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Ultrafast energy transfer dynamics resolved in isolated spinach light-harvesting complex I and the LHC I-730 subpopulation

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Abstract

The energy migration in isolated bulk LHC I and its subpopulations LHC I-680 and LHC I-730 have been studied by fluorescence spectroscopy and femtosecond absorption spectroscopy. LHC I-680 and LHC I-730 display significant differences in both their steady-state and time-resolved fluorescence properties. The steady-state emission maximum is located at 680 nm for LHC I-680 and at 730 nm for LHC I-730. It was also found that different kinetic models were required to fit the time-resolved fluorescence data of the two subpopulations, two components of 374 ps and 3.9 ns were required for LHC I-680 and three components of 157 ps, 510 ps and 2.8 ns for LHC I-730, respectively. Using femtosecond absorption recovery spectroscopy we were able to resolve a fast 200–400 fs depolarization in bulk LHC I at 655, 665 and 670 nm. The results indicate a fastest hopping time between individual chlorophylls of 200–400 fs in bulk LHC I. In the subpopulation LHC I-730, on the other hand, both the isotropic absorption recovery decay and the depolarization process occurred with a 15 ps lifetime, while in the subpopulation LHC I-680 we could not resolve any ultrafast relaxation process. The 15 ps phase is assigned to a transfer of excitation energy to the pigment giving rise to far-red emission component F735 of PS I.

Keywords: Photosystem I; Light-harvesting complex I; Femtosecond spectroscopy; Fluorescence spectroscopy; Anisotropy; F690; F735; Energy transfer

1. Introduction

Photosystem I (PS I) of higher plants mediates light-driven electron transport from plastocyanin to ferredoxin which then reduces $NADP^+$ to NADPH. It is composed of a multi-subunit core complex (PS I core) and an outer antenna, the light-harvesting complex I (LHC I). The PS I core complex in higher plants consists of 13 polypeptides [1]. The reaction centre polypeptides PsaA and PsaB (80 and 84 kDa) harbour the reaction centre chlorophyll P700, most of the co-factors needed for primary charge separation and an inner antenna which binds approximately 100 chlorophyll *a* (Chl *a*) molecules [2].

LHC I was first identified by Mullet et al. [3] and subsequently isolated by Haworth et al. [4] using sucrose density gradient centrifugation of native PS I solubilised with dodecyl maltoside and Zwittergent-16. LHC I is composed of four polypeptides of about 21–24 kDa which together bind approximately 100 Chl *a* and Chl *b* molecules in the ratio of 3.0–3.5. Electron microscopy studies led Boekema et al. [5] to propose that eight LHC I polypeptides surround each core complex in a shell-like structure. This implies that there are two copies of each LHC I polypeptide per reaction centre. Further fractionation of LHC I, using either an additional overnight sucrose density centrifugation [6] or mild SDS-PAGE [7], resulted in the isolation of two pigment-binding sub-complexes; LHC I-680 (23 and 24 kDa) and LHC I-730 (21 and 21.5 kDa) with 77 K fluorescence maxima at 680 and 730 nm, respectively.

A special feature of native PS I is the fluorescence at 720 nm (F720) and 735 nm (F735), due to long-wavelength-absorbing pigments. It is believed that the low-energy pigments, especially the F720-pigments, localized on a lower energy level than the reaction centre chlorophyll P700, act as energy sinks concentrating excitons in the vicinity of the reaction centre [8], F735 could also have a more protective role against over-excitation especially at low temperatures [9].

We have in a previous report investigated the energy

Abbreviations: Chl, chlorophyll; fs, femtosecond; LHC, light-harvesting complex; PS, photosystem; ps, picosecond; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; SPC, single photon counting.

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transfer dynamics and the role of these far-red emission components in native PS I and PS I core using fluorescence spectroscopy [10]. There is to our knowledge only one previous study on isolated LHC I using time-resolved fluorescence. This study, by Mukerji and Sauer [11], resulted in a rather complex model of the excited state dynamics, consisting of five components. However, no measurements have been performed on isolated LHC I using femtosecond spectroscopy. Furthermore, no time-resolved study has been made on the two subpopulations LHC I-680 and LHC I-730. On the PS I core antenna of a photosynthetic green alga there is, however, one report using femtosecond spectroscopy with the aim to study the first energy transfer events. In that study, Du et al. [12] were for the first time able to measure the energy transfer hopping time between adjacent chlorophyll molecules using the fluorescence up-conversion technique. There are, furthermore, two other studies on the PS I core antenna of using femtosecond spectroscopy [13,14]. In one of these studies, Hastings et al. [13] observed a sub-picosecond component, but it was not interpreted by the authors [13].

In the present communication we have used femtosecond absorption spectroscopy in combination with steady-state and picosecond fluorescence spectroscopy to examine the primary energy transfer events occurring within LHC I and its subpopulations, LHC I-680 and LHC I-730.

2. Materials and methods

2.1. Isolation and sub-fractionation of LHC I

Spinach (*Spinacia oleracia* L.) was grown under artificial light in nutrient solution as described in [15]. Native PS I particles were isolated by sucrose density gradient centrifugation of destacked thylakoids solubilised in Triton X-100 (0.75% (w/w), 0.8 mg Chl/ml). This was done as described by Mullet et al. [3], but with 0.05% Triton X-100 included in the gradients instead of 0.02%, which was shown to minimize the LHC II contamination. After 20 h of centrifugation in an SW 28 rotor (Beckman), aggregated native PS I particles were collected with a syringe from the bottom of the gradients on top of the 2 M sucrose cushion, diluted three times with ice-cold water and pelleted by centrifugation at $120000 \times g$ for 1 h.

Further fractionation of PS I into PS I core and LHC I was performed essentially as in [4] and sub-fractionation of LHC I as in [6] with modifications as specified below.

PS I particles of 12 mg chlorophyll were diluted with 50 mM sorbitol, 5 mM EDTA (pH 7.5) to 0.5 mg Chl/ml and solubilised with dodecyl β-D-maltoside (1.8 mg/ml) and Zwittergent-16 (2.4 mg/ml) for 60 min with slow stirring at 4° C. 6 ml aliquots of detergent-treated PS I particles were layered on top of 26 ml 0.1–1.0 M sucrose density gradients supplemented with 20 mM Tricine (pH 7.8) and 0.1% dodecyl β-D-maltoside and underlaid with 8 ml sucrose cushions. Ultracentrifugation for 24 h at $90000 \times g$ yielded three green bands that were collected dropwise from the bottom of the tubes. The two lower bands contained unfractionated native PS I and PS I core devoid of LHC I. The upper band from four tubes representing LHC I, were pooled and diluted from 30 to 200 ml with ice-cold water and concentrated in an AMICON cell with a YM 10 filter to 3 ml. Concentrated LHC I was then applied in 1.5 ml samples to 12 ml 0.1–1.0 M sucrose density gradients containing 20 mM Tricine (pH 7.8) and 0.05% Triton X-100 on top of 1.0 ml 2.0 M sucrose. Centrifugation at $180000 \times g$ for 35 h resulted in two close green bands in the upper part of the gradient that were collected dropwise from the bottom of the tubes. The upper band represented LHC I-680 and the lower one LHC I-730.

SDS-PAGE was performed using the buffer system of Laemmli [16], an acrylamide gradient of 12–22.5% and a stacking gel of 6%. Solubilisation of the samples was carried out at room temperature for 10 min and electrophoresis was run at 0° C. Silver staining of the SDS-PAGE was performed according to [17].

As an additional purity control of the result of the isolation procedure, the steady-state fluorescence at 77 K was measured, using a Perkin Elmer LS 50 spectrometer. Excitation wavelength was 435 nm and 60% (w/w) glycerol was added to the sample solution.

2.2. Spectroscopy

Steady-state fluorescence measurements at room temperature were carried out on a Spex Fluorolog 112 equipped with Glan Thompsson polarizers. Fluorescence spectra were recorded at the magic angle polarization relative to the excitation light and corrected for the sensitivity of the detection system. Integrated steady-state spectra were corrected for the sensitivity of the detection system. The quantum yields integrated over the emission spectra were calculated using a reference substance, Oxazine 1 (Lambda Physik), dissolved in methanol, whose quantum yield was previously determined to be 0.14 [10]. The sample quantum yield was then determined according to the relation:

$$\phi_f = \phi_{ref} \cdot \frac{(1 - 10^{-A_{ref}})}{(1 - 10^{-Af})} \cdot \frac{S_f}{S_{ref}}$$

where ϕ_{ref} denotes the quantum yield of the reference, A_{ref} and A_f the absorption of the reference and sample, respectively. S_{ref} and S_f are the integrated fluorescence of reference and sample, respectively.

In the picosecond experiment the light source consisted of a mode-locked continuous wave Nd:YAG laser (Spectra Physics model 3000) synchronously pumping a cavity dumped kiton red dye laser (Spectra Physics model 375). The duration of the optical pulses were typically in the order of 10 ps. For the single photon counting (SPC)

detection system, a cooled Hamamatsu 12 micron R 156401 micro channel plate photomultiplier tube was used. Typical response function of the electronic system was 70 ps which in principle allows a time resolution of ~ 15 ps after deconvolution. The fluorescence was collected at magic angle polarization to that of the excitation light and in a geometry perpendicular to the excitation direction. Typically, 50 000 counts were collected in the peak channel in order to obtain a high signal-to-noise ratio. The fluorescence decays were analyzed with the Globals Unlimited program package, developed at the Laboratory for Fluorescence Dynamics at the University of Illinois at Urbana-Champaign. The fluorescence decays were fitted to a sum of free running exponentials,

$$F(\lambda_{em},t) = \sum_i A_i(\lambda_{em}) \cdot e^{-t/\tau_i}$$

where $A_i(\lambda_{em})$is the fluorescence amplitude associated with lifetime τ_i and $F(\lambda_{em},t)$ is the fluorescence decay at a certain emission wavelength λ_{em}. Decay associated spectra (DAS.) were then constructed from this analysis procedure. The quality of the fit was judged by the χ^2 parameter.

In the femtosecond experiments the light-source consisted of a continuous wave mode-locked Nd:YAG laser (Spectra Physics model 3800) synchronously pumping a cavity dumped DCM dye laser (Spectra Physics model 375). To produce femtosecond pulses, fibre prism compressors were used in front of (Spectra Physics model 3690) and after (home constructed) the dye laser. The duration of the optical pulses were typically around 300 fs (fwhm) with a bandwidth of 3 nm (fwhm). In a few experiments another dye laser was used (Spectra Physics model 3500) and in this case the optical pulses were considerably shorter, about 90 fs (fwhm) with a band width of 5 nm (fwhm). The average power of both laser systems varied between 5 and 15 mW as measured at the position of the sample, with a pulse repetition rate of 4 MHz, corresponding to a pulse energy of 2–3 nJ/pulse, i.e., $8 \cdot 10^{13}$ photons/pulse per cm^2. This intensity could further be reduced by using OD gray filters. The maximum time span of the transients was typically around 50 ps. The measurements were performed with the well-known one-colour pump/probe technique with an Ithaco PC lock-in amplifier in the detection system. The experiments were carried out at room temperature and the polarization of the probe pulse was set at parallel, perpendicular or at the magic angle polarization with respect to the pump pulse using a Fresnl romb.

The time-resolved anisotropy was then calculated directly from a transient with parallel polarization and one with perpendicular polarization according to the standard expression:

$$r(t) = \frac{I(t)_\perp - I(t)_\parallel}{I(t)_\perp + 2 \cdot I(t)_\parallel}$$

The time-resolved data of the magic angle polarization experiments were analyzed with the program Spectra Processor (S. Savikhin Software). The anistropys were analyzed either manually or by using a standard curvefitting procedure without deconvuluting with the instrument response function. The quality of the fit was judged by standard deviation calculation of the fit. Conclusions were based upon measurements from at least 10 different fresh samples.

The sample material was contained in a medium consisting of 20 mM Tricine (pH 7.8), 40 mM sucrose and 4 mM NaCl. In the fluorescence experiments the maximal absorption of the sample in the region of the Q_y transition, in the 670 nm region, was around 0.2 per cm in order to avoid self absorption of the emission within the cuvette. The typical absorbance of the sample in the pump/probe experiments was about 0.6–0.7 at the excitation wavelengths. A high-speed rotating cell was used in order to avoid damage to the sample due to high light intensities and accumulation of photoproducts, e.g., chlorophyll triplets. The pathlength in the rotating cell was 1 mm and the rotation speed was typically around several hundred revolutions per minute. This means that the same spot in the rotating cell is not exposed to laser radiation twice within ~ 3 ms.

3. Results

3.1. Isolation of LHC I and its subpopulations LHC I-680 and LHC I-730

Sucrose density gradient centrifugation of PS I particles solubilised with dodecyl maltoside yielded three chlorophyll containing bands. The upper band contained the four LHC I polypeptides detached from the PS I core (Fig. 1, lane 2), from now on referred to as bulk-LHC I. Upon further fractionation by sucrose density gradient centrifugation of LHC I, two green bands with roughly similar chlorophyll content were resolved. The upper band was identified by SDS-PAGE to be enriched in the LHC I-680 polypeptides of 23 and 24 kDa (Fig. 1, lane 3) and showed a chlorophyll *a/b* ratio of 3.1. The lower band with a chlorophyll *a/b* ratio of 3.2 was dominated by LHC I-730 consisting of two closely migrating polypeptides of 21 and 21.5 kDa (Fig. 1, lane 4). 77 K fluorescence measurements showed exclusively emission at 680 and 730 nm for the upper and lower bands, respectively (data not shown), which further confirmed their identities.

The chlorophyll concentration was typically around 0.1–0.2 mg/ml for all of the three different preparations.

The absorption spectra of bulk-LHC I and its subpopulation LHC I-680 were very similar with maxima around 670 nm for the Q_y transition. The Q_y transition of the chorophylls in LHC I-730 was, however, located around 674 nm as seen in Fig. 2.

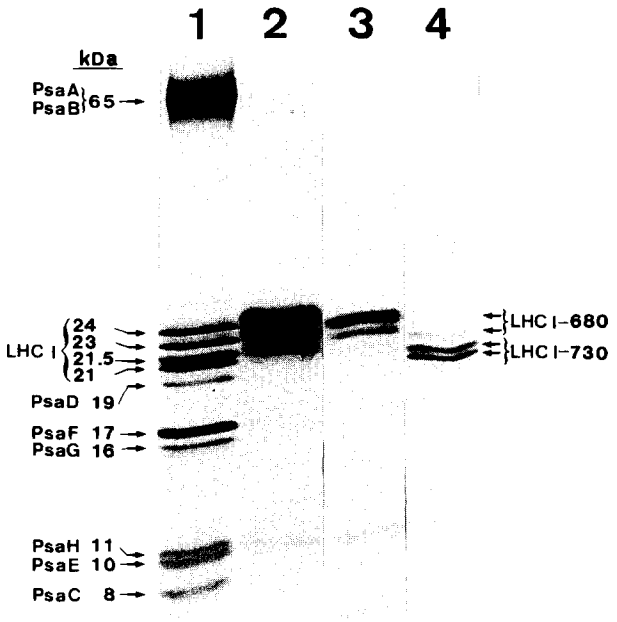

Fig. 1. SDS-PAGE of solubilised native PS I subjected to sucrose density gradient centrifugation. (1) Native PS I. (2) Bulk LHC I from first gradient. Its four polypeptides are slightly shifted to higher molecular weight and not so well resolved due to presence of detergents. (3) Upper band from second gradient representing LHC I-680. (4) Lower band from second gradient representing LHC I-730.

3.2. Steady-state and time-resolved fluorescence spectroscopy

Steady-state fluorescence spectra of the two LHC I subpopulations were distinctly different even when recorded at room temperature (Fig. 3). The LHC I-680 fraction had a fluorescence maximum at 680 nm, while LHC I-730 had maxima at both 680 and 730 nm, with the latter clearly dominating. Estimates of the fluorescence quantum yields were also made and were found to be about 9% for bulk LHC I, 28% for LHC I-680 and 11% for LHC I-730. With respect to the quantum yield of the subpopulation LHC I-680, it should be mentioned for a comparison that the quantum yield for free chlorophyll is approx. 30% [18].

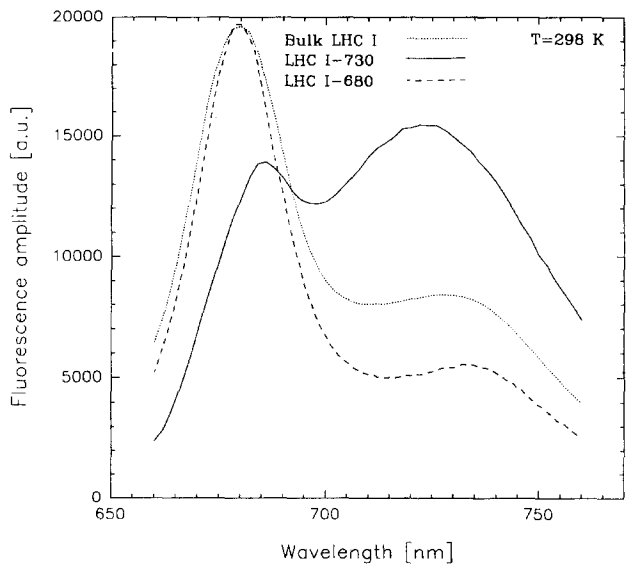

Fig. 3. Steady-state fluorescence spectra of bulk LHC I and its subpopulations LHC I-680 and LHC I-730 at room temperature. Excitation wavelength is 650 nm.

Decay-associated spectra (DAS.) were constructed from time-resolved fluorescence data on unfractionated (bulk) LHC I (Fig. 4) and its two subpopulations LHC I-680 and LHC I-730 (Figs. 5 and 6). To fit the data of bulk LHC I, at least three components were required, of 108 ps (τ_1), 578 ps (τ_2) and 3.0 ns (τ_3), respectively. All of them

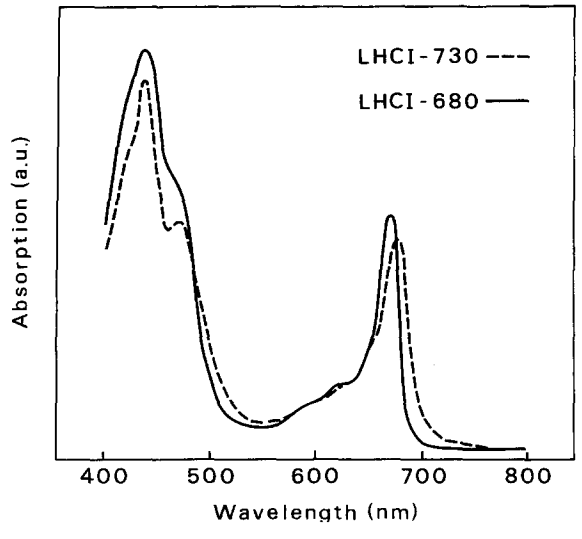

Fig. 2. Absorption spectra of the sub-populations LHC I-680 and LHC I-730 at room temperature. The spectra of bulk LHC I is very similar to the spectra of LHC I-680 with respect to the position of the Q_y transition.

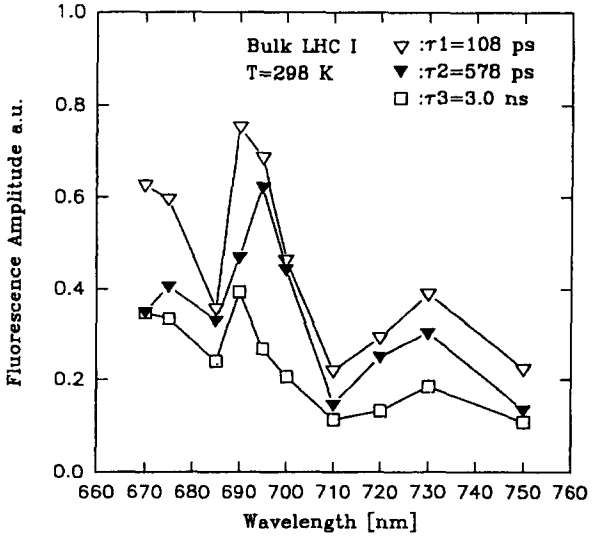

Fig. 4. Decay associated spectra (DAS) of bulk LHC I at room temperature. Global χ^2 for this fit is 1.10. Excitation wavelength is 650 nm.

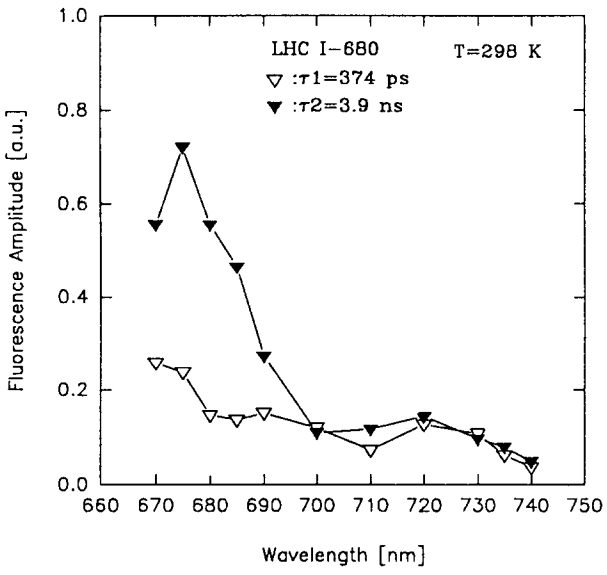

Fig. 5. Decay associated spectra (DAS) of LHC I-680 at room temperature. Global χ^2 for this fit is 0.98. Excitation wavelength is 650 nm.

showed emission maxima around 680 nm but also showed distinct emission around 735 nm. It was also necessary to use three time components for LHC I-730 where the best fit resulted in a 157 ps component (τ_1), a 510 ps component (τ_2) and a 2.8 ns component (τ_3) (Fig. 5). The 157 ps component exhibited a maximum at 675 nm, while the 510 ps component fluoresced maximally at 735 nm (Fig. 5). The LHC I-680 complex only required two lifetimes in order to obtain a good fit, namely 374 ps (τ_1) and 3.9 ns (τ_2) as seen in Fig. 6. The fluorescence decay of LHC I-680 is clearly dominated by the 3.9 ns component with a maximum at 675 nm. Time-resolved fluorescence data of the subpopulation LHC I-680 should in principle be possible to fit with a single exponential decay. Within the inhomogenously broadened pigment pool of LHC I-680 it is reasonable to assume that redistribution of excitation energy is very fast and therefore difficult to detect in an SPC experiment. The 374 ps component is therefore regarded as either a result of the fitting procedure, necessary to introduce to obtain a good fit, or due to a fraction of partly disconnected chlorophylls transferring energy with very low efficiency.

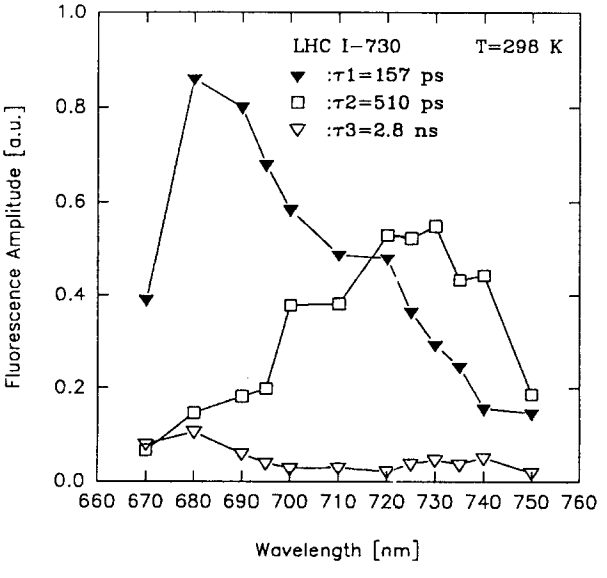

Fig. 6. Decay associated spectra (DAS) of LHC I-730 at room temperature. Global χ^2 for this fit is 0.94. Excitation wavelength is 650 nm.

The steady-state emission anisotropy was measured for all samples and it was found that the anisotropy of the subpopulations LHC I-680 and LHC I-730 has a rather constant value of ~ 0.1 in the range from 660 to 720 nm where the anisotropy could be accurately determined (data not shown). The steady-state emission anisotropy of bulk LHC I had a slightly lower value of ~ 0.07 in the same wavelength region (data not shown).

3.3. Femtosecond pump-probe spectroscopy

The absorption recovery kinetics of bulk LHC I at 665 nm consisted of a hardly resolvable decay shorter than 100 fs, using parallel polarization between pump and probe pulses (Fig. 7a). The duration of the optical pulses in these experiments was about 90 fs. Depolarization at this wavelength occurred in 150–250 fs as seen in Fig. 7b. At 670 and 655 nm we used a slightly different laser system which produced optical pulses of about ~ 300 fs (fwhm). Also at these wavelengths a fast depolarization in bulk-LHC I occurred on the time-scale of 200–500 fs (data not shown). These transients most likely represent incompletely time-resolved depolarization processes, since the optical pulses in these experiments were of similar duration as the depolarization processes. Performing the pump and probe experiment at 655 nm, where in principle both chlorophyll *a* and *b* absorb, did not result in any new depolarization times. A rough estimate based upon the absorption profiles of chlorophyll *a* and *b* and using the fact that the Chl *a*/*b* ratio is 3.0–4.0 for bulk LHC I [10,11], shows that only 10–20% of the absorption is due to chlorophyll *b* at 655 nm. It is therefore reasonable to assume that any relaxation processes exclusively associated with chlorophyll *b* could be very difficult to observe with the signal-to-noise level of this study.

It was furthermore observed that similar results were obtained even when the excitation intensity, i.e., photons/pulse per cm^2, was reduced by ~ 50%. Nor were these results affected by changing the rotation velocity of the rotating cell. These observations indicate singlet-triplet annihilation to be negligible in our experiments.

The subpopulation LHC I-730 was devoid of a fast depolarization on the 100 fs time-scale at 670 nm. In this case the relaxation phase is dominated by a 15 ps component, which is observed both in the isotropic absorption recovery decay (data not shown) and in the depolarization

decay, as illustrated in Fig. 8a and b. It was observed that the relaxation phase became slower, i.e., 30–50 ps, when the sample was exposed to laser light for several hours. We think that this is due to an aging process, which is also confirmed by comparing the absorption spectra of a fresh and a used sample where the absorption at 674 nm had decreased significantly, 10–15%. It also had shifted slightly towards the blue, indicating a larger fraction of free chlorophyll. Our conclusion is that the laser irradiation has induced some kind of irreversible photodamage. We have accordingly chosen not to consider the results of these aged samples. Notably, for bulk LHC I such an aging process was not observed.

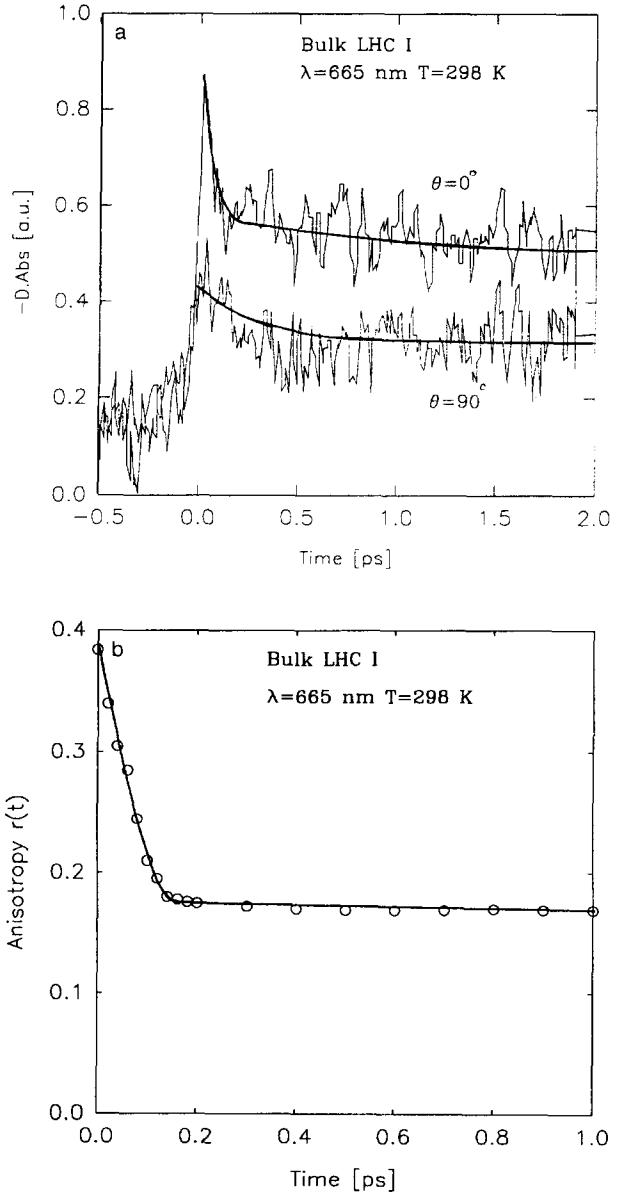

Fig. 7. (a) Parallel and perpendicular transients of bulk LHC I at 665 nm using 90 fs pulses at room temperature. (b) Anisotropy of Bulk LHC I at 665 nm and at room temperature. The depolarisation time in this particular experiment is 210 fs.

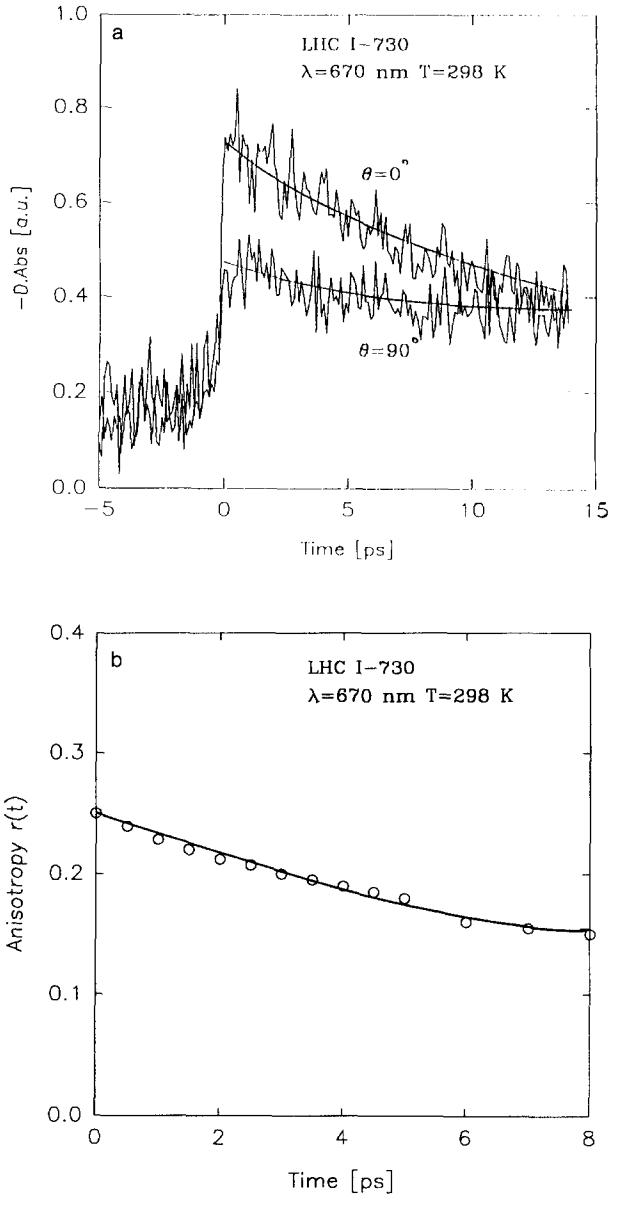

Fig. 8. (a) Parallel and perpendicular absorption recovery decays of LHC I-730 at 670 nm and at room temperature. The isotropic relaxation phase is about 15 ps. (b) Anisotropy decay of LHC I-730 at 670 nm and at room temperature. The depolarisation occurs in 15 ps.

One-colour pump/probe experiments were also performed on the subpopulation LHC I-680. However, this system did not exhibit any fast relaxation phenomena, the absorption recovery signal had a lifetime of ~ 100 ps (data not shown) as obtained in an experiment over the first 50 ps. In view of the high quantum yield we are concerned about the integrity and intactness of this subpopulation. The possibility can not be excluded that a fraction of the chlorophylls is no longer bound to the protein and we have therefore chosen not to discuss the time-resolved results of this system at this stage.

4. Discussion

4.1. Fluorescence properties of bulk LHC I and its subpopulations

The steady-state fluorescence spectra of the LHC I-730 subpopulation exclusively exhibited 730 nm emission at 77 K (data not shown). At room temperature LHC I-730 showed apart from the 730 nm emission, significant emission around 685 nm (Fig. 3). This emission probably reflects minor contamination by LHC I-680 as judged from the SDS-PAGE (Fig. 1, lane 4) or it might partly be due to thermally activated F690 emission of LHC I-730. A minor contamination of LHC I-730 as detected by the sensitive silver staining of the SDS-PAGE (Fig. 1, lane 3) could also explain the small shoulder around 730 nm in the steady-state emission spectra of LHC I-680 (Fig. 3). Part of this shoulder, however, also results from a vibronic transition of chlorophyll *a* itself.

The time-resolved fluorescence data indicate a complex excited state relaxation kinetics for LHC I-730, possibly due to redistribution of the excitation energy within LHC I-730 (Fig. 5). This results in energy transfer to the pigment emitting at 735 nm designated F735 [19]. The origin of the 157 ps component (Fig. 5) with maximum at 680 nm as observed in LHC I-730 is associated to the main pool of chlorophylls absorbing at 674 nm. The intermediate component of 510 ps (Fig. 5) is associated with the far-red emission around 730 nm.

In the DAS of bulk LHC I we were not able to find a component which could represent energy transfer between the LHC I-680 and LHC I-730 subpopulations (Fig. 4). Instead, it appears as if the combination of the DAS of LHC I-680 (Fig. 5) and LHC I-730 (Fig. 6), respectively, yields a DAS resembling that of bulk LHC I. This notion of limited energy migration is consistent with the electron microscopy imaging by Boekema et al. [5]. As judged by this study, there appears to be little structural contact between the polypeptides and it is therefore reasonable to assume that they might easily be disconnected from each other after isolation.

The only previous time-resolved fluorescence study on isolated LHC I is the work by Mukerji and Sauer [11]. Although that study was performed on a preparation which appears to be very similar to the bulk LHC I of this study, the excited state kinetics observed resulted in a substantially different model [11]. The important differences, as compared to our study, are two components found at 300 K of 30 and 200 ps, respectively, that at 77 K appear as energy transfer components [11]. In our study an extensive search for the χ^2-minima was performed over the whole parameter surface in the global analyses procedure. We found that a three-component model is the simplest but also the best model to describe the data satisfactorily (Fig. 4). In this analysis procedure we do not observe the 30 ps component, which in the study of Mukerji and Sauer [11] was a very strong component. Since the experiments seem to have been performed in a similar way on similar samples, it is at present not clear why there is such a difference.

4.2. Ultrafast depolarization in bulk LHC I

Bulk LHC I exhibits a fast depolarization in the range of 200–400 fs (Fig. 7) For bulk LHC I the fast depolarization was observed at three wavelengths, 655, 665 and 670 nm. For the experiments at wavelengths 655 nm and 670 nm, ~ 300 fs pulses were used and the result was an fast pulse limited relaxation phase (data not shown). We interpret the data as a depolarization time of 300 ± 100 fs seems to be appropriate in spite of the insufficient time-resolution in two of the experiments.

At 655 nm an estimate showed that the probability of chlorophyll *b* excitation was small and we therefore conclude that the pump/probe experiment at this wavelength essentially shows the absorption recovery of chlorophyll *a*. We want to remark, however, that energy transfer in LHC II between chlorophyll *b* and chlorophyll *a* also occurs on the 0.3–05 ps time-scale [20–22]. LHC I, on the other hand, exhibits some differences in initial polarization as compared to LHC II [21] since the initial anisotropy for LHC I is equal to 0.4 (Fig. 7b). In conclusion, we are therefore reluctant to interpret the ~ 300 fs depolarization at 655 nm as a chlorophyll *b* to chlorophyll *a* energy transfer.

The anisotropy at zero time delay between excitation and probe pulse is hard to determine, since the anisotropy determinations require a very high signal-to-noise ratio. There could also be complications due to the coherent interaction between the pump and probe pulse at zero delay. We find, however, that a value in the range of 0.38–0.42 for the anisotropy at time zero is justified, based on the average over a number of data sets from different samples.

In principle, an initial anisotropy of 0.7 might be observed for a pair of strongly coupled chromophores according to a theoretical study by Knox and Gülen [23]. This has also been observed for a highly symmetric system, a tetraphenylporphyrin molecule [24] and more relevant for this study, in the bacteriochlorophyll *a* protein antenna of the green bacterium *Chlorobium tepedium* [25]. Since the anisotropy measured in the present study at time zero did not exceed 0.4, the system is clearly within the incoherent limit. It then remains to interpret the fast depolarization as the incoherent hopping time of excitation energy between two neighbouring chlorophyll *a* molecules. In a very recent study, Du et al. [12] measured the single step energy transfer time in the PS I core antenna of a mutant of the green alga *Chlamydomonas reinhardtii*. In the present study we report essentially similar observations as Du et al. [12], namely a 200–400 fs depolarization time and no excitation wavelength dependence in the relaxation phase.

A similar hopping time was also estimated by Owens et al. [26] using a random walk model. A hopping time of approx. 200 fs was an essential parameter in order to simulate the energy transfer in the core antenna, as observed by Jia et al. [8].

The residual anisotropy of ~ 0.10 (Figs. 7b) as observed in the pump-probe experiments on bulk LHC I and in the steady-state fluorescence anisotropy of all the LHC I preparations most likely represents an intrinsic anisotropy due to local order. One can calculate the relative orientation angle θ between the normal of the membrane plane and the Q_y transition of the chromophores in using the relation,

$$r = r_0 \cdot \left(\frac{3 \cdot \cos^2 \theta - 1}{2} \right)^2$$

where $r_0 = 0.4$ and θ is the orientation angle. Inserting $r = 0.1$ results in an angle $\theta = 35°$ between the transition of the chromophores and the normal of the membrane plane. The anisotropy of $r = 0.1$ is typical of a system where the Q_y transitions are in a plane [27].

4.3. Energy transfer within LHC I-730

LHC I-730 did not exhibit any fast depolarization on the femtosecond time-scale at 670 nm, the isotropic signal and the anisotropy decayed with a 15 ps time-constant (Fig. 8). The initial anisotropy was is in this case only about 0.25. The reason for the unresolved anisotropy decay, from 0.4 to 0.25 in less than 100 fs, must originate from ultrafast energy transfer within the chlorophyll pool absorbing at 674 nm. (Fig. 8b). The 15 ps component clearly must represent the energy transfer process between the main pool of chlorophylls absorbing at 674 nm and the pigments responsible for the far-red emission around 730 nm designated F735 [19]. Assuming that this energy transfer is a Förster type of transfer, there are mainly two factors that influence the transfer rate, namely distance and spectral overlap. There are homologies between the LHC I and LHC II polypeptides in the whole amino acid sequence where the chlorophyll ligands are situated, which in turn strongly indicate structural similarities between LHC I and LHC II [28,29]. It is therefore reasonable to assume that the chlorophyll packing in LHC I is equal to that of LHC and distances of 10–20 Å between chromophores could be expected. Such distances have in other systems resulted in sub-picosecond energy transfer processes [30]. The limiting factor in LHC I-730 is, however, the weak spectral overlap between emission dipoles of the F690 chlorophylls and absorption dipoles of F735 molecules. The absorption associated with the F735 emission has recently been determined to be centered around 716 nm and is accordingly called C716 [31]. The bandwidth of this spectral component might be as large as 15–20 nm [31] and the overlap integral J, defined as

$$J = \int \epsilon_A(\nu) \cdot f_D(\nu) \cdot d\nu,$$

will have a relatively low value as, for instance, comparing the J value between the Chl b and Chl a system (a very rough estimate in assuming two gaussian spectral profiles shows that the J value for F690 and C716 overlap could only be $\sim 10\%$ compared to the Chl b and Chl a system). Accordingly, the energy transfer to F735 will occur on a picosecond time-scale.

The fluorescence quantum yield of LHC I-730 was found to be only about a third of that of LHC I-680. This is probably due to the fact that a large part of the LHC I-730 emission of has its origin in a 157 ps component and that free chlorophylls with a long fluorescence lifetime are associated with the LHC I-680 subpopulation. When determining the quantum yield using steady-state fluorescence spectroscopy, mainly nanosecond fluorescence components will contribute to the quantum yield.

We could not observe the 15 ps component in isolated bulk LHC I in any of the time-resolved experiments. In bulk LHC I this could be due to the fact that there are other processes apart from the energy transfer to F735. With respect to the time-resolved fluorescence experiments we remark that 15 ps is on the limit of the time-resolution of our spectrometer. The absorption spectra of bulk LHC I and its subpopulation LHC I-680 are more or less identical with respect to the position of the Q_y transition. Both systems have maxima at 670 nm, while LHC I-730 has a maximum at 674 nm (Fig. 2). It is thus reasonable to assume that the absorption recovery signal in bulk LHC I is dominated by the signal from its subpopulation LHC I-680 and that the 15 ps absorption recovery transient of the LHC I-730 subpopulation is too weak to be detected in the bulk LHC I within the sensitivity of our measurements. Our results are in fair agreement with some conclusions reached in earlier studies. Wittmershaus [32] observed for instance, at low temperatures (77 K), a biphasic rise of the F735 emission in intact chloroplasts with a fast component in the range of 10–20 ps. Other studies have revealed equilibration times in the order of 10 ps in intact PS I preparations [33,34]. Although the time resolution of our SPC apparatus should in principle allow us to detect a 15 ps component, we were not able to resolve the rise of the 730 nm emission in the subpopulation LHC I-730 or bulk LHC I. We are, however, confident in our conclusions based upon our observations in the absorption recovery experiments, since the time-resolution in this case is more than sufficient to resolve picosecond components.

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References

[1] Ikeuchi, M. (1992) Plant Cell Physiol. 33, 669–676.
[2] Golbeck, J.H. and Bryant, D.A. (1991) in Current Topics in Bioenergetics (Lee C.P., ed.), Vol. 16, pp 83–107, Academic Press, San Diego.
[3] Mullet, J.E., Burke, J.J. and Arntzen, C.J. (1980) Plant Physiol. 65, 814–822.
[4] Haworth, P., Watson, J.L. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 151–158.
[5] Boekema, E.J., Wynn, R.M. and Malkin, R. (1990) Biochim. Biophys. Acta 1017, 49–56.
[6] Lam, E., Ortiz, W. and Malkin, R. (1984) FEBS Lett. 168, 10–14.
[7] Bassi, R. and Simpson, D. (1987) Eur. J. Biochem. 163, 221–230.
[8] Jia, Y.W., Jean, J.M., Werst, M.M., Chan, C. and Fleming, G.R. (1992) Biophys. J. 63, 259–273.
[9] Trissl, H.-W., Becks, B. and Wulf, K. (1993) Photochem. Phtobiol. 57, 108–112.
[10] Pålsson, L.O., Tjus, S.E., Andersson, B. and Gillbro, T. (1994) J. Chem. Phys. Submitted.
[11] Mukerji, I. and Sauer, K. (1993) Biochim. Biophys. Acta 1142, 311–320.
[12] Du, M., Xie, X., Jia, Y., Mets, L. and Fleming, G.R. (1993) Chem. Phys. Lett. 201, 535–542.
[13] Hastings, G., Kleinherebrink, F.A.M., Lin, S. and Blankenship, R.E. (1994) Biochemistry 33, 3185–3192.
[14] Schweitser, G. and Holzwarth, A.R. (1994) unpublished results.
[15] Andersson, B., Åkerlund, H.E. and Albertsson, P.Å. (1976). Biochim. Biophys. Acta 432, 122–132.
[16] Laemmli, U.K. (1970) Nature 227, 680–685.
[17] Wray, W., Boulikas, T., Wray, V.P. and Hancock, R. (1981) Anal. Biochem. 118, 197–203.
[18] Karukstis, K.K. (1991) in Chlorophylls (Scheer, H., ed.), pp. 769–787, CRC Press, Boca Raton.
[19] Wittmershaus, B.P., Woolf, V.M. and Vermaas, W.F.J. (1992) Photosynth. Res. 31, 75–87.
[20] Eads, D.D., Castner, E.W., Randall, S.A., Mets, L. and Fleming, G.R. (1989) J. Phys. Chem. 93, 8271–8275.
[21] Pålsson, L.O., Gulbinas, V., Spanfort, M.D. and Gillbro, T. (1994) FEBS Lett. 339, 134–138.
[22] Du, M., Xie, X., Mets, L. and Fleming, G.R. (1994) J. Phys. Chem. 98, 4736–4741.
[23] Knox, R.S. and Gülen, D. (1993) Photochem. Photobiol. 57, 40–43.
[24] Galli, G., Wynne, K., LeCours, S.M., Therien, M.J. and Hochstrasser, R.M. (1993) Chem. Phys. Lett. 206, 493–499.
[25] Savikhin, S. and Struve, W.S. (1994) Biochemistry 33, 11200–11208.
[26] Owens, T.G., Webb, S.P., Mets, L., Alberte, R.S. and Fleming, G.R. (1987). Proc. Natl. Acad. Sci. USA 84, 1532–1536.
[27] Miller, M., Gillbro, T. and Olsson, J.M. (1993) Photochem. Photobiol 57, 98–102.
[28] Kühlbrandt, W., Neng Wang, D. and Fujioshi, Y. (1994) Nature 367, 614–621.
[29] Jansson, S. (1994) Biochim. Biophys. Acta 1184, 1–19.
[30] Sharkov, A.V., Kryukov, I.V., Khorosilov, E.V., Kryukov, P.G., Fisher, R., Scheer, H. and Gillbro, T. (1992) Chem. Phys. Lett. 191(6) 633–638.
[31] Goebts, B., Van Amerongen, H., Monshouwer, R., Kruip, J., Rögner, M., Van Grondelle, R. and Dekker, J.P. (1994) Biochim. Biophys. Acta 1188, 75–85.
[32] Wittmershaus, B.P. (1987) in Progress in Photosynthesis Research (Biggins J., ed.), Vol. I, pp 75–82, Martin Nijhoff, Dordrecht.
[33] Causgrove, T.P., Yang, S. and Struve, W.S. (1989). J. Phys. Chem. 93, 6844–6850.
[34] Owens, T.G., Webb, S.P., Mets, L., Alberte, R.S. and Fleming, G.R. (1989) Biophys. J. 56, 95–106.