Photosystem I Activity Is Increased in the Absence of the PSI-G Subunit*

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PSI-G is a subunit of photosystem I in eukaryotes. The function of PSI-G was characterized in Arabidopsis plants transformed with a psaG cDNA in antisense orientation. Several plants with significantly decreased PSI-G protein content were identified. Plants with reduced PSI-G content were indistinguishable from wild type when grown under optimal conditions, despite a 40% reduction of photosystem I. This decrease of photosystem I was correlated with a similar reduction in state transitions. Surprisingly, the reduced photosystem I content was compensated for by a more effective photosystem I because the light-dependent reduction of NADP⁺ in vitro was 48% higher. Photosystem I antenna size determined from flash-induced P700 absorption changes did not reveal any significant effect on the size of the photosystem I antenna in the absence of PSI-G, whereas a 17% reduction was seen in the absence of PSI-K. However, nondenaturing green gels revealed that the interaction between photosystem I and the light-harvesting complex I was less stable in the absence of PSI-G. Thus, PSI-G plays a role in stabilizing the binding of the peripheral antenna. The increased activity of PSI-G in the absence of PSI-G suggests that PSI-G could have an important role in regulation of photosystem I.

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§ The abbreviations used are: PS, photosystem; LHC, light-harvesting complex; Chl, chlorophyll; HPLC, high pressure liquid chromatography.

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Photosystem (PS) catalyzes the light-driven electron transfer from reduced plastocyanin to oxidized ferredoxin and is composed of a chlorophyll a-binding core complex and a chlorophyll a- and b-binding peripheral antenna called LHCI. PSI from higher plants is a supramolecular complex consisting of at least 17 different polypeptides located in the nonapressed thylakoid membranes (1). The core of PSI consists of at least 13 different subunits (A–N). The PSI-A and PSI-B subunits are homologous and form a heterodimer, which binds the primary electron donor P700 (a chlorophyll dimer) and the electron acceptors A₀ (a chlorophyll a molecule), A₁ (a phylloquinone), and Fреш (a [4Fe-4S] iron-sulfur cluster) (1–3). The remaining cofactors, F₈₅₀ and F₆₇₃ (both [4Fe-4S] iron-sulfur clusters), are bound to PSI-C. The other subunits of PSI do not bind electron acceptors. In PSI of the cyanobacterium Synechococcus elongatus, the PSI-A/B dimer, together with some of the smaller membrane-intrinsic subunits, binds the 90 Chl a and 22 β-carotene molecules that constitutes the core antenna system (3).

The subunits PSI-G, PSI-H, and PSI-N are unique to higher plants and algae, and in the absence of PSI-G, whose function of PSI-G in the interaction with LHCI is likely.

To investigate the role of PSI-G, we transformed Arabidopsis plants with a psaG cDNA in antisense orientation under the control of a constitutive promoter. Transformants with no or very low levels of PSI-G protein were obtained, and the plants were analyzed at both the biochemical and leaf level.

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EXPERIMENTAL PROCEDURES

Plant Material—Arabidopsis thaliana (L.) Heyn cv. Columbia was used for all experiments. Plants were grown in peat in a controlled environment Arabidopsis Chamber (Percival AR-60L, Boone, IA) at a photosynthetic flux of 100–120 μmol photons m⁻² s⁻¹, 20 °C, and 70% relative humidity. The photoperiod was 12 h for plants used for transformation, whereas the photoperiod was 8 h for plants used for biochemical and physiological analysis to suppress the induction of flowering.

Vector Construction and Plant Transformation—A 550-bp fragment containing the entire coding region of PSI-G was amplified from a full-length cDNA clone (279G17T; Arabidopsis Biological Resource Cen-
ter DNA Stock Center, Columbus, OH) by PCR using primers based on a genomic sequence for the PsaG gene (GenBank accession number AC002328). The fragment was cloned in antisense orientation between the enhanced cauliflower mosaic virus 35S promoter and 35S terminator in the pPZP111 vector (15). The vector construct was transformed by electroporation followed by the antisense confirmed by sequencing. Subsequently, a fragment containing the enhanced 35S promoter followed by the antisense AC002328). The fragment was cloned in antisense orientation between XbaI and ligated into the binary vector pPZP111 (15). The vector construct was transformed by electroporation into Agrobacterium tumefaciens strain C58 (17). Arabidopsis plants were transformed by the floral dip method using Silwet L-77 (Lehle Seeds, Round Rock, TX) (18).

Isolation of Thylakoid Membranes and Purification of PSI—Leaves from 8–10-week-old plants were used for isolation of thylakoids as described previously (19). Total Chl and Chl a/b ratio in thylakoids were determined in 80% acetone according to the method of Lichtenthaler (20). For preparation of PSI complexes, thylakoid membranes (1 mg Chl ml⁻¹) were solubilized for 10 min with 1% dodecyl-β-D-maltoside (Sigma) at 0 °C. After 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), and 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 full-resolution cystine leaves harvested before bolting.

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### RESULTS

**Generation of Arabidopsis PSI-G Antisense Plants**—A total of 110 kanamycin-resistant plants derived from the original transformed plants were screened by immunoblotting analysis of total leaf protein extracts (data not shown). Approximately one-third of these had either no detectable PSI-G protein or significantly reduced amounts of PSI-G. The detection limit was about 3% of wild type levels of PSI-G. Under the growth conditions used, there was no obvious visible difference between plants lacking PSI-G and the wild type, and plants without PSI-G had a normal life cycle and produced seeds. Plants were screened by immunoblotting for the presence of PSI-G before further analysis. All experiments were performed with plants or thylakoids that had either no detectable PSI-G protein or <8% residual PSI-G protein. Several independent lines were analyzed to rule out any effects from positioning the inserted DNA.

**Pigment Composition, P700, and In Vitro NADP⁺ Photoreduction**—In thylakoids from plants with no detectable PSI-G or reduced amounts of PSI-G, the Chl a/b ratio was 2.76 ± 0.08 (±S.D.; n = 12), whereas in wild type plants, the ratio was 2.83 ± 0.09 (±S.D.; n = 6) (Table I). Although this difference is not significant, it suggests that plants without PSI-G have a lower PSI/PSII ratio due to an increased peripheral antenna.

To analyze this further, the amount of P700 was determined with plants or thylakoids that had either no detectable PSI-G protein or <8% residual PSI-G protein. Several independent lines were analyzed to rule out any effects from positioning the inserted DNA.

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### Table I

| Thylakoids | Chl a/b ratio | No PSI-G | No PSI-K |
|------------|---------------|----------|----------|
| Wild type  | 2.83 ± 0.09   | 2.76 ± 0.08 | 3.20 ± 0.15⁺ |
| PSI-200    | 9.0 ± 0.2     | 9.1 ± 0.6  | 10.6 ± 0.3³ |
| PSI-200    | 11.1 ± 0.2    | 11.0 ± 0.8 | 9.4 ± 0.3³ |

⁺ Value is significantly different (p < 0.05) from the wild type value.
of PSI-G or PSI-K clearly leads to different compensatory responses.

To visualize directly on the protein level that plants with reduced amounts of PSI-G have a lower content of the PSI subunits and also to analyze changes in the amounts of light-harvesting chlorophyll a/b proteins of PSI and PSII, thylakoid proteins were separated as described above, and the blots were incubated with antibodies as indicated. The Lhca2 antibody also detects Lhcb4 (CP29). Quantification was performed on two independent preparations of both wild type (WT) and PSI-G-deficient (−G) thylakoids, and the standard error was 10% or less.

To analyze whether PSI-G has any effect on electron transfer, NADP⁺ photoreduction was determined using thylakoids purified from plants without PSI-G and wild type plants. With thylakoids from wild type plants, an activity of 33.1 ± 2.3 μmol NADPH s⁻¹ (μmol P700)⁻¹ (±S.E.; n = 9) was determined, and with thylakoids devoid of PSI-G, an activity of 49.1 ± 6.2 (±S.E.; n = 9) was determined. These values are significantly different (p < 0.02). Hence, the activity is 48% higher in the absence of PSI-G. The relatively high standard deviation on the measurements performed on thylakoids devoid of PSI-G is related to the varying amounts of residual PSI-G protein in the preparations used.

Low-temperature Fluorescence and PSI Antenna Size—To analyze the role of PSI-G in antenna function, fluorescence

![Image](http://www.jbc.org/Downloaded from http://www.jbc.org)
emission at 77 K after excitation at 435 nm was recorded. The fluorescence emission spectra using thylakoids of both wild type and plants devoid of PSI-G are shown in Fig. 3. The spectra revealed a consistent 1-nm blue-shift from 734 nm to 733 nm in plants lacking PSI-G, which could suggest that the interaction between LHCII and the PSI core is affected in the absence of PSI-G. The 1-nm blue-shift was also seen when measurements were performed on intact leaves but was not seen when measurements were performed on purified PSI-200 (results not shown). To investigate the antenna function further, the PSI antenna size was estimated by determining P700 absorption change as a function of flash intensity. The excitation laser flash was initially set at saturating intensity to ensure quantitative excitation of all P700, and the intensity was then successively lowered with the use of neutral gray filters. At each flash intensity, the amplitude of P700 absorption change was monitored (Fig. 4). We have previously demonstrated that plants devoid of PSI-K have less Lhca2 and Lhca3 and are impaired in energy transfer from the peripheral antenna to the core (12). It was therefore of interest to estimate the actual antenna size in thylakoid samples from plants devoid of PSI-K as well (Fig. 4). To estimate the relative antenna cross-section, the light response curves were fitted to a single hit Poisson distribution (A/I = A_sat × (1 − exp(−kI)), where A/I is the signal at the given flash intensity, A_sat is the maximum signal with a saturating flash, I is the flash intensity, and k is the relative antenna cross-section). With this analysis, it was shown that the cross-section of PSI in the absence of PSI-K was 17.6 ± 10.7% (±S.D.; n = 8) smaller than that in the wild type (p = 0.001). In good agreement with this reduction in functional antenna size, the content of Chl b, which is only found in the peripheral antenna, is 15–16% lower in the absence of PSI-K. In contrast with these differences in antenna composition and function in the absence of PSI-K, the P700 light saturation curve for thylakoids devoid of PSI-G is essentially identical to that of the wild type (Fig. 4), and the Chl b content of PSI-200 particles devoid of PSI-G also does not differ from that of the wild type (Table 1). Therefore, we conclude that PSI-G has no direct effect on the PSI antenna. Furthermore, PSI-G and PSI-K have very different effects on PSI, despite their homology.

**Pigment-Protein Complexes of PSI-G-deficient and Wild Type Plants**

Pigment-protein complexes were solubilized from wild type, PSI-G-less, and PSI-K-less thylakoid membranes using octylglucoside and separated by nondenaturing gel electrophoresis. This separation revealed seven major pigment-protein complexes in the wild type (Fig. 5), whereas the PSI-associated antenna proteins are separated into trimeric LHCII, CP47, CP43, and LHC monomers consisting of CP29, CP26, CP24, and monomeric LHCII. Plants devoid of PSI-G had reduced amounts of PSI complexes in a green band that migrates with the same electrophoretic mobility as wild type CPI*. In addition, a PSI band with significantly higher electrophoretic mobility appears, indicating that PSI complexes devoid of PSI-G have lost light-harvesting proteins, probably due to their instability during electrophoresis in the absence of PSI-G. The behavior of PSI devoid of PSI-K is quite different from that of PSI devoid of PSI-G (Fig. 5). Apparently, the absence of PSI-G causes greater instability of the complex during electrophoresis in this system than the absence of PSI-K.

**State Transitions**

State 1-state 2 transitions are a dynamic mechanism that enables plants to respond rapidly to changes in illumination and involve the dissociation of a mobile pool of the LHCII from PSII and concomitant association of this LHCII with PSI. State transitions are detected as differential changes in fluorescence at room temperature from PSII in leaves that are exposed to alternating PSII and PSI light, i.e., blue light or blue light together with far-red light (Fig. 6). Expressed as relative fluorescence changes (Fr), plants devoid of PSI-G have only 45% of the state transitions observed in the wild type plants. Thus, the capacity for redistribution of absorbed excitation energy between the two photosystems is significantly reduced in plants without PSI-G.

**DISCUSSION**

We have successfully produced *Arabidopsis* plants with highly reduced or no detectable PSI-G protein using the antisense technique and thereby obtained an important source for investigating the role of the PSI-G protein in vivo as well as in vitro.

**FIG. 3.** Low-temperature fluorescence emission spectra reveal a blue-shift in plants without PSI-G. The spectra of a wild type plant (WT) and a plant without any detectable PSI-G protein (No PSI-G) are shown. Excitation wavelength was 435 nm.

**FIG. 4.** Light-saturation curves for P700 oxidation in thylakoids devoid of PSI-G or PSI-K compared with wild type. The curves were acquired by flashing cuvettes containing mildly solubilized thylakoids with laser light of varying intensity and measuring the resulting P700 oxidation. The absorbances are plotted relative to saturation (ΔA_sat = 100). Each point is the average obtained with seven to eight thylakoid samples. For clarity, the error bars have been omitted, but the standard errors were less than 2% for all points.
PSI-G does not interact directly with LHCl—PSI-G has been suggested to be positioned on the periphery of the PSI core and has therefore been suggested to interact with the peripheral antenna (9). However, the data presented here clearly demonstrate that PSI-G is not required for function of the PSI antenna. The antenna size measurements obtained using flash-induced P700 oxidation showed that the functional size of the peripheral antenna was unaffected in the absence of PSI-G, whereas in the absence of PSI-K, a clear reduction in antenna size was measured. This is further supported by the pigment analysis of PSI-200 particles, in which a clear decrease in Chl b content was seen in the absence of PSI-K, and no change was seen in the absence of PSI-G. However, this is somewhat in contrast to the separation of the protein/pigments complexes by mildly denaturing gel electrophoresis (green gels), in which samples from plants devoid of PSI-G revealed reduced amounts of PSI-LHClI complexes (CPI*) and the appearance of a new green band with higher electrophoretic mobility at the same time. The appearance of this second PSI band indicates that PSI proteins are lost from the PSI holocomplex, most probably due to a destabilization of the structural organization of the antenna in the absence of PSI-G. A similar but not as pronounced effect is observed in samples from plants without PSI-K. Although the absence of PSI-G caused a destabilization of PSI under the mildly denaturing conditions of the green gels, there is no indication that PSI was destabilized in vivo or during the preparation of PSI with nonionic detergents.

In leaves and thylakoids from plants lacking PSI-G, the low-temperature fluorescence emission spectra revealed a 1-nm blue-shift in the far-red emission peak from 734 nm to 733 nm. The fluorescence emission peak at 734 nm in plants is thought to arise from the Lhca1/Lhca4 dimer (8), and apparently binding of the dimer to the reaction center core rather than heterodimerization gives rise to the far-red fluorescence (26, 27). However, when any of the four Lhca proteins are completely missing or unable to interact with PSI, a large blue-shift of about 7 nm is seen (28–30). Thus, a blue-shift in the long-wavelength fluorescence emission should indicate that the interaction between the Lhca complexes and the PSI core is perturbed. However, no blue-shift was found in the purified PSI-200 particles devoid of PSI-G. This suggests that the blue-shift observed in thylakoids and intact leaves lacking PSI-G is due to fluorescence from Lhca complexes that are not bound to the core. The immunoblotting analysis indicates a 20–40% decrease in the amounts of most PSI core subunits. A similar reduction in the amounts of the Lhca2 and Lhca3 subunits in the absence of PSI-G was also observed. However, the Lhca1 and Lhca4 proteins were only reduced by 10%. Thus, Lhca1-Lhca4 complexes that are not connected with a PSI core are present, and this will result in fluorescence emission that is more blue-shifted than that seen when all the LHCI complexes are connected. It is known that deficiency in PSI does not necessarily lead to a similar deficiency in LHCI because the barley mutant viridis-zb63, which contains less than 5% of the PSI core proteins, still accumulates wild type amounts of the LHCI complexes (31).

In the case of PSI-K, a clear reduction in the amounts of Lhca2 and Lhca3 was shown by immunoblot analysis, and this correlated with a 2-nm blue-shift, although there was no significant change in the amounts of Lhca1/Lhca4 (12). This can be explained by the fact that the Lhca complexes are located on one side of the PSI core (10) and are thereby in contact with each other. Thus, a change in the binding environment of one of the antenna complexes is likely to affect the fluorescence properties of the other. In the absence of PSI-G, the situation is more subtle because the more severe destabilization during green gel electrophoresis suggests an interaction, whereas functional antenna size, Chl b content, and the low-temperature fluorescence data do not support a role for PSI-G in direct contact with the peripheral antenna. The data presented demonstrate that PSI-G is not required for attachment of the Lhca complexes to the core.

PSI-G affects the amount of PSI—The amount of PSI was reduced by 40% in the absence of PSI-G. This was independently verified by the state transition measurements, in which only 55% less state 1-state 2 transition than did the wild type. Thus, the energy distribution to PSI at the expense of PSI is significantly reduced in these plants. PSI is required for state transitions (32); hence, the lower amount of PSI in the absence of PSI-G explains the decreased capacity for redistribution of absorbed energy.

PSI-G attenuates PSI electron transport—The absence of PSI-G has a positive effect on electron transport because the in vitro NADP+ photoreduction was stimulated by 48% in thylakoids with reduced amounts of PSI-G.
Removal of PSI-N, PSI-H, or PSI-L results in a less efficient PSI, and the plants compensate for this by increasing the amount of PSI, whereby a normal phenotype is maintained at least under optimal growth conditions (19, 24, 33). This is explained by a mechanism in which the amounts of the two photosystems will adjust individually to maintain optimal electron transport. Plants with a reduced amount of PSI-G have 40% less PSI but display a normal phenotype under our growth conditions. This can be explained if PSI in the absence of PSI-G is more efficient and is supported by the higher in vitro PSI activity measured in the absence of PSI-G.

Under light-limited conditions, it would not be possible to significantly increase the efficiency of PSI electron transport. However, under high irradiance levels when PSII photoprotective thermal dissipation is engaged, PSI will be absorbing many more photons than it is receiving electrons from PSII. Thus, PSI will have to deal with this excess excitation energy. One way is cyclic electron flow around PSI, but the capacity of this pathway is modest in comparison with the excess photon load when zeaxanthin/ΔpH-dependent energy dissipation is fully engaged in PSI (34). The oxidized primary donor of PSI, P700+*, is a strong quencher of excited states in the PSI antenna and can accumulate when PSI photochemistry outpaces PSI. We suggest that PSI-G plays an important role in this process. PSI-G is present in one copy per P700, and it is assumed that PSI-G is always attached to PSI. If PSI-G has a regulatory role, then PSI-G will have to be able to execute this function while attached to the core complex. One hypothesis is that PSI-G changes conformation in response to changes in light quantity/quality or changes in pH and that this conformational change results in increased electron transfer to ferredoxin. Under normal conditions, PSI-G will maintain the electron transfer rate through PSI at a level that is below the potential maximum rate, but the amounts of the two photosystems will have been adjusted to maintain optimal electron transport. In high light or under other photoinhibitory conditions, PSI-G changes conformation and thereby allows higher electron transfer rates via more efficient electron transfer to ferredoxin. This will lead to overoxidation of P700*, and P700+* will be the prevailing species. P700+* will stay oxidized until an electron arrives from PSI-G and will act as an efficient quencher of excitation energy in PSI. Thus, the role of PSI-G could be to shift PSI back and forth between a normal working mode and a quenching mode. This means that the PSI-G antisense plants will be in constant quenching mode. If this hypothesis is correct, then PSI without PSI-G should be better protected against PSI photoinhibition but would be more poorly adapted to variations in light intensity. Another way that PSI-G could affect PSI activity would be to uncouple at least part of the peripheral antenna, whereby excess excitation energy is prevented from reaching the core antenna and reaction center. In this way, PSI-G could perhaps be thought to cause a nonphotochemical de-excitation of pigments. However, no difference in PSI cross-section was found under almost the same conditions of solubilization at which an increased NADP+ reduction was seen. Hence, we think that PSI-G most likely acts on electron transport rather than on light harvesting. The mechanism behind the conformational change or uncoupling in response to light or pH changes is not known. This will require detailed knowledge about putative interactions with pigments (carotenoids and/or Chl a) and the location of PSI-G in the PSI complex.

In conclusion, PSI-G and PSI-K are integral membrane proteins of 11 and 9 kDa, respectively. The two euaryotic subunits are equally similar to the cyanobacterial PSI-K subunit, and they have clearly evolved from the same ancestral protein. However, the functions of the two proteins in higher plants are quite different. PSI-K has a role in interaction with Lhca2 and Lhca3, whereas PSI-G does not have an important role in interaction with the peripheral antenna. PSI-G is not necessary for attachment of the light-harvesting complexes to the core and is probably not in direct contact with LHCl. More importantly, PSI-G seems to regulate PSI electron transport, and this could be important for photoprotection of PSI. Future investigations will focus on this aspect of PSI-G with a dynamic role in PSI and on the molecular mechanism by which PSI-G exerts its proposed function.

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