Identification of N- and C-terminal Amino Acids of Lhca1 and Lhca4 Required for Formation of the Heterodimeric Peripheral Photosystem I Antenna LHCI-730[†]

Volkmar H. R. Schmid,* Harald Paulsen, and Jens Rupprecht

Institut für Allgemeine Botanik, Johannes Gutenberg-Universität, Müllerweg 6, 55099 Mainz, Germany

Received December 13, 2001; Revised Manuscript Received May 24, 2002

ABSTRACT: Apoproteins of higher plant light-harvesting complexes (LHC) share considerable amino acid sequence identity/similarity. Despite this fact, they occur in different oligomeric states (i.e., monomeric, dimeric, and trimeric). As a step toward understanding the underlying structure requirements for different oligomerization behavior, we analyzed whether amino acids at the N- and C-termini of Lhca1 and Lhca4 are involved in the formation of the heterodimeric LHCI-730. Using altered proteins produced by deletion or site-directed mutagenesis for reconstitution, we were able to identify amino acids required for the assembly of LHCI-730. At the N-terminus of Lhca1, W4 is involved in heterodimerization. This interaction probably depends on aromatic properties because only replacement of W4 by F resulted in dimer formation. Also, at the C-terminus of Lhca1, W seems to play a crucial role for interaction with Lhca4. A detailed analysis by point mutants revealed the importance of an aromatic residue at position 185. One or more other amino acid(s) located downstream of position 188 may exert additional stabilizing effects, presumably in a cooperative way. The scenario for Lhca4 is different. Dimerization broke down only after the deletion of the entire extrinsic N- or C-terminal region, demonstrating that the termini of Lhca4 are not involved in strong interactions with Lhca1 decisive for dimerization. At the N-terminus, dimerization was abolished after the removal of the same number of amino acids at which monomer formation failed. Site-specific mutagenesis of the amino acid decisive for LHC-formation in a deletion study demonstrated that its character is of no importance for dimerization and, therefore, that abolition of dimer formation may be the consequence of a loss in monomer formation. At the C-terminus of Lhca4, an even higher number of amino acids than required for monomer formation could be removed without the loss of dimerization. The decisive position is I168, located in the third transmembrane region. Because all point mutants of I168 in the full-length protein yielded dimers, failure of dimerization may be caused by either falling below a critical length of the polypeptide chain, resulting in the loss of too many weak interactions, or by too strong an impairment of Lhca4-folding. Interestingly, N- and C-terminal mutants of Lhca4 not able to form stable monomers formed stable dimers, indicating stabilization of labile monomeric complexes by the Lhca1 subunit in dimerization. Finally, the significance for dimer formation of amino acids in other parts of Lhca1 and Lhca4 which may be involved, besides the amino acids identified here in the specific assembly of the heterodimeric LHCI-730, is discussed. Their identification will result in a better understanding of structure characteristics determining the different oligomerization behavior of LHCs.

Transient as well as permanent protein associations are of exceptional importance for biological function. Beginning with the structure of hemoglobin by Perutz, a growing number of proteins was identified whose functional unit consists of two or more subunits. Among those, dimers and tetramers are especially frequent, and more complex organizational forms are often based on dimeric substructures (*1*). Interface properties of the proteins, such as size, shape, and complementarity, which are superimposed by charge and hydrophobicity properties are considered, besides other parameters, as important factors governing dimer formation (*2*−*4*).

Oligomeric protein complexes are also found in the thylakoid membrane of plant chloroplasts, the location of the photosynthetic apparatus. They exist on the level of multisubunit complexes; photosystem (PS)[1] II (*5*) and the cytochrome b_6f complex (*6*) occur at least predominantly as dimers and the cyanobacterial PSI in a trimeric state (*7*). On the other hand, oligomeric forms assembled by individual proteins are found among the light-harvesting complexes (LHC). These are composed of a protein moiety to which chlorophylls and carotenoids are ligated. Their function consists in the collection of solar radiation and transmission of the energy to the photochemical active reaction centers. According to their association with either PSII or PSI, they are called Lhca1-4 (PSI) or Lhcb1-6 (PSII). The 10 apoproteins share considerable amino acid sequence homology, which is 35% or more (*8*). As a consequence, it is assumed that all LHCs possess a similar structure as LHCIIb (composed of Lhcb1 through Lhcb3), whose structure was

[†] This project was supported by the Deutsche Forschungsmeinschaft (Schm 1203/2-1 and 2-3).

* Corresponding author. Phone: 0049-6131-3924203. Fax: 0049-6131-3923787. E-mail: vschmid@mail.uni-mainz.de.

[1] Abbreviations: Lhc, apoprotein of a light-harvesting complex; LHC, light-harvesting complex; LHCIIb, major light-harvesting complex of photosystem II comprised of protein subunits Lhcb1, Lhcb2, and Lhcb3; PS, photosystem; WT, wild-type (unchanged protein).

10.1021/bi016042x CCC: $22.00
Published on Web 06/25/2002

obtained at near atomic resolution (*9*). LHCIIb binds at least 12 chlorophyll molecules, has three transmembrane helices connected by extrinsic loop regions, and has N- and C-terminal extrinsic extensions. Against the background of the high sequence similarity, the different oligomerization behavior of the Lhc proteins is unexpected. While Lhcb4−Lhcb6 form only monomeric complexes, the Lhcb1−Lhcb3 form trimers (*10*−*12*). For the Lhc associated with PSI, it was demonstrated that Lhca1 and Lhca4 form a heterodimer called LHCI-730 (*13*−*15*). It was shown that dimerization of Lhca1 and Lhca4 is lipid-dependent (*16*) and very specific; neither Lhca1 nor Lhca4 give rise to dimers with Lhcb1 (*14*) or Lhca3 (Schmid et al., unpublished data). The situation with the two other proteins Lhca2 and Lhca3 remains equivocal. A homodimeric as well as a heterodimeric organization of these proteins has been suggested (*13, 17, 18*).

The extensive amino acid sequence conformity of the Lhc proteins is especially pronounced in the first and third transmembrane region. This may be indicative for an involvement of extrinsic protein portions at the N- and C-terminus, the loop regions, as well as the second transmembrane helix in the different oligomerization behavior. Indeed, for Lhcb1, being the major component of LHCIIb, it could be demonstrated that a stretch of six amino acids at the N-terminus including a W, Y, and R is necessary for trimerization (*19*). This result was obtained by use of deletion and points mutants for reconstitution experiments. Also, at the C-terminus, it was possible to identify a W required for assembly of the LHCIIb trimer (*20*). Detailed dimerization analyses were also performed with another light-harvesting complex, the core antenna LH1 of purple bacteria, which is made up of heterodimeric entities composed of an α and β subunit (*21, 22*). By use of point-mutated and chemically synthesized β subunit proteins for reconstitution experiments, the importance for dimerization could be demonstrated for several aromatic amino acids located either in the hydrophilic membrane interior or in the extrinsic region (*22, 23*). Deletion mutagenesis studies additionally demonstrated the involvement of N- (*24*) and C-terminal (*25*) extrinsic amino acids in subunit or LH1 formation.

Toward a better understanding regarding the structure elements responsible for different oligomerization behavior of higher plant LHCs, we analyzed the significance of the N- and C-terminal regions of Lhca1 and Lhca4 for formation of heterodimeric LHCI-730. To avoid misinterpretation of dimer-forming capability resulting from a failure in monomer formation, we previously identified the protein segments dispensable for monomer formation (*26*). Starting out from these results, we first identified, by deletion mutagenesis, protein segments at the N- and C-terminus of Lhca1 and Lhca4 not required for heterodimerization. Subsequently, we exchanged, in full-length proteins, the amino acids whose removal in the deletion series caused a failure in dimerization. Substitution by amino acids with different side-chain properties provided insight in the importance and required properties of the amino acid at this position for dimerization and additionally allowed a distinction between the effects caused by the polypeptide falling below a critical length and the requirement of the special amino acid.

EXPERIMENTAL PROCEDURES

Construction of Deletion and Point Mutants. Deletion and point mutants were generated using the pDS expression plasmids with *lhca1* and *lhca4*, as described in ref *26*. Construction of deletion mutants followed the procedure of Rupprecht et al. (*26*). Site-directed mutagenesis of *lhca1* and *lhca4* was performed by the PCR-based method described by Chen and Przybyla (*27*). Primers were designed in a way which allowed nucleotide substitution(s) for the desired amino acid and additionally insertion or erasure of a unique restriction site, which facilitated identification of positive clones. All clones used for protein expression were checked by DNA sequencing (GENterprise, Mainz, Germany) to ensure precise PCR-mediated DNA amplification.

Deletion mutants are termed by the number of missing amino acids as compared to the mature tomato apoproteins and a N or C for N- and C-terminal mutants. The name of the point mutants is based on the original amino acid, its location in the apoprotein, and the new amino acid.

Production of Materials Used for Reconstitution. Overexpression of recombinant proteins in *Escherichia coli* strain JM 101, isolation of inclusion body protein, and isolation of total pigment extract from tomato thylakoids was as described recently (*28*). Examination of inclusion body preparations by SDS−PAGE demonstrated that deletion or site-specific mutagenesis did not affect protein overexpression adversely, resulting in strongly accumulated Lhca1 and Lhca4 proteins.

Reconstitution, Examination of Dimerization, and Determination of Dimerization Efficiency. Reconstitution of monomeric LHCIs from overexpressed apoproteins and total pigment extract was as in ref *26*. Dimer reconstitutions were performed accordingly with the exception that reconstitutions with total pigment extract equivalent to 30 μg of chlorophyll were carried out with equal amounts of both proteins (12.5 μg of each). The ability for dimer formation was checked by partially denaturing gel electrophoresis (*29*) of the reconstitution mixtures. Following electrophoresis, the gels were scanned with an Epson GT-7000 flat bed scanner. The intensity of the dimer bands was then determined with the AIDA 1000/1D Image Analyzer software, version 1.02 (Raytest Isotopenmessgeräte, Straubenhardt, Germany). The intensity of the wild-type (WT) band of each gel was set to 100%, and the intensity of the mutant band is given as percentage thereof.

RESULTS

In previous experiments, monomeric Lhca1 and Lhca4 separated in sucrose density gradients containing dodecylmaltoside did not associate to form dimeric LHCI-730 (*14*). We concluded that these two proteins have to fold simultaneously to give rise to LHCI-730. However, in the course of the present work, we observed that mixing the reconstitution solutions for monomeric Lhca1 and Lhca4 prior to density gradient centrifugation or lithium dodecylsulfate−PAGE separation results in the formation of dimeric LHCI-730 indistinguishable from that described in Schmid et al. (*14*; not shown). Thus, it can be assumed that already folded Lhca1 and Lhca4 subunits can interact to form LHCI-730.

Identification of Amino Acids at the N-Terminus of Lhca1 and Lhca4 Involved in Dimerization. To analyze the

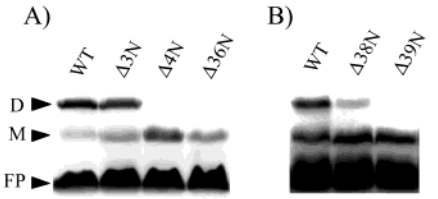

FIGURE 1: Influence of N-terminally shortened proteins on formation of dimeric LHCI-730 as revealed by partially denaturing gel electrophoresis. (A) Wild-type (WT) and given N-terminally shortened Lhca1 were reconstituted together with wild-type Lhca4. (B) Indicated N-terminally truncated Lhca4 and Lhca4-WT were reconstituted with the wild-type of Lhca1. D, dimer; M, monomer; FP, free pigment.

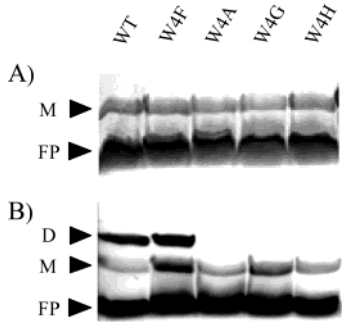

FIGURE 2: Effect of point mutations in the full-length Lhca1 of the last amino acid required for dimer formation as determined by N-terminal deletion mutants. Lhca1 wild-type (WT) or indicated Lhca1 point mutants were reconstituted either alone for analysis of monomer formation (A) or together with Lhca4 wild-type for examination of dimer forming capability (B). Shown are partially denaturing gels after separation of the respective reconstitution mixtures. D, dimer; M, monomer; FP, free pigment.

possible involvement of the N-terminus of Lhca1 in dimerization, we reconstituted the last deletion mutant of Lhca1 that still forms monomers (Δ36N; *26*) with the unchanged Lhca4 wild-type protein. As is obvious from Figure 1A, this abolished dimerization. Stepwise shortening of the N-terminus (Lhca1Δ34N, Lhca1Δ10N, Lhca1Δ5N, Lhca1Δ4N) shows that only three amino acids (Lhca1Δ3N) can be deleted without the loss of dimer formation. Thus, W at position 4 is of special importance for the connection of the two protein subunits. Dimerization efficiency of Lhca1Δ3N is only slightly reduced in comparison with the Lhca1-WT (Figure 1A).

Next, the participation of the Lhca4 N-terminus was analyzed. Therefore, the last N-terminal deletion mutant of Lhca4 achieving monomer formation (Lhca4Δ38N) was reconstituted together with the Lhca1-WT. In this case, dimers were still obtained, although the dimer yield declined substantially (Figure 1B). Use of further shortened Lhca4 demonstrated that the removal of the following amino acid in Lhca4Δ39N already resulted in the complete absence of a dimer band.

To gain insight into the mode of interaction which may come from the W in Lhca1 and Lhca4, we exchanged these amino acids in the full-length proteins against amino acids of varying hydrophobicity and shape. Because of the premise that proteins used for investigation of dimerization have to be able to form monomers, we first checked the monomer formation capability of the produced point-mutated proteins. As is shown in Figure 2A, replacement of W4 by the indicated amino acids did not impair monomer formation.

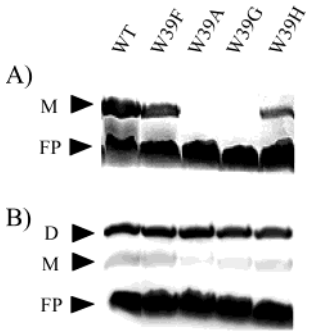

FIGURE 3: Significance of W39 in full-length Lhca4 for LHC reconstitution. Lhca4 wild-type (WT) or given Lhca4-point mutants were reconstituted to test monomer formation (A). In B, the same Lhca4-proteins were combined with the Lhca1-WT in the reconstitution mixtures to analyze dimer formation. The band patterns obtained from reconstitution mixtures following partially denaturing gel electrophoresis are presented. D, dimer; M, monomer; FP, free pigment.

Table 1: Dimer Formation of C-terminal Deletion Mutants of Lhca1 with Lhca4 Wild-Type (WT)[a]

protein	dimerization	dimerization frequency [%]	number of experiments	dimerization efficiency [%]
Lhca1-WT	+	100	10	100
Lhca1Δ13C	+	100	8	21 ± 6
Lhca1Δ14C	+	78	9	18 ± 9
Lhca1Δ15C	+	89	9	25 ± 17
Lhca1Δ16C	+	70	10	15 ± 10
Lhca1Δ17C	−	0	6	0

[a] For the last four dimerizing deletion mutants, the dimerization frequency and the dimerization efficiency as determined by partially denaturing gel electrophoresis are given. For dimerization efficiency values, only the samples that dimerized were considered. Data are means ± SD from the given numbers of experiments.

The following dimerization experiments revealed that only F with its hydrophobic property effected heterodimerization (Figure 2B). By contrast, amino acids with aliphatic residues (A, G) or less hydrophobic character (H) disturbed dimer formation.

Subsequently, the same experiments were performed with the point mutants of W39 of Lhca4. In this case, however, not all mutants were able to form monomeric complexes (Figure 3A). Only mutants with W substituted by F and H gave monomers, whereas exchanges against A or G abolished monomer formation. Nevertheless, we examined all exchange mutants of W39 with regard to dimerization. As depicted in Figure 3B, all mutants dimerized to a comparable extent, indicating that the amino acid at position 39 is not decisive for dimer formation. Besides, this result reveals that proteins not able to form stable monomers can potentially still achieve dimer formation, possibly because they are stabilized by the other protein.

Amino Acid Requirements for Dimerization at the C-Terminus of Lhca1 and Lhca4. For identification of amino acids involved in interactions at the C-terminus, we started again with the last deletion mutants still allowing monomer formation (Lhca1Δ20C, Lhca4Δ12C; *26*). Using Lhca1Δ20C together with Lhca4-WT, no formation of dimers was observed. Therefore, less-truncated proteins were produced and used for reconstitution experiments. The first deletion mutant giving rise to a dimer was Lhca1Δ16C (Table 1). However, the dimer yield was very low, reflecting formation

Table 2: Quantitative Analysis of Dimer Formation of C-terminal Deletion Mutants of Lhca4 with Lhca1 Wild-Type[a]

protein	dimerization	dimerization frequency [%]	number of experiments	dimerization efficiency [%]
Lhca4-WT	+	100	14	100
Lhca4Δ26C	+	100	4	64 ± 24
Lhca4Δ28C	+	100	5	75 ± 25
Lhca4Δ29C	+	100	7	68 ± 29
Lhca4Δ32C	+	71	14	34 ± 23
Lhca4Δ33C	−	0	9	0

[a] The dimerization frequency and the dimerization efficiency determined by partially denaturing gel electrophoresis are shown for the last four dimerizing deletion mutants. Only samples that dimerized were included in the dimerization efficiency values. Data are means ± SD from the given numbers of experiments. WT, wild-type.

of a rather unstable dimer, which resulted in a band intensity of only 15% as compared to the band obtained with the Lhca1-WT. Probably as a consequence of this instability, a dimer band was not always detectable with Lhca1Δ16C. Also, deletion of up to 14 amino acids at the C-terminus impaired dimer formation significantly, and the first mutant which formed dimers throughout was Lhca1Δ13C (Table 1). Because Lhca1Δ16C is the first mutant which allowed dimerization in the majority of the experiments, we consider W185 as the amino acid decisive for interaction.

Removal of 12 amino acids (Lhca4Δ12C) at the C-terminus of Lhca4 results not only in proper formation of monomeric but also of dimeric complexes. Consequently, further amino acids were removed. It was interesting that dimerization could be achieved when even more amino acids were missing than for formation of stable monomers (Table 2). As for the C-terminus of Lhca1, there was a gradual decrease in the yield of dimers when consecutively shortened proteins were used. Deletion of up to 32 amino acids (Lhca4Δ32C) resulted in a dimer band in 71% of the experiments. Removal of an additional amino acid (Lhca4Δ33C) abolished dimer assembly completely (Table 2), pointing to the amino acid at position 168 as possibly important for dimer formation. The intensity of the dimer band of Lhca4Δ32C was 34% and the intensity of the dimer band of the last monomer forming mutant Lhca4Δ12C was 91% in comparison to the wild-type, indicating a stabilizing effect by the amino acids located in between.

To clarify whether the loss of dimerization following C-terminal deletions is caused by falling below a critical length of the polypeptide or rather by the removal of the property of the first amino acid indispensable for dimer formation, point mutations at the relevant positions were introduced in the full-length proteins. W185 of Lhca1 was exchanged against the same amino acids as used for point mutations at the N-terminus. The point mutations did not have a significant effect on monomer formation (Figure 4A). By contrast, dimerization was differentially affected by these mutations. Reconstitutions of Lhca4-WT with Lhca1-W185F resulted in a dimer band almost as strong as with the Lhca1-WT (Figure 4B). Substitutions of W185 by A, G, and H resulted in a decline of dimer yield, revealing the importance of an aromatic amino acid at this position.

At the C-terminus of Lhca4, I168 was exchanged against A, F, H, L, and W. All these mutations did not disturb monomer formation (Figure 5A). Also, dimerization occurred in all instances. Dimerization for all mutants is comparable to the WT as is obvious from the comparable dimer yield (Figure 5B).

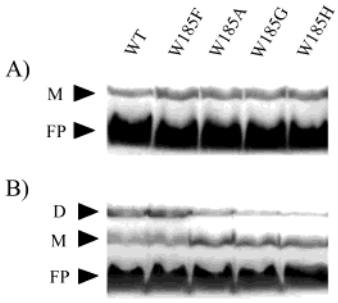

FIGURE 4: Analysis of the significance of W185 in full-length Lhca1 for reconstitution of monomeric Lhca1 and dimeric LHCI-730. For examination of monomer formation given, Lhca1 proteins were reconstituted alone (A). In B, dimer formation of these Lhca1 mutants was analyzed by reconstitutions with Lhca4 wild-type (WT). Samples were separated by partially denaturing gel electrophoresis, and the resulting band patterns are depicted. D, dimer; M, monomer; FP, free pigment.

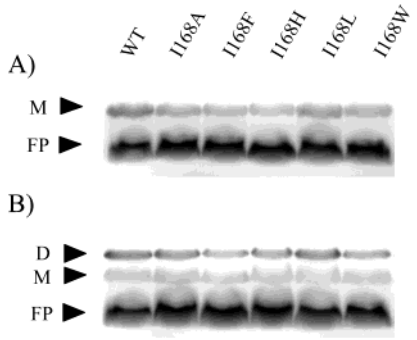

FIGURE 5: Gel electrophoretic analyses of the effect of I168 point mutations in full-length Lhca4 on monomer and dimer formation. Labeled point mutants were reconstituted alone to check monomer formation (A). Reconstitutions of these mutants together with Lhca1 wild-type (WT) were performed to test dimer formation (B). Reconstitution mixtures were subjected to partially denaturing gel electrophoresis, and the resulting band patterns are shown. D, dimer; M, monomer; FP, free pigment.

DISCUSSION

The structural prerequisites underlying different oligomerization behaviors of higher plant LHCs are largely unknown. Therefore, we analyzed whether amino acids at the N- and C-terminus of Lhca1 and Lhca4 participate in heterodimerization of LHCI-730. By removal of protein segments at the termini and subsequent point mutations of the first nonremovable amino acid in the full-length proteins, it was possible to gain insight into amino acids involved in and properties required for the formation of recombinant LHCI-730. Former experiments regarding monomer formation of N- and C-terminally truncated Lhca1 and Lhca4 (*26*) allowed for the effects based on the instability of monomeric complexes and the effects on the interaction of the subunits to be distinguished. Interestingly, at the N- and C-terminus of Lhca1, tryptophan residues were identified as essential for dimerization by deletion mutagenesis. W and other aromatic amino acids are frequently found in protein−protein interfaces (*30*) and can represent a "hot spot" within interacting amino acid patches (*31*, *32*). An explanation for this phenomenon might be the large contribution of W to association constants, which was found repeatedly (e.g., refs *2* and *23*).

N-Terminus of Lhca1. Deletion of the three N-terminal amino acids resulting in a slightly reduced dimer yield and site-specific mutagenesis of W4 of Lhca1 demonstrate the involvement of W4 and the upstream located amino acids in LHCI-730 heterodimerization. Whether this interaction with Lhca4 is direct or indirect cannot be decided yet. The latter could be the consequence of a local conformational change which, in turn, results in a special alignment of the N-terminus facilitating the interaction with Lhca4. Anyway, the interaction of W4 appears to depend on its aromatic side chain because only substitution by the aromatic F allows for dimer formation. Because of the capability of W to form hydrophobic interactions and hydrogen bonds simultaneously (*33*), the interaction may be caused by hydrophobic interaction. Interestingly, a W is conserved at corresponding positions in all dimer-forming Lhc of PSI but not in those associated with PSII (*8*) which form monomers or trimers with the exception of Lhcb4, which is a monomer in vivo (*34*) but shows a tendency to oligomerize in vitro (*35*). This may indicate that W at this position is generally important for dimerization of LHCI but that specific heterodimerization by Lhca1 and Lhca4 depends on additional specific interactions. In Lhcb1, too, a W at the N-terminus was identified as an important component of a hexapeptide involved in trimerization (*19*). Therefore, W may generally play a role in the formation or stabilization of oligomeric LHC of higher plants at the N-terminus.

Monomer yields of Lhca4-WT and Lhca4Δ38N are comparable (*26*). On the other hand, reconstitution of Lhca1-WT with Lhca4Δ38N results in a significantly reduced dimer yield as compared to experiments with both wild-type proteins. This suggests that amino acids in the N-terminal extrinsic region of Lhca4 undergo dimer stabilizing interactions with Lhca1. Because, in accord with the structure suggestion of Green and Pichersky (*36*), these 38 amino acids comprise the entire hydrophilic N-terminal domain of Lhca4, additional interaction(s) of the Lhca1 N-terminus with amino acids in the stromal loop of Lhca4 can be postulated. The involvement of amino acids of loop regions was also suggested for the lattice formation of bacteriorhodopsin (*37*), and for Lhcb1 an unexpected significance of single amino acids in the stromal loop for assembly of stable monomeric LHCIIb was observed (*38*).

N-Terminus of Lhca4. The abolishment of dimer formation in reconstitutions with Lhca4Δ39N is apparently caused by the instability of monomeric complexes because failure of dimer and Lhca4 monomer formation occur upon the removal of the same number of N-terminal amino acids. Membrane proteins generally possess a high content of W, which is especially accumulated in the region of membrane surfaces (*33*, *39*) leading to the conclusion that it anchors the protein to the lipid headgroup (*33*). When a comparable structure for LHCIIb (*9*) and Lhca4 is assumed, W39 would be located at the surface of the membrane. However, because all point mutants of W39 in full-length Lhca4 achieved dimer formation, such an effect is improbable to occur in Lhca4 or is at least superimposed by other stabilizing effects. Because all point mutants of W39 dimerized to a comparable extent, the property of the residue at this position seems to be of no importance regarding LHCI-730 formation, supporting the assumption that dimerization failure results from abolished monomer formation. Interestingly, also the mutants W39A and W39G formed dimers to the same extent as the other mutants, although they did not yield monomers following partially denaturing electrophoresis. This indicates that monomers not stable enough to withstand electrophoretic separation can still be stabilized by structure components of the other interaction partner.

C-Terminus of Lhca1. Also at the C-terminus of Lhca1 a W is crucial for LHCI-730 formation as judged from deletion mutagenesis. In Lhcb1, C-terminal shortening resulted in a failure of monomer formation when the 11th amino acid (W) was removed (*40*). Detailed trimerization analysis of full-length Lhcb1 with point mutations of this W demonstrated its involvement in oligomerization (*20*). Only exchange against F resulted in trimerization, whereas A, G, or H at this position did not. Thus, the situation at the C-terminus of Lhcb1 is comparable to that of W4 at the N-terminus of Lhca1. At the C-terminus of Lhca1, we observed a somewhat different pattern. F readily formed dimers as opposed to A, G, and H exhibiting weaker dimer bands. Because of the influence of this single-point mutation on dimerization efficiency, it appears that the loss of dimer formation coinciding with the removal of W in Lhca1Δ17C results from falling below a critical length of the polypeptide chain which is accompanied by the loss of a strong, quite specific interaction. This interaction depends largely on the aromatic property of the introduced amino acid. Therefore, the interaction of this amino acid with Lhca4 may be the consequence of hydrogen bonding or hydrophobic interaction. When a direct interaction of W185 with Lhca1 is assumed, the site of interaction is probably located in the lumenal loop region connecting helices 1 and 2 because dimerization still occurred when the entire extrinsic C-terminal region of Lhca4 was removed. Because of the significant impairment of dimer formation by the deletion mutants Lhca1Δ14C−Δ16C, W185 could represent a "hot spot" within a patch of amino acids involved in dimer formation/stabilization. Such hot spots in protein interfaces are frequently formed by aromatic residues including W (*31*, *32*). Also, in the β subunit of the LH1 of *Rhodobacter sphaeroides*, aromatic residues at the C-terminus are very important for heterodimerization (*23*). These are located either close to the surface of the membrane or in the hydrophilic portion of the membrane and probably stabilize the complex by hydrogen bonds to bacteriochlorophyll. At the C-terminus of Lhca1, additional dimer-stabilizing effects arise from amino acid(s) located downstream N188. This is evident from the strongly reduced dimer yield obtained with Lhca4Δ13C (Table 1). A good candidate for such a cooperatively acting amino acid might be F199 due to its aromatic properties.

C-Terminus of Lhca4. Shortening of the C-terminus of Lhca4 by 33 amino acids resulted in the complete collapse of dimer formation. Thus, the removal of I168 is crucial, which is the 11th amino acid in the third transmembrane helix counted from the C-terminus, according to the structure proposed by Green and Pichersky (*36*). Interestingly, dimer formation occurred with even more extensively truncated Lhca4 than required for the formation of stable monomers, reflecting a stabilization effect by the other subunit as was also observed for N-terminal point mutants of Lhca4. The significance of amino acids in transmembrane regions for oligomerization was reported recently for LHCIIb (*41*). Lhcb1 did not trimerize upon exchanges of H68 in the first and Q131 in the second helices. However, point mutations

of I168 in full-length Lhca4 demonstrated that I168 is not decisive for dimerization because no effect was observed for the introduced amino acids. Because of the lacking impact of point mutations of I168 in the full-length protein, failure of dimerization with the deletion mutant could be the consequence of a too extensive shortening of the protein. As a consequence, the loss of several weak interactions between the two subunits may occur, resulting in the observed gradual decrease of dimer yield following a more extensive shortening of the protein. However, also a strong impairment of Lhca4 folding or a too strong destabilization of the resultant monomer could be the reason for the disruption of dimerization. Interestingly, strong reduction in dimer yield and failure in dimerization occurred in the mutants (Δ32C, Δ33C) in which the putative L1 lutein binding site (*8*) is removed. Because of this coincidence, it is conceivable that the loss of this binding site could be decisive for failure in the assembly of a labile monomer and that way causes the abolition of dimer formation.

Outlook. Because interface areas of monomers in a dimer are usually in the range between 6.5% and 29% of the surface area (*1*), additional contact sites besides those identified in this study may participate in the formation of LHCI-730. Because the amino acid sequence in the second helix deviates most strongly among the Lhc proteins (*8*), it appears to be a promising target for a more detailed analysis regarding specific interactions. As a first step, it will be interesting to substitute the second helix of Lhca1 or Lhca4 by the corresponding helix of Lhca2 or Lhca3. A dimerization motif as GxxxG in the homodimeric membrane protein glycophorin A (*42*) is not present in helical regions of Lhca1 or Lhca4 but only in Lhcb1-3 and Lhca2. However, the second helix of Lhca1 has a similar AxxxH motif which is also found in the α and *β* polypeptides of LH1 and LH2 of purple bacteria (*21*, *23*). The presence of a similar motif in Lhca4 (HxxxI) in the opposite direction and the identification of I and A as contact points between helices in bacteriorhodopsin (*37*) makes them a particularly interesting target for mutational analyses. A detailed analysis of these areas and amino acids will provide further insight in the structure prerequisites for specific heterodimerization and will pave the way for understanding the structure basis for different oligomerization behaviors of LHCs.

ACKNOWLEDGMENT

We thank Dominik Corbet for his help with some of the experiments and Dr. Stephan Hobe for critically reading the manuscript.

REFERENCES

1. Jones, S., and Thornton, J. M. (1995) *Prog. Biophys. Mol. Biol. 63*, 31−65.
2. Stites, W. E. (1997) *Chem. Rev. 97*, 1233−1250.
3. Popot, J.-L., and Engelman, D. M. (2000) *Annu. Rev. Biochem. 69*, 881−922.
4. Valdar, W. S. J., and Thornton, J. M. (2001) *Proteins 42*, 108−124.
5. Santini, C., Tidu, V., Tognon, G., Ghiretti Magaldi, A., and Bassi, R. (1994) *Eur. J. Biochem. 221*, 307−315.
6. Breyton, C., Tribet, C., Olive, J., Dubacq, J.-P., and Popot, J.-L. (1997) *J. Biol. Chem. 272*, 21892−21900.
7. Kruip, J., Boekema, E. J., Bald, D., and Rögner, M. (1994) *Photosynth. Res. 40*, 279−286.
8. Pichersky, E., and Jansson, S. (1996) in *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R., and Yocum, C. F., Eds.), pp 507−521, Kluwer Academic Publishers, Dordrecht, The Netherlands.
9. Kühlbrandt, W., Wang, D. N., and Fujiyoshi, Y. (1994) *Nature 367*, 614−621.
10. Peter, G. F., and Thornber J. P. (1991) *J. Biol. Chem. 266*, 16745−16754.
11. Bassi, R., and Dainese, P. (1992) *Eur. J. Biochem. 204*, 317−326.
12. Jackowski, G., Kacprzak, K., and Jansson, S. (2001) *Biochim. Biophys. Acta 1504*, 340−345.
13. Jansson, S., Andersen, B., and Scheller, H. V. (1996) *Plant Physiol. 112*, 409−420.
14. Schmid, V. H. R., Cammarata, K. V., Bruns, B. U., and Schmidt, G. W. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 7667−7672.
15. Knoetzel, J., Bossmann, B., and Grimme, L. H. (1998) *FEBS Lett. 436*, 339−342.
16. Schmid, V. H. R., Beutelmann, P., Schmidt, G. W., and Paulsen, H. (1998) in *Photosynthesis: Mechanisms and Effects* (Garab, G., Ed.) Vol. I, pp 425−428, Kluwer Academic Publishers, Dordrecht, The Netherlands.
17. Croce, R., and Bassi, R. (1998) in *Photosynthesis: Mechanisms and Effects* (Garab, G., Ed.) Vol. I, pp 421−424, Kluwer Academic Publishers, Dordrecht, The Netherlands.
18. Ganeteg, U., Strand, A., Gustafsson, P., and Jansson, S. (2001) *Plant Physiol. 127*, 150−158.
19. Hobe, S., Förster, R., Klingler, J., and Paulsen, H. (1995) *Biochemistry 34*, 10224−10228.
20. Kuttkat, A., Hartmann, A., Hobe, S., and Paulsen, H. (1996) *Eur. J. Biochem. 242*, 288−292.
21. Zuber, H., and Cogdell R. J. (1995) in *Anoxygenic photosynthetic bacteria* (Blankenship, R. E., Madigan, M. T., and Bauer, C. E., Eds.), pp 315−348, Kluwer Academic Publishers, Dordrecht, The Netherlands.
22. Davis, C. M., Bustamante, P. L., Todd, J. B., Parkes-Loach, P. S., McGlynn, P., Olsen, J. D., McMaster, L., Hunter, C. N., and Loach, P. A. (1997) *Biochemistry 36*, 3671−3679.
23. Kehoe, J. M., Meadow, K. A, Parkes-Loach, P. S., and Loach, P. A. (1998) *Biochemistry 37*, 3418−3428.
24. Richter, P., Brand, M., and Drews, G. (1992) *J. Bacteriol. 174*, 3030−3041.
25. McGlynn, P., Westerhuis, W. H. J., Jones, M. R., and Hunter, C. N. (1996) *J. Biol. Chem. 271*, 3285−3292.
26. Rupprecht, J., Paulsen, H., and Schmid, V. H. R. (2000) *Photosynth. Res. 63*, 217−224.
27. Chen, B., and Przybyla, A. E. (1994) *BioTechniques 17*, 657−659.
28. Paulsen, H., and Schmid, V. H. R. (2002) in *Heme, Chlorophyll and Bilins: Methods and Protocols* (Smith, A. G., and Witty, M., Eds.), pp 235−253, Humana Press, Totowa, NJ.
29. Schmid, V. H. R., and Schäfer, C. (1994) *Planta 192*, 473−479.
30. Jones, S., and Thornton, J. M. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 13−20.
31. Bogan, A. A., and Thorn, K. S. (1998) *J. Mol. Biol. 280*, 1−9.
32. Sengchanthalangsy, L. L., Datta, S., Huang, D. B., Anderson, E., Braswell, E. H., and Ghosh, G. (1999) *J. Mol. Biol. 289*, 1029−1040.
33. Schiffer, M., Chang, C.-H., and Stevens, F. J. (1992) *Protein Eng. 5*, 213−214.
34. Barber, J., and Kühlbrandt, W. (1999) *Curr. Opin. Struct. Biol. 9*, 469−475.
35. Giuffra, E., Cugini, D., Croce, R., and Bassi, R. (1996) *Eur. J. Biochem. 238*, 112−120.
36. Green, B. R., and Pichersky, E. (1994) *Photosynth. Res. 39*, 149−162.
37. Krebs, M. P., and Isenbarger, T. A. (2000) *Biochim. Biophys. Acta 1460*, 15−26.
38. Heinemann, B., and Paulsen, H. (1999) *Biochemistry 38*, 14088−14093.
39. Yau, W.-M., Wimley, W. C., Gawrisch, K., and White, S. H. (1998) *Biochemistry 37*, 14713−14718.
40. Paulsen, H., and Kuttkat, A. (1993) *Photochem. Photobiol. 57*, 139−142.
41. Yang, C., Kosemund, K., Cornet, C., and Paulsen, H. (1999) *Biochemistry 38*, 16205−16213.
42. Russ, W. P., and Engelman, D. M. (2000) *J. Mol. Biol. 296*, 911−919.

BI016042X