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Excited State Dynamics in Photosystem I: Effects of Detergent and Excitation Wavelength

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ABSTRACT Femtosecond transient absorption spectroscopy has been used to investigate the energy transfer and trapping processes in both intact membranes and purified detergent-isolated particles from a photosystem II deletion mutant of the cyanobacterium *Synechocystis* sp. PCC 6803, which contains only the photosystem I reaction center. Processes with similar lifetimes and spectra are observed in both the membrane fragments and the detergent-isolated particles, suggesting little disruption of the core antenna resulting from the detergent treatment. For the detergent-isolated particles, three different excitation wavelengths were used to excite different distributions of pigments in the spectrally heterogeneous core antenna. Only two lifetimes of 2.7–4.3 ps and 24–28 ps, and a nondecaying component are required to describe all the data. The 24–28 ps component is associated with trapping. The trapping process gives rise to a nondecaying spectrum that is due to oxidation of the primary electron donor. The lifetimes and spectra associated with trapping and radical pair formation are independent of excitation wavelength, suggesting that trapping proceeds from an equilibrated excited state. The 2.7–4.3 ps component characterizes the evolution from the initially excited distribution of pigments to the equilibrated excited state distribution. The spectrum associated with the 2.7–4.3 ps component is therefore strongly excitation wavelength dependent. Comparison of the difference spectra associated with the spectrally equilibrated state and the radical pair state suggests that the pigments in the photosystem I core antenna display some degree of excitonic coupling.

INTRODUCTION

Photosystem one (PS I) is one of the two reaction centers found in oxygenic photosynthetic organisms. It is a pigment-protein complex that uses light energy to drive electron transfer from a soluble electron donor, plastocyanin or soluble cytochrome c_6, to the electron acceptor $NADP^+$. PS I contains about 13 protein subunits. The functional characteristics of many of these subunits have been determined (for reviews see Golbeck and Bryant, 1991; Almog et al., 1992; Golbeck, 1994). The x-ray structure of PS I from a cyanobacterium has been determined at 6 Å resolution, revealing the positions of many α-helical regions of the proteins, the iron sulfur centers that serve as early electron acceptors, and some of the chlorophylls and other cofactors (Krauss et al., 1993).

The PS I core complex in higher plants, cyanobacteria, and green algae consists of a protein heterodimer of the PsaA and PsaB gene products that bind the primary electron transfer components and about 60–100 chlorophyll-*a* (Chl-*a*) spectral forms. The PsaA and PsaB apoproteins show more than 96% homology in higher plants, cyanobacteria, and green algae (Cantrell and Bryant, 1987; Mühlenhoff et al., 1993), suggesting strong conservation of both structure and function of PS I from the different species. In higher plants and green algae a peripheral, Chl-*b*-containing, antenna complex called light-harvesting complex one is coupled to the core (Mullet, 1980). Cyanobacteria lack this peripheral Chl-*b*-containing light-harvesting complex one. The simplified antenna structure makes cyanobacteria particularly useful for the study of energy transfer and trapping processes in the PS I core antenna (Hastings et al., 1994a,b; Kleinherenbrink et al., 1994a; Woolf et al., 1994).

Pure PS I particles, free from PS II and chlorophylls associated with PS II antenna complexes, have been isolated by a variety of detergent treatment protocols (Shiozawa et al., 1974; Mullet, 1980; Rögner et al., 1990). Pure and intact PS I membrane fragments from cyanobacteria and green algae have also been obtained by generating mutants that lack PS II and its associated peripheral antenna complexes (Surzycki, 1971; Vermaas et al., 1987, 1988).

Although it is possible to obtain intact PS I membrane fragments that have not been prepared with detergents (Hastings et al., 1994a,b), the light-scattering properties of these intact samples make optical experiments difficult and it is preferable for many experiments to use relatively scatter-free purified detergent-isolated particles. Nechushtai et al. (1986) have shown that the 77K fluorescence emission spectrum from a variety of PS I particles depends on both the type and concentration of detergent used to solubilize the particles. In the absence of detergents it is suggested that the 77K fluorescence emission in PS I core particles from higher plants and cyanobacteria peaks at ~720 nm. β-Dodecyl maltoside (β-DM) had little effect on the position of the emission maximum. However, the detergents Triton X-100 (TX-100) and sodium dodecyl sulfate both resulted

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in a shift of the emission maximum from 720 nm to 670–685 nm. The origin of this detergent-induced wavelength shift of the emission maximum is not entirely clear, but it indicates that the detergent environment should be carefully controlled in the preparation of purified PS I.

Preparation of PS I membranes from mutants that lack PS II avoids questions concerning detergent-induced distortion of the kinetics in either time-resolved fluorescence or absorption experiments. Generation of PS I-only mutants also allows the direct comparison of non-detergent-treated and detergent-treated PS I particles and therefore allows an assessment of the extent that detergents may influence the excited state dynamics in the PS I core antenna.

In a previous study using intact PS I membrane fragments from *Synechocystis* sp. PCC 6803, we showed that the excitation energy equilibrated between the various antenna spectral forms in about 4 ps (Hastings et al., 1994a). This time is in good agreement with the results of Du et al. (1993), who measured an isotropic decay lifetime of 5 ps in PS I-60 particles from *Chlamydomonas reinhardtii*. In PS I-100 particles from *Synechococcus* sp., which were prepared by using a relatively harsh detergent treatment (we note, however, that the thermophilic cyanobacteria have a very high tolerance to TX-100), the corresponding process was found to have a lifetime of 8–12 ps (Holzwarth et al., 1993). Previously, we suggested that the difference in time constants could be due to subtle changes in structure that may result from detergent treatment or to the use of different excitation wavelengths (Hastings et al., 1994a). Below we show that neither of these hypotheses is likely and that the differences are probably associated with real differences in the preparations from the different organisms.

Most computer simulations of the kinetics in the PS I core antenna assume a 2D or 3D lattice of weakly coupled pigments with no clustering (Jia et al., 1992; Trinkunas and Holzwarth, 1994; Laible et al., 1994). Some experiments appear to favor this type of modeling (Du et al., 1993), whereas Causgrove et al. (1988, 1989) and Struve (1990) suggest that some of the pigments may be clustered because of excitonic interactions or spatial proximity. The available x-ray structure of PS I is not of sufficient resolution to resolve this question, in that only about half of the pigments can be visualized and the presence or absence of closely coupled pigments cannot be determined with any confidence (Krauss et al., 1993). Below we shall show that at least some of the pigments in the PS I core antenna display strong excitonic interactions, leading to questions concerning the appropriate structural model that should be adopted in computer simulations of energy trapping dynamics.

In this paper we considerably extend our studies of the energy and electron transfer processes in PS I particles from *Synechocystis* sp. PCC 6803. We have investigated what effect, if any, the detergent β-DM may have on the excited state dynamics by comparing the results of identical measurements performed on both intact membrane fragments and on PS I particles prepared by using the relatively mild detergent β-DM. We show that detergent-isolated PS I particles reflect rather closely the more intact system. The isolation of a relatively scatter-free, intact detergent-isolated particle allows the use of spectrally overlapped excitation and probe wavelengths, which is of considerable use in the study of the excited state dynamics of the spectrally heterogeneous PS I core antenna. We have used three different excitation wavelengths, which has allowed a detailed investigation of the energy transfer processes in the PS I core antenna.

MATERIALS AND METHODS

Membrane fragments from the mutant psbDI/C/DII of *Synechocystis* sp. PCC 6803, which contain only the PS I reaction center, were prepared as described previously (Hastings et al., 1994a). Purified PS I particles were prepared from the membranes using a protocol similar to that described by Rögner et al. (1990). Briefly, membrane fragments were thawed and centrifuged for 10 min at 10,000 rpm in a Sorvall SS-34 rotor (about 12,000 × *g*). The pelleted membranes were suspended in buffer containing 20 mM HEPES (pH 7), 10 mM $CaCl_2$, 10 mM $MgCl_2$, 0.5 M mannitol, 20% glycerol, 1% β-DM (w:v), and protease inhibitor phenylmethylsulfonyl fluoride for 30 min. The mixture was centrifuged for 30 min at 10,000 rpm in a Sorvall SS-34 rotor. The supernatant was loaded onto a 8–32% (w/v) sucrose gradient containing 0.04% β-DM (w:v) and centrifuged at 150,000 × *g* for 20 h in a Beckman 45 Ti rotor. Two green bands were found (in addition to an orange band at the top of the gradient that is due to carotenoids). Only the lower green band was used in the experiments described here. The lower green band corresponds to the trimeric form of the PS I core as described by Rögner et al. (1990) and Kruip et al. (1994). Sucrose was exchanged for buffer containing 20 mM HEPES (pH 7), 10 mM $CaCl_2$, 10 mM $MgCl_2$, 0.5 M mannitol, and 0.03% β-DM (w:v) by using an Amicon YM-100 filter.

Femtosecond transient absorption measurements

All experiments described here were performed at room temperature. Samples were suspended in buffer as described above, and approximately 20 mM ascorbate and 10 μM phenazine methosulfate (PMS) were added. The sample was loaded in a spinning cell with an optical pathlength of 2.5 mm and a diameter of 18 cm. The absorbance of the sample in the cell was ~1–1.2 at the peak of the Q_y absorption band (about 680 nm for membrane fragments and 678 nm for the detergent-treated particles).

The laser system is nearly identical to that described before (Hastings et al., 1994a). Previously, we used attenuated, 200-fs, 590-nm excitation pulses, at a repetition rate of 540 Hz. In the experiments described here these 590-nm pulses were used to generate a second spectral continuum. A portion of this spectral continuum was selected by using interference filters with spectral half-widths of 8–12 nm. The selected portion of the spectral continuum was then amplified with a prism-type flowing dye cell (Santa Ana), pumped by a portion of the intense, 532-nm, 100-ps pulses from the regenerative amplifier. Here we describe experiments performed using 590-nm, 678-nm (full width at half-maximum (FWHM) = 12 nm), and 710-nm (FWHM = 12 nm) excitation.

All experiments were performed with the pump and probe beams set at the magic angle. Only low levels of excitation were used (typically less than 25% of the reaction centers are excited by each pulse). Data were collected between 0–90 ps and 0–12 ps, in the 640–780 nm spectral region. Analysis procedures are identical to those described previously (Hastings et al., 1994a). Decay-associated spectra (DAS) with lifetimes in the 24–28 ps range (see table) were obtained from analysis of data collected on a 90-ps timescale, and DAS with lifetimes of 2.7–4.3 ps were obtained from analysis of data collected on a 12-ps timescale, where the shorter lifetimes are better resolved.

Micro/millisecond transient absorption measurements

Absorption difference spectra were obtained ~10 μs after a saturating, broad-band xenon flash by using a spectrometer equipped with a multichannel detector as previously described (Hastings et al., 1994b). Single-wavelength (4-nm bandwidth) transient absorption measurements on the hundreds of microseconds to millisecond timescale were performed on a single-beam spectrophotometer that has been described (Kleinherenbrink et al., 1994b). Difference spectra are built up by performing measurements at a number of wavelengths. Before each set of femtosecond transient absorption measurements, the kinetics of $P700^+$ re-reduction were monitored on this spectrometer. The concentration of PMS and ascorbate were adjusted such that the ground state had completely recovered in less than 80 ms. Therefore, in the femtosecond transient absorption experiments, we expect insignificant build-up of reaction centers with oxidized P700.

RESULTS

Comparison of the kinetics in membranes and detergent-isolated particles

For each PS I particle, data were collected under a variety of experimental conditions (Table 1). For intact PS I membrane fragments, only 590 nm excitation was used (Hastings et al., 1994a). For detergent-isolated PS I particles three different excitation wavelengths were used. Table 1 summarizes the lifetimes that were obtained from global analysis of data collected on each timescale, for each particle, using the different excitation wavelengths. Lifetimes of 24–28 ps are well resolved in data collected on a 90-ps timescale. In the analyses of data collected on a 12-ps timescale these 24–28-ps lifetimes are not considered explicitly but are fitted as time-independent parameters. We have performed a number of global simulations, including analyses in which a longer component is specifically included, and we find that this procedure does not greatly affect either the lifetime or the DAS of the faster components. The spectrum of the nondecaying component obtained on the 12-ps timescale is similar in shape to the sum of the 24–28-ps and nondecaying spectra obtained from analyses of data collected on a 90-ps timescale.

Similar lifetimes are obtained after 590 nm excitation of the PS I membrane fragments or the detergent-isolated PS I particles (Table 1). The overall similarity in lifetimes obtained using either of the two PS I particles suggests that the detergent treatment does not appear to significantly alter the excited state dynamics within the PS I core antenna.

Fig. 1, *A*, *B*, and *C*, shows the DAS of the 3.7 ps, 24 ps, and nondecaying components respectively, obtained from global analysis of transient absorption data, collected after 590 nm excitation of purified, detergent-isolated, PS I particles (dotted lines). Also shown for comparison in Fig. 1, *A*, *B*, and *C*, are the corresponding spectra of the 4 ps, 28 ps, and nondecaying components found for the membrane fragments, under comparable experimental conditions (solid lines, taken from figure 2 of Hastings et al., 1994a). The spectra of the 3.7 ps and 24 ps components for the detergent-isolated particles have been scaled by a factor of 0.65 for ease of comparison with the 4 and 28 ps spectra. The nondecaying spectra in Fig. 1 *C* have not been normalized because the amplitudes are coincidentally very similar at 700 nm.

Excitation wavelength dependence of energy transfer in the PS I core antenna

The PS I membrane fragments scatter laser light strongly and transient absorption measurements could only be performed with excitation wavelengths that are well shifted relative to the probed spectral region (590 nm excitation was used while absorption changes were monitored in the 660 to 720 nm spectral region). The relatively scatter-free, detergent-isolated PS I particles allow the use of overlapped excitation and probe wavelengths and thus the excitation of specific groups of pigments in the core antenna.

For transient absorption experiments performed with the detergent-isolated PS I particles from *Synechocystis* sp. PCC 6803, three different excitation wavelengths were chosen. Excitation at 590 nm was chosen so that a direct comparison could be made with data collected using the membrane fragments from the same species (Fig. 1). In the detergent-isolated cyanobacterial PS I particles the main Q_y peak is at 678 nm, suggesting that there is a large group of pigments that absorb near this wavelength. In addition, Woolf et al. (1994) have shown that there is also a group of "long wavelength" pigments that absorb maximally between 703 and 708 nm. Excitation at 710 nm was chosen with the intent of directly exciting mainly the longer wave-

TABLE 1 Lifetimes obtained from global analysis of transient absorption data

	Membrane fragments*		Purified detergent-treated particles					
	590 nm		590 nm		678 nm		710 nm	
	12 ps time scale	90 ps time scale	12 ps time scale	90 ps time scale	12 ps time scale	90 ps time scale	12 ps time scale	90 ps time scale
Lifetimes (ps)	0.56	—	0.2	—	0.18	—	0.07	—
	4	5	3.7	3.9	2.7	2	4.3	5.7
	ND‡	28	ND‡	24	ND‡	27	ND‡	26
	—	ND‡	—	ND‡		ND‡	—	ND‡

*Data taken from Hastings et al. (1994a).

‡The spectra are nondecaying (ND) on the timescale considered here.

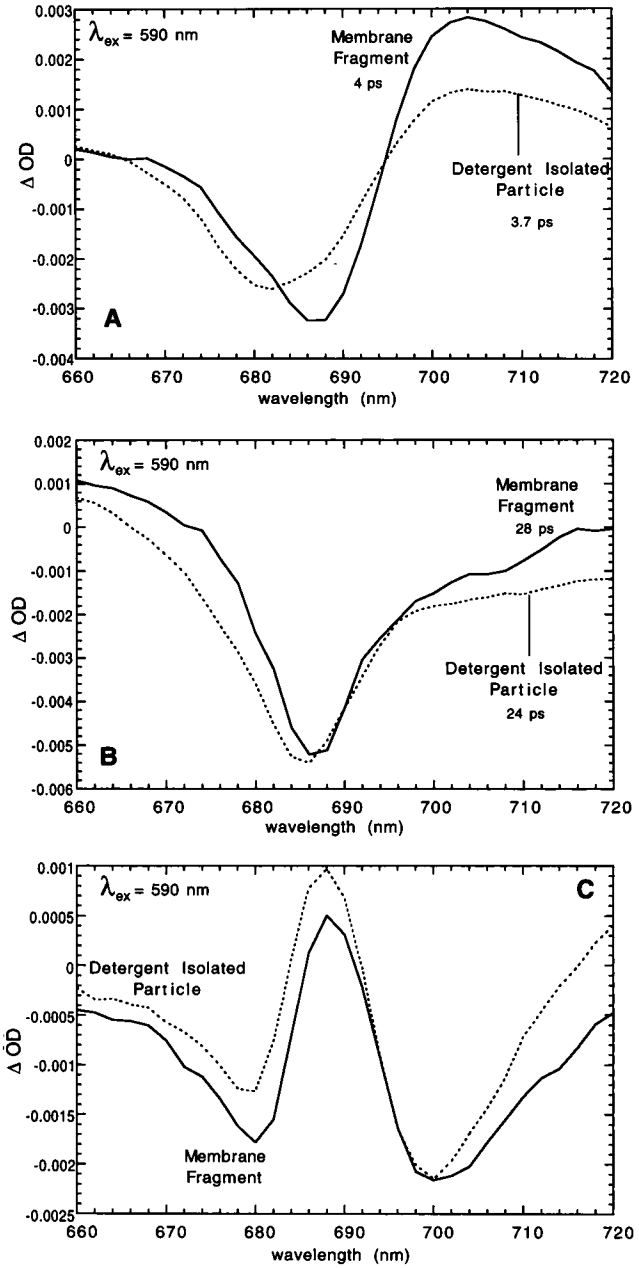

FIGURE 1 Decay-associated spectra of the (*A*) 3.7–4 ps; (*B*) 24–28 ps; and (*C*) nondecaying components obtained from global analyses of transient absorption data collected after 590 nm excitation of detergent-isolated PS I particles (· · · ·) or PS I membrane fragments (——) from *Synechocystis* sp. PCC 6803. In (*C*) the spectra have not been normalized. In (*A*) and (*B*) the spectra for the detergent-isolated PS I particle have been scaled by a factor of 0.65, for ease of comparison with the spectra of the membrane fragment.

length group of pigments, and 678 nm excitation was used to excite mainly the shorter wavelength group of pigments. The use of 678 nm excitation also allows a more direct comparison with transient absorption data obtained from the thermophilic cyanobacterium *Synechococcus* sp. (Holzwarth et al., 1993).

We expect that 590 nm excitation will excite the pigment distribution in the core antenna relatively homogeneously. Because the vast majority of the pigments absorb near 680 nm, we anticipate that the initially excited distribution of pigments formed after 590 nm excitation shall be similar to that formed after 678 nm excitation.

Fig. 2 shows the kinetics of the absorption changes observed at 680 and 704 nm, after 590, 678, and 710 nm excitation of the detergent-isolated PS I particles from *Synechocystis* sp. PCC 6803. The kinetics have been normalized to the data obtained after 678 nm excitation (see below). After 678 nm excitation, at probe wavelengths near 680 nm, the data can also be described for four exponential components. We find that the 2.7 ps component splits into two components with lifetimes of 1.7 and 5.3 ps (data not shown). The observation of two lifetimes may indicate two different equilibration processes; however, because fitting to an extra exponential component significantly improves the fits only near 680 nm we shall discuss only the 2.7 ps component, obtained from three exponential fits, and simply note that this lifetime may be a combination of two different lifetimes. Our analysis thus represents a starting point for more complicated models.

The kinetics at 680 nm, after 678 nm excitation (Fig. 2, middle), show very little noise associated with scattering of the excitation beam and, as expected, the kinetics observed after either 590 or 678 nm excitation are very similar (Fig.

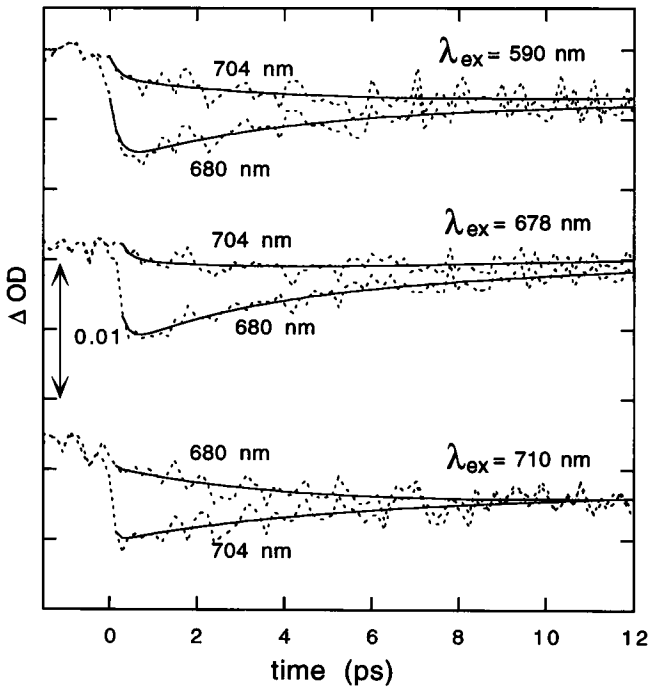

FIGURE 2 Kinetics of the absorption changes observed between 0 and 12 ps, at 680 and 704 nm, after 590 (*top*), 678 (*middle*), and 710 nm (*bottom*) excitation of detergent-isolated PS I particles from *Synechocystis* sp. PCC 6803. The dotted lines are the data and the solid lines are the fitted functions. The lifetimes associated with the fitted functions are given in Table 1. The curves obtained after 678 and 710 nm excitation have been offset for clarity. The kinetic traces obtained after 590 and 710 nm excitation have been scaled to the data obtained after 680 excitation, as described in the text.

2, top and middle curves). The initial absorption changes appear to be strongly excitation wavelength dependent; after 678 nm excitation, an initial bleaching followed by a recovery of ground state absorption (or decay of an initial bleaching) is observed in the 680 nm spectral region. In direct correspondence, a rise or grow-in of a bleaching is observed near 704 nm. When the excitation wavelength is changed to 710 nm the opposite behavior is observed. A grow-in or rise of a bleaching is observed at 680 nm, and a corresponding decay of an initial bleaching is observed at 704 nm.

This wavelength-dependent behavior is clearly demonstrated in Fig. 3, where the difference spectra at three delay times, after 678 (*A*), 590 (*B*), and 710 nm (*C*) excitation, are shown. All spectra in Fig. 3 were obtained from data collected on a 12 ps timescale. Similar spectra were also obtained from data collected on a 90 ps timescale (not shown). The 400 fs and 6 ps spectra essentially show the spectra before and after the 2.7–4.3 ps process is complete. After 590 or 678 nm excitation, between 400 fs and 6 ps, we see a clear recovery of the initial bleaching in the 680 nm region and corresponding rise or grow-in of a bleaching on the longer wavelength side. After 710 nm excitation, between 400 fs and 6 ps, a clear recovery on the longer wavelength side and corresponding rise on the shorter wavelength side is observed. Between 6 and 11 ps the bleaching decays further at all wavelengths in the 680–720 nm spectral region. The spectra at 11 ps, after excitation at all three wavelengths, are very similar, with peaks at ~682 and 700 nm.

The 400 fs spectra in Fig. 3, *A* and *B*, confirm that the initially excited distribution of pigments is similar at 590 and 678 nm excitation. Both spectra show a negative peak at 682 nm and have a FWHM that is similar to the excitation pulse width. The 400 fs spectrum observed after 590 nm excitation is slightly broader on the long wavelength side compared to the corresponding spectrum observed after 678 nm excitation. This may be due to some direct excitation of lower energy pigments with 590 nm excitation. This may suggest that there are a relatively large number of pigments that absorb between 690 and 700 nm. Holzwarth et al. (1993) have calculated that there are about 15 pigments that absorb at 695 nm in PS I particles from *Synechococcus* sp. The 400 fs spectrum in Fig. 3 *C* peaks at 702 nm with a shoulder at 684 nm and is considerably broader than the 400 fs spectra in Fig. 3, *A* and *B*. This suggests that absorption at 710 nm is dominated by pigments that absorb near 698 nm (assuming a 4 nm red shift due to stimulated emission), further indicating that there may be a relatively large number of pigments (10–15) that absorb between 690 and 700 nm. This result also follows from examination of the 11 ps spectra in Fig. 3 (spectra after the equilibration process is complete). It can be shown that two pools of pigments with peaks at 680 (near the absorption peak) and 708 nm (Woolf et al., 1994) cannot adequately describe the 11 ps spectra. From equilibrium thermodynamics, assuming 98 pigments per reaction center, we find that there would have to be at least 18 pigments that absorb at 708 nm, very much higher than the number estimated by Woolf et al. (1994) or Gobets et al. (1994). The 11 ps spectra in Fig. 3 suggest that there are also a significant number of pigments absorbing near 700 nm.

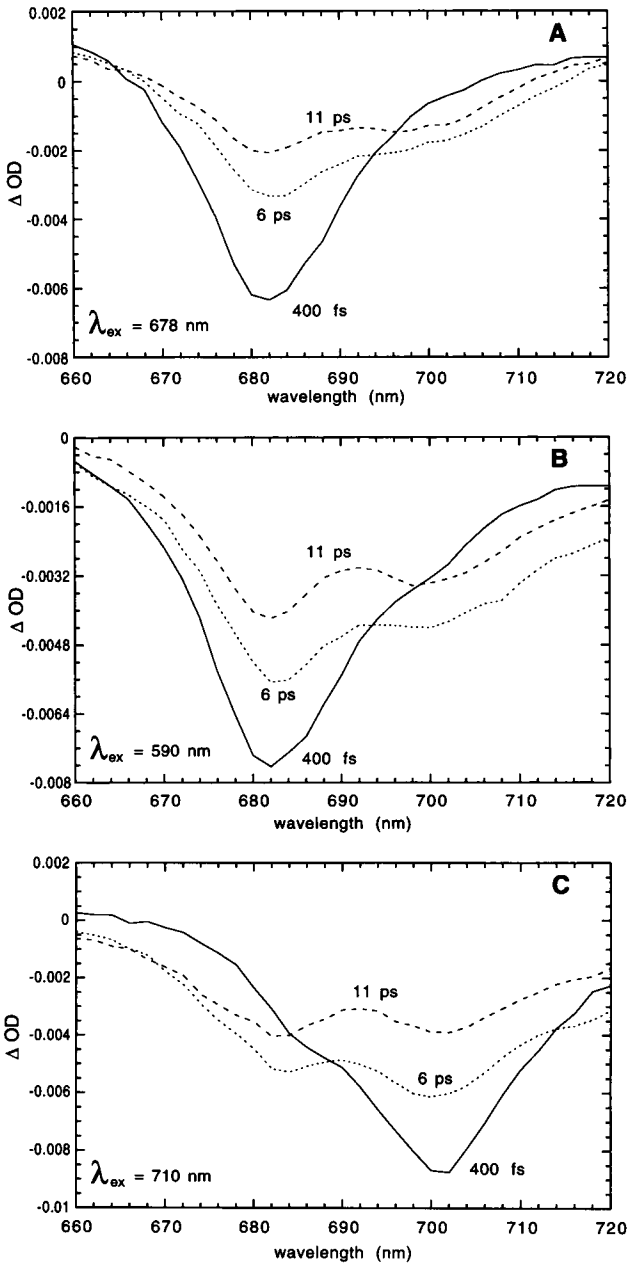

FIGURE 3 Room temperature absorption difference spectra at three different time delays, after (*A*) 678 nm; (*B*) 590 nm; and (*C*) 710 nm excitation of detergent-isolated PS I particles from *Synechocystis* sp. PCC 6803. The spectra obtained after 590 and 710 nm excitation have been scaled to the spectra obtained after 680 excitation, as described in the text.

The fact that the 400 fs spectrum in Fig. 3 *C* displays a distinct shoulder at 684 nm may suggest an ultrafast uphill energy transfer process from longer (695 nm) to shorter (680 nm) wavelength-absorbing pigments, which in turn may indicate different couplings between different pigment pools. We have not performed detailed calculations to determine whether this possibility is realistic. Alternatively, it could be the case that the ~680 nm absorbing pigments are

directly excited (the rise at 680 nm, after 710 nm excitation, is within our time resolution), which seems reasonable given that the number of 680 nm absorbing pigments is far in excess of the longer wavelength absorbing pigments. The 400 fs spectra in Fig. 3, *B* and *C*, demonstrate clearly that we are able to excite very different distributions of pigments, although we were not able to excite the longest wavelength absorbing pigments selectively. We have also performed experiments with 720 nm excitation pulses with 8 nm bandwidth. We find very similar results (data not shown) to the data shown in Fig. 3 *C*.

From Table 1 it appears that the data can be described by two lifetimes of 2.7–4.3 ps (equilibration) and 24–28 ps (trapping), as well as a long-lived or nondecaying component, independent of excitation wavelength and the type of PS I particle used. Fig. 4 shows the DAS of the 2.7–4.3 ps (*A*), 24–27 ps (*B*), and nondecaying spectra (*C*) obtained from global analysis of data collected after 590 (long dashed line), 678 (short dashed line), and 710 nm (solid line) excitation of detergent-isolated PS I particles from *Synechocystis* sp. PCC 6803 at room temperature.

At this point it is important to outline the data normalization procedures used. First, for each excitation wavelength, data collected on the two different timescales were normalized by comparing the spectra obtained ~1 ps after excitation. Second, the data obtained by using different excitation wavelengths were normalized by scaling the nondecaying difference spectra such that the absorption changes are the same at 700 nm (see Fig. 4 *C*). The data collected after 590 and 710 nm excitation were scaled to the data collected after 678 nm excitation. The nondecaying difference spectrum, obtained after 590/710 nm excitation (Fig. 4 *C*), has been scaled by a factor of 0.95/0.87. The spectra of the 2.7–4.3 ps and the 24–27 ps components have also been scaled accordingly (this normalization was not applied to the 3.7 ps, 24 ps, and nondecaying spectra in Fig. 1). We shall show below that the nondecaying spectra in Fig. 4 *C* are due to P700 oxidation. Therefore, assuming that all of the absorption changes are associated with single photon events, this normalization accounts for differences in excited state concentration that occur because of differences in excitation intensity.

The three 24–27 ps spectra in Fig. 4 *B* are all very similar in shape. The negative amplitude of the 27 ps spectrum, obtained from data collected after 678 nm excitation, is less than those of the other two spectra, which may result from a slight shift in the spectrum due to incomplete resolution in the global analysis. The fact that the nondecaying spectrum associated with 678 nm excitation in Fig. 4 *C* may appear to be shifted more negatively (relative to the other two spectra) agrees with this. Although there may be small shifts in the spectra, we are careful to interpret our data only within the limits set by the noise level in our measurements. The solid line in Fig. 5 *A* shows the mean of the three 24–27 ps spectra in Fig. 4 *B*. The error bars are plus or minus the standard error of the three measurements in Fig. 4 *B*. Similarly, the three nondecaying spectra in Fig. 4 *C* appear to be

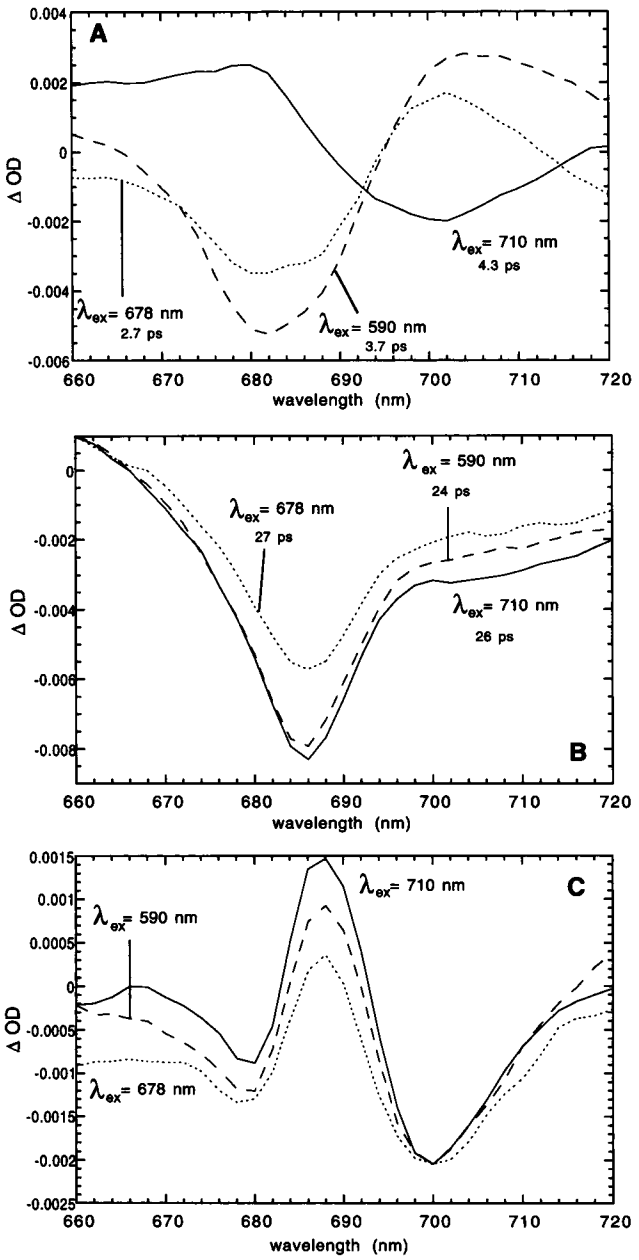

FIGURE 4 Decay-associated spectra of the (*a*) 2.7–4.3 ps; (*b*) 24–27 ps; and (*c*) nondecaying components, obtained from global analysis of transient absorption data collected after 590 (– – –), 678 (····), and 710 (——) nm excitation of detergent-isolated PS I particles from *Synechocystis* sp. PCC 6803.

similar. The mean of the three spectra in Fig. 4 *C* is also shown in Fig. 5 *A* (dashed line), with the error bars again representing plus or minus the standard error of the three measurements.

The nondecaying spectrum found from experiments performed on the membrane fragments (Fig. 1 *C*, solid line) is due to P700 and some other pigments with long-lived excited states, for example residual phycobilins (Hastings et al., 1994b). By using another experimental approach, the P700 difference spectrum for the PS I membrane fragments

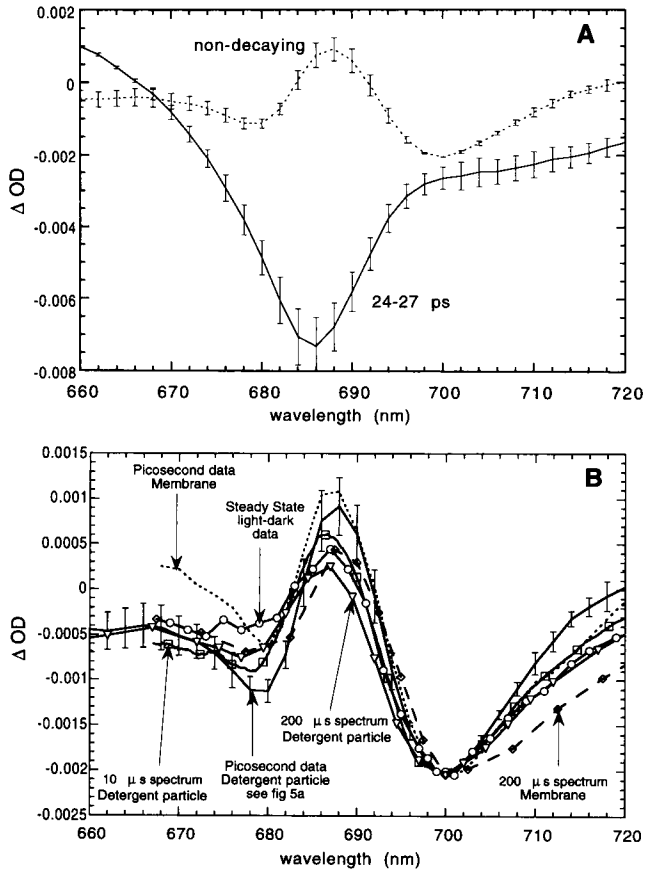

FIGURE 5 (*A*) ——, Average of the three 24–27 ps spectra shown in Fig. 4 *B*; · · · · ·, average of the three nondecaying spectra shown in Fig. 4 *C*. The error bars are plus or minus the standard error. The standard error is the standard deviation divided by the square root of the number of measurements, which in this case is three. (*B*) (solid line with error bars, also shown in *A*), averaged nondecaying spectrum for the detergent-isolated PS I particles. · · · · ·, ($P700^+$ − P700) difference spectrum obtain from picosecond, oxidized minus reduced experiments on the intact PS I membrane fragments (also shown in Fig. 4 of Hastings et al., 1994b); □, difference spectrum obtained 10 μs after saturating xenon flash excitation of the detergent isolated PS I particles; ▽, difference spectrum obtained 200 μs after saturating 532 nm laser flash excitation of the detergent isolated PS I particles or, ◇, PS I membrane fragments; ○, steady-state light-minus-dark difference spectrum for the detergent-isolated PS I particles. All spectra have been normalized at 700 nm.

was obtained and is presented in figure 4 of Hastings et al. (1994b). This spectrum is reproduced in Fig. 5 *B* along with the mean nondecaying spectrum from Fig. 5 *A*. For comparison, we also present four other difference spectra in Fig. 5 *B*. These spectra were obtained from either microsecond transient absorption or steady-state light-minus-dark difference measurements on either the PS I membrane fragments or the detergent-isolated particles. There appear to be small differences in the six spectra in Fig. 5 *B*, especially in the 690 nm spectral region. However, these differences are probably due to noise associated with the use of four different spectrometers. Aside from these minor differences, the overall similarities are striking. All six spectra show a positive absorption change near 688 nm and display bleaching peaks at 678–680 and 700 nm (all spectra are normalized at 700 nm).

DISCUSSION

Detergent-isolated versus nondetergent preparations

In all of the experiments described here we have used only the trimeric form of the PS I core (Rögner et al., 1990; Kruip et al., 1993). The spectroscopic properties of the monomeric form are strikingly similar (van der Lee et al., 1993; Gobets et al., 1994). The trimeric form has been isolated from the thermophilic cyanobacterium *Synechococcus* sp., and its 3-D crystal structure has been determined to a resolution of 6 Å (Krauss et al., 1993). It has been suggested recently that both the monomer and trimer exist in vivo (Hladík and Sofrová, 1991; Shubin et al., 1993; Kruip et al., 1994). The relative amounts of monomer or trimer that can be extracted depend on the ion concentrations used in the isolation procedure (Kruip et al., 1994).

Rögner et al. (1990) and Kruip et al. (1994) have indicated that detergent-isolated PS I particles from *Synechocystis* sp. PCC 6803 contain ~75 Chl-*a*/P700. We showed previously that intact membranes from the PS II deletion mutant from *Synechocystis* sp. PCC 6803 contain ~98 Chl-*a*/P700. After solubilization of the membranes in the detergent β-DM we find that the Q_y peak of the absorption spectrum blue shifts by about 2 nm, which may suggest the loss of some chlorophyll pigments. By directly comparing the millisecond kinetics in both membrane fragments (taking into account scattering) and detergent-isolated particles we find that under saturating light conditions the bleaching at 700 nm due to P700 oxidation is about 20% higher in the detergent-isolated particles, which may suggest a slightly decreased chlorophyll-to-P700 ratio of ~80 Chl-*a*/P700 in the detergent-isolated particles. Although some pigments appear to be lost upon detergent treatment, the spectral nature of these pigments is not obvious from the data in Fig. 1.

Nechushtai et al. (1986) indicated that the detergents TX-100 and sodium dodecyl sulfate could cause pronounced shifts in the peak position of the fluorescence emission of different PS I particles. The detergent β-DM had a much less pronounced effect on the steady-state fluorescence properties of the different PS I particles, compared to TX-100. This would suggest that the detergent-isolated PS I particles used here are probably relatively intact, because we only used β-DM in our isolation procedures. It is not our intent to fully characterize the effects that a variety of detergents may have on picosecond kinetics; we intend only to show that the detergent-isolated particles used here are intact and have not been significantly altered because of the detergent treatment.

The origin of the differences in the nondecaying spectra associated with the membrane fragments and the detergent-isolated particles (Fig. 1 *C*) is unclear. In the membrane fragments, excess phycobilisomes that were not removed

during the isolation procedure may be excited and may contribute to some extent to the membrane fragment nondecaying spectrum in Fig. 1 *C*. Below we show that the nondecaying spectrum associated with the detergent-isolated particle is due only to P700 oxidation. It therefore appears likely that the nondecaying spectrum associated with the membrane fragment (Fig. 1 *C*, solid line) also contains some bleaching that is associated with uncoupled pigments that are not present in the purified detergent particle. This fact is also apparent from our previous studies (Hastings et al., 1994a,b).

Because the spectra in Fig. 1 *C* contain different contributions from different pigments it is not clear if they can be properly normalized relative to each other. We have chosen not to scale the spectra in Fig. 1 *C*. Because we cannot normalize the data obtained by using the different PS I particles to the final yield of radical pair state, it is not obvious how to normalize the DAS in Fig. 1, *A* and *B*, or which wavelength should be used for this normalization. In view of the above we have scaled the 3.7 and 24 ps spectra by a factor of 0.65 for ease of comparison.

The fact that the 24 ps spectrum associated with the detergent-isolated particles (Fig. 1 *B*) has been scaled by a factor of 0.65 but is comparable in amplitude to the 28 ps spectrum associated with the membrane, whereas the nondecaying spectra in Fig. 1 *C* have not been scaled, is very hard to explain assuming only single photon processes. Either there are multi-photon processes in the core antenna of the detergent-isolated particles (but not in the membrane), which evolve on a timescale similar to that of the trapping process, or the antenna pigments are (excitonically) coupled in some way that is obscured in the membrane data (see below).

The similarity in lifetimes found from measurements on membrane fragments and detergent-isolated particles suggests conservation of function of the PS I core antenna after detergent treatment. The shape of the spectra demonstrate more conclusively that this is indeed the case. For example, both spectra in Fig. 1 *A* clearly demonstrate, by virtue of their positive/negative lobes, that the faster 3.7–4 ps components are associated with energy transfer from shorter to longer wavelength absorbing pigments. We also note that the spectra further indicate that long wavelength pigments exist and are functional in both the membrane fragments and the detergent-isolated PS I particles. The 24 and 28 ps spectra in Fig. 1 *B* are also broadly similar in shape, again suggesting that the same process is observed in both the membrane fragments and the detergent-isolated particles. Small differences in the shape of the DAS in Fig. 1, *A* and *B*, may be due to slightly different pigment stoichiometries in the two PS I particles as discussed above.

Previously, we assigned the 4 ps spectrum in Fig. 1 *A* to equilibration among the various spectral forms in the core antenna of the membrane fragment. The 28 ps spectrum in Fig. 1 *B* was assigned to overall decay of the equilibrated distribution of excitations because of trapping at the reaction center (Hastings et al., 1994a). The above discussion indicates that the same processes are observed with almost identical lifetimes in the detergent-isolated PS I particles.

Trapping and radical pair formation in the detergent-isolated PS I particles

The DAS in Fig. 4 *B* are all associated with trapping and are clearly all very similar. The mean of the three spectra in Fig. 4 *B* is shown in Fig. 5 *A* (solid line). Within the limits set by the error bars in Fig. 5 *A* the spectrum associated with trapping is independent of excitation wavelength. Trapping results in the formation of a nondecaying state, characterized by the difference spectrum in Fig. 5 *A* (dotted line). Again, within the limits set by the error bars in Fig. 5 *A*, trapping results in the formation of the same state independent of excitation wavelength. In Fig. 5 *B* we compare the nondecaying difference spectrum of Fig. 5 *A* with a number of other time-resolved and steady-state difference spectra. The bleaching at 700 nm decays in several tens of milliseconds. On a millisecond timescale, the flash-induced difference spectrum is due to $P700^+F^-$, where F is an iron sulfur center. Because F does not absorb in this wavelength region (Golbeck and Bryant, 1991), the micro/millisecond flash-induced difference spectra are due only to P700 oxidation, as is the steady-state light-minus-dark difference spectrum. Because all of the spectra in Fig. 5 *B* are similar in shape it follows that the nondecaying spectrum found on the picosecond timescale, from measurements of the detergent-isolated PS I particles, is also due only to P700 oxidation and does not include absorption changes associated with an electron acceptor. The differences in the spectra in Fig. 4 *C*, prior to normalization, are relatively small. Therefore, the same radical pair state is formed with similar yield, independent of excitation wavelength. Quantitative comparison of the bleaching at 700 nm in the picosecond and microsecond difference spectra (Fig. 5 *B*) indicates that the excitation intensities in the picosecond measurements are such that each femtosecond laser pulse excites less than 25% of the PSI complexes. The similarity in the shape of the spectra in Figs. 4, *B* and *C*, and 5 *B* provides strong evidence for the appropriateness of the analysis procedures employed here.

Even with the present detailed information, it is still not possible to distinguish conclusively whether a trap-limited or pseudo-trap-limited model provides a more appropriate description of trapping in the PS I core antenna (Hastings et al., 1994a). To determine which model is more appropriate it is necessary to selectively excite P700, and as we described previously, this is not possible in the PS I particles used in the present study. Selective excitation does appear to be possible in the studies of Kumazaki et al. (1994a,b), who used a PS I particle with significantly reduced chlorophyll content. In essence, if P700 can be directly excited and the excitation energy still ends up on antenna pigments, then the excited state dynamics are likely to be more accurately described by a trap-limited kinetic model.

Coupling between pigments in the PS I core antenna

There is clearly a large difference in the area under the two spectra in Fig. 5 *A*. Direct integration of the curves yields a ratio of 3.6 ± 0.8 for the area under the 24–27 ps spectrum relative to the area under the nondecaying spectrum. The error in the ratio was estimated by using the error bars in Fig. 5 *A*. The 24–27 ps spectrum contains contributions from absorption changes associated with radical pair formation, so the ratio of 3.6 represents a lower limit. Even allowing for stimulated emission, this ratio is rather large. From femtosecond transient absorption measurements on PS I particles from *Synechococcus* sp. it was found that the area under the spectrum associated with trapping was comparable to the area under the spectrum associated with radical pair formation (Holzwarth et al., 1993). In contrast, Kumazaki et al. (1994a,b) have performed measurements on PS I particles from spinach containing either 12 or 30 Chl-*a*/P700, and they find a large difference in the area under the 0.5–2 ps spectrum compared to the area under the 230–500 ps spectrum, similar to what is observed here.

When low excitation intensities are used (only single photon processes) and if the pigments in the antenna are viewed as weakly coupled chromophores, this large difference in area is very difficult to explain. One possible explanation is that some of the reaction centers may be in the closed state (P700 oxidized), and we therefore observe absorption changes mainly associated with the antenna pigments. Our samples are rotated at 2 Hz, and thus the same sample volume is excited every 500 ms. We routinely monitor the ground state recovery kinetics before femtosecond measurements, and in all of our experiments the concentrations of PMS and ascorbate are adjusted such that full recovery occurs within 80 ms. This argues against substantial build-up of $P700^+$.

We can also rule out the contribution of multiple photon processes. The same kinetics were obtained from single photon counting measurements (Hastings et al., 1994a), and we observed essentially the same kinetics and spectra when the excitation intensities were increased by more than a factor of 2 (data not shown).

Finally, we have performed measurements using only the trimeric PS I unit. If these units are well connected by energy transfer then it may be that we have underestimated our excitation intensities (note that the number of antenna pigments per primary electron donor does not change). However, we have performed similar measurements on PS I reaction centers from *C. reinhardtii* and from spinach, both of which are known not to form trimeric units, and we find very similar results to those reported here (G. Hastings et al., manuscript submitted for publication).

The relatively large ratio in the area under the spectra associated with trapping and radical pair formation has also been observed in purple bacterial chromatophores (Xiao et al., 1994). Using a mutant from *Rb. capsulatus* that lacks LH II, Xiao et al. (1994) found at least a fivefold decrease in oscillator strength as the system evolved from the antenna excited singlet state to the radical pair state. In certain purple bacterial chromatophores it is thought that the basic antenna unit (B820) is a dimer of interacting BChl's (van Mourik et al., 1991; van Grondelle et al., 1994; Koolhaas et al., 1994). Xiao et al. (1994) further suggested, based on their observations, that the basic dimeric units must also display strong excitonic interactions.

In PS II reaction centers a factor of twofold increase in area under the time-resolved difference spectra is observed as energy is transferred from chlorophyll pigments (C670) to P680, the primary electron donor in PS II. This change in area was used to indicate that P680 is at least dimeric (Durrant et al., 1992). Thus, large changes in the area under difference spectra associated with different states has been used as evidence to suggest excitonic interactions between pigments (see also Gobets et al., 1994; Xiao et al., 1994; Novoderezhkin and Razjivin, 1993, 1994). In a similar vein, we suggest that the large change in area under the spectra associated with trapping and radical pair formation indicates some degree of (excitonic) coupling among the chlorophyll pigments in the core antenna. The number of pigments that interact or aggregate is difficult to determine. If it is assumed that stimulated emission accounts for 50% of the area under the 24–27 ps spectrum, then the area under the difference spectrum decreases by about twofold by going from the equilibrated excited state to the radical pair state. This difference could be explained by the formation of dimers in the PS I core antenna (assuming the nondecaying spectrum is due to a monomeric species). Very recently, Gobets et al. (1994) have investigated the nature of the long wavelength pigment(s) in PS I particles from *Synechocystis* sp. 6803. The longest wavelength pigment(s) display a Stokes shift that is more than double that observed for monomeric Chl-*a* in detergent (Kwa et al., 1994) and is similar to that observed for the primary electron donors in purple bacteria. This led Gobets et al. (1994) to the conclusion that the long wavelength pigment(s) have to be at least a dimer of strongly coupled pigments, as is the case for the primary electron donors in purple bacteria. Further indications of excitonically coupled pigment units in the PS I core antenna have been obtained from CD experiments on PS I particles from spinach containing 7–10 Chl-*a*/P700 (Ikegami and Itoh, 1986).

Strongly coupled pigments or aggregates in the PS I core antenna will likely affect the energy migration kinetics and may have serious implications as far as computer simulations using lattice type models are concerned (Jia et al., 1992; Laible et al., 1994; Trinkunas and Holzwarth, 1994). For instance, Fetisova (1990) has shown that the formation of pigment clusters (with strong coupling between pigments in single clusters, and weak coupling between pigments in different clusters) can increase the efficiency of energy transfer from antenna to reaction center. A special arrangement or orientation of groups of interacting pigments will influence energy transfer rates and has not been considered in any computer simulations so far. Unfortunately, the struc-

tural information available from the 6 Å resolution x-ray structure is not sufficient to resolve this issue, although some significant indications may be possible with a moderate increase in resolution.

Coupling between pigments also raises questions concerning the origin of spectral heterogeneity in the PS I core antenna. Spectral heterogeneity may be a result of pigment-protein interactions or/and pigment-pigment interactions (spectral heterogeneity results from different cluster types bound to the protein). Resonance Raman studies of a number of Chl-*a* and -*b* containing protein complexes from higher plants favored the idea that chlorophyll does not form oligomers or clusters in higher plant pigment-protein complexes and that it is only monomeric pigment-protein interactions that give rise to spectral heterogeneity (Lutz, 1977; Lutz et al., 1979; see Lutz and Robert, 1988, for a review), in contrast to the data presented here and the conclusions of Causgrove et al. (1988) and Struve (1990).

Equilibration in the PS I core antenna

After the processes that are responsible for the 2.7–4.3 ps component are complete the observed absorption changes no longer depend on the excitation wavelength, within the limits set by the error bars in Fig. 5 *A*. However, the absorption changes over the first few picoseconds depend strongly on the excitation wavelength (Figs. 2, 3, 4). After 590 (678) nm excitation, the shape of the 3.7 (2.7) ps spectrum in Fig. 4 *A* suggests that they are associated with energy transfer from pigments that absorb in the 680 nm spectral region to pigments that absorb near and beyond 700 nm. The 3.7 and 2.7 ps spectra in Fig. 4 *A* clearly indicate that long wavelength pigments are present and functional in the core antenna of the detergent-isolated particles. The 4.3 ps spectrum in Fig. 4 *A*, obtained after 710 nm excitation, appears to be inverted relative to the other two spectra, suggesting that this component is a result of energy transfer processes from longer to shorter wavelength absorbing pigments. The shape of the 4.3 ps spectrum demonstrates that we can directly excite these longer wavelength pigments, which leaves little doubt about their existence, and that we can observe equilibration processes from the initially prepared distribution, which leaves little doubt about their functionality.

The fact that we can observe energy transfer in either direction, depending on which distribution of pigments we initially excite, and that we find similar lifetimes independent of excitation wavelength suggests that the 2.7–4.3 ps process is not influenced by vibrational relaxation or Q_x to Q_y conversion within a single spectral form. The 2.7–4.3 ps spectra are probably associated entirely with energy transfer processes between vibrationally cool excited singlet states.

Many authors have considered equilibration in the PS I core antenna. Downhill energy transfer has been observed directly (Holzwarth et al., 1993; Turconi et al., 1993, 1994; Hastings et al., 1994a), as have rapidly red-shifted absorption changes (Klug et al., 1989). Owens et al. (1989) and McCauley et al. (1989) have also observed blue-shifted fluorescence after excitation in the red edge of the absorption spectrum. In the studies in which the excitation wavelength was varied (Trissl et al., 1993; Owens et al., 1989) equilibration was inferred from the fact that the overall trapping time appeared to be independent of excitation wavelength, as we have also shown here. All of these studies taken together suggest rather convincingly that equilibration occurs among the various spectral forms before the trapping process, although the actual dynamics of the equilibration process were not fully revealed in any of the previous studies.

Recently Laible et al. (1994) have performed computer simulations, using 3D, space-filling lattice models. They introduced the term "transfer equilibrium" to describe the equilibrated distribution of pigments that occur in the presence of photoactive reaction centers, and to distinguish from the term "Boltzmann equilibration," which they use to refer to the distribution of excitations that would occur at infinite time in the absence of a photochemical trap. In their PS I model the two distributions can differ significantly. Laible et al. (1994) assume trap-limited type models but did not test models in which specific energy transfer processes could limit the trapping time.

Based on a simple Gaussian deconvolution of the room temperature absorption spectrum we can estimate the pigment content of the detergent-isolated PS I particles (data not shown). From these estimates we can calculate the spectral distribution at (Boltzmann) equilibrium and hence estimate the difference spectrum that would be associated with the equilibrium distribution. This calculation takes into account both excited state absorption as a Gaussian centered at the same wavelength as the absorption but with half the height and twice the width and stimulated emission, assuming a 6 nm Stokes shift. Within the levels set by our signal-to-noise ratio, the difference spectra we observe after the faster 3–4 ps processes are complete are in agreement with those expected for the Boltzmann distribution (data not shown). We are unable to observe what differences, if any, trapping may have on the equilibrated excited state distribution.

Boltzmann equilibration depends purely on the number and spectral type of pigments and is independent of the relative structural details of these pigments. The fact that the equilibrated distribution we observe is similar to the Boltzmann distribution indicates that we would be unable to distinguish between a funnel and a random model on the basis of room temperature data, as also shown by Laible et al. (1994). At lower temperatures the kinetics clearly slow down in the sense that energy is trapped for longer periods of time on the red pigments (Woolf et al., 1994; Gobets et al., 1994). Thus it may be possible to investigate the structural details of the PS I core more fully at lower temperatures.

Heliobacterial reaction centers contain Fe-S clusters as terminal acceptors and are broadly similar to PS I (Trost and Blankenship, 1989; Nitschke et al., 1990; Liebl et al., 1993). Compared to heliobacteria, the lifetimes that characterize

equilibration in the PS I core antenna appear to be rather long. Reaction centers from heliobacteria are spectrally heterogeneous and contain about 35 BChl-*g* per primary electron donor (van Dorssen et al., 1985; Smit et al., 1989). Equilibration is found to occur in less than 1 ps (van Noort et al., 1992; Lin et al., 1994). In PS I the effective antenna size (Trissl, 1993) is about 17 pigments (Hastings et al., 1994a), depending on the pigment stoichiometries, compared to 12 in heliobacteria (Lin et al., 1994). Therefore, even accounting for the slightly different effective antenna size, the lifetime characterizing the equilibration process(es) in heliobacterial reaction centers is exceptionally short, compared to the corresponding lifetimes found here for PS I. The reason for the very short equilibration times in heliobacteria, compared to PS I, has not been resolved but may be related to the fact that some of pigments in the PS I core antenna appear to be excitonically coupled (see above), which may not be the case in heliobacteria. We note, however, that time constants of 2–3 ps for equilibration in the PS I core have been obtained in simple lattice simulations without any assumptions concerning clusters of pigments (Jia et al., 1992).

In PS I particles from the cyanobacterium *Synechococcus* sp., Holzwarth et al. (1993) found that equilibration was characterized by a time constant of 8–12 ps, about 2–3 times longer than observed here. Previously (Hastings et al., 1994a), we suggested that this difference could be due to the use of different excitation wavelengths, or that it could also be the result of some artifact caused by the detergent treatment. Here we show that neither the detergent treatment nor the excitation wavelength can explain the different equilibration times in the different cyanobacterial PS I particles. The differences are almost certainly due to basic differences in the preparations: PS I particles from *Synechococcus* sp. are unusual in that they clearly contain an increased number of long wavelength pigments, relative to other species (in PS I particles from *Synechococcus* sp. the room temperature steady-state fluorescence maximum is at 720 nm, in contrast to most other PS I core particles, which show a peak at ~690 nm). The different pigment composition may give rise to the different observed equilibration lifetimes. However, it is not clear if there are also structural deviations in the core antenna of the different cyanobacterial preparations, which may also contribute to the different equilibration lifetimes. Our data suggest that a strong coupling exists between a few or larger groups of pigments in the PS I core antenna of *Synechocystis* sp. PCC 6803, which may not be the case for detergent-isolated PS I particles from *Synechococcus* sp.

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