A new potential light-harvesting protein, named Lhca5, was recently detected in higher plants. Because of the low amount of Lhca5 in thylakoid membranes, the isolation of a native Lhca5 pigment-protein complex has not been achieved to date. Therefore, we used in vitro reconstitution to analyze whether Lhca5 binds pigments and is actually an additional light-harvesting protein. By this approach we could demonstrate that Lhca5 binds pigments in a unique stoichiometry. Analyses of pigment requirements for light-harvesting complex formation by Lhca5 revealed that chlorophyll b is the only indispensable pigment. Fluorescence measurements showed that ligated chlorophylls and carotenoids are arranged in a way that allows directed energy transfer within the light-harvesting complex. Reconstitutions of Lhca5 together with other Lhca proteins resulted in the formation of heterodimers with Lhca1. This result demonstrates that Lhca5 is indeed a protein belonging to the light-harvesting antenna of photosystem I. The properties of Lhca5 are compared with those of previously characterized Lhca proteins, and the consequences of an additional Lhca protein for the composition of the light-harvesting antenna of photosystem I are discussed in view of the recently published photosystem I structure of the pea.

Photosystem I (PSI) of higher plants is a multi-protein complex located in the thylakoid membrane and can be separated into two moieties, namely the core complex containing the primary donor (P700) that performs the charge separation and the external antenna, the so-called light-harvesting complex I (LHCl). All LHCs consist of a protein backbone that coordinates pigments not covalently in a way that allows directed energy transfer to the reaction center chlorophylls (Chl). In this way, LHCs increase the quantum yield of photosynthesis. The recently published crystal structure of pea PSI showed at a resolution of 3.4 Å the presence of four pigmented Lhca proteins attached to one side of the PSI core complex (1). The PSI structure clearly revealed the orientation of the LHCl backbone and the presence of three transmembrane and two small extrinsic helices in each Lhca protein. Additionally, the number (12 per monomer) and orientation of the coordinated Chls were visible. However, the exact assignment of the resolved LHCl to Lhca1 to Lhca4 could not be achieved, leaving room for speculation about their identity. To date, the LHCs resolved in this structure were assigned to Lhca1–Lhca4 according to previously performed biochemical and mutagenic studies (2–6). Lhca1 and Lhca4 form the heterodimeric LHCl complex (2, 3, 7) whereas Lhca2 and Lhca3 apparently form either homodimers or heterodimers (2, 3, 5).

Genomic data, however, have shown a greater heterogeneity of LHCl. In some species, one Lhca protein is encoded by several genes. In tomato, for example, there exist isoforms of Lhca1 (cab6a and cab6b, respectively) (8) and Lhca4 (cab11 and cab12, respectively) (9). Arabidopsis thaliana contains only one gene each for Lhca1–Lhca4. However, genes encoding a fifth and sixth Lhca-like protein (lhca5 and lhca6) have been identified (10). Lhca6 shows very high sequence similarity to Lhca2 and can therefore be assumed an Lhca2 isoform. The expression of Lhca5 homologues has been confirmed in various plants by expressed sequence tag data base searches (11). According to the amino acid sequences of tomato Lhca proteins, the closest relatives of Lhca2 and Lhca4, which share an identity of ~46% with Lhca5. Under normal growth conditions the Lhca5 mRNA level is <10% that of the other Lhca proteins in A. thaliana (10, 11). This might be the reason why the Lhca5 protein could not be detected for a long time, despite detailed studies of PSI. Only recently did refined analytical methods allow the identification of Lhca5 in two higher plants (11, 12). In A. thaliana the Lhca5 apoprotein was detected in isolated thylakoids and PSI by immunoblotting (11). Using tandem mass spectrometry, we showed that the gene product of lhca5 is present in PSI as well as in the LHCl-730 of the tomato (12). The same approach led to the identification of a possible Lhca5 homologue in Chlamydomonas reinhardtii (13).

In Lhca5, the amino acids known to be involved in chlorophyll binding in other Lhca proteins (14, 15) are conserved (11). Consequently, one may assume that Lhca5 binds pigments too. However, isolation of a pigment-protein complex of Lhca5 has not yet been achieved, which is most probably due to the small amount of this LHC in PSI and LHCl preparations compared with that of other Lhca proteins. Therefore, the question arises as to whether Lhca5 actually binds pigments and, thus, is a “real” Lhca apoprotein. In the case that it could be identified as chlorophyll-binding protein it would be interesting to learn more about its biochemical and spectral properties, which might give hints towards its physiological significance.

Because of the low amount of Lhca5 in thylakoid membranes, it has yet not been possible to address these questions by analysis of the native pigment-protein complex. Therefore, in vitro reconstitution, which proved to yield native-like LHCs from other Lhc proteins, appears to be a suitable approach (5, 7, 16–21). Pigment analyses of native LHCIs (e.g. 5, 19, 21–23) and reconstituted Lhca1–Lhca4 (5, 7, 19, 21, 24) revealed that all four Lhca proteins coordinate Chl a, Chl b,
lutein (lut), violaxanthin (vio), and β-carotene (β-car) at different ratios. In addition, the Chl ab ratio of LHCs seems to correlate with the chlorophyll species required for LHC formation (17–19, 24–27). Spectral characterizations demonstrated differences between the four LHCl species (5, 7, 19, 24). Two of them (r-Lhca3 and r-Lhca4) show a pronounced long wavelength fluorescence emission, which is attributed to the presence of an asparagine residue at the Chl-binding site A5 (nomenclature according to Ref. 28) (19, 29). By contrast, Lhca1 and Lhca2 show fluorescence in the 680–690-nm range, comparable with that of the LHCl proteins of PSII (5, 7, 18, 19, 24–26).

In this study, we aimed to clarify whether Lhca5 actually is an LHC apoprotein that binds pigments in a way that is suitable for directed energy transfer. To achieve this goal, we employed the reconstitution technique using bacterially expressed Lhca5 proteins. The reconstituted Lhca5 was analyzed with regard to its pigment binding properties, fluorescence properties, and oligomerization behavior.

**EXPERIMENTAL PROCEDURES**

Construction of an Lhca5 Expression Plasmid—The cDNA sequence corresponding to the mature A. thaliana Lhca5 apoprotein (10) was amplified by PCR using the primers 5′-TGAGAGTCGACGCGAGGAGGCATCAACCC-3′ (forward) and 5′-AATCTTCTGACCTAGATG-TAGAGTGAAGGAGG-3′ (reverse). These primers generated a Sall and PstI restriction site at the 5′-end and the 3′-end, respectively. The amplified sequence was cloned into a pDS12 expression plasmid as described (19). Amplification and insertion into the expression plasmid was verified by DNA sequencing of the complete coding region.

**Production of Materials Required for Reconstitution—** For Lhca5 expression, the expression plasmid was transformed into E. coli strain JM101. Overexpression was done following the protocol described for Lhca1, Lhca3, and Lhca4 (19). Subsequently, the proteins were isolated as inclusion bodies as reported previously (30). Protein quantification was performed using the Bio-Rad dye binding assay according to the manual. Accumulation of the expressed protein was analyzed by fully denaturing sodium dodecyl sulfate (SDS)-PAGE followed by either immunodetection with an Lhca5 antibody or Coomassie staining to determine the apparent molecular mass of the expressed protein (12). Total pigment extract and individual pigments were isolated from thylakoids as described (30).

**LHC Reconstitution—** Reconstitution of Lhca5 was performed using the detergent exchange method (7). For subsequent analysis of the reconstitution mixture with partly denaturing lithium dodecyl sulfate-PAGE (Li-PAGE), a 10-μg inclusion body protein and pigment equivalent to 40 μg of Chl a + b were used. For sucrose density gradient separation (7), these amounts were increased to 160 and 200 μg, respectively. To analyze the pigment composition of Lhca5, reconstitution was performed with total pigment extracts. To determine the significant individual pigments for LHCl formation, pigment mixtures lacking individual pigments were used. Individual pigments were mixed corresponding to the ratio found in native LHCl (Chl a/b = 3; Chl a/Chl b/lut/vio/β-car = 9:3:1:0.5:0.5:0.45). When one pigment species was omitted from the mixture, it was replaced by another Chl or carotenoid species to maintain a Chl to carotenoid ratio of 6. Analysis of dimer formation was done with reconstitution mixtures containing half of the protein amount of both proteins.

**Pigment Analysis—**Photometric Chl quantification was performed in 80% acetone using the equations of Porra et al. (32). To determine the pigment composition of Lhca5, pigments equivalent to 1 μg of Chl were extracted from sucrose density gradient bands with sec-butyl alcohol (33) diluted with two volumes of 80% acetone and subjected to high pressure liquid chromatography analysis as in (19).

**Fluorescence Measurements—**77 K fluorescence emission measurements were performed with a Fluoromax3 (ISA, John Yvon-Spes, Grazbrunn, Germany). Sucrose density gradient bands were adjusted to 2 μg/ml Chl, 60% glycerol, 5 mM Tricine/NaOH (pH 7.8), and 0.05% β-dodecylmaltoside. The samples were excited at 410 and 470 nm, and the emission was recorded in 1-nm steps using a bandwidth of 2 nm for excitation and emission light. To gain insight into the contribution of different pigment species to fluorescence emission, excitation spectra were measured. The bandwidth was 4 nm for excitation and 2 nm for emission. Excitation spectra were recorded at fluorescence emission maxima of 684 nm (Lhca1 and Lhca5 monomer and Lhca1/Lhca5 dimer) and 735 nm (Lhca1/Lhca4 dimer).

**RESULTS**

The apoprotein corresponding to the mature form of Lhca5 of A. thaliana was expressed in E. coli and isolated as inclusion bodies. Separation of inclusion body protein in a fully denaturing gel resulted in the resolution of two intensely stained bands exhibiting apparent molecular masses of 24.2 and 25.1 kDa, respectively, as deduced from comparison with molecular mass standard proteins (Fig. 1, middle lane). Immunoblot analyses with Lhca5 antibodies raised against a peptide of the N terminus of the protein of A. thaliana detected both bands (Fig. 1, right lane). The apparent molecular mass of the lower band corresponds well to the theoretical mass of 24.3 kDa, deduced from translated cDNA sequence using the default values of the ExPASy peptide mass tool at www.expasy.org/tools/peptide-mass.html. Because of its size, the upper band is most probably the consequence of translational readthrough of the first stop codon and termination by the next stop codon located six codons downstream. This phenomenon was also observed in Lhca4 (7).

To test whether Lhca5 actually is a pigment-binding protein we reconstituted the protein with total pigment extract and separated the reconstitution mixture using partially denaturing gel electrophoresis. Fig. 2 reveals the formation of a stable r-Lhca5, proving that Lhca5 is indeed an LHC apoprotein. Former studies with other Lhc proteins demonstrated that the omission of individual pigments from the reconstitution mixture had an impact on the formation of stable LHCs. To elucidate which pigments are necessary for the formation of stable r-Lhca5, the apoprotein was reconstituted with different pigment mixtures, each lacking an individual pigment species, and the mixtures were separated on partially denaturing gels. From Fig. 2 it is obvious that omission of Chl a or individual carotenoids resulted in an r-Lhca5 yield comparable with that obtained from reconstitutions with all pigments or total pigment extract. By contrast, loss of r-Lhca5 formation was observed when reconstitutions were performed in the absence of Chl b.

To analyze the pigment stoichiometry of r-Lhca5, reconstitution mixtures were separated by sucrose density gradient centrifugation to achieve separation of pigments not bound to protein from r-Lhca5. The band containing monomeric LHC was collected and used for high pressure liquid chromatography measurements (Fig. 3). According to the recently published crystal structure of pea PSI (1), each Lhca protein binds 12 Chl molecules. Therefore, the pigment content of r-Lhca5 is presented on the basis of 12 Chls. Pigment binding of Lhca5 is rather specific, as the pigment ratio differs significantly from that in the total pigment extract used for reconstitution (Fig. 3). On average, r-Lhca5 binds 8.6 Chl a and 3.4 Chl b, resulting

![FIG. 1. Accumulation of the Lhca5 protein expressed in E. coli.](http://www.jbc.org)

Shown is a fully denaturing polyacrylamide gel of an Lhca5 inclusion body preparation stained with Coomassie Blue (left) and the corresponding immunoblot decorated with an Lhca5 antibody (right). M, molecular mass standard; a5, Lhca5; anti-a5, antibody raised against Lhca5.

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| Chl a | Chl b | Carotenoids | Total |
|------|------|-------------|-------|
| 8.6  | 3.4  |             | 12    |

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*Fig. 2. Analysis of dimer formation.*
in a Chl a/b ratio of 2.6. The carotenoid content is quite high and amounts to 2.7. Lut is the most abundant xanthophyll present in almost two (1.9) copies per 12 Chls. The other carotenoids are all present in substoichiometric amounts. Vio is found at a ratio of 0.3 per 12 Chls. Small amounts of neoxanthin (neo; 0.35) as well as β-car (0.2) were also associated with r-Lhca5.

To assure the functionality of r-Lhca5, sucrose density gradient bands containing monomeric r-Lhca5 were used to record 77 K fluorescence emission spectra after excitation at 410 and 470 nm. Both spectra (Fig. 4) exhibit one distinct emission peak at 684 nm. A long wavelength fluorescence emission peak is not present. The fluorescence peak originating from uncoupled Chl b at 650 nm is rather small, demonstrating proper energy transfer from Chl b to Chl a.

The presence of only one band in partially denaturing gels (Figs. 2 and 5A) indicates that r-Lhca5 only occurs in a monomeric form. To confirm this assumption, r-Lhca5 was additionally separated by more gentle sucrose density gradient centrifugation. This also yielded only one band co-sedimenting with monomeric Lhca1 to Lhca4, which indicates that Lhca5 forms monomeric LHCs but no homodimers (Fig. 5, A and B). The possible formation of heterodimers, such as the one formed by Lhca1 and Lhca4, was investigated by reconstitution of Lhca5 with each of the other Lhca proteins. Neither in partially denaturing gels nor in sucrose density gradients could a dimer band comparable with that of LHCI-730 (Fig. 5, A and B) be resolved. However, in sucrose density gradients an additional band migrating lower than the monomeric band was detectable when Lhca5 was reconstituted together with Lhca1. Analysis of these two bands by denaturing SDS-PAGE revealed that both bands contain the Lhca1 and Lhca5 apoprotein (not shown), which indicates interaction between Lhca5 and Lhca1. To analyze this finding in more detail, the ratio of the two apoproteins in the reconstitution mixture was varied. Fig. 6A shows that the yield of the lower LHC band was highest at Lhca5/Lhca1 ratios of 1:1 and 5:1. Analyses of the protein composition of the sucrose density gradient bands by fully denaturing SDS-PAGE (Fig. 6B) demonstrated that only the upper band reflected the protein ratio of the reconstitution mixture, whereas the lower band contained the two proteins in approximately equal amounts. This strongly indicates the formation of an Lhca1 and Lhca5 heterodimer.

Interestingly, the fluorescence emission spectrum of the Lhca1/Lhca5 heterodimer is very similar to the spectra of the monomeric constituents but strongly deviates from that of the Lhca1/Lhca4 dimer (Fig. 7A). To analyze potential differences between the excitation energy transfer in the Lhca1/Lhca5 dimer versus that in the Lhca1/Lhca4 dimer, 77 K excitation spectra of both dimers were recorded (Fig. 7B). The excitation spectrum obtained for the Lhca1/Lhca5 dimer...
In panel A, used at the indicated ratio and reconstituted with total pigment extract. In panel A, the band pattern of reconstitution mixtures separated by sucrose density gradient ultracentrifugation is shown. Panel B depicts the protein composition of sucrose gradient bands analyzed by SDS-PAGE. M, monomers; D, dimers; u, upper band; l, lower band; a1–a5, Lhca1–Lhca5.

**FIG. 6.** Influence of the Lhca5/Lhca1 ratio in the reconstitution mixture on dimer formation. Lhca5 and Lhca1 apoproteins were used at the indicated ratio and reconstituted with total pigment extract. In panel A, the band pattern of reconstitution mixtures separated by sucrose density gradient ultracentrifugation is shown. Panel B depicts the protein composition of sucrose gradient bands analyzed by SDS-PAGE. M, monomers; D, dimers; u, upper band; l, lower band; a1–a5, Lhca1–Lhca5.

...clearly differs from that of the Lhca1/Lhca4 dimer in the absorption region of Chl a and the carotenoids. In the Lhca1/Lhca5 dimer, pigments absorbing at 465 and 472 nm contribute stronger to Chl a fluorescence emission. To elucidate whether these differences arise from dimerization, excitation spectra of Lhca1 and Lhca5 monomers were additionally measured. Fig. 7B reveals that in Lhca5, Chl b and the carotenoids make stronger contributions to fluorescence emission than in Lhca1. Comparison of the excitation spectra of the Lhca1 and Lhca5 monomers and the Lhca1/Lhca5 dimer shows that the difference in the Chl b and carotenoid absorption region is partially caused by the Lhca5 monomer (inset in Fig. 7B). However, the curves of monomeric Lhca1 and Lhca5 do not sum up to that of the Lhca1/Lhca5 dimer, as is obvious from comparison with the calculated average spectrum of the monomers. Therefore, a slight increase in the contribution of Chl b and the carotenoids in the Lhca1/Lhca5 dimer appears to be caused by dimerization.

**DISCUSSION**

In this study, we demonstrate that Lhca5 binds pigments and is actually an additional LHC apoprotein. Reconstitutions of Lhca5 led to the formation of pigment-protein complexes that could be isolated by partially denaturing gel electrophoresis and sucrose density gradient centrifugation. The high reproducibility of its pigment composition indicates that pigment binding is specific. In addition, the observed energy transfer from Chl b to Chl a corroborates the light-harvesting function of Lhca5.

**Lhca5 Binds Pigment—**Despite the high degree of amino acid sequence similarity, the different Lhc proteins bind pigments in a unique stoichiometry, although there are similarities to other r-Lhcs. The Chl a/b ratio of 2.6 is identical or at least comparable with that of r-Lhca4 (2.6), r-Lhca2 (2.3) (5, 7, 19, 24), and r-Lhcb5 (2.2) (25). By contrast, r-Lhcb1 and r-Lhcb6 exhibit significantly lower Chl a/b ratios of 1.3 and 1, respectively (17, 26), whereas r-Lhcb4, r-Lhca1, and r-Lhca3 have a significantly higher Chl a/b ratio of 3, 3.5, and 6.1, respectively (5, 7, 18, 19, 24).

However, there are not only differences in preferential Chl binding by the various Lhc proteins but also in carotenoid binding with regard to the amount and stoichiometry of coordinated carotenoids. Recombinant Lhca2 and Lhca4 bind only two carotenoids per monomer (5, 7, 19, 21), in contrast to three in Lhca3 (19, 21, 24). Whether Lhca1 binds two or three carotenoids is still under debate (5, 15, 19). For r-Lhca5 we obtained 2.7 carotenoids per monomer, indicating that it usually binds three molecules and would thus be comparable with r-Lhca3. A high carotenoid content would be beneficial to a function of Lhca5 in heat dissipation and thereby in the photoprotection of PSI as proposed by Ganeteg et al. (11), who showed that Lhca5 mRNA is up-regulated under high light conditions in contrast to the other Lhca proteins.

Regarding the carotenoid composition, lut is the main xanthophyll in Lhca5. It is present in 1.9 copies, comparable with that of r-Lhca3 (19, 21, 24). Whether Lhca1 binds two or three carotenoids is still under debate (5, 15, 19). For r-Lhca5 we obtained 2.7 carotenoids per monomer, indicating that it usually binds three molecules and would thus be comparable with r-Lhca3. A high carotenoid content would be beneficial to a function of Lhca5 in heat dissipation and thereby in the photoprotection of PSI as proposed by Ganeteg et al. (11), who showed that Lhca5 mRNA is up-regulated under high light conditions in contrast to the other Lhca proteins.

**FIG. 7.** 77 K fluorescence emission and excitation spectra of monomeric and dimeric LHCI. In panel A the emission spectrum of the Lhca1/Lhca5 dimer is compared with those of its monomeric constituents and the Lhca1/Lhca4 dimer. Excitation wavelength was 410 nm. In panel B excitation spectra of monomeric Lhca1 and Lhca5, a calculated average spectrum of these monomers, and the Lhca1/Lhca5 and Lhca1/Lhca4 dimers are shown. The inset shows the region between 435 and 478 nm at higher magnification. Excitation was recorded at the wavelength of maximum fluorescence emission (684 nm for Lhca1 and Lhca5 monomers and Lhca1/Lhca5 dimers; 735 nm for Lhca1/Lhca4 dimers). Spectra were normalized to the maximum peak at 436 nm.
reconstituted proteins (0.5–1.1) investigated by Croce et al. (5) and Castelletti et al. (24) and indicates that a fraction of vio can be replaced by other carotenoids in vitro, depending on the reconstitution and purification procedure. Interestingly, neo is bound to Lhca5 in a substoichiometric amount (0.34), which is in contrast to data on native LHCl where no neo has been detected (5, 7, 19, 22, 23). However, it has been shown that recombinant Lhcas of tomato bind neo when it is present in the reconstitution mixture (19). It is possible that in reconstituted recombinant Lhcas of tomato bind neo when it is present in the reconstitution mixture (19). It is possible that in reconstituted LHCIIs neo replaces β-car, which is bound at a much higher ratio in native LHCI (0.3–0.5) (5, 19) than in reconstituted LHCI (0.05 in Lhca1, Lhca2, and Lhca4; 0.2 in Lhca3 and Lhca5) (19). Thus, pigment binding of at least one carotenoid-binding site of each LHCI protein seems to be flexible, allowing occupation by different carotenoids depending on their presence during reconstitution. Such flexibility of some carotenoid-binding sites has already been described for minor LHCIIIs and LHCIIb (37–40).

Summarizing the pigment data, Lhca5 seems to have a unique pigment composition. The carotenoid content and composition resembles that of Lhca3, and the Chl composition resembles that of Lhca4.

Chlorophyll b Is Required for Formation of r-Lhca5—It has been shown for the minor (Lhcb4 and Lhcb5) and major LHCII (Lhcb1) proteins as well as for LHCl proteins that the Chl \( a/b \) ratio correlates with the Chl species required for LHC formation (17–19, 25, 27). Reconstitution of LHCs with a relatively high content of Chl \( b \) (Lhcb1, Lhcb5, Lhca2, and Lhca4) requires the presence of Chl \( b \). By contrast, Chl \( a \) is dispensable for the formation of these LHCs. For complexes with a high Chl \( a \) content (Lhcb4, Lhca1, and Lhca3), the presence of Chl \( a \) is necessary for LHC formation. Lhca5 fits into this picture, as it has a relatively low Chl \( a/b \) ratio (2.6) and the omission of Chl \( b \) from the reconstitution mixture impairs complex formation completely (Fig. 2), whereas Chl \( a \) or individual carotenoids (lut, vio, and β-car) can be omitted from the reconstitution mixture without a significant impact on the yield of LHC. Thus, the pigment requirement of Lhca5 is comparable with that of Lhca2 (19). In both r-Lhca5 and r-Lhca2 the reconstitution yield is not affected by omitting lut, which, in contrast, results in strongly reduced yields of r-Lhca1, r-Lhca3, and r-Lhca4 (19). Regarding the requirement of individual carotenoids for complex formation, Lhca5 and Lhca2 seem to resemble Lhcb1 in that lut can be substituted by other xanthophylls (40, 41).

Reconstituted Lhca5 Is a Functional Light-harvesting Complex—Fluorescence excitation and emission measurements of r-Lhca5 showed that energy transfer from Chl \( b \) to Chl \( a \) and from carotenoids to Chls is established after reconstitution, confirming that r-Lhca5 is a functional LHC. The emission spectrum is similar to that of r-Lhca1 (5, 7) and r-Lhca2 (19, 24) which exhibit one distinct fluorescence emission peak at 684 and 688 nm, respectively. In contrast to the latter, r-Lhca5 does not have a shoulder at the long wavelength flank. Neither does r-Lhca5 show pronounced long wavelength fluorescence like r-Lhca3 and r-Lhca4 (5, 19, 24). The occurrence of long wavelength fluorescence depends on the presence of an asparagine residue as the ligand for the Chl at binding site A5 (19, 29).

Because Lhca5 has a histidine as Chl ligand at position A5, the existence of such long wavelength Chl was not to be expected. These data agree with room temperature fluorescence measurements of the Lhca5 knock-out plants of \( A. \ thaliana \) (11), which revealed no significant change in the fluorescence spectra of \( A. \ thaliana \) wild type and \( L. \hca5 \), either in whole leaves or in PSI preparations. These results provide strong evidence that Lhca5 does not contribute to the long wavelength fluorescence of PSI. This is also valid for the dimer formed by Lhca1 and Lhca5, because its fluorescence emission almost does not differ from that observed for the monomeric subunits. There was no shift in the fluorescence maximum as can be seen when r-Lhca1 and r-Lhca4 interact. This result indicates that in LHCII-730 a closer interaction between the subunits may exist that causes pigment rearrangement.

Lhca5 Forms Heterodimers—Apart from pigment binding, another feature specific to members of the LHC-family is their different oligomerization behavior. Whereas LHClIIs exists in vivo in a trimeric form (42), the minor LHCIIIs (Lhcb4–6) occur as monomers (43). Concerning the LHCI proteins, it is known that Lhca1 and Lhca4 form a heterodimer (2, 3, 7). Lhca2 and Lhca3 dimers has not been achieved (19, 24). This suggests that the interactions between Lhca2 and Lhca3 monomers must be much weaker than the one between Lhca1 and Lhca4. The same might be true for Lhca5.

To test the heterodimerization of Lhca5, we reconstituted it together with each of the other Lhca proteins. Reconstitution of Lhca5 with Lhca1 yielded two distinct bands in sucrose density gradients (Figs. 5A and 6A). The second band migrated lower than the bands of monomeric LHCIs but did not comigrate with the LHCII-730 heterodimers. A weaker interaction between Lhca1 and Lhca5 as compared with that of Lhca1 and Lhca4 may be an explanation for this different sedimentation behavior. Consequently, Lhca1/Lhca5 heterodimers would adopt a less compact structure than LHCII-730, resulting in dimers with lower density. Analysis of the protein composition in the upper and lower density gradient bands after reconstitutions with different Lhca1/Lhca5 ratios revealed that in the lower band the two apoproteins are present in a fixed, approximately equimolar ratio (Fig. 6). This finding provides evidence that the lower band contains heterodimers formed by Lhca1 and Lhca5. The in vitro interaction of Lhca5 with Lhca1 additionally supports the assignment of Lhca5 to PSI.

The formation of an Lhca1/Lhca5 dimer also seems to be feasible, as Lhca5 is the closest relative of Lhca4, the “normal” interaction partner of Lhca1, and possesses an amino acid sequence identity of 46%. Additionally, Lhca4 knock-out plants show an up-regulation of Lhca5, which might indicate that Lhca5 can replace Lhca4 under certain environmental condi-
tions (11). In contrast to this hypothesis, the amount of Lhca1 is strongly reduced in ΔLhca4 plants. Thus, Lhca4 may stabilize Lhca1 in vivo and cannot be completely replaced by Lhca5 (11). Data on Lhca2 and Lhca3 knock-out plants additionally show that the amount of Lhca5 correlates with the amount of Lhca2. This finding suggests that the binding of Lhca5 to PSI is stabilized by Lhca2 rather than by Lhca1, as the latter is present in normal amounts in both knock-out plants. In contradiction to an interaction of Lhca5 with Lhca2 or Lhca3 is the detection of Lhca5 in LHCI-730 but not in LHCI-680 (Lhca2 + Lhca3) in a mass spectrometric analysis (12), which substantiates our finding that Lhca5 interacts with Lhca1.

Consequence of an Additional Lhca Protein on PSI Composition—Despite the fact that there is good evidence now that Lhca5 is an LHCI protein present in various higher plants, the question remains as to how this additional LHCI fits into the PSI antenna—

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REFERENCES

1. Ben-Shem, A., Frolow, F., and Nelson, N. (2003) Nature 420, 630–635
2. Knoetzel, J., Svendsen, I., and Simpson, D. J. (1992) Eur. J. Biochem. 206, 209–215
3. Jansson, S., Andersen, B., and Scheller, H. V. (1996) Plant Physiol. 112, 409–420
4. Ganeteg, U., Strand, A., Gustafsson, P., and Jansson, S. (2001) Plant Physiol. 127, 150–158
5. Croce, R., Morosinotto, T., Castelletti, S., Breton, J., and Bassi, R. (2002) Biochim. Biophys. Acta 1556, 29–40
6. Schmid, V. H. R., Paulsen, H., and Ruppenrecht, J. (2002) Biochemistry 41, 9126–9131
7. Schmid, V. H. R., Cammarata, K. V., Bruns, B. U., and Schmitt, G. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7667–7672
8. Fichersky, E., Hoffen, N. F., Bernatovsky, R., Piechulla, B., Tankelsky, S. D., and Cashmore, A. R. (1987) Plant Mol. Biol. 9, 205–216
9. Schwartz, E., Shen, D., Aschersold, R., McGrath, J. M., Pichersky, E., and Green, B. R. (1991) FEBS Lett. 280, 229–234
10. Jansson, S. (1999) Trends Plant Sci. 4, 236–240
11. Ganeteg, U., Klimmek, F., and Jansson, S. (2004) Plant Mol. Biol. 54, 641–651
12. Storf, S., Stauber, E. J., Hippler, M., and Schmid, V. H. R. (2004) Biochemistry 43, 9214–9224
13. Stauber, E. J., Fink, A., Markert, C., Kruse, O., Johannsmeier, U., and Hippler, M. (2003) Eur. J. Biochem. 270, 978–984
14. Melkozernov, A. N., Lin, S., Schmitt, V. H. R., Lago-Places, P., Paulsen, H., and Blankenship, R. E. (2001) in Proceedings of the 12th International Congress on Photosynthesis, August 18–23, Brisbane, Australia, article S1-055 (on-line and CD-ISSN 064907116). CSIRO Publishing, Melbourne, Australia
15. Morosinotto, T., Castelletti, S., Breton, J., Bassi, R., and Croce, R. (2002) J. Biol. Chem. 277, 36253–36261
16. Paulsen, H. G., and Schmidt, G. W. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 146–150
17. Paulsen, H., Rümler, U., and Rügiger, W. (1990) Planta 181, 204–211
18. Guffra, E., Cugini, D., Croce, R., and Bassi, R. (1996) Eur. J. Biochem. 238, 112–120
19. Schmid, V. H. R., Potthast, S., Wiener, M., Bergauer, V., Paulsen, H., and Storf, S. (2002) J. Biol. Chem. 277, 37307–37314
20. Melkozernov, A. N., Schmid, V. H. R., Schmidt, G. W., and Blankenship, R. E. (1998) J. Phys. Chem. 102, 8183–8189
21. Wehner, A., Storf, S., Jahns, P., and Schmid, V. H. R. (2004) J. Biol. Chem. 279, 26823–26829
22. Dann, I., Steinmetz, D., and Grimme, L. H. (1999) in Current Research in Photosynthesis (Ballishefsky, K. M., ed) Vol. II, pp. 607–610, Kluwer Academic Publishers, Norwell, MA
23. Lee, A. I.-C., and Thornton, J. P. (1995) Plant Physiol. 107, 565–574
24. Castelletti, S., Morosinotto, T., Robert, B., Caffarri, S., Bassi, R., and Croce, R. (2003) Biochemistry 42, 4226–4234
25. Ros, F., Bassi, R., and Paulsen, H. (1998) Eur. J. Biochem. 253, 653–658
26. Pagano, A., Croce, G., and Bassi, R. (1998) J. Biol. Chem. 273, 17154–17165
27. Schmid, V. H. R., Thomé, P., Rühle, W., Paulsen, H., Kühlbrandt, W., and Rogl, H. (2001) FEBS Lett. 499, 27–31
28. Kühlbrandt, W., Wang, D. N., and Fujisuyo, Y. (1994) Nature 367, 614–621
29. Morosinotto, T., Breton, J., Bassi, R., and Croce, R. (2003) J. Biol. Chem. 278, 49223–49229
30. Paulsen, H., and Schmid, V. H. R. (2002) in Heme, Chlorophyll and Bilins: Methods and Protocols (Smith, A. G., and Witty, M., eds) pp. 253–255, Humana Press, Totowa, NJ
31. Schmid, V. H. R., and Schafer, C. (1994) Planta 192, 473–479
32. Porra, R. J., Thompson, W. A., and Kriedemann, P. E. (1989) Biochim. Biophys. Acta 975, 384–394
33. Martinsson, T. A., and Plumley, F. G. (1995) Anal. Biochem. 238, 123–130
34. Peter, G. F., and Thornber, J. P. (1991) J. Biol. Chem. 266, 16745–16754
35. Bassi, R., Pineau, B., Dainese, P., and Marquardt, J. (1993) Eur. J. Biochem. 214, 297–303
36. Ruban, A. V., Lee, P. J., Wentworth, M., Young, A. J., and Horton, P. (1999) J. Biol. Chem. 274, 10458–10465
37. Croce, R., Canino, G., Ros, F., and Bassi, R. (2002) Biochemistry 41, 7334–7343
38. Croce, R., Remelli, R., Varotto, C., Breton, J., and Bassi R. (1999) FEBS Lett. 456, 1–6
39. Croce, R., Weiss, S., and Bassi, R. (1999) J. Biol. Chem. 274, 29613–29623
40. Hohe, S., Niehuis, H., Bender, A., and Paulsen, H. (2000) Eur. J. Biochem. 267, 616–624
41. Janssen, S., Paulsen, H., and Hobe, S. (2001) J. Biol. Chem. 276, 22154–22159
42. Butler, P. J. G., and Kühlbrandt, W. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3797–3801
43. Bassi, R., and Dainese, P. (1992) Eur. J. Biochem. 204, 317–326
44. Jansson, S., Steffansson, H., Nyström, U., Gustafsson, P., and Albertsson, P. A. (1997) Biochim. Biophys. Acta 1320, 297–309
45. Bosmann, B., Knoetzel, J., and Jansson, S. (1997) Photosynth. Res. 52, 127–136
46. Bailey, S., Walters, R. G., Jansson, S., and Horton, P. (2001) Planta 213, 794–801
47. Allen, J. F., and Forsberg, J. (2001) Trends Plant Sci. 6, 317–326
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