* 29413 FD (10) was used as the wild-type strain in the experiments described here. Cyanobacterial cultures were grown either in liquid BG11 (11) on a rotary shaker or on BG11 plates at 30°C under white light [60 $\mu E \cdot m^{-2} \cdot s^{-1}$; 1 E (einstein) = 1 mol of photons]. Solid medium was supplemented with 1.5% (wt/vol) agar, 0.3% sodium thiosulfate, and 10 mM Tes/KOH (pH 8.2). Stock solutions of neomycin (Nm), spectinomycin (Sp), streptomycin (Sm), and fructose were filter-sterilized and added to both the liquid and the solid media at final concentrations of 50 μg/ml, 2.5 μg/ml, 2 μg/ml, and 5 mM, respectively. To measure cell growth, filaments were sonicated to generate two- to six-cell fragments and used to determine scattering at 730 nm on a DW2000 spectrophotometer (SLM Aminco).

E. coli cells were grown at 37°C in Luria broth (12).

Southern Hybridization. Genomic DNA from *Anabaena* 29413 cells was isolated essentially as described (10). Plasmid DNA was isolated by an alkaline lysis method (12). For Southern hybridization analysis, DNA fragments fractionated in 0.8% agarose gels were transferred onto nitrocellulose membrane (Schleicher & Schuell, 0.45 μm) and hybridized to random-primed, ^{32}P-labeled probe (13). Hybridization was at 58°C in 6× NET (900 mM NaCl/180 mM Tris Cl, pH 7.5/6 mM Na_2EDTA) and was followed by four washes in 2× NET at room temperature. Other routine DNA manipulations were performed essentially as described (12).

Mutagenesis of *psaC* Gene. Conjugal transfer of exogenous DNA was carried out according to Elhai and Wolk (9). The conjugation involved triparental mating between two *E. coli* strains, SL399 and SL374, and *Anabaena* cells. SL399 contained the donor plasmid pSL398 and a helper plasmid pRL528 whereas SL374 contained the helper plasmid pRL443 (9). In the *psaC* interruption mutant of *Anabaena* 29413, the *psaC*::Nm^r construct replaced the wild-type *psaC* gene in the chromosome via a double reciprocal recombination event (see *Results*).

Protein Analysis. Thylakoid membranes were isolated essentially as described (14). Polypeptide profiles of thylakoid membranes from wild-type and T398-1 cells were analyzed by SDS/PAGE in 16–22% gradient gels containing urea (15). Samples were heated at 70°C for 5 min before they were loaded on the gel. Protein blotting onto nitrocellulose filters and incubation with polyclonal antibodies were performed according to ref. 14.

EPR Analysis. EPR measurements were performed on a Bruker ER 200D X-band spectrometer equipped with an Oxford ESR10 helium-flow cryostat. The frequency was measured with a Systron-Donner 6245A frequency counter and the field was calibrated with a weak pitch sample. The *g* values are within ±0.005 of the quoted values.

Transient Absorption Spectroscopy. Flash-induced absorption transients were measured using a laboratory-built, time-resolved single-beam spectrophotometer (16). The sample was placed in a 1-cm square cuvette and protected from the measuring beam by a shutter, except during and 500 ms prior to recording of a trace. The photomultiplier was protected from scattered actinic light by two red blocking filters (CS 2-64 and CS 2-58, Corning). Actinic flashes were provided by a xenon flash lamp (FX200, EG & G Vactec, St. Louis) filtered by a broad interference filter (DT Blau, Balzers) and a blocking filter (CS 4-96). The photomultiplier was placed 12 cm from the sample to decrease the flash-induced artifact due to Chl fluorescence.

Estimation of Pigment Composition. Chl estimation in the isolated thylakoid membranes was made in 80% acetone (17). The Chl/P700 ratio of the thylakoid membranes was determined according to Mullet *et al.* (18), with the following modifications. Thylakoid membranes were resuspended in 20 mM Tris Cl, pH 7.5/20 mM KCl/1% (vol/vol) Triton X-100 to a final Chl concentration of 25 μg/ml. $K_3Fe(CN)_6$ and sodium ascorbate (Asc) were used at final concentrations of 0.5 mM and 5 mM, respectively. The extinction coefficient for P700 in the presence of Triton X-100 determined by Sonoike and Katoh (19) was used.

Measurement of Photosynthetic Electron Transfer Reactions. A Clark-type oxygen electrode was used to measure the rates of photosynthetic electron transfer reactions at 30°C with saturating red (CS 2-63, Corning) light (3500 $\mu E \cdot m^{-2} \cdot s^{-1}$). Filaments from exponentially growing cultures of wild-type and T398-1 strains were pelleted by centrifugation at 3000 × *g* for 5 min (room temperature) and the pellets were resuspended in 20 mM Tris Cl (pH 7.0). Whole-chain electron transport (PSII and PSI) was measured in the presence of 10 mM $NaHCO_3$. PSII-specific electron transport was measured in the presence of 0.1 mM 2,6-dichloro-*p*-benzoquinone (DCBQ) and 1 mM $K_3Fe(CN)_6$. PSI electron transport was measured in medium containing 50 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea, 1 mM Asc, 2 mM methyl viologen (MV), and 1 mM 3,6-diaminodurene (DAD, Fluka). The samples were adjusted to a final Chl concentration of 10 μg/ml (20).

RESULTS

Insertional Inactivation of the *psaC* Gene. In *Anabaena* 29413 the *psaC* gene, encoding the apoprotein for the two [4Fe-4S] centers, F_A and F_B, is present in a single copy in the chromosome and is transcribed as a monocistronic mRNA. Molecular cloning and sequencing of *psaC* as well as analysis of its transcription will be reported elsewhere (34). Here we describe a site-directed mutant in which *psaC* has been inactivated.

The scheme for the inactivation of *psaC* is shown in Fig. 1*A*. A 1.1-kbp *Sal* I fragment from the plasmid pRL442, containing the Nm^r gene cartridge (21), was inserted at a *Bsm* I site in the coding region of *psaC*. The resultant plasmid, pSL377, was digested with *Kpn* I and *Hin*dIII, and the 4.6-kbp fragment containing the interrupted *psaC* gene was cloned at the unique *Bst*EII site of the shuttle vector pRL277 (a gift from Y. Cai and C. P. Wolk, Michigan State University), thus creating the plasmid pSL398. The *E. coli* strain SL399 containing this plasmid and a second *E. coli* strain, SL374, were then used in triparental mating with *Anabaena* 29413 cells and the resultant Nm^r cyanobacterial colonies were further analyzed to find the desired mutant. pSL398, a derivative of pRL277, cannot replicate in *Anabaena* cells, since it lacks any cyanobacterial replicon (9). Hence, the Nm^r cells must have the plasmid (or a part of it) integrated into the chromosomal DNA via homologous recombination with the chromosomal fragment in the plasmid (10). If there were a single reciprocal recombination event, the resulting exconjugant would be resistant to Nm as well as Sp/Sm. On the other hand, double reciprocal recombination would yield Nm^r colonies that are sensitive to Sp/Sm. This latter class of exconjugants is expected to have the wild-type *psaC* gene replaced by the insertionally inactivated copy of the gene. From three different conjugation experiments, we obtained a total of 68 exconjugants. Among these, 16 were double recombinants and 52 were single recombinants as determined by the antibiotic selection criterion described above. One of the double recombinants, T398-1, was selected for further analysis.

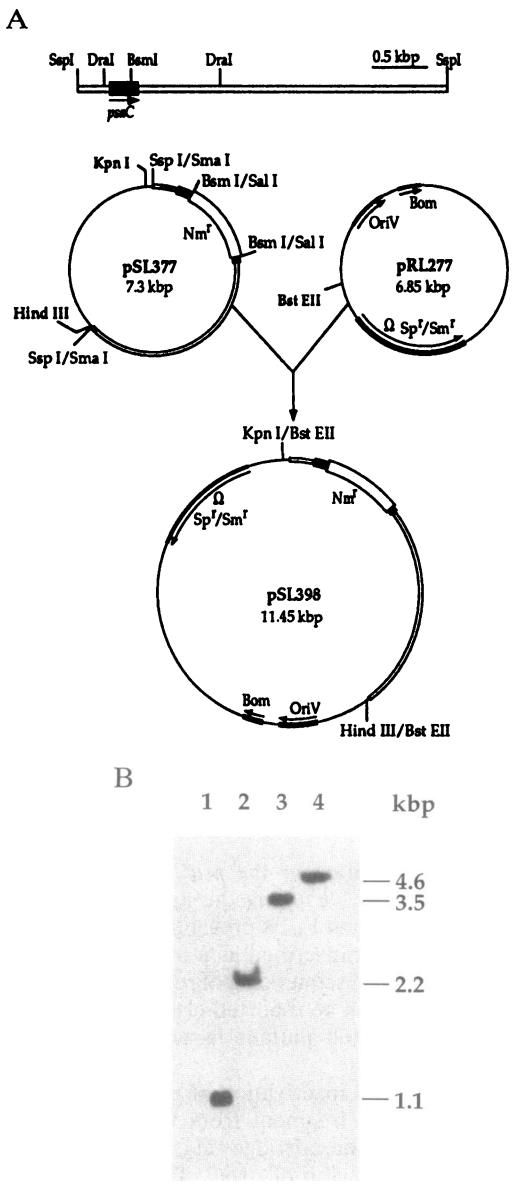

FIG. 1. (*A*) Construction of a clone for insertional inactivation of the *psaC* gene. The bar at the top represents a 3.5-kilobase-pair (kbp) *Ssp* I fragment of *Anabaena* 29413 genomic DNA containing the 243-bp coding region of *psaC* (black rectangle). A 1.1-kbp *Sal* I fragment (C.K3; ref. 21) containing a Nm^r gene cartridge (white rectangle) was inserted at a *Bsm* I site, 190 bases 3′ to the beginning of this open reading frame, to form pSL377. A 4.6-kbp *Kpn* I–*Hin*dIII fragment from pSL377 was then cloned at the single *Bst*EII site of the shuttle vector pRL277 (9) to create the donor plasmid pSL398. Two restriction sites separated by a slash (e.g., *Bsm* I/*Sal* I) indicate blunt-end ligations and are thus not recognized by either enzyme. (*B*) Southern blot of genomic DNA from wild-type (lanes 1 and 3) and mutant T398-1 (lanes 2 and 4) cells of *Anabaena* 29413. DNA was digested with *Dra* I (lanes 1 and 2) or *Ssp* I (lanes 3 and 4), electrophoresed in a 0.8% agarose gel, transferred to nitrocellulose filter, and probed with a ^{32}P-labeled *psaC* probe.

Cyanobacteria contain multiple copies of their chromosome in each individual cell (22). Repeated sonication and plating of T398-1 cells on medium containing Nm and fructose under illuminated condition failed to segregate the mutant allele of *psaC* to a homozygous condition, as evidenced from Southern analysis of chromosomal DNA (data not shown). However, dark incubation of these cells in the presence of Nm and fructose resulted in complete segregation of the interrupted allele. Southern analysis (Fig. 1*B*) showed that the *psaC* probe hybridized to a 1.1-kbp *Dra* I (lane 1) and a 3.5-kbp *Ssp* I (lane 3) fragment of chromosomal DNA from wild-type cells. The same probe hybridized to a 2.2-kbp *Dra* I (lane 2) and a 4.6-kbp *Ssp* I (lane 4) fragment of DNA from T398-1 mutant cells, as expected for an interrupted *psaC* gene (see Fig. 1*A*). Thus, the wild-type allele of *psaC* is absent from the T398-1 cells.

Growth Properties of the T398-1 Cells. The mutant cells could grow in the dark when fructose was added to the growth medium (Table 1). Growth rate of the mutant cells under these conditions was not significantly different from that of wild-type cells. However, the T398-1 cells did not show any photoautotrophic growth, indicating that its photosynthetic activities were inhibited. The mutant cells were extremely light-sensitive. Cultures of T398-1 cells inoculated at a low cell density failed to grow under illumination. When started with a larger inoculum (OD_{730} = 0.1), the mutant cells could grow under light in the presence of fructose, although their growth rate was significantly lower than that of the same cells grown in darkness. In comparison, the wild-type cells grew twice as fast under illumination as in the dark in fructose-supplemented medium.

Protein Composition of the Mutant. In the construction of the mutant strain T398-1, the Nm^r gene cartridge was inserted 53 bases upstream of the 3′ end of the *psaC* coding region. To ensure that there was no modified *psaC* gene product in the mutant, we used immunoblot analysis of membrane proteins of wild-type and T398-1 strains. Probing with polyclonal antibodies raised against spinach PSI-C polypeptide showed that no PSI-C-like protein was present in the membrane from T398-1 cells (Fig. 2*A*, lane 2). The same antibodies, however, recognized the 9-kDa PSI-C protein in the wild-type membrane (Fig. 2*A*, lane 1). When different strips of the same blot were probed with polyclonal antibodies raised against the PSI-A/B polypeptides from *Synechococcus* 7942 (Fig. 2*B*) and the D1 polypeptide of spinach PSII (Fig. 2*C*), these polypeptides were detectable in the mutant thylakoid membranes. Interestingly, the amount of the PSI reaction-center proteins, PSI-A/B, was almost the same in wild-type and mutant cells. Thus the absence of the PSI-C protein did not affect the synthesis, membrane insertion, and stability of the PSI complex. In contrast, the amount of D1, a component of the PSII reaction center, was significantly increased in the mutant membranes.

EPR Analysis of Iron-Sulfur Centers F_A and F_B. Fig. 3*A* shows the EPR spectrum of iron-sulfur centers in membranes from wild-type cells. Upon dark chemical reduction of this membrane sample, EPR signals with *g* values of 2.04, 1.94, 1.92, and 1.88 were observed, indicating the presence of F_A and F_B (1, 23). Under identical conditions, none of these four signals was observed in membranes from T398-1 (Fig. 3*B*).

Table 1. Growth rate, Chl/P700 ratio, and photosynthetic electron transport activities of wild-type and T398-1 strains

	Wild type	T398-1
Doubling time, hr		
Light	36 ± 3	No growth
Light + fructose	18 ± 2	61 ± 5
Dark + fructose	38 ± 3	44 ± 4
Chl/P700, mol/mol*	141 ± 11	133 ± 15
Electron transport†		
H_2O to $NaHCO_3$	226 ± 55	−242 ± 40
DAD/Asc to MV	−941 ± 40	−814 ± 40
H_2O to DCBQ/$Fe(CN)_6^{3-}$	538 ± 20	557 ± 20

Each value is the mean ± SD of at least five independent experiments.
*Determined from chemical difference spectra.
†Determined by polarography and expressed as μmol of O_2 per mg of Chl per hr. Negative numbers indicate O_2 uptake.

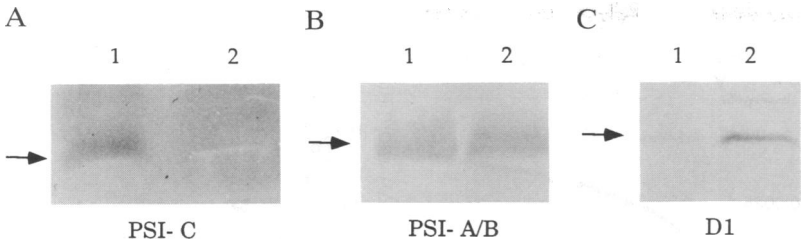

FIG. 2. Immunoblot detection of thylakoid proteins from wild-type (lane 1) and T398-1 (lane 2) strains of *Anabaena* 29413. Membrane samples were fractionated by SDS/16–22% PAGE and blotted on nitrocellulose filters. Different strips of the same blot were incubated with polyclonal antibodies against the PSI-C protein (*A*), the PSI-A/B proteins (*B*), and the D1 protein of PSII (*C*). Each sample contained 10 μg of Chl.

The Chl concentration of the T398-1 sample was 2.2-fold higher than that of the wild-type sample. We conclude that both F_A and F_B are missing from the membranes of T398-1. A small signal at $g = 1.91$ was observed in the mutant membrane, which is presumably hidden under the larger signals from F_A and F_B in the wild-type membrane. A signal at $g = 1.92$ has been observed in whole cells of *Aphanocapsa* and has been ascribed to an iron-sulfur center in an NAD(P)H:quinone oxidoreductase (24).

Composition of Pigments. Chemical difference spectra of membrane samples indicated that the Chl/P700 ratio was similar in the wild-type and mutant cells (Table 1). Thus, the absence of the PSI-C protein and F_A/F_B did not affect the concentration of PSI reaction centers in the mutant cells.

Measurement of Photosynthetic Electron Transfer Reactions. Table 1 shows the rates of whole-chain (PSII and PSI) as well as partial (PSII or PSI) reactions of photosynthetic electron transport in intact cells of wild-type and mutant strains. In the mutant cells, the whole-chain electron transport reaction measured in terms of light-mediated, bicarbonate-dependent O_2 evolution was completely absent. Instead we observed O_2 uptake, presumably due to respiration. When DCBQ and $Fe(CN)_6^{3-}$ were present as electron acceptors for PSII, similar rates of O_2 evolution in the wild-type and mutant strains were observed. Thus, PSII was not affected in these mutant cells. In addition, rates of light-induced electron transfer from DAD/Asc to MV, mediated by the PSI reaction center, were similar in the wild-type and mutant cells. These data are consistent with the presence of a functional electron transport system that includes F_X in PSI of T398-1.

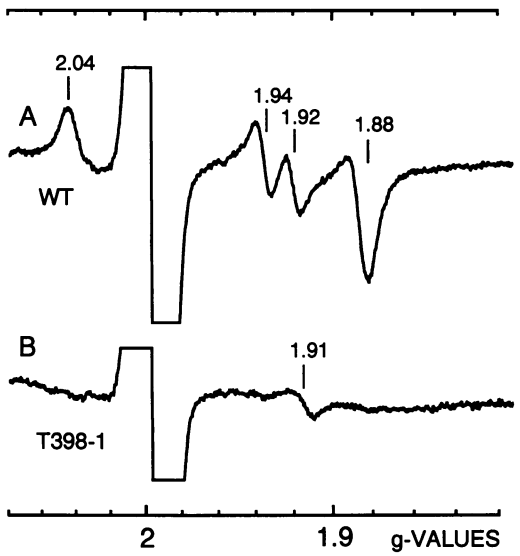

FIG. 3. EPR spectra of thylakoids from *Anabaena* 29413 wild type (WT) cells (*A*) and T398-1 mutant cells (*B*) showing signals from chemically reduced iron-sulfur centers. Wild-type (0.67 mM Chl) or T398-1 (1.45 mM Chl) membranes were in a reaction medium containing 0.2 M glycine/NaOH (pH 11.0), 250 μM MV, and a few crystals of sodium dithionite. Samples in the EPR tubes were dark-adapted for 6 min at room temperature and then cooled to 19 K. Spectrometer conditions were as follows: microwave power, 2 mW; modulation amplitude, 10 G; receiver gain, 1×10^5. Each spectrum is the average of 32 scans (20 s per scan).

Time-Resolved Optical Spectroscopic Analysis. The ability of P700 to undergo photooxidation and subsequent rereduction due to back reactions between reduced donor(s) and $P700^+$ was investigated by monitoring flash-induced absorbance transients in the near infrared (25). The rapid flash-induced absorbance decrease at 706 nm (due to the formation of $P700^+$) recovered with a half time of 45–50 ms (Fig. 4*A*). These recovery kinetics correspond to those of the back reaction between F_A^-/F_B^- and $P700^+$ (1). In comparison, the kinetics of P700 recovery were significantly faster ($t_{1/2} = 1$–3 ms) in the T398-1 membrane sample. This rapid rereduction is consistent with the absence of both F_A and F_B in the mutant membranes, resulting in a faster back reaction between $P700^+$ and an earlier acceptor, presumably F_X (1).

The flash-induced absorbance decrease was smaller in the mutant sample as compared to the wild-type sample, although chemical difference spectra indicate that the Chl/P700 ratio is similar in the wild-type and mutant cells. This apparent discrepancy may be explained by the presence of a rapidly decaying component of the flash-induced signal that is not detected under our experimental conditions.

Fig. 4*C* shows the spectrum of the flash-induced absorption changes in the wild-type and T398-1 mutant membranes, respectively. The position of the peak was between 705-706 nm in the wild-type membranes, whereas it was at 704 nm in the mutant sample.

DISCUSSION

In this study we demonstrate that it is possible to create PSI-deficient mutants in the filamentous cyanobacterium *Anabaena* 29413. The ability of this organism for dark chemotrophic growth makes it an ideal candidate for the creation of photosynthesis-deficient mutants. Availability of a conjugation-mediated gene-transfer system for this cyanobacterium has allowed us to create a targeted mutation to inactivate the *psaC* gene. The resultant mutant T398-1 lacked the PSI-C protein and its associated cofactors F_A and F_B and consequently lost its PSI-mediated electron transfer activity from P700 to ferredoxin and subsequent electron acceptors. These genetic studies complement the biochemical reconstitution experiments of Golbeck and coworkers (1, 6) that have led to a definition of the PSI-C protein in the organization of the F_A and F_B iron-sulfur centers. Among the filamentous cyanobacteria, most of the gene inactivation experiments in the past have been performed in *Anabaena* 7120 (26–29). Unfortunately, this organism is an obligate photoautotroph and is not suitable to create mutants that are deficient in photosynthetic electron transfer processes. Recently, a light-activated heterotrophic growth condition has been established for the unicellular strain *Synechocystis* 6803 (30) that should be suitable for the creation and maintenance of PSI-deficient mutants. However, in contrast to *Synechocystis* 6803, *Anabaena* 29413 offers the opportunity to study the relationship between nitrogen fixation and photosynthetic electron transport by using PSI-deficient mutants (31).

Interruption of *psaC* by the Nm^r gene cartridge led to the complete loss of the PSI-C protein from the membrane, even

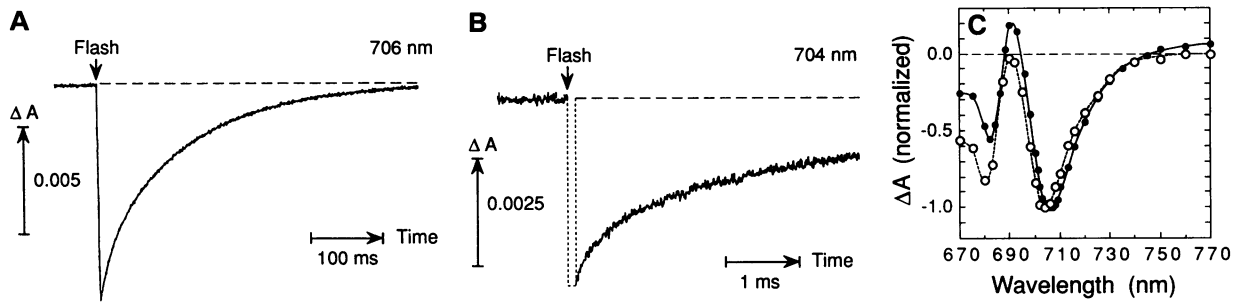

FIG. 4. (*A* and *B*) Kinetics of flash-induced absorption change of thylakoid membranes from *Anabaena* 29413 wild-type cells at 706 nm (*A*) and T398-1 mutant cells at 704 nm (*B*). Thylakoids were suspended at a Chl concentration of 25 μM (wild type) or 19 μM (T398-1) at a temperature of 20°C in 50 mM Tris Cl, pH 8.3/1.7 mM Asc/33 μM 2,6-dichloroindophenol. The samples were dark-adapted at least 20 s prior to the flash. (*C*) Flash-induced absorption spectra of thylakoid membranes isolated from *Anabaena* 29413 wild-type (solid line) and T398-1 mutant (dashed line) cells. Conditions were as described above.

though 79% of the gene toward the 5′ end was still unmodified. The reaction center of PSI was assembled in the thylakoid membranes of the mutant strain in the absence of PSI-C, the apoprotein for the terminal electron acceptors in PSI. The evidence supporting this are (*i*) the presence of PSI-A/B polypeptides detected through immunoblot analysis (Fig. 2*B*), (*ii*) time-resolved optical spectroscopic data showing formation of $P700^+$ (Fig. 4), and (*iii*) light-induced PSI-mediated electron transfer from DAD/Asc to MV (Table 1). The majority of the PSI mutants of other organisms, generated in the past by random mutagenesis, lack the PSI-A/B proteins. For example, a large number of PSI-deficient mutants of *Chlamydomonas reinhardtii* have been characterized by the lack of the reaction-center proteins (PSI-A and PSI-B) and a set of six low molecular weight proteins of PSI (32, 33). On the other hand, targeted *psaD* and *psaE* mutants of *Synechocystis* 6803 have assembled PSI complexes (4, 5). However, both of these mutants are capable of photoautotrophic growth, implying that they have PSI-mediated electron transfer activities. Although PSI-D and PSI-E polypeptides are components of PSI, they do not bind any of the known electron-transport intermediates. In contrast, the PSI-C protein binds two cofactors, F_A and F_B. Past studies on inactivation of different PSII proteins that bind various electron-transport intermediates in *Synechocystis* 6803 indicate that in all of these mutants, PSII reaction-center activity is absent (3). To our knowledge, T398-1 is the first cyanobacterial photosynthesis-deficient mutant in which complete loss of an apoprotein for one or more cofactors did not affect the formation and stability of the reaction center. A possible reason is that the PSI-C protein is an extrinsic membrane protein, whereas all of the PSII cofactor-binding proteins studied are membrane-integral.

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