The PSI-K Subunit of Photosystem I Is Involved in the Interaction between Light-harvesting Complex I and the Photosystem I Reaction Center Core*

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PSI-K is a subunit of photosystem I. The function of PSI-K was characterized in Arabidopsis plants transformed with a psaK cDNA in antisense orientation, and several lines without detectable PSI-K protein were identified. Plants without PSI-K have a 19% higher chlorophyll a/b ratio and 19% more P700 than wild-type plants. Thus, plants without PSI-K compensate by making more photosystem I. The photosystem I electron transport in vitro is unaffected in the absence of PSI-K. Light response curves for oxygen evolution indicated that the photosynthetic machinery of PSI-K-deficient plants has less capacity to utilize light energy. Plants without PSI-K have less state 1-state 2 transition. Thus, the redistribution of absorbed excitation energy between the two photosystems is reduced. Low temperature fluorescence emission spectra revealed a 2-nm blue shift in the long wavelength emission in plants lacking PSI-K. Furthermore, thylakoids and isolated PSI without PSI-K had 20–30% less Lhca2 and 30–40% less Lhca3, whereas Lhca1 and Lhca4 were unaffected. During electrophoresis under mildly denaturing conditions, all four Lhca subunits were partially dissociated from photosystem I lacking PSI-K. The observed effects demonstrate that PSI-K has a role in organizing the peripheral light-harvesting complexes on the core antenna of photosystem I.

Photosystem I (PSI)1 from higher plants is a supramolecular complex consisting of 17 different polypeptides located in the non-appressed thylakoid membranes (1). The complex catalyzes the light-driven electron transfer from plastocyanin to ferredoxin and is composed of a chlorophyll a binding core complex and a chlorophyll a/b binding peripheral antenna called LHCI. The core of PSI is a heterodimer consisting of the 82–83-kDa PSI-A and PSI-B subunits. At the interface between the two homologous subunits, the primary electron donor P700 (a chlorophyll a dimer) and the electron acceptors A6 (a chlorophyll a molecule), A1 (a phylloquinone), and F4 (a [4Fe-4S] iron-sulfur cluster) are bound (1–3). The remaining cofactors, F4 and F8 (both [4Fe-4S] iron-sulfur-clusters), are bound to PSI-C. The other subunits of PSI do not bind electron acceptors. Almost all of the ~90 Chl a and 14 β-carotene molecules are bound to the PSI-A/B dimer. However, recent data have shown that some pigment molecules might be bound to some of the smaller subunits (3). Higher plants and green algae contain the additional subunits PSI-G, PSI-H, and PSI-N, which have not been identified in the PSI complexes from cyanobacteria. All the subunits of PSI appear to be present in one copy per P700 (1). Cyanobacterial PSI occurs as trimers of functional PSI, whereas such trimers have not been reported for eukaryotic PSI.

The LHCI moiety is arranged around the core (4) and is composed of the products of four nuclear genes, Lhca1–4, with molecular masses of 20–24 kDa. LHCI binds about 110 chlorophyll a and b molecules and approximately 20 xanthophyll molecules per P700 (5, 6). LHCI can be separated into three subcomplexes providing a nomenclature based on the 77-K fluorescence emission of each subcomplex; the LHCI–730 complex is a heterodimer composed of Lhca1 and Lhca4, and LHCI–680A and LHCI–680B are homodimers of Lhca3 and Lhca2, respectively (7–9). The dimer complexes associate independently with the reaction center (10).

The structure of PSI from the thermophilic cyanobacterium Synechococcus elongatus has been analyzed at 4-Å resolution by x-ray crystallography (3, 11). In the structural model six transmembrane α-helices were located in the region facing adjacent monomers. Three of the helices defining the connection domain near the trimer axis were assigned to PSI-L and PSI-I. The other two α-helices were assigned to PSI-K, which contains two transmembrane α-helical regions (12), and the remaining helix was assigned to PSI-M. The presence of PSI-K is not sufficient for trimer formation when PSI-L is absent (13) suggesting that PSI-L is required for trimerization in cyanobacteria (3). Furthermore, six α-helices in the amino-terminus of PSI-A and PSI-B together with the α-helices of the peripheral subunits PSI-L, -I, -K, -J, -F, and -M describe a roughly continuous ring of α-helices around the core antenna system, which indicates that the role of the peripheral subunits may be primarily structural by providing a partially enclosing and stabilizing framework to the core antenna system (3). The authors predict that loss of any of the peripheral subunits, and in particular PSI-F, -K, and -L, which possess a large membrane integral surface area, could lead to the partial disruption of the core antenna system. Besides a role in delimiting the core antenna system PSI-L, -K and -F are also assigned a role in co-ordination of chlorophyll a molecules and/or in providing a suitable hydrophobic environment to accommodate such molecules (3). This is supported by spectroscopic data obtained with

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1 The abbreviations used are: PS, photosystem; LHC, light-harvesting complex; Chl, chlorophyll; CPI, PSI reaction center with light-harvesting complexes; Tricine, N-(2-hydroxy-1,1-bis(hydroxymethyl) ethyl)glycine; Fm, maximum fluorescence.

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a PSI-K-deficient PSI mutant of Synechocystis PCC6803, which suggest that the absence of PSI-K results in loss of core antenna chlorophyll(s) absorbing near 675 nm (14).

PSI-K from spinach may be tightly associated with the PSI-A/B heterodimer (15, 16). However, PSI-K from spinach, pea, and barley was depleted from the PSI core by methods used for separation of LHCl from PSI (17, 18). Treatment of thylakoids with proteinase K resulted in degradation of PSI-K (19), indicating that part of the PSI-K polypeptide is exposed on the stromal side of the thylakoid membrane. It has therefore been proposed that the membrane-spanning PSI-K subunit is located near the rim of the PSI complex between the PSI and LHCl and is thus easily lost upon detergent treatment (18).

There is significant sequence similarity between PSI-G and PSI-K from eukaryotes (18). A computer comparison of PSI-G and PSI-K from Arabidopsis displays approximately 30% amino acid identity. In fact, the cyanobacterial PSI-K is equally similar to plant PSI-G and PSI-K. However, there is no evidence that cyanobacterial PSI contains more than one copy of PSI-K. In the genome sequence of Synechocystis PCC6803 two open reading frames have been assigned as potential psaK genes. The deduced primary sequences of the two open reading frames show only 42% overall identity. The role of one of the psaK genes, which encoded a peptide with an amino terminus corresponding to that of the Psak peptide purified from PSI, has recently been analyzed, and it was shown that the gene product was dispensable for growth, photosynthesis, and the formation of PSI trimers in Synechocystis (20). The role of the other potential psaK gene remains unknown. However, this gene does not encode a protein with higher similarity to PSI-G. Thus, the role of PSI-K in higher plants is still unclear.

In plants, PSI occurs as a monomer, and a role of PSI-K in stabilization of a trimer is excluded. The indications from the cyanobacterial structural model of a continuous ring of α-helices around the core antenna system where the role of the peripheral subunits is primarily structural may also be valid for the plant PSI. Furthermore, light-harvesting chlorophyll a/b-binding proteins are only present in plants and not in cyanobacteria, and one other function of PSI-K could be in the interaction with LHCl as indicated from cross-linking studies in which PSI-K was found to interact with PSI-A/PSI-B and Lhca3 (10).

To investigate the role of PSI-K we transformed Arabidopsis plants with a psaK cDNA in antisense orientation under the control of a constitutive promoter. Transformants with a single copy of the antisense psaK gene and the 35 S terminator were analyzed with several methods at both the biochemical and physiological levels. The vector construct was transformed by electroporation (24) into the Agrobacterium tumefaciens strain C58 (25). Plasmid integrity in A. tumefaciens cultures used for plant transformation was verified by polymerase chain reaction analysis on colonies. Arabidopsis plants were transformed by the floral dip method using Silwet L-77 (26).

Seeds harvested from transformed plants were germinated on MS medium (Sigma) containing 50 mg l−1 of kanamycin sulfate, and 0.8% agar for 2 weeks, and kanamycin-resistant Arabidopsis plants were selected. Seedlings were then transplanted to soil. All biochemical and physiological experiments were performed with fully expanded rosette leaves harvested prior to stem elongation from leaf-grown plants.

Isolation of Thylakoid Membranes and Preparation of PSI Complexes—Leaves from 6–8-week-old plants were used for isolation of thylakoids as described previously (27). Total Chl and Chl a/b ratio were determined in 80% acetone according to Lichtenthaler (28). The samples were frozen in liquid nitrogen and stored at −80 °C. For preparation of PSI complexes, thylakoid membranes were prepared as described by Mullet et al. (29). Thylakoid membranes (1 mg of Chl ml−1) were solubilized for 10 min with 1% dodecyl-β-D-maltoside (Sigma) at 0 °C. Following centrifugation (5 min, 20,000 × g), aliquots of the supernatant were applied on sucrose gradients. The sucrose gradients were prepared by freezing and subsequent thawing at 4 °C of 11 ml of 0.4 M sucrose, 20 mM Tricine-NaOH (pH 7.5), 0.06% dodecyl-β-D-maltoside. The gradients were centrifuged for 20 h at 285,000 × g. The PSI band was collected with a syringe, and its protein composition was analyzed by fully denaturing SDS polyacrylamide gel electrophoresis. Immunoblot Analysis—Plants lacking the PSI-K subunit were identified by immunoblotting. Chl leaf extracts were prepared as described in Haldrup et al. (27). Immunoblotting was carried out by transferring electrophoresed proteins to nitrocellulose membranes followed by incubation with polyclonal rabbit antibodies raised against barley PSI-K and PSI-F proteins (30) and visualization using secondary antibodies conjugated with alkaline phosphatase (DAKO, Copenhagen, Denmark). Isolated thylakoids were analyzed in similar immunoblotting procedures using antibodies raised against PSI-G, -H, and -L and Lhca1, Lhca2, Lhca3, and Lhca4 from barley. These were detected using a chemiluminescent detection system (SuperSignal, Pierce, Rockford, IL and ECL, Amersham Pharmacia Biotech) according to the instructions of the manufacturers. The Lhca1 and Lhca3 antibodies were a kind gift from Dr. Stefan Jansson, University of Umeå, Umeå, Sweden.

NADP+- Photoreduction Measurements—NADP+ photoreduction activity of PSI was determined from the absorbance change at 340 nm as described by Naver et al. (31) using thylakoids equivalent to 5 µg of Chl. The thylakoids were solubilized in 0.1% n-decyl-β-D-maltopyranoside prior to the measurement.

P700 Flash Absorption Spectroscopy—Flash-induced P700 absorption change was measured at 834 nm, essentially as described previously (31, 32). The saturating actinic pulse (532 nm, 6 ms) was produced by a Nd:YAG laser. Thylakoids (32 µg of Chl ml−1) were dissolved in 250 µl of 20 mM Tricine (pH 7.5), 40 mM NaCl, 8 mM MgCl2, 0.1% decanal, and 60 µM 2,6-dichlorophenolindophenol. The solution was centrifuged three times for 20 s at 200 × g to remove starch grains prior to measurement. The sample (200 µl) was transferred to a cuvette with 1-cm path length. A diode laser provided the measuring beam, which was detected using a photodiode. P700 content was calculated from the absorption change at 834 nm using an absorption coefficient of 5000 m−1 cm−1 for P700 (33).

Photon-yield and Fluorescence Measurements—Rates of photosynthetic O2 exchange were measured at 20 °C and CO2 saturation with a system incorporating a leaf-disc electrode (Model LD-2/2, Hansatech, Norfolk, United Kingdom) and a Schott KL-1500 lamp as described by Haldrup et al. (27). The leaf area was determined using a Gel Doc scanner (Bio-Rad) and measuring the area using the Gel Doc software. Determination of conventional fluorescence parameters was performed with a pulse amplitude modulation 101–103 fluorometer (Walz, Effeltrich, Germany) by using a standard setup as reported previously by Haldrup et al. (27).

State 1-State 2 Transitions in Leaves—State transitions were measured with a pulse amplitude modulation 101–103 fluorometer (Walz, Effeltrich, Germany). A detached leaf from 30-min dark-adapted plants was illuminated with the maximum fluence rate (FM) of 800 µmol photons m−2 s−1 for 60 s. A pulse of 1.5 ms at 25% of FM was measured during exposure to a saturating flash (0.5 s, 6000 µmol m−2 s−1). The leaf was then illuminated for 20 min with 100 µmol photons m−2 s−1 blue light (which favors PSI) from a Schott KL-1500 lamp equipped with a Corning 4–96 filter. The maximum fluorescence yield (Fm2) was then measured in this state (state 2). Subsequently state 1 was induced by switching on a far-red light (Walz 102-PR) (which
favors PSI), and after 20 min the maximum fluorescence yield (Fm1) was measured. The far-red light was then switched off, and the kinetics of state 1 to state 2 transition was measured.

Low Temperature Fluorescence Measurements—The fluorescence spectrum at 77 K was recorded for intact leaves that were dark-adapted for 30 min before measurement using a bifurcated light guide connected to a Perkin-Elmer LS50B spectrofluorometer. The excitation light had a wavelength of 435 nm, and emission was detected from 650 to 800 nm.

PSI Antenna Function and Cyclic Electron Transport—PSI antenna function was determined from light-induced P700 absorption changes at 810 nm using the Walz dual-wavelength emitter detector unit ED-P700DW-E connected via a pulse amplitude modulation fluorometer to a Tektronix TDS420 oscilloscope. A leaf from a dark-adapted plant was fixed to the light fiber, and the leaf was illuminated for 5 min using the state 1 light conditions described under “State 1-State 2 Transitions in Leaves.” Subsequently, the leaf was only illuminated by far-red light (actinic light from a Walz 102-FR source) for 30 min before measurement using a bifurcated light guide connected to a Perkin-Elmer LS50B spectrofluorometer. The excitation light had a wavelength of 435 nm, and emission was detected from 650 to 800 nm.

The postillumination change in absorption was resolved into exponential decays by a Levenberg-Marquardt nonlinear regression procedure (34).

Green Gel Electrophoresis—Non-denaturing green gel electrophoresis was carried out as described by Knoetzel and Simpson (35), except that a Bio-Rad Mini-Protean II electrophoresis cell was used for the separation of pigment proteins.

RESULTS

Construction of Arabidopsis Lines Lacking PSI-K by Antisense—The original transformed plants were self-fertilized, and the seeds produced were germinated and selected on kanamycin-containing plates. A total of 99 kanamycin-resistant plants were analyzed. Total leaf protein was prepared from all lines and analyzed by immunoblotting using an antibody raised against barley PSI-K, and as an internal control an antibody against barley PSI-F was used (Fig. 1). In 37 of the 99 analyzed plants no PSI-K protein could be detected. The detection limit was about 3% of wild-type PSI-K content.

There was no obvious visible difference between wild-type plants and plants lacking PSI-K, and plants without PSI-K had a normal life cycle and seed set. All experiments were carried out with plants that were screened by immunoblotting for the presence of PSI-K prior to further analysis.

To test the stability of the PSI-K suppressed phenotype, eight lines that had no detectable PSI-K when analyzed in the T1 generation were selected at random for analysis of T2 offspring. For each line, eight offspring plants were analyzed by immunoblotting (data not shown). In only two lines all offspring plants had no detectable PSI-K, and two other lines had offspring with wild-type PSI-K amounts. The remaining four lines revealed offspring with mixed phenotypes, i.e. some had no detectable PSI-K, some had reduced levels of PSI-K, and others had wild-type levels of PSI-K.

 Pigment Composition, P700 and Antenna Function, and in Vitro NADP⁺ Photoreduction—In plants with no detectable PSI-K the Chl ab ratio was 3.20 ± 0.15 (± S.D., n = 7), whereas in plants with wild-type levels of PSI-K the ratio was 2.70 ± 0.08 (± S.D., n = 5). These ratios are significantly different (Student's t test, p < 0.001). Thus, compared with wild-type, plants without PSI-K have a 19% higher Chl ab ratio, which indicates either an increased PSI/PSII ratio or a decreased antenna size.

The amount of P700 was determined by flash-induced absorption changes at 834 nm. These measurements show that plants with no detectable PSI-K have 19% more P700 (when compared on a chlorophyll basis) to plants with normal PSI-K levels. The number of chlorophylls per P700 reaction center was 573 ± 28 for wild-type and 476 ± 15 (± S.D., n = 4) for plants devoid of PSI-K. These ratios are significantly different (Student's t test, p < 0.001). This indicates that plants without PSI-K compensate by making more PSI.

To analyze the importance of PSI-K for electron transfer, NADP⁺ photoreduction was determined on thylakoids purified from four lines without PSI-K and four lines with wild-type levels of PSI-K. With thylakoids from wild-type an activity of 15.0 ± 1.4 μmol NADPH sec⁻¹ (μmol P700)⁻¹ (± S.D., n = 4), and with thylakoids devoid of PSI-K an activity of 12.7 ± 2.6 μmol NADPH sec⁻¹ (μmol P700)⁻¹ was obtained. The difference between these values is not significant suggesting that plants without PSI-K are not affected in in vitro steady-state PSI activity.

The effect of PSI-K on the amounts of other PSI core polypeptides was investigated by immunoblot analysis of thylakoid preparations from four plants without PSI-K and four plants with wild-type levels of PSI-K. The samples were loaded on a chlorophyll basis (Fig. 2). The thylakoids from the four antisense PSI-K plants are clearly devoid of PSI-K protein, whereas there are no drastic changes seen in the amounts of PSI-F, -G, -H, and -L. Therefore, the PSI core appears to be intact in plants devoid of PSI-K.

Leaf Level Photosynthesis (O₂ Curves), State Transition, and Fluorescence Quenching—Oxygen evolution was determined in detached leaves from wild-type plants and plants without PSI-K (Fig. 3). The relative quantum yield (ΦO₂) for O₂ evolution was determined from the linear part of the curves to be 0.071 in wild-type plants and 0.068 in PSI-K-deficient plants.
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Flux densities. Each point compensation point, and quantum yield were deduced. PFD in A determinations.

Plants without PSI-K a value of 0.065 ± 0.006 (± S.D., n = 5) was determined, and with plants without PSI-K a value of 0.065 ± 0.009 (± S.D., n = 5) was obtained. These values are significantly different (Student’s t test, p < 0.001). Thus, plants without PSI-K have less state 1-state 2 transition suggesting that the redistribution of absorbed excitation energy between the two photosystems is reduced in the PSI-K-deficient plants.

After the far-red light was switched off a rapid increase in fluorescence due to over-excitation of the plastoquinone pool is evident in both wild-type and plants without PSI-K (Fig. 4, Far-red light off). However, the kinetics with which this fluorescence reached the steady-state level during the transition from state 1 to state 2 differs between wild-type and plants lacking PSI-K. Expressed as the time it takes to reach 50% of the steady-state fluorescence in state 1, a value of 1.6 min was obtained for wild-type, whereas a value of 2.7 min was obtained for plants lacking PSI-K. The values are significantly different (Student’s t test, p < 0.05). Thus, state 1-state 2 transition is not only reduced, but the transition that takes place is also slower in plants without PSI-K.

Both photochemical and non-photochemical fluorescence quenching showed the same light response in plants without PSI-K and in wild-type plants (data not shown). Thus, despite the decreased state transition and PSI antenna function, the plants are able to maintain normal redox conditions in the intersystem chain.

Low Temperature Fluorescence and PSI Antenna Composition and Function—Because PSI-K has been proposed to interact with LHCl (10) it was of interest to investigate the low temperature fluorescence properties of the PSI-K-deficient plants. The fluorescence emission spectra at 77 K of several plants that were shown to have no PSI-K by immunoblot analysis were recorded. The spectra revealed a 2-nm blue shift from 734 to 732 nm in plants lacking PSI-K (Fig. 5), which suggests that the interaction between LHCl and the PSI core is affected by the lack of PSI-K.

To investigate whether any of the light-harvesting proteins were affected by the absence of PSI-K, the amounts of the four individual Lhca1–4 proteins and the two Lhcb1–2 proteins were estimated by immunoblot analysis of thylakoid preparations (Fig. 6). Lhcb1-2 are part of the mobile LHCII pool that migrate during state transition. Plants without PSI-K had substantially less Lhca2 and Lhca3, whereas the amounts of Lhca1, Lhca4, and the Lhcb proteins were unaffected (Fig. 6). Quantification of the protein amounts showed that plants lacking PSI-K had 20–30% less Lhca2 and 30–40% less Lhca3 than wild-type.

To directly analyze the content of the Lhca proteins and the...
core proteins in PSI complexes of plants devoid of PSI-K. PSI complexes were obtained by mild solubilization of the thylakoid membrane with dodecyl-β-D-maltoside and ultracentrifugation in sucrose gradients. Both wild-type and plants devoid of PSI-K revealed one pigment-protein band in the gradient that could be identified as the PSI complex. The protein composition of the PSI complex is shown in Fig. 7. It is clear that the PSI complex from plants devoid of PSI-K has reduced amounts of Lhca2 and Lhca3, whereas the other PSI subunits are present in amounts equal to those observed in PSI complexes from wild-type. The reduction in Lhca2 and Lhca3 corresponds well to the reduced amounts of these two proteins estimated by immunoblotting of thylakoid membranes (Fig. 6). Thus, the absence of PSI-K does not completely prevent binding of Lhca2 and Lhca3 to the PSI core.

The function of the PSI antenna was determined from light-induced P700 absorption changes at 810 nm and expressed as the time ($t_{0.9}$) it takes to reach 90% of the maximal absorption change (Fig. 8). For wild-type and PSI-K-deficient plants, the $t_{0.9}$ value of 5.4 ± 0.9 s (± S.D., n = 5) and for plants without PSI-K a value of 6.6 ± 0.9 s (± S.D., n = 8) was determined. The values are significantly different ($p < 0.05$), and it is therefore concluded that plants lacking PSI-K have a less efficient PSI antenna.

From the same traces, cyclic electron transport was estimated from the re-reduction of P700. The change in P700 absorption after the illumination period could be fitted with a double exponential curve with a fast and a slow time constant. The fast time constant, which corresponds to cyclic electron transport, was 1.64 and 1.70 s for wild-type and PSI-K-less plants, respectively. The two values are not significantly different. The change in absorption during the fast time phase corresponded to approximately 50% in both wild-type and PSI-K-deficient plants. Thus, the absence of PSI-K has no effect on cyclic electron transport, whereas the antenna function estimated from the same measurements shows a clear difference.

The separation of the pigment-protein complexes solubilized from thylakoid membranes of wild-type and PSI-K-less plants revealed seven major pigment proteins (Fig. 9A). PSI is found in the green band called CPI*. The PSII-associated antenna proteins are separated into trimeric LHClI, CP47, CP43, CP29, CP26, and CP24. Monomeric LHClII co-migrates with CP26. However, plants devoid of PSI-K had reduced amounts of PSI absorption.
complexes with attached light-harvesting proteins in a green band that migrates with the same electrophoretic mobility as wild-type CPI*. In addition, a PSI band with slightly higher electrophoretic mobility appears, indicating that PSI com-
plexes from PSI-K-less plants revealed that the latter had reduced amounts of PSI-LHCI complexes (CPI*) compared with wild-
type. CPI* from wild-type contains PSI complexes with the full complement of Lhca proteins. A substantial part of the corre-
sponding PSI complexes from the plants devoid of PSI-K has been shown that the Lhca dimers associate directly to the interaction between the Lhca1/Lhca4 dimer and the core. It has been shown that the Lhca1–4 proteins after green gel electrophoresis indicated Lhca proteins are lost because of a destabilization of the structural organization of the entire LHCI antenna in the absence of PSI-K.

DISCUSSION

We have successfully produced Arabidopsis plants with no detectable PSI-K protein using the antisense technique and thereby obtained an efficient tool for investigating the role of the PSI-K protein in vivo, as well as in vitro. The frequency of substantial down-regulation of the PSI-K protein was approx-
imately one-third of the analyzed T1 plants. However, when offspring from plants that were found to have a “no PSI-K” phenotype were tested by immunoblotting analysis the phenotype was found not to be stably inherited. Only two of the eight tested lines gave offspring consistently devoid of PSI-K. Appar-
ently, the no PSI-K phenotype is stable throughout the lifetime of the plant, because the first screening of the plants by immu-
noblotting could be performed at the 6–8-leaves stage, and selected plants were analyzed again after preparation of thylakoids from fully expanded rosette leaves.

The Effect of PSI-K on LHCI—PSI-K is positioned on the periphery of the PSI core complex and is as such likely to interact with LHCI. Cross-linking studies have suggested that PSI-K is adjacent to Lhca3 (10). Immunoblotting showed that lack of PSI-K resulted in a 20–30% reduction of Lhca2 and a 30–40% reduction of Lhca3. Purification of PSI complexes from plants devoid of PSI-K confirmed that PSI complexes from PSI-K-deficient plants had unaffected levels of Lhca1 and Lhca4, whereas the levels of Lhca2 and Lhca3 were reduced to a similar extent as deduced from the immunoblot analysis of thylakoid membranes. The PSI complexes revealed that light-
harvesting complexes consisting of Lhca2 and Lhca3 can be attached to the PSI complexes in the absence of PSI-K. Thus, PSI-K is not necessary for attachment of light-harvesting complexes but is needed for stable interaction. This is supported by green gel electrophoresis where the separation of the pigment-
protein complexes from thylakoid membranes of wild-type and the PSI-K-less plants revealed that the latter had reduced amounts of PSI-LHCI complexes (CPI*) compared with wild-
type. CPI* from wild-type contains PSI complexes with the full complement of Lhca proteins. A substantial part of the corre-
sponding PSI complexes from the plants devoid of PSI-K has reduced levels of Lhca proteins. The appearance of a second PSI band with higher electrophoretic mobility than CPI* and the corresponding immunoblot analysis showing increased release of Lhca1–4 proteins after green gel electrophoresis indicated that Lhca proteins are lost because of a destabilization of the structural organization of the entire LHCI antenna in the absence of PSI-K.

In plants lacking PSI-K, the low temperature fluorescence emission spectra revealed a 2-nm blue shift in the far-red emission peak from 734 to 732 nm. The fluorescence emission peak at 734 nm in plants arises from LHCI-730, a dimer consisting of Lhca1 and Lhca4 (9). In Arabidopsis plants that had no Lhca4 protein, a reduction in the long wavelength emission and a 6-nm blue shift of the peak was seen (37) suggesting that Lhca4 is responsible for most of the long wavelength fluorescence emission. In barley leaves it was recently shown that there are two long wavelength emission peaks, one at 732 nm and one at 742 nm, which emanate from Lhca1 and Lhca4, respectively, when the proteins are bound to the reaction cen-
ter of PSI (38). It was furthermore suggested that binding to the reaction center core rather than heterodimerization leads to the red shift from 732 to 742 nm (38). Thus, the observed blue shift in the long wavelength fluorescence emission in the plants devoid of PSI-K most likely involve the Lhca1/Lhca4 dimer. A change in the amounts of Lhca1 and Lhca4 was not observed in the thylakoids or in isolated PSI. Furthermore, a complete detachment of Lhca1/Lhca4 from the core in the PSI-K-deficient plants is unlikely because of the relatively small blue shift in the long wavelength fluorescence. Finally, cross-
linking studies do not support a direct interaction between PSI-K and the Lhca1/Lhca4 dimer (10). A likely explanation for the observed blue shift is that the absence of PSI-K and/or the Lhca2 and Lhca3 dimers around some of the PSI core affects the interaction between the Lhca1/Lhca4 dimer and the core. It has been shown that the Lhca dimers associate directly to
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PSI-A/PSI-B without the requirement of each other but also that one Lhca protein is in contact with two others (10). Thus, it is tempting to suggest that the four dimeric Lhca complexes are located on one side of the PSI core and thereby are in contact with each other. If the two Lhca3 and Lhca2 homodimers are located next to each other the loss of PSI-K primarily results in loss of the Lhca3 dimer, and this in turn leads to loss of the Lhca2 dimer.

**PSI-K-less Plants Have an Antenna Whose Function Is Less Efficient**—In accordance with the decrease in the amount of Lhca2 and Lhca3 we observed a less efficient antenna in the absence of PSI-K. However, the method used to estimate antenna function uses a strong far-red light-emitting diode with a peak around 735 nm as the actinic light source. This light selectively excites PSI through the far-red-absorbing chlorophylls of the PSI core antenna and the outer LHCl-730 antenna. In our estimation of antenna function in the plants lacking PSI-K we observe a significant reduction compared with wild-type. Because of the far-red light source used, this reduction is most likely not directly related to the decreased content of Lhca2 and Lhca3 in the plants devoid of PSI-K. Instead, it suggests that the perturbation of the outer antenna as evidenced by the blue shift in the long wavelength emission is the cause of the reduced antenna function. Alternatively, the core antenna system has been partially disrupted as predicted to be a consequence of the loss of PSI-K in *S. elongatns* (3) or long wavelength-absorbing chlorophyll(s) bound to the PSI core at the interface between the core and LHCl have been lost because of the absence of PSI-K.

**The Absence of PSI-K Has No Effect on Electron Transport**—In contrast to the pronounced effect on antenna function and composition, PSI-K is not directly involved in electron transport, because the *in vitro* NADP+ photoreduction was not affected in the absence of PSI-K. This is in agreement with PSI-K being located away from the symmetry plane of the PSI complex (10) and also that PSI-K is found to be dispensable for growth and photosynthesis in the cyanobacterium *Synechocystis* PCC6803 (20).

**Plants Devoid of PSI-K Have Reduced State 1-State 2 Transition—Arabidopsis** plants lacking PSI-K had approximately 33% less state 1-state 2 transition than wild-type. Regulation of the distribution of excitation energy between the two photosystems on a short time scale is correlated with the redox state of the bulk plastoquinone pool (39, 40). Reduction of plastochlorophyll activates a LHClII kinase that phosphorylates some peripheral LHClII protein that in turn is thought to cause a change in its attachment, from PSIII in stacked grana domains to PSI located in non-stacked membrane domains. This results in energy distribution to PSI at the expense of PSIIII, thus tending to oxidize the quinone pool.

In *Arabidopsis* devoid of PSI-N the NADP+ photoreduction was reduced by almost 50%, whereas the state 1-state 2 transition was only slightly reduced (27). In this case the reduced state transition most likely reflects that the mobile LHClII interacts with a less efficient PSI. The plants devoid of PSI-K are not significantly reduced in NADP+ photoreduction but exhibit a significant reduction in state transition. We therefore suggest that the reduced state transition in the PSI-K-deficient plants is because of less efficient interaction between the mobile LHClII and PSI. The observed reduction in state transition in plants without PSI-K suggests that a full complement of Lhca complexes around PSI is needed to obtain efficient contact to or energy transfer from LHClII. Alternatively, the Lhca2 and/or Lhca3 dimers are directly involved in the interaction between the mobile LHClII pool and the PSI core.

The increased Chl a/b ratio indicated either an increased PSI/PSII ratio or a decreased antenna size, and the Chl/P700 ratios obtained for wild-type and PSI-K-less plants indicated an increased PSI/PSII ratio. However, the finding that plants devoid of PSI-K have less Lhca2 and Lhca3 also suggests a decrease in antenna size. Thus, part of the increased Chl a/b ratio can be explained by a decrease in antenna size, and the rest can be explained by an increased PSI content. Therefore, plants without PSI-K have compensated, at least in part, by making more PSI.

It appears that an increase in PSI is a general response to a less efficient PSI. In two recent studies where either PSI-N or PSI-H were depleted in *Arabidopsis* plants, a 15–17% increase in PSI was observed (27, 41). A PSI deficiency might be detected by the plant through more reduced conditions in the plastoquinone pool and the cytochrome b/f complex. It has recently been shown that the *psaA* and *psaB* genes, which encode the reaction center apoproteins of PSI, are induced when plastoquinone is reduced and repressed when it is oxidized (42). We did not observe a more reduced quinone pool in the PSI-K-deficient plants, but it should be noted that measurements of leaf-level fluorescence were performed on plants that were adapted to growth without PSI-K.

In conclusion, PSI-K is significant for efficient function and organization of the PSI antenna. Plants grown under optimal conditions are not adversely affected by the lack of PSI-K. However, it can be predicted that PSI-K gives a clear selective advantage particularly under conditions of low light or light of fluctuating spectral composition.

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