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Energy transfer and charge separation kinetics in Photosystem I. 2. Picosecond fluorescence study of various PS I particles and light-harvesting complex isolated from higher plants

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Abstract

Two different Triton X-100 isolated photosystem (PS) I particle preparations as well as isolated light-harvesting complex (LHC) I from spinach have been studied by steady state and time-resolved fluorescence with a time resolution of about 5 ps. The dependence of the fluorescence kinetics and spectra on two parameters was investigated in detail: firstly, the solubilizing detergent used in the measurements was varied between Triton X-100, octylglucoside and SB12 (zwitterionic detergent). Secondly, the PS I particle size and type were varied, studying what is believed on the basis of literature data to be PS I-native and PS I core, respectively. All of these changes had pronounced effects on the kinetics. By global analysis procedures, we generally find four and in some cases five lifetime components in all data sets. Our results indicate that all PS I preparations studied, including the PS I-core complex lacking LHC I, are heterogeneous, displaying at least a bimodal distribution in antenna size. We attribute this size distribution to the action of Triton X-100 used in the isolation procedure. The fastest lifetime component in the range of 6–14 ps, depending on antenna size, appears as a rise term at wavelengths above 710 nm in all particles and is attributed to an energy transfer in the antenna system from the main pool of short wavelength absorbing pigments (F690) to pool(s) of long wavelength absorbing and fluorescing pigments (F720 and/or F735). The observation of such an antenna equilibration component at room temperature is crucial for the understanding of the excitation kinetics. Furthermore, for the sample that is believed to be "native" PS I, we found two lifetime components of 31–49 ps (depending on the detergent) and 130 ps. We attribute both of them to an overall charge separation, but originating from different particle types in the sample. We thus propose a fundamental antenna size heterogeneity. The shorter time most probably corresponds to intact PS I cores, whereas only the longer one originates from "native" PS I with antenna size of about 200 Chl. The data suggest that the excitation kinetics in all of these PS I particles is close to the trap-limit.

Keywords: Time-resolved fluorescence; Photosystem I; Kinetic modeling; Energy transfer; Global analysis; Detergent effect

1. Introduction

1. Introduction

Photosystem (PS) I is the photosystem of oxygen-evolving organisms that receives electrons from PS II via the linear electron transport chain and reduces NADP$^+$ to NADPH. Despite a substantial amount of work on the primary reactions in PS I (for reviews, see e.g. Refs. [1,2]), the structure and function of this

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Abbreviations: PS I (II), Photosystem I (II); LHC I, light harvesting complex I; OG, octylglucoside; SB12, *N*-dodecyl-*N*,*N*-dimethylammonio-3-propane sulfonate; Mes, 2-[*N*-morpholino]ethanesulfonic acid; PMS, phenazine methosulfate; SPT, single-photon timing; Chl, chlorophyll; DAS, decay-associated spectrum; DCM, 4-dicyanomethylene-2-methyl-6-(dimethylaminostyril)-4*H*-pyran.

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pigment-protein complex are not understood in detail. From a structural point of view, a great deal of information concerning the composition of the pigment protein complex is available (see Ref. [3] for a review), though a detailed X-ray determination of the PS I structure is still missing although a map with about 6 Å has been obtained [4]. The antenna system is made up of peripheral units (light harvesting complex, LHC I) and the PS I core antenna. In the former, the pigments are bound to four different polypeptides, in the latter all pigments are bound to the psaA and psaB protein subunits [5,6]. It is widely accepted that native PS I particles from higher plants contain a total of approx. 200 Chl/P700, about 100 of which are located in the LHC I, whereas the rest is bound to the core antenna [6].

The origin of the spectral components in the fluorescence emission of native PS I particles is a matter of discussion, in particular with respect to the role of the long wavelength emitting pigments that have been found in basically all PS I preparations [7–9]. The major part of the chlorophyll antenna pigments absorb around 678 nm and fluoresce around 690 nm (F690). In addition, two pigment pools with red-shifted emissions are found, which have been called F720 and F735 according to their fluorescence maxima [10]. The nature, exact location, size and function of these red pools within the antenna are not known, though there exists some evidence that the F720 pool is located in the core antenna system while the F735 pool is thought to be part of the LHC I [10–13] (for a recent attempt to address the localization of red pigments in the PS I core see Ref. [14]). Thus even in preparations of the PS I core at least two pigment pools have to be taken into account in order to get a consistent picture of the excitation dynamics [15]. Several decompositions of spectral contributions (pools) of PS I antenna pigments have been proposed in the literature [16–19], but most of these deconvolutions suffer from the lack of independent experimental support from, e.g., time-resolved measurements. A first attempt for a kinetic characterization of these pools has been published recently [19] (see also simulation of PS I excitation kinetics [14,18]). Quite generally the existence of several spectral antenna pools is expected to give rise to complex kinetics in time-resolved measurements. Thus advanced measuring and data analysis techniques are required, preferentially in cooperation with kinetic modeling [14,18, 20] to unravel the structure/function relationship of excitation dynamics in such systems. In view of these difficulties, it is not surprising that no general understanding of the primary processes in PS I has been achieved so far. A principal complication is that for the solubilization and isolation of PS I particles various detergents have to be used which might actually give rise to uncontrolled detachment of chlorophyll and/or peripheral polypeptides. Several standard preparation methods have been proposed in the literature [10,21,22] but the isolates have never been scrutinized with respect to their actual antenna homogeneity or inhomogeneity but were mostly characterized with stationary methods that can only provide information about their average properties. The occurrence of antenna size heterogeneity in such PS I preparations has recently been demonstrated by Zipfel and Owens [23]. It is also interesting to note that very recently it has been shown that PS I antenna heterogeneity occurs already in intact thylakoids [24].

An important and powerful tool to get insight into the primary steps occurring in PS I is provided in principle by kinetic studies in the picosecond time range. For this reason numerous time-resolved investigations have been carried out during the recent years (see Refs. [1,2] for reviews). Thus it is known that the very first steps of the energy transfer and charge separation take place within the time scale of picoseconds. However, the energy transfer between different chlorophyll antenna pools, which is one important aspect in characterizing and understanding the primary reactions, has hardly been resolved up to now in PS I isolated from higher plants. A better characterization of such processes has been provided in PS I isolated from cyanobacteria at low temperatures [25] and recently also at room temperature [19,26,27]. Direct energy transfer between chlorophylls on the femtosecond time scale has been resolved recently for the first time in PS I antennae [28,29]. Up to now only few studies on native PS I particles from higher plants have been performed. Mukerji and Sauer [16] and Lyle and Struve [30] reported complex kinetics in such particles. Several preliminary kinetic models were proposed in which various pools of pigments were accounted for in order to rationalize the experiments but the time resolution in most of these studies was insufficient to kinetically resolve the energy transfer between these pools [5,10,25,31]. Thus, Mukerji and Sauer [16] did not find any direct evidence for an energy transfer process between different pools in the antenna system of large (PS I-200) PS I particles. Struve and coworkers [30,32] found several very fast (several picoseconds) kinetic components in transient absorption measurements on various PS I preparations which may actually be indicative of such energy transfer processes between pools. However, since their experiments were of the one-color pump-probe type and did not extend into the red part of the spectrum it is difficult to make a precise assignment of the kinetics to specific pools.

The aim of this study is to get further insight into the excitation kinetics of the PS I antenna system from higher plants and their relationship to particle structure and antenna size. An essential aspect of this work is to test the integrity and structure variation of the PS

I complexes under various conditions of isolation and measurement, in particular with reference to the effects of detergents. We will show that preparations that were considered homogeneous so far [10,16,22] are in fact heterogeneous in composition and antenna size.

2. Materials and methods

2.1. Preparation of samples

Two different PS I particles have been isolated from spinach thylakoids using methods described previously [22] with slight modifications. Thylakoid membranes were isolated as described by Strotmann et al. [33] setting the chlorophyll concentration to 2 mg/ml, subsequently incubated with Triton X-100 (25 mg Triton/mg Chl) for 30 min at room temperature and then centrifuged at 48000 × *g* for 30 min. The resulting supernatant was diluted 5:1 in Tricine buffer (20 mM, pH 7.5, 0.2% Triton), layered on a DEAE column (DE52, Whatman) and washed with 60 mM NaCl. The column was eluted with a linear NaCl gradient (60–500 mM NaCl). All PS I containing fractions, as judged by a Chl *a*/Chl *b* ratio > 6 (6–8 was actually found), were collected yielding a sample with an antenna size of 100–120 Chl/P700 (all antenna sizes were determined following the method described by Markwell et al. [34]). In order to separate PS I core and LHC I, this preparation was further incubated with Triton X-100 (0.5 mg Chl/ml, 1% Triton) for 4 h at 4°C, layered on an acrylamide agarose column (ACA34, Pharmacia) and eluted with Mes buffer (25 mM Mes, pH 7.3, 0.1% Triton, 0.25 mM NaCl). As a result, we obtained another sample type showing an antenna size of 60 Chl/P700 as well as LHC I particles. All preparations described so far were resolubilized in Triton. In order to get samples in SB12 and octylglucoside (OG), we diluted the concentrated Triton solution (1:50, v/v) by adding buffer containing either SB12 or OG. The final concentration was adjusted to 0.05% SB12 (w/v) or 0.73% OG (w/v), respectively. All samples were characterized by SDS gel electrophoresis and Western blotting (data not shown). The integrity of the samples was checked regularly by steady state absorption, fluorescence emission and excitation spectra before and in most cases after each kinetic measurement. For fluorescence measurements, all preparations were diluted to 8 μg Chl/ml in Tricine buffer (20 mM Tricine, pH 7.5 and the respective detergent, i.e. 0.73% OG, 0.05% SB12 or 0.1% Triton). 10 mM ascorbate and 10 μM phenazine methosulfate (PMS) were freshly added.

Our preparations yield two types of PS I particles which are in close similarity to those isolated by Mullet et al. [10]. The particle called PS I-110 by these authors corresponds closely to our large sample type, whereas the preparation which they called PS I-65 has properties very close to our small sample type. In order to avoid further confusion, we will use the same notation as Mullet et al. by naming our preparations PS I-110 and PS I-65 throughout this text.

At this point it is also useful to address the problem of the determination of the antenna size (Chl/P700 ratio). This quantity can be measured by two different methods, one of which is based on chemical oxidation with ferricyanide, while the other detects the photooxidation kinetics directly using actinic light sources [23]. Often these two methods yield distinctly different results. All values for antenna size in this paper have been determined by chemical oxidation according to the method described by Markwell et al. [34] using low ferricyanide concentration.

2.2. Spectroscopic methods

Stationary fluorescence and excitation spectra were recorded on a Spex-Fluorolog spectrometer as described previously both at room temperature and at 77 K (60% glycerol added). All steady state spectra are normalized to the largest band.

Time-resolved fluorescence data were measured applying the single-photon timing (SPT) technique using a synchronously pumped cavity-dumped dye laser with a repetition rate of 800 kHz as described previously [35]. Sample fluorescence was spectrally resolved by a double monochromator (DH10, Jobin Yvon) with slits of 1 mm giving a spectral resolution of about 4 nm (spectra not shown). A MCP-type photomultiplier was used as detector. Two slightly different detection setups were applied. For PS I-110 an IR-intensified MCP detector was employed (Hamamatsu R2809U-05) giving a system prompt response of about 70 ps (FWHM). For measuring PS I-65 particles and LHC I complexes a VIS-type MCP (R2809U-07) providing a system response of 32 ps was used. Either DCM or Sulforhodamin 101 were used as laser dyes. In all decay measurements a minimum of 40000 counts was accumulated in the peak channel. The liquid samples were pumped through a flow measuring cuvette (1.5 mm × 1.5 mm) generally at a rate of 12 ml/min. Pumping at 120 ml/min, however, yielded the same results. All measurements were done at 12 ± 1°C, thermostating both the sample and the cuvette. Data were analyzed either by single decay or global analysis methods by fitting to sums-of-exponentials models as described previously over a complete set of measurements taken at different detection wavelengths [35]. These results are presented as decay-associated fluorescence spectra (DAS).

3. Results

PS I-110, PS I-65 and isolated LHC I complex samples have been chracterized by steady state and time-resolved fluorescence. In one series of measurements we studied the influence of the detergent upon the stationary and kinetic spectral properties of PS I-110 using Triton X-100, OG, or SB12 as detergent. In a second series of measurements we investigated the influence of the particle size and composition upon the fluorescence steady state and kinetic properties.

3.1. Stationary spectra

The results of the steady state measurements of all sample types in two different detergents are summarized in Fig. 1. Fig. 1a–c show spectra taken at room temperature, while in Fig. 1d–f the data corresponding to 77 K are presented. The wavelengths of the maxima and shoulders in the fluorescence spectra of all preparations are compiled in Table 1. At room temperature the maxima of all particles are at about 680 nm and the larger size particles show pronounced fluorescence bands in the 720–740 nm region. This long wavelength fluorescence is substantially increased (relative to the short wavelength maximum) when changing the detergent to OG. All PS I particles show a drastic increase in fluorescence quantum yield upon cooling to 77 K. Essentially all of this emission increase occurs at wavelengths above 700 nm. PS I-110 shows a low temperature emission maximum at 735 nm with a large ratio of the fluorescence intensities of approx. 690 nm to 740 nm while PS I-65 shows a low temperature emission at approx. 720 nm.

Table 1
Wavelengths of maxima and corresponding amplitude ratios in steady state spectra of various PS I preparations in different detergents at room temperature (top) and 77 K (bottom)

	PS I-110		PS I-65		LHC I
	Triton	OG	Triton	OG	OG
RT					
λ_1 (nm)	$\underline{675}$	$\underline{685}$	$\underline{674}$	$\underline{675}$	$\underline{675}$
λ_2 (nm)	730	725	725	(729)	726
$A_1:A_2$	4.35	1.26	4.35	4.97	3.66
77 K					
λ_1 (nm)	$\underline{672}$	677	$\underline{671}$	(691)	$\underline{675}$
λ_2 (nm)	730	$\underline{735}$	720	$\underline{722}$	730
$A_1:A_2$	4.95	0.10	3.23	0.22	4.50

λ_1 (λ_2) is the position of the short (long) wavelength maximum; A_1, A_2 the corresponding amplitudes; underlined, main maximum; in parentheses, shoulder.

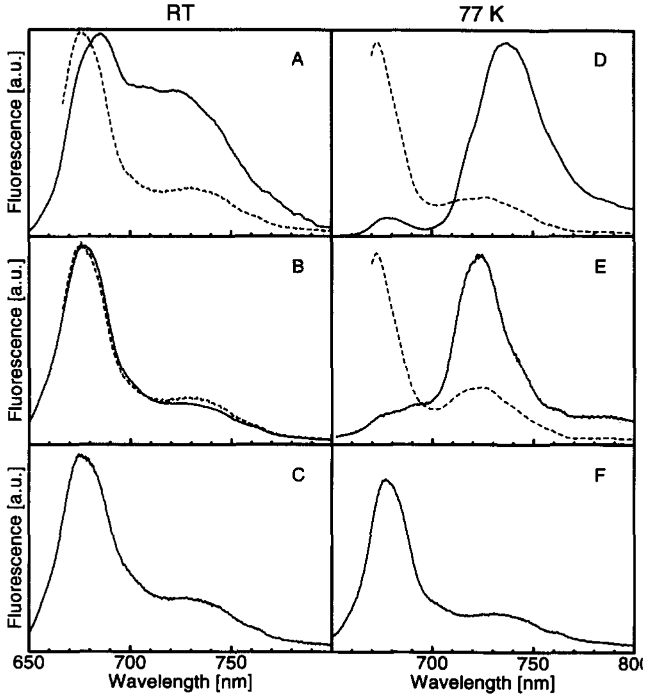

Fig. 1. Corrected stationary fluorescence spectra of the three preparations investigated. All spectra have been normalized to their relative maximum. The left column (a–c) shows data taken at room temperature, the three spectra on the right (d–f) were recorded at 77 K. The three rows represent the preparations that were investigated: PS I-110 (top), PS I-65 (middle), LHC I (bottom). The spectra were taken in Triton X-100 (dashed lines, excitation wavelength 650 nm, not recorded for LHC I) and in octylglucoside (solid lines, excitation wavelength 435 nm).

3.2. Time-resolved measurements

All time-resolved measurements have been performed at 285 K with an excitation wavelength of $\lambda_{exc} = 670$ nm. Decays have generally been recorded for detection wavelengths from $\lambda_{det} = 680$ nm to 736 nm in intervals of 7 or 10 nm. Samples were flowed at 12 ml/min in order to prevent photooxidation of P700, although we have previously found in other PS I preparations that oxidizing P700 had no effect on the lifetimes [27]. Data were analyzed by a global analysis procedure which was applied to complete sets of decay measurements over the whole emission wavelength range, the quality of the fits was judged by weighted residual plots and a χ^2 criterion. For all combinations of samples and detergents a sum of four exponentials was necessary to describe the kinetics adequately over a fitting range of 900 ps. This yielded the DAS and corresponding lifetimes τ_i ($i = 1, \ldots, 4$) for each of the samples. Figs. 2a–c show DAS exemplifying the effect of detergent for PS I-110 in Triton X-100, OG, and SB12, respectively. In Table 2 all lifetime data and the relative amplitudes for the longest-lived component (denoted A_{long}) are summarized. In order to analyze properly the long lifetime we had to use a longer fitting time window of 9.2 ns in addition to the normally used shorter fitting window of 900 ps. The smaller window serves for precise determination of the short lifetime components τ_1, τ_2 and τ_3 which are at the focus of our attention here, whereas the longest lifetime τ_4 can be determined precisely only in the large fitting window. For PS I-65 in OG actually five exponentials were necessary to give a good fit which resulted in a splitting of the long lifetime into two new ones as compared to a four-exponential fit (cf. Table 2). In Fig. 3, the DAS of all long-lived components of PS I-110 in the various detergents are shown in their proper relative contribution in their respective decay analysis.

The time-resolved fluorescence of LHC I was also measured at 680 nm and 730 nm upon excitation at 670 nm in Triton, OG and SB12 (same detergent concentrations as for other samples). These measurements were carried out in order to check whether any of the lifetime components in PS I particles, in particular the long-lived approx. 5 ns component was actually due to free LHC I. The single decay analysis of both measurements yielded one major lifetime (relative amplitude more than 90%) with time constants in the range of 5.1–5.6 ns and up to three minor components (cf. Table 2 for summary of data). Because detailed lifetime studies would be necessary to assign the faster components in LHC I, we do not comment further on internal processes in isolated LHC I.

3.3. Kinetics in dependence on antenna size and structure

In the second part of this study we investigated the kinetics of the two different PS I particles with OG as detergent. All other measurement conditions were identical to those described above. Fig. 4 shows the DAS resulting from the global analysis of these data. The DAS depend strongly on the type of particle. In particular lifetime component $\tau_2 = 18$ ps for PS I-65 (Fig. 4b) has a spectrum distinctly different from all the others. It shows very little long wavelength fluorescence in contrast to the larger particles.

4. Discussion

4.1. Stationary spectra

A very pronounced difference can be seen between the spectrum of PS I-110 and that found for PS I-65 lacking LHC I. At room temperature, a shift of the stationary emission maximum to 675 nm along with a pronounced increase of the ratio of short to long wavelength emission intensity as compared to the other PS I particles is seen in OG (cf. Table 1 and Fig. 1) and also in SB12 (data not shown). For the OG particles, at 77 K the emission maximum shifts from 735 nm in PS I-110 down to 722 nm for PS I-65. The changes induced by removal of LHC I when preparing the PS I-65 as well as those induced by lowering the temperature from 300 K to 77 K are in good agreement with the results of Arntzen and coworkers [10], who investigated very similar PS I preparations as we studied here.

Table 2

Lifetimes (ps) resulting from global analysis for PS I samples and from single decay analysis for LHC I samples investigated in various detergents

	PS I-110			PS I-65	LHC I		
	Triton	OG	SB12	OG	Triton	OG	SB12
Lifetime determined in small window							
τ_1	8	14	14	6	–	5	–
τ_2	31	44	49	18	–	54	–
τ_3	135	123	124	52		483	254
τ_4	4210	4190	1460	2260		7115	5315
Lifetime determined in large window							
τ_1	8	21	23	13	–	19	–
τ_2	45	72	90	39	129	192	–
τ_3	285	182	251	470	–	1444	1354
τ_4	5580	4750	3040	5180	5603	5167	5273
5-exponential analysis							
τ'_4	–	–	–	1476	–	–	–
τ'_5	–	–	–	5331	–	–	–
Rel. amplitude A_{long} (%)							
680 nm	42.2	2.7	0.1	4.9	94	66	98
722 nm	18.7	0.9	0.03	3.4	93	34	93

For precise analysis, two different fitting time windows of 900 ps (small window) and 9200 ps (large window) were used (refer to text for details). The lifetimes τ_1, τ_2 and τ_3 are determined more precisely in the small window, whereas τ_4 is determined better in the large window. A_{long} is the amplitude of the longest-lived component in the corresponding decay; its relative amplitude at 680 nm and 722 nm is listed. Errors in lifetimes in the small window analysis for τ_1 ± 2 ps, τ_2 and τ_3 $\pm 10\%$, in the large window τ_4 $\pm 10\%$.

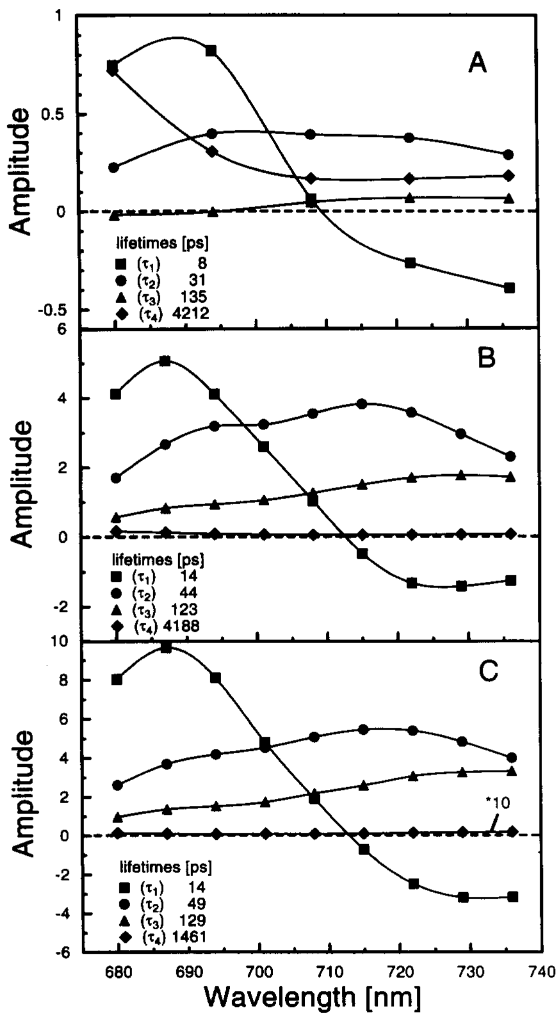

Fig. 2. Decay-associated spectra (DAS) of PS I-110 in three different solubilizing detergents as calculated by global analysis. (a) Triton X-100 (0.1%); (b) octylglycoside (0.73%); (c) SB12 (0.05%). The excitation wavelength was $\lambda_{exc} = 670$ nm. For errors in lifetimes refer to Table 2.

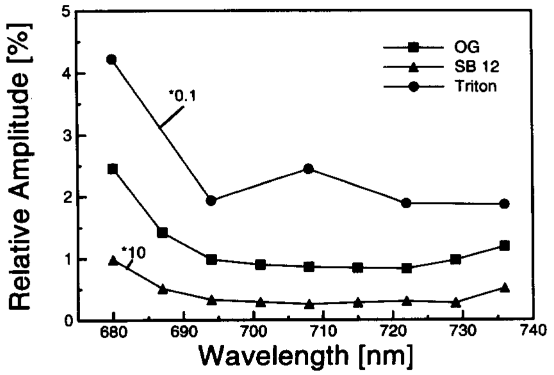

Fig. 3. Decay-associated spectra (DAS) of the longest-lived component (A_4) of PS I-110 particles as determined in three different detergents; circles: Triton X-100; squares: OG; triangles: SB12. The y-axis represents the relative contribution of the amplitude A_4 to the respective total decay amplitude. Both the spectra of PS I-110 in Triton and in SB12 have been rescaled by factors as indicated.

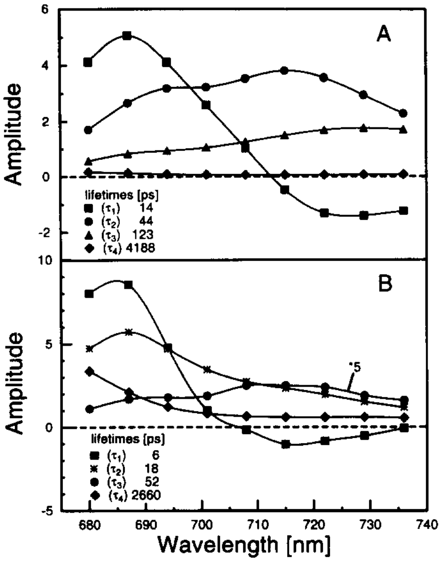

Fig. 4. Decay-associated spectra (DAS) of different PS I preparations. (a) PS I-110; (b) PS I-65. All measurements were done with octylglycoside (0.73%) as detergent. The excitation wavelength was $\lambda_{exc} = 670$ nm

4.2. Time-resolved measurements

The time-resolved data exhibit several characteristic and conspicuous features both in lifetimes and amplitudes of the DAS. First of all, the spectral shape of DAS of the lifetime component τ_1 shows a positive amplitude for wavelengths below approx. 710 nm and a clearly developed *negative amplitude* beyond that wavelength in all samples and all detergents (the exact zero-crossing wavelength depends somewhat on the particle type). This is an indication for an energy transfer process occurring within the antenna system of the PS I particles. The time constant of τ_1 increases with the antenna size: we find values of 6 ps for PS I-65 and 14 ps for PS I-110. Such rise terms in DAS as well as in τ_1 are clearly indicative of energy transfer from short wavelength emitting pool(s) (F690) to long wavelength emitting pool(s) (F720 and F735) [19,27]. They have not been reported previously for detergent isolated PS I particles from higher plants. The detection of this energy transfer process and also the corresponding time constants, however, are quite analogous to our previous results for PS I particles isolated from Synechococcus [19,27] and for detergent-free PS I preparations from spinach (Y100 stroma thylakoids) [26]. Mukerji and Sauer [16] did not resolve the fast energy transfer process in their measurements on very similar particles at the higher temperatures but only in some preparations at 77 K. After the time required for the τ_1 equilibration process all spectral developments in the excited states of the particles are finished and the excitation decays in a multi-exponential fashion without any further spectral changes in the emission spectra of the independently emitting particles, as can be judged from the absence of any other rise terms (negative amplitude components) in the DAS. (Note that all energy transfer components would have to show a negative amplitude in some part of the spectrum [14,36].)

4.3. Kinetics of PS I-110 particles

The two lifetime components τ_2 and τ_3 for the PS I-110 particles show distinctly different DAS in all detergents which allow us to safely exclude kinetic schemes where the τ_2 and τ_3 components arise from the same particle. Such a case has been found, e.g., for the charge recombination processes in PS II [1,37,38] which give rise to biexponential kinetics with identical DAS. We have in fact tested by target analysis various kinetic models on the data which assumed that the three fastest lifetime components derive from the same particle. These models all proved to be unsuitable for explaining the data, as could have been guessed already qualitatively from the DAS and corresponding lifetimes. The crucial point here is that two prominent components with all positive amplitude throughout the spectrum exist. From the basic definition of a DAS we can thus exclude that they arise from the same particle (see e.g. Refs. [14,39]). Rather these components must derive from different particles. In view of all these observations we propose that the most reasonable explanation for components τ_2 and τ_3 is that particles with different antenna size and/or structure coexist in solution, i.e., that the preparation is in fact heterogeneous. From their DAS, which display a large red contribution in fluorescence (F720 and F735), and their short lifetimes it is clear, however, that both components are related to functional PS I particles [27,40]. We can exclude that these components are due to isolated LHC I which has quite a different spectrum [12] and lifetime [41] (cf. Table 2 and Fig. 1). We thus suggest that all the preparations of PS I particles studied here and obtained with isolation procedures which are in fact quite similar to well established procedures [10], represent vastly heterogeneous particles which differ in total antenna size as well as in structure and/or in the ratio of blue/red pigments. The component τ_3 observed in our PS I-110 preparations is in fact reminiscent in lifetime and DAS to data reported before for stroma thylakoids from spinach [26,42]. Futhermore, for Synechococcus PS I core particles [19,27] the lifetime of about 36–40 ps and their DAS are rather close to those found here for component τ_3 in PS I-65 and τ_2 in PS I-110. This confirms the assignment of the τ_2 component to a population of particles with reduced antenna size as a consequence of the isolation procedure.

Since the core antenna size in higher plants is believed to be about 100 Chl and in view of the generally assumed similarity between the core antenna of higher plants and cyanobacteria [6], it is reasonable to suggest that the τ_2 component in the DAS of PS I-110 arises from more or less intact PS I core particles. This suggestion is based on the similarity of lifetime and DAS of τ_2 in PS I-110 and cyanobacterial PS I core [19,27]. The lifetime τ_2 will then be determined by the overall excitation decay due to charge separation as determined from previous work [19,27]. The energy transfer process indicated by the τ_1 component in these core particles also appears to have overall kinetics very similar to those from Synechococcus [27]. We can of course not be sure whether the observed spectral features are entirely representative for the intact core system, since we cannot exclude that the detergent also randomly removed some core chlorophyll during the isolation in addition to removing LHC I.

The DAS of the τ_3 component with a lifetime in the order of about 120 ps in PS I-110 is close to that found in intact pea thylakoids [2] and in detergent-free stroma thylakoids from spinach [26]. Arguing along the same line as above and comparing component τ_3 to component τ_2, we have to conclude that the former should reflect the overall excitation decay time and DAS of larger PS I particles than those giving rise to τ_2 (note that the overall lifetime should scale to some extent with the antenna size [43]). These larger particles then most likely represent particles with antenna size and structure close to the actual native PS I particles which are believed to have an antenna size of about 200 Chl (see e.g. Refs. [6,44] for reviews). This suggestion is strongly supported by the similarity of PS I lifetimes and DAS in intact thylakoids to those of the τ_3 component [42]. A very interesting feature of this DAS is the fact that the maximum is shifted to approx. 735 nm for this subpopulation. This is also seen in the steady state spectra at low temperatures (Fig. 1) and somewhat less pronounced also at room temperature. From the comparison with component τ_2 we conclude that the addition of the LHC I complex to the PS I core shifts the spectrum to long wavelengths. Thus the F735 emitting pigments should be located in the LHC I, as has already been proposed previously [12,13,45] on the basis of a steady state study on isolated LHC I and more recently by a time-resolved study [46]. Our time-resolved data fully support this view.

Judging from the lifetimes alone and assuming a simple linear relationship between lifetime and antenna size [47], we would have to conclude that the particles giving rise to component τ_3 are by about a factor of 3 larger than those responsible for component

τ_2. However, such a simple linear relationship is in fact not expected, since clearly the relative amount of red pigments is substantially higher in the particles (subpopulation) responsible for component τ_3 than in those responsible for component τ_2. These additional red pigments are located in the LHC I complex and tend to lengthen the lifetime substantially more than what could be expected from the mere increase in Chl/P700 ratio (see Ref. [14] for a detailed discussion). For this reason one may expect that linear antenna size/lifetime relationships as proposed earlier by Owens et al. [43,47] will not generally hold for all PS I particles, since a change in antenna size will in most cases also affect the relative content of red pigments (see below for a further discussion). Assuming that the antenna sizes of the two subpopulations are about 100 (component τ_2 corresponding to PS I core) and 200 (component τ_3 corresponding to native PS I) Chl/P700 [44] we can, from the area under the respective DAS and the average (bulk) antenna size of about 110 Chl/P700, estimate that our PS I-110 preparation contains in fact about 83% PS I core and about only 17% probably "native" PS I particles with functionally coupled LHC I. (Note that the integrated amplitude under the DAS is proportional to the total number of chlorophylls contributing to a certain pool and not to the number of particles [42,48], and that we further assume the absorption cross-section of all chlorophylls to be similar at the excitation wavelength. This is, however, quite reasonable, as we only find absorption changes in the long wavelength regions.) Calculation of the average antenna size in this distribution then yields 117 Chl/P700. This value is in very good agreement with the results of our measurements using chemical oxidation (100–120 Chl/P700).

It is interesting at this point to compare our lifetime results with those of Mukerji and Sauer [16] who measured a preparation that they called PS I-(200), assuming that it represented an intact "native" PS I antenna complex with LHC I attached. When we analyze the data of our PS I-110 preparation with only four components over a long time window, we do in fact get almost exactly the same lifetimes and DAS as reported by Mukerji and Sauer. However, under such analysis conditions we are also unable to resolve the fastest lifetime component which in optimal analysis shows a rise term in our case. The further observation that under such conditions we also get very similar amplitude ratios of the various components as compared to Mukerji and Sauer leads us to conclude that their PS I-(200) preparation was probably very similar to our PS I-110 preparation, which we now show to be highly heterogeneous and to contain only a small amount of actually intact "native" PS I. Thus the only difference with our results consists in their inability to resolve the fast equilibration component which can be attributed to the limited time resolution of that experiment, as the authors pointed out [16].

4.4. Kinetics of PS I-65 particles

The analysis of the PS I-65 preparation yields lifetimes and DAS that are quite distinct from those of the PS I-110 preparation discussed above. The fastest component shows again a rise term at long wavelengths, but the relative amplitude of the negative part as compared to the positive part of the DAS is much lower than in the other particles studied here. The corresponding lifetime is also shorter (6 ps as compared to 12–14 ps). The τ_2 component is quite short-lived (18 ps) and shows very little long wavelength fluorescence. In fact this DAS is very similar to the one observed by Owens et al. [43] for particles of antenna size of about 60 Chl/P700 and is also compatible with the data by Werst et al. [49] (antenna size 40–50). Our lifetimes are quite different and substantially shorter for the main component, however, than those reported in these previous publications. We attribute these differences to the fact that both groups had a smaller signal/noise ratio and did not apply global analysis procedures, so they simply could observe the average lifetime of the three short-lived components which we can resolve in our measurements due to the better component resolving power of the global analysis. Due to their lower time resolution the average value obtained by those authors is mainly determined by the contribution of the longer-lived component, which is also present in our preparations (τ_3, approx. 50 ps), albeit at a small percentage. We attribute the fastest (τ_1) component again to excitation equilibration within the antenna similar to the large PS I particles discussed above and the τ_2 and τ_3 components to the overall excitation decay due to charge separation in particles with different antenna sizes. Given the (bulk) average antenna size of about 60 Chl and the small amount of red pigments, as judged from the DAS and $\tau_2 = 18$ ps, this appears to be quite a reasonable charge separation time for such a small particle [47]. Furthermore, the lifetimes τ_1 and τ_2 can be compared to data measured by Causgrove et al. [32], who investigated PS I preparations of the same size (Chl/P700 approx. 60) by means of single-color transient absorption experiments. For an excitation wavelength of 670 nm, they find average values of approx. 6 ps and 18 ps. Taking into account the limitations given by their two-exponential single decay analysis, these values agree very well with the fluorescence data reported here. The τ_3 component in our PS I-65 preparation in both its spectrum and lifetime (52 ps) is close to the τ_2 component in PS I-110 (44 ps). A comparison thus points to the suggestion that it represents again more or less intact PS I cores. The slight increase from 44 ps to 52 ps may be indicative of a more complex antenna size distribution than just bimodal (see below). An interesting aspect is the relative amount of red pigments which is much smaller in the small PS I particles than for the τ_2 component found in the PS I-110 preparation. Obviously it is possible to extract a substantial amount of the red pigments by detergent. This addresses the so far unresolved question where these pigments are located. From kinetic modeling studies both we [14,20] and Jia et al. [18] have recently proposed that such red pigments should be located close to the RC. Our results might in fact suggest that most, but perhaps not all, of the red pigments are not located in the center but more at the periphery of the particles, possibly organized in a small cluster where they can be easily removed by detergent. The feasibility of such a scheme has been addressed by kinetic modeling studies [14]. This poses again the so far unresolved question of the actual role of red pigments in the function of this and other photosystems.

At this point a reconsideration of the lifetime and DAS component τ_1 in each of the decays is in order. In view of the above discussion of heterogeneity in the samples and the observation that this lifetime actually depends sensitively on the antenna size, we would expect to find two different rise components, one for each positive DAS, rather than just one as was actually observed. However, resolving so many components of similar shape and lifetime is not possible with the present data analysis techniques and the achievable signal/noise ratio. We suggest to consider the experimentally resolved τ_1 component as a mixture of two components (see Refs. [50] and [14,20] for a more detailed discussion of the excitation equilibration process).

Beyond the considerations discussed above, one further central point should be stressed. Independent of particle type or detergent, in all our DAS we find only one energy transfer process in the antenna system associated with lifetimes in the order of 10 ps. Though there might still be faster unresolved components, we do not find any spectral evolution with longer time constants than about 14 ps, which inevitably means that no further antenna equilibration processes go on after that time. If we compare these equilibration times to the charge separation times we measured (minimum 45 ps and up to 130 ps), we find a ratio between 3:1 and 10:1 in these lifetimes in the various particles. This clearly fulfills the condition of a trap-limited kinetics, which is in good agreement with our results with Synechococcus [27], but seems to be at variance with the nearly diffusion limited kinetics as suggested previously by Owens et al. [43,47]. However, more recent direct measurements of the energy transfer of PS I antennae in the femtosecond range clearly show that much of the antenna equilibration process indeed occurs in less than about 1 ps [19] while equilibration with the long wavelength pigments takes about 10 ps in PS I cores [14,19].

Finally, we should mention that our finding of a heterogeneity comprised of two systems might in fact still be a simplified picture, since even in global lifetime analysis it is generally difficult to resolve lifetimes that differ by less than a factor of 2, in particular when their spectral shapes are similar. Due to these limitations we cannot exclude a more complex distribution of antenna sizes rather than two discrete ones. There could be higher modal distributions and even a continuous range of lifetimes and thus antenna sizes cannot be excluded a priori.

4.5. Detergent effects on the kinetics

Based on the above discussion it is now interesting to look at the detergent effects in detail. When using Triton we observe a very large amplitude component with lifetime τ_4 in the order of 5 ns. The corresponding DAS has its maximum below 680 nm. The lifetime is very similar to that of isolated LHC I (cf. Table 2) and also that of free chlorophyll [51]. We can, however, exclude free chlorophyll as the main origin of this component, since this would most likely have been separated by the isolation procedure on the column. If the same sample (with Triton as detergent) is diluted with buffer that contains OG instead of Triton, the amplitude of this component is reduced drastically from about 40% down to about 3% (see Fig. 3). It is important to note that no chlorophylls are lost or gained during this detergent exchange. The decrease in the amplitude of τ_4 is accompanied by an increase in lifetime of τ_1 from 8 to 14 ps, in an increase of τ_2 from 31 to 44 ps and in a large increase in the amplitude of component τ_3 relative to that of component τ_2. This trend is further strengthened upon using to SB12 as detergent. All of these observations can be rationalized by assuming that LHC I, which is functionally almost completely decoupled from the core when using Triton X-100, actually gets recoupled in the other detergents upon simple detergent exchange. This represents in fact an in situ LHC I/PS I-65 reconstitution experiment. Very clearly Triton functionally detaches LHC I and thus does not represent a good detergent for obtaining intact PS I, despite the fact that it is used in most isolation protocols for PS I described in the literature (see Materials and methods). The effect of exchange of Triton by milder detergents on the stationary spectra has already been observed by Nechushtai et al. [52], who reported pronounced spectral changes at 77 K upon exchanging Triton for dodecylmaltoside detergent. Our results are completely in line with their interpretation. These experiments also suggest that the τ_3 component belongs to PS I particles that carry the

LHC I complex. Of all the detergents that we have tried SB12 gives by far the best functional coupling of LHC I and possibly also of the other chlorophylls to the PS I particle, as can be judged from the almost negligible amount of long-lived components in that detergent.

5. Conclusions

Our results show that detergents have a dramatic influence on the integrity and structure of PS I antennae. The preparation procedures used here are very similar to protocols considered so far to yield uniform preparations. We now show that in fact they result in heterogeneous distributions of antenna sizes. We suspect that this is mainly due to the effect of Triton X-100 which is common to all these procedures, i.e., the extraction of PS I from thylakoids. We propose that SB12 represents a much better detergent for PS I than Triton or even OG. It remains to be tested whether SB12 is suitable to directly extract PS I from thylakoids. The detergent treatment has also a severe influence on the content of red pigments which are determining to a large extent the spectral shape, the excitation equilibration time and last but not least the overall trapping lifetime of the particles [14]. We have presented here a simplified preliminary picture of the excitation kinetics in isolated PS I from higher plants. It is clear from our results that any meaningful detailed and more exact kinetic models, including possible linear or non-linear antenna size relationships of overall lifetimes and more detailed kinetic models, must await the development of improved isolation procedures that yield more homogeneous particles both in antenna size and in pigment composition and structure. We suspect that unnoticed heterogeneity problems of the kind reported here are also responsible for some of the unexpected results reported by Owens et al. [43] who observed a break in the antenna size/lifetime relationship between small and large (LHC I containing) particles. Since their measurements were carried out with Triton, it may be assumed that also in their case LHC I was not functionally coupled. The proposed antenna size heterogeneity of all our preparations is not very surprising in view of the observations reported by Owens and coworkers [23], who studied the photooxidation kinetics of P700 in detail and came to similar conclusions with regard to antenna size distributions in isolated PS I particles. Finally, for all the particles our data indicate that antenna equilibration occurs on a time scale several times faster than the overall excitation decay. Thus we can conclude that the overall excitation decay kinetics is not limited by excitation diffusion in the antenna but is closer to be trap-limited, as already proposed by us previously [19,26,27].

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