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Lhca5 interaction with plant photosystem I

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Abstract In the outer antenna (LHCI) of higher plant photosystem I (PSI) four abundantly expressed light-harvesting protein of photosystem I (Lhca)-type proteins are organized in two heterodimeric domains (Lhca1/Lhca4 and Lhca2/Lhca3). Our cross-linking studies on PSI-LHCI preparations from wild-type *Arabidopsis* and pea plants indicate an exclusive interaction of the rarely expressed Lhca5 light-harvesting protein with LHCI in the Lhca2/Lhca3-site. In PSI particles with an altered LHCI composition Lhca5 assembles in the Lhca1/Lhca4 site, partly as a homodimer. This flexibility indicates a binding-competitive model for the LHCI assembly in plants regulated by molecular interactions of the Lhca proteins with the PSI core.

Keywords: Lhca5; Light-harvesting complex I; Photosystem I; Cross-linking

1. Introduction

The core of higher plant photosystem I (PSI) consists of at least 15 subunits (PsaA–L and PsaN–P) [1,2] and provides two interactions sites for peripherally associated chlorophyll (chl) *a*/*b*-binding (*cab*) proteins. These light-harvesting antenna proteins (LHC proteins) are encoded by nuclear genes of the LHC multi-gene family coding for proteins that contain one to four trans-membrane helices and share a number of conserved chlorophyll- and xanthophyll-binding motifs [3,4]. In higher plants 14 different types of three-helix LHC proteins (Lhca1–Lhca6 and Lhcb1–Lhcb8) are expressed [5]. Four of the Lhca-type proteins (Lhca1–Lhca4) assemble at subtype-specific positions with one copy each as an external antenna (LHCI) with the PSI core on the PsaF-site of the complex [6]. The depletion of one protein-type affects the association of the others [7,8] and this interdependence was shown to be strongest between Lhca1/a4 and Lhca2/a3. While heterodimers of Lhca1/a4 have been isolated from native PSI-LHCI preparations [9,10] and reconstituted in vitro [11] this has not been achieved for Lhca2/a3, yet. Opposite to the LHCI-binding site the subunits Psa-H, -L, and -O provide a contact site for mobile trimers of light-harvesting complex II (LHCII) consisting of Lhcb1 and Lhcb2 [12].

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Abbreviations: Lhca, light-harvesting protein of photosystem I; ΔLhca, depleted in Lhca protein; LHCI, light-harvesting complex I; PSI, photosystem I; wt, wildtype; chl, chlorophyll

In plants Lhca1–Lhca4 are abundantly expressed while Lhca5 and Lhca6 are present at much lower levels [5] under controlled standard laboratory growing conditions (i.e. 18–23 °C temperature, 150–200 μE light intensity, controlled humidity, sufficient water and nutrient supply). Lhca6 has not been localized so far but for Lhca5 an association with PSI-LHCI has been demonstrated in *Arabidopsis* and tomato [13,14]. Studies of Lhca5 reconstituted in vitro have revealed a pigment-binding pattern typical for Lhca proteins and indicated heterodimer formation with Lhca1 [15]. Comparisons of *Arabidopsis* lines lacking Lhca5 with the wildtype (wt) indicated that Lhca5 is present only in substoichiometrical amounts and might not play a major role for higher plant light-harvesting, at least under standard conditions [13]. However, increased amounts of Lhca5 in PSI particles from plants depleted in Lhca1 and Lhca4 indicated a binding flexibility dissimilar to those of the abundantly expressed Lhca1 to Lhca4 proteins as these never were found in elevated amounts compared to wt [8]. Here, we have used chemical cross-linking of PSI-LHCI particles prepared from wt and ΔLhca lines to characterize the molecular binding pattern of Lhca5 with the aim to pinpoint its localization.

2. Material and methods

PSI-LHCI particles were isolated from wt (ecotype colombia) and Lhca-deficient (Δa1–Δa5) lines of *Arabidopsis thaliana* as described in [8]. PSI-LHCI from pea was isolated as described for tomato in [11]. Particles were diluted in 20 mM Tricine–NaOH, pH 7.5, to final concentrations of 0.2–0.4 μg Chl per μl. Chemical cross-linking of primary amines at distances of 12 Å was performed for 30 min at room temperature after adding 1:10 volumes of dithiobis[succinimidylpropionate] (DSP; Pierce, Rockford) in dimethyl-sulfoxide (100% v/v) with final concentrations of DSP of 0.25–5 mM. Cross-linking was terminated by adding 1:11 volumes of 0.5 M Tris–HCl, pH 7.5, and incubation for 15 min at room temperature. After adding 1 volume of non-reducing sample buffer (50 mM Na_2CO_3, 15% v/v sucrose, 2.5% v/v SDS) assays were frozen at −20 °C.

Cross-linked proteins were separated with SDS–PAGE containing 16% or 21% polyacrylamide in the resolving gel followed by Coomassie-staining or transfer of proteins to nitrocellulose filters (Nitropure, 22 micron; GE Osmonics, Minnetonka). Immunoblot detection using mono-specific primary antibodies against Lhca1–Lhca5 (Agrisera, Vännäs) was performed according to [7].

3. Results and discussion

After separation with SDS–PAGE the amount of Lhca proteins resolved between 20 and 25 kDa is reduced in the cross-linked sample (0.5 mM DSP) and additional protein bands

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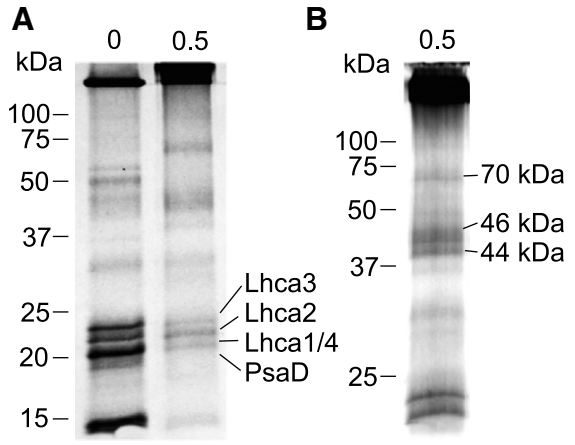

Fig. 1. PSI-LHCI (wt, 5 μg chl/lane) cross-linked with 0 (control) and 0.5 mM DSP separated on SDS–PAGE with (A) 16% and (B) 21% acrylamide in the resolving gel followed by Coomassie-staining.

can be detected between 37 and 75 kDa compared to the control (Fig. 1A). Separation in a 21% SDS gel resolves three major abundant bands containing cross-linking products with masses of about 44, 46, and 70 kDa, respectively (Fig. 1B). Immunoblotting identifies three bands containing Lhca5 (Fig. 2A) in the wt: the monomeric Lhca5 at ~22 kDa and cross-linking products of ~46 and ~70 kDa and these products are also identified in cross-linked PSI particles prepared from pea (Fig. 2B). The Lhca5 antibody reacts strongest with the product at ~70 kDa but this signal is also present in a cross-linked PSI preparation lacking Lhca5 (Δa5; Fig. 2A). Therefore, the ~70 kDa band cannot represent a genuine Lhca5 cross-linking product and we conclude that the band of ~46 kDa represents the only genuine Lhca5-specific cross-linking product in PSI-LHCI from *Arabidopsis* and pea. The artificial cross-reactivity of the antibody might be explained by de novo generation of intra-molecular epitopes induced by the cross-linker.

The size of the genuine Lhca5 cross-linking product of ~46 kDa already indicated that it presumably had been cross-linked to one of the other Lhca proteins. Lhca1–Lhca4 are the only proteins of matching weight present in the preparation and their amounts are clearly reduced in the cross-linked sample (Fig. 1A). To identify possible cross-linked partners of Lhca5 within the band of ~46 kDa we separated cross-linked PSI-LHCI particles from wt *Arabidopsis* using gels with broad slots spanning 80% of the width of the gel. This subsequently allowed parallel probing of the blots with antibodies against all Lhca proteins using the MiniPROTEAN II multiscreen apparatus (Biorad, Hercules). As the Lhca proteins differ in apparent molecular masses (Fig. 1A) cross-linking products containing different combinations of Lhca proteins can be distinguished accordingly. If two antibodies specific to different Lhca proteins do not give a clear reaction to a product of the same size it is indicative that those proteins do not form a cross-linking product. From this analysis (Fig. 3) it becomes clear that the antibodies specific for Lhca1, Lhca4, and Lhca5 do not recognize a product of the same size. In fact, antibodies against Lhca1 and Lhca4 both react with a cross-linking product of slightly lower molecular weight (B, Fig. 3) than the band recognized by antibodies against Lhca2, Lhca3 and Lhca5 (A, Fig. 3). This band contains cross-linked dimers of Lhca2/a3 but also Lhca5 cross-linked either to Lhca2, Lhca3, or another Lhca5. We favour a Lhca2-Lhca5 dimer as PSI particles prepared from plants genetically depleted in Lhca2 contain less than 5% of wt-levels of Lhca5 [8]. The combination of both findings strongly indicate that Lhca5 interacts with PSI via Lhca2 at the Lhca2/a3-site of PSI. The finding of cross-linking products of Lhca1/a4 and Lhca2/a3 is in line with earlier data obtained from chemical cross-linking [16].

As the Lhca5 content is reduced in plants lacking Lhca2 and Lhca3 but elevated in plants lacking Lhca1 and Lhca4 [8] we analyzed the cross-linking products containing Lhca5 in PSI particles prepared from these lines (Δa1–Δa4; Fig. 4). The pattern of bands recognized by the Lhca5 antibody of the particles prepared from the Δa1 plants is similar to the wt pattern although the amount of Lhca5 in particles from the Δa1 line is elevated compared to the wt. Very little amounts of cross-linking products were detected in cross-linked particles prepared from the Δa2 line which accumulates only decreased amounts of Lhca5 protein compared to the wt. But as PSI-

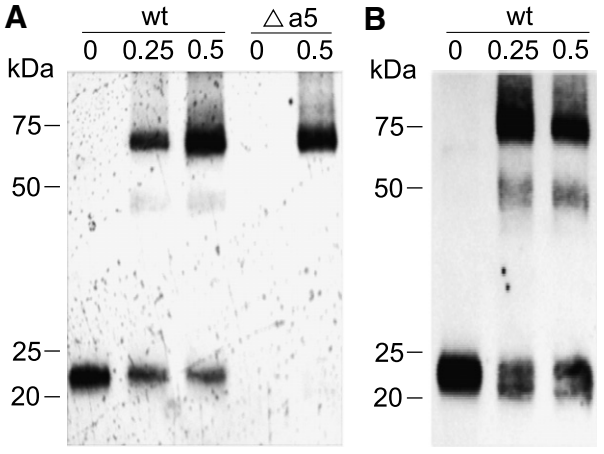

Fig. 2. Lhca5 immunoblot analysis of PSI-LHCI (3 μg chl/lane) prepared from (A) wt and Δa5 *Arabidopsis* (B) and wt pea after cross-linking with 0 (control), 0.25, and 0.5 mM DSP.

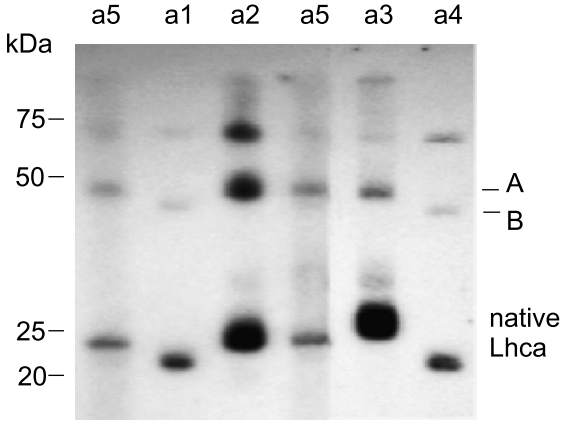

Fig. 3. Immunoblot analysis of PSI-LHCI (wt, 15 μg chl/lane) from *Arabidopsis* cross-linked with 0.25 mM DSP; parallel immunodecoration with antibodies against Lhca1 to Lhca5 (a1–a5) identifies cross-linking products containing Lhca5/Lhca2/Lhca3 (band A) and Lhca1/Lhca4 (band B).

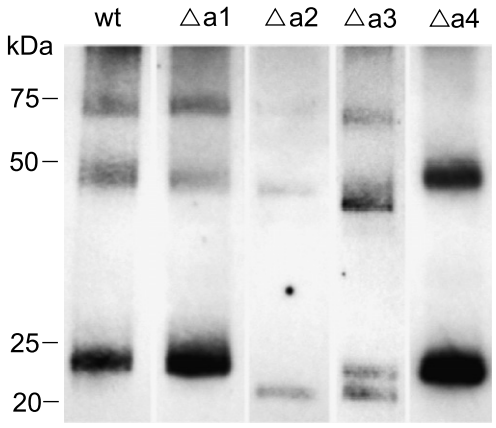

Fig. 4. Lhca5 immunoblot analysis of PSI-LHCI (2 μg chl/lane) from wt and Lhca-deficient lines (Δa1–Δa4) after cross-linking at 0.25 mM DSP.

LHCI particles from the Δa2 line do not contain any Lhca2 or Lhca3 protein [8] those low amounts of cross-linking product of 46 kDa might also indicate a weak interaction of Lhca5 with Lhca1 or Lhca4 in this line. In these particles the cross-linking results in a modified form of Lhca5 with slightly lower apparent molecular mass. It is also detected in the particles prepared from the Δa3 line (where the non-cross-linked form of wt apparent molecular mass is also present). Both bands have also been detected by the Lhca5 antibody in preparations of non-crosslinked wt PSI-LHCI in this (data not shown) and earlier studies [13] indicating that both actually represent Lhca5. In cross-linked PSI-LHCI prepared from the Δa4 line high amounts of a product of 46 kDa were obtained in addition to the monomeric Lhca5. PSI particles from the Δa4 line do not contain relevant amounts of any other Lhca protein [8] so this product consists of a cross-linked dimer of Lhca5. As this requires docking of Lhca5 to adjacent positions we conclude that interactions with the PSI core partly facilitate the formation of Lhca5 homodimers in the Lhca1/a4 binding site when Lhca1 and Lhca4 are absent. In addition, no artificial Lhca5 product at ~70 kDa is detected in this line. This band detected in cross-linked particles from the other lines therefore presumably contains Lhca proteins modified by the cross-linking resulting in the recognition with the Lhca5 antibody.

We conclude from the data presented that Lhca5 assembles with wt PSI-LHCI peripherally at the Lhca2/a3 site where it seems to be in close contact to Lhca2 (Fig. 5A). When the amount of Lhca2 is reduced (in the Δa2 and Δa3 lines) total Lhca5 amounts decrease accordingly. Lhca5 does not bind to the empty Lhca3 binding site even when substantial amounts of Lhca2 are present (Δa3 line), which again indicates a peripheral location of Lhca5. In PSI particles depleted in Lhca1/a4 (Δa1 and Δa4 line) elevated amounts of Lhca5 are found and high amounts of cross-linking product at 46 kDa are detected in the Δa4 line. The latter indicates a binding of Lhca5 in the empty Lhca1/a4 binding site where the interactions with the core partly facilitate the stabilization of an Lhca5 homodimer (Fig. 5B). This is obviously not possible in the Lhca2/a3 site even if it is empty (Δa2 line). Homodimerization has not yet been reported for any of the other Lhca proteins, neither in vivo nor in vitro. However, in vitro reconstitution has demonstrated a heterodimerization of Lhca5 with Lhca1 [15] and Lhca5 was found together with Lhca1 and Lhca4 in dimeric fractions of LHCI-730 [14] implicating the possibility for Lhca1/a5 heterodimers or Lhca5 homodimers in vivo. But as Δa4 plants which show highest amounts of Lhca5 do not even accumulate Lhca1 on a thylakoid level [9] the presence of Lhca5/a1 heterodimers in *Arabidopsis* in vivo is unlikely. Obviously, interaction of Lhca proteins with PSI core proteins plays a major role in determining the interaction properties of some Lhca proteins. This could be in line with the finding that the Lhca2/a3 heterodimer present in native LHCI has not been successfully reconstituted from the individual Lhca proteins, yet [17]. The elevated amounts of Lhca5 in the Δa1 line presumably are also partly present as homodimers in the Lhca1/a4 binding sites not occupied by Lhca1/a4 heterodimers. We cannot exclude that Lhca5 might interact with Lhca2 as a homodimer even in the wt (Fig. 5A). Cross-linked homodimers of Lhca5 will have a similar apparent molecular mass as heterodimers of Lhca2/a3, hardly distinguishable in our study (Fig. 3). But as we did not identify any cross-linking product containing Lhca5 in the 100 kDa range (2 cross-linked dimers) the Lhca5 homodimer might be exclusively formed in the Lhca1/a4 binding site in the absence of Lhca1 and Lhca4.

The flexible binding behavior of Lhca5 could illustrate a regulatory principle in the assembly of the PSI-LHCI antenna: during evolution a co-adaptation of each of the Lhca1–Lhca4 proteins to a single specific interaction site of the PSI core took

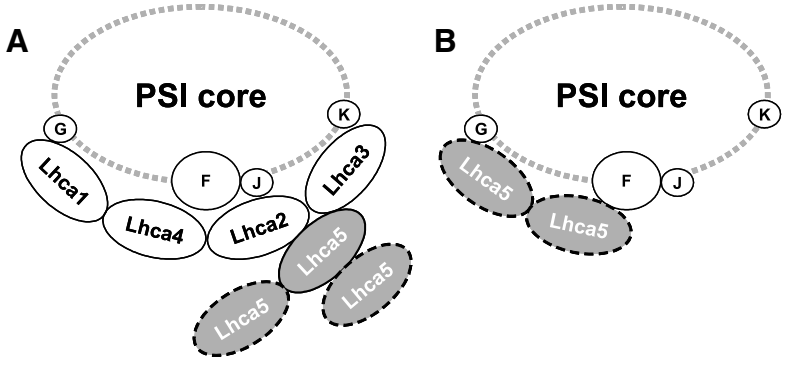

Fig. 5. Schematic model of the LHCI-binding site of plant PSI indicating positions of Lhca1 to Lhca4 relative to the core subunits Psa-G, -F, -J, and -K. Lhca5 binds (A) as a monomer (solid line) or homodimer (dotted line) close to Lhca2 in the Lhca2/Lhca3 site (situation in native PSI-LHCI complex in the wt) or (B) as a homodimer directly to the core in the Lhca1/Lhca4 site in the absence of Lhca1/Lhca4 (situation in Δa4 and Δa1 line).

place. Proteins that can bind more flexible but less specific to some of the binding-positions (like Lhca5) are out-competed by the abundant Lhca proteins, at least under controlled standard laboratory growing conditions. In antenna systems where the composition of LHC proteins is altered by means of genetic modification this flexibility can be brought out: plants depleted in Lhcb1 and Lhcb2 assemble elevated amounts of Lhcb5 which was found to replace Lhcb1 and Lhcb2 [18]. Based on these findings it is tempting to speculate if even wt plants are able to regulate the composition of their antennas accordingly by means of regulation of expression or assembly under different physiological conditions.

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