PICOSECOND TIME-RESOLVED FLUORESCENCE FROM DETERGENT-FREE PHOTOSYSTEM I PARTICLES

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ABSTRACT Picosecond time-resolved fluorescence measurements have been taken on a detergent-free P700-enriched complex at room temperature isolated from the blue-green alga *Phormidium luridum* with a chlorophyll *a* to reaction center ratio of 100. Emission at greater than 665 nm is characterized by two exponential-decay components. A fast component, which dominates the initial decay with an average lifetime of 16 ps and 87% amplitude, is attributed to excitations in the core antenna chlorophyll-proteins, which are rapidly trapped by the primary electron donor, P700. A second component, with an average lifetime of 106 ps and 13% amplitude, is attributed to the peripheral antenna proteins. For 532-nm, 30-ps pulse excitation the results are virtually independent of fluence in the range of 2×10^{12} to 4×10^{16} photons/cm^2 and the oxidation state of P700. Addition of sodium dodecyl sulfate to 0.1% causes the second component's lifetime to increase by an average of a factor of 2.5. Only minor changes are observed in the first component's lifetime and the relative amplitudes of the two components. Two fractions isolated from the detergent-treated samples have also been examined. Our results indicate that excitation energy transfer within photosystem I is very efficient and that the excitation kinetics of the antennae may be limited by the trapping rate of P700 or strongly affected by the heterogeneity of the antennae.

INTRODUCTION

For photosynthesis to proceed efficiently, a complex structural organization of light-harvesting antenna pigments has evolved which transfers absorbed energy to the reaction center complexes of photosystems I and II (PSI and PSII) on a picosecond time scale. The small fraction of this energy lost as fluorescence provides a major probe for investigating the kinetics of excitation transfer in the initial stages of the photosynthetic process. Much has been learned from time-resolved fluorescence studies of intact green plant chloroplasts and algae (see reviews 1–6), but improved procedures for isolating the individual photosystems provide the means for examining the parts to help elucidate the very complex whole system.

Multi-exponential analyses of photon-counting fluorescence measurements from green plants and algae have revealed a component with a decay time ranging from 40 to 130 ps that has been attributed to PSI (3, 4, 7). Recently, a 53-ps decay from a mutant strain lacking PSII has also been reported (8). Early investigations of PSI-enriched fractions of varying purity and chlorophyll *a* to primary electron donor of PSI (P700) ratios (usually ~40–100 Chl/P700) reported single-exponential decays from 10 to 130 ps (2, 9). Current studies have resolved multi-exponential decays with estimates of the lifetime of the prominent fast component in the 45 (10, 11) to 20 ps (12) range (see review 3). A recent transient absorption study of a Triton X-100 PSI preparation with a Chl/P700 ratio of 60 reported a 20–45-ps decay component (13), while a 10-ps decay has been measured from a highly enriched PSI preparation with an 8–10 Chl/P700 ratio (14). All past studies have examined PSI-enriched fractions prepared with detergents, a process that can disrupt the native Chl-protein structure essential for efficient energy transfer to P700 (13, 15, 16).

Our intent in this work is to understand the effect that detergent has on the excitation transfer network formed by the interacting PSI antenna groups and accurately measure the rapid decay component characterizing excitation transfer from the core antennae to P700. We are studying a unique PSI preparation that does not use detergents (15, 17). These photoactive, detergent-free PSI particles have a Chl/P700 ratio and spectral characteristics similar to those of the whole cells and are free of PSII contamination, making them in all likelihood an excellent reflection of PSI in the intact cells. The results of this work update a previous report in abstract form (18).

MATERIALS AND METHODS

1. Sample Preparation

The blue-green alga *Phormidium luridum* was grown as described previously (19). Isolation from this alga of a Chl-protein complex

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enriched in P700 was performed without detergent (15–17). Cells in 50 mM Tris buffer (pH 8.0 at 22°C) were lysed by lysozyme overnight, frozen, and then thawed. After filtering the material, it was passed through a Sepharose 4B column, eluting a green solution which was passed through the column again to remove phycobiliproteins. The green solution was separated into five fractions using sucrose density-gradient centrifugation. The orange-green fraction was collected, placed on a second sucrose gradient, and centrifuged separating it into two fractions. The green fraction, highly enriched in P700, was collected and suspended in buffer or lyophilized and kept at −15°C until needed.

Room temperature time-resolved fluorescence measurements were performed on (*a*) freshly prepared samples, frozen and then thawed before use, and (*b*) lyophilized samples resuspended in buffer solution. Lyophilized samples were also examined in buffer with (*a*) 10 mM sodium dithionite as a reducing agent, (*b*) 10 mM potassium ferricyanide as an oxidizing agent, and (*c*) sodium dodecyl sulfate (SDS) detergent to a concentration of 0.1% added. Sample solutions were prepared to a variety of concentrations but with an absorbance of ≤0.15 at 532 nm in a 1- or 0.5-mm path-length glass cuvette to ensure uniform excitation. Between measurements the sample was checked for settling and occasionally stirred. No sample degradation was observed during the time of the measurements as checked by comparing results taken at the beginning and end of a run. Room temperature absorption spectra for each sample were measured using an absorption spectrophotometer (Lambda-3; Perkin-Elmer Corp., Norwalk, CT). These P700-enriched complexes had a Chl/P700 ratio of ~100 (15). The ratio of Chl to P700 was ~111 in the broken cells. The PSI fraction accounted for 49% of the total Chl of the whole cells. In buffer solution at room temperature, this PSI preparation had an extinction coefficient of 68.7 mM^{-1} cm^{-1} at 680 nm, which was also the peak of absorption in the intact cells. Large spectral shifts resulted from the addition of detergent (15). At 295 K, the detergent-free PSI particles had a fluorescence peak at 687 nm which after treatment with SDS shifts to 678 nm. The addition of detergent also caused the Chl/P700 ratio to drop to 51. Isolation procedures and spectral characteristics for all the above PSI preparations have been reported in references 15 and 17.

Time-resolved fluorescence measurements at room temperature have also been taken on two fractions, H (heavy) and L (light), isolated from the detergent-free PSI preparations (20). These fractions were prepared by adding the anionic detergent SDS to the originally detergent-free PSI particles (SDS/Chl = 6:1, wt/wt), then passing them through a Sephacryl S-200 column and eluting with 0.05% SDS-Tris buffer at pH 8.0. The two resulting fractions were then collected and dialyzed in Tris buffer at 4°C with several changes to remove the SDS detergent (20). The samples were kept frozen and were thawed just before use. The H fraction contained ~43 Chls per P700 while the L fraction had a Chl/P700 ratio >300 and appeared to be made up of mostly peripheral light-harvesting Chl-proteins (20). The room temperature emission peak for the H and L fractions was 677 nm. The physical and photochemical properties of these fractions have been reported in reference 20.

2. Excitation and Fluorescence Detection

The samples were excited with single 30-ps 532-nm light pulses from a frequency-doubled active-passive mode-locked Nd^{3+}/YAG (neodymium^{3+}/yttrium aluminum garnet) laser at a repetition rate of 0.5 Hz. Time-resolved fluorescence was detected using a low-jitter (<2 ps) streak camera, an image intensifier, and a gated silicon-intensified target (SIT) vidicon detector read by an optical multichannel analyzer (21) (OMA-II; EG&G Princeton Applied Research Corp., Princeton, NJ). Details on excitation and collection optics, data acquisition, and instrument response corrections have been described elsewhere (22, 23). Measurements on malachite green in methanol (Lin, S., and B. P. Wittmershaus, unpublished data) and on DNA (24) with this apparatus have been successfully deconvoluted to resolve fluorescence lifetimes as small as 4 ps.

The excitation pulses were best described as having a spatial intensity distribution which was a two-dimensional Gaussian profile across the sample's surface and which remained uniform as it passed through the thickness of the sample. The excitation fluence was defined as equal to $N/\pi r_0^2$, where N is the number of excitation photons and r_0 is the radial distance from the peak of the excitation profile to the e^{-1} point. Excitation fluence ranged from 2×10^{12} photons/cm^2 to 4×10^{16} photons/cm^2.

Fluorescence was collected through glass long-pass cutoff filters (Schott Glass Technologies Inc., Duryea, PA), usually an RG-665 (665-nm cutoff) and/or interference filters. On average, 100–400 shots were taken for each measurement. No fluorescence from the cuvette or buffer at wavelengths >590 nm was observed. The diameter of the focused spatial image ranged from 8 to 17 OMA channels, depending on the excitation optics. Three separately prepared lyophilized PSI samples, one fresh-frozen PSI sample, and one preparation of the H and L fractions were examined.

3. Data Analysis

A simple least-squares routine was developed to fit the data to one or two kinetically isolated components by iterative convolution. Theoretical curves were generated by numerical integration of the appropriate kinetic equations (23) using excitation profiles taken directly from excitation light scattered from each sample. The data were effectively fit to a sum of exponential decays. The amplitudes before the exponential terms ($\%A$) and the lifetime (τ) for each population were varied in a search to minimize the variance of the fit (25) similar to the technique used in other studies (7, 8). At the end of each run the results were used as the starting parameters until no further improvement in the fit could be found. The pulse profile was shifted within ±2 OMA channels to determine the location that gave the minimum fit variance. A plot of the normalized residuals $R(i)$, $R(i) = [\text{Data}(i) - \text{Fit}(i)]/\text{Data}(i)$ for the ith OMA channel was also considered in judging the quality of the fit. To estimate the "acceptable" range of parameters, the curve-fitting program was run with one parameter held fixed away from its minimum-variance fit value while the others were allowed to change. The resulting parameters and residual curve were then compared with those of the best fit. Systematic deviations above the noise level for typical measurements were observed in the residual plots when the fixed parameter or the variable ones were not within approximately ±12% of their best-fit values.

RESULTS

Fig. 1 shows a typical plot of the time-resolved fluorescence intensity from detergent-free PSI particles in buffer solution at 22°C for emission of wavelengths >665 nm under low excitation intensity at 532 nm. Also shown is the least-squares fit obtained to these data with the sum of two exponential-decay components and the normalized residuals plot, which is typical of most of the fits. Table I lists the averaged results of analysis on three resuspended lyophilized samples. The total average values are $\tau_1 = 15.7 \pm 0.7$ ps, $\tau_2 = 106 \pm 8$ ps, $\%A_1 = 87 \pm 1$, and $\%A_2 = 13 \pm 1$. Sample variations show up mostly in the lifetime of the second component. The latter being a small part of the initial time-resolved fluorescence, the observed changes between samples are relatively minor, yet clearly resolved. The rapid initial decay remains fairly constant from sample to sample. No significant changes in fluorescence decay were observed when the fluorescence was filtered through a 685-nm, 14-nm bandwidth interference filter or a 695-nm long-pass cutoff filter. The addition of sodium dithionite to 10 mM as a reducing agent or potassium ferricyanide to 10 mM as an oxidizing agent also did not measurably change the fluorescence decay observed. The average

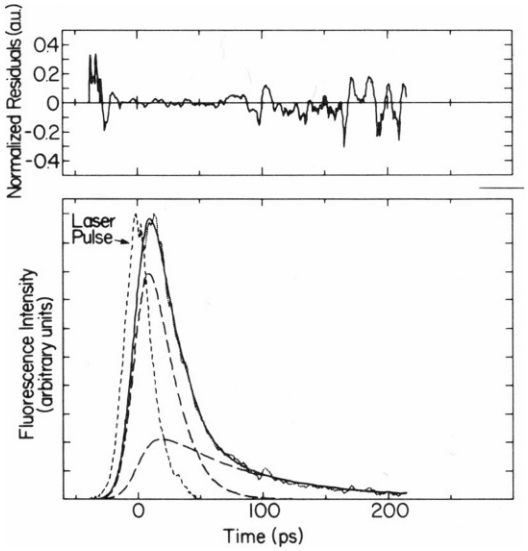

FIGURE 1 Time-resolved fluorescence at wavelengths >665 nm from detergent-free PSI particles at room temperature (Chl/P700 ≃ 100). The experimental curve (·····) has a two-component least squares fit (——) of τ_1 = 15.83 ps, $\%A_1$ = 87.41, τ_2 = 76.21 ps, and $\%A_2$ = 12.59. The individual fit components are also plotted (——). Above is a plot of the normalized residuals, $R(i)$. The sample is excited with 532-nm light pulses of fluence 2.3 × 10^3 photons/cm^2 (-----). The experimental data are a sum of 100 shots. One OMA channel equals 0.64 ps. The lyophilized sample was suspended in a 50 mM Tris buffer solution (pH 8, 22°C) to an optical density at 532 nm equal to 0.14 in a 1-mm cuvette. Refer to Table I for average fit parameters.

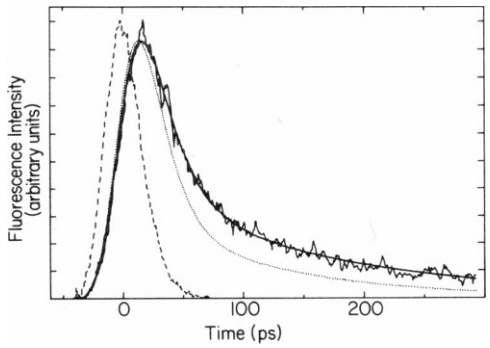

FIGURE 2 Time-resolved fluorescence at wavelengths >665 nm from a non-lyophilized (fresh) detergent-free PSI sample (——). The two-component fit (smooth, ——) has parameters τ_1 = 21.36 ps, $\%A_1$ = 79.68, τ_2 = 159.6 ps, and $\%A_2$ = 20.32. The excitation pulse (-----) fluence is 4.7 × 10^{13} photons/cm^2 (100 shots averaged). One OMA channel equals 0.74 ps. Also shown for comparison is the average fit for the resuspended lyophilized samples (·····), τ_1 = 15.7 ps, $\%A_1$ = 86.8, τ_2 = 106 ps, and $\%A_2$ = 13.2.

parameters for measurements taken on a fresh preparation of PSI particles which was frozen then thawed before use are also given in Table I. A representative data curve for this sample is shown in Fig. 2 with the average fit to the three lyophilized samples plotted for comparison.

The great majority of our data are fit adequately with two components within the noise of the measurement. We cannot strictly rule out the presence of a third small component with a long lifetime because of fitting limits imposed by the limited time duration and signal-to-noise ratios of our measurements. This is particularly relevant to data from SDS-treated samples discussed later. While the average results for each of the three lyophilized samples are in good agreement, two-component least-squares fittings on individual curves give ranges of τ_1 from 9 to 23 ps, v_2 from 60 to 200 ps, and $\%A_1$ from 81 to 93. Sample variation and fitting error are the major causes. Table I lists the standard deviations for each set of measurements.

To check for distortion of the fluorescence decay caused by exciton annihilation (26), the excitation fluence at 532 nm was varied within the range of 2 × 10^{12} to 4 × 10^{16} photons/cm^2. In all cases no change was observed in the decay curves for fluences of 7 × 10^{13} photons/cm^2 or less. Above this value ~70% of our measurements showed no detectable change as illustrated in Fig. 3. In a few sets of measurements the lifetime and to a lesser extent the amplitude of the second component was observed to

TABLE I
RESULTS OF TWO-COMPONENT LEAST-SQUARES FITTING OF FLUORESCENCE >665 nm FROM ROOM-TEMPERATURE DETERGENT-FREE PSI PARTICLES AND ISOLATED FRACTIONS FROM *P. LURIDUM**

Sample	τ_1	τ_2	$\%A_1$	$\%A_2$	No. of measurements
	ps	*ps*			
Lyophilized					
A	17.7 (1.7)	142 (43)	90.3 (3.2)	9.7 (3.2)	6
B	17.6 (4.0)	84 (14)	84.7 (4.7)	15.3 (4.7)	8
C	13.6 (2.8)	103 (47)	86.4 (5.3)	13.6 (5.3)	13
Average	15.7 (3.5)	106 (43)	86.8 (5.0)	13.2 (5.0)	27
0.1% SDS treated‡	×1.25 (0.22)	×2.50 (0.63)	×1.02 (0.07)	×0.98 (0.29)	10§
Fresh	21.8 (1.1)	180 (22)	81.7 (2.1)	18.3 (2.1)	3
Heavy fraction	21.6 (2.6)	426 (45)	91.7 (1.1)	8.3 (1.1)	5
Light fraction	21.3 (2.4)	1242 (116)	52.3 (5.5)	47.7 (5.5)	4

*Values in parentheses are standard deviations.
‡Values given are multiplicative factors related to changes relative to the detergent-free PSI sample.
§Ten pairs of measurements (PSI particles with and without SDS) using all three of the lyophilized samples were performed.

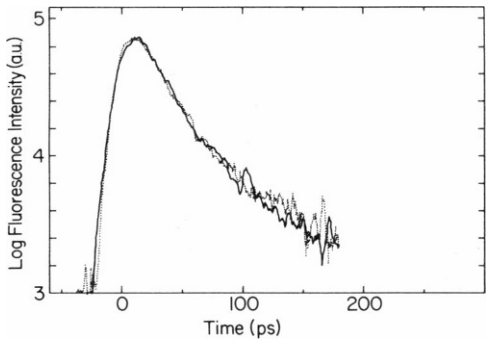

FIGURE 3 Dependence of the fluorescence decay from detergent-free PSI particles on the fluence of excitation at 532 nm. Log_{10} of fluorescence at wavelengths >665 nm is plotted for excitation at 2.3×10^{13} photons/cm^2 (——) and 2.7×10^{15} photons/cm^2 (· · · · ·). The curves are normalized and each is an average of 100 shots. One OMA channel equals 0.64 ps.

decrease slightly (≤30%) as the fluence level increased above 1×10^{14} photons/cm^2, with changes observed in τ_1 being well within the fitting uncertainty.

The time-resolved fluorescence data from PSI particles with and without SDS detergent added to 0.1% are compared in Fig. 4. Ten pairs of measurements, with identical experimental conditions within each set, covering all three lyophilized samples were performed. The average results of SDS addition (see Table I) are that τ_1 remains nearly constant (increases by a factor of 1.25 ± 0.07), τ_2 becomes significantly larger (increases by a factor of 2.5 ± 0.2), and the relative amplitudes of component one and component two are unchanged (the ratio of $\%A_1$ to $\%A_2$ = 1.02 ± 0.02 times the non-detergent result). No effect of exposure time was observed from 0.5 to 3 h before measurement, the typical range of periods of exposure for these experiments. One SDS-treated sample was examined for excitation intensity dependence over a fluence range of 2×10^{12} to 3×10^{15} photons/cm^2 with no change in the fluorescence decay seen. It was observed that adding SDS to this detergent-free preparation had the immediate effect of changing a moderately turbid solution to a more translucent one. This was clearly characterized by changes in the absorption spectra (15).

The time-resolved fluorescence measurements on one preparation of heavy and light fractions separated from the SDS-treated PSI particles (20) are shown in Fig. 5, with the fitting results given in Table I. Two-component fittings were again found satisfactory in representing the data. The heavy fraction, which contains most of the P700 (20), exhibited no change in fluorescence decay in the fluence region examined, 2×10^{13} to 7×10^{14} photons/cm^2. Fluorescence detected through a 570- or 665-nm long-pass cutoff filter gave identical curves. Use of a 695- or 715-nm long-pass cutoff filter produced decays with a slightly larger τ_1 (~20% or a 4 ps increase) and a small decrease in $\%A_2$ (~10%). The light fraction, which was mostly antenna Chl-proteins, was insensitive to fluence in the range 2×10^{13} to 9×10^{15} photons/cm^2. Fluorescence measured through a 570-, 665-, or 715-nm long-pass cutoff filter gave the same results.

DISCUSSION

The fluorescence decay from this detergent-free preparation of PSI particles is characterized by two exponential-decay components: a very rapidly decaying component (≃16 ps), representing a majority of the Chl population, and a smaller, longer-lived component (≃106 ps) (Fig. 1, Table I). In terms of the model of the antenna Chl-protein organization in PSI proposed by Mullet et al. (16), the fast

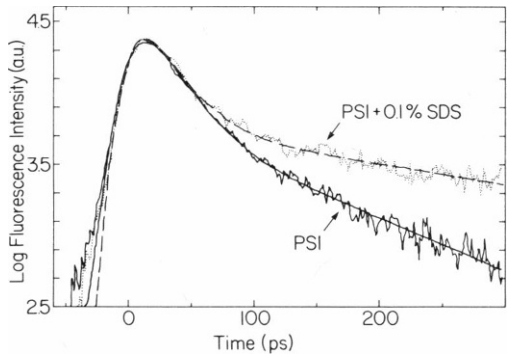

FIGURE 4 The effect of addition of SDS detergent to a concentration of 0.1% on the time-resolved fluorescence from originally detergent-free PSI particles. Log_{10} of fluorescence at wavelengths >665 nm from the detergent-free sample (——) is plotted with the two-component fit (smooth, ——) of τ_1 = 18.23 ps, $\%A_1$ = 88.29, τ_2 = 118.1 ps, and $\%A_2$ = 11.71. Log_{10} of fluorescence from the PSI sample after the addition of SDS to 0.1% (· · · · ·) is plotted with the two-component fit (——) of τ_1 = 23.03 ps, $\%A_1$ = 87.33, τ_2 = 305.6 ps, and $\%A_2$ = 12.67. The excitation pulse (-----) fluence is 8.8×10^{13} photons/cm^2. The experimental curves are normalized and each is an average of 200 shots. One OMA channel equals 1.28 ps. Notice the large change in slope in the 100–300 ps region, indicating an increase in τ_2.

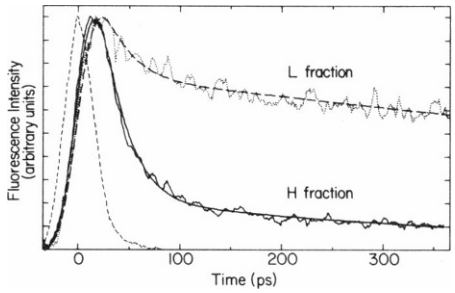

FIGURE 5 Time-resolved fluorescence at wavelengths >665 nm from the heavy (H) and light (L) fractions isolated from detergent-free PSI particles after detergent treatment (20). The H fraction (——), a highly P700-enriched particle with a Chl/P700 = 43, has a two-component fit (smooth, ——) of τ_1 = 20.97 ps, $\%A_1$ = 90.20, τ_2 = 432.9 ps, and $\%A_2$ = 9.80. The excitation fluence is 6.5×10^{13} photons/cm^2. The L fraction (· · · · ·), composed of PSI particles and antenna Chl-proteins not coupled to PSI units (Chl/P700 > 300), has a two-component fit (— —) of τ_1 = 19.96 ps, $\%A_1$ = 56.52, τ_2 = 1360 ps, and $\%A_2$ = 43.48. The excitation pulse (-----) fluence is 8.3×10^{14} photons/cm^2. For both data curves, one OMA channel equals 1.03 ps. The experimental curves are normalized and each is an average of 200 shots.

decay component can be attributed to the core and internal antennae which rapidly transfer excitations to P700. These protein complexes remain fairly intact during sample preparation. The longer-lived second component can then be identified with the peripheral antenna Chl-proteins (16), which may be bound to the core antennae and/or directly to P700. These assignments are supported by comparing the removal of peripheral antennae by detergent treatment of PSI (16) with our detection of a large effect by detergent on the lifetime of the second component and our measurements on the H and L fractions. This is discussed in detail below. The framework of the organizational scheme of Mullet et al. (16) is applicable to PSI preparations from algae (6). The core and internal antenna complexes of PSI (16) are similar in algae and higher plants (27, 28). There are, however, differences among the Chl-proteins comprising the peripheral antenna complex (i.e., C705, which is a spectral form of Chl *a* absorbing maximally at 705 nm [1]) (6, 28, 29).

Though a large majority of our data are fit well with the sum of two exponential-decay components, we do not believe this accurately describes the interactions between the protein groups making up PSI. It is more likely that after isolation procedures the peripheral antennae have been disrupted and are left with varying degrees of excitation-transfer coupling[1] to the rest of the PSI units. This results in sample-to-sample fluctuations reflected in the uncertainty of τ_2 and $\%A_2$ (see Table I). The peripheral Chl-proteins are the first removed upon further P700 enrichment (15, 16, 29) and the addition of moderate amounts of SDS dramatically affects their excitation-transfer efficiency (Fig. 4, Table I). Freezing and thawing of the fresh sample has the similar effect of disrupting the peripheral antennae as illustrated by the results of Table I and Fig. 2. Therefore the second component in our analysis represents an average over the distribution of lifetimes for these sensitive peripheral antennae. The curve shown in Fig. 1 is from a sample in which the peripheral antenna Chls were generally more efficient in transferring their excitations to the PSI units (τ_2 = 76 ps) than for the average sample (τ_2 = 106 ps). The smallest estimate for τ_2 ($\simeq$60 ps) is indicative of the lowest limit of coupling between the peripheral antennae and PSI in vivo. In view of their sensitivity to isolation procedures, rates of energy transfer in excess of 1.7×10^{10} s^{-1} are likely.

Considering the rapid quenching of excitations in the core antenna Chls by P700, it is not surprising that exciton–exciton annihilation has a smaller effect on the measured rate of decay (Fig. 3) in comparison with experiments on whole chloroplasts (23, 26). Minor changes (decreases of $\leq$3 ps) in the decay of a 16-ps lifetime population under an excitation fluence up to 4×10^{16} photons/cm^2 are observed after solving the appropriate kinetic equations numerically (23) with a 6×10^{-9} cm^3/s annihilation rate, an 8×10^{19} cm^{-3} chromophore density, and a 1.5×10^{-17} cm^2 ground-state absorption cross-section. These parameters are typical of those derived from similar measurements and analysis on chloroplasts (23). This illustrates that under these excitation conditions, the fast decay component is not significantly altered by exciton annihilation. This has been noted in many other investigations (9, 13, 14). The later two studies did indicate a decrease in fluorescence yield with an increasing intensity, which was attributed to saturation and possible rapid annihilation within the time scale of excitation (30). A minority of our measurements indicate that exciton annihilation may have affected the fluorescence decay of the second component at high excitation intensities. This has been observed by Il'ina et al. (13).

The fluorescence decay of the detergent-free PSI particles at room temperature (Fig. 1) is mostly from protein-bound Chls with emission maxima ~685 nm. These are found in the core, internal, and peripheral complexes (16). There are several observations that support this assignment. The largest contribution to the steady-state fluorescence spectra is from components centered approximately at 685 nm (15, 17). Only small changes are observed in the time-resolved emission as measured through a 685-nm interference filter or a 665- or 695-nm cutoff filter. After removing a considerable amount of the peripheral antennae, the resulting H fraction (20) still has a fluorescence decay very similar to that of the intact detergent-free PSI preparation (Figs. 5 and 1, respectively). This predominance of emission from the 685-nm component at 295 K has obscured resolution of the weak fluorescence from the long-wavelength Chls, responsible for 720-nm (15, 17) and 735-nm (31) emission at 77 K.

In the absence of detergent, the hydrophobic proteins of PSI (15, 16) tend to clump together in aqueous solution, producing a turbid sample. Peripheral Chl-proteins disturbed during preparation do not become separated but cling to the larger PSI complexes, permitting excitation transfer to occur. The addition of a strong anionic detergent, like SDS, even at the moderate level of 0.1%, removes peripheral Chl-proteins from the PSI particles and may cause dispersion of the clumped PSI units into separate particles. This is supported by the successful use of detergents in isolating separate antenna complexes from our initially detergent-free PSI particles (the H and L fractions [20]), and from other similar PSI preparations (16, 29, 32, 33). Our results indicate that the initial addition of SDS to this originally detergent-free PSI preparation does not change the amount of antenna pigment making up the more weakly coupled second component, but rather decreases its efficiency of excitation transfer to the rest of the PSI unit, resulting in an average increase in lifetime of a factor of 2.5 (Fig. 4, Table I). In solution these freed

[1]"Coupled" and "coupling" will be used to refer only to interaction among chlorophyll proteins which results in excitation energy transfer between their respective chromophores. It shall not necessarily imply physical binding or contact between them.

Chl-proteins may still have enough contact with the remaining PSI units to be loosely coupled for energy transfer such that their average lifetime remains less than the 1.2 ns observed for noncoupled antennae (see discussion for the L fraction below). The core and internal Chl-proteins are only slightly affected (τ_1 increases by a factor of 1.25), indicating that the detergent acts mostly on a definable group of proteins (the peripheral complex) as suggested by the work of Mullet et al. (16). The observations of Il'ina et al. (13) after addition to 1.5% of Triton X-100 detergent to their sample are qualitatively similar to ours, but their analysis revealed an increase in the relative amplitude of their long component ($\tau > 500$ ps) and no change in the lifetimes. The important difference between the two experiments is that Il'ina's detergent-prepared PSI particles had a Chl/P700 ratio of 60 and therefore had most of the peripheral antennae removed initially. We presume that the core and internal Chl-proteins were being acted on by the detergent in their study.

After addition of SDS to the detergent-free PSI particles, two complexes, the light and heavy fractions, can be separated (20). The light fraction contains a mixture of a fast ($\simeq$21 ps, 52%) and a very long-lived component ($\simeq$1.2 ns, 48%) (Fig. 5, Table I). The fast decay component indicates that some PSI units, presumably without much peripheral antennae attached to them, are present. The second population is clearly peripheral antennae stripped away from the PSI particles and energetically noncoupled. They therefore have a fluorescence lifetime indicative of isolated Chl-proteins, which agrees very well with the 1.2-ns lifetime measured from spinach light-harvesting Chl-proteins (34).

The heavy fraction may be similar to other PSI samples of (13, 27–29), and it is probably missing most of the peripheral antennae which end up isolated in the L fraction (16, 20). The H fraction contains mostly PSI units with relatively intact core Chl-proteins as indicated by the presence of a predominant fast decay component (Fig. 5, Table I). Though this fraction contains only half the original Chl in the PSI particles with a Chl/P700 of 43, its fluorescence decay is very similar to that obtained for the whole PSI preparation with 0.1% SDS added (Fig. 4) and in both cases only a small increase in τ_1 occurs (Table I). The increase in τ_1 observed for the H fraction when using a 695-nm or 715-nm cutoff filter may be evidence of longer lifetime emission from Chls fluorescing in the 720-nm region. Their fluorescence contribution should be more significant in the H fraction than in whole detergent-free PSI particles due to a decrease in the relative amount of Chls with fluorescence maxima around 685 nm in the H fraction.

Qualitatively our lifetime measurements agree with the majority of recent time-resolved studies on PSI-enriched fractions prepared by detergent methods, coinciding with the lower values of previous estimates of 20–45 ps for fast decay component and 100–300 ps for the smaller, longer-lived population (10, 12, 13). Rise-time measurements for P700 oxidation of 15–30 ps (13) and 25 ps (35) are also in agreement. Our result of $\tau_1 = 16$ ps is one of the smallest lifetimes assigned to the fast-decaying fluorescence associated with a large, isolated PSI particle (Chl/P700 $\simeq$ 100). Moya et al. (12) have reported a $\tau_1 = 15$–20 ps and the work of Il'ina et al. (13) has also implied that excitation transfer in PSI may be occurring this quickly. The difficulty of quantitatively comparing these reported lifetimes is compounded by the possibility that for some measurements the rapid decay component is shorter than the resolution of the experiment. Differences in sample preparation and starting material are also concerns. The core Chl-proteins seem to maintain their integrity fairly well as indicated by only minor changes in τ_1 after initial exposure to detergent (Fig. 4, and reference 13), further isolation procedures (Table I), and heat treatment (14). However, our observation of a slight increase in τ_1 for the heavy fraction and fresh sample (Table I) and the longer lifetimes observed by others for detergent-treated PSI particles (2–4, 9) could be explained by a degradation in the energy transfer coupling of the inner core antennae to P700.

In reviewing the time-resolved studies of PSI, we find no definite correlation between a decrease in the fluorescence lifetime reported and a decrease in Chl/P700. In fact, the larger lifetime estimates generally come from detergent-treated preparation with Chl/P700 ratios <100. These observations are consistent with the small change of τ_1 we detect in comparing detergent-free PSI to the smaller Chl/P700 H fraction (Table I).

For a simple model of a single trap fed by a homogeneous pool of Chl-proteins, the quenching of excitations in the antennae by the reaction center will depend on the size of the chromophore pool; the smaller the pool, the greater the quenching (5, 36; Gülen, D., unpublished report). How the quenching varies with pool size depends on the rate of trapping at the reaction center. For a trapping rate much less than the diffusion rate of excitations in the antennae (trap-limited case), the quenching of excitations is weakly dependent on the size of the antenna pool in comparison to the reaction center having a trapping rate much greater than excitation diffusion (diffusion-limited case) (Gülen, D., unpublished report). In terms of this simple model the lack of evidence for a decrease in τ_1 with a decrease in Chl/P700 indicates that exciton trapping in PSI is not strongly diffusion limited and may tend towards the trap limited case. This supports Il'ina et al.'s (13) conclusion that excitation transfer among the antenna Chls does not limit photo-oxidation of P700, and the work of Gulotty et al. (8), which gives evidence that an exciton visits P700 1.4–3.7 times before being trapped.

The homogeneous antenna model is an approximation for the known spectral heterogeneity of Chl-proteins in PSI (6, 16, 28, 29, 33). General models proposed for the organization of antennae in PSI (6, 16) lack the detail and

certainty for determining how much the excitation kinetics of PSI deviate from predictions of the homogeneous model. Certain configurations of a heterogeneous antenna network might explain some of our experimental observations. Within the peripheral complex are small amounts of long-wavelength spectral forms of Chl proposed to act as intermediate traps for excitations from the majority of Chls in the core, internal, and peripheral antennae (1, 4, 6, 16). Upon enrichment of a PSI preparation to Chl/P700 values significantly smaller than 100, the long-wavelength Chls of C705 in PSI from higher plants and of C690 in PSI from green algae are removed and can be isolated from the remaining PSI units (references 16, 32, and 33, and 28 and 29, respectively). Removal of these long-wavelength Chls could mean the loss of excitation traps for the core antenna proteins, resulting in an increase in the fluorescence lifetime of Chls in the core proteins. Another possibility is that Chls located near the reaction center and of energy below most of the antenna-Chls could funnel excitations towards the reaction center. The rapid localization of excitations into a small antenna pool may cause the excitation quenching rate to be less sensitive to the number of peripheral antennae.

If all the excitations in PSI went through such a small proximal antenna group before reaching the reaction center, the fluorescence lifetime of the large number of other Chl-proteins would not change with a change in the trapping rate at the reaction center. Only the proximal antennae's quenching rate would be affected. This scheme is consistent with our observation that the redox state of P700 does not affect the lifetime of 685-nm fluorescence from the bulk antenna-Chls. We did not, however, observe any increase in fluorescence after closure of the reaction centers attributable to a proximal antenna pool which might be expected to occur. Previous time-resolved work (11, 13, 14) and earlier studies of fluorescence yield from PSI (37) have shown that photo-oxidation of P700 causes little change in fluorescence lifetime or yield at room temperature. Other researchers have proposed $P700^+$ (37) or one of the reduced primary acceptors (38) as other possible quenching sites of nearly equal effectiveness as P700, but the cause of PSI's fluorescence being insensitive to redox state is still considered an open question. We hope that future work examining closely the effect of the redox state of acceptors on PSI's fluorescence decay and looking for further evidence for a proximal antenna pool may help to explain this issue.

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REFERENCES

1. Butler, W. L. 1961. A far red absorbing form of chlorophyll *in vivo*. *Arch. Biochim. Biophys.* 93:413–422.
2. Breton, J., and N. E. Geacintov. 1980. Picosecond fluorescence kinetics and fast energy transfer processes in photosynthetic membranes. *Biochim. Biophys. Acta.* 594:1–31.
3. Holzwarth, A. R. 1986. Excited state kinetics of chlorophyll antenna pigments. *In* Encyclopedia of Plant Physiology. Vol. 19. L. A. Staehelin and C. J. Arntzen, editors. Springer-Verlag, New York. 299–309.
4. Karukstis, K. K., and K. Sauer. 1983. Fluorescence decay kinetics of chlorophyll in photosynthetic membranes. *J. Cell. Biochem.* 23:131–158.
5. Van Grondelle, R. 1985. Excitation energy transfer, trapping and annihilation in photosynthetic systems. *Biochim. Biophys. Acta.* 811:147–195.
6. Bose, S. 1982. Chlorophyll fluorescence in green plants and energy transfer pathways in photosynthesis. *Photochem. Photobiol.* 36:725–731.
7. Holzwarth, A. R., J. Wendler, and W. Haehnel. 1985. Time-resolved picosecond fluorescence spectra of the antenna chlorophylls in *Chlorella vulgaris*. Resolution of photosystem I fluorescence. *Biochim. Biophys. Acta.* 807:155–167.
8. Gulotty, R. J., L. Mets, R. S. Alberte, and G. R. Fleming. 1985. Picosecond fluorescence study of photosynthetic mutants of *Chlamydomonas reinhardii:* origin of the fluorescence decay kinetics of chloroplasts. *Photochem. Photobiol.* 41:487–496.
9. Searle, G. F. W., J. Barber, L. Harris, G. Porter, and C. J. Tredwell. 1977. Picosecond laser study of fluorescence lifetimes in spinach chloroplast photosystem I and photosystem II preparations. *Biochim. Biophys. Acta.* 459:390–401.
10. Holzwarth, A. R., W. Haehnel, J. Wendler, G. W. Suter, and R. Ratajezak. 1984. Picosecond fluorescence kinetics and energy transfer in antennae chlorophylls of green algae and membrane fractions of thylakoids. *Adv. Photosyn. Research.* 1:73–76.
11. Shuvalov, V. A., A. V. Klevanik, A. V. Sharkov, P. G. Kryukov, and B. Ke. 1979. Picosecond spectroscopy of photosystem I reaction centers. *FEBS* (*Fed. Eur. Biochem. Soc.*) *Lett.* 107:313–316.
12. Moya, I., J. E. Mullet, J.-M. Briantais, and R. Garcia. 1981. Comparison between lifetime spectra of chloroplasts and subchloroplasts particles at −196 and 20 C. *Proc. Int. Congr. Photosynthesis, 5th.* G. Akoyunoglou, editor. Balaban Int. Sci. Services, Philadelphia. 163–172.
13. Il'ina, M. D., V. V. Krasauskas, R. J. Rotomskis, and A. Y. Borisov. 1984. Difference picosecond spectroscopy of pigment-protein complexes of photosystem I from higher plants. *Biochim. Biophys. Acta.* 767:501–506.
14. Kamogawa, K., J. M. Morris, Y. Takagi, N. Nakashima, K. Yoshihara, and I. Ikegami. 1983. Picosecond fluorescence studies of P700-enriched particles of spinach chloroplasts. *Photochem. Photobiol.* 37:207–213.
15. Huang, C., and D. S. Berns. 1983. Partial characterization of six chlorophyll *a*-protein complexes isolated from a blue-green alga by a non-detergent method. *Arch. Biochem. Biophys.* 220:145–154.
16. Mullet, J. E., J. J. Burke, and C. J. Arntzen. 1980. Chlorophyll proteins of photosystem I. *Plant Physiol.* 65:814–822.
17. Huang, C., and D. S. Berns. 1981. Isolation of P700-chlorophyll-protein complex from a blue-green alga by a nondetergent method. *Biochim. Biophys. Res. Commun.* 101:351–358.
18. Wittmershaus, B. P., and C. Huang. 1982. Time dependence of the fluorescent emission of detergent-free photosystem-I particles. *10th Meeting of the American Society for Photobiology.* Vancouver, BC, Paper No. TAM-C7.
19. MacColl, R., J. J. Lee, and D. S. Berns. 1971. Protein aggregation in C-phycocyanin. *Biochem. J.* 122:421–426.
20. Huang, C., D. S. Berns, and D. U. Guarino. 1984. Characterization

of components of P700-chlorophyll *a*-protein complex from a blue-green alga, *Phormidium luridum*. *Biochim. Biophys. Acta.* 765:21–29.
21. Knox, W., and G. Mourou. 1981. A simple jitter-free picosecond streak camera. *Optics Comm.* 37:203–206.
22. Knox, W. 1983. Picosecond time-resolved spectroscopic study of vibrational relaxation processes in alkali halides. Ph.D. thesis, University of Rochester.
23. Wittmershaus, B. P., T. M. Nordlund, W. H. Knox, R. S. Knox, N. E. Geacintov, and J. Breton. 1985. Picosecond studies at 77K of energy transfer in chloroplasts at low and high excitation intensities. *Biochim. Biophys. Acta.* 806:93–106.
24. Georghiou, S., T. M. Nordlund, and A. M. Saim. 1985. Picosecond fluorescence decay time measurements of nucleic acids at room temperature in aqueous solution. *Photochem. Photobiol.* 41:209–212.
25. Bevington, P. R. 1969. Data Reduction and Error Analysis for the Physical Sciences. McGraw-Hill Book Co., New York.
26. Geacintov, N. E., and J. Breton. 1982. Excitation annihilation and other nonlinear high-intensity excitation effects. *In* Biological Events Probed by Ultrafast Laser Spectroscopy. R. R. Alfano, editor. Academic Press, Inc., New York. 157–191.
27. Brown, J. S., R. S. Alberte, and J. P. Thornber. 1974. Comparative studies on the occurrence and spectral composition of chlorophyll-protein complexes in a wide variety of plant material. *Proc. Int. Photosynth. Congr., 3rd, Israel.* M. Avron, editor. Elsevier North-Holland Biomedical Press, Amsterdam. 1951–1962.
28. Tapie, P., Y. Choquet, J. Breton, P. Delepelaire, and F.-A. Wollman. 1984. Orientation of photosystem I pigments: investigation by low-temperature linear dichroism and polarized fluorescence emission. *Biochim. Biophys. Acta.* 767:57–69.
29. Wollman, F.-A., and P. Bennoun. 1982. A new chlorophyll-protein complex related to photosystem I in *Chlamydomonas reinhardii*. *Biochim. Biophys. Acta.* 680:352–360.
30. Gülen, D., B. P. Wittmershaus, and R. S. Knox. 1986. Theory of picosecond-laser-induced fluorescence from highly excited complexes with small numbers of chromophores. *Biophys. J.* 49:469–477.
31. Tusov, V. B., B. N. Korvatovskii, V. Z. Pashchenko, and L. B. Rubin. 1980. Nature of 735-nm fluorescence of chloroplasts at room and low temperatures. *Dokl. Biophys. (Engl. Transl. Dokl. Akad. Nauk SSSR).* 252:112–115.
32. Haworth, P., J. L. Watson, and C. J. Arntzen. 1983. The detection, isolation and characterization of a light-harvesting complex which is specifically associated with photosystem I. *Biochim. Biophys. Acta.* 724:151–158.
33. Lam, E., W. Ortiz, and R. Malkin. 1983. Chlorophyll a/b proteins of photosystem I. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 168:10–14.
34. Nordlund, T. M., and W. H. Knox. 1981. Lifetime of fluorescence from light-harvesting chlorophyll a/b proteins. Excitation intensity dependence. *Biophys. J.* 36:193–201.
35. Kamogawa, K., A. Kamiki, N. Nakashima, Y. Yoshihara, and I. Ikegami. 1981. Picosecond transient behavior of reaction-center particles of photosystem I isolated from spinach chloroplasts. Energy and electron transfer upon single and multiple photon excitation. *Photochem. Photobiol.* 34:511–516.
36. Pearlstein, R. M. 1982. Excitation migration and trapping in photosynthesis. *Photochem. Photobiol.* 37:835–844.
37. Butler, W. L. 1978. Energy distribution in the photochemical apparatus of photosynthesis. *Annu. Rev. Plant Physiol.* 29:345–378.
38. Ikegami, I. 1976. Fluorescence changes related in the primary photochemical reaction in the P-700-enriched particles isolated from spinach chloroplasts. *Biochim. Biophys. Acta.* 449:245–258.