Energy transfer and trapping in the Photosystem I complex of *Synechococcus* PCC 7942 and in its supercomplex with IsiA

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**Abstract**

The cyanobacterium *Synechococcus* PCC 7942 grown under iron starvation assembles a supercomplex consisting of a trimeric Photosystem I (PSI) complex encircled by a ring of 18 CP43 or IsiA complexes. It has previously been shown that PSI of *Synechococcus* PCC 7942 contains less special long-wavelength (‘red’) chlorophylls than PSI of most other cyanobacteria. Here we present a comparative analysis by time-resolved absorption difference and fluorescence spectroscopy of the processes of energy transfer and trapping in trimeric PSI and PSI–IsiA supercomplexes from *Synechococcus* PCC 7942. All experiments were performed with the primary electron donor of PSI (P700) in the oxidized state. Our data suggest that in the PSI complex the excitation energy is equilibrated with a lifetime of 0.6 ps among the so-called bulk chlorophylls, is distributed in 3–4 ps between the bulk and red chlorophylls, and is trapped in the reaction center in 19 ps. This trapping time is shorter than that observed for other cyanobacteria, which we attribute to the lower content of red chlorophylls in PSI of this organism. In the PSI–IsiA supercomplexes, the distribution of excited states is blue-shifted compared to that in PSI, leading to a lengthening of the equilibration processes. We attributed a phase of about 1 ps to initial energy equilibration steps among the IsiA and PSI core bulk chlorophylls, a 5–7 ps phase to equilibration between bulk and red chlorophylls within the PSI core, and a 38 ps phase to trapping in the reaction center. The data suggest that the excitation energy is equilibrated among the IsiA and PSI core antenna chlorophylls before trapping occurs. Data analysis based on a simple kinetic model revealed an intrinsic rate constant for energy transfer from IsiA to PSI in the range of 2–F 1 ps. Based on this value we suggest the presence of one or more linker chlorophylls between the IsiA and PSI core complexes. These results confirm that IsiA acts as an effective light-harvesting antenna for PSI.

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**Keywords:** Photosystem I; PSI–IsiA; IsiA; *Synechococcus* PCC 7942; Transient absorption; Time-resolved fluorescence

1. Introduction

PSI–IsiA supercomplexes were found in the cyanobacteria *Synechocystis* PCC 6803 and *Synechococcus* PCC 7942 grown under conditions of iron limitation [1,2]. According to the structural models presented in Refs. [1–4], each PSI–IsiA supercomplex consists of a trimeric Photosystem I (PSI) core complex encircled by 18 IsiA or CP43’ subunits. These models were based on the known crystal structure of the PSI complex of the thermophilic cyanobacterium *Thermosynechococcus elongatus* [5] and of the model of the structure of the IsiA-related CP43 subunit of PSI [6]. Recent spectroscopic studies have shown that IsiA increases the absorption cross-section of PSI and acts as an additional antenna complex for PSI [7,8]. Physiological studies, however, assigned a photoprotective function to IsiA because it was suggested to remove excess excitation energy [9]. It was recently reported that IsiA not only accumulates under conditions of iron stress, but also under conditions of oxidative stress [10].

The processes of energy transfer and trapping in PSI of different cyanobacteria were extensively studied during last decades (see Refs. [11–13] for recent reviews). The equilibration of excitation energy in the PSI core complex starts with the fast relaxation among excitonic states in the antenna, followed by random hopping of the localized
excitations between nearest neighbors in the bulk antenna. Within a short time (200–600 fs) a group of chlorophylls (chls) absorbing at 685 and 700 nm becomes populated. Then, the excitation energy is distributed between the bulk and long-wavelength absorbing chlorophylls (red chlorophylls) in 3–8 ps, and trapped by charge separation in the reaction center (RC) in 23–50 ps. The trapping time was shown to depend on the amount and spectral composition of the red chlorophylls [14].

PSI from Synechococcus PCC 7942 occupies a unique position among the various species of cyanobacteria regarding the properties of the red chlorophylls. In this complex, the red chls give rise to 5 K absorption and fluorescence maxima at 703 and 713–714 nm, respectively, while their oscillator strength corresponds to that of two chl molecules [7]. In contrast, all cyanobacterial PSI complexes that were spectroscopically analyzed in detail thus far contain red-most chlorophylls with a 5 K absorption band peaked at least at 708 nm with the oscillator strength of several chl molecules [14–18]. One could thus expect shorter trapping times for Synechococcus PCC 7942 than for most other cyanobacteria [14].

Time-resolved studies on isolated IsiA particles have not yet been performed. Because the structure and spectroscopy of this complex resembles that of the CP43 complex of PSII, similar energy equilibration kinetics may be expected for both complexes. For CP43 it was shown that at 77 K the excitation energy fully equilibrates among all ~13 chls in about 2 ps [19]. Recently, Melkozernov et al. [8] reported a first analysis of the excited state dynamics of PSI–IsiA supercomplexes of Synechocystis PCC 6803. The authors concluded that there is a rapid and efficient energy transfer between the outer antenna ring and the PSI core complex. They attributed a 0.2 ps component to energy transfer processes within or between IsiA complexes, a 1.7 ps component to energy transfer from the IsiA antenna ring to the PSI core and a 10 ps component to the overall excitation transfer from IsiA to PSI. Based on a model of the three-dimensional structure of the PSI–IsiA supercomplexes [4], the shortest distance between chls in neighboring IsiA subunits was estimated to be about 10 Å, which suggests that energy transfer within the IsiA ring could be extremely rapid. It was also suggested that some chls of the PSI core are located in the vicinity (22–33 Å) of chls of the IsiA ring, thus allowing fast energy transfer between IsiA and the PSI core.

In our previous work [7] we have studied PSI trimers and PSI–IsiA supercomplexes from Synechococcus PCC 7942 by various types of steady state absorption and fluorescence spectroscopy. It was shown that the IsiA ring increases the absorption cross-section of PSI by about 100% and functions as an efficient light-harvesting complex for PSI. In this work we analyze these complexes by subpicosecond absorbance difference and fluorescence techniques in order to find out how the increase of the antenna affects the performance of PSI in terms of rates of energy equilibration and trapping.

2. Materials and methods

2.1. Sample preparation

PSI–IsiA, PSI and IsiA particles were isolated and prepared from the cyanobacterium Synechococcus PCC 7942 as described before [7]. For the spectroscopic measurements, all samples were diluted in a buffer containing 20 mM Bis-Tris (pH 6.5), 10 mM MgCl2, 10 mM CaCl2 and 0.03% n-dodecyl-β-D-maltoside (β-DM). The optical density (OD) of the samples used for the transient absorption and fluorescence measurements was about 0.7 mm−1 and 0.8 cm−1, respectively, at the Qy absorption maximum.

2.2. Transient absorption

Absorption difference spectra were recorded with a femtosecond spectrophotometer, described in detail elsewhere [20]. In brief, the output of Ti:Sapphire oscillator (Coherent Mira) was amplified by means of chirped pulse amplification (Alpha-1000 US, B.M. Industries), generating 1 kHz, 800 nm, 60 fs pulses. Single-filament probe white light was generated in a 2 mm sapphire plate. Pump light at 400 nm was obtained by doubling the 800 nm fundamental. The energy of excitation was 10 nJ/pulse. The excitation beam was focused in a spot with 400 μm diameter. We estimated that about one to two photons were absorbed by each complex per laser shot. Transient absorption difference spectra were collected with probe and excitation beams oriented at magic angle. The cuvette (1 mm pathlength) was shaken in order to refresh the sample from shot to shot. The time resolution was 100 fs and the spectral resolution was 3 nm. The steady state absorption of the sample before and after measurements did not show any changes. The data were corrected for white light group velocity dispersion and instrument response, and fitted globally as described in Ref. [21].

2.3. Time-resolved emission

Time-resolved fluorescence emission spectra were recorded with a Hamamatsu C5680 synchroscan streak camera as described in Ref. [14]. In short, the output of a Ti:Sapphire oscillator (Coherent Mira-Rega), generating 125 kHz, 800 nm, 150 fs pulses, was doubled via an OPA (Coherent), producing 125 kHz, 400 nm, 150 fs pulses. The sample was placed in a spinning cell (diameter 10 cm) with rotation frequency of 75 Hz. The excitation energy was 1–2 nJ/pulse and the excitation beam was focused in a spot with 150 μm diameter, corresponding to about 0.25–0.5 absorbed photons per complex. Fluorescence was collected...
at magic angle with respect to the polarization of the excitation beam. The time resolution was 4 ps and the spectral resolution was 4 nm. The steady state absorption of the PSI complexes did not show any differences before and after the measurements. In some cases, the steady state absorption of the PSI–IsiA complexes lost up to 2–3% of its initial amplitude, while the absorption maximum shifted slightly to the blue (always less than 1 nm), suggesting that a small fraction of the PSI–IsiA supercomplexes decomposed during the measurements.

The fluorescence data were corrected for white light group velocity dispersion and instrument response, and fitted globally as described in Ref. [14].

3. Results

3.1. Transient absorption

Fig. 1 shows the room temperature absorption spectra of the isolated PSI–IsiA, IsiA and PSI complexes of Synechococcus PCC 7942. In the Qy absorption region of the chls, the spectra peak at 674, 669 and 679 nm, respectively. The absorption of the Qy band of the PSI core complex is clearly red-shifted compared to that of IsiA, suggesting that excitation of IsiA in the PSI–IsiA supercomplex will initiate downhill energy flow towards the PSI core. However, the strong overlap of the Qy bands of PSI and IsiA makes it impossible to excite IsiA selectively within a supercomplex. We thus applied nonselective (400 nm) laser excitation to record ultrafast absorption changes in the PSI and PSI–IsiA complexes.

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Global analysis of the transient absorption spectra revealed five components for both PSI and PSI–IsiA complexes. The decay-associated difference spectra (DADS) are shown in Fig. 3. We note that most absorption upon 400 nm excitation originates from the Soret transitions of the chls, while only a
A small fraction is absorbed by the \( \beta \)-carotene molecules in the PSI core or IsiA complex (see Ref. [22] for an analysis of transient absorption changes in the PSI core complex of \( T. \) elongatus upon selective \( \beta \)-carotene excitation). We also note that under our experimental conditions, P700 occurs almost exclusively in the oxidized state. The underlying mechanisms for the quenching of excitation are significantly different with P700 in the reduced and oxidized state. When P700 is reduced (open RC) quenching occurs due to photochemistry while with oxidized P700 (closed RC) the photochemistry is blocked and excitations are quenched by P700\(^+\). The mechanism of this quenching is unclear at present. The trapping kinetics of cyanobacterial PSI complexes, however, have been shown to be very similar with reduced and oxidized P700 [23,24].

In the PSI core complex, the fastest component has a lifetime of 90 fs. The positive amplitude of this DADS means that the excited states of the \( Q_y \) absorption band are populated during this time, and we associate this phase with Soret to \( Q_y \) relaxation processes. The small negative band on the blue side of the positive amplitude suggests some additional complexity in the DADS possibly due to the presence of some ultrafast relaxation within the \( Q_y \) band. The next DADS has a lifetime of 0.6 ps and is characterized by a negative part with minimum at 672 nm and positive part with maximum at 687 nm. This phase can be attributed to downhill energy transfer between chls absorbing maximally at about 670 and 680–690 nm. The next component of 3.2 ps has a minimum around 680 nm and maximum around 700–703 nm. We attribute this phase to energy transfer from the bulk chlorophylls to the red species. However, this component is non-conservative, i.e. the negative part of this component has a larger area than the positive part. This means that a small part of the excitations disappear from the \( Q_y \) range during the 3.2 ps phase, most probably by trapping in the reaction center. The next component has a lifetime of 19 ps and is the last component with a large amplitude. Its negative amplitude implies that the ground state of the chlorophylls is largely recovered during this phase. This phase can therefore be attributed to the trapping time of the excitation energy by oxidized P700. The last DADS has a very small amplitude and a lifetime of at least 4 ns, and can be attributed to the decay of excited states of unconnected chlorophylls.

In PSI–IsiA, the 400 nm excitation will induce an about equal distribution of the excitations between the IsiA and PSI core chls. The first DADS (Fig. 3B) has a lifetime of 100 fs and its mostly positive amplitude suggests that this phase originates from Soret to \( Q_y \) relaxation. There is again a negative band on the blue wing of the amplitude, indicating some ultrafast relaxation within the \( Q_y \) band of PSI and/or IsiA. The next component has a lifetime of 1.2 ps and a rather complicated shape. It is likely that several processes contribute to this phase. The equilibration of excitation energy within the PSI core antenna is expected to occur in about 0.6 ps (see above), whereas the equilibration of excitation energy within IsiA may also proceed in this time range, because in the related CP43 protein of PSII, at 77 K, two equilibration phases were observed with lifetimes of about 0.2–0.4 and 2–3 ps [19]. In addition, initial energy transfer steps between IsiA subunits and between IsiA and PSI are also possible during this time. The next DADS has lifetime of 6.6 ps, and shows a minimum at 680 nm and a maximum at 700–703 nm. This phase can be attributed to the energy transfer from bulk chls to red forms. This DADS has an additional bleaching at 662 nm compared to the 3.2 ps component in PSI, from which we conclude that the transfer of energy from the IsiA antenna to the PSI core complex also contributes to this phase. The IsiA to PSI energy transfer could also suggest the longer time of this component in the PSI–IsiA supercomplex compared to that of the corresponding phase in the PSI core complex. Again, like in the PSI core complex, this component has a non-conservative amplitude, which suggests that a small part of the excitation energy is trapped in the RC already during this 6.6 ps phase. The next DADS represents the trapping component. It has a lifetime of 38 ps, which is two times longer than in the isolated PSI core complex.
In Fig. 3C, we compare the 38 ps component of PSI–IsiA with the 19 ps trapping component of the PSI core complex. This representation makes clear that in the supercomplex higher energy levels contribute more strongly to the trapping by the reaction center than in the PSI core complex. This in turn leads to the conclusion that the excitation energy is not fully transferred from IsiA to PSI before trapping occurs, but is distributed between the peripheral and core antenna complexes. Thus the presence of the IsiA antenna leads to a blue shift of the excited states distribution and to a lengthening of the lifetimes of various equilibration and trapping processes.

3.2. Time-resolved fluorescence

We have also measured fluorescence decays of the PSI and PSI–IsiA complexes upon 400 nm excitation. Compared to the transient absorption measurements, these measurements were carried out with about 40 times lower time resolution, four times less energy per pulse but a 125 times higher laser frequency. This higher frequency probably explains why with these types of experiments a slight damage of the PSI–IsiA complexes was observed after the measurements (see Materials and methods).

Fig. 4 shows decay-associated emission spectra (DAES) obtained from the global analysis. Four components were sufficient to obtain a good fit of the data, because the time resolution did not permit a good resolution of the subpicosecond processes. The fastest component is fitted with lifetimes of 0.4 ps in the PSI core complex (Fig. 4A) and 0.5 ps in the PSI–IsiA supercomplex (Fig. 4B) and due to its positive amplitude could be mainly attributed to the Soret to Qy relaxation. The next DAES has a positive amplitude at 660–700 nm and a negative amplitude at 700–740 nm for both complexes and reflects energy transfer from bulk to red spectral forms. The 5.2 ps lifetime of this process in PSI–IsiA is probably prolonged compared to the corresponding 3.7 ps phase in the PSI core complex because of additional energy transfer processes from the IsiA antenna to the PSI core complex. Similar to the pump-probe data, these components have a non-conservative character, meaning that part of the excitations disappears from the system, most probably by trapping in the reaction center. The third DAES has a lifetime of 19.6 ps in the PSI core complex and 31.3 ps in the PSI–IsiA supercomplex. Within these times, the excitations disappear from the Qy range because of trapping in the reaction center by quenching by P700+. The next DAES (about 5 ns) has a minor amplitude in the PSI core, but a larger amplitude in the supercomplex. We assign this component to the fluorescence of uncoupled chlorophylls. The larger amplitude of this component in the supercomplex can be explained by a slight sample degradation during these particular measurements, which also resulted in a <1 nm blue shift of the Qy absorption maximum. This slight degradation means that some amount of disconnected IsiA and PSI units were accumulated in the sample during the
measurements. This fact can also explain the shortening of the lifetime of the trapping component in the time-resolved fluorescence measurements (31 ps) compared to that observed in the transient absorption measurements (38 ps), where sample degradation did not occur.

In Fig. 4C, the trapping components of the PSI core and the PSI–IsiA supercomplex are shown together for comparison. Obviously, the maxima of the PSI–IsiA DAES are blue-shifted compared to those of PSI, meaning that the excited state distribution in PSI–IsiA is blue-shifted due to the presence of excited IsiA chls. The trapping time in the PSI–IsiA supercomplex is about two times longer than in the PSI core complex.

### 4. Modelling

Our data reveal that the additional IsiA antenna leads to a blue shift of the excited state distribution and a lengthening of the trapping time in the PSI–IsiA supercomplex by a factor of about 2.

A simple kinetic model was applied to achieve a better understanding of these processes and to estimate the intrinsic rate of energy transfer from IsiA to PSI. The model is similar to the compartmental model described by Gobets et al. [14], which was used to successfully describe the observed fluorescence kinetics in cyanobacteria with different contents of red chlorophylls.

In the case of the PSI core complex, the model constitutes two compartments (Fig. 5A): “Bulk” and “Red” are associated with the average energy levels of the bulk and red chls of the PSI core complex, respectively. The energy gap between the two levels is expressed by the parameter $\Delta_{BR}$ (see Table 1). The intrinsic rate constants of energy transfer from “Bulk” to “Red” ($k_{BR}$) and backward ($k_{RB}$) are connected via the Boltzmann factor: $k_{RB} = k_{BR}(N_B/N_R)\exp (-\Delta_{BR}/kT)$, in which $N_x$ is the number of chlorophylls in compartment $x$, $\Delta_{xy}$ is the energy difference between compartment $x$ and compartment $y$, $k$ is Boltzmann’s constant, $T$ is the absolute temperature. We assume that the excitation energy is irreversibly trapped by the RC (P700) from both “Bulk” and “Red” with rate constants $k_{TB}$ and $k_{TR}$, which both will define the average trapping time in the PSI core.

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<th>Parameters of the compartmental model shown in Fig. 5</th>
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<td>$\Delta_{AB}$ (cm$^{-1}$)</td>
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<td>$\Delta_{BR}$ (cm$^{-1}$)</td>
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Fig. 6. Integrated fluorescence (A,C) and delta absorption (B,D) signals for the PSI and PSI–IsiA together with a fit obtained on the basis of the compartmental model shown in Fig. 5.
complex. For the PSI–IsiA supercomplex an additional energy level (compartment) belonging to IsiA is added (Fig. 5B). It has a higher average energy than “Bulk” as it follows from the absorption properties of IsiA. “IsiA” is connected with “Bulk” via the intrinsic transfer rate constants $k_{AB}$ and $k_{BA}$ and indirectly contributes to the process of trapping by transferring excitation energy to “Bulk”. The model was described mathematically by a system of linear differential equations. The solution could be expressed through the eigenvalues of the system. The reciprocals of these eigenvalues indicate the lifetimes observed in the experiments.

In order to exclude the influence of apparatus functions on the experimentally measured kinetics, we used data obtained from global analysis of the transient absorption and fluorescence measurements, and integrated over all wavelengths. We excluded components belonging to free pigments (∼5 ns) and Soret to Q_y relaxation (∼100 fs). The model presented in Fig. 5A gives a good fit to the fluorescence decay in the PSI core (Fig. 6A). The parameters of the fit are listed in Table 1 and are compared with those obtained for Synechocystis 6803 [14]. The values are comparable for both species except that the lifetime of trapping from “Red” ($k_{TR}$) is longer for the latter species, which can be explained by the higher content of red pigments in Synechocystis 6803. Also note that ($k_{BR}$)$^{-1}$ is longer than ($k_{TB}$)$^{-1}$. This means that trapping from the “Bulk” starts earlier than transfer “Bulk” to “Red”. This explains the non-conservative character of the 3.2 and 6.6 ps (pump-probe) and 3.7 and 5.2 ps (time-resolved fluorescence) components. Slight fluctuations of the value for $\Delta BR$ do not affect the quality of the fit. We used the value 422.6 cm$^{-1}$, which corresponds to a position of “Bulk” at 678 nm and of “Red” at 698 nm (at room temperature). The model describes the overall decay processes for both the fluorescence and transient absorption experiments rather well, but do not give a perfect fit in the fast time range of the transient absorption experiments (Fig. 6B). This could be explained by the contribution of the dynamics of higher vibration levels (see above) and the presence of excited states absorption and stimulated emission.

Fluorescence kinetics and transient absorption of the PSI–IsiA supercomplexes were fitted reasonably well (Fig. 6C, D) with all corresponding parameters fixed as for PSI, while the values of the energy gap between “IsiA” and “Bulk” ($\Delta BR$) and the rate constant of energy transfer from “IsiA” to “Bulk” ($k_{AB}$) were adjusted to fit the experimental results (Table 1). It turned out that the best fit could be obtained with $k_{AB}$ in the range of 2 ± 1 ps. The relatively small energy gap between “IsiA” and “Bulk” provides the possibility of back transfer from the PSI core chls to the IsiA antenna, which explains the blue shift of the excited states distribution upon addition of IsiA and keeps IsiA excited states populated during the time excitations occur in the system.

We conclude that a very fast intrinsic rate constant of energy transfer from IsiA to PSI is required to explain the measured kinetics in PSI–IsiA supercomplexes.

5. Discussion

5.1. Energy equilibration and trapping in the PSI from Synechococcus PCC 7942

In this contribution we present a comparative analysis of the processes of energy transfer and trapping in trimeric PSI complexes and PSI–IsiA supercomplexes from the cyanobacterium Synechococcus PCC 7942, based on time-resolved difference absorption and fluorescence spectroscopy. Both methods revealed a main trapping time of 19 ps in the (trimeric) PSI core complex. As pointed out in our previous work [7], the PSI complex from Synechococcus 7942 occupies a unique position among the various species of cyanobacteria regarding the properties of the red chlorophylls. Not only the number of red chlorophylls in this cyanobacterium is smaller than in Synechocystis 6803, T. elongatus or Spirulina platensis, also the quality differs, because the red-most chlorophyll absorbs maximally at 703 nm (at 4 K) in Synechococcus 7942, whereas the other three cyanobacterial species have at least a red chlorophyll peaking at 708 nm. Even the qualification of ‘red’ chlorophyll is questionable in Synechococcus 7942, because the 703 nm absorption wavelength of this chlorophyll is about isoenergetic with that of the primary electron donor P700. Only chlorophylls that absorb at longer wavelengths than P700 should probably be considered as ‘red’ chlorophylls. The observed trapping time is slightly faster than the 20–23 ps observed in the PSI core complex from Chlamydomonas reinhardtii [25], which also has a low content of red chlorophylls.

The trapping times for the trimeric PSI complexes of Synechocystis 6803, T. elongatus and S. platensis were found to be about 23, 34 and 50 ps, respectively, and by means of a target analysis, Gobets et al. [12,14] suggested that the trapping time for a PSI complex without any red chlorophyll would be 18 ps. Our finding that a trapping time in PSI of Synechococcus PCC 7942, in which there is only one dimer of red chlorophylls that is almost iso-energetic with P700, is only slightly longer than 18 ps agrees very well with this suggestion. Our data also support the conclusion that the amount and spectral properties of the red chlorophylls are important factors that determine the trapping time in the PSI core complex.

In general, PSI can be considered as a well-organized network of energy transfer pathways [11,16,26–30]. Monte Carlo stimulations performed for the PSI core antenna system [17,30] revealed that the number of transfer steps of an excitation can be several hundreds before it is trapped in the reaction center. Initially, an excitation in a high-lying exciton state relaxes into lower-lying states, then hopping of localized excitons in an inhomogeneous distribution of lowest states is occurring until a Boltzmann distribution is reached. The rates of these processes are determined by a number of factors, such as the spectral heterogeneity of the antenna system, pigment–pigment
coupling, pigment–protein interactions and the orientation of the transition dipole moments.

In the PSI core complex from *Synechococcus* PCC 7942 the process of Soret to Qy relaxation takes place in about 90 fs. Then in 0.6 ps the excitation energy equilibrates between the chlorophylls of the core antenna absorbing in the range of 660–690 nm (bulk chlorophylls), then in 3–4 ps the excitation is distributed between the bulk chlorophylls and C703, after which the excitation is trapped by the reaction center in 19 ps.

In our kinetic model the 19 ps trapping time is an average of the lifetimes of two separate trapping processes from “Bulk” and “Red”. According to the model the trapping starts as soon as the excitation reaches either the “Bulk” or “Red” pools. This competes with a significant loss of excited states already at early times (see Fig. 6A, B). Among the pathways for loss of excitation from excited chlorophylls in photosynthetic unit there are fluorescence, non-radiative decay, formation of triplet states and trapping by reaction center. During the 60 ps in which excitations are observed in PSI the loss of excitations due to fluorescence will contribute only 1% from the total (assuming a 5 ns lifetime of free chls), which is negligibly small. In PSI–IsiA, excitations are observed during 120 ps and thus the fluorescence loss is about 2%, which is still negligibly small. This means that fluorescence losses do not play a significant role on the general decay of the excited states in a time range of 10–100 ps. Upon low-light excitation (up to 1–2 photons per complex, see Materials and methods) triplet formation and non-radiative decay also give a small contribution to the total decay of excited chls [31]. Apparently, the observed fast decay of excited states could be mostly explained by the process of trapping in the reaction center. Trapping in the RC at times earlier than the average trapping time was also observed for PSI from *T. elongatus* with reduced P700 [22]. This is in line with the fast (1–1.5 ps) lifetimes of charge separation proposed in the literature [12,17,35].

### 5.2. Role of IsiA in the excited-state dynamics of PSI

The IsiA antenna has a blue-shifted absorption compared with that of PSI. This initiates downhill energy transfer towards PSI. Our data revealed that the presence of the IsiA antenna ring around a trimeric PSI complex leads to (i) a lengthening of the lifetimes for the energy equilibration and trapping processes and (ii) a shift of Boltzmann equilibrium towards higher energies. In the PSI–IsiA supercomplexes, the IsiA molecules participate in energy transfer processes during the time excited states occur in the system. This suggests that energy transfer between IsiA and PSI occurs all the time that excited states exist in the system. Global analysis, however, did not allow us to estimate the rate of this process, but revealed two major equilibration phases of 1.2 and 5–6 ps. The fastest phase can be attributed to (mainly) initial energy equilibration steps within the individual IsiA and PSI complexes, which are overlapped with initial transfer steps among neighboring IsiA units and from IsiA to PSI. The 5–6 ps phase combines energy transfer from IsiA to PSI and equilibration in PSI (involving the red chlorophylls), and during this time IsiA loses a considerable part of the excited states. This means that in the PSI–IsiA supercomplexes, the excitation energy is delocalized over the IsiA and PSI chlorophylls during the 38 ps trapping phase by the reaction center.

The 38 ps trapping time in the PSI–IsiA complex is increased two times compared with the 19 ps observed in the PSI core complex, proportional to the doubling of the antenna size. This can be expected for both trap- and transfer-to-the-trap-limited kinetics of charge separation, and would suggest fast energy equilibration within and between the peripheral and core antenna chlorophylls before the excitations are trapped in the RC. On the basis of calculations which take into account spectral/structural heterogeneity of the antenna pigments in *Synechococcus elongatus* it was suggested [17] that the kinetics of the excited state decay are balanced between the trap- and transfer-limited cases and that PSI is optimized rather for robustness than for extremely fast trapping. It would be reasonable to attribute these properties to all PSI including *Synechococcus* PCC 7942.

Our fit based on a kinetic model in which IsiA was represented as an additional compartment revealed an intrinsic lifetime for the energy transfer from IsiA to PSI core 2 ± 1 ps. Based on this value we can estimate the distance between neighboring pigments in the IsiA and PSI connected by energy transfer, using the Förster equation (see, e.g. Ref. [19]), in which the rate of energy transfer from a donor (D) to an acceptor (A) is given by

$$k_{DA} = \frac{C_{DA}}{n^{4}} \frac{k^{2}}{R_{DA}^{6}}$$

where $C_{DA} = 32.26$ ps$^{-1}$ nm includes the overlap integral [19,32], $n = 1.55$ [19,32,33] is the refractive index, and $k = 2$ is the orientation factor for the most optimal orientation for energy transfer. As not all of the IsiA chls participate in the process of energy transfer to PSI but only those which are located closer to PSI (linker chls), the value $k_{DA}$ must be multiplied by a factor $\frac{x}{z}$, where $x$ is a total number of chls in IsiA and $z$ is a number of linker chls [34]. Taking this into account and assuming optimal orientations (head to tail) of the transition dipole moments of the donor and acceptor chls we can estimate that the distance between neighboring pigments is maximally $13–14$ Å assuming $x = 16$ and $z = 2$. Distances of $22–33$ Å were suggested for the PSI–IsiA supercomplexes from *Synechocystis* PCC 6803 [4]. The value of $13–14$ Å implies two possibilities: either the IsiA ring is located closer to PSI than thought before [4], or there are additional chls located between the IsiA ring and PSI. The first possibility is probably unlikely because of the sizes of the PSI and IsiA subunits. The second possibility may be...
in line with our estimation of 16–17 chls per IsiA subunit [7] compared with 13 chls reported for the IsiA-related CP43 subunit of PSI [6]. The additional chls could function as linker chls, which provide the possibility of fast and efficient transfer from IsiA to PSI. A good example of existence of linker chls is found in a recently published crystal structure of plant PSI [36]. The plant PSI complex consists of a PSI core complex with a very similar structure as that observed for cyanobacterial PSI and of four membrane-associated antenna complexes (LHCI) that arise from a different family of antenna proteins as IsiA. About 10 chls were found to be positioned between the different LHCl subunits and 10 other chls were found in the cleft between LHCl and the PSI core. All these chls shorten the inter pigment distances between the nearest chls in the peripheral and core antenna complexes from 18 to 10–15 Å and thus will speed up the energy transfer in the PSI complex.

In a previous study, Melkozernov et al. [8] determined the excited-state dynamics of the PSI–IsiA supercomplex from the cyanobacterium *Synechocystis* PCC 6803. The authors observed lifetimes of 0.2, 1.7, 10 and 43 ps, which they attributed to energy transfer within or between IsiA complexes (0.2 ps), energy transfer from the IsiA ring to the PSI core complex (1.7 ps), the overall excitation decay from IsiA to PSI (10 ps) and trapping in the reaction center (43 ps). The value of 1.7 ps for the energy transfer from IsiA to PSI is in agreement with our results 2 ± 1 ps. The different lifetimes may be inherent to differences in both PSI systems and/or to the applied experimental conditions (e.g., 665 nm excitation in Ref. [8] and 400 nm excitation in our study).

For instance, the slight shortening of the trapping time of 43 ps for PSI from *Synechocystis* 6803 to 38 ps for PSI from *Synechococcus* 7942 may very well originate from the lower content of red chlorophylls in the latter species. Furthermore, a Soret to Qy relaxation process (our 0.1 ps phase) does not occur in the analysis described in Ref. [9] because 665 nm excitation was used.

5.3. Function of IsiA

On one hand, the IsiA antenna increases the cross-section of absorbed light, thus under similar illumination, the PSI–IsiA supercomplex will absorb two times more photons than PSI. But on the other hand, the time before the trapped quantum of energy will be processed by the RC (trapping time) is two times slower in PSI–IsiA supercomplex than in PSI core complex. Which of these two factors (cross-section or trapping time) will determine the biological functionality of the supercomplex? As it follows from our data the excitation is indeed efficiently transferred from IsiA to RC; i.e. there are no unprocessed excitations left in the PSI–IsiA after trapping by RC has occurred. At the same time fluorescence and non-radiative losses in the PSI–IsiA are comparable with those in PSI in spite of the two times longer trapping time. This leads to the conclusion that the processing of excitations without significant losses is more important than as-fast-as-possible processing. And thus the increased light-harvesting ability due to enlarged cross-section plays the most important role in the biological functionality of the PSI–IsiA supercomplex. This principle seems to be common for different types of PSI organization. For example, it was shown that in PSI–LHCl complexes of green plants, there is also efficient energy trapping, even though the longest excited state lifetimes are about three times longer than in the PSI–IsiA complexes [37].

We note that IsiA may only harvest light for PSI when it is bound to PSI in a PSI–IsiA supercomplex. Recent evidence has shown that under some experimental conditions IsiA can accumulate in cyanobacteria without being bound to PSI (N. Yeremenko et al., unpublished observations). Under such conditions, IsiA may fulfill other roles, for instance as a quencher of excess excitation energy or as a chlorophyll storage device.

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