

Kinetics of excitation trapping in intact Photosystem I of *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*

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Abstract

We measured picosecond time-resolved fluorescence of intact Photosystem I complexes from *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*. The antenna system of *C. reinhardtii* contains about 30–60 chlorophylls more than that of *A. thaliana*, but lacks the so-called red chlorophylls, chlorophylls that absorb at longer wavelength than the primary electron donor. In *C. reinhardtii*, the main lifetimes of excitation trapping are about 27 and 68 ps. The overall lifetime of *C. reinhardtii* is considerably shorter than in *A. thaliana*. We conclude that the amount and energies of the red chlorophylls have a larger effect on excitation trapping time in Photosystem I than the antenna size.

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1. Introduction

Photosystem I (PSI) is one of the four major complexes responsible for the light reactions in oxygenic photosynthesis. It is bound to the thylakoid membranes of chloroplasts and uses light to catalyze the oxidation of plastocyanin and the reduction of NADP⁺. The electron transfer reactions take place in the PSI core complex, which in green plants consists of 14 subunits and binds about 100 chlorophyll (Chl) molecules [1]. The structure of the PSI core complex from the thermophilic cyanobacterium *Thermosynechococcus elongatus* is known at 2.5-Å resolution [2].

Green plants and green algae both bind a membrane-intrinsic peripheral antenna complex called LHCI. Green plant light-harvesting complex I (LHCI) consists of four main gene products, known as Lhca1–4, and a minor one

(Lhca5), which all bind both Chl *a* and Chl *b* molecules as well as several carotenoids [3,4]. Recently, the structure of the PSI–LHCI complex from a higher plant (*Pisum sativum* var. *alaska*) was resolved with 4.4-Å resolution [5]. The PSI core part of this complex shows a great similarity with that of the cyanobacterial PSI core complex. The structure also confirmed earlier electron microscopy studies that LHCI locates on the PSI-J side of the PSI-core complex [6]. In the structure, 167 chlorophylls were resolved, from which 56 are located in the Lhca region and 10 between the Lhca proteins and the core complex.

In the green alga *Chlamydomonas reinhardtii*, LHCI consists of at least nine gene products and shows a larger diversity than plant peripheral light-harvesting systems [7–11]. Recent electron microscopy experiments have revealed two slightly differently sized PSI–LHCI complexes in *C. reinhardtii* [12,13]. A model of this structure suggests that most of the Lhca proteins are bound in two rows at the PSI-J side of the complex, and that a single Lhca protein is located between the PSI-K, PSI-B, PSI-L, and PSI-H subunits [14]. If the pigment stoichiometry of the *C. reinhardtii* LHCI is similar as in higher plants, about 230 chlorophylls are

Abbreviations: β-DM, *n*-dodecyl-β,D-maltoside; PSI-LHCI, Photosystem I-light-harvesting complex I particle; DAS, decay-associated spectra; SAS, species-associated spectra

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located in the *C. reinhardtii* PSI–LHCI complex (personal communication with M. Hippler).

One of the special features of PSI is the existence of the so-called red chlorophylls. These chlorophylls absorb light of longer wavelength than the primary electron donor P700. In green plants, there are at least two pools of red chlorophylls, one located in the core complex, the other in LHCI. These red pigment pools are responsible for 77-K emission bands peaking at around 720 nm (PSI-core) and 735 nm (LHCI) [15]. In *C. reinhardtii*, the reddest emission peaks at about 715 nm, which presumably arises from the PSI core complex. An isolated LHCI complex of *C. reinhardtii* has been reported to have a 77-K emission maximum at 705 nm [8], which is near the emission maximum observed for the Lhca2 protein of green plants [16–19].

Recently, several groups have studied the excitation dynamics of PSI [20–26]. The single-step energy transfer times between chlorophylls in the PSI antenna have been estimated to occur in about 100–200 fs [27,28]. The equilibration with the red chlorophylls occurs between 2 and 15 ps in the various systems [23]. The overall trapping of the excitation energy occurs in one major phase of 20–50 ps in isolated PSI core complexes, the exact kinetics being dependent on the amount and energies of the red chlorophylls [22], and in two phases of about 50 and 120 ps in green plant PSI–LHCI particles [21,25,29]. In iron-stressed cyanobacteria, PSI–IsiA complexes are formed and IsiA also constitutes a “peripheral antenna” around the PSI-core complex. The energy transfer from IsiA proteins to the PSI core complex was shown to be relatively fast, resulting in a monophasic excitation trapping, about 40 ps [30,31]. In contrast to LHCI, IsiA does not bind red chlorophylls.

The effect of the size of the peripheral antenna on the trapping kinetics has hardly been studied. A number of earlier studies suggested a linear relation between antenna size and trapping time [32,33]. In order to understand the effects of the size of the LHCI antenna and the energy levels of the red chlorophylls on the excitation dynamics in PSI–LHCI complexes, we investigated isolated PSI–LHCI complexes from a green alga (*C. reinhardtii*) and a green plant (*Arabidopsis thaliana*) by time-resolved fluorescence spectroscopy under similar experimental conditions. The results indicate that the energy levels of the red chlorophylls are more important for the excitation trapping kinetics than the size of the peripheral antenna.

2. Materials and methods

PSI–LHCI particles from *A. thaliana* were prepared by *n*-dodecyl- β ,D-maltoside (β -DM) solubilization and sucrose density gradient centrifugation as described by Jensen et al. [34]. The PSI–LHCI particles from *C. reinhardtii* (strain CC-2137) were prepared also by β -DM solubilization and sucrose gradient centrifugation as described in Germano et

al. [12] with an additional FPLC run through a Superdex 200 column after the ultracentrifugation.

For the spectroscopic measurements, the isolated *A. thaliana* PSI–LHCI particles were diluted in 20 mM Bis-Tris (pH 6.5), 20 mM NaCl, and 0.06% β -DM. A buffer of 20 mM HEPES (pH 7.5), 5 mM MgCl₂, 0.03% β -DM was used for *C. reinhardtii* PSI–LHCI particles. In the time-resolved experiments, 10 mM sodium ascorbate and 10 μ M phenazine metasulfate (PMS) were added and in the case of the low-temperature experiments 66% glycerol was used. In the steady state emission experiments, the samples were placed in a 1-cm cuvette, whereas in the time-resolved experiments the samples were placed into a 2-mm-thick spinning cell with a diameter of 10 cm and a rotation speed of 30 Hz in the case of green plant and 25 Hz in the case of green algal PSI–LHCI particles. In both cases, the OD of the samples was about 0.1 at 680 nm.

The steady state fluorescence emission measurements were performed with a 1/2 m spectrograph (Chromex 500IS) and a CCD camera (Chromex Chromcam I). The spectral resolution was about 0.5 nm. A tungsten halogen lamp (Oriol) with bandpass filter transmitting at 420 nm (bandwidth of 20 nm) was used for excitation.

The time-resolved measurements were performed with a streak camera described in detail in Ref. [22]. Excitation pulses of 400 nm (~100 fs) with repetition rates of 150 kHz and 125 kHz for green plant and green algal PSI–LHCI particles, respectively, were used. The pulse energy was about 0.7 nJ for green plant and about 0.4 nJ for green alga PSI–LHCI particles, which resulted in about 0.3 and 0.2 photons per complex from *A. thaliana* and *C. reinhardtii*, respectively. The excitation light was collimated with a 15-cm focal length lens, resulting in a focal diameter of 150 μ m in the sample. The fluorescence was detected through a polarizer, set to magic angle with respect to the polarization of excitation light, at right angles with respect to the excitation beam, using a Hamamatsu C5680 synchroscan streak camera and a Chromex 250IS spectrograph. The streak images were recorded with a cooled Hamamatsu C4880 CCD camera. The exposure times were 10 and 15 min for 550–800 ps and 200 ps time bases, respectively.

For each particle, the images from both time bases were integrated together and analyzed by using a global analysis method that estimates decay-associated spectra (DAS) [35]. These DAS correspond to the amplitudes of the exponential fluorescence decay components. The instrument response function was modelled as a Gaussian with FWHM of about 3.2–4.2 ps and 5.5–8.8 ps for the 200 and 550–800 ps time bases, respectively.

3. Results

Fig. 1A shows 5-K fluorescence emission spectra of isolated PSI particles from *A. thaliana* (solid line) and *C. reinhardtii* (dashed line) after Chl *a* Soret-band excitation at

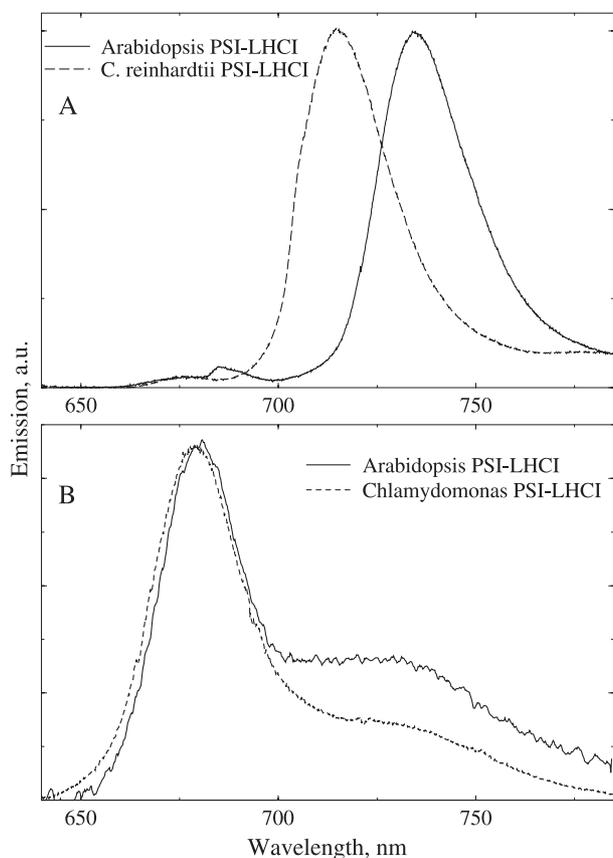


Fig. 1. Low-temperature, 5 K, (A) and room temperature (B) emission spectra of isolated PSI-LHCI particles from *Arabidopsis thaliana* (solid line) and *Chlamydomonas reinhardtii* (dashed line) after Chl *a* Soret-band excitation at 420 nm.

420 nm and demonstrates the energy difference of the red pigments of *A. thaliana*, where an emission band appears around 735 nm, and *C. reinhardtii*, with an emission band around 715 nm (see also the Introduction). Fig. 1B shows the effect of the red pigment composition of the two particles to the room temperature steady state emission spectra. Both spectra show a main band around 680 nm together with a long-wavelength tail. Chlorophylls, which do not transfer energy to the reaction centre, have a considerably longer lifetime (about 5 ns), and are mainly responsible for the 680 nm band (see also below). The long wavelength tail originates mainly from the red pigments of the complexes.

Excitation dynamics in isolated green plant and green algae PSI-complexes was studied by measuring time-resolved fluorescence spectra after ultrashort 400 nm excitation pulses. Using this wavelength, mainly Chl *a* molecules are excited and according to the stoichiometry of the pigments of green plant PSI-LHCI, about 70% of the excitations go to the PSI core and about 30% to LHCI, if the linker pigments between the PSI core and LHCI are considered as “PSI core pigments”. In the case of *C. reinhardtii*, this ratio is about 55/45, because in this organism PSI-LHCI contains more Lhca proteins (see above and Refs. [12–14]).

Fig. 2 shows selected decay traces from the integrated fluorescence streak images. From the traces, one can see the energy transfer processes by the decay component on the blue side of the detection wavelengths (686 nm, bulk pigments), which is accompanied by a rise component in the traces detected on the red side (713 nm and 727 nm, red pigments) of the spectrum. It can also be seen, mainly from the decay traces with detection wavelengths at 713 nm and 727 nm, that in the case of *A. thaliana* the overall decay takes more time than in the case of *C. reinhardtii*. However, all observations from the raw data need to be treated with care, because in the *A. thaliana* samples, more long living (5 ns, see below) pigments exists, which do not necessarily belong to the PSI-LHCI systems. Therefore, in order to determine the lifetimes of the decay components with corresponding DAS of PSI-LHCI particles, global analysis of the data was performed. The quality of the fit is seen as dashed and dotted lines following the experimental traces. One constraint was used in the analysis: the “time-zero”-spectrum (the 1-ps component), which describes the transition from the Soret-states to the Chl *a* Q_y -states, was set to zero in the Chl *a* emission region. Emission in the Chl *b* region during this fast time scale was allowed in case that Chl *b* molecules gain some excitation either directly via the excitation pulse or via the Chl *a* Soret- to Chl *b* Soret-transition. The energy transfer rate from Chl *b* to Chl *a* molecules is known to be ultrafast, mainly within 500 fs and 2 ps [36]. The estimated DAS of each complex are shown in Fig. 3. The time constants of the spectra, together with their trapping proportions, are gathered in Table 1. The subpicosecond component represents the rise of excitation on the first singlet state (Q_y -state) from the laser excited higher states (Soret-states). The second component (5 ps) represents energy equilibration between bulk chlorophylls and the red pigments of the PSI-LHCI. As the 5-ps component is mainly conservative, hardly any trapping takes place in green plant PSI particles on this time scale (Fig. 3A). The first trapping component of the *A. thaliana* particle has a lifetime of about 20 ps and contributes with 52% to the overall decay of the system. This component can be assigned as a trapping time of most of the PSI core pigments (Table 1). The trapping time of 82 ps has a 45% contribution to the overall decay and is mainly from the LHCI pigments. The spectrum with about 5-ns lifetime (observed in both samples) originates from pigments that do not initiate electron transfer processes. These pigments are mainly responsible for the room temperature steady state emission at around 680 nm, as shown in Fig. 1B.

The DAS of the fluorescence decay of isolated PSI-LHCI particles from *C. reinhardtii* (Fig. 3B) show similarities with that of *A. thaliana*. Again, the rise of the fluorescence of the Q_y -state is observed to take place in about 1 ps. The second spectrum with a time constant of 5 ps shows a positive amplitude between 660 nm and 700 nm and a shallow negative part between 700 nm and 715 nm. This spectrum describes energy equilibration in the antenna,

as in the case of green plants. However, contrary to the green plant equilibration spectrum, the nonconservativity of the spectrum indicates that also some trapping takes place

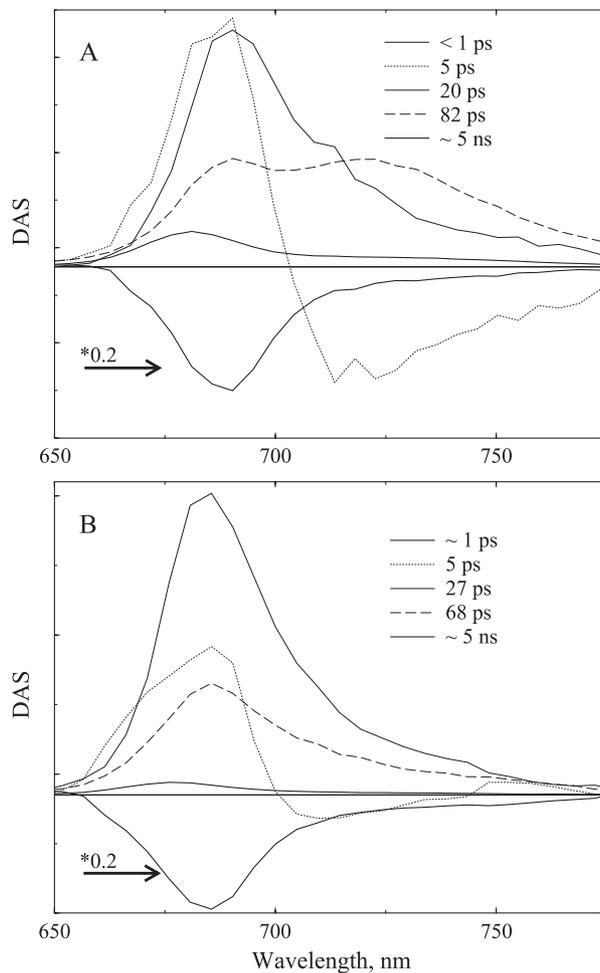
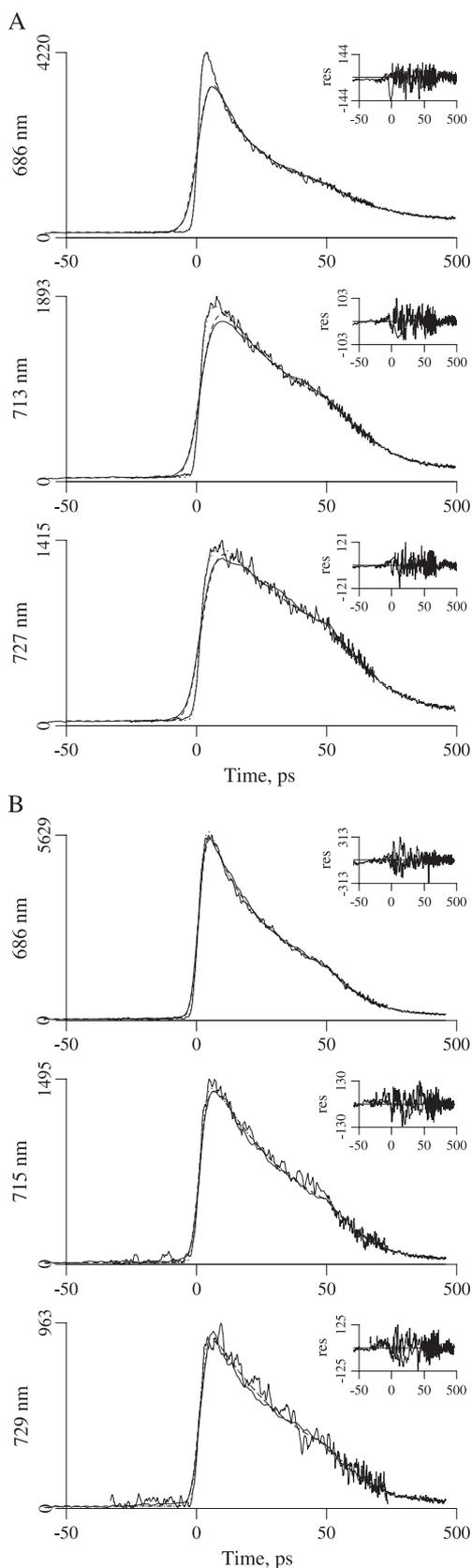


Fig. 3. Room temperature decay-associated spectra (DAS) of fluorescence decay of isolated PSI particles from *Arabidopsis thaliana* (A) and from *Chlamydomonas reinhardtii* (B) after 400 nm short pulse excitation. In both cases, the excitation takes place via Chl *a* Soret-transition.

on this time scale (see also Table 1). The maximum of the negative part is located at 705 nm, which is blue-shifted from that of *A. thaliana*, in line with the shorter wavelength of the red pigments in *C. reinhardtii*. The next two components (27 ps and 68 ps) in Fig. 3B correspond to the trapping of excitations by charge separation in PSI–LHCI particles. The shapes of the spectra are similar to each other, only slightly more red emission proportional to bulk emission can be observed in the latter spectrum. The time constants are faster than those of PSI–LHCI from

Fig. 2. The fluorescence decay traces of *Arabidopsis thaliana* (A) and *Chlamydomonas reinhardtii* (B) at detection wavelengths of 686 nm, 713–715 nm and 727–729 nm. The two traces represent data at two different time bases, i.e., lower time resolution follows measurement with longer time base (200 ps vs. 800 ps in the case of *A. thaliana* (A) and 200 ps vs. 550 ps in the case of *C. reinhardtii* (B)). The dotted and dashed lines correspond to the global fits of data measured with short and long time bases, respectively, and the insets correspond to the residuals of the traces. Note that the time axes in the decay traces are linear between –50 and 50 ps and logarithmic at later delay times.

Table 1

Time constants of isolated PSI–LHCI particles from *Arabidopsis thaliana* and *Chlamydomonas reinhardtii* obtained by global analysis from time-resolved fluorescence measurements

| Description | <i>A. thaliana</i> | | <i>C. reinhardtii</i> | |
|---|--------------------|-----|-----------------------|-----|
| Soret-Q _y -transition ^a | <1 ps | | <1 ps | |
| Energy transfer/trapping | 5 ps | 3% | 5 ps | 18% |
| Trapping | 20 ps | 52% | 27 ps | 57% |
| Trapping | 82 ps | 45% | 68 ps | 25% |
| Average lifetime <τ> | 47 ps | | 33 ps | |

The error of the lifetime analysis is about 10%. The excitation wavelength was 400 nm and mainly Chl *a* molecules were excited. The free Chl *a* contribution (~5-ns component) is not shown in the table. The average lifetime of a particle was calculated with $\langle\tau\rangle = \sum_i \alpha_i \tau_i$, where α is the integrated area of the DAS (Fig. 3), shown in percentages on the right columns.

^a Below the limit of the time-resolution of the apparatus (~4 ps).

A. thaliana and both trapping spectra show clearly less red emission. The main trapping component has a time constant of 27 ps and contributes 57% of the total decay of the system. The slowest trapping phase has a decay time constant of about 68 ps with about 25% of decay proportion. Compared to the 82-ps component spectrum of *A. thaliana*, the spectrum of the 68-ps component lacks the reddest band. The 27-ps trapping component can be assigned to the pigments of the core antenna and part of the peripheral antenna, whereas the latter component reflects excitation trapping from the chlorophylls of LHCI proteins only. A recent time-resolved study of similar *C. reinhardtii* particles, where both isolated PSI–LHCI and PSI core particles were studied [37], confirms this idea. It was observed that PSI core without LHCI has only one trapping component with lifetime of about 26 ps. A difference between lifetimes in the study in Ref. [37] and this study was the longest trapping component of PSI–LHCI particle being about 100 ps lifetime in Ref. [37] instead of about 70 ps as in our study. This kind of difference in the decay lifetimes can be caused by slightly different measurement conditions or particle preparation. However, the spectra of the decay components were rather similar in both studies and conclusions shown below could, in principle, be drawn from both sets of experimental data.

4. Discussion

Emission kinetics of similar PSI–LHCI particles from *A. thaliana* under 475 nm and 710 nm excitation have been reported by us in Ref. [25]. In the present study, however, we did not use the six-component analysis for *A. thaliana* particles as in Ref. [25], but a five component analysis for both systems, because the excitation kinetics from PSI LHCI particles *C. reinhardtii* could be analyzed only with five components. A comparison between the two types of particles is more straightforward when the same number of components is used. We checked that a six-component analysis of the data in this study gave similar results as those

in Ref. [25]. The quality of the fit in the case of *A. thaliana* lowers only with about 6% when a five-component instead of six-component fit is used. Most likely, the improvement from five- to six-component analysis reflects the inhomogeneity of the PSI–LHCI systems, both in terms of antenna size and energy of the red pigments. Then, in the case of six-component analysis, the large inhomogeneity is taken into account more accurately, and the longest component in the six-component fit (about 120 ps) describes trapping from the complexes that occupy the ‘reddest’ red pigments. We note, that the average lifetime of the emission decay does not depend on the number of components used in the analysis.

By comparing the excitation kinetics of the studied particles, it becomes immediately clear that the fluorescence lifetimes of the isolated PSI–LHCI complexes from the green algae *C. reinhardtii* are shorter than those from the green plant *A. thaliana*. Although the antenna of *C. reinhardtii* is about 30–60 chls larger than the one of *A. thaliana*, the average lifetime in the case of *C. reinhardtii* is 33 ps whereas in the case of *A. thaliana* it is 47 ps (Table 1). Thus, the factor that determines the overall lifetime is in the first place the energy and amount of the red pigments and in the second place the size of the antenna.

In order to demonstrate the ‘physical origin’ of the observed decay time differences between the green plant and green alga PSI–LHCI particles, we modelled our time-resolved data by the spectrotemporal model shown in Fig. 4A. A comprehensive description of this way of modelling can be found in Ref. [35].

To obtain species-associated spectra (SAS) by this method, similar constraints as in the study of Gobets et al. [22] on cyanobacterial PSI core complexes were used. By assuming that the oscillator strengths of all the emitting species are the same, the areas of the emission spectra of each compartment were restricted to be equal (within 10%). This restriction influences the excitation proportions and trapping times of each compartment. The first spectrum describes the transition from the Soret-states, and from carotenoids and Chl *b* molecules, to Chl *a*. As in the global analysis, this spectrum has an amplitude only in the Chl *b* Q_y-emission range, whereas the spectrum was set to zero in the Chl *a* emission range. The emission of the red pigment compartment was set to zero for wavelengths below 680–690 nm. The back transfer rates between compartments were forced to follow detailed balance with the Boltzmann factor $k_B T$: ($k_b = k_f (N_b/N_f) \exp(-\Delta E_{bf}/k_B T)$), where k_b and k_f are the backward and forward rates, N_f and N_b are the degeneracy factors estimated for each compartment (noted in percentage of the total chlorophyll content of the particles in the ‘compartment-boxes’, Fig. 4A) and the ΔE_{bf} is the mean energy difference between each compartment, obtained from the emission spectra of the compartments (Fig. 4B,C). The excitation energy trapping and equilibration time constants were estimated from the obtained time-resolved fluorescence data while the spectral restrictions

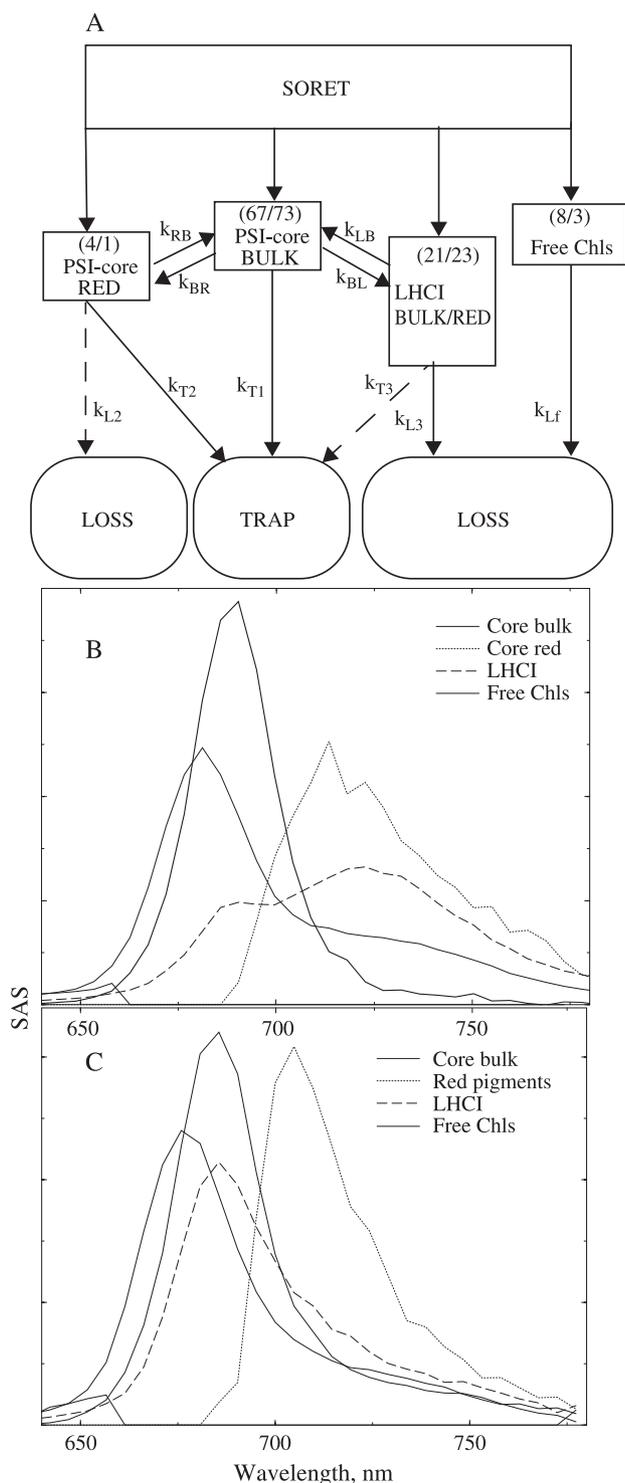


Fig. 4. Emission decay pathway model in isolated PSI–LHCI particles after 400 nm excitation. (A) The obtained energy equilibration and trapping rates are noted in Table 2 and amplitudes of the lifetimes in Tables 3A and 3B. Numbers in parentheses denote the initial excitation distribution in percentage (%), which effectively denotes the number of Chl *a* molecules, in each compartment for the two preparations (*Arabidopsis thaliana*/*Chlamydomonas reinhardtii*). Emission spectra of the compartments (species associated spectra) of the isolated PSI–LHCI particles from *A. thaliana* (B) and from *C. reinhardtii* (C).

were fulfilled. Naturally, the observable lifetimes and the DAS (not shown) resulting from the target analysis must resemble those from the global analysis (Table 1 and Fig. 3). The obtained values from the compartmental analysis are collected in Tables 2, 3A and 3B, and the resulting SAS are shown in Fig. 4B,C.

In the modelling, the “PSI-core bulk” compartment gains about 67% and 73% of the initial excitation distribution for *A. thaliana* and *C. reinhardtii*, respectively, which, after subtraction of the free chlorophyll part (8% and 3%) accounts for about 123 and 163 Chl *a* molecules, respectively. Thus, the compartment “PSI-core bulk” is not only the core antenna that is introduced in the structural PSI–LHCI model [5], but rather the bulk pigments in the core antenna, between the region of LHCI and core complex, and also some Lhca-pigments that are in close vicinity to the core complex and far away from the red pigments. The trapping times of this compartment (about 21 ps and 23 ps, for *A. thaliana* and *C. reinhardtii*, respectively, $1/k_{T1}$ in Table 2) are slightly longer than those estimated for cyanobacterial PSI with no red chlorophylls [22,31], as expected, because of the larger compartments in the present case. However, if the trapping time would reflect linearly the pigment content, the trapping times would be 22 ps and 29 ps for *A. thaliana* and *C. reinhardtii*, respectively. A possible reason for this discrepancy may be in the different molar absorption coefficients and spectra of P700 from different species, as reported by Witt et al. [38]. The second compartment “PSI-core red” collects about 4% and 1% of excitation, which corresponds to about six and two Chl *a* molecules in *A. thaliana* and *C. reinhardtii*, respectively. In the case of *A. thaliana*, this compartment can safely be attributed to the red pigments of the core complex. The emission maximum of the spectrum is around 713 nm and the energy transfer rates between the bulk antenna and the red pigments (e.g., k_{BR} and k_{RB} in Table 2) and the trapping rate (k_{T2}) are similar to those found by Gobets et al. [22] for cyanobacterial PSI particles. In the case of *C. reinhardtii*, the location of this red pigment pool is not as clear as in the case of green plants (see

Table 2

Decay and excitation equilibration rates (in ns^{-1}) obtained from the target analysis

| Description | <i>A. thaliana</i> | <i>C. reinhardtii</i> |
|-------------|--------------------|-----------------------|
| k_{T1} | 47.8 | 44.3 |
| k_{T2} | 26.6 | 0 |
| k_{T3} | 0.43 | 0.43 |
| k_{L2} | 0.52 | 0 |
| k_{L3} | 6.81 | 11.0 |
| k_{LF} | 0.19 | 0.19 |
| k_{BR} | 62.1 | 21.3 |
| k_{RB} | 108 | 151 |
| k_{BL} | 14.8 | 3.27 |
| k_{LB} | 7.08 | 4.83 |

The descriptions of the symbols are shown in Fig. 4A.

Table 3A

Amplitude matrix of the lifetimes as a result of the target analysis of PSI–LHCI particle from *Arabidopsis thaliana* (see also Refs. [25,34])

| Lifetimes | Soret | Bulk | Red (core) | LHCI | Free | Trap |
|-----------|-------|-------|------------|-------|-------|-------|
| 1.0 ps | 1.0 | −0.76 | 0.01 | −0.20 | −0.08 | 0.04 |
| 5 ps | 0 | 0.36 | −0.29 | −0.03 | 0 | −0.04 |
| 21 ps | 0 | 0.36 | 0.26 | −0.15 | 0 | −0.48 |
| 80 ps | 0 | 0.05 | 0.05 | 0.38 | 0 | −0.24 |
| 5 ns | 0 | 0 | 0 | 0 | 0.08 | 0 |
| ∞ | 0 | 0 | 0 | 0 | 0 | 0.72 |

Data are to be compared with the values from global analysis (Table 1).

Introduction). The higher energy of the red pigments of *C. reinhardtii* is reflected by the maximum of the emission band at about 705 nm and the uphill energy transfer from the red pigments to the bulk pigments being faster than in the case of *A. thaliana*. In the case of *C. reinhardtii*, no direct decay from the “red”-compartment could be observed. The final spectrum of the PSI–LHCI particle, noted as a “LHCI bulk/red”-compartment, collects about 21% and 23% of the initial excitation in the case of *A. thaliana* and *C. reinhardtii*, respectively. This corresponds to about 33 Chl *a* molecules in *A. thaliana* (i.e., two to three monomeric units most likely the ones containing the red chlorophylls, Lhca3 and Lhca4 [18,39]) and about 51 Chl *a* molecules in *C. reinhardtii* (i.e., about four to five monomeric Lhca units [8,12–14]). The spectra show clear differences between the two species, as expected from the known energies of the red pigments (see Fig. 1 and Introduction). The excitation equilibration rates are rather long, mainly because of the large pigment contents of the compartments “PSI-Core bulk” and “LHCI bulk/red”. We note that the k_{nm} values do not imply a single energy transfer step between the chlorophylls in “PSI-core bulk” and “LHCI”, but rather the overall time, which is required for equilibration processes between and within the compartments and involve many single energy transfer steps. Most likely, single energy-transfer steps between closely contacted chlorophylls of different subunits (say 10–20 Å, as resolved from the PSI–LHCI structure [5]) are ultrafast. Then, in both cases, in order to get similar residuals as in the case of global analysis, a direct decay of the “LHCI bulk/red”-compartment was needed. We divided this decay into two channels: a direct trapping to P700 from the “LHCI” and a loss channel. The direct trapping rate was estimated by analyzing all distances of the antenna chlorophylls to the P700

Table 3B

Amplitude matrix of the lifetimes as a result of the target analysis of PSI–LHCI particle from *Chlamydomonas reinhardtii* (see also Refs. [25,34])

| Lifetimes | Soret | Bulk | Red | LHCI | Free | Trap |
|-----------|-------|-------|-------|-------|-------|-------|
| 1.0 ps | 1.0 | −0.78 | 0 | −0.23 | −0.03 | 0.03 |
| 6 ps | 0 | 0.17 | −0.12 | −0.03 | 0 | −0.04 |
| 25 ps | 0 | 0.56 | 0.11 | −0.08 | 0 | −0.61 |
| 63 ps | 0 | 0.05 | 0.01 | 0.31 | 0 | −0.15 |
| 5 ns | 0 | 0 | 0 | 0 | 0.03 | 0 |
| ∞ | 0 | 0 | 0 | 0 | 0 | 0.77 |

chlorophylls using the green plant PSI–LHCI structure from Ref. [5]. A constant term, which reflects the common spectral overlap and the orientation of the molecules, was estimated by averaging the ‘trapping’ rates of all core antenna pigments to P700 to be 21 ps, when divided by the distance-term (R^6). The average ‘direct’ trapping time (k_{T3})^{−1} from “LHCI bulk/red” then amounts to about 2.3 ns, which is close to the average emission lifetime of isolated LHCI particles, in line with the fact that the average distance (72 Å) of the LHCI chlorophylls to P700 is approximately equal to the Förster-radius for Chl *a* to Chl *a* energy transfer. By using this information, the loss channel (k_{L3})^{−1} is described by lifetimes of about 150 ps and 91 ps for *A. thaliana* and *C. reinhardtii*, respectively. We also implemented a loss channel (k_{L2}) for the core red pigments and estimated that to be in the order of the emission lifetime of the PSI-core at low temperatures (~2 ns, J. Ihalainen, unpublished data).

The effect of the lower amount and the higher excitation energies of the red pigments in LHCI of *C. reinhardtii* on the excitation decay lifetimes is most clearly demonstrated in Tables 3A and 3B, where the amplitudes of each compartment for each lifetime are tabulated. Two main points can be observed. First, the PSI–LHCI particle from *A. thaliana* (Table 3A) shows a stronger excitation flow from the “PSI-core bulk” antenna to the “red” pigments during the 5-ps phase than that of *C. reinhardtii* (Table 3B). Second, during the ~20-ps phase, a rather large increase of excitations in “LHCI bulk/red” compartment can be seen in the case of *A. thaliana*, whereas in the case of *C. reinhardtii*, larger decay of excitation from “PSI-core bulk” and a minor increase of excitation in “LHCI bulk/red” is obtained. Consequently, the “trap” compartment of *C. reinhardtii* gains more excitation during the ~20 ps than the one in *A. thaliana*, which in turn has a higher amplitude

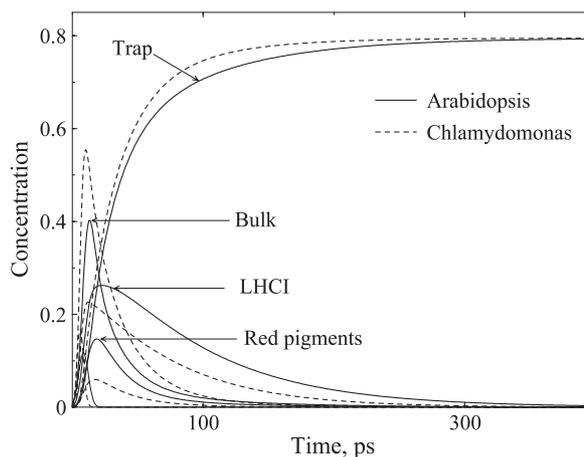


Fig. 5. The evolution of the excitation concentration of each compartment. For sake of clarity, the ‘Free Chlorophyll’ compartment has been left out and the excitation contribution of the free chlorophylls (8% and 3% in *Arabidopsis thaliana* and *Chlamydomonas reinhardtii*, respectively) are scaled away. Thus, the concentration of the ‘trap’ compartment describes the ‘photosynthetic yield’ of PSI–LHCI system in our experiment.

during the slow trapping phase (80 ps in *A. thaliana* and 63 ps in *C. reinhardtii*, Tables 3A and 3B).

The observed faster trapping time in case of *C. reinhardtii* does not, however, influence the overall trapping yield of PSI–LHC complex. As seen from Fig. 5 and Tables 3A and 3B ('Trap' columns, infinite lifetimes), regardless of the faster excitation rise of the trap in case of *C. reinhardtii*, the yield is about the same (about 80%, after subtraction of the free pigment contribution) in both cases. We notify that this value is slightly lower than the commonly assumed (>90%) photochemical yield of PSI systems. We could not, however, find a trustworthy study on the absolute photochemical yield of isolated and purified PSI–LHCI particles. As seen from the model in Fig. 4 and from that in Tables 3A and 3B, the main loss channel locates in the "LHCI bulk/red" compartment. This is in line with time-resolved studies with isolated LHCI proteins, where several decay components with lifetimes between 20 and 600 ps have been observed [36,40]. On the other hand, we cannot completely rule out that the 'missing' 10–15% of the photochemical yield in our model is a modelling artefact.

We estimated that in both of our experiments, emission from about 5% closed P700, due to partial second excitation in the laser spot, is detected. On the other hand, we estimate that about 11–13% of the particles can receive a second excitation, which most likely effects to our data similarly as 'closed' P700, because of the large size of the PSI–LHCI particle. In our model, P700+ then act similarly as a 'true' P700 trap.

We note that similar target analysis methods for fluorescence data have been published for cyanobacterial PSI core particles [22], and for pump-probe data of *C. reinhardtii* PSI core particles [26]. We tested the model published by Müller et al. [26] with the presented data and observed that introducing back transfer from the reaction centre back to antennae does not significantly improve the description of the data. Therefore, the kinetics between antennae and the reaction centre are modelled with a unidirectional loss/trapping of excitation, as described by Gobets et al. [22].

5. Concluding remarks

In time-resolved studies of PSI systems, two main trapping times have been observed, if the system has a peripheral light-harvesting antenna with red pigments [21,25; this study]. If the PSI core complex has several red pigment pools (like PSI systems from *Synechococcus elongatus* and *Spirulina plantesis*) or a peripheral antenna without red pigments (like the PSI–IsiA complex), the trapping time increases but stays monophasic when compared to the PSI core particles without red pigments [22,30,31]. Thus, the trapping times have been shown to depend on the excitation energy level and the number of red pigments, but also on the size of the antenna complex. In

this study, we show that in PSI–LHCI particles from *C. reinhardtii*, which have a large peripheral antenna and a low (but not zero) amount of red pigments, "some" lifetimes are longer than in the cyanobacterial PSI systems, where no LHCI with red chlorophylls protein is present. On the other hand, PSI–LHCI particles from *C. reinhardtii* show clearly faster average excitation decay time than that of *A. thaliana*, where a smaller LHCI is present but with a higher amount of red pigments. By using a spectrotemporal model, we demonstrate why the size of the peripheral antenna is not as important as the number, position, or energy of the red pigments on the excitation lifetime of the system. However, because the trapping times are in both cases very fast, the observed lifetime difference does not influence the overall photochemical yield of the studied particles.

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