The PSI-O Subunit of Plant Photosystem I Is Involved in Balancing the Excitation Pressure between the Two Photosystems*

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PSI-O is a subunit of photosystem I in eukaryotes. The function of PSI-O was characterized in Arabidopsis plants using RNA interference. Several transformants with the psaO-RNAi construct were generated, and a high proportion of the plants contained only very little or virtually no residual PSI-O. Plants lacking PSI-O have a 50% reduction in state transitions indicating a role for PSI-O in the balancing of excitation energy between the two photosystems. PSI-H and -L have been shown previously to be involved in state transitions, and immunoblot analysis revealed that plants devoid of PSI-L or -H also have 80–90% reduction in the abundance of PSI-O. In contrast, down-regulation of PSI-O has no negative effect on the content of PSI-H and -L. The interaction between PSI-O and the PSI-L was confirmed by chemical cross-linking. A model of PSI is proposed in which PSI-L as the most ancient subunit is closest to the reaction center, and PSI-O is positioned close to PSI-L on the PSI-H/L-I side of the PSI complex. PSI-H, -L, -O, and possibly -I are all involved in forming a domain in PSI that is involved in the interaction with light-harvesting complex II.

Photosystem (PS)I 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 light-harvesting complex (LHC) I. PSI from higher plants is a supramolecular complex consisting of at least 18 different polypeptides located in the non-appressed thylakoid membranes (1). The core of PSI consists of at least 14 different subunits named PSI-A–L, PSI-N, and PSI-O. The PSI-A and -B subunits are homologous and form a heterodimer, which binds the primary electron donor P700 (a chlorophyll a dimer) and the electron acceptors A0 (a chlorophyll a molecule), A1 (a phytolquinone), and F645 (a [4Fe-4S] iron-sulfur cluster) (1–3). The remaining cofactors, F680 and F800 (both [4Fe-4S] iron-sulfur clusters), are bound to PSI-C. The other subunits of PSI do not bind electron acceptors. The PSI-A/B dimer together with some of the smaller membrane-intrinsic subunits bind ~90 Chl a and 22 β-carotene molecules, which constitutes the core antenna system (3, 4).

The PSI-O subunit was discovered in Arabidopsis thaliana recently (5). The mature PSI-O is a 10-kDa protein with two transmembrane helices. PSI-O has no counterpart in PSI of cyanobacteria but seems to be present in higher plants, in mosses, and in green algae. However, the primary structure of PSI-O does not give any indications about its specific function, because there is no significant sequence similarity to any known protein in the data bases.

Together with PSI-O, the subunits PSI-G, -H, and -N are unique to higher plants and algae. In the recent x-ray crystallographic structure of plant PSI at 4.4 Å, the structure and position of the PSI-G and -H subunits within the PSI complex are revealed (4). However, PSI-N and -O are either not resolved at the current resolution or are lost from the complex during preparation, and their position and exact position in the PSI complex are therefore not known.

LHCI is also specific for higher plants and algae. This peripheral antenna is, at least in higher plants, composed of the products of four nuclear genes, Lhca1-4, with molecular masses of 20–24 kDa. The four proteins form two heterodimers consisting of Lhca1/Lhca4 and Lhca2/Lhca3 (4). The two dimers are arranged so that the individual proteins are in a series creating a crescent-shaped belt adjacent to the core complex on the PSI-F side. This arrangement maximizes the number of LHCI chlorophylls facing the core (4). LHCII binds 56 Chl a and b molecules/P700 (4) besides lutein, violaxanthin, and small amounts of β-carotene (6, 7).

The PSI-G, -H, and -N fulfill functions in PSI that are unique to eukaryotic PSI. PSI-H has been shown to be involved in state 1–state 2 transitions probably in the interaction with LHCII (8), PSI-N is involved in interaction with plastocyanin (9), and PSI-G is involved in the stabilization of LHCI and regulation of PSI activity (4, 10, 11). It is therefore likely that PSI-O plays a role in the interaction between the PSI core and other complexes in the thylakoid membrane such as LHCI or LHCII. Alternatively PSI-O is involved in the regulation or fine tuning of PSI activity.

To investigate the role of PSI-O we undertook a reverse genetic approach by transforming Arabidopsis plants with an RNA interference (RNAi) construct containing the psaO EDNA under the control of a strong constitutive promoter. Transformants with no or very low levels of PSI-O protein were obtained, and the plants were analyzed both at the biochemical and physiological level.

EXPERIMENTAL PROCEDURES

Plant Material—A. thaliana L. Heyn cv. Columbia was used for all of the experiments. Plants were grown in compost in a controlled environment chamber (Percival AR-80L) at a photosynthetic flux of 100–120 μmol of photons m⁻² s⁻¹, 20 °C, and 70% relative humidity. The photoperiod was 12 h for plants used for transformation, whereas

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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 270-bp fragment containing the coding region of the mature PSI-O was amplified from a full-length cDNA clone (AY533754, Kazusa DNA Research Institute, Japan (12)) by PCR using primers that were based on a cDNA sequence for the psaO gene (GenBank™ accession number AJ493060). The fragment was cloned in sense and antisense orientation separated by a 220-bp fragment containing the intron coding region from a nitrite reductase gene from Phaseolus vulgaris (GenBank™ accession number U10419 (13)). The psaO hairpin construct (see Fig. 1A) was cloned behind the enhanced CaMV 35 S promoter in the pBS48 vector (14, 15) and orientation of the insert was confirmed by sequencing. Subsequently, a fragment containing the enhancer of the 35 S promoter followed by the psaO hairpin construct and the 35 S terminator was excised with XbaI and ligated into the binary vector pZPP111 (16). The vector construct was transformed by electroporation (17) into the Agrobacterium tumefaciens strain C58 (18). Arabidopsis plants were transformed by the floral dip method using SilkWet L-77 (Lehle Seeds) (19).

Seeds harvested from transformed plants (T0) were germinated on Murashigue and Skoog medium (Sigma) containing 2% sucrose, 50 mg l⁻¹ kanamycin sulfate, and 0.8% agar for 2 weeks, and kanamycin-resistant Arabidopsis plants were selected. Seedlings (T1) were then transplanted to compost. All biochemical and physiological experiments were performed with fully expanded rosette leaves harvested prior to bolting.

Isolation of Thylakoid Membranes and Cross-linking and Isolation of PSI—Leaves from 8–10-week-old plants were used for isolation of thylakoids as described previously (9). Total Chl and Chl a/b ratio in thylakoids were determined in 80% acetone according to Lichtenthaler (20). PSI for cross-linking was prepared by solubilizing thylakoids (0.5 mg/ml Chl/ml) in 5 mg/ml digitonin. After stirring for 30 min at 4 °C the mixture was centrifuged at 48,000 × g for 30 min to remove unsolubilized material. The supernatant was spun subsequently in an Ultracentrifuge at 257,000 × g for 1 h at 4 °C. The pellet enriched in PSI particles was resuspended in 20 mM Tricine, pH 7.5, 20% glycerol, frozen in aliquots in liquid nitrogen, and stored at −80 °C. Chemical cross-linking was performed with diithiothreitol (Dithiothreitol) (DTSP) according to Fling and Oliver (21). The digitonin PSI preparation was diluted to 0.3 mg of Chl/ml in 20 mM Tricine, pH 7.5, 3 mg/ml N-dodecyl-β-maltoside. DTSP was dissolved in dimethylformamide and added to a final concentration of 0.015 mg/ml. After incubation of the PSI particles and DTSP at room temperature for 30 min in darkness, the reaction was stopped by adding 1/7 volume of 10 mM Tris, pH 7.4, 1 mM EDTA. Subsequently 1 volume of non-reducing sample buffer (50 mM Na₂CO₃, 15% sucrose, 2.5% SDS) was added, and the solution was incubated for 20 min at room temperature before loading the samples onto a 12% SDS-polyacrylamide gel. After electrophoresis, the gels were cut out and incubated for 30 min in reducing sample buffer (50 mM Na₂CO₃, 15% sucrose, 2.5% SDS, 50 mM dithiothreitol) to obtain complete reductive cleavage of the cross-linked products. The gel slice was placed on top of a 2–5% gradient gel and re-electrophoresed. After electrophoresis the proteins were transferred to nitrocellulose and analyzed with different combinations of antibodies. SDS-polyacrylamide gels were prepared according to Fling and Gregerson (22).

Immunoblotting—Plants lacking the PSI-O subunit were identified by immunoblotting. Crude leaf extracts were prepared, and immunoblotting was carried out as described previously (23). Isolated thylakoids were analyzed in similar immunoblotting procedures using antibodies as indicated in the figure legends. Primary antibodies were detected using a chemiluminescent detection system (ECL, Amersham Bioscience, or Immuno-Star, Bio-Rad) according to the instructions of the manufacturer.

NADP⁺ Photoreduction Measurements—The NADP⁺ photoreduction activity of PSI was determined from the absorbance change at 340 nm as described by Naver et al. (24) using thylakoids equivalent to 5 μg of Chl. Thylakoids were solubilized in 0.1% N-dodecyl-β-maltoside prior to the measurements. The total P700 content was determined from the ferricyanide-oxidized minus ascorbate-reduced difference spectrum using an extinction coefficient of 64,000 M⁻¹ cm⁻¹ at 700 nm. The thylakoids were solubilized with 0.2% Triton X-100, and the measurements were repeated 4–5 times on several independent thylakoid preparations.

Fluorescence Measurements—Fluorescence emission spectra were recorded by excitation of leaves at 77 K with a SpectraMax M5 FL (Molecular Devices). The relative change in fluorescence was calculated as \( F_r = \frac{I_{77} - I_{295}}{I_{295}} \) for the control of a strong constitutive promoter (Fig. 1). Standard fluorescence parameters (1–3, \( \Phi_{PSII} \), \( \Phi_{NPQ} \)) under growth light conditions and in darkness were performed as in Lunde et al. (25), and the parameters were calculated using the following equations, 1–3, \( \Phi_{PSII} \) (PSII excitation efficiency) = \( \frac{F_r - F_s}{F_m - F_s} \), \( \Phi_{PSII} \) (quantum yield of PSII) = \( \frac{F_m - F_o}{F_m - F_s} \), \( \Phi_{NPQ} \) (dark-adapted) = \( \frac{F_m - F_s}{F_m} \). Standard fluorescence measurements were performed as described previously for standard fluorescence.

RESULTS

Generation of Arabidopsis Plants without PSI-O—PSI-O is encoded by a single gene in Arabidopsis, and to address the function of PSI-O suppression of the psaO gene was performed using RNAi. A DNA construct containing the sense cDNA and antisense cDNA separated by an intron-coding region under the control of a strong constitutive promoter (Fig. 1A) was introduced into the Arabidopsis genome by transformation. A total of 66 kanamycin-resistant plants derived from the original transformed plants were screened by immunoblotting analysis of total leaf protein extracts using a polyclonal antibody against PSI-O (Fig. 1B). Fifty-nine plants contained very low amounts of or no detectable PSI-O protein. The detection limit was about 3% of wild type levels of PSI-O. Under the growth conditions used there was no obvious visible difference between plants lacking PSI-O (psaO-RNAi) and the wild type (Fig. 1C) except that the psaO-RNAi plants flowered ~5–7 days later than the wild type. Otherwise the psaO-RNAi plants had a
normal life cycle and were fully fertile.

To analyze whether the double-stranded (ds) RNA-mediated suppression of PSI-O was transmitted to the next generation, seeds from 14 T1 lines that had no detectable PSI-O protein were analyzed. Seven T2 plants from each line were tested by immunoblotting of the total leaf protein extracts using the PSI-O antibody (data not shown). In 8 of the 14 lines, all 7 offspring plants had no immunodetectable PSI-O protein, in 3 lines all of the offspring plants had wild type levels of PSI-O protein, and in 2 lines there were individuals both with wild type levels of PSI-O protein and no detectable PSI-O protein.

Plants were screened by immunoblotting for the presence of PSI-O prior to further analysis. All experiments were performed with plants or thylakoids that had no detectable amounts of PSI-O protein. Several independent psaO-RNAi lines were analyzed to rule out any effects from position of the inserted T-DNA.

**Pigment Composition, P700, and in Vitro NADP⁺ Photoreduction**—In thylakoids from psaO-RNAi plants with no detectable PSI-O the Chl a/b ratio was 3.11 ± 0.05 (± S.D., n = 3), whereas in wild type plants the ratio was 3.05 ± 0.07 (± S.D., n = 3). Although the difference between these numbers is not significant, there is a tendency that the Chl a/b ratio is slightly higher in the absence of PSI-O, which suggests that plants without PSI-O have a slightly higher PSI/PSII ratio or a slightly decreased peripheral antenna.

To analyze this further the amount of P700 was determined in solubilized thylakoids using chemical oxidation. The number of chlorophylls/P700 reaction center was 656 ± 43 (± S.D., n = 7) for wild type and 664 ± 31 (± S.D., n = 4) for thylakoids from the psaO-RNAi plants with no detectable content of PSI-O. The numbers are not significantly different and indicate that the lack of PSI-O does not affect the PSI/PSII ratio.

To analyze whether PSI-O has any effect on electron transfer, NADP⁺ photoreduction was determined using thylakoids purified from plants without PSI-O and wild type plants. With thylakoids from wild type an activity of 50.6 ± 0.05 μmol of NADPH s⁻¹ (μmol of P700⁻¹ s⁻¹) (± S.D., n = 4) and with thylakoids devoid of psaO-RNAi plants an activity of 54.7 ± 10.6 μmol of NADPH s⁻¹ (μmol of P700⁻¹ s⁻¹) (± S.D., n = 4) was determined. These values are not significantly different.

Immunoblotting analysis of thylakoid proteins was performed to analyze whether the absence of PSI-O caused changes in the other major thylakoid complexes (Fig. 2). The lanes on the gel were loaded with proteins corresponding to equal amounts of chlorophyll. This analysis showed that the tested subunits of PSII, cytochrome b₆f, and ATPase are present in normal amounts in the thylakoids of psaO-RNAi plants. This is also the case for almost all of the PSI subunits; however the PSI-L subunits are clearly present in greater amounts in thylakoids devoid of PSI-O.

**Low Temperature Fluorescence**—A putative role of PSI-O in the interaction between the PSI core and the peripheral LHCl was analyzed by measuring fluorescence emission at a low temperature. This has been shown previously (10, 23, 26–28) to be a very sensitive method to reveal perturbations in the peripheral PSI antenna. Fluorescence emission between 650 and 800 nm after excitation at 435 nm was recorded at 77 K. The fluorescence emission spectra using intact leaves of both wild type plants and plants devoid of PSI-O are shown in Fig. 3. It is clear that there is no significant difference in the wavelength of the fluorescence emission maximum between spectra obtained with the wild type and the plants with no detectable PSI-O protein. Therefore the absence of PSI-O does not cause perturbations of the peripheral antenna, and PSI-O is not likely to be involved in the binding or function of the LHCl antenna. This is also supported by the immunoblot analysis (Fig. 2), which shows no significant changes in the amounts of the four Lhca proteins.

**State Transitions**—State 1–state 2 transitions are dynamic mechanisms that enable plants to respond rapidly to changes in illumination and involve the dissociation of a mobile pool of LHClII from PSII and concomitant association of this LHClII with PSI (for a review see Ref. 29). State transitions can be detected as differential changes in fluorescence from PSII in leaves at room temperature that are exposed to alternating PSII and PSI light, i.e. blue light or blue light together with far-red light. Expressed as relative fluorescence changes (Fr), plants devoid of PSI-O have only 54% of the state transitions observed in the wild type plants (Table I). The fluorescence parameters 1-qP and ΦP₉₁₀₉ under alternating PSII and PSI light were estimated, and even in PSI light where the mobile pool of LHClII is associated with PSII, the PSII excitation pressure is higher in the plants devoid of PSI-O when compared with wild type (Table I). After exposure to PSI light, the excitation pressure was about twice as high for the PSI-O-less plants (Table I). Thus, the capacity for redistribution of ab-
sorbed excitation energy between the two photosystems is significantly reduced in plants without PSI-O. This suggests a role for PSI-O in this process.

The PSII excitation pressure (estimated as 1-quin) was also measured directly in the growth chamber under the light conditions to which the plants were adapted (Table I). Under these conditions 1-quin was increased 2-fold in the plants lacking PSI-O, and the photochemical efficiency (ΦPSII) was reduced by 4% compared with wild type. The redox level of QA, as indicated by the PSII excitation pressure, reflects the balance between the light-mediated reduction by the PSII reaction centers and the rate of oxidation through interchain electron transport, i.e. PSI activity. Thus, even under constant growth conditions the flow of electrons through the two photosystems is restricted in the absence of PSI-O.

**Localization of PSI-O in the PSI Complex**—It has been shown previously that in plants devoid of PSI-H there are essentially no state transitions, and PSI-H has been suggested to be part of a docking site on PSI for the mobile LHCII (8). Plants lacking PSI-H have a concomitant reduction in PSI-L to ~50% of wild type, and plants devoid of PSI-L have only ~20% PSI-H compared with wild type. Plants devoid of PSI-L but still retaining 20% PSI-H display slightly more state transitions than plants completely lacking PSI-H (25). This strongly confirms the close association between these two subunits as already indicated by cross-linking studies (21). It was of interest therefore to find out whether the association of PSI-O with the PSI core complex was dependent on the presence of PSI-H and -L or other peripheral PSI subunits. Thylakoid samples from a range of antisense and co-suppression lines lacking PSI-H, -L, -N, -G, -K, -F, or -E were analyzed by immunoblotting to see whether the absence of any of these PSI subunits affects the presence of PSI-O (Fig. 4). The amount of PSI-O is reduced with 70–90% in thylakoids devoid of PSI-H or -L compared with wild type, whereas the absence of any of the other subunits does not result in a specific loss or reduction of PSI-O. It therefore seems that PSI-O is highly dependent on the presence of PSI-L and -H, and it is likely that PSI-O is located near these two subunits in the PSI complex. This is also in agreement with the observed reduction in state transitions in the absence of PSI-O.

To verify the interaction of PSI-O with either PSI-H or -L directly, chemical cross-linking was performed. The diithiothreitol-cleavable cross-linker DTSP was added to PSI-enriched fractions. After cross-linking the sample was separated by non-reducing SDS-PAGE. The lane with cross-linked proteins was cut out and reduced with dithiothreitol to cleave the cross-linker. Subsequently the gel strip was placed on top of a second SDS gel, and the individual proteins were separated according to size in a second fully reductive SDS-PAGE step. After this the non-cross-linked proteins were in the diagonal of the gel, and cross-linked proteins were underneath each other in the same lane separated according to size. Identification of proteins was done by immunoblotting (Fig. 5). A cross-linked product consisting of PSI-H and -L is identified easily (Fig. 5A) thus confirming the close association between these two proteins. Smaller products consistent with the previously identified products of PSI-L+PSI-I and PSI-H+PSI-I (21) are also seen (Fig. 5A). A cross-linking product of 25 kDa, which clearly is resolved into proteins that react with antibodies against PSI-L and -O, is also seen (Fig. 5B). This confirms that PSI-O is located on the PSI-L/H side of the PSI complex. We have not been able to determine the composition of the other cross-linking products that contain PSI-O.

**DISCUSSION**

**Generation of Plants Lacking PSI-O**—We have used dsRNA-mediated genetic interference to effectively induce specific inhibition of the PSI-O subunit of photosystem I. A high number of T1 plants (59 of 66 or 89%) did significantly reduced amounts of or no PSI-O protein. Similar frequencies of T1 plants deficient in a specific protein have been obtained by dsRNA-mediated genetic interference of genes involved in, for instance, flower development (30). The fraction of individuals with inhibition of psaO gene expression is significantly higher than similar experiments in which we have used antisense suppression or co-suppression of other PSI subunits (9, 10, 23, 25, 27, 31). In these cases, we only observed 25–35% of the T1 plants with reduced or no detectable amounts of the suppressed gene product.

In our experience, the suppression of a gene using antisense or co-suppression is not necessarily retained in subsequent generations. In a recent study (25) with antisense suppression of psaL in Arabidopsis we found that in only 4 of 14 T1 lines all of the offspring plants had a high proportion of individuals with no detectable PSI-L. In the case of antisense suppression of psaK or psaG we found 3 of 9 lines and 1 of 11 lines, respectively, which had a high proportion of individuals with no detectable protein of the suppressed gene. In contrast to this, 8 of 14 Psao-RNAi lines all produced offspring plants without any detectable PSI-O protein. Thus, the dsRNA interference of PSI genes appears to be much more efficient in causing an inhibition of gene expression, and it also seems to be more persistent and inherited compared with antisense and co-suppression.

**The Absence of PSI-O Affects Accumulation of PSI-L in the Thylakoid Membrane**—Plants down-regulated in the PSI-L protein retain only 10–20% PSI-H (8) and ~10% PSI-O. Primary down-regulation of PSI-H results in 50% PSI-L and 10–20% PSI-O. This indicates that binding of PSI-O to the PSI complex is dependent on the presence of both PSI-H and -L, and direct interaction between PSI-O and -L was clearly demonstrated by chemical cross-linking. In contrast, the primary

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**TABLE I**

Fluorescence parameters measured on psao-RNAi and wild type plants

| Condition | Wild type | psao-RNAi |
|-----------|-----------|-----------|
| State transitions, Fs | 0.75 ± 0.06 | 0.41 ± 0.09* |
| (1-quin) in PSI light (state 1) | 0.08 ± 0.02 | 0.10 ± 0.03** |
| (1-quin) in PSI light (state 2) | 0.068 ± 0.02 | 0.12 ± 0.02* |
| ΦPSII (state 1) | 0.71 ± 0.04 | 0.69 ± 0.05 |
| ΦPSII (state 2) | 0.67 ± 0.05 | 0.62 ± 0.04** |
| (1-quin) (growth light) | 0.032 ± 0.01 | 0.057 ± 0.01* |
| ΦPSII (growth light) | 0.74 ± 0.01 | 0.71 ± 0.01* |
| NPQ (growth light) | 0.18 ± 0.04 | 0.13 ± 0.05 |
| ΦPSII (dark-adapted) | 0.81 ± 0.01 | 0.81 ± 0.01 |
The PSI-O Subunit of Photosystem I

Fig. 4. PSI-O is dependent on the presence of PSI-H and -L. Immunoblot analysis of thylakoids from knock-out, antisense-, or cosuppression lines essentially devoid of PSI-K, -G, -L, -F, -H, -E, or -N. Control samples are thylakoids from wild type (WT) and psaO-RNAi plants (ΔO). In each lane proteins corresponding to 1 μg of Chl are separated by SDS-PAGE and subjected to immunoblot analysis using antibodies against PSI-F, PSI-O, and PSI-C. Numbers below the immunoblot panels indicate the ratios of PSI-O/PSI-F, the dash indicates not determined.

Fig. 5. Cross-linking of PSI-O with PSI-L. Diagonal electrophoresis and immunoblotting of digitonin-PSI cross-linked with DTSP. The first dimension was run from left to right and the second dimension was run from top to bottom. The apparent molecular mass in the first dimension is indicated on the top, and the migration of the different proteins in the second dimension is shown on the left. The left lane is digitonin-PSI without DTSP treatment, and the diagonal containing proteins that were not cross-linked in the first dimension is indicated (dashed line). A, using antibodies against PSI-L and PSI-H the product at 25–30 kDa containing PSI-L+PSI-H is shown. B, using antibodies against PSI-L and PSI-O it is seen that the ~25-kDa product contains PSI-L+PSI-O.

down-regulation of PSI-O has no negative effect on the content of PSI-H and -L. This behavior of the three proteins fits very well with the PSI-L protein being the most ancient and conserved subunit closest to the reaction center and the eukaryotic PSI-H and -O proteins being later additions located more peripherally in the complex.

Surprisingly, there is significantly more of the PSI-L subunit in thylakoids devoid of PSI-O (Fig. 2). From a quantification of dilution series, a 50–60% increase in abundance of PSI-L was estimated in plants without PSI-O (data not shown). This increase in abundance of PSI-L was only seen in thylakoids and not in sucrose gradient-purified PSI-200 (data not shown). This suggests that PSI-L is either synthesized at a higher rate or is more stable than most of the other PSI subunits. Alternatively the entire PSI complex is turned over faster than the PSI-L subunit in the absence of PSI-O. Whatever the reason, it might be that PSI-L has a particular role in the assembly and/or turnover of PSI. Unfortunately PSI-O is not present or not resolved in the recently published 4.4 Å structure of plant PSI (4). A more detailed structure and further studies are needed to learn more about the interaction between PSI-L and -O.

PSI-O Is Involved in State Transitions—In the absence of PSI-O there is a 50% reduction of state transitions. We have characterized previously A. thaliana plants lacking PSI-H that were essentially unable to perform state transitions (8). The PSI-O-deficient plants do not show any secondary loss of PSI-H or -L. This strongly suggests that PSI-O is directly involved in state transitions. PSI-O could be necessary for the binding or stabilization of LHCII during state transitions. We have shown recently that LHCII does in fact interact physically with exactly the PSI-L-H-O-I side of PSI (32) as suggested from studies of plants devoid of PSI-H or PSI-L. In this study, PSI particles were isolated under very mild conditions, and it was shown that LHCII could be chemically cross-linked with PSI-H, -L, and -I. No cross-linking of LHCII to PSI-O was found, but the absence of a cross-linking product cannot be used to conclude that the two proteins are not in contact. In support of PSI-O being involved in an interaction with LHCII, it was shown that PSI particles isolated from mutants lacking PSI-H, -L, or -O had a much decreased content of LHCII and was similar to PSI particles isolated from the wild type in state 1 (32). Thus, PSI-H, -L, -O, and possibly -I are all involved in forming a domain in PSI that is responsible for state transitions. Some of the proteins interact directly with LHCII, and others may be required for the stability or assembly of the domain.

PSI-O Affects Electron Transport through PSI—In the absence of PSI-H a 30–40% decrease of the steady state electron transport (measured as in vitro NADP⁺ photoreduction activity) through PSI is seen (8, 25, 31). The most likely cause for this is that the absence of PSI-H affects the binding of ferredoxin (31). Thus, the absence of PSI-H causes a slower electron transport through PSI and consequently leads to a more reduced plastoquinone pool both under PSIII light and under normal growth-light conditions (8, 25). The plants devoid of PSI-H clearly attempt to compensate for this by an increased PSI/PSII ratio (25, 31).

The PSI-O-less plants also have a more reduced plastoquinone pool (i.e. increased PSI excitation pressure) under PSI light and under normal growth-light conditions suggesting a decreased electron transport through PSI in the absence of PSI-O. The PSI/PSII ratio is not significantly changed in the absence of PSI-O or at least the ratio is not changed to the same extent as seen in the absence of PSI-H, which was reported to be ~15–20% (31). However, the Chl a/b ratio is slightly increased in the absence of PSI-O and could indicate a small increase in PSI over PSII. The increase is small, probably less than 10%, which can explain why the increase is not easily detected either with the estimates of Chl/P700 or with the immunoblot analysis.

Steady state electron transport in vitro showed no decrease in the absence of PSI-O, and most likely PSI-O does not affect subunits on the stromal side of PSI. The increased excitation pressure in the absence of PSI-O is therefore unlikely to be a result of the differences in the electron transport processes. Most likely, the binding of LHCII to PSI, to varying degrees, is taking place under normal growth-light conditions, i.e. not only during extreme transitions to state 2. In agreement with this, PSI particles isolated from plants in state 1 contained significant amounts of LHCII (32). This could be important for maintaining optimal electron transport through PSI even under constant environmental conditions, a feature that is missing in the absence of PSI-O.
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