Excitation energy trapping in photosystem I complexes depleted in Lhca1 and Lhca4

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Abstract We report a time-resolved fluorescence spectroscopy characterization of photosystem I (PSI) particles prepared from *Arabidopsis* lines with knock-out mutations against the peripheral antenna proteins of Lhca1 or Lhca4. The first mutant retains Lhca2 and Lhca3 while the second retains one other light-harvesting protein of photosystem I (Lhca) protein, probably Lhca5. The results indicate that Lhca2/3 and Lhca1/4 each provides about equally effective energy transfer routes to the PSI core complex, and that Lhca5 provides a less effective energy transfer route. We suggest that the specific location of each Lhca protein within the PSI–LHCI supercomplex is more important than the presence of so-called red chlorophylls in the Lhca proteins.
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1. Introduction

In oxygenic photosynthetic organisms, photosystem I (PSI) oxidizes plastocyanin and reduces $NADP^+$. This reaction is driven by light, collected by chlorophylls and carotenoids. These pigments are located in two distinct antenna complexes, the PSI core complex and the peripheral light-harvesting complex I (LHCI). The PSI core binds about 100 Chl *a* and more than 20 β-carotene molecules, while LHCI binds about 70 Chl *a* + *b* molecules and 12 xanthophylls [1]. The function of LHCI is to increase the absorption cross-section of PSI and to deliver excitation energy for the PSI core complex, in which photochemical charge separation takes place. Green plant LHCI normally consists of four different proteins Lhca1–4 [2] that each have been shown to be present with one copy per PSI [3]. Another protein (Lhca5) is present in substoichiometric

amounts [4], but this amount is increased under high-light conditions and in plant lines where Lhca1 and Lhca4 have been genetically depleted [4,5]. In vitro reconstitution of Lhca5 yielded a typical light-harvesting protein [6], but a significant functional contribution of Lhca5 to light harvesting has not yet been proven.

A number of studies have been reported on the trapping of excitation energy in PSI systems with or without peripheral antennae [7–9]. Most studies conclude that at room temperature the trapping time from the core antenna is between 18 and 50 ps, depending on the content and energy levels of the Chls that absorb at longer wavelengths than the primary electron donor [10]. Müller et al. [11] concluded that this observation can be explained by a reversible charge separation reaction. In PSI of green plants and algae, one or two additional trapping lifetime(s) of about 70 ps and/or 130 ps have been observed [12–15]. The additional lifetimes in LHCI-containing systems originate from a slow equilibration between LHCI and the PSI core complex [14], which probably arises from distinct structural compartments with several 'gap' pigments in between [3] and the presence of red pigments in LHCI. In PSI–IsiA supercomplexes from iron-stressed cyanobacteria, where the red pigments locate in the core complex, only one trapping lifetime of about 40 ps was observed [16,17], which suggests ultra-fast equilibration phase between PSI core and IsiA complex, implying good connectivity between the PSI core and IsiA pigments.

Recently, we have reported biochemical and steady-state spectroscopic properties of isolated PSI–LHCI particles obtained from *Arabidopsis* lines lacking a specific light-harvesting protein of photosystem I (Lhca) protein [5]. Here, we report a detailed analysis by time-resolved fluorescence spectroscopy of the particles obtained from knock-out mutants of the Lhca1 and Lhca4 genes. In the particles from the first mutant, the results provide information on energy transfer characteristics of Lhca2 and Lhca3, whereas in those from the second mutant the results give details on an Lhca protein without red chlorophylls, most likely Lhca5.

2. Materials and methods

PSI–LHCI particles were isolated from *Arabidopsis thaliana* plant lines depleted in the expression of distinct Lhca protein subtypes as described in Klimmek et al. [5]. The samples were diluted with a buffer containing 20 mM Bis–Tris (pH 6.5), 20 mM NaCl, and 0.06%

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β-DM to an OD680 of about 0.1. Samples for low temperature steady-state measurements contained 66% (v/v) glycerol and were placed in a helium-bath cryostat (Utreks, Ukraine), which was then cooled down to 5 K. Samples for time-resolved measurements contained 10 mM sodium ascorbate and 10 μM phenazine metasulphate (PMS) and were placed into a 2 mm thick spinning cell with a diameter of 10 cm and a rotation speed of 25 Hz.

The *steady-state* fluorescence emission spectra were measured with a 1/2 m imaging spectrograph and a CCD camera (Chromex Chromcam I) with a spectral resolution of about 0.5 nm. For broadband excitation, a tungsten halogen lamp (Oriel) was used with a band-pass filter transmitting at 420 nm (bandwidth of 20 nm). The obtained emission spectra were corrected for the wavelength-dependent sensitivity of the detection system.

The *time-resolved* measurements were performed with a Streak camera setup. In short, excitation pulses of 400 nm (~100 fs) with vertical polarization were generated using a titanium:sapphire laser (Coherent, VITESSE) with regenerative amplifier (Coherent, REGA) and a double pass optical parametric amplifier (Coherent, OPA) and a Berek compensator. The repetition rate was 150 kHz with pulse energy of 0.6 nJ in the sample, which resulted in less than 25% excited protein complexes per pulse. The fluorescence was detected at right angle with respect to the excitation beam through a polarizer at magic angle using a Chromex 250IS spectrograph and a Hamamatsu C5680 synchroscan streak camera. The streak images were recorded with a cooled Hamamatsu C4880 CCD camera. The exposure times per image were 15 and 10 min for 200 ps and 1 ns time bases, respectively. The detected streak images were analyzed globally and the decay-associated spectra (DAS) were estimated [18]. The instrument response function was modeled as a Gaussian with FWHM of about 3 and 8 ps for the 200 ps and 1 ns time bases, respectively.

3. Results

The PSI–LHCI particle preparations used in this study have been previously subjected to a comprehensive characterization of the Lhca protein and pigment contents, functional antenna size, and steady-state spectroscopic features [5]. In that study, we showed that the overall LHCI composition is affected in particles prepared from Lhca-depleted lines, most likely due to interactions between the Lhca proteins in the PSI–LHCI complex. In the case of Lhca1 suppression (denoted below as ΔLhca1), PSI–LHCI particles not only lack about 90% of Lhca1 but also Lhca4, resulting in Lhca2 and Lhca3 as main LHCI proteins in that sample (Table 1). The antenna size in the ΔLhca1 samples was found to be 17% smaller when compared to the wild type (WT) [5]. In the case of the Lhca4 knock-out mutation (denoted below as ΔLhca4), basically all Lhca1–4 proteins are missing, and only very small traces of Lhca2 and Lhca3 could be detected (Table 1). In both lines elevated amounts of Lhca5 were observed. Lhca5 seems to

be the most abundant Lhca protein in the ΔLhca4 sample and based on HPLC analysis and antenna size determinations we suggested that the ΔLhca4 samples contain one Lhca protein per PSI core complex [5]. However, no corresponding protein bands could be identified by silver or coomassie staining and efforts to isolate significant amounts of native Lhca5 from PSI–LHCI preparations from the ΔLhca4 line have not yet been successful (Schmid and Klimmek, unpublished).

Fig. 1 demonstrates the effect of the Lhca mutations on the 4 K emission spectra of the PSI–LHCI particles. Green plant PSI–LHCI exhibits a red emission maximum at about 735 nm [19], caused by the red-most pigments in Lhca3 and Lhca4 [20]. In ΔLhca1, the red emission maximum locates at about 732 nm, which is slightly red-shifted from the emission maximum of reconstituted Lhca3 (at about 724 nm) [20]. In ΔLhca4, the red emission maximum locates at about 720 nm, which is the emission maximum of the PSI-core antenna at low temperatures [21], in line with the almost complete absence of all conventional Lhca proteins, especially Lhca3 and Lhca4 (Table 1). The shoulder at 750 nm in ΔLhca1 can be attributed to a vibrational band of the main emission at 685 nm, while the bands near 670 and 680 nm in all samples may be attributed to unconnected chlorophylls and Lhca proteins, respectively.

By comparing time-resolved fluorescence spectra from WT and Lhca-mutated samples the effect of particular Lhca proteins on the excitation kinetics can be studied. In the case of WT, about 70% of the 400 nm excitation light is absorbed by the PSI core and about 30% by LHCI if the linker pigments between the PSI core and LHCI are considered as PSI core pigments. In the case of ΔLhca1 and ΔLhca4, these ratios are about 80/20 and 93/7, respectively. The estimated DAS of each studied complex are shown in Fig. 2. The time constants and trapping proportions are listed in Table 2. The WT data are very similar to those observed previously with PSI–LHCI samples from WT *Arabidopsis* plants [13,14], which were fitted with five [13] or four [14] (sub)ps and two ns components. In [14], we explained that the (sub)ps decay of PSI–LHCI particles can be described sufficiently by four components. The five component fit, which results in a final lifetime of PSI–LHCI particles to be around 120 ps with about

Table 1
Functional LHCI antenna sizes (%) and Lhca protein content in PSI–LHCI preparations of WT and Lhca1/Lhca4-depleted (ΔLhca1, ΔLhca4) plants used in this study according to Klimmek et al. [5]; n.d., not detectable

	wt	ΔLhca1	ΔLhca4
antenna (%)	100.0	83.0	67.6
Lhca1	1.0	0.1	n.d.
Lhca2	1.0	0.8	<0.1
Lhca3	1.0	1	<0.1
Lhca4	1.0	0.1	n.d.
Lhca5[a]	x	2x	3x
LHCII content[b]	0.07–0.08	0.07–0.08	0.125

[a]The number of Lhca5/PSI is not known for the wt.
[b]As LHCII trimers per PSI-core.

Fig. 1. Steady-state emission of WT (dotted), ΔLhca1 (dashed), and ΔLhca4 (solid) mutant at 4 K after 420 nm excitation.

Fig. 2. Decay-associated spectra of globally analyzed time-resolved fluorescence data of WT (A) and ΔLhca1 (B), and ΔLhca4 (C) mutant at room temperature after 400 nm excitation.

bulk and red chlorophylls of PSI–LHCI, as well as some trapping (Fig. 2A, Table 2), most likely from the bulk pigments located close to the reaction centre. The size and shape of this spectrum is similar in the PSI particles from the WT and both mutants. The third spectrum has in all investigated particles a lifetime of about 21 ps and an all-positive and similar shape, which indicates trapping of excitations at all wavelengths. Its contribution to the overall decay of the system increases from about 37% in WT to 70% in ΔLhca4 (Table 2). This phase was assigned to trapping from PSI core pigments [14] and the fact that the relative amplitude of this phase increases with smaller LHCI antenna size is consistent with this idea.

The fourth component has a lifetime of about 80–130 ps, but a rather different spectral shape and amplitude in the three investigated particles. This component has been assigned to trapping from the LHCI [12,14]. The relative trapping proportions of this phase decrease from about 44% in WT to about 26% in ΔLhca1 and about 10% in ΔLhca4, consistent with a contribution of lower amounts of each Lhca protein in the mutants (Table 1). Fig. 3 shows the LHCI trapping spectrum of all three studied particles normalized to their maxima of the emission at around 685 nm. We note that the DAS are not the physical emission spectra of the species (Species Associated Spectra, SAS), but actually linear combinations thereof [18,22] and therefore DAS are not necessarily always comparable. In this case, however, we can compare directly the final trapping spectra obtained from global analysis, because the next two long lifetimes (1 and 7 ns) are unconnected to the final trapping state (Fig. 2). The spectrum of ΔLhca1 (dashed line in Fig. 3) is slightly blue-shifted compared to that of the WT (dotted line in Fig. 3), consistent with the absence and presence of red-most Chl-containing Lhca4 and Lhca3 complexes, respectively. The spectrum of ΔLhca4 (solid line in Fig. 3) does not contain a significant contribution around 730 nm, which suggests that 95% of the remaining Lhca protein in this complex does not bind a red chlorophyll.

In all samples, two components with lifetimes of about 1 and 7 ns were needed for a sufficient fit (Fig. 2). These components can be assigned to uncoupled pigments or LHCI proteins, and it is likely that these components give rise to the emission bands at 670 and 680 nm in Fig. 1.

4. Discussion

Previous research has indicated that there are significant differences in the energy transfer kinetics in PSI–LHCI supercomplexes from green plants and PSI–IsiA supercomplexes from iron-stressed cyanobacteria. In the latter complexes, most of the trapping of excitation energy takes place with one time constant, which is almost twice as long as in the PSI core complex without peripheral antenna [16,17] and which has the characteristics of a system in which the excitation energy is fully equilibrated between core and peripheral antenna before it is trapped by charge separation. In PSI–LHCI complexes from green plants, however, two main trapping components are generally observed [12–15], of which the fastest one arises from excitations that are absorbed in the core antenna and have a higher probability to get trapped by charge separation in the reaction centre than to 'escape' to the peripheral antenna, whereas the slowest one arises from excitations that are slowly

20% decay amplitude [13], takes slightly better into account the inhomogeneity, both in terms of antenna size and energy of the red pigment of the particles [14]. The subpicosecond component represents the transfer of excitation energy from higher excited states (Soret-states) to the Q_y-state [14]. The second component (6–9 ps) represents energy equilibration between

Table 2
Lifetimes and trapping proportions (integrated areas under each DAS spectrum proportional to the total area of DAS, shown in percentage, %) of WT and mutants of PSI–LHCI particles estimated from the global analysis of the time-resolved fluorescence data

Sample	WT		ΔLhca1		ΔLhca4	
	τ	A_{tot}/A_{PSI} (%)	τ	A_{tot}/A_{PSI} (%)	τ	A_{tot}/A_{PSI} (%)
Soret-Q$_y$-transition[a]	<1 ps	–	<1 ps	–	<1 ps	–
Trap 1	6 ps	14/16	6 ps	20/23	9 ps	14/16
Trap 2	21 ps	35/38	24 ps	43/49	23 ps	61/73
Trap 3	78 ps	42/46	90 ps	25/28	127 ps	9/11
U. LHCI	1.2 ns	5	1.6 ns	7	2.2 ns	8
U. Chl a	6.6 ns	4	7.2 ns	5	7.1 ns	8

The description of the components (the left-most column) has the following link with the description in the text (see also [14]). Trap 1: the EET-component, which obtains a small amount of trapping, mainly from the core 'bulk' pigments, Trap 2: a trapping component from the PSI core and the linker pigments, Trap 3: trapping from the pigments in LHCI, U. LHCI: unconnected LHCI-proteins, U. Chl a: unconnected Chl a pigments.
[a]Below the limit of the time-resolution of the apparatus (~3 ps).

equilibrated between the peripheral antenna and the PSI core complex while trapping of excitation occurs.

The ΔLhca1 particles investigated in this work are largely devoid of Lhca1 and Lhca4, but retain most of Lhca2 and Lhca3. These particles have an almost two-times lower Lhca content than WT particles. The experiments described here show that the slow phase has an about two times smaller amplitude in the ΔLhca1 particles than in the WT, but has about the same kinetics. These results are consistent with parallel routes for energy transfer from Lhca1/4 and Lhca2/3 to PSI, each with similar spectrum and kinetics. If there is efficient energy transfer between Lhca1/4 and Lhca2/3 (which is expected because of the presence of linker chlorophylls between Lhca4 and Lhca2 [3]), then there are in principle three possibilities, i.e., energy transfer from LHCI to PSI proceeds predominantly via Lhca2/3, or predominantly via Lhca1/4, or about equally via both. The first possibility predicts a faster energy transfer if Lhca1/4 is absent, the second a slower, the third about equal kinetics (the faster kinetics of the first possibility arise from the higher probability that an excitation arrives at the linker chlorophyll needed for energy transfer to

PSI [23]). The experiments also show that the kinetics and spectrum of the fast trapping phase(s) is (are) almost equal in ΔLhca1 and WT, in agreement with its attribution to trapping from the core antenna.

The ΔLhca4 particles investigated in this work retain about one Lhca protein, which is the main origin of the 127 ps trapping phase in globally analyzed time-resolved fluorescence data. The results described here indicate that 95% of the remaining Lhca complexes do not bind red chlorophylls (Fig. 3), so it cannot be Lhca3. Lhca4 is naturally absent, as it is genetically knocked out 100% in this line. It is unlikely that this protein is Lhca2, because the very small amounts of retained Lhca2 and Lhca3 seem to be similar [5]. It is also unlikely that the retained protein is Lhca1, because it was not detected in the immunoblots of the PSI particles from the ΔLhca4 mutant, and because the contents of Lhca1 and Lhca4 usually correlate. The retained protein can not be LHCII either, because its content in the PSI–LHCI preparation is too low (one LHCII per 8–15 PSI-particles in the sample [5]) to give such a rise of emission in our global analysis. In addition, the low-temperature emission and absorption spectra of the ΔLhca4 particles are not consistent with significant amounts of LHCII [5]. We cannot completely rule out that there is no Lhca protein bound to PSI in this mutant and that the additional lifetime in the ΔLhca4 particles arise from gap pigments that remained bound, but in defected configuration, to the PSI core complex during purification. However, if an additional Lhca protein is present, the most tentative candidate is Lhca5, which is present in elevated amounts in the ΔLhca4 PSI particles and which was shown not to bind red chlorophylls [6]. So, although the biochemical characterization of this protein has not been successful, it has clear spectroscopic signatures of the Lhca5 protein.

From the observation of the 127 ps decay phase in the ΔLhca4 sample in this study, we conclude that the protein, presumably Lhca5, is coupled to the PSI core and delivers excitation energy to P700. However, this lifetime is longer than the corresponding lifetime in WT particles with a full Lhca content, despite the absence of red chlorophylls in Lhca5. Red chlorophylls generally retard the energy transfer, because they lower the probability that the excited state resides on the chlorophylls from which excitations are transferred to the reaction centre (see also [10]). This suggests that the functional coupling between Lhca5 and the PSI core is not as good as in the case of Lhca1–4. Whether Lhca5 is located at a position with a less
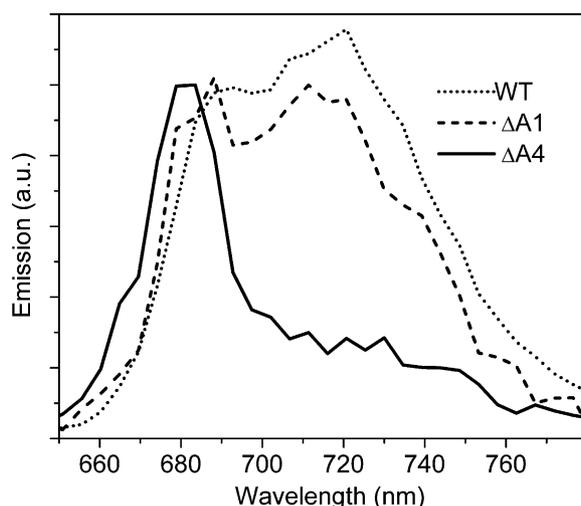
Fig. 3. The final trapping component of PSI–LHCI particles of WT (dotted) and ΔLhca1 (dashed), and ΔLhca4 (solid) mutant after global analysis of the time-resolved fluorescence data at room temperature after 400 nm excitation. The decay lifetimes of the components are 78, 90, and 127 ps for WT, ΔLhca1, and ΔLhca4 samples, respectively.

efficient energy transfer route to the PSI core, or it has another type of orientation than Lhca1–4, or the gap pigments between Lhca5 and the PSI core are blue-shifted compared to the bulk chlorophylls, so that excitations under their way to the PSI core have to pass a high-energy barrier remains to be answered in future studies.

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