Light-harvesting Complex II Binds to Several Small Subunits of Photosystem I*

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Mobile light-harvesting complex II (LHCII) is implicated in the regulation of excitation energy distribution between Photosystem I (PSI) and Photosystem II (PSII) during state transitions. To investigate how LHCII interacts with PSI during state transitions, PSI was isolated from Arabidopsis thaliana plants treated with PSI or PSII light. The PSI preparations were made using digitonin. Chemical cross-linking using diethio-bis(succinimidylpropionate) followed by diagonal electrophoresis and immunoblotting showed that the docking site of LHCII (Lhcb1) on PSI is comprised of the PSI-H, -L, and -I subunits. This was confirmed by the lack of energy transfer from LHCII to PSI in the digitonin-PSI isolated from plants lacking PSI-H and -L. Digitonin-PSI was purified further to obtain an LHCII-PSI complex, and two to three times more LHCII was associated with PSI in the wild type in State 2 than in State 1. Lhcb1 was also associated with PSI from plants lacking PSI-K, but PSI from PSI-H, -L, or -O mutants contained only about 30% of Lhcb1 compared with the wild type. Surprisingly, a significant fraction of the LHCII bound to PSI in State 2 was not phosphorylated. Cross-linking prior to sucrose gradient purification resulted in copurification of phosphorylated LHCII in the wild type, but not with PSI from the PSI-H, -L, and -O mutants. The data suggest that migration of LHCII during state transitions cannot be explained sufficiently by different affinity of phosphorylated and unphosphorylated LHCII for PSI but is likely to involve structural changes in thylakoid organization.

In oxygenic photosynthesis two photosystems, PSI and PSII, work in series to convert light energy into chemical energy. PSI is also involved in cyclic electron transport without the participation of PSII, and this process serves to produce additional ATP and to regulate the transthylakoidal proton gradient (1), but in plants this is a minor part of the electron transport. In linear electron transport, PSI and PSII operate with the same rate, but natural environmental conditions, such as the quality and quantity of light, are constantly fluctuating, and this may alter the balance between the two photosystems. The two photosystems have different absorption spectra, and therefore a change in light quality may favor one photosystem over the other. However, plants can balance the excitation energy distribution between the two photosystems via a mechanism known as state transitions, which was discovered more than 30 years ago (2–4). If PSII is overexcited relative to PSI, the plastoquinone pool becomes overreduced, and this will activate a kinase that phosphorylates a mobile pool of light-harvesting complex II (LHCII), leading to the lateral movement of LHCII in favor of PSI. This is the so-called “State 2,” in which the PSI antenna is smaller and the PSI antenna is larger than in State 1 (5, 6). State 1 is obtained when PSI is preferentially excited, which leads to oxidation of the plastoquinone pool and inactivation of the LHCII kinase. The phospho-LHCII is then dephosphorylated by a redox-independent phosphatase and moves back to PSII. Although the phenomenon of state transitions has been recognized for a long time, there is still considerable uncertainty about the mechanism. Two models have been proposed to explain the movement of LHCII. According to one model, alteration in the surface charge upon phosphorylation leads to structural changes of the thylakoid membrane and results in the movement of phospho-LHCII away from grana stacks (7–9). According to another model, the net movement of LHCII toward PSI in State 2 is caused by PSII with higher affinity for unphosphorylated LH-CII and PSI with higher affinity for phospho-LHCII, therefore movement of phospho-LHCII is a question of molecular recognition (6). Although the models differ in the way of explaining state transitions, they both involve phosphorylation of LHCII as a prerequisite for the initiation of State 1–State 2 transitions. A search for kinases involved in the phosphorylation of thylakoid proteins has been carried out by many workers for more than 20 years. A family of proteins, thylakoid-associated kinases, was identified as good candidates for LHCII kinases (10, 11). The antisense Arabidopsis plants with low amounts of thylakoid-associated kinase 1 showed a lower level of LHCII phosphorylation and were deficient in state transitions, but the phosphorylation of LHCII was distributed equally between PSI and PSI under white light, which was also the case in wild type plants (11). This indicates that the correlation between LHCII phosphorylation and state transition is complex. Recently, Depege et al. (12) reported a novel kinase in Chlamydomonas reinhardtii, thylakoid-associated serine-threonine protein kinase, and demonstrated that it is required for the phosphorylation of LHCII and for state transitions.

LHCII consists of three different proteins, Lhcb1, Lhcb2, and Lhcb3. Lhcb1 and Lhcb2 are the most abundant and can form Lhcb1 homotrimers and Lhcb1/2 heterotrimers, which are believed to be a mobile complex (13, 14). Both proteins usually
exist in several very similar isoforms, but specific isoforms are not well conserved between species, indicating that they probably are not redundant rather than having specific functions. However, this is another unclear point because it is not known whether there are biochemical differences between the mobile and nonmobile LHCII apart from the reversible phosphorylation described above. Electron microscopy studies have revealed that PSI core complexes are found as dimers surrounded by LHCII trimers (15–18). There are specific binding sites for the trimers, but the number of trimers/PSII complex differs depending on species and growth conditions. Generally, strongly bound, intermediately bound, and loosely bound trimers can be recognized. In contrast to the situation with PSII, the binding site of LHCII on PSI is not known.

PSI is composed of a multisubunit core complex (PSI core) and outer antenna, the light-harvesting complex I (LHC1). The PSI core complex in higher plants consists of 14 different subunits (PSI-A to PSI-L, PSI-N) (19, 20) and a recently discovered subunit PSI-O (21). LHC1 is composed of four different subunits of about 21–24 kDa, Lhc1 to Lhca4. The association between LHC1 and PSI is relatively weak, and a stable complex is difficult to purify. Early studies (22) suggested that LHCII was bound to PSI rather than directly to the PSI core, but this conclusion was based on the inability to reconstitute a LHCII-PSI complex using PSI devoid of LHCII-680 (composed of Lhca2 and Lhca3). We now know that preparation of a PSI complex devoid of LHCII is likely to cause the loss of additional small core subunits that were unknown at the time. Electron microscopy studies have revealed that all LHCII subunits bind at the side of PSI-F and PSI-J subunits of the PSI core complex (23). Arabidopsis plants lacking PSI-H were highly deficient in state transitions and have identical PSI antenna size in both States 1 and 2, whereas in wild type the antenna size of PSI was found to increase about 33% during transition to State 2 (5). Therefore we suggested that LHCII binds directly to the PSI core and that PSI-H is a part of a docking site (5). Based on cross-linking and x-ray crystallography data, the PSI-H subunit is positioned close to PSI-L and PSI-O on the opposite side of the PSI core complex compared with PSI-P, PSI-J, and LHC1 (24, 25). However, until now, no direct biochemical evidence has ever been found for the association of LHCII to PSI during state transitions.

To investigate how LHCII interacts with PSI during state transitions, we purified LHCII-PSI complex from wild type Arabidopsis in State 1 and State 2, and we also used mutants lacking the PSI-H, -L, -O, or -K subunits to determine the docking sites for LHCII. The results indicate that PSI-H, -L, -O, and -I all participate in forming the docking site. Furthermore, the results show that both phosphorylated and unphosphorylated LHCII are associated with PSI in State 2.

EXPERIMENTAL PROCEDURES

Plant Materials—Arabidopsis thalaina (L.) Heynh ecotype Col-0 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, 70% relative humidity. The photoperiod was 8 h. Transformants lacking specific PSI subunits were obtained by antisense or cosuppression and have been reported before (5, 26–28, PSI-O mutant).²

Light Treatment—Colored filters were used in the experiments to provide PSI light (120) and PSI light (11) as described by Pfannschmidt et al. (29). PSI light was obtained with an orange filter (Rosco, 105 orange, Teadon Aps, Slenlose, Denmark), and PSI light was obtained with a red filter (HT 027 medium red, LEE Filters, Andover, UK). Gray filter (209 neutral density, LEE Filters) was used to adjust the light coming through the orange filter to a level similar to that through the red filter (50–70 μmol photons m⁻² s⁻¹). The filters were mounted in a controlled environment chamber equipped with 400-Watt Powertone HPI-T Plus lamps (Philips). Six-week-old wild type plants were exposed to PSI or PSII light for 1 h prior to harvesting of leaves. Plants lacking PSI-H, -L, -O, or -K subunits were treated with PSI light for 1 h.

State Transitions in Leaves—State transitions were measured with a pulse amplitude modulation 101–103 fluorometer (Walz, Effeltrich, Germany) in the growth chamber equipped with filters as described above. Plants were dark-adapted for 30 min before the measurements. A detached leaf from a wild type plant was fixed to the light fiber, which was positioned so the leaf was horizontal and received the same irradiation as the plants used for preparation of thylakoids. Maximum fluorescence yield (Fₘ) was determined by exposing the leaf to a fluo-
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² P. E. Jensen, A. Haldrup, S. P. Zhang, D. Leister, and H. V. Scheller, unpublished data.
**RESULTS**

**State Transitions in the Growth Chamber**—To make sure that we chose the right light conditions to do the following experiments, we first tested whether the plants performed state transitions in the growth chamber. To induce state transitions, we equipped a growth chamber with orange and red filters for preferential excitation of PSII and PSI, respectively. The light intensity passing through the filters was about 50–70 μmol photons m⁻² s⁻¹. The plants were dark-adapted for 30 min before measuring the maximum fluorescence signal (Fₘₐₓ). The figure is a representative trace of one leaf. Similar results were obtained with leaves from different wild type plants.

In this way the two sections could be aligned exactly. To determine the relative content of Lhcb1, Lhcb2, and phosphothreonine in the LHClI/PSI complexes each sample was electrophoresed together with a dilution series of known amounts of LHClI to ensure that the response on the immunoblots was in a linear range. Quantitation was carried out by scanning the x-ray films and analyzing them using the ImageQuant software (Molecular Dynamics).

Polyclonal antibodies against PSI proteins were prepared in rabbits and have been described before (21, 35). Antibodies against Lhcb1 and Lhcb2 were kind gifts of S. Jansson, Umeå University, Sweden. Antibodies against phosphothreonine were obtained from Cell Signaling Technology, Inc. (Beverly, MA).

**Diagonal Electrophoresis**—The digitonin-PSI preparations (treated with PSI light) were diluted to 0.3 μg of Chl/ml in 20 mM Tricine, pH 7.5, 0.3% (w/v) DM, and 10 mM NaF. Chemical cross-linking with DTSP was carried out as described above. The cross-linked samples were mixed with 1 volume of nonreducing sample buffer (50 mM Na₂CO₃, 15% w/v sucrose, 2.5% (w/v) SDS), the solution was incubated for 20 min at room temperature, and loaded on the first dimension 12% 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, and 50 mM dithiothreitol) to obtain complete reductive cleavage of the cross-linked products. The gel slice was placed on top of a second 8–25% gradient gel and reelectrophoresed.

**P700 Photooxidation Measurements**—The photoreaction kinetics of P700 were monitored with the absorbance changes at 810 nm with 880 nm as reference (ΔA₈₁₀→₈₈₀) using the dual wavelength unit ED-P700DW of a PAM 101–103 fluorometer (Walz) as described by Bukhov et al. (36) with some modifications. Actinic light from a KL1500 halogen lamp (Schott, Mainz, Germany) with different light intensities was controlled by an electronic shutter, which opened for 1 s with 40-s lamp (Schott, Mainz, Germany) with different light intensities was controlled by an electronic shutter, which opened for 1 s with 40-s

**Binding Site for Light-harvesting Complex II on Photosystem I**

**Pectate Lyase**

**Chemical Cross-linking and Diagonal Electrophoresis**—To test this possibility, digitonin-PSI was cross-linked with DTSP, subjected to SDS-PAGE under nonreducing conditions, and electrophoretically to nitrocellulose membranes. Two cross-linked products could be clearly identified with an antibody against Lhcb1 (Fig. 3A, lane 1). One cross-linked product (Fig. 3A, lane 2) with an apparent molecular mass of ~30 kDa, was mostly a product of PSI-I and Lhcb1 because an antibody against PSI-I identified a product of the same size.

Previous work has shown that cross-linking products migrate with apparent molecular masses corresponding to the combined mo-

**Fig. 2. Polypeptide composition of the DM-PSI and digitonin-PSI preparations.** A, SDS-polyacrylamide gel stained with Coomassie Brilliant Blue. Lane 1, DM-PSI from State 1; lane 2, DM-PSI from State 2; lane 3, digitonin-PSI from State 1; lane 4, digitonin-PSI from State 2. B, phosphoprotein content in digitonin-PSI from State 1 (L1) and State 2 (L2) as determined by immunoblotting using a phosphothreonine antibody. Samples were loaded on chlorophyll basis (2 μg of Chl/lane).
Lhcb1 (25 kDa) and PSI-I (4 kDa). The other cross-linked molecular masses of the individual proteins (24), and 30 kDa is in each lane on the nitrocellulose blot (except for the first lane) was cut into two sections and incubated with two different antibodies. This allows exact alignment of the blots. The antibodies used are indicated above each lane. A, analysis of cross-linking products involving Lhcb1. Lane 1 and the right half of lanes 2–4 were incubated with Lhc1 antibody. The cross-linking products of 30 kDa (†) and 55–60 kDa (*) are indicated. B, the same as lane 2 in A but with shorter exposure time. C, same as in A for analysis of cross-linking products involving Lhcb2. The cross-linking product of 30 kDa is 57 kDa, and the data indicated above each lane was cut into two sections and incubated with two different antibodies. The combined molecular mass of Lhcb1, PSI-I, PSI-H, and PSI-L is 57 kDa, and the data therefore indicate that the immuno-reactive cross-linking product is a product of these four proteins. The previously described (24) cross-linking products involving PSI-L/PSI-H (28 kDa), PSI-L/PSI-I (22 kDa), and PSI-H/PSI-I (14 kDa) are also clearly seen on the blot. With an antibody against Lhcb2 a cross-linking product with apparent molecular mass of around 50 kDa (†) was observed (Fig. 3C). However, this product is clearly smaller than the cross-linking product involving PSI-I, -L, and -H, and it may correspond to an Lhcb2 dimer.

A well known problem with using antibodies against cross-linked proteins is that epitopes may be lost because of the cross-linking (24). However, with a cleavable cross-linker such as DTSP this problem can largely be overcome by electrophoresing the cleaved cross-linked products in a second dimension. To confirm the above conclusions further, the gel slices after the first dimension were therefore cut out, incubated with reducing sample buffer to cleave the cross-linked products completely, and reelectrophoresed. In the first dimension, cross-linked products will migrate approximately according to the combined apparent molecular mass, but after cleavage the previously linked proteins will migrate separately according to their normal apparent molecular masses and therefore form vertically aligned spots in the second dimension (24). Proteins that are not cross-linked will migrate to the same position in both dimensions and thus form a diagonal in the final gel. The blot in Fig. 4A confirms that Lhcb1 formed two cross-linked products of 30 and 55–60 kDa. The 55–60-kDa product is clearly resolved into Lhcb1 (25 kDa) and PSI-L (14 kDa; note that the protein migrates faster than the actual molecular mass of 18 kDa). PSI-H (10 kDa) is also vertically positioned under Lhcb1 and PSI-L corresponding to 55–60 kDa in the first dimension (Fig. 4B), and the spots of PSI-L and PSI-H can be resolved into two cross-linking products. One might be Lhcb1/PSI-L/PSI-H, and another might be Lhcb1/PSI-L/PSI-H plus PSI-I (4 kDa). The presence of PSI-I in the 60-kDa product could not be confirmed in the second dimension, probably because of the lower sensitivity of the PSI-I antibody. The product of ∼30 kDa is seen to consist of large amounts of Lhcb1 together with PSI-I (Fig. 4C). The presence of PSI-L and PSI-H at slightly higher position is evidence of a different product of those two proteins, which was described previously (24). These results clearly demonstrate that Lhcb1 binds to the PSI core in the digitonin-PSI preparation in State 2, and the docking site involves PSI-L, PSI-H, and PSI-I. Similar diagonal electrophoresis using antibody against Lhcb2 did not reveal the nature of the 50-kDa cross-linking product (data not shown). Thus, the product is either a dimer of Lhcb2 or a product between Lhcb2 and a non-PSI protein.

Kinetics of P700 Photooxidation—To see whether the LHClII was functionally attached to PSI in the digitonin-solubilized PSI, energy transfer was determined directly as time course of photooxidation of P700 in vivo. Fig. 5 shows the curves of P700 photooxidation of digitonin-PSI from the wild type and PSI-H- and L-mutants. It can be clearly seen that the rate of photooxidation was much slower in the mutants than in the wild type. The τ50 for saturation and the relative antenna size of these different samples were calculated from such curves (Table I). The results indicate that in the absence of PSI-H, PSI-L, or PSI-O, the antenna size in State 2 was about 15–16% smaller than in the wild type.

Purification and Characterization of a LHClII Complex—To get more direct evidence about the docking site of LHClII on PSI, we further purified the digitonin-PSI preparations from both wild type and mutant plants on sucrose gradients containing DM. This procedure is milder than direct solubilization of thylakoid membranes with relatively high concentrations of DM. PSI was purified in this manner both directly using the digitonin-PSI preparations and after cross-linking of the digitonin-PSI with DTSP. Three main bands were obtained from the sucrose gradients and characterized by Chl α/β ratio and SDS-PAGE. The upper band was highly enriched in LHClII but did not contain any PSI, the middle band was LHClII-PSI complex, and the lower band was aggre-
Fig. 5. Photooxidation of P700 determined as light-induced absorbance changes at 810 nm with absorbance at 860 nm as reference. Digitonin-PSI preparations from the wild type (WT) and mutants lacking PSI-L or PSI-H were illuminated in the presence of 0.5 mM ascorbate and 0.05 mM methyl viologen. Each curve is the average of three measurements. The light intensity was 13 μmol photons m\(^{-2}\) s\(^{-1}\).

Table I  
Time course of P700 photooxidation in digitonin-PSI preparations

|       | Antenna size Statistical significance |
|-------|--------------------------------------|
| t\(_{1/2}\) | % of WT | p     |
| WT    | 146 ± 9   | 100    |
| ΔH    | 169 ± 5   | 84.6 ± 1.5 | 0.0004 |
| ΔL    | 174 ± 6   | 83.1 ± 0.5 | 0.003  |
| ΔO    | 171 ± 13  | 84.6 ± 0.8 | 0.005  |

gates containing several different components including PSI, LHCII, and PSII. The polypeptide composition of the LHCII-PSI complex from wild type (State 2) is shown in Fig. 6. LHCII was clearly bound to PSI when isolated after cross-linking with DTSP, but it is difficult to determine the amount of LHCII in the non-cross-linked samples from Coomassie-stained gels.

Fig. 7 shows immunoblotting analysis of the LHCII-PSI complex. Using an antibody against Lhcb1 (Fig. 7A), LHCII was also detected when DTSP was not used. Furthermore, the quantitation showed that Lhcb1 was present in State 1 at a level of about 40% compared with State 2 (Table II). LHCII-PSI was prepared in the same way from plants lacking PSI-H, -L, -O, or -K, in all cases after treatment of the plants with PSI light. Compared with the wild type, with less than half the amount of Lhcb1 was bound to PSI lacking PSI-H, -L, -O, or -K, in all cases after treatment of the plants with PSI light. Compared with the wild type, with less than half the amount of Lhcb1 was bound to PSI lacking PSI-H, -L, or -O, whereas the content of Lhcb1 was higher in the absence of PSI-K than in the wild type (Fig. 7A and Table II). When the preparations were cross-linked with DTSP prior to sucrose gradient centrifugation, the resulting LHCII-PSI preparations contained about 5-fold more Lhcb1 (Fig. 7B and Table II). Even with cross-linking the PSI preparations were essentially devoid of Lhcb1 for the PSI-O mutant. In the PSI-H and PSI-L less plants, some Lhcb1 was purified with the PSI, although about 60–65% less than in the wild type in State 2 (Fig. 7B and Table II). Using an antibody against Lhcb2, the results were quite different from those found with the Lhcb1 antibody both without cross-linking (Fig. 7C) and with cross-linking (Fig. 7D). The quantitation showed that the content of Lhcb2 in the PSI-H or -L less PSI was about 35–45% higher than that in the wild type when DTSP was used (Table II). Without cross-linking, PSI from the PSI-H or -O mutants contained about 30–40% less Lhcb2 than the wild type, whereas in the PSI-L less PSI, the content of Lhcb2 was almost the same as in the wild type (Table II).

The content of Lhcb1 and Lhcb2 as determined above is relative. An estimate of the absolute amounts was made by comparing immunoblots of the purified LHCII-PSI and known amounts of the isolated LHCII from the sucrose gradients. Assuming that the isolated LHCII was composed of Lhcb1 and Lhcb2 in a 1:1 ratio we could estimate the molar ratio of Lhcb1 to P700 to 0.24 and 1.5 in the non-cross-linked and the cross-linked LHCII-PSI preparations, respectively. For Lhcb2 the corresponding numbers were 0.19 and 0.45. These numbers give a rough indication of the amount of LHCII in the preparations. An average content of two Lhcb monomers/P700 corresponds to an antenna size of about 15% of the LHCI level of about 40% compared with State 2 (Table II). LHCII was clearly bound to PSI when isolated after cross-linking with DTSP prior to sucrose gradient centrifugation. Using an antibody against Lhcb1 (Fig. 7A), LHCII was also detected when DTSP was not used. Furthermore, the quantitation showed that Lhcb1 was present in State 1 at a level of about 40% compared with State 2 (Table II). LHCII-PSI was prepared in the same way from plants lacking PSI-H, -L, -O, or -K, in all cases after treatment of the plants with PSI light. Compared with the wild type, with less than half the amount of Lhcb1 was bound to PSI lacking PSI-H, -L, or -O, whereas the content of Lhcb1 was higher in the absence of PSI-K than in the wild type (Fig. 7A and Table II). When the preparations were cross-linked with DTSP prior to sucrose gradient centrifugation, the resulting LHCII-PSI preparations contained about 5-fold more Lhcb1 (Fig. 7B and Table II). Even with cross-linking the PSI preparations were essentially devoid of Lhcb1 for the PSI-O mutant. In the PSI-H and PSI-L less plants, some Lhcb1 was purified with the PSI, although about 60–65% less than in the wild type in State 2 (Fig. 7B and Table II). Using an antibody against Lhcb2, the results were quite different from those found with the Lhcb1 antibody both without cross-linking (Fig. 7C) and with cross-linking (Fig. 7D). The quantitation showed that the content of Lhcb2 in the PSI-H or -L less PSI was about 35–45% higher than that in the wild type when DTSP was used (Table II). Without cross-linking, PSI from the PSI-H or -O mutants contained about 30–40% less Lhcb2 than the wild type, whereas in the PSI-L less PSI, the content of Lhcb2 was almost the same as in the wild type (Table II).

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Fig. 6. Polypeptide composition of the LHCII-PSI complex obtained from sucrose gradient centrifugation of digitonin-PSI in the presence of low amounts (0.05% w/v) of DM. The wild type (WT) material was obtained from plants treated with PSI light (L2). Samples were loaded on Chl basis (2.5 μg of Chl). The samples in A and C were prepared without DTSP, and the samples in B and D were treated with DTSP prior to sucrose gradient centrifugation. The wild type (WT) material was obtained from plants treated with PSI light (L2) or PSI light (L1). The material from mutants lacking PSI subunits was from plants treated with PSI light. All samples were loaded on Chl basis (1 μg of Chl).
ments, whereas the non-cross-linked PSI had obviously lost a large fraction of the associated LHCII.

Phosphorylation of LHCII is believed to be a prerequisite for the initiation of state transitions, so we also analyzed the phosphorylation level in the LHCII-PSI complexes. Fig. 8 shows the immunoblotting analysis of LHCII-PSI complexes using a phosphothreonine antibody. With DTSP cross-linking, phosphorylated LHCII could be detected in the wild type but was not detectable in PSI-H, -L, or -O mutant plants in State 2 (Fig. 8A and Table II). Without DTSP, a very small amount of phosphorylated LHCII could be detected in the wild type and in PSI prepared from the PSI-K mutant in State 2 (Fig. 8B). Notably, only a very small fraction of LHCII was phosphorylated in the wild type in State 2 even though both Lhcb1 and Lhcb2 were present in the preparation (Table II). Phospho-LHCII has been shown to be more resistant to thylakoid proteases than unphosphorylated LHCII (37). Therefore, it was important to ensure that the use of DTSP did not differentially affect proteolysis of LHCII or phospho-LHCII. We therefore compared the content of phospho-LHCII relative to total Lhcb1 after incubation of the digitonin preparations with and without DTSP. During the incubation no degradation of phospho-LHCII was detected, and the relative content of phospho-LHCII was the same with and without DTSP (data not shown).

Chemical phosphate determination of the purified LHCII showed the presence of 0.9 phosphate group per Lhcb monomer in State 2 (data not shown). Using immunoblotting with the phosphothreonine antibody and comparing with dilution series of the LHCII, we estimated that the LHCII-PSI preparation in State 2, which was isolated after cross-linking with DTSP, contained about 3 phosphate groups in LHCII/psaE. This number is somewhat higher than the about two monomers/psaE determined with the antibodies against Lhcb1 and Lhcb2. The difference may reflect multiple phosphorylations or inaccuracy in quantitation from immunoblots but indicate that in the cross-linked sample, most of the LHCII bound to PSI in State 2 was phosphorylated.

DISCUSSION

The majority of LHCII trimers are bound to PSII (15), but a fraction of mobile LHCII is able to dissociate from PSII. The reversible dissociation of LHCII is responsible for state transitions, which serve to balance excitation energy distribution between PSI and PSII. Plants without PSI-H do not perform state transitions, the PSI antenna does not increase in State 2, and LHCII remains with PSII (5). Therefore, PSI-H was proposed as docking site of LHCII on PSII (5). However, the mutants with less state transitions also show changes in thylakoid structure (fewer stacks in grana) (38), and indirect effects could not be excluded. Until now, there still are some controversies about whether the mobile LHCII actually docks to PSI. No direct biochemical evidence has been provided yet about the docking site of LHCII to PSI. We therefore wanted to investigate the direct association between LHCII and PSI.

Several workers have made efforts to obtain a purified LHCII-PSI complex, but so far the existence of a LHCII-PSI complex was mostly based on indirect evidence. Bassi and Simpson (22) purified a significant amount of LHCII that energy transfer from LHCII to PSI seemed to take place, PSI preparations reflected the situation in stacked thylakoids. Recent results (39) obtained similar preparations from pea. Although they showed that energy transfer from LHCII to PSI seemed to take place, it is questionable whether the large amount of LHCII in the PSI preparations reflected the situation in stacked thylakoids. Recently, Pesaresi et al. (40) reported a stable LHCII-PSI aggregate present in low light-adapted thylakoids of Arabidopsis mutant knocked out in one of the psae genes. However, because state transitions in the mutant are almost completely suppressed, this may be an abnormal LHCII-PSI complex, and no evidence for its functionality in energy transfer has been obtained.
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state transitions, so we thought that the PSI-O subunit might be involved in state transitions has been shown in many studies (6–9, 43, 44), a demonstration that LHCII phosphorylation is directly involved in state transitions has not been presented. It is also not clear whether phosphorylation of LHCII causes a change in the thylakoid structure, which promotes LHCII migration, or causes a lower affinity for PSI and/or a higher affinity for PSI. Furthermore, phosphorylation of LHCII might have a direct role in regulation LHCII turnover because phospho-LHCII is more resistant to proteolysis by proteases in the thylakoid membrane (37). Hence, the significance of phosphorylation of LHCII during state transitions could be in the protection of exposed LHCII in the stroma membranes against proteases. A few studies have indicated a lack of correlation between phosphorylation and state transitions. Snyders and Kohorn (11) found that LHCII associated with PSI in State 2 was not phosphorylated. Furthermore, LHCII phosphorylation is very low at high light intensities (45), whereas at least some studies have suggested significant state transitions at higher light intensities (31, 46). On the other hand, state transitions are normally determined by indirect methods, and it is possible that they do not really take place at high light intensity (47). The data presented here show that the LHCII-PSI complex isolated from wild type plants contains unphosphorylated LHCII in State 2. The LHCII-PSI complex isolated without chemical cross-linker contained about 24% LHCII compared with the preparation with cross-linker, but the content of phosphothreonine was less than 5% (Table II), indicating that most of the LHCII was unphosphorylated in the non-cross-linked samples. Based on the content of phosphate we can estimate that after cross-linking about 80% of the LHCII in the PSI preparations was phosphorylated in State 2. In State 1 phosphorylated LHCII was not detectable even after cross-linking. This indicates that phosphorylated LHCII is bound to PSI in State 2 but more weakly than the unphosphorylated LHCII. The antibodies used to determine phosphorylation levels have limited sensitivity, and they cannot distinguish between different phosphorylation sites. Additional studies in the future are needed to determine more accurately the ratio between phosphorylated and unphosphorylated LHCII bound to PSI under different conditions. According to the molecular recognition model, LHCII would move from PSI to PSII during transition to State 2 because the phosphorylated LHCII has a higher affinity for PSI and/or a lower affinity for PSII. However, the data reported here indicate that phosphorylated LHCII does not have a higher affinity for PSI. Maybe phoso-LHCII is more loosely bound to PSI than LHCII without phosphorylation, but if this were the case why would unphosphorylated LHCII also move away to PSI? The data presented here are actually more compatible with a variant of the surface charge model. We propose that LHCII moves in state transitions not because the phoso-LHCII has higher affinity for PSI but because the phosphorylation causes structural changes in the thylakoid membranes, which promote movement of LHCII, i.e. both in its phosphorylated and unphosphorylated form. However, we find it unlikely that the charges of the phosphate group contribute significantly to an electrostatic driving force and find it more likely that simple diffusion describes the actual movement. The LHCII appears to bind to a specific site on PSI, and if this site is nonfunctional (as in mutants lacking PSI-H) LHCII tends to remain associated with PSII. In this sense both the molecular recognition and the surface charge models are needed to explain the migration of LHCII.

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