A Large Fraction of PsaF Is Nonfunctional in Photosystem I Complexes Lacking the PsaJ Subunit†

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Received December 1, 1998; Revised Manuscript Received February 18, 1999

ABSTRACT: PsaJ is a small hydrophobic subunit of the photosystem I complex (PSI) whose function is not yet fully understood. Here we describe mutants of the green alga *Chlamydomonas reinhardtii*, in which the *psaJ* chloroplast gene has been inactivated either in a wild-type or in a PsaF-deficient nuclear background. Cells lacking one or both subunits grow photoautotrophically and contain normal levels of PSI. Flash-absorption spectroscopy performed with isolated PSI particles isolated from the PsaJ-deficient strain indicates that only 30% of the PSI complexes oxidize plastocyanin (Pc) or cytochrome c_6 (Cyt c_6) with kinetics identical to wild type, whereas the remaining 70% follow slow kinetics similar to those observed with PsaF-deficient PSI complexes. This feature is not due to partial loss of PsaF, as the PsaJ-less PSI complex contains normal levels of the PsaF subunit. The N-terminal domain of PsaF can be cross-linked to Pc and Cyt c_6 indicating that in the absence of PsaJ, this domain is exposed in the lumenal space. Therefore, the decreased amount of functional PsaF revealed by the electron-transfer measurements is best explained by a displacement of the N-terminal domain of PsaF which is known to provide the docking site for Pc and Cyt c_6. We propose that one function of PsaJ is to maintain PsaF in a proper orientation which allows fast electron transfer from soluble donor proteins to $P700^+$.

Photosystem I (PSI)[1] is a multisubunit complex located in the thylakoid membranes of chloroplasts and cyanobacteria. It acts as a light-driven oxido-reductase to transfer electrons from plastocyanin (Pc) or cytochrome c_6 (Cyt c_6) to ferredoxin. The PSI complex consists of five chloroplast-encoded subunits (PsaA, -B, -C, -I, and -J) and at least six nuclear-encoded subunits (PsaD, -E, -F, -G, -K, -L) (*1, 2*). All the redox components of PSI are bound to the core subunits PsaA and PsaB except for the two terminal electron acceptors which are liganded by the PsaC subunit (*1, 3*). PsaD and PsaE are located on the stromal side of PSI and together with PsaC provide the docking site for soluble ferredoxin (*4*−*9*). In *C. reinhardtii*, the PsaF subunit is required for complex formation and fast electron transfer between Pc or Cyt c_6 and PSI, but is dispensable for photoautotrophic growth (*10, 11*). The interaction between PsaF and donor proteins is mediated by its positively charged N-terminal end which interacts electrostatically with the acidic residues of the soluble electron carriers, as shown by chemical cross-linking and mutagenesis studies (*11, 12*). The lack of this N-terminal domain of PsaF in cyanobacteria correlates with the absence of complex formation and fast electron transfer (*11, 13, 14*). PsaF also contains a transmembrane domain which has been proposed to interact with PsaE (*15*). PsaI and PsaL form the cyanobacterial PSI trimerization domain. Loss of PsaL prevents PSI trimer formation, and inactivation of PsaI reduces PsaL accumulation by 80% and destabilizes PSI trimers (*16*−*18*). PsaJ is a small hydrophobic protein of 4.4 kDa that can be cross-linked to PsaF and is predicted to have a single transmembrane domain (*15*). In cyanobacteria, the *psaF* and *psaJ* genes are cotranscribed (*19, 20*). Deletion of the *psaJ* coding sequence in *Synecocystis* PCC 6803 leads to the destabilization of the *psaF* mRNA which accumulates to 20% of wild-type levels. PSI particles isolated from this mutant lack PsaJ and contain about 20% of the PsaF subunit compared to wild type. The PsaF subunit from the PsaJ-less complex was found to be extracted more easily by chaotropic agents or by Triton X-100, indicating that PsaJ stabilizes PsaF (*20*). These two subunits have been localized at the periphery of PSI by comparative electron microscopy of isolated PSI particles from the mutant lacking both subunits and wild type (*21*). This result allowed for the assignment of electron densities to PsaF and PsaJ in the PSI structure solved at 4 Å (*22*). In algae and higher plants, the *psaJ* gene is located in the chloroplast genome whereas *psaF* is a nuclear gene. In this case it is therefore possible to inactivate *psaJ* without affecting the expression of *psaF*. Here we present the characterization of mutant strains of the green alga *Chlamy-*

† This work was supported by Grant 3100-050895.97 from the Swiss National Science Foundation and by Deutsche Forschungsgemeinshaft Grant SFB 388-A1. E.B. was supported by a postgraduate scholarship from the Natural Science and Engineering Research Council of Canada.

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[1] Abbreviations: PSI, photosystem I; Cyt c_6, cytochrome c_6; Pc, plastocyanin.

10.1021/bi982821a CCC: $18.00
Published on Web 04/03/1999

domonas reinhardtii with inactivated *psaJ* genes either in a wild-type or in a *psaF*-deficient nuclear background. We show that PSI complexes lacking PsaJ or both PsaJ and PsaF accumulate to wild-type levels and that in the absence of PsaJ, the PSI complex contains wild-type levels of PsaF. Our results further suggest that the role of the PsaJ subunit is to maintain PsaF in a proper orientation so that its N-terminal domain can serve as a docking site for Pc or Cyt c_6 for mediating fast electron transfer to $P700^+$.

EXPERIMENTAL PROCEDURES

Strains and Media. C. reinhardtii wild-type and mutant strains were grown as described (*23*). When necessary, the media [Tris acetate phosphate medium (TAP) and high-salt minimal medium (HSM)] were solidified with 2% Bacto agar (Difco) and supplemented with spectinomycin (Sigma).

Nucleic Acid Techniques. Standard procedures were used for the preparation of recombinant plasmids (*24*). The bacterial host used was *E. coli* DH5α. *C. reinhardtii* total DNA was isolated as described previously (*25*). Southern blotting and hybridization were carried out as described (*24*). To disrupt the *psaJ* gene, a 2.1 kb *Kpn*I fragment from the chloroplast DNA *Eco*RI restriction fragment R11 was cloned into the pBluescribe vector (Stratagene) (*26*). The resulting plasmid was linearized by partial digestion with the *Alw*NI restriction endonuclease, and the ends were subsequently blunted. The 1.9 kb *aadA* expression cassette that confers spectinomycin and streptomycin resistance to *C. reinhardtii* (*27*) was excised from pUC-atpX-aad by digesting with *Eco*RV and *Sma*I and inserted in either orientation into the *Alw*NI site located 34 nucleotides downstream of the *psaJ* initiation codon.

Chloroplast transformation in *C. reinhardtii* wild-type and 6F cells was carried out as described (*28*) with a helium-driven particle gun adapted from the one described by Finer et al. (*29*). Transformants were selected on TAP agar plates containing 150 μg/mL spectinomycin and recloned 3 times on TAP−spectinomycin plates.

SDS−PAGE and Western Blot Analysis. Protein separation and blotting were carried out according to standard procedures. The ECL chemiluminescence system was used for detection of the immunoreactions (Amersham).

Purification of Thylakoid Membranes, PSI, Pc, and Cyt c_6. Thylakoid membranes and PSI complex purification were performed as described in Fischer et al. (*30*). Pc and Cyt c_6 isolation was accomplished according to published procedures (*31*, *32*) with modifications as described (*11*). Protein concentrations were determined spectroscopically by using an extinction coefficient of 4.9 mM^{-1} cm^{-1} at 597 nm for the oxidized form of Pc (*33*) and 20 mM^{-1} cm^{-1} at 552 nm for the reduced form of Cyt c_6 (*34*).

Cross-linking between Pc or Cyt c_6 and PSI was performed as described (*11*).

Laser-Flash Absorption Spectroscopy. Kinetics of flash-induced absorption changes at 817 nm were measured essentially as described (*35*). The measuring light was provided by a luminescence diode [Hitachi HE8404SG, 40 mW, full-width at half-maximum (fwhm) 30 nm] supplied with a stabilized battery-driven current source. The light passed through a cuvette containing 200 μL of the sample with an optical path length of 1 cm and a 817 nm interference filter (fwhm 9 nm) before detection with a Si-photodiode. Confidence intervals for the fitted parameters were estimated by systematically altering each parameter and allowing for adjustment of all other fitting parameters. From plots of the minimized sum of squared residuals against the parameter value, confidence intervals were determined within one standard deviation [for further details, see (*36*)]. Asymmetrical confidence intervals are generally obtained. For half-lives, the absolute range of the confidence interval is given. For amplitudes, the average of slightly differing upper and lower intervals is given.

RESULTS

Insertional Inactivation of the psaJ Gene. The *psaJ* gene was previously identified (*37*) in the *C. reinhardtii* 1.47 kb *Hinc*II/*Kpn*I chloroplast DNA sequence comprising the ribosomal protein gene *rps12* [(*38*) accession number M29284]. The *C. reinhardtii psaJ* gene encodes a 41 amino acid polypeptide that displays 50−71% sequence identity with its homologues from algae, land plant, and cyanobacteria (Figure 1A). The *psaJ* gene is located 310 bp upstream of *rps12* and 335 bp downstream of the *atpI* gene encoding the ATP-synthase complex subunit IV (accession number AF061851). These three genes are transcribed from the same DNA strand (Figure 1B).

A 2.1 kb *Kpn*I restriction fragment containing *psaJ* from the chloroplast DNA *Eco*RI restriction fragment R11 (*26*) was subcloned. The *aadA* expression cassette which confers spectinomycin and streptomycin resistance to *C. reinhardtii* when expressed in the chloroplast (*27*) was inserted in either orientation into the *psaJ* coding sequence (Figure 1B; see Experimental Procedures for details). Constructs with the *aadA* cassette were introduced in the chloroplast genome via biolistic transformation of the wild-type or the 6F strain. The latter was obtained by crossing the PsaF-deficient 3bF strain which is cell-wall-deficient to wild type in order to partially restore the cell-wall [(*10*) and Farah, unpublished results]. Several independent transformants were selected on TAP plates supplemented with spectinomycin and kept in dim light (5 μE m^{-2} s^{-1}). To verify the inactivation of *psaJ* in the chloroplast genome, total DNA isolated from the transformants and parental strains was analyzed by Southern blot analysis and PCR amplification. Figure 1C shows a Southern blot analysis of *Eco*RI-digested DNA from wild type, 6F, and transformants probed with the *psaJ* coding sequence. A 4.9 kb fragment is detected in the wild type and 6F lanes whereas a fragment of 6.8 kb is present in the transformants containing the *aadA* cassette in either orientation. The increase in size of 1.9 kb corresponds to the size of the *aadA* cassette. To assess the level of homoplasmicity of the transformants, we performed PCR amplifications on their respective DNAs with two distinct sets of primers in the same reaction: one specific for the *psaJ* gene and one specific for the *psaC* gene as an internal control. The absence of amplification product corresponding to *psaJ* in the transformants demonstrates that the strains are homoplasmic and that no wild-type *psaJ* is left in their genome (Figure 1D). Under the conditions used, amplification of *psaJ* was undetectable when wild-type type DNA was mixed with a 100-fold excess of DNA from the J− transformant (data not shown). Fluorescence transients of dark-adapted transformants were similar to transients obtained with wild-type cells (*39*). These

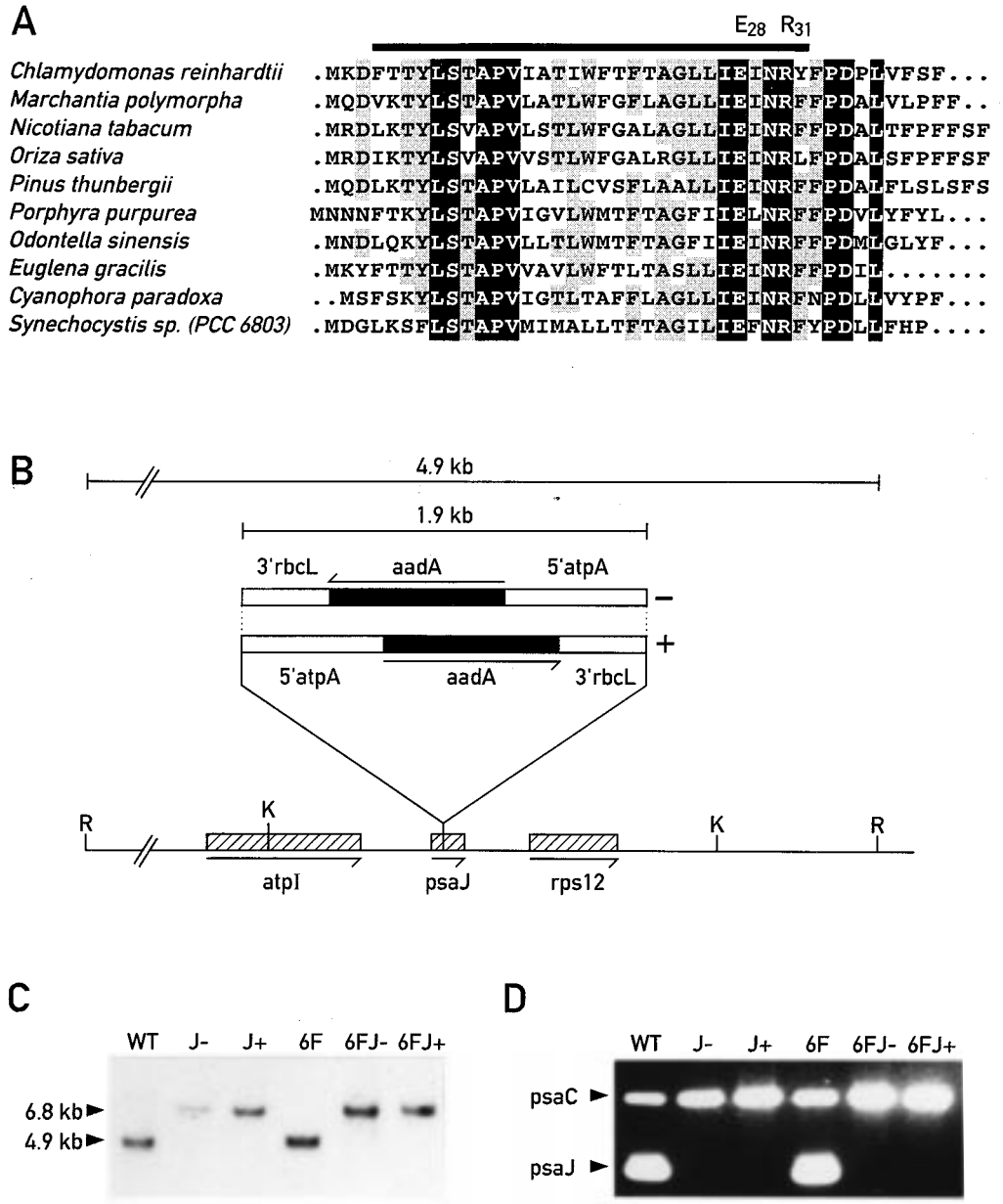

FIGURE 1: (A) Sequence alignment of the PsaJ polypeptide from *C. reinhardtii*, *Marchantia polymorpha* (P12191), *Nicotiana tobacum* (P12193), *Oryza sativa* (P12192), *Pinus thunbergii* (P41613), *Porphyra purpurea* (P51194), *Odontella sinensis* (P49485), *Euglena gracilis* (P30094), *Cyanophora paradoxa* (P48117), and *Synechocystis* sp. strain PCC 6803 (Q55329). The amino acids which are identical to corresponding residues among all PsaJ proteins are in black boxes. Amino acids which are conserved in at least six sequences are in gray boxes. The PsaJ putative transmembrane helix, calculated with the TMAP program (*45*), is indicated by a solid line over the alignment. The conserved residues E_{28} and R_{31} are shown; they could form an ionic pair allowing E_{28} to serve as a side chain ligand to the central Mg atom of a chlorophyll molecule. (B) Schematic map of the 4.9 kb chloroplast genomic fragment containing the *atpI*, *psaJ*, and *rps12* genes. The *aadA* cassette is inserted at an *Alw*NI site within the coding sequence of *psaJ* in the sense and antisense orientations, indicated by + and −, respectively. Restriction sites are: K, *Kpn*I; R, *Eco*RI. Arrows indicate the direction of transcription. (C) Southern blot analysis of total genomic DNA. After *Eco*RI digestion, the DNA was separated by agarose gel electrophoresis, blotted, and hybridized with a ^{32}P-labeled probe spanning the *psaJ* coding sequence. The J+ and 6FJ+ transformants contain the cassette in the sense orientation whereas in the J− and 6FJ− transformants the cassette is inserted in the antisense orientation. (D) PCR amplification of total genomic DNA with primers specific for the *psaC* and *psaJ* genes. Both sets of primers were added to the reactions. After amplification, the products were separated by agarose gel electrophoresis and stained with ethidium bromide. The positions of the expected amplification products are indicated.

mutant strains were able to grow photoautrotrophycally on minimal medium, indicating that the overall photosynthetic activity is not significantly affected and that functional PSI accumulates in these cells.

Inactivation of psaJ Does Not Affect the Expression of the Flanking Genes. We performed a Northern blot analysis on total RNA isolated from the wild-type, 6F, J+, J−, 6FJ+, and 6FJ− strains to determine the accumulation of the *psaJ* mRNA. Figure 2 shows that the 0.7 kb *psaJ* transcript, present in the wild-type and 6F strains, is undetectable when the *aadA* cassette is inserted in either orientation, thus confirming the inactivation of the *psaJ* gene. It has been reported that the downstream *rps12* gene is transcribed into a major 0.8 kb mRNA, but that it is also cotranscribed with *psaJ*, giving rise to two minor transcripts of 1.9 and 2.9 kb (*38*). Hybridization of the blot with a probe specific for *rps12* showed that the major 0.8 kb transcript is unaffected in the transformants, suggesting the existence of a promoter

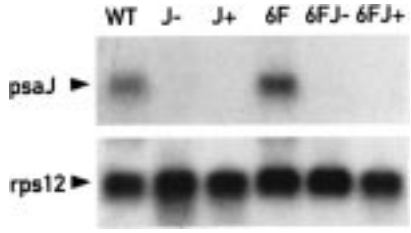

FIGURE 2: RNA blot analysis. Total RNA (6 μg per lane) isolated from wild type and transformants was separated on a 1.2% agarose−formaldehyde gel and blotted. The same probe as in Figure 1 was used for detection of the *psaJ* transcript. A *Hin*fI fragment containing the 5′UTR and part of the coding region of *rps12* was used for probing the *rps12* transcript.

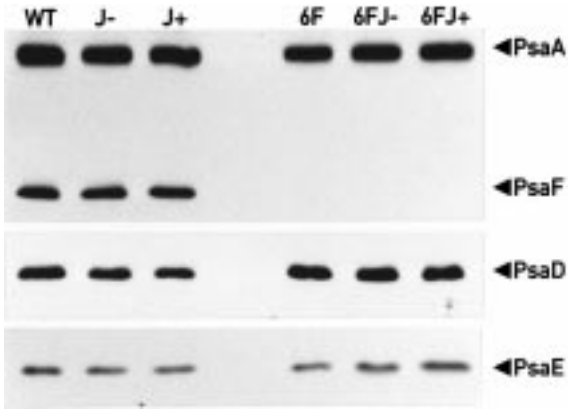

FIGURE 3: Immunoblot analysis performed on total cells extracts from wild type and transformants growing exponentially in TAP liquid medium under low light (5 μE m^{-2}/s^{-1}). 15 μg of total proteins was separated by SDS−PAGE, electroblotted on a nitrocellulose membrane, and probed with antibodies raised against the PsaA, -D, -E, and -F subunits of *C. reinhardtii*.

downstream of *psaJ* that drives expression of *rps12* (Figure 2). However, the longer transcripts were undetectable, suggesting that the insertion of the *aadA* cassette affects the formation and/or stability of these mRNAs (data not shown). The fact that the transformants are able to grow on minimal medium at rates similar to wild type already suggests that the insertion of the *aadA* cassette did not alter the expression of the upstream *atpI* gene which encodes an essential subunit of the C−F_0 ATP synthase complex (*40*). This was also confirmed by Northern blot analysis (data not shown). We conclude that the insertion of the *aadA* cassette into the coding sequence of *psaJ* inactivates its expression without markedly affecting the expression of the flanking genes.

PSI Accumulation Is Not Affected by the Absence of PsaJ and/or PsaF. To determine the amount of PSI complex in the transformants, total cells extracts were analyzed by immunoblotting with antibodies raised against the PsaA, -D, -E, and -F polypeptides of *C. reinhardtii.* These subunits accumulate to wild-type levels in the J+, J−, 6F, 6FJ+, and 6FJ− strains except for the PsaF subunit which is absent from the three latter strains (Figure 3). These results show that PSI complexes lacking PsaF and/or PsaJ can stably assemble in vivo in *C. reinhardtii* and that PsaF accumulation does not require PsaJ.

Isolated PSI Complex Lacking PsaJ Contains Normal Levels of PsaF Subunit. We isolated PSI particles devoid of PsaJ by solubilization of thylakoid membranes from the J− strain and by sucrose density gradient centrifugation. Because this mutant PSI complex might be more sensitive to

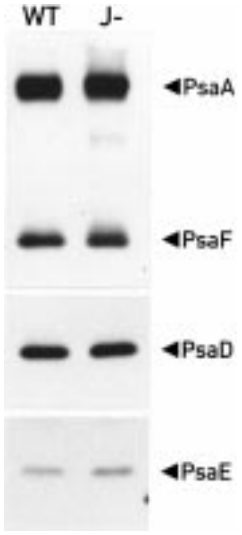

FIGURE 4: Immunoblot analysis of PSI particles purified from wild type and the J− strain. 1 μg of chlorophyll *a* was loaded per lane, and immunoblot analysis was performed as described in Figure 3.

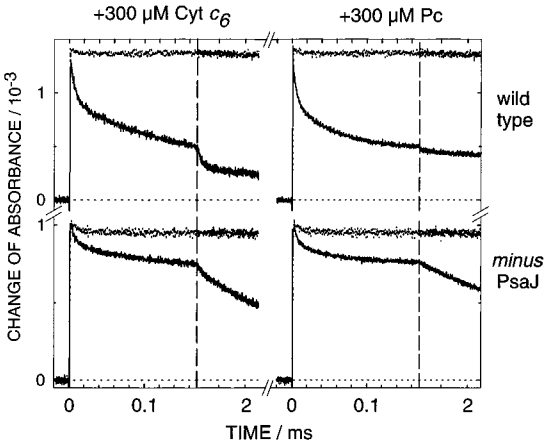

FIGURE 5: Kinetics of $P700^+$ reduction by Pc or Cyt c_6. Absorbance transients at 817 nm were induced by a laser flash. Experiments were done with PSI particles isolated from wild type or from the J− strain, in the presence of 300 μM Pc (right) or 300 μM Cyt c_6 (left). The absorbance transients measured in the absence of donor proteins are shown by dotted traces. Vertical dashed lines separate regions recorded with a different time base. The cuvette contained PSI at a concentration of 90 μg/mL, in 0.05% (w/v) β-dodecyl maltoside, 10 mM $MgCl_2$, 30 mM 3-(*N*-morpholino)propanesulfonic acid (pH 7.0), 1 mM sodium ascorbate, 0.1 mM methyl viologen, and 0.2 mM diaminodurene.

detergents, we monitored its subunit composition by immunoblot analysis of isolated PSI particles (Figure 4). It appears that the wild-type and mutant complexes accumulate similar levels of PsaA, -D, -E, and -F, indicating that during the purification procedure these subunits remain associated with PSI in the absence of PsaJ.

Electron Transfer from Pc or Cyt c_6 to PSI Lacking PsaJ. The electron transfer from soluble Pc or Cyt c_6 to PSI was investigated in vitro using time-resolved laser-flash absorption spectrometry by measuring absorbance changes at 817 nm. Figure 5 (left and right panels) shows the transients of PSI particles isolated from wild type and from the J− strain in the presence of 300 μM Cyt c_6 and Pc, respectively. The kinetics have been analyzed by least-squares fits to a three-exponential decay. The fastest kinetic component has an invariant half-life of 3−5 μs in all measurements and can

Table 1: Properties of the Kinetic Phases of Electron Transfer from Pc or Cyt c_6 to Wild-Type and Mutant PSI Complexes[a]

	soluble electron donor [300 μM]	WT PSI			J− PSI		
		half-life (μs)	amplitude	relative amplitude	half-life (μs)	amplitude	relative amplitude
first-order phase	Pc	3.7 (2.5−6.3)	$a_1 = 0.46$ (±0.1)	0.34 (±0.07)	4 (2−9)	$a_1 = 0.16$ (±0.12)	0.15 (±0.12)
	Cyt c_6	4.6 (2.8−7.5)	$a_1 = 0.48$ (±0.15)	0.34 (±0.11)	5 (2−10)	$a_1 = 0.18$ (±0.12)	0.17 (±0.11)
second-order phase	Pc	45 (39−53)	$a_2 = 0.43$ (±0.05)	0.32 (±0.04)	33 (20−70)	$a_2 = 0.11$ (±0.05)	0.11 (±0.05)
	Cyt c_6	101 (80−125)	$a_2 = 0.6$ (±0.07)	0.43 (±0.05)	93 (60−150)	$a_2 = 0.13$ (±0.03)	0.13 (±0.03)
total amplitude (a_{tot})	Pc		1.36	1		1.04	1
	Cyt c_6		1.4	1		1.05	1
% of sub-ms amplitudes relative to total amplitude $(a_1 + a_2)/a_{tot}$	Pc			66			26
	Cyt c_6			77			30

[a] Amplitude units: change of absorbance × 10^3 (see Figure 5). The relative amplitudes are defined as the ratios of a_1 and a_2 to the total amplitude corresponding to total photooxidized P700. The total amplitude a_{tot} is the sum of the amplitudes of the two submillisecond phases and of the slow phase which is independent of PsaF (see text). Absolute range of confidence and upper and lower limit of the confidence intervals are given for half-lives and amplitudes, respectively.

be attributed to electron transfer within a preformed complex between the donor protein and PSI (*11*, *35*). The intermediate kinetic component follows a pseudo first-order time course with half-lives of 33−45 and 93−101 μs for Pc and Cyt c_6, respectively. The dependence of these half-lives on the concentration of the soluble protein (data not shown) indicates a second-order reaction with rate constants (k_2) of 6×10^7 and 2×10^7 M^{-1} s^{-1} for Pc and Cyt c_6, respectively. The very slow component of the $P700^+$ reduction has been attributed to electron transfer to PSI which is either not accessible to the soluble electron carrier or lacking PsaF (*11*, *35*). The effect of the PsaJ disruption is obvious from a comparison of the transients in Figure 5. While in the wild-type PSI the amplitudes of the two fast components constitute 77% of the total amplitude with Cyt c_6, this contribution is reduced to 30% in the PsaJ-less PSI (Table 1). The slightly lower amplitudes found with Pc are partly caused by the increasing absorption of oxidized Pc superimposed to the decreasing absorption of $P700^+$ at 817 nm. These results indicate that although the PsaJ-deficient PSI complexes contain normal amounts of PsaF subunit, as shown by immunoblotting (Figure 4), 70% of these complexes feature only the third slow kinetic component which is similar to that observed with PsaF-deficient PSI complexes. Thus, it appears that in the absence of PsaJ, 70% of the PsaF subunits are unable to interact efficiently with Pc or Cyt c_6 for fast electron transfer to $P700^+$. To exclude the possibility that this alteration could be due to the solubilization of the thylakoid membranes with β-dodecyl maltoside during PSI purification, we performed the same measurements with thylakoid membranes isolated from wild type and from the J− strain. When $P700^+$ reduction was mediated either by residual Pc that was trapped in the lumen or by external 5 μM Pc that was added after a short sonication of the thylakoids, the amplitude of the PsaF-dependent second-order kinetics was 76% and 42% of the total amplitude with wild-type and mutant membranes, respectively (data not shown). At this Pc concentration, the first-order component is undetectable. Hence, the large loss of PsaF function is not due to detergent treatment of the PSI complex during preparation, but is representative of the state of PSI within the thylakoid membrane.

Chemical Cross-Linking of Pc or Cyt c_6 to PSI Is Not Affected by the Absence of PsaJ. Chemical cross-linking between Pc or Cyt c_6 and PSI has been achieved with PSI from higher plants and *C. reinhardtii* (*11*, *41*, *42*). The cross-link is formed between lysine residues present in the N-terminal domain of PsaF and carboxyl groups provided by acidic residues of the soluble proteins. We performed cross-linking experiments with wild-type and PsaJ-lacking PSI particles together with Pc or Cyt c_6. After cross-linking, the samples were separated by SDS−PAGE and analyzed by immunoblotting with antibodies raised against the PsaF polypeptide of *C. reinhardtii* (Figure 6). Clearly, the absence of the PsaJ subunit does not affect the cross-linking between PsaF and Cyt c_6 and only slightly alters the cross-linking between PsaF and Pc. This indicates that the lysines present in the N-terminal region of PsaF are accessible and can react with the carboxyl groups of Pc or Cyt c_6.

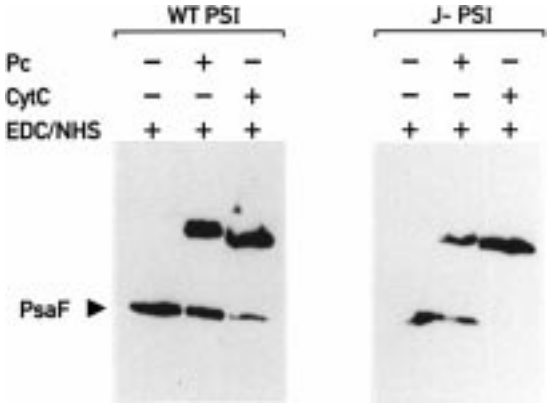

FIGURE 6: Chemical cross-linking of Pc or Cyt c_6 to PSI. After SDS−PAGE and electroblotting, cross-linking products obtained with PSI isolated from wild type (left) or from the J− strain (right) were probed with antibodies raised against PsaF. 2 μg of Chl was loaded per lane. Pc and Cyt c_6 were used at a concentration of 20 μM for the cross-linking experiments. The position corresponding to the free PsaF subunit is indicated.

DISCUSSION

In this work we have taken advantage of the physical separation of the *psaF* and *psaJ* genes, which are located in the nuclear and chloroplast genomes, respectively, to generate *C. reinhardtii* strains in which the *psaJ* gene is inactivated. The availability of a PsaF-deficient strain also allowed us to generate strains lacking both PsaF and PsaJ subunits. We have shown that insertional inactivation of the *psaJ* gene with the *aadA* cassette inserted in either orientation does not markedly affect the expression of its flanking genes *atpI* and *rps12*.

Steady-state accumulation of PSI does not require the PsaJ subunit. PsaF is present at wild-type levels in PsaJ-less PSI, indicating that, although these two subunits interact, PsaF is not destabilized by the absence of PsaJ. Therefore, the reduction of PsaF observed by Xu et al. (*20*) in *Synecocystis* PCC 6803 strain lacking the *psaJ* coding sequence is most probably due to the reduced *psaF* mRNA level in this strain. However, differences between PSI of *C. reinhardtii* and *Synecocystis* PCC 6803 cannot be excluded. Analysis of the *C. reinhardtii* strains in which the *psaJ* and *psaF* genes have been deleted reveals that functional PSI accumulates stably in the absence of both subunits as shown by the immunoblot analysis, the fluorescence transients, and the photoautotrophic growth of the mutant strains.

In vitro reduction of $P700^+$ by Pc or Cyt c_6 follows identical kinetics with the mutant and wild-type PSI complexes, indicating that PsaJ does not participate directly to the binding site for either protein. Interestingly, however, the amplitude of the two fast components of electron transfer is reduced to 30% of the total amplitude in the mutant complex although PsaF is fully retained during PSI purification and is accessible to Pc and Cyt c_6 for chemical cross-linking. Recently, mutagenesis of lysine residues from the N-terminal part of PsaF of *C. reinhardtii* has shown that this domain provides a precise recognition site for binding and fast $P700^+$ reduction (*12*). Displacement of this domain and alteration of the distance or orientation between the redox partners would have dramatic effects on electron-transfer kinetics. The most striking observation is that the absence of PsaJ does not alter the half-lives of the different kinetic phases, but leads to the formation of two PSI complex subpopulations with respect to electron transfer to $P700^+$: one behaves like wild type with fully functional PsaF whereas the other features characteristics similar to a PsaF-deficient strain. Our data suggest that in 70% of the PSI complexes lacking PsaJ, the N-terminal domain of PsaF is unable to provide a binding site for either Pc or Cyt c_6 that leads to electron transfer to $P700^+$, which can be best explained by a displacement of this domain. The observation that 23−34% of wild-type complexes also appear to be damaged and incapable of fast electron transfer suggests that the binding site for Pc and Cyt c_6 is rather sensitive. This supports the view that the lack of a stabilizing PsaJ subunit leads to an increased proportion of PSI complexes incompetent for fast electron transfer. The fact that similar results were obtained when measurements were performed on thylakoids, either with endogenous Pc or with added Pc, suggests that the reduction of functional PsaF also takes place in vivo, although we cannot formally exclude the possibility that this alteration is due to the cell breakage step during thylakoid preparation. Interestingly, the predicted transmembrane helix of PsaF appears to be shorter and less hydrophobic than typical transmembrane helices, which is consistent with the need for an additional subunit for its stabilization (*43*). We propose that the function of the PsaJ subunit is to maintain PsaF in a proper orientation so that its N-terminal domain can fulfill its function. Whether the "empty space" created by the absence of PsaJ also modifies the positioning of PsaE and alters the reducing side of PSI is currently under investigation. Other small hydrophobic subunits of PSI might have similar functions to PsaJ. The structural similarities between PsaI, a 4 kDa protein predicted to form a single transmembrane domain, and PsaJ suggest that they both stabilize the transmembrane helices of their respective partners PsaL and PsaF.

Interestingly, PsaJ contains two conserved charged residues in its putative transmembrane helix, E_{28} and R_{31} (Figure 1A). These residues could form an ionic pair allowing E_{28} to serve as a side chain ligand to the central Mg atom of a chlorophyll molecule as described in the atomic model for the light harvesting complex II (*44*). Thus, another function of the PsaJ subunit could be the binding of chlorophyll in the periphery of PSI. This hypothesis is consistent with the presence of chlorophyll molecules in the vicinity of the transmembrane helices ascribed to PsaF and PsaJ in the PSI structure (*22*).

ACKNOWLEDGMENT

We thank N. Roggli for preparing the figures.

REFERENCES

1. Golbeck, J. H. (1992) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 43*, 293−324.
2. Golbeck, J. H. (1994) in *The Molecular Biology of Cyanobacteria* (Bryant, D. A., Ed.) pp 319−360, Kluwer Academic Publishers, Dordrecht, The Netherlands.
3. Brettel, K. (1997) *Biochim. Biophys. Acta 1318*, 322−373.
4. Lelong, C., Setif, P., Lagoutte, B., and Bottin, H. (1994) *J. Biol. Chem. 269*, 10034−10039.
5. Xu, Q., Jung, Y. S., Chitnis, V. P., Guikema, J. A., Golbeck, J. H., and Chitnis, P. R. (1994) *J. Biol. Chem. 269*, 21512−8.
6. Hanley, J., Setif, P., Bottin, H., and Lagoutte, B. (1996) *Biochemistry 35*, 8563−8571.
7. Sonoike, K., Hatanaka, H., and Katoh, S. (1993) *Biochim. Biophys. Acta. 1141*, 52−57.
8. Rousseau, F., Setif, P., and Lagoutte, B. (1993) *EMBO J. 12*, 1755−1765.
9. Fischer, N., Hippler, M., Setif, P., Jacquot, J. P., and Rochaix, J. D. (1998) *EMBO J. 17*, 849−858.
10. Farah, J., Rappaport, F., Choquet, Y., Joliot, P., and Rochaix, J. D. (1995) *EMBO J. 14*, 4976−4984.
11. Hippler, M., Drepper, F., Farah, J., and Rochaix, J. D. (1997) *Biochemistry 36*, 6343−6349.
12. Hippler, M., Drepper, F., Haehnel, W., and Rochaix, J. D. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 7339−7344.
13. Hippler, M., Reichert, J., Sutter, M., Zak, E., Altschmied, L., Schroer, U., Herrmann, R. G., and Haehnel, W. (1996) *EMBO J. 15*, 6374−6384.
14. Hervas, M., Ortega, J. M., Navarro, J. A., De la Rosa, M. A., and Bottin, H. (1994) *Biochim. Biophys. Acta 1184*, 235−241.
15. Jansson, S., Andersen, B., and Scheller, H. V. (1996) *Plant Physiol. 112*, 409−420.
16. Chitnis, V. P., and Chitnis, P. R. (1993) *FEBS Lett. 336*, 330−334.
17. Xu, Q., Hoppe, D., Chitnis, V. P., Odom, W. R., Guikema, J. A., and Chitnis, P. R. (1995) *J. Biol. Chem. 270*, 16243−16250.
18. Schluchter, W. M., Shen, G., Zhao, J., and Bryant, D. A. (1996) *Photochem. Photobiol. 64*, 53−66.
19. Xu, Q., Yu, L., Chitnis, V. P., and Chitnis, P. R. (1994) *J. Biol. Chem. 269*, 3205−3211.
20. Xu, Q., Odom, W. R., Guikema, J. A., Chitnis, V. P., and Chitnis, P. R. (1994) *Plant Mol. Biol. 26*, 291−302.
21. Kruip, J., Chitnis, P. R., Lagoutte, B., Rogner, M., and Boekema, E. J. (1997) *J. Biol. Chem. 272*, 17061−17069.
22. Krauss, N., Schubert, W. D., Klukas, O., Fromme, P., Witt, H. T., and Saenger, W. (1996) *Nat. Struct. Biol. 3*, 965−973.
23. Harris, E. H. (1989) *The Chlamydomonas Source Book: a Comprehensive Guide to Biology and Laboratory Use*, Academic Press, Inc., San Diego, CA.

24. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
25. Rochaix, J.-D., Mayfield, S., Goldscmidt-Clermont, M., and Erickson, J. (1988) in *Plant molecular biology: A practical approach* (CH, S., Ed.) pp 253−275, IRL Press, Oxford.
26. Rochaix, J.-D. (1978) *J. Mol. Biol. 126*, 597−617.
27. Goldschmidt-Clermont, M. (1991) *Nucleic Acids Res. 19*, 4083−4089.
28. Boynton, J. E., Gillham, N. W., Harris, E. H., Hosler, J. P., Johnson, A. M., Jones, A. R., Randolph-Anderson, B. L., Robertson, D., Klein, T. M., Shark, K. B., et al. (1988) *Science 240*, 1534−1538.
29. Finer, J. J., Vain, P., Jones, M. W., and McMullen, M. D. (1992) *Plant Cell Rep. 11*, 323−328.
30. Fischer, N., Setif, P., and Rochaix, J. D. (1997) *Biochemistry 36*, 93−102.
31. Merchant, S., and Bogorad, L. (1986) *J. Biol. Chem. 261*, 15850−15853.
32. Kerfeld, C. A., Anwar, H. A., Interrante, R., Merchant, S., and Yeates, O. T. (1995) *J. Mol. Biol. 250*, 627−647.
33. Katoh, S., Shiratori, I., and Takamiya, A. (1962) *J. Biochem. 51*, 32−40.
34. Wood, P. M. (1978) *Eur. J. Biochem. 87*, 9−19.
35. Drepper, F., Hippler, M., Nitschke, W., and Haehnel, W. (1996) *Biochemistry 35*, 1282−1295.
36. Venturoli, G., Drepper, F., Williams, J. C., Allen, J. P., Lin, X., and Mathis, P. (1998) *Biophys. J. 74*, 3226−3240.
37. Boudreau, E., Otis, C., and Turmel, M. (1994) *Plant Mol. Biol. 24*, 585−602.
38. Liu, X. Q., Gillham, N. W., and Boynton, J. E. (1989) *J. Biol. Chem. 264*, 16100−16108.
39. Bennoun, P., and Delepelaire, P. (1982) in *Methods in chloroplast molecular biology* (Edelman, Ed.) pp 25−38, Elsevier Biomedical Press, New York.
40. Lemaire, C., and Wollman, F. A. (1989) *J. Biol. Chem. 264*, 10235−10242.
41. Hippler, M., Ratajczak, R., and Haehnel, W. (1989) *FEBS Lett. 250*, 280−284.
42. Wynn, R. M., and Malkin, R. (1988) *Biochemistry 27*, 5863−5869.
43. Chitnis, P. R., Purvis, D., and Nelson, N. (1991) *J. Biol. Chem. 266*, 20146−20151.
44. Kühlebrandt, W., Wang, D. N., and Fujiyoshi, Y. (1994) *Nature 367*, 614−621.
45. Persson, B., and Atgos, P. (1994) *J. Mol. Biol. 237*, 182−192.

BI982821A