Deregulation of Electron Flow within Photosystem II in the Absence of the PsbJ Protein*

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The photosystem II (PSII) complex of photosynthetic oxygen evolving membranes comprises a number of small proteins whose functions remain unknown. Here we report that the low molecular weight protein encoded by the psbJ gene is an intrinsic component of the PSII complex. Fluorescence kinetics, oxygen flash yield, and thermoluminescence measurements indicate that inactivation of the psbJ gene in Synechocystis 6803 cells and tobacco chloroplasts lowers PSII-mediated oxygen evolution activity and increases the lifetime of the reduced primary acceptor QA (more than a 100-fold in the tobacco ΔpsbJ mutant). The decay of the oxidized S2,3 states of the oxygen-evolving complex is considerably accelerated, and the oscillations of the Qp/Sn recombination with the number of exciting flashes are damped. Thus, PSII can be assembled in the absence of PsbJ. However, the forward electron flow from QA to plastocyanine and back electron flow to the oxidized Mn cluster of the donor side are deregulated in the absence of PsbJ, thereby affecting the efficiency of PSII electron flow following the charge separation process.

The photosystem II complex (PSII)1 of the thylakoid membrane is involved in the photochemical process resulting in water oxidation, oxygen evolution, and reduction of plastocyanine. PSII comprises a core complex formed by a few proteins binding the ligands required for primary photochemistry and electron transfer and the chlorophyll-binding antennae as well as a number of low molecular weight proteins whose functions have not yet been identified (1, 2). The process of light-induced charge separation and reduction of a quinone acceptor, similar to that performed by PSII, is also carried out by photosynthetic bacteria that do not evolve oxygen and perform cyclic electron flow around the bacterial photochemical reaction center (RC) (3). While in both cases a small number of proteins binding the appropriate ligands are capable of light-driven charge separation and electron transfer, the linear electron flow performed by PSII using water as a source of electrons is more complex and may require regulatory steps that are not demanded by the cyclic electron flow of the bacterial RC. Thus it is plausible that proteins of PSII, besides those acting as energy-transferring antennae (4), may play some role in the regulation of the forward and backward electron flow within the PSII core complex. Recombination of the primary charge-separated pair due to back electron flow may lead to the generation of singlet oxygen considered to be the cause of PSII oxidative stress and turnover of its core proteins (5, 6). Indeed cytochrome b559, an essential component of the PSII core complex is considered to play such a regulatory role by diverting electrons from the reducing side of PSII and channeling them to the oxidized donor side of the complex (7). This protein is missing from the RC complex of purple anoxygenic photosynthetic bacteria. The psbE and psbF genes encoding the α and β subunits of cytochrome b559, respectively, are with a few exceptions members of the psbEFLJ operon (8), suggesting that the products of the remaining genes of this operon, psbL and psbJ, may be involved in regulatory processes as well. Synechocystis 6803 cells in which psbL has been inactivated do not have a functional PSII despite the fact that components of the complex are present (9). Recently, inactivation of each of the genes in the psbEF-LJ gene cluster in tobacco plastids was successfully performed.2 The ΔpsbL tobacco mutant lacked functional PSII, while the ΔpsbJ mutant had impaired but detectable PSII activity. In the present study, functional analysis of both the cyanobacterial and tobacco ΔpsbJ-less mutants demonstrate that the psbJ-encoded protein plays a regulatory role in the electron flow within the PSII complex.

MATERIALS AND METHODS

Transformation of Tobacco Chloroplasts—A 3,675-base pair SalI/Eco47III fragment of the plastid chromosome of Nicotiana tabacum cv. Petit Havanna, nucleotide positions 65,310–68,985 (GenBankTM/EBI data bank accession number Z00044), containing the entire psbEFLJ operon was inserted into Smal/SalI double-digested Bluescript SK vector and cloned in E. coli XL1-Blue (Stratagene, Heidelberg, Germany). A terminator-less, chimeric adaA gene conferring spectinomycin resistance to chloroplastic mutants (10) was fused to the homologous psbA promoter. This construct was integrated into psbA modified by site-specific mutagenesis to generate an artificial PsbA restriction site at the 5‘-end of the structural gene using the synthetic oligonucleotide

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1 The abbreviations used are: PSII, photosystem II; RC, reaction center; DCMU, 3-(3,4-dichlorophenyl)1,1-dimethyleurea; TL, thermoluminescence signal; LHClI, light harvesting protein complex associated with PSII; PSI, photosystem I; PQ, plastocyanine; PQH2, plastohydroquinol.

2 R. E. Regel, N. B. Ivleva, H. Zer, J. Meurer, S. V. Shestakov, R. G. Herrmann, H. B. Pakrasi, and I. Ohad, manuscript in preparation.
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5'-GGGGTAAATGGCCGATACTGCAGGAAGGATTCCTC-3'. This operon-conform insertion interrupts the gene N-terminally between the two threonine residues at positions 4 and 5. This aadA cassette with terminator was also placed into an EcoRV site located 3' in the untranslated region of the operon (nucleotide position 66,055). This site was chosen on the basis of data presented for tobacco plants recombined and transformed throughout this article as RV5 control plants. Transformation of N. tabacum chloroplasts, selection, and culture of transformed material and assessment of the homoplastic state were performed essentially as described in De Santis-Maciossek et al. (11). The material was grown for 4–5 weeks in 12 h dark/12 h light cycles at 10 μmol of photons m⁻² s⁻¹ and 200 μmol m⁻² s⁻¹ actinic light and 50 μg/ml spectinomycin under selective conditions. As opposed to the leaves of the wild type control plants that continued to grow and maintained PSII activity most of the ∆psbJ leaves gradually lost photosynthetic activity and senesced within several weeks. The material chosen for analysis was homoplastic and fairly green when grown in dim light. A detailed description of the psbE operon mutants will be presented elsewhere.2

Cyanobacterial Cells—Synechocystis 6803 wild type, the strain in which psbJ was inactivated (13) (T203 mutant; referred to throughout this article as the psbJ-stop mutant) and the CP47 histidine-tagged HT-3 cells (14) were grown at 30 °C in BG11 medium (15). For the growth of psbJ-stop cells, the medium was supplemented with 5 μg ml⁻¹ of chloramphenicol. The liquid cultures were grown under continuous fluorescent white light (50 μmol of photons m⁻² s⁻¹) with vigorous bubbling with filtered room air.

Measurement of Photosynthetic Activity: Oxygen Evolution—PSII activity of cyanobacterial or isolated tobacco thylakoids using DBCQ and potassium ferricyanide as artificial electron acceptors was measured using a Clark-type oxygen electrode as described (16, 17).

Flash-induced Oxygen Yield—Flash-induced oxygen yield of cyanobacterial cells was measured after dark adaptation for 7 min using a home-built, bare-platinum, Joliot-type electrode as described (18). For the estimation of the rates of decay of the S₁ or S₂ states, the cells were exposed to 1 or 2 single-turnover exciting flashes (EG&G, LS-11340, 6 μs duration), respectively, followed by additional flashes after increasing time intervals, and the oxygen yield at the third flash was measured.

Chlorophyll a Fluorescence Induction Kinetics—Chlorophyll a fluorescence induction kinetics of the tobacco wild type and mutant plant leaves was measured using a Pulse Modulated Fluorometer (PAM-101, Walz, Germany).1 Leaves were dark-adapted for 15–30 min prior to the measurements. The light intensity of the modulated measuring beam (5.5 μmol of photons m⁻² s⁻¹) was 0.1 of the saturating light pulse (1 s) used for the measurement of the Fm level at saturating light intensity. The light intensity of the modulated measuring beam for dark-adapted leaves (30 μmol m⁻² s⁻¹) was 10 μmol m⁻² s⁻¹.

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Thylakoid Membranes—Thylakoid membranes were obtained by centrifugation of tobacco leaf homogenates according to Zer et al. (21). For preparation of thylakoids, 8–12 leaves that exhibited variable fluorescence in the range of 0.3–0.4 for the ∆psbJ mutant and >0.60 for the wild type plants were collected from four to six individual plants. PSII Preparations—PSII preparations exhibiting high rates of oxygen evolution were obtained from the CP47 histidine-tagged HT-3 strain of Synechocystis 6803 mutant cells as described by Bricker et al., (14).

Thermoluminescence Measurements—Charge recombination of Q₀/S₂,3 states of the manganese electron donor cluster or of the Q₄/S₄ state in presence of DCMU (10 μM) were measured as described (5, 22). The emission due to the recombination of a mixed population of Q₄/S₄ or Q₄/S₃ states could be resolved into two distinct TL signals (23). For TL signal measurements, tobacco thylakoids (5–10 μg Chl ml⁻¹) were suspended in buffer containing 20 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 20 mM NaCl, 200 μM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 100 mM sorbitol. For TL measurements of cyanobacteria, cells were suspended in fresh BG11 medium (200 μg Chl ml⁻¹) and dark-adapted at room tempera-

2 FIG. 1. Localization of PsbJ in the PSII-complex of Synechocystis 6803 thylakoid membranes. Thylakoid membranes of the wild type and psbJ-stop cells and a purified His-tagged PSII-core preparation from Synechocystis 6803 HT-3 strain were resolved by denaturing SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane, and immunodecorated with anti-PsbJ antibodies. A, membranes from wild type cells; B, membranes from psbJ-stop cells; C, His-tagged PSII core preparation from HT-3 cells. Lanes A and B had 15 μg and lane C had 1 μg of Chl-containing material.

ture for 2 h followed by storage on ice until use (24). Samples (10–15 μg of Chl) were dark adapted on the thermoluminescence apparatus stage at 20 °C for 3 min and then cooled to −10 °C and excited by one or multiple single-turnover light flashes (Xenon arc discharge lamp, EG&G, 0.05 microfarads capacitor, charged at 1000 v) as indicated followed by rapid cooling to −30 °C. The samples were then heated at a constant rate (0.5 °C s⁻¹), and light emission versus temperature was measured by photon counting. The decay time course of the Qₐ/S₃ charge separated state was measured at 20 °C. The samples were exposed to two consecutive exciting flashes (0.3 Hz) and further incubated in darkness for times as indicated before rapid freezing to −30 °C and activation of the heating and photon counting processes. Freezing from 20 °C to 0 °C was 3 s, and the data were corrected for this value.

Phosphorylation Assay—Redox-dependent LHCl phosphorylation of wild type and ∆psbJ tobacco thylakoids was carried out as described (21). The phosphorylation mixture (100 μl) contained 50 mM Tris-HCl, pH 8.0, 10 mM NaCl, 10 mM MgCl₂, 0.2 mM ATP (25 μCi γ-32P[ATP], 10 mM NaF and thylakoid membranes equivalent to 20 μg of chlorophyll. All incubations were carried out in Eppendorf tubes at 25 °C and in darkness or under room light as indicated. Activation of the protein kinase in darkness by duroquinol (1 mM) was as described (21). Phosphorylation was terminated by addition of denaturing SDS/polyacrylamide gel electrophoresis sample buffer, and the membrane polytopesides were resolved as described (25). The extent of phosphorylation was assayed by autoradiography.

Measurements of Chlorophyll, PSI Activity, and Immunodetection of PsbJ—Chlorophyll concentration was measured in methanolic extracts of cyanobacterial cells (26) and in 80% acetone extracts for tobacco thylakoids. Photosystem I activity in intact leaves was estimated by measuring absorption changes related to F₇₀₀ oxidoreduction using the PAM-101-dedicated PSI attachment. Immunodetection of PsbJ on Western blots was carried out as described using monospecific polyclonal α-PsbJ antibodies (13).

RESULTS

Localization of PsbJ in PSI—We have previously shown that PsbJ is a membrane-associated protein in Synechocystis 6803 (13). To ascertain that the changes in PSI activity described in this work are due to the loss of a PSI intrinsic component, we have tested the presence of the PsbJ protein in oxygen evolving PSI preparations. The data presented in Fig. 1 demonstrate that PsbJ is a component of the PSI complex in Synechocystis 6803 and is absent in the psbJ-stop cyanobacterial mutant cells. PsbJ was also detected in the PSI crystals of Synechococcus elongatus (2). Since this gene is present in the chloroplast genome of higher plants, and its inactivation drastically affects PSI electron flow in tobacco thylakoids (see below), we infer that PsbJ is also a component of the higher plant PSI.

Loss of PsbJ Affects the Oxygen Evolution Activity and the Kinetics of Qₐ Oxidation by Forward Electron Flow—Oxygen evolution was impaired in both the ∆psbJ and psbJ-stop mutants. The ∆psbJ thylakoids evolved 20–40 μmol of oxygen Chl⁻¹ h⁻¹ (mg) when measured with DBCQ as an electron acceptor as compared with 120 μmol of oxygen (mg) Chl⁻¹ h⁻¹ for the control thylakoids. However, the impairment of oxygen evolution activity on a chlorophyll basis in the cyanobacterial psbJ-stop mutant was less pronounced allowing slow autotrophic growth and compatible at least partially with a lower PSI/PSI ratio in these cells (13). The Fₐ/Fₐ₋₁ values were in the range of 0.74–0.76 for the control RV5 tobacco leaves and 0.57 (± 0.1) for the ∆psbJ mutant. The variable fluorescence parameter of the tobacco mutant were due to increasing senescence with the time of growth of the plants in the plastic containers. The ∆psbJ mutant of tobacco exhibited F₇₀₀ oxidoreduction activity as measured by the PAM-101 attachment. However, the rate of F₇₀₀ reduction in the mutant was considerably slower as compared with that of the control plants indicating that the reduction of the PQ pool is impaired

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in the mutant plants (not shown). Fluorescence quenching \((F_0/F_s)\) in the control tobacco leaves after 2–3 min exposure to red actinic light was about 0.3. LHCCI phosphorylation in the RV5 thylakoids was light activated and inhibited by DCMU. The \(\Delta psbJ\) mutant did not exhibit fluorescence quenching activity and the redox-dependent LHCCI phosphorylation in isolated thylakoids \((21)\) was not activated by light (Fig. 2). However, redox-dependent protein kinase activity could be elicited in the \(\Delta psbJ\) thylakoids by addition of duroquinol. These results can be explained by a low rate of PQ reduction in the mutant. The ratio PQH/\(PQ\) induced by white light illumination used in the phosphorylation assay could be low due to the activation of PSII and plastoquinone oxidation by ambient oxygen and thus may not be sufficient to activate the redox-dependent LHCCI kinase. Addition of far red light \((710\ \text{nm}, 20\ \mu\text{mol m}^{-2}\ \text{s}^{-1})\) slightly elevated the oxidation of \(Q_A\) in the wild type (Fig. 2) as well as in the \(\Delta psbJ\) leaves \((\text{not shown})\) indicating that quinone/quinol exchange at the \(Q_A\) site occurs also in the mutant.

In both the tobacco and cyanobacterial mutants, the oxidation of \(Q_A^*\) as measured by fluorescence kinetics was slower as compared with that of the wild type \((\text{Figs. 2 and 3})\). The oxidation of \(Q_A\) in the tobacco \(\Delta psbJ\) plants exhibits an initial relatively fast phase accounting in various leaves from 10–40% of the signal followed by an extremely slow phase \((t_{1/2}\ \text{ranging between} 20–60\ \text{s})\) \((\text{Figs. 2 and 3A})\). The fluorescence decay in the absence of DCMU is due mostly to forward electron flow from of \(Q_A\) to \(Q_B\). The above results indicate that this path of electron flow is significantly hindered in the mutant. Inhibition of electron flow from \(Q_A^*\) by DCMU and acceptance of electrons from \(Q_B^*\) by DBCQ indicates that binding of ligands at the \(Q_A\) site and thus, PQ/PQH\(_2\) exchange is not significantly hindered in the mutant. Inhibition of electron flow to \(Q_B\) indicates that \(Q_A\) electrons from \(Q_A\) as it does to the artificial electron acceptor, DBCQ.

Measurements of the decay time of \(Q_A\) in the cyanobacterial \(psbJ\)-stop cells showed a slower rate as compared with that of the wild type cells. However, the difference between the mutant and the wild type cells was significantly smaller than that in the tobacco plants and could not be accurately resolved by the \(PAM\) 101 fluorometer. This difference was, however, clearly resolved when the oxidation of \(Q_A\) was measured using a dual modulation kinetic fluorometer that has a time resolution in the range of 10 \(\mu\text{s}\). The oxidation of \(Q_A\) was about 40% slower in the cyanobacterial mutant cells as compared with that of the wild type cells \((\text{Fig. 3B})\).

**Alteration of Back Electron Flow in the \(\Delta psbJ\)-less PSI of Cyanobacterial Cells**—Back electron flow from the acceptor to the donor side of PSII was measured by several methods. The decay of \(Q_A\) by back electron flow in the presence of DCMU as estimated by fluorescence kinetics showed a doubling of the oxidation time in the \(psbJ\)-stop cyanobacterial cells \((t_{1/2}, \ 1.2\ \text{s}\ \text{as compared with} 0.5\ \text{s for the wild type cells})\) \((\text{Fig. 3B})\). The most striking difference between the \(psbJ\)-stop and wild type strains of \(\text{Synechocystis} 6803\) cells was found in the decay of the \(S_2\) state \(\text{via} \ \text{back electron flow from the acceptor side as evidenced by measurements of oxygen flash-yield} (t_{1/2} \ \text{about} 3\ \text{s} \ \text{and} 60\ \text{s}, \ \text{respectively})\) \((\text{Fig. 4A})\). The decay of the TL signal resulting from the charge recombination of the \(Q_{A}/S_2^*\) state was also significantly faster in the \(psbJ\)-stop mutant as compared with the wild type cells \((5.6\ \text{s} \ \text{and} 22\ \text{s}, \ \text{respectively})\) \((\text{Fig. 4B})\). The maximal photon emission due to charge recombination of \(Q_{A}/S_2^*\) was shifted to a slightly lower temperature for the cyanobacterial mutant as compared with the wild type cells \((26^\circ \text{C} \ \text{and} 30^\circ \text{C}, \ \text{respectively})\), while the temperature of the maximal emission resulting from the recombination of \(Q_{A}/S_2^*\) in the presence of DCMU was slightly increased in the mutant relative to the wild type cells \((11^\circ \text{C} \ \text{and} 9^\circ \text{C}, \ \text{respectively})\). This is in agreement with the increase in the \(Q_A\) oxidation time in the presence of DCMU by the same route as measured by fluorescence kinetics \((\text{Fig. 3B})\). The intensity of both the \(Q_A\) and \(Q_B\) thermoluminescence emission bands of the \(psbJ\)-stop cells on a Chl basis was significantly lower as compared with that of the wild type cells \((\text{Figs. 5A} \ \text{and} 5B)\) and amounts to about 11 and 25% of the intensity of wild type TL signals for the \(Q_{A}/S_2^*\) and the \(Q_{B}/S_2^*\) recombination, respectively. The oscillations of the TL signals intensity with the number of exciting flashes showed a pattern of maximal emission following excitation by two and six consecutive flashes for the mutant and wild type cyanobacterial cells. However, the signal from the mutant was slightly damped \((\text{Fig. 5C})\).

**Alteration of the Back Electron Flow in the \(\Delta psbJ\)-less PSI of Tobacco Thylakoids**—The TL signal resulting from the recombination of the \(Q_{A}/S_2^*\) states was downshifted by about 10 °C in the \(\Delta psbJ\) thylakoids relative to that of the control RV5 membranes, while the emission maximum due to the recombination of \(Q_{A}/S_2^*\) state in the presence of DCMU was upshifted by about 4 °C \((\text{Fig. 5B})\). The TL signals intensity of the tobacco \(\Delta psbJ\) mutant did not oscillate with the number of exciting flashes \((\text{Fig. 5D})\). The decay of the \(Q_{A}/S_2^*\) states at

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**Fig. 2.** Fluorescence kinetics, fluorescence quenching and LH-CII phosphorylation. Upper panel, fluorescence kinetics of tobacco control RV5 \(\text{left side traces} \) and \(\Delta psbJ\) mutant leaves \(\text{right side traces} \) indicating absence of fluorescence quenching and slow oxidation of \(Q_A\) in the control leaves. Lower panel, LHCCI phosphorylation in tobacco control and \(\Delta psbJ\) thylakoids; upward and downward arrows, saturating light pulse on/off, respectively; \(AL\), actinic light; \(FR\), far red light (710 nm).

**Fig. 3.** Fluorescence decay kinetics in tobacco leaves \(A\) and \(\text{Synechocystis} 6803\) cells \(B\). Note the high level of \(F_0\) in the \(\Delta psbJ\) leaves \(A\). \(\Delta psbJ\) traces normalized to the \(F_0\) level demonstrating the slow fluorescence decay in the \(\Delta psbJ\) tobacco mutant; \(B\), triangles, traces in the absence of DCMU; circles, traces in the presence of DCMU; open and closed symbols, wild type and \(psbJ\)-stop cells, respectively.
20 °C as estimated by TL measurements in the thylakoids of ΔpsbJ tobacco mutant was basically similar to that of the psbJ-stop cyanobacterial mutant. However, the shape of the curve clearly indicates presence of two major phases, an initial fast phase accounting for about 60% of the signal with a $t_{1/2}$ roughly estimated to be less than 2 s and a second slow phase extending up to 33 s with an estimated $t_{1/2}$ of about 18 s (Fig. 4B). The decay of the above TL signal in thylakoids of the RV5 tobacco thylakoids exhibited a $t_{1/2}$ of about 25 s (not shown) as also reported for higher plant thylakoids (27). The slow phase of the TL signal decay in the ΔpsbJ tobacco mutant could explain the slow phase of fluorescence decay due to the oxidation of QA in the absence of DCMU observed in intact leaves (Figs. 2 and 3).

**DISCUSSION**

The findings presented in this work demonstrate that functional PSII can be assembled in absence of PsbJ. However, the electron flow within the PsbJ-less PSII complex is deregulated and characterized by a slower oxidation of the primary electron acceptor quinone QA via forward electron flow and a faster reduction of the oxidized S states. While the changes of fluorescence decay, oxygen flash yield and charge recombination detected by TL, are common to both, the cyanobacterial and tobacco mutants, the extents of these changes are more accentuated in the tobacco mutant as compared with the cyanobacterial mutant.

**Electron Flow within PSII**—The major difference between the psbJ-stop cyanobacterial and ΔpsbJ tobacco mutants appears in the oxidation of QA via forward electron flow to QB. This process is slower by about 40% in the psbJ-stop cells as compared with a slowing by almost 2 orders of magnitude in the tobacco ΔpsbJ mutant. This large increase in the life time of QA explains the suppression of the TL signal oscillations with the number of exciting flashes in the tobacco thylakoids (D), respectively. The data are representative of several experiments, and the variation between different batches of cyanobacterial cultures or tobacco thylakoids preparations was within 15%. Wt, wild type Synechocystis 6803 cells; RV5, control tobacco thylakoids.

**FIG. 5. Thermoluminescence signals from Synechocystis 6803 cells and tobacco thylakoids.** A, thermoluminescence signals due to recombination of QA/S2 and QB/S2,3 states (QA and QB traces, respectively) in Synechocystis 6803. B, thermoluminescence signal due to recombination of QA/S2 (QA trace) and oscillation of the QB/S2,3 signal with flash number (QB traces) in tobacco thylakoids. The shifts in the emission peak temperature as a function of the flash number is due to alternating changes in S2/S3 ratio with the number of flashes from 1 to 6. C and D, oscillation of TL intensities with the number of flashes calculated from data similar to those shown in B for Synechocystis cells (C) and tobacco thylakoids (D), respectively. The data are representative of several experiments, and the variation between different batches of cyanobacterial cultures or tobacco thylakoids preparations was within 15%. Wt, wild type Synechocystis 6803 cells; RV5, control tobacco thylakoids.

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Since the TL signal due to charge recombination in the absence of DCMU does not resolve the contributions of back electron flow from QA from that from QB, the photon emission peak is broader and is shifted to a temperature lower than that of the wild type thylakoids. Accordingly, the decay of the TL signal resulting from this recombination is also considerably faster (Fig. 5).

The back electron flow due to the Q2/S2,3 charge recombination in the cyanobacterial psbJ-stop cells as measured by thermoluminescence shows a small downshift of the TL signal emission temperature (−4–5 °C) and a marginal upshift of the TL signal (<2 °C) for the recombination of the Q2/S2 state in presence of DCMU. This is in agreement with the small degree of damping of the TL signal oscillation with the number of exciting flashes that maintains a normal 2/6 pattern (5, 27) in the wild type cells. However, the extent of the TL signal on a chlorophyll basis as compared with that of the wild type cyanobacterial cells is extremely low. Considering that the PSI/PSII ratio in this mutant cells on a chlorophyll basis is about 0.4 (13), one would expect a proportional loss of the TL signal intensity. The lower TL emission of the psbJ-stop cells as compared with the wild type strain observed in this work indicates that in a large fraction of the PSI1 population back electron flow and reduction of the S2,3 states occurs via a different route that may not involve the recombination of the primary charge-separated state and the related photon emission. Back electron flow measured by the oxygen flash yield technique in the psbJ-stop cells in the absence of DCMU indeed shows a very fast decay of the S2 and S3 states as compared with that in the wild type cells with a lifetime compatible with that measured by the TL method in the absence of DCMU. These results indicate that the electron donor for this process could be QA. However, the pathway of electron transfer possibly involves an intermediate electron transfer carrier reducing S2 and S3, yet avoiding the recombination of the primary charge-separated pair. The pathway for this route of back electron flow could include cytochrome b559 directly (28) or in combination with Chlα (29) and possibly via a carotenoid cation radical (6, 30).

Oxygen Evolution—The interpretation of the experimental results presented above is supported by the oxygen evolution measurements. The rate of electron flow in the thylakoids of the tobacco mutant using DCBQ as an electron acceptor that binds to the QA site and accepts electrons from QA was significantly lower than that of the wild type thylakoids. The reduction of PQ was even slower as indicated by the extremely slow oxidation of QA in the absence of artificial electron acceptors. These results possibly indicate that the loss of PsbJ induces a change in the redox potential of the QA/QA pair relative to DCBQ bound at the QB site that is more negative than that relative to PQ bound at this site. Binding of ligands at the QB site has been reported to affect the redox potential of QA (31). The fact that the QA/QA potential can be significantly altered following interactions of ligands with the QA site of the D1 protein in proximity to the QA site points toward the possibility that the QA potential may be influenced by interactions between the D2 protein harboring the QA binding site and adjacent proteins.

The possibility that the absence of PsbJ induces changes in the QA site affecting the rate of PQ/QH2 turnover at the QB site could also explain the slow oxidation of QA in the absence of DCMU. However, changes in the PQ turnover at the QA site cannot explain the observed changes in the lifetime of the S states, nor those of the shifts in the TL signal temperature for the recombination of both the Q4P/S2,3 and QA/S2 states.

The question arises as to the difference in the extent of PSII activity induced by the loss of PsbJ between tobacco and cyanobacteria. A priori in view of the similarity of the activity of PSI between higher plants and cyanobacteria, one would not expect such a difference. However, one should consider that PSII in plants differs in terms of its polypeptide composition from that of cyanobacteria. The 17- and 23-kDa oxygen evolution-enhancing polypeptides as well as the tightly bound light-harvesting complexes encoded by the lheb3,4 genes are present in plant PSI1 but are absent from the cyanobacterial PSI1. It is possible that the PSI1 complexes differ in their plasticity at both structural and functional levels. The degree of PSI1 alteration by inactivation of PSI1 related genes between cyanobacteria and higher plants has been reported to differ, possibly indicating a more stringent control of PSI1 activity in the cyanobacterial cells as compared with the eukaryotic PSI1 (28, 32). This may be related to the fact that in the cyanobacterial thylakoids the redox sate of the PQ pool, and thus back electron flow, is affected by both the light-driven as well as the respiratory pathways.

Mechanism—The phenomena described in this work may be explained assuming that the QA potential of the mutants is more positive relative to that of the wild type PSI1. The upshift of the TL peak emission temperature by about 4 °C for the recombination of Q2/S2 state in the presence of DCMU and the shorter lifetime of the S states is in agreement with this suggestion. Changes observed in these parameters are considered as being related to changes in the redox potentials of the above electron donors/acceptors (29, 33–35). The experimental evidence in support of the above-offered hypothesis is circumstantial. Additional experiments including measurements of the QA/QA potential of the mutants need to be performed to further elucidate the mechanism of the observed changes in the electron flow within PSI1 induced by the absence of PsbJ. The highly hydrophobic PsbJ protein with a single transmembrane helix is not known to participate in the binding of any electron carrier ligand. Recently it was proposed that the interaction between cytochrome b559 and QA is involved in the setting of the redox potential of both these PSI1 components (30, 36). The absence of PsbJ may affect the interaction of additional proteins in the PSI1 complex that may be involved in this process. Alternatively, PsbJ may interact directly with and affect the proteins involved in the electron transfer process. A close interaction between cytochrome b559 and the D2 protein that forms the QA binding niche has been suggested (6, 35, 37) and supported by the recently reported x-ray crystal structure of PSI1 at 3.8 Å resolution (2). It is possible that an association of PsbJ with the above-mentioned two proteins regulates the interaction between these electron carriers, thus affecting the electron flow path. Among the unidentified transmembrane α-helices resolved by x-ray analysis of the S. elongatus PSI1 (2), one is located in close vicinity to cytochrome b559 and may belong to PsbJ. Further work using cross-linking methods may provide support for this hypothesis. While it is established that cytochrome b559 may assume a high or low potential in response to factors affecting the protein environment (29, 31), no such change has been reported to occur for the QA site of functional PSI1. Possibly, the H subunit of the bacterial RC may play a similar role in the regulation of electron flow via QA (38). Based on the results presented in this work it is conceivable that PsbJ functions in conjunction with the other components encoded by the psbEFLJ operon in the regulation of the electron traffic within PSI1 and thus plays an important role in the efficiency of forward electron flow following the charge separation process.

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