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The light-harvesting complexes of higher plants Photosystem I: Lhca1/4 and Lhca2/3 form two red-emitting heterodimers

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Running title: Dimeric antenna complexes of Photosystem I

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The outer antenna of higher plants Photosystem I is composed of four complexes (Lhca1-4) belonging to the light-harvesting protein family. Difficulties in their purification have so far prevented the determination of their properties and most of the knowledge about Lhcas has been obtained from the study of the *in vitro* reconstituted antennas. In this work we were able to purify the native complexes, showing that Lhca2/3 and Lhca1/4 form two functional heterodimers. Both dimers show red-fluorescence emission with maxima around 730 nm, as in the intact PSI complex. This indicates that the dimers are in their native state and that LHCI-680, which was previously assumed to be part of the PSI antenna, does not represent the native state of the system. The data show that the light-harvesting properties of the two dimers are functionally identical, concerning absorption, long-wavelength emission and fluorescence quantum yield, while they differ in their high-light response. Implications of the present results for the understanding of the energy transfer process in PSI are discussed. Finally, the comparison of the properties of the native dimers with those of the reconstituted complexes demonstrates that all the major properties of the Lhcas are reproduced in the *in vitro* systems.

Introduction

In the first steps of photosynthesis light energy is captured and converted into chemical energy. In higher plants and algae this process takes place in the thylakoid membrane, where Photosystem II (PSII) and Photosystem I (PSI) work in concert with cytochrome *b₆f* and ATP-synthase to harvest the light and store its energy by generating ATP and NADPH.

Higher plant PSI is a multi-protein-pigment complex, composed of two moieties: the core and the peripheral light-harvesting antenna (LHCI). The core complex harbours the reaction centre (RC), all the electron transport cofactors, ~100 Chlorophylls (Chl) *a* and 22 β -carotenes [1, 2]. LHCI coordinates Chl *a*, Chl *b*, and the carotenoids (Car) β -carotene, violaxanthin, and lutein [3]. It is composed of four light-harvesting complexes (Lhc), called Lhca1-4. The Lhca proteins are encoded by the Lhc gene family, which also includes the Lhcb antenna complexes of PSII. All Lhc's show high sequence conservation, with 43-55% sequence identity between the different Lhca's and up to 75% sequence homology [4]. The Lhca's are located on one side of the core and are assembled into two dimers: Lhca1/4 and Lhca2/3 [5, 6]. It has been suggested that under certain growth conditions Lhca2 and Lhca3 might be able to form homodimers [1]. However, it has been shown that all four Lhca proteins are present in a one to one ratio with the PSI core under standard [7] and high- and low-light conditions [8], in agreement with the exclusive presence of a heterodimeric Lhca2/3 complex.

The main function of LHCI is to harvest light and transfer the excitation energy to the RC, where it is used for charge separation. In addition, it has been shown that the LHCI moiety is the first target of high-light damage of PSI-LHCI, thereby protecting the core complex against photodamage [9-11].

A peculiar feature of PSI is the presence of red forms: Chls that absorb at energy lower than that of the primary electron donor P700 and which have extremely broad and red-shifted fluorescence emission spectra [12]. They are conserved in plants, algae and bacteria. Still, their function is not fully understood. It has been suggested that they: (i) focus the energy to the primary electron donor, (ii) have a role in protection against light-stress, or (iii) absorb light efficiently in a dense vegetation system where light is enriched in wavelengths above 690 nm [13, 14]. It is shown that the red forms have an important effect on the excitation energy transfer of PSI [12, 15, 16]. Being at low energy these Chls have a high probability to be populated [17] and their excitation energy must be transferred energetically up-hill to P700, in order to be used for photochemistry [16]. In higher plants the red forms are associated with LHCI. Although several studies have analysed the trapping kinetics in PSI, no general agreement has been reached [15, 18, 19]. This is mainly because PSI is a very large and complex system and little information is at present available about the properties of the individual PSI building blocks

(e.g. Lhca dimers). This information is necessary to be able to disentangle the contribution of the individual complexes from the analysis of the whole system.

In the past, LHCI was often separated into two fractions upon isolation: LHCI-680 and LHCI-730, named after their low temperature at 77K (LT) fluorescence emission maxima [20-24]. LHCI-680 lacks the red-shifted emission, and consists mainly of monomeric Lhca2 and Lhca3, while LHCI-730 is strongly enriched in the Lhca1/4 heterodimer [21, 23, 24]. Based on these results it was assumed that only the Lhca1/4 dimer contains "red" Chls. However, the LT 680 nm fluorescence was absent in preparations containing all four Lhca complexes in their native dimeric form [25,26]. Furthermore, dimeric fractions enriched in Lhca1/4 or Lhca2/3 both showed a red-absorption tail with similar amplitude, indicating that red forms are also present in Lhca2/3 [27].

Due to the very similar biochemical properties of the two dimeric LHCI complexes, full separation has not been achieved yet and most of the available information on the individual Lhca complexes was obtained by *in vitro* reconstitution of monomeric Lhca1-4 and dimeric Lhca1/4 antenna complexes [23, 27-29]. The LT fluorescence emission maxima were found at: 690nm (Lhca1), 702nm (Lhca2), 725nm (Lhca3), and 733nm (Lhca4) [27-29]. It has been shown that the red forms in the Lhca complexes originate from a strongly excitonically coupled Chl dimer, involving Chl603 and Chl609 (nomenclature as in [30]). It has also been demonstrated that an asparagine as ligand for Chl603, as it is in Lhca3 and Lhca4, is needed for this strong coupling. Indeed, all other Lhc complexes have a histidine at this position and do not show red-shifted emission [31-35]. To account for the extreme red-shift and the broad fluorescence emission spectra it was suggested that the lowest exciton state of the dimer mixes with a charge-transfer state [36-38], which was recently demonstrated to be correct in the case of the Lhca4 monomer [39].

In this work we purified and fully characterized the Lhca2/3 and Lhca1/4 heterodimers, obtaining a complete picture of their biochemical, spectroscopic and functional properties, thus opening the way to a full understanding of the excitation energy transfer in PSI. We also show that LHCI-680 is not a native state of the antenna complexes of PSI. Finally, the availability of native purified complexes allows for a comparison with the recombinant antenna complexes.

Experimental

Plant material – The WT, Lhca1 T-DNA knock-out ($\Delta a1$) and Lhca2 anti-sense ($\Delta a2$) *Arabidopsis thaliana* (WT-col-0) plants (described before [40, 41]) were grown at a day(8h)/night(16h) regime of 22 °C/19 °C, with a light intensity of 130 $\mu\text{E m}^{-2} \text{s}^{-1}$ and 70% relative humidity.

LHCI isolation and analysis. Thylakoids were isolated as described elsewhere [20]. PSI-LHCI isolation was as described in [42]. LHCI isolation was adapted from [25]. In short: PSI-LHCI with a Chl concentration of 0.3mg/ml was solubilised with 1% n-dodecyl- β ,D-maltoside (β -DM) and 0.5% Zwittergent-16. After 1 min of vortexing, the sample was loaded onto a 0.1-1M sucrose gradient, containing 10mM tricine, pH 7.8 and 0.03% β -DM and centrifuged at 41.000rpm for 22h in a Beckman SW41 rotor. The fractions were analysed by a modified Laemmli SDS-PAGE system and Coomassie staining, as described in [7]. Quantities loaded on a Chl basis were: 3 μg PSI-LHCI, 1 μg dimeric fraction of solubilised PSI-WT and 0.5 μg for the dimeric fraction of PSI- $\Delta a1$ and $\Delta a2$. Quantification of Coomassie stain was done as described in [7] after digitizing the gel with a Fujifilm LAS 3000 scanner. Immunoblot was done as described in [42]. Pigment analysis was done as in [43].

Steady-state spectroscopy – Absorption spectra were recorded on a Varian Cary 4000UV-Vis-spectrophotometer. For 77K measurements a home-built liquid N₂ cooled low-temperature device was used. Fluorescence spectra were recorded at 77K and 283K on a Fluorolog 3.22 spectrofluorimeter (Jobin Yvon-Spex). Samples were diluted to an OD of 0.04 cm^{-1} at the Q_y maximum. Circular-dichroism (CD) spectra were recorded at 283K on an AVIV 62ADS spectropolarimeter. All measurements were performed in 10mM tricine pH 7.8, 0.03% α -DM

and 0.5M sucrose (room temperature (RT) and 283K) or 67% (w/v) glycerol for 77K measurements.

Fluorescence quantum yield – Fluorescence quantum yields (Φ_{FI}) at 283K were calculated by dividing the ratio of integrated fluorescence intensities on a wavenumber scale by the ratio of their total absorption factor (1- Transmission) for the spectral region around 630nm, where the sample was excited. The emission of Chl *a* in acetone, with $\Phi_{FI} = 0.30$ [44] was used as a reference.

Photobleach assay – Lhca dimers were illuminated for 20 minutes in the presence of oxygen at RT with a Schott 200 cold white light source at stage 3, giving a light intensity of $5.5\text{mE m}^2\text{ s}^{-1}$. Light was passed through a 1cm water filter. Samples in a 4x10mm cuvette, with an initial optical density at Q_y maximum of 0.3 in the light path of 4mm, were homogenized every 2 minutes. Control samples were kept at RT in the dark for 20 minutes. Samples were concentrated, loaded on a 0.1-1M sucrose gradient and centrifuged at 44,000rpm for 19h in a Beckman SW60 rotor.

Results

Isolation of the native LHCI dimers – LHCI of wild type (WT) plants is suggested to be composed of two dimers: Lhca2/3 and Lhca1/4 [6]. However, homogeneous purification of these dimers has never been achieved from PSI-WT because of the highly similar properties of the complexes [27]. It has been shown recently [42] that PSI complexes coordinating only Lhca2/3 or Lhca1/4 can be obtained from plants lacking either Lhca1 or Lhca2 ($\Delta a1$ and $\Delta a2$ *Arabidopsis* mutants). These preparations thus represent a good starting point for the homogeneous preparation of each dimer. PSI- $\Delta a1$, $\Delta a2$ and WT were solubilised and subjected to sucrose gradient density ultracentrifugation (fig1A). Five pigment-containing fractions were obtained and identified by their mobility in the gradient, by SDS-PAGE and by absorption spectroscopy (data not shown) as: free pigments, dimeric Lhca, PSI core, and PSI supercomplexes with reduced (PSI-LHCI^r) or with full (PSI-LHCI) antenna size. In the gradient of PSI- $\Delta a1$, which in addition to Lhca2 and 3 also retained part of the Lhca4 [42], some monomeric Lhca4 was present between the free pigments and dimeric Lhca. The SDS-PAGE and immunoblot analysis (fig1BC) of the dimeric Lhca fraction purified from the $\Delta a1$, $\Delta a2$ and WT PSI preparations shows that they contain respectively Lhca2/3, Lhca1/4 and a mixture of the two dimers (in the following named LHCI). The SDS-PAGE shows that the Lhca1/4 dimer is 100% clean, while the purity of the Lhca2/3 dimer is 95% (SI1 for details), the impurity being Lhca1/4, as expected because a small amount of Lhca1/4 dimer is present in the starting PSI preparation, as previously shown [42]. This is due to the fact that the T-DNA insertion of the $\Delta a1$ plants is located in the promoter region, and thus a small part of the PSI complexes in these plants retains Lhca1 [45] and thus the Lhca1/4 dimer. The analysis of the gel (see SI1 for details) also showed that Lhca2 and Lhca3 are present in equal amounts, confirming that they are present as a heterodimer.

Pigment composition – The pigment composition of the Lhca1/4 and Lhca2/3 dimers is very similar (Table 1), both having a Chl *a/b* ratio of 3.7 and a Chl/Car ratio of 4.7-4.8. The main difference concerns the ratio between the Cars: the amount of β -carotene is higher in Lhca2/3 than in Lhca1/4, while the opposite is true for violaxanthin and lutein.

LT absorption – The 77K absorption spectra (fig2A) of the two dimers show clear differences reflecting the different environment in which the pigments are embedded as can be appreciated from the second derivative analysis of the spectra (fig2BC). It is important to underline that in several regions in which the second derivative of Lhca1/4 shows a signal, no signal is present for Lhca2/3, indicating that the 5% impurity (Lhca1/4) in the latter preparation is below the detection sensitivity and does not influence our measurements.

The most peculiar feature of Lhca complexes is the presence of low-energy absorption forms. Interestingly, the absorption in the red tail is extremely similar for both dimers. In order to get more details about the red forms, the Q_y region of the spectra was described in terms of

Gaussians (see SI2 for details). The red-most bands show a maximum at 706-707nm and a FWHM of 25nm and they represent 8.5% and 8.9% of the total oscillator strength in the Q_y region (630-750nm) for Lhca1/4 and Lhca2/3, respectively. The high similarity of the absorption properties indicates that the organization of the Chls responsible for the red absorption is also very similar in the two dimers.

Circular Dichroism – In order to compare the pigment-pigment interactions in Lhca1/4 and Lhca2/3, the CD spectra were recorded (fig3A). In the Q_y region, the main components of both dimers have the characteristic (-+-) signal like all other members of the Lhc family [46], indicating a similar structural arrangement of the pigments. In the blue region the spectra are rather different, indicating specific pigment-pigment or pigment-protein interactions in the two dimers.

Steady-State Fluorescence – The fluorescence emission spectra of the two dimers, recorded at 283K and 77K, are presented in figure 3BC. At 283K the Lhca1/4 spectrum has maxima at 685.5nm and 721nm. Lhca2/3 has a similar spectral shape with maxima at 687 and 713nm. Upon lowering the temperature to 77K the Lhca1/4 dimer loses nearly all 685.5nm emission, while the maximum shifts to 731.5nm. The maximum of Lhca2/3 also shifts to lower energies (728.5nm), however a shoulder remains around 697 nm. An emission band around 700nm was observed previously for an LHCI preparation containing both Lhca dimers [25, 26], and was assigned to Lhca2 [28]. This is puzzling, because for an equilibrated, intact Lhca2/3 dimer at 77K nearly all excitation energy is expected to be located on the lowest-energy form of Lhca3 [28]. A possible explanation for the observed emission could be that some dissociation of dimers has occurred, giving rise to Lhca2 that cannot transfer its energy to Lhca3. To investigate whether this is the case, the oligomeric state of the sample was analysed on a sucrose gradient. Only one band was observed (not shown), and its LT fluorescence spectrum was recorded directly after harvesting. The 697 nm emission was still present, indicating that it is an intrinsic property of the dimeric complex.

Previous data on recombinant complexes have shown that the fluorescence yield of Lhca complexes is far lower than that of LHCI, thus indicating that Lhca complexes are in a partially quenched state in solution [37]. This is particularly interesting because a quenched conformation can be related to the mechanisms of energy dissipation in the antenna (for a review see [47]), which protect the plants against high light damage. To check if this is also the case for native dimeric Lhca, the fluorescence yield (Φ_F) at 283K was calculated (for details see section: Experimental). Φ_F was 0.15 (+/- 0.01) for Lhca2/3 and 0.14 (+/- 0.01) for Lhca1/4. These values for the native dimers are significantly higher than the value of 0.063 reported for the reconstituted Lhca1/4 complex [37], but lower than that of LHCI ($\Phi_F = 0.22$ [48]), indicating that the Lhca dimers are indeed in a partially quenched state in solution.

Comparing the properties of Lhca1/4 and Lhca2/3 with the one of LHCI

The peripheral antenna complex of PSI (LHCI) is composed of equal amounts of Lhca1/4 and Lhca2/3 [7]. Therefore the normalized spectra of the purified Lhca1/4 and Lhca2/3 dimer should add up to the spectra of LHCI. Figure 4 shows the comparison of the sum of the absorption, CD and fluorescence spectra of the two dimers with the spectra of the LHCI fraction purified from WT plants. The excellent agreement shows that indeed the spectroscopic properties of LHCI can be explained by those of the two purified dimers.

Comparing the dimers with the intact system – In order to investigate whether the properties of the dimers change during purification, their LT emission is compared to those in isolated PSI systems and PSI in the thylakoid membranes. In $\Delta a3$ -PSI Lhca3 is completely lacking, therefore Lhca4 coordinates the lowest-energy Chls and is responsible for most of the LT emission. This allows comparison of the spectroscopic properties of the red forms of Lhca4 in PSI with those of Lhca1/4 (fig5A). The properties of the red forms in Lhca3 (of Lhca2/3) can be compared with those of $\Delta a4$ -PSI, where Lhca4 is replaced by Lhca5 [42]. In this complex Lhca3 is responsible for most of the LT emission (fig5B). In both cases there is a good match between the compared spectra. This means that the properties of the red forms remained

unchanged during the purification of the Lhca1/4 and Lhca2/3 dimer. This is further confirmed by the comparison of the spectra of the dimers with that of the thylakoid membrane (fig5C). Also in this case there is a good overlap between the spectra of the dimers and the reddest peak of the thylakoid spectrum which originates from PSI. Because the red forms are very sensitive to changes in the environment of the corresponding pigments [49], the fact that LT emission does not change upon isolation indicates that the dimers are in their native state.

Photobleaching – It has been shown that the LHCI moiety is the first target of high-light damage in PSI-LHCI [9-11]. This was explained by the concentration of excitation energy on the red forms, thus giving rise to the highest probability of generating dangerous triplets on the corresponding Chls [9], which are mainly located in LHCI [25]. However, it has also been reported that triplets formed on the red forms are quenched with 100% efficiency by a nearby Car located in the 621 site [50], which would provide excellent photoprotection to the whole system. Thus there is a discrepancy: on the one hand the red forms are proposed as a site of photodamage, on the other hand they were shown to be fully protected. To clarify this point we investigated the photodamage for isolated dimers.

Both dimers were subjected to high-light treatment. The absorption spectra of the treated and untreated samples are presented in figure 6. The total absorption in the Q_y region decreased demonstrating that photobleaching had occurred. The difference spectra untreated-treated of both dimers show a peak at 682nm, a shoulder at 672nm and a tail extending into the red, meaning that the red forms are not fully protected.

A light-induced trimer to monomer transition has been observed for the major light-harvesting complex of PSII (LHCII) [51]. Therefore, it was investigated whether light-induced monomerization also occurs in the Lhca dimers. The complexes were exposed to strong light followed by density-gradient centrifugation. Interestingly, light-induced monomerization was observed for Lhca2/3, but not for Lhca1/4 (fig 7).

Monomerization affects pigment organization – For recombinant (r) Lhca1/4 it has been shown that dimerization affect the pigment interactions, as indicated by the fact that the CD spectrum of the dimer differs from the sum of the CD spectra of the monomers [27]. The light induced monomerization of the Lhca2/3 dimer (fig 7) allows to investigate if a similar effect occurs in these complexes. Figure 8 shows that the CD spectra of the monomeric band, containing Lhca2 and Lhca3, is rather different from the dimer. Therefore, it can be concluded that dimerization affects the pigment organization in both dimers and/or create new interactions between pigments of different monomers.

Reconstituted vs native Lhca complexes – The availability of native dimers and monomers Lhca allows for a comparison of their properties with those of the reconstituted complexes (SI3), which have been widely used for the study of the PSI antenna complexes.

Based on the pigment composition of the monomers, identical Chl *a/b* ratio and Chl/Car ratio's are expected in both dimers, as is indeed the case (table SI3). The Car composition of the sum of the monomers is similar to that of the dimer, indicating that the specificity for the Car binding is maintained in the reconstituted complexes. The only difference is the higher amount of β -carotene (at the cost of lutein) in the dimers. This was also observed for the reconstituted Lhca1/4 dimer, indicating that dimerization stabilises the coordination of β -carotene, or that the binding of β -carotene is required for dimerization, but that this difference is not due to the reconstitution procedure.

The data also show high similarity between the spectroscopic properties of the native and reconstituted Lhca1/4 dimer (figSI3.1). The properties of the red most bands are identical, although the reconstituted complex shows somewhat smaller intensity in the red.

So far it has not been possible to reconstitute the Lhca2/3 dimer [23], but the individual monomers were obtained [23, 28]. In figure SI3.2 the sum of the reconstituted Lhca2 and Lhca3 spectra is compared with the mixture of native Lhca2 and Lhca3 obtained by light-induced monomerization. The striking similarity of the spectra shows that also in this case the pigment organization in the reconstituted complexes is virtually identical to that of the native ones.

Discussion

LHCI-680 is not a natural component of the PSI antenna complex – For a long time it has been thought that LHCI is composed of two fractions, one without red forms: LHCI-680 (enriched in Lhca2 and Lhca3) and one with red forms: LHCI-730 (enriched in Lhca1 and Lhca4). However, it was also shown that fractions containing all four Lhca complexes in dimeric state did not show emission at 680 nm at LT and it was inferred that the LHCI-680 fraction was composed of partially denatured complexes in a monomeric state [27, 52]. Therefore, it was suggested that native LHCI is composed of dimeric complexes which all contain red forms [27]. Unfortunately, these complexes could never be fully separated. Here, by using PSI complexes from mutant plants, we were able for the first time to purify these dimeric complexes: Lhca1/4 and Lhca2/3. The properties of these dimers can fully explain the pigment composition (Table1), CD, absorption and fluorescence spectra (fig 4) of LHCI. Moreover, both dimers show red emission forms with maxima around 730 nm, the same as in the PSI complex, implying that the purification does not alter the properties of the dimers. These results present the final proof that LHCI-680 is not a natural component of the PSI antenna complex.

Lhca2 and Lhca3 form a functional heterodimer – It is well established that Lhca1 and Lhca4 form a heterodimer, while the heterodimeric association state of Lhca2 and Lhca3 could not be confirmed by either reconstitution *in vitro* [23] or purification of the complex [27]. Several results indicate that Lhca2 and Lhca3 are not associated to PSI core as a homodimer: (i) In Lhca2 anti-sense plants Lhca3 is completely lacking at the PSI level, while its mRNA is present [41, 42, 45, 53], thus demonstrating that an Lhca3 homodimer is not being formed. (ii) In the Lhca3 anti-sense plants 35% of Lhca2 (as compared to the WT level) is still associated with the core, but exclusively in its monomeric form as is evident from both electron-microscopy and sucrose density ultracentrifugation, where Lhca2 was only found in the monomeric fraction [42]. In this study we were able to isolate Lhca2/3 and to show that it is a functional heterodimer in which energy-transfer between the monomers occurs, as indicated by the fact that most of the emission is red-shifted, thus originating from Lhca3 [27-29].

Four different Lhca complexes form two nearly identical dimers –The four monomeric Lhcas obtained by *in vitro* reconstitution strongly differ from each other in their biochemical and spectroscopic properties [37, 54]. However, it was noticed previously [53] that the sum of the absorption spectra of reconstituted monomeric Lhca1 and Lhca4 was similar to the sum of Lhca2 and Lhca3, therefore it was expected that the two dimers would also have similar absorption properties. The analysis of the native complexes shows that this is indeed the case: the two dimers have identical Chl *a/b* ratio's and rather similar absorption spectra. What is surprising is that this similarity is not limited to the absorption, but it extends to most of the spectroscopic properties including long-wavelength emission and fluorescence quantum yield Φ_F . From a functional point of view PSI thus has two identical antenna units, thereby extending the pseudo binary symmetry that characterizes the core complex [2] to the outer antenna system.

Although light harvesting represents the main task of Lhca complexes, they are also involved in photoprotection: in high-light conditions they can act as a “fuse” and dissipate excess energy to minimize photodamage to the core complex [9]. In this respect, the data show a clear difference in functionality for the two dimers: light treatment leads to the monomerization of Lhca2/3 but not of Lhca1/4. In PSI-LHCI monomerization of Lhca2/3 might induce the dissociation of the dimer from the core complex. In this case Lhca2/3 cannot transfer its excitation energy to P700, which leads to an increase of the excited-state lifetime and thus to an increased probability to form Chl triplets. This can thus explain why Lhca2 and Lhca3 are the first antennas to be degraded upon light treatment of PSI-LHCI [11, 55, 56].

The domain harbouring the red forms is conserved in the two dimers – A peculiar feature of the antenna complexes of PSI is the presence of Chls that absorb above 700 nm and which are associated with Lhca3 and Lhca4 [36]. The properties of these forms could be studied for native and functional dimers, allowing a direct comparison of Lhca2/3 with Lhca1/4 (SI4). The data show that the red forms have nearly identical spectroscopic properties in both dimers, indicating that their environment and organization must be very similar.

The two dimers have identical light-harvesting properties: implications for energy transfer in PSI-LHCI – The study of excitation energy transfer and trapping in PSI is extremely complex because the system is composed of 170 Chl molecules, making the modelling of the fluorescence kinetics very challenging. The system is usually described using compartment models in which the major building blocks (Lhca complexes, core complex, RC) are considered. Knowledge about the spectroscopic properties of the compartments is required for the evaluation of these models [18]. Until now this information was not available. The newly obtained data for the dimers open the way to design and evaluate a model which truly describes the energy trapping and transfer in PSI.

The reconstituted complexes are good replicas of the native system – In this study the biochemical and spectroscopic properties of native and reconstituted complexes were compared. The data clearly demonstrate that all the major properties of the monomeric Lhcas are reproduced in the *in vitro* systems, testifying once more that the reconstituted complexes are valuable replicas of the native systems.

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Tables**Table1 Pigment binding properties of Lhca dimers.**

The pigment compositions of LHCI, Lhca1/4, Lhca2/3 and the average of Lhca1/4 and Lhca2/3 are presented normalized to 100Chls. Values are averages of 3 measurements on independent preparations for Lhca1/4 and Lhca2/3 and of 2 repetitions for LHCI; standard deviations are indicated between brackets.

	Chl a/b	Chl/Car	Vio	Lut	β -car
LHCI	3.71 (0.01)	4.65 (0.01)	4.74 (0.04)	11.14 (0.02)	5.39 (0.03)
Lhca1/4	3.74 (0.09)	4.82 (0.04)	5.30 (0.05)	11.31 (0.13)	4.23 (0.13)
Lhca2/3	3.70 (0.12)	4.69 (0.07)	4.07 (0.15)	10.55 (0.35)	6.66 (0.46)
Average	3.72	4.77	4.69	10.9	5.45

Figures

Fig1 PSI solubilisation.

Panel A: Sucrose gradients of solubilised PSI from WT, $\Delta a1$, and $\Delta a2$ plants. In addition a sucrose gradient of mildly solubilised WT thylakoids is presented, to show the migration behaviour of Lhc complexes in monomeric and trimeric aggregation state. Panel BC: SDS-PAGE (B) and immunoblot (C) of PSI-LHCI (I) and dimeric Lhca fraction of solubilised PSI from WT (II), $\Delta a1$ (III) and $\Delta a2$ (IV) plants. Note that in the case of PSI-LHCI, PsaD and Lhca3 overlap on this gel.

Fig2 LT Absorption of Lhca1/Lhca4 and Lhca2/Lhca3.

Panel A: 77K Absorption spectra of Lhca1/4 (dash) and Lhca2/3 (solid). Spectra are normalized to Chl content. Panel BC: Second derivatives of the absorption spectra in the Soret (B) and Q_y region (C).

Fig3. Circular dichroism and fluorescence of the dimeric Lhca complexes.

A: CD spectra of Lhca1/4 (dash) and Lhca2/3 (solid) at 283K. Spectra are normalized to the Chl concentration. BC: Fluorescence emission spectra of Lhca1/4 (dash) and Lhca2/3 (solid) at 283K (B) and 77K (C). Excitation was at 475nm, spectra are normalized to the maxima.

Fig 4. Comparison of the spectra of Lhca1/4 and Lhca2/3 (solid) with those of LHCI (dash).

A: Sum of LT absorption spectra of Lhca1/4 and Lhca2/3, compared with LHCI. Spectra are normalized in the Q_y region to the number of Chls. B: Sum of CD spectra of Lhca1/4 and Lhca2/3 compared to that of LHCI. C: 283K fluorescence emission spectra of average Lhca1/4 and Lhca2/3 and LHCI. Spectra are normalized on an energy scale to their fluorescence quantum yield. For LHCI a value of 14.5% was used. D: LT fluorescence emission spectra of average from Lhca1/4 and Lhca2/3 and LHCI. Spectra are normalized to the same fluorescence quantum yield.

Fig5 Fluorescence emission of Lhca dimers compared to PSI.

LT emission spectra of Lhca1/4 (grey solid) and $\Delta a3$ -PSI (black dash) (A), Lhca2/3 (grey solid) and $\Delta a4$ -PSI (black dash) (B), and Lhca1/4 (black solid), Lhca2/3 (grey solid), and thylakoids (C). Thylakoids were of Lhcb2 antisense plants which have a reduced LHCII antenna size and therefore the PSI emission is less obscured by PSII emission. Spectra are normalized to the maxima.

Fig 6 Photobleaching of native Lhca dimers.

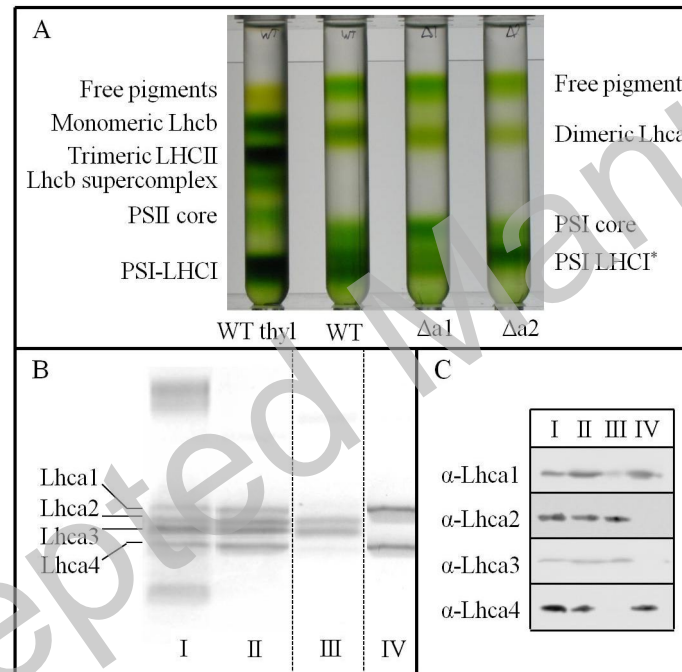
Absorption spectra of Lhca1/4 (A) and Lhca2/3 (B), control (solid bold), light-treated sample (dash), difference (solid thin), and the ratio difference/control (dash-dot).

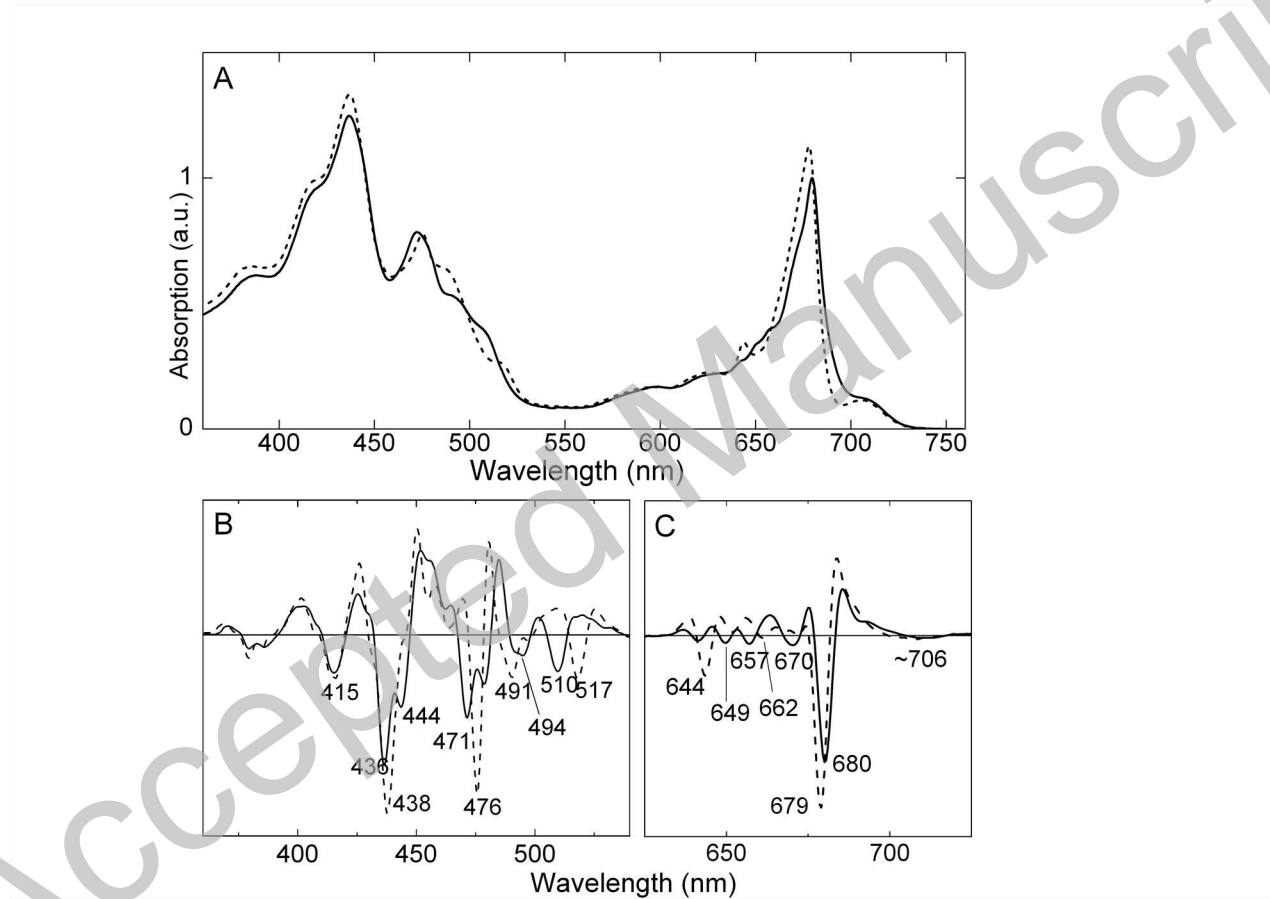
Fig 7. Analysis of oligomeric state of light-treated Lhca complexes

Sucrose density gradient loaded with Lhca2/3 and Lhca1/4, kept for 20 minutes at RT in the dark or in strong light. The left gradient was loaded with a mixture of monomeric and trimeric Lhcb complexes.

Fig 8. CD spectra of monomeric vs dimeric Lhca2/3

CD spectra of dimeric Lhca2/3 before (dot) and after (solid) light treatment, and a light induced mixture of monomeric Lhca2 and Lhca3 (dash). Spectra are normalized to the absorption in the Q_y region.





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