Spectral Inhomogeneity of Photosystem I and Its Influence on Excitation Equilibration and Trapping in the Cyanobacterium *Synechocystis sp.* PCC6803 at 77 K

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ABSTRACT Ultrafast transient absorption spectroscopy was used to probe excitation energy transfer and trapping at 77 K in the photosystem I (PSI) core antenna from the cyanobacterium *Synechocystis sp.* PCC 6803. Excitation of the bulk antenna at 670 and 680 nm induces a subpicosecond energy transfer process that populates the Chl *a* spectral form at 685–687 nm within few transfer steps (300–400 fs). On a picosecond time scale equilibration with the longest-wavelength absorbing pigments occurs within 4–6 ps, slightly slower than at room temperature. At low temperatures in the absence of uphill energy transfer the energy equilibration processes involve low-energy shifted chlorophyll spectral forms of the bulk antenna participating in a 30–50-ps process of photochemical trapping of the excitation by P_{700}. These spectral forms might originate from clustered pigments in the core antenna and coupled chlorophylls of the reaction center. Part of the excitation is trapped on a pool of the longest-wavelength absorbing pigments serving as deep traps at 77 K. Transient hole burning of the ground-state absorption of the PSI with excitation at 710 and 720 nm indicates heterogeneity of the red pigment absorption band with two broad homogeneous transitions at 708 nm and 714 nm (full-width at half-maximum (fwhm) $\sim$ 200–300 cm^{-1}). The origin of these two bands is attributed to the presence of two chlorophyll dimers, while the appearance of the early time bleaching bands at 683 nm and 678 nm under excitation into the red side of the absorption spectrum (>690 nm) can be explained by borrowing of the dipole strength by the ground-state absorption of the chlorophyll *a* monomers from the excited-state absorption of the dimeric red pigments.

INTRODUCTION

Photosystem I (PSI) of oxygen-evolving cyanobacteria, algae, and higher plants is a membrane supramolecular complex with ferredoxin-NADP-reductase activity sensitized by chlorophyll *a*-dependent conversion of solar energy into chemical energy of transmembrane charge separation. In cyanobacteria, PSI is found as trimers (Boekema et al., 1987; Schubert et al., 1997), while in higher plants it is organized as a monomer of the PSI core complex surrounded by eight peripheral chlorophyll (Chl) *a/b*-containing light-harvesting I (LHCI) complexes (Boekema et al., 1990).

The chromophore part of the monomeric PSI core antenna consists of ≈100 chemically identical Chl *a* molecules. The protein environment modifies the site energies of Chl *a* molecules, resulting in a distribution of spectral forms in the Q_y region. In the highly heterogeneous absorption spectrum, each spectral form is a result of either pigment-protein or pigment-pigment interaction. Different parts of the PSI core perform different functions. The bulk antenna captures light and equilibrates the chlorophyll excitation over the whole complex. The primary donor P_{700} in the central part of the complex traps the excitation and transfers an electron to a monomeric Chl redox carrier as the primary charge separation. Electron transfer to secondary acceptor A_1 (phylloquinone) and iron-sulfur centers F_X and $F_{A/B}$ (Brettel, 1997) follows the primary event.

Extensive studies of excitation transfer and photochemical trapping in the PSI core antenna (see van Grondelle et al., 1994 and van Amerongen et al., 2000, for reviews) demonstrated that the decay of excited Chl *a* in the PSI core is multiexponential and results from the spectral inhomogeneity and specific structural organization of the Chl *a* spectral forms in the complex. The distances between Chl *a* molecules in the core antenna predict subpicosecond energy transfer rates based on the Förster inductive resonance mechanism with an average single-step transfer time of ~200 fs (Du et al., 1993; Schubert et al., 1997). The subpicosecond energy transfer was recently detected at room temperature in the PSI core antenna from cyanobacteria *Synechocystis sp.* PCC 6803 (Melkozernov et al., 1998, 2000a; Savikhin et al., 1999, 2000) and *Spirulina platensis* (Holzwarth et al., 1998). Excitation into the blue edge of the absorption around 660 nm induces downhill energy transfer with lifetimes of 0.3–0.6 ps between bulk antenna chlorophylls, which was interpreted as a major equilibration phase in the antenna. On a picosecond time scale the equilibration process involves a broad inhomogeneous band of low energy (red) pigments absorbing below 700 nm. This well-studied process takes 2–5 ps for PSI core antenna from the cyanobacterium *Synechocystis sp.* PCC 6803 (Hastings et al., 1994, 1995a, b; Turconi et al., 1996; Melkozernov et al., 1998, 2000a; Gobets et al., 1998a; Savikhin et al., 1999, 2000). Slower equilibration times for PSI from cyanobac-

Received for publication 19 October 2000 and in final form 10 May 2001.

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0006-3495/01/08/1144/11 $2.00

teria *Synechococcus elongatus* (8–15 ps) (Turconi et al., 1993; Byrdin et al., 2000) and *Spirulina platensis* (9 ps) (Dorra et al., 1998) are probably associated with additional pools of low-energy pigments in these species. At physiological temperatures the excitation of the equilibrated antenna in the PSI core is trapped by P_{700} within 20–30 ps in *Synechocystis* PSI (Hecks et al., 1994; Hastings et al., 1994, 1995a; Dimagno et al., 1995; Melkozernov et al., 1998), and 35–50 ps in cyanobacteria *S. elongatus* and *S. platensis* (Holzwarth et al., 1998; Gobets et al., 1998b; Karapetyan et al., 1999). If the Förster-type energy transfer between pigments dominates excitation dynamics in the bulk core antenna of the PSI, similar well-separated time scales (subpicosecond equilibration between individual pigments and pigment clusters, picosecond energy redistribution to red spectral forms and 20–40 ps photochemical trapping) would be expected at lower temperatures. To date, no detailed study of excitation dynamics in the PSI core antenna at low temperature is available.

Pigment-pigment interactions in the clusters of pigments could contribute to excitonic spectral forms shifted to the red relative to the absorption band of the bulk antenna (Melkozernov et al., 2000a, b). A nonphotochemical hole-burning study of PSI from *Synechocystis sp.* PCC6803 (Rätsep et al., 2000) suggests excitonic interactions in the antenna based on observations of nonphotochemical holes at 708 and 714 nm supplemented with high-energy satellite holes at 699, 695, and 692 nm coinciding with the features of the low-temperature absorption spectrum. A fluorescence line narrowing study (Gobets et al., 1994) and Stark hole-burning experiments at 4 K (Rätsep et al., 2000; Hayes et al., 2000) showed that the optical transition of Chl *a* molecules in the PSI from *Synechocystis* absorbing with $\lambda >$ 700 nm is characterized by strong electron-phonon coupling. Increased electron-phonon coupling is thought to result from a significant suppression of the Chl *a* zero-phonon line at 4 K due to strong pigment-pigment interactions, which is consistent with the formation of dimers (Gobets et al., 1994; Pålsson et al., 1996; Rätsep et al., 2000; Hayes et al., 2000; Melkozernov et al., 2000a, b). Our recent finding of direct coupling of the longest-wavelength absorbing pigments with transition at 683 nm on the subpicosecond time scale (Melkozernov et al., 2000b) suggests strong excitonic interactions in the dimeric red pigments. The transient band at 683 nm decaying within 300 fs was present even though the PSI reaction center (RC) was oxidized at low temperature, indicating that the transitions at 708 and 683 nm reflect the antenna exciton state. This is in agreement with recent data of Savikhin et al. (2000) who found in experiments on anisotropy decay at room temperature that the anisotropy pattern of the bands around 680 nm and 710 nm is independent of oxidation state in the RC.

This paper presents details of excitation equilibration and excitation trapping in the PSI core antenna at 77 K based on femtosecond transient spectroscopy using excitation of the bulk antenna at 670 nm and the lower energy spectral forms at 695, 700, 710, and 720 nm. It is shown that the mechanisms of the energy equilibration and trapping by the reaction center in the PSI at low temperature are similar to that at physiological temperatures. The origin of the longest-wavelength absorbing spectral forms at 708 and 714 nm in PSI from *Synechocystis sp.* PCC 6803 is discussed based on the detailed transient hole-burning study of the longest-wavelength absorbing spectral forms.

MATERIALS AND METHODS

Isolation of photosystem I

Photosystem I particles were isolated from the photosystem II-less mutant psbDI/C/DII of the cyanobacterium *Synechocystis sp.* PCC 6803 as described earlier (Hastings et al., 1994). The Chl a/P_{700} ratio in a mixture of PSI monomers and trimers was ~90.

Transient absorption spectroscopy

For time-resolved absorption spectroscopy at 77 K the sample was resuspended in 20 mM Tris HCl buffer, pH 8.0, 100 mM $MgCl_2$, 0.03% β-dodecyl maltoside, 20 mM sodium ascorbate, 15 μM phenazine methosulfate (PMS), 67% glycerol (v/v), and frozen to 77 K using a liquid nitrogen optical cryostat Optistat (Oxford, UK).

The measurements of transient absorption spectra of the photosystem I core antenna were performed using the femtosecond laser spectrometer described earlier (Melkozernov et al., 2000a). Laser pulses of 100 fs full-width at half-maximum (fwhm) at 790 nm, 800 mW power, and 1 kHz repetition rate were generated from a regeneratively amplified Ti-S system (Clark-MXR, Dexter, MI). One part of the laser pulses is used to generate a white continuum, which is then split into the probe and reference beams and focused on the sample. The other part is used to pump an optical parametric amplifier (OPA), which provides excitation in a broad spectral range. The output of the OPA is further filtered with a narrow interference filter (fwhm = 5 nm) at specific wavelengths, sent via a computer-controlled translation delay line and focused on the sample as the pump beam. After the filtering of the pump beam the actual pulse duration was 150 fs. The differential absorbance of the sample at different delays of the pump beam was monitored by a dual diode array detector (Princeton Instruments, PDPA-1024, Trenton, NJ) over a 150-nm spectral range.

Transient absorption spectra were measured both on the 2- and 200-ps time scales in the 600–750 nm spectral region with spectral resolution of 0.14 nm per channel and excitations at 670, 695, 710, and 720 nm (fwhm = 5 nm). The kinetics of each data set were analyzed globally and decay-associated spectra (DAS) were constructed after deconvolution of the observed kinetics with the excitation pulse and correction for the spectral dispersion of the probe beam. The intensity of the pump pulses was typically 1–3 μJ/pulse to ensure that <1 photon was absorbed per RC to avoid the singlet-singlet annihilation.

RESULTS

Energy equilibration in the bulk antenna at 77 K

Spectral evolution of absorption changes of the PSI core at 77 K within 200 ps after the excitation at 670 nm are shown in Fig. 1. On a short time scale (Fig. 1 *A*) the absorption changes induced by relatively narrow laser pulses (fwhm of ~100 cm^{-1}) include transient hole burning of the ground-

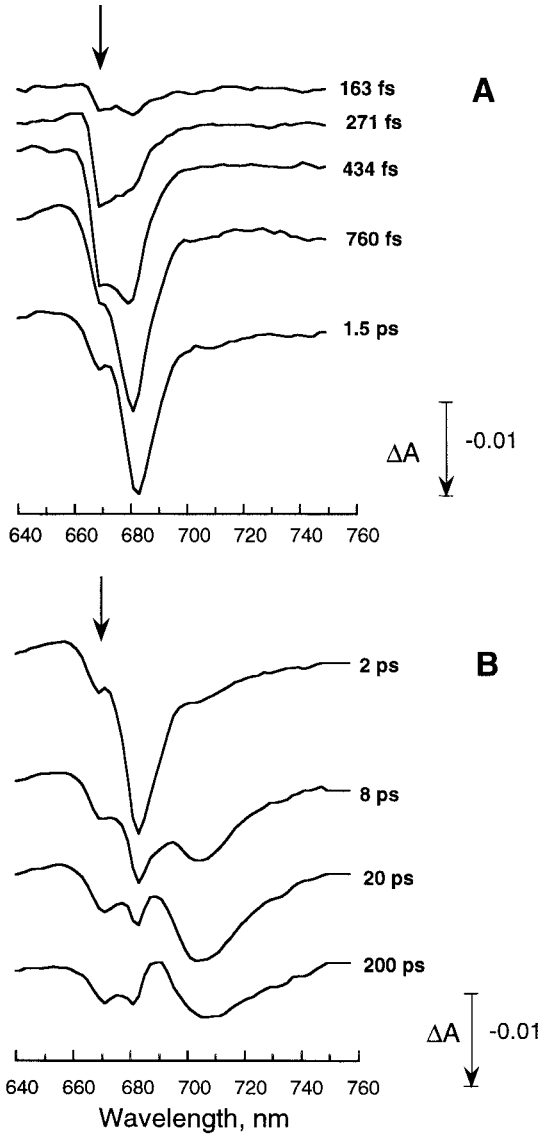

FIGURE 1 Transient absorption spectra of the photosystem I core from *Synechocystis sp*. PCC 6803 measured at 77 K on a 2-ps time scale (*A*) and 200-ps time scale (*B*) with excitation at 670 nm (fwhm = 100 cm^{-1}). Transient spectra are shown at different representative pump-probe delays. The wavelength axis in the spectra is offset for clarity.

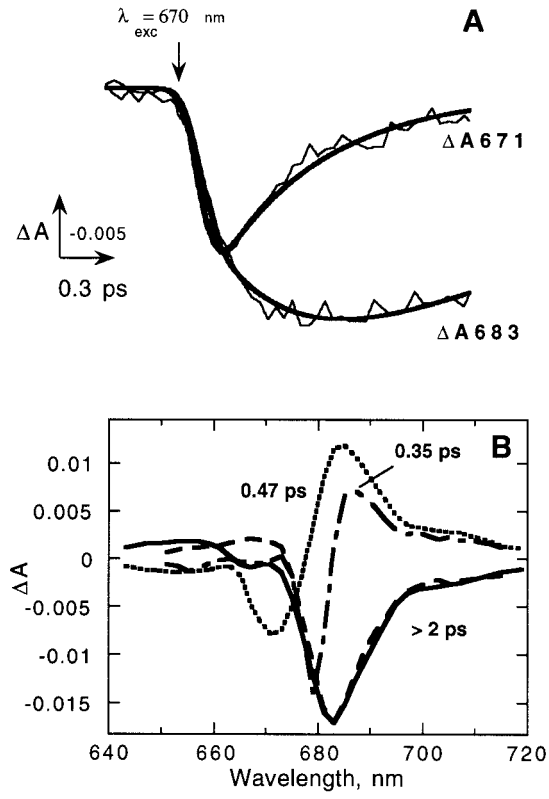

FIGURE 2 (*A*) Transient kinetics at 671 and 683 nm of the PSI core at 77 K and excitation at 670 nm. (*B*) DAS obtained after global analysis of transient kinetics on the 2-ps time scale with excitation at 670 and 680 nm; 0.47-ps DAS (*dotted line*) and 0.35-ps DAS (*dash-dotted line*) are subpicosecond energy transfer induced by excitation at 670 and 680 nm, respectively. DAS represented by unresolved long-lived excited states decaying with lifetimes longer than 2 ps are shown by solid (670 nm excitation) and dashed lines (680 nm excitation).

state absorption centered at the wavelength of the excitation followed by a subpicosecond energy transfer from Chls absorbing at 670 nm to the spectral pool around 683 nm. The initial photobleaching/stimulated emission (PB/SE) at 670 nm (see the 163-fs spectrum) is accompanied by a prompt excitation of a major pool of bulk Chl *a* at 683 nm excited via its broad vibronic band. It is likely that the vibrational relaxation of the majority of Chl *a*-683 evolves on a time scale not resolved in this study. Alternatively, the buildup of the PB at 683 nm in the early transient spectra might indicate the presence of unresolved energy transfer to the C-683 transition. Shapes of transient spectra within 1.5 ps after the excitation are consistent with the subpicosecond energy transfer from spectral forms of Chl *a* absorbing at 670 nm to the major pool of Chls in the PSI bulk antenna. The lifetime of subpicosecond decay of the ΔA band at 670 nm is 470 fs (see kinetics in Fig. 2*A*). A kinetic rise with similar lifetime is observed at 683 nm. DAS of this subpicosecond process, obtained after global analysis of the kinetics measured in the 620–760 nm spectral region with excitation at 670 and 680 nm, are shown in Fig. 2 *B*. Negative amplitudes of the DAS with subpicosecond lifetimes illustrate the decay of PB/SE with the maximum coinciding with the excitation wavelength. This is in agreement with the inhomogeneous nature of the broad absorption band of the bulk antenna (major part of ~100 monomeric Chl *a* of the PSI core). Additionally, the negative part of the 0.47 ps DAS observed with excitation at 670 nm is broadened by either vibronic relaxation of the C-683 band or unresolved ultrafast energy transfer processes. Vibronic relaxation of the C-683 band might occur due to excitation of these pigments via tails of 0–1 vibrational band at 630 nm of the major Q_y-transition band or the phonon wing of

the major absorption band, which is an intrinsic property of the optical transitions of monomeric antenna pigments (Reinot et al., 2001). The positive amplitudes of the subpicosecond DAS indicate that excitation of the pigments of the bulk antenna results in a subpicosecond energy transfer process to the Chl *a* spectral forms in the 685–700-nm region, with a maximum around 685–687 nm.

Fig. 1 *B* illustrates the excitation equilibration process at low temperature among the Chl *a* spectral forms in the antenna within 200 ps after excitation. Significant changes in absorption occur between 2 and 20 ps, including decay of the excitation centered at 683 nm and appearance of a new broad transient absorption band with maximum ΔA at 708 nm. This band reflects excitation population of low-energy Chls (red pigments) in the PSI core. At 200 ps, the broad ΔA band at 708 nm shows a decay. The long-lived excitation remaining around 670 nm in the 200-ps spectrum is most likely due to an uncoupled/nonequilibrated antenna.

To probe the role of the low-energy Chl *a* spectral forms in the excitation equilibration and trapping at low temperatures, the PSI core antenna was excited at 695 and 710 nm (Fig. 3). Excitation at 695 nm (Fig. 3 *A*) induces a narrow transient absorption band with a maximum photobleaching 4 nm shifted to the blue relative to the excitation wavelength. At 2 ps after excitation, this ΔA band shows a significant red-shifted broadening, indicating an equilibration process similar to that shown in Fig. 1 *B*. From 10 to 200 ps, the absorption changes include appearance of the long-lived ΔA band at 683 nm along with a broad shoulder at 675 nm. Appearance of the latter band in transient spectra with time delays from 10 to 70 ps suggests that at least part of this band in the 200-ps spectrum obtained with excitation at 670 nm (Fig. 1 *B*) is equilibrated at low temperature. Within 180 ps, the low-energy ΔA band at 708 nm exhibits a decay of photobleaching. Shift of the excitation to 710 nm does not significantly change the spectral profile of this decay (Fig. 3 *B*). A decrease of ΔA683/ΔA708 ratio within 200 ps illustrates a dominating long-lived excitation decay process in the pool of red pigments. The 180-ps spectrum in Fig. 3 *A* is generally similar to the 200-ps spectrum in Fig. 1 *B*. However, the ΔA683/ΔA708 ratio increases with the shift of the excitation wavelength to 670 nm, indicating a long-lived contribution by the decay of red pigments.

All transient spectra measured with excitation at 710 nm (Fig. 3 *B*) include spectral structure in the blue region with two pronounced bands at 675 nm and 683 nm. A change of the ΔA683/ΔA675 ratio within 180 ps suggests that longer-lived decaying processes in this spectral region are associated with longest-wavelength absorbing pigments in the antenna. Earlier, we found that excitation of the PSI core at 77 K in the spectral region from 695 to 710 nm induces the appearance of a transient absorption band at 683 nm decaying within 300 fs (Melkozernov et al., 2000b).

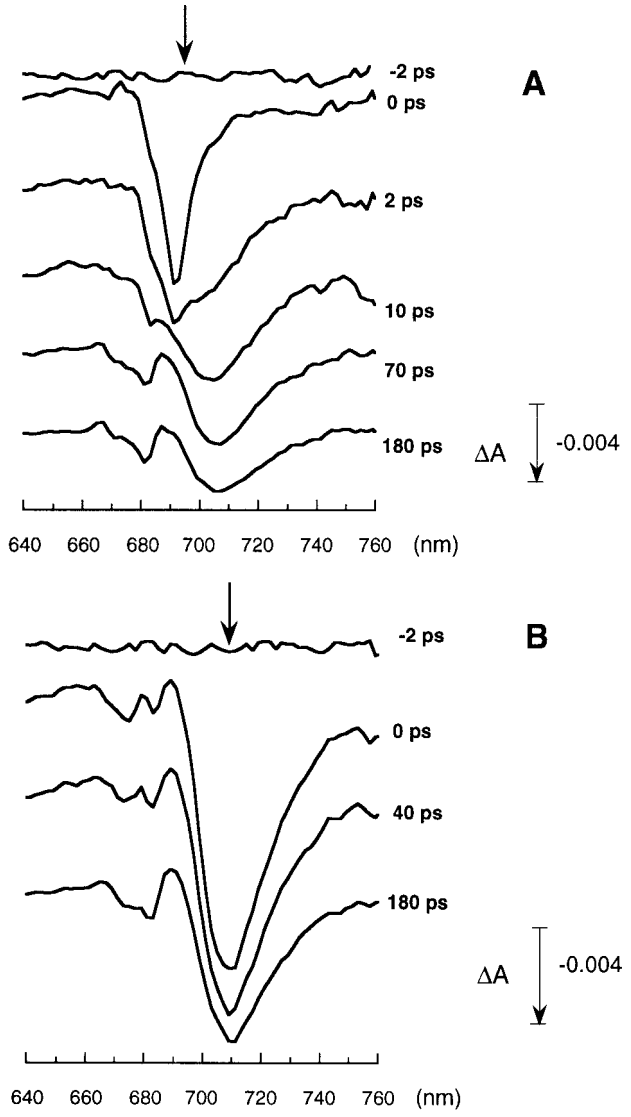

FIGURE 3 Transient absorption spectra of the PSI core measured at 77 K on a 200-ps time scale with excitation at 695 (*A*) and 710 nm (*B*). Transient spectra are shown at different representative pump-probe delays. Time 0 is defined at maximum ΔA. The wavelength axis in the spectra is offset for clarity.

Global analysis of 77 K transient spectra

Three sets of transient spectra with excitation at 670, 695, and 710 nm (Figs. 1 and 3) were analyzed globally. DAS with a three-exponential fit of kinetics measured on a 200-ps time scale are presented in Fig. 4. Excitations at 670 and 695 nm induce similar equilibration processes in the antenna with lifetimes varying from 4 to 6 ps (Fig. 4, *A* and *B*). The shape of DAS of this shortest exponential component resolved on this time scale indicates an energy transfer process between the bulk antenna pigments and red pigments in the PSI core antenna. The negative part of the DAS obtained with excitation at 670 nm is significantly broader, indicating participation of the excited bulk antenna Chls in

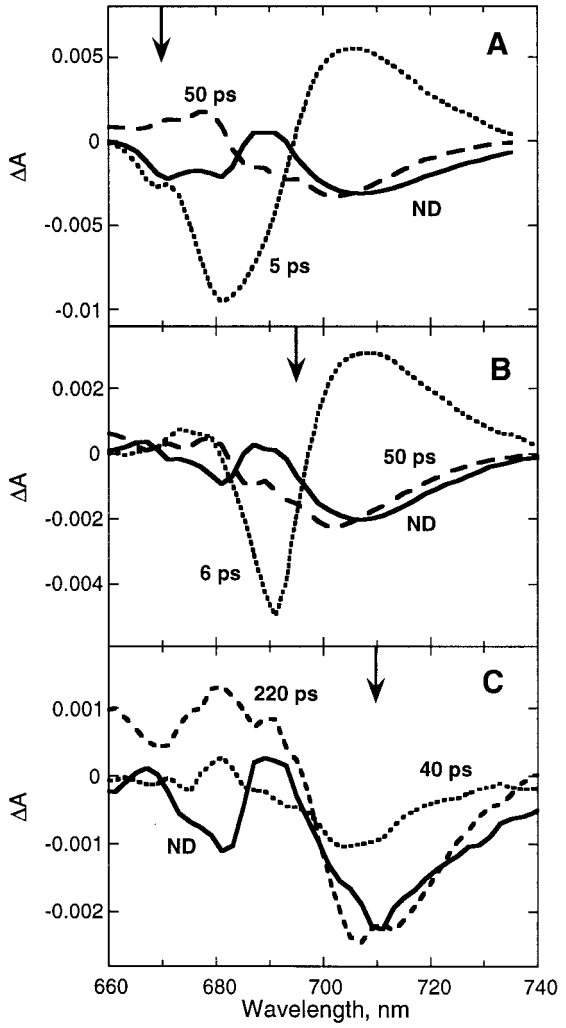

FIGURE 4 Decay-associated spectra obtained after global analysis of transient kinetics on the 200-ps time scale with excitation at 670 nm (*A*), 695 nm (*B*), and 710 nm (*C*).

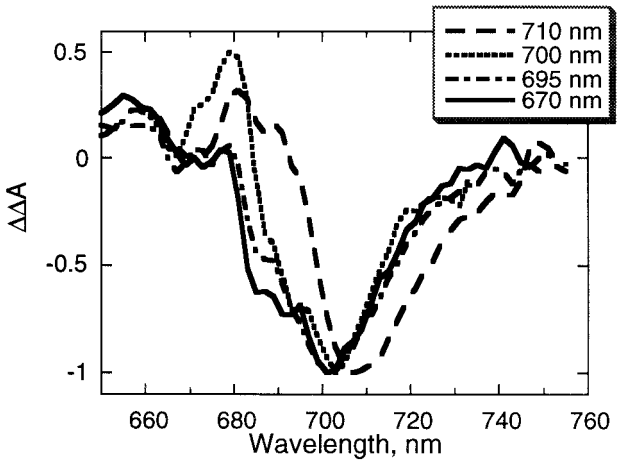

FIGURE 5 Difference between transient absorption difference spectra (20–200 ps) measured at excitation at 670, 695, 700, and 710 nm. Spectra are normalized to the maximum ΔA.

the equilibration. With excitation at 695 nm and the absence of uphill energy transfer, the equilibration involves only Chl spectral forms absorbing in the 680–700 nm region with a dominant contribution of the spectral form at 690 nm. Data of Fig. 2 *B* suggest that these spectral forms are populated on the subpicosecond time scale following the energy transfer from the bulk antenna Chls. Similar positive parts of the 4.8-ps DAS in Fig. 4 *A* and 6-ps DAS in Fig. 4 *B* indicate a rise of PB in the 700–720 nm spectral region attributed to excitation of the pool of low-energy pigments in the PSI core under excitations at 670 and 695 nm. A similar 5-ps energy transfer process is detected with excitation at 700 nm (data not shown).

The decay of the excitation in the equilibrated antenna at 77 K is best described by two components, an intermediate component with a lifetime of 40–50 ps and a long-lived component substantially nondecaying on this time scale (ND component in Fig. 4). Spectral profiles of the intermediate DAS are largely similar. They have a major negative band peaked around 705 nm and a broad shoulder around 685–695 nm. The presence of the shoulder around 685–695 nm indicates that part of the pigments excited as far as at 710 nm might participate in the excitation decaying process in the core antenna with the lifetime of 40–50 ps. Taking into account the data on subpicosecond and picosecond equilibration in the antenna (subpicosecond DAS in Fig. 2 *B* and picosecond DAS in Fig. 4, *A* and *B*), it is reasonable to ascribe this process to photochemical trapping in the antenna by P_{700}. Earlier, it was shown for PSI from cyanobacterium *S. elongatus* that ~45% of the RCs at low temperature perform a reversible photochemistry with $P^+A_1^-$ recombination time of ~170 μs (Schlodder et al., 1998; Pålsson et al., 1998). Under our experimental conditions (1 kHz repetition rate of pump pulses) this could ensure that P_{700} is reduced for each excitation pulse. However, with shift of the excitation wavelength to the red (Fig. 4 *C*), the long-lived ΔA band in the antenna becomes largely associated with the excitation trapping on longest-wavelength absorbing pigments. A heterogeneous decay of the red pigment's excitation is presented in Fig. 4 *C* by 200-ps DAS and an ND component.

Fig. 5 further proves that the 40–50-ps process represents a real decay of the excitation in the antenna at low temperature. The shape of the difference absorption spectra (20–200 ps) obtained with excitations at 670, 695, and 700 nm indicates two decaying ΔA bands at 700–705 and 690 nm, suggesting that the excitations within these spectral forms are equilibrated and participate in trapping. The difference spectrum obtained with excitation at 710 nm shows a red shift that can be explained by a low-temperature trapping on red pigments acting as deep traps.

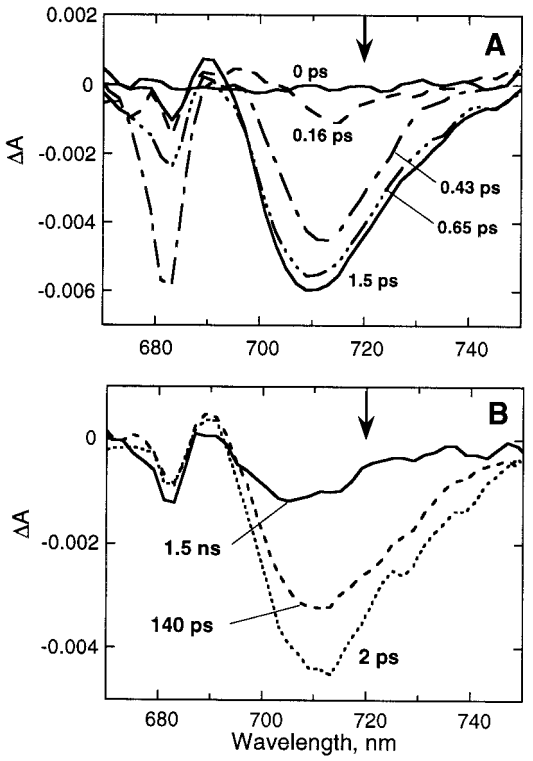

FIGURE 6 Transient absorption spectra of the PSI core measured at 77 K on a 2-ps (*A*) and 2-ns time scale (*B*) with excitation at 720 nm (fwhm = 100 cm^{-1}) shown at different representative pump-probe delays.

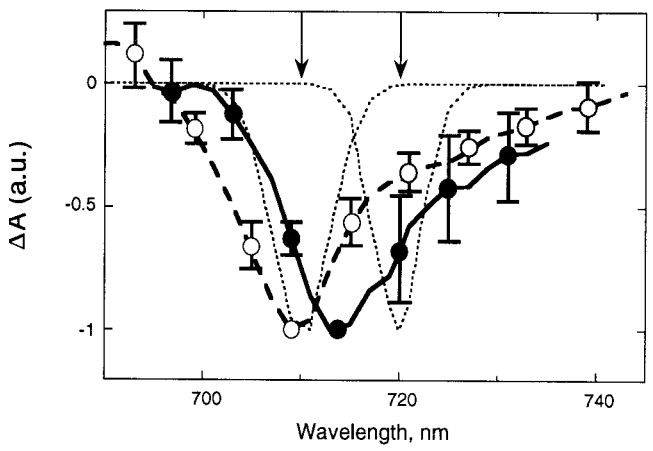

FIGURE 7 Comparison of the 77 K transient absorption spectra measured at time delay of ~250 fs and excitation at 710 nm (*dashed line*, *open circles*) and 720 nm (*solid line*, *closed circles*). All spectra are normalized to 1. The error bars indicate the standard errors of independent measurements. Spectral profiles of pump pulses are shown by dotted lines.

Heterogeneity of the red pigment band

To get insight into the heterogeneity of the spectral band of the red pigments at low temperature, the PSI core antenna was excited at 720 nm into the very red tails of the absorption band of the red pigments. Representative transient spectra on a 2-ps time scale are shown in Fig. 6 *A*. The data show that the maximum of ΔA in the early transient spectra is blue-shifted to 714 nm with respect to the maximum of the excitation pulse at 720 nm (fwhm = 100 cm^{-1}). A broad PB/SE band centered at 714 nm is accompanied by a transient band at 683 nm and a satellite band at 675 nm. We previously found this structure in the early transient spectra at excitation at 700 and 710 nm (Melkozernov et al., 2000b). We observed that shift of the excitation from 700 nm further to the red results in a splitting of the band into 683- and 675-nm bands. Within 300 fs, this band starts to decay. Between 400 fs and 1.5 ps the band decays substantially (only 3% of the band is left at 1.5 ps).

A broad ΔA band excited at 720 nm and centered at 714 nm at 260 fs delay has a width of 250 cm^{-1}. Within 2 ps the band broadens to 495 cm^{-1} and shifts to 708 nm, the maximum of the main pool of red pigments. This dramatic shift of the transient ΔA bands indicates two transitions at 714 and 708 nm. Fig. 7 compares the widths of the transient holes excited at 720 and 710 nm before the spectral broadening. Within the absorption band of the bulk antenna chlorophylls the width of the transient hole is limited by the width of the excitation pulse (~100 cm^{-1}). As a result, the maximum of the early transient spectrum coincides with the excitation wavelength. The data of Fig. 7 indicate that the widths of the early transient spectra excited at 720 and 710 nm at 77 K are not limited by the width of the excitation pulse, suggesting that the transient holes are homogeneously broadened. Both spectra are blue-shifted relative to the excitation. For the main band of red pigments (C-708), this shift is more pronounced at room temperature (705 nm). This is consistent with the temperature-dependent shift of the absorption maximum of red pigments from 704 nm at room temperature to 708 nm at low temperatures (Rätsep et al., 2000). The transition at 714 nm (C-714) is weaker and broader than 708 nm transition.

Excitation decay dynamics of the red pigments that are not resolved on the 200-ps time scale with excitation at 710 nm (Figs. 3 *B* and 4 *C*) are resolved on a 2-ns time scale, as shown in Fig. 6 *B*. The difference spectrum (2 ps–1.5 ns) (not shown) accounts for a trapping of the excitation on red pigments at low temperature and trapping on P_{700}.

DISCUSSION

Excitation energy transfer in the PSI core at low temperature

Our experimental data with the excitation of the bulk antenna pigments of the PSI core at 670 nm (Fig. 1) show that the excitation populates the major Chl *a* spectral form of the PSI core on the subpicosecond time scale. Peaks of the decaying parts of corresponding 300–500 fs DAS detected with excitation at 670 and 680 nm (Fig. 2 *A*) coincide with

the excitation wavelengths, in agreement with the transient hole-burning data (Melkozernov et al., 2000b) and the inhomogeneous nature of the broad absorption band of antenna Chls. However, the rising part of the DAS is independent of the excitation wavelength and is centered at 685–687 nm, with extension to 700 nm. Similar subpicosecond processes were detected at room temperature in neutral PSI (Melkozernov et al., 2000a) and oxidized PSI complexes (Savikhin et al., 1999, 2000). The data indicate that the excitation equilibration of the pigments in the bulk antenna takes a few transfer steps to populate the Chl *a* spectral form at 685–687 nm.

At 77 K, excitation of the bulk antenna at 670 nm and selective excitation of Chls absorbing at 695 nm result in an equilibration of excitation energy with red pigments within 4–6 ps (Fig. 3, *A* and *B*). Similar results were obtained earlier with excitation of P_{700} at 700 nm at 77 K (Melkozernov et al., 2000b). A slight increase of an equilibration time compared to that at room temperature (2–4 ps) is due to a decrease of spectral overlap between Chl spectral forms at low temperatures. Due to the significant decrease of uphill energy transfer at 77 K, the majority of the bulk antenna Chls do not participate in the equilibration upon excitation into the red edge of the Q_y absorption band of the PSI core.

The bleaching transient absorption band at the initial times upon 695-nm excitation is evidently much narrower than in other cases, and is 4-nm blue-shifted relative to the excitation wavelength (see Fig. 3 *A*). This can be explained by assuming that a homogeneous band is initially excited. If this band belongs to absorption of monomeric Chls in the electron transfer pathway (Melkozernov et al., 2000b), a fast energy transfer to P_{700} unresolved on the time scale of 200 fs may be expected. As a result, mixed kinetics of the charge separation and excitation equilibration with the surrounding antenna might be responsible for broadening of the bleaching band in the transient spectra and for the energy transfer to the longest-wavelength absorbing pigments (see Fig. 3 *A*).

At low temperature, the population of the antenna under excited-state equilibrium is expected to be shifted toward the red spectral forms because of decreased uphill energy transfer (Melkozernov et al., 2000b). The data of Fig. 5 show that in PSI from *Synechocystis sp*. at 77 K, difference transient absorption spectra (20–200 ps) obtained with excitations at 670, 695, and 700 nm are peaked at 705 nm, with a broad flanking shoulder at 690 nm. This indicates the participation of the red-shifted spectral forms of the core antenna absorbing at 685–700 nm populated on subpicosecond time scale (Fig. 2 *B*) and the longest-wavelength absorbing spectral forms at 708 nm populated on a picosecond time scale (Fig. 3, *A* and *B*). It is reasonable to assign this transient band with a lifetime of 40–50 ps to photochemical trapping at low temperature. The photochemical trapping is consistent with a high quantum yield for charge separation observed in the cyanobacterial PSI (Pålsson et al., 1996, 1998; Brettel, 1997; Schlodder et al., 1998). Regardless of the excitation wavelength, the trapping involves Chl spectral forms absorbing in the 685–700 nm range. These spectral forms include C-685, C-692, C-695, and C-699 (Rätsep et al., 2000). The origin of these spectral forms, except C-699, which most probably belongs to P_{700}, is an open question. The homogeneous C-691 band (C-692 in Rätsep et al., 2000) was suggested to originate from absorption of monomeric Chls in the RC (Melkozernov et al., 2000b). The presence of 3–4 Chls absorbing around 695 nm (C-695) that are coupled with P_{700} was suggested based on thermodynamic analysis of the absorption and fluorescence spectra of PSI from higher plants (Croce et al., 1996). According to the current structure of PSI (Schubert et al., 1997), the candidates for the other pigments that contribute to the absorption in this spectral region might be clusters of "tightly packed" pigments in the core with center-to-center distances of 6–9 Å located around the RC (Melkozernov et al., 2000a, b) and characterized by stronger pigment-pigment interactions. The strongest pigment-pigment interactions are expected in a group of longest-wavelength pigments. Based on a transient absorption spectroscopy study of the PSI core from *Synechocystis sp.* PCC 6803 at room temperature and 77 K (Melkozernov et al., 2000a, b) C-708 pigments were assigned to dimers located close to the RC. It is interesting to suggest that at low temperature a smaller antenna size involving only the "clustered" pigments may participate in photochemical trapping by P_{700}.

At low temperature, the photochemical trapping in the PSI core from *Synechocystis sp*. is competing with the trapping on longest-wavelength absorbing pigments with optical transitions at 708 and 714 nm. Pålsson et al. (1998) reported that at 5 K ~50% of the excitation in the PSI core from *Synechococcus* are not at equilibrium with P_{700}. Such an incomplete equilibration in the cyanobacterial PSI antenna at low temperatures suggests that part of the red pigments at 77 K serve as deep excitation traps (Figs. 3 *B* and 4 *C*). Difference absorption spectra of PSI at long delay times obtained with excitations at 670, 695, 710, and 720 nm (Figs. 1, 3, and 6) contain the long-wavelength PB ΔA band at 710 nm with minor bleaching ΔA bands at shorter wavelengths (around 680 nm). The relative intensity of the short-wavelength ΔA band revealed excitation wavelength dependence that might be attributed to spectral inhomogeneity among the PSI complexes. The decay of excitation on the longest-wavelength absorbing pigments at 77 K takes several hundreds of picoseconds (Figs. 3 *B* and 4 *C*). The P_{700} photooxidation spectrum observed at 1.5-ns delay centers at 705 nm at 77 K (703 nm at room temperature) (Fig. 6 *B*, see also Melkozernov et al., 2000b), indicating that some complexes containing the red spectral forms still can deliver the excitation to the RC. The data suggest that C-714 pigments might serve as deep excitation traps at low temperature.

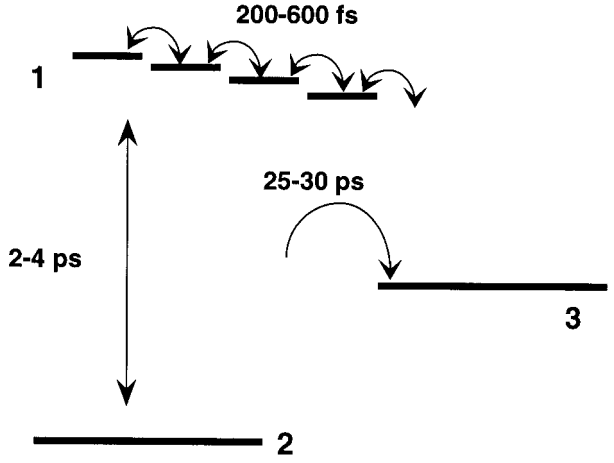

FIGURE 8 Simplified scheme of the excitation energy transfer in the PSI core antenna. Level 1 refers to the core antenna chlorophylls (bulk antenna) including the low energy bands populated within several hopping steps (200–600 fs). Within 2–4 ps the excitation spreads toward the longest-wavelength absorbing pigments (level 2). Level 3 determines the state of photochemical trap P_{700}. The rates shown correspond to the room temperature measurements (Melkozernov et al., 2000a).

General scheme of the excitation energy decay in PSI

Based on recent experimental data on ultrafast absorption spectroscopy of the PSI core antenna at room temperature (Holzwarth et al., 1998; Savikhin et al., 1999; 2000; Melkozernov et al., 1998, 2000a), the excitation dynamics in the PSI core antenna has to contain at least three levels with distinct kinetics (see scheme in Fig. 8): 1) bulk antenna chlorophylls (equilibration times 200–600 fs); 2) long-wavelength chlorophylls absorbing at ~708 nm (equilibration time with bulk antenna of 2–4 ps at room temperature); 3) photochemical trap, P_{700} (trapping time of 25–30 ps at room temperature). The data of Figs. 1–4 and our earlier data (Melkozernov et al., 2000b) show that these three time scales are also well-expressed at lower temperatures. The qualitative description of the major kinetic components in the system is presented below. Development of a more quantitative model of the excitation dynamics in the PSI is under progress (Valkunas, L., A. N. Melkozernov, S. Lin, and R. E. Blankenship, manuscript in preparation).

Equilibration in the bulk antenna

Fast equilibration kinetics in the main bulk antenna (level 1 in Fig. 8) result from the energy transfer from the "blue" pigments to the "red" ones within the inhomogeneously broadened Q_y absorption band of the core antenna Chls. The majority of the core antenna Chls (also termed as bulk antenna) with mean interpigment distances of 16 Å largely contribute to major sub-bands in the ground-state absorption at 673 and 682 nm (Melkozernov et al., 2000a). Earlier experimental observations (Hastings et al., 1995a) suggest that the pigments in the PSI core display some degree of excitonic coupling. The current structural model of the PSI reaction center (Schubert et al., 1997) allows identification of ~25 antenna pigments with pairwise center-to-center distances shorter than 10 Å (Melkozernov et al., 2000a, b), suggesting that pigment-pigment interactions in these clusters of Chls could contribute to excitonic spectral forms shifted to the red relative to the absorption band of the bulk antenna. The data of Fig. 2 *B* and ultrafast spectroscopy data of PSI at room temperature (Melkozernov et al., 2000a; Savikhin et al., 1999) suggest that these spectral forms absorb at 685–700 nm. The subpicosecond lifetimes indicate a local character of the Chl *a* excitation evolution, taking only a few transfer steps for the excitation to migrate to these spectral forms. Estimation of the excitation transfer rates between pigments of the bulk antenna based on the structural data and the Förster theory gives subpicosecond transfer times.

Transfer to longest-wavelength absorbing pigments

The longest-wavelength absorbing pigments (red pigments) are part of clustered antenna pigments (dimers or oligomers) around the RC. Strong pigment-pigment interactions in the longest-wavelength absorbing pigments could be responsible for the origin of red-shifted spectral forms absorbing below 700 nm (Pålsson et al., 1998; Melkozernov et al., 2000a, b). Because excitation equilibration in the bulk antenna is much faster than the transfer to the longest-wavelength absorbing pigments (300–500 fs versus 4–6 ps), the effective delivery rate of the excitation to the red pigments (W_{del}) and the detrapping rate (W_{detr}) to the bulk antenna can be evaluated based on the perturbed two-level model (Somsen et al., 1996; van Amerongen et al., 2000):

$$W_{del} = \frac{z}{N-1} W_{a-r} \quad (1)$$

$$W_{detr} = z W_{r-a} \quad (2)$$

where N is the total (effective) amount of antenna pigments, z is a coordination number of the longest-wavelength absorbing pigments (relative to the major core antenna pigments), and W_{a-r} and W_{r-a} are the excitation single (average) step transfer rate to the longest-wavelength absorbing pigments and back to the core antenna, respectively. The last values can be attributed to each other via the detailed balance relationship (Laible et al., 1998); however, it should be noted that the additional entropy factor $1/(N-1)$ in the effective delivery rate of the excitation to the longest-wavelength absorbing pigments (W_{del}) compensates the Boltzmann factor followed from the detailed balance relationship. This makes both values, the excitation delivery to the longest-wavelength absorbing pigments (Eq. 1) and its detrapping to the core antenna (Eq. 2) relatively close to

each other. Our room temperature absorption transient data with excitation of either bulk antenna or longest-wavelength absorbing pigments support this conclusion (Hastings et al., 1995b; Melkozernov et al., 2000a). However, if we take into account that the bulk antenna is also inhomogeneous and contains several populations of pigments, with the lower energy pool (clustered pigments) probably distributed more closely to the center of the structure (Melkozernov et al., 2000a, b), this diminishes the effective number of the bulk pigments *N* and thus makes the excitation more concentrated on the pigments, contributing to the lower energy-shifted spectral forms of the bulk antenna (685–700 nm) upon equilibration. This is supported by our observation of a 30–50-ps decaying process in the antenna at 77 K (Figs. 4 and 5) involving these spectral forms.

Excitation trapping by P_{700}

The photochemical trapping proceeds on a much longer time scale (25–30 ps at room temperature and 30–50 ps at 77 K) in comparison with the equilibration of the excitation between the bulk antenna and the red pigments. In the case of very fast charge separation the excitation lifetime of the PSI antenna is determined by the excitation delivery process, either from the longest-wavelength absorbing pigments or from the bulk pigments of the core. The slow excitation transfer from the bulk antenna can result from the distance factor, while the slow excitation delivery from the red pigments can be caused by a decrease of the spectral overlap and by unfavorable orientations of corresponding transition dipole moments of red pigments. The temperature dependence of the excitation decay kinetics and/or the structural data of the PSI organization has to be used to determine the limiting steps of the excitation trapping.

Molecular origin of longest-wavelength absorbing pigments.

Our data show that for long wavelength excitations at 710 and 720 nm (Fig. 7) different transient absorption bands are generated at the initial times (~200–250 fs), both being shifted to the blue relative to the excitation wavelengths. The blue-shift of the C-708 band with increasing temperature is consistent with an excitonic nature of the band (Rätsep et al., 2000). The bandwidths of these bleaching bands are of the order of 250 cm^{-1} (in the case of excitation at 710 nm) and 300–400 nm (at 720 nm of excitation) at ~250 fs delay after the excitation. The fact that both transient holes are broader than the bandwidth of the excitation pulses used in the experiments indicates under conditions of the experiments that there are two homogeneous transitions of the red pigments in the PSI antenna from *Synechocystis sp.* PCC 6803 at 708 and 714 nm. This spectral region is characterized by a strong electron-phonon coupling (Gobets et al., 1994; Rätsep et al., 2000), indicating the presence of Chl *a* dimers. Inspection of the current 4 Å resolution structural model of the cyanobacterial PSI allows us to suggest that two to three strongly coupled dimers observed in the structure can be candidates for the site of these two transitions (Melkozernov et al., 2000a, b). Both of the transient holes start to broaden very rapidly, turning into the broad bleaching bands shown in Figs. 3 and 6. The C-714 state can be easily missed because the fast broadening of the band is accompanied by a shift of the photobleaching maximum from 713–715 nm at a time delay of 200 fs to 708–710 nm at a time delay of 500–600 fs (Fig. 6 *A*). The C-714 pigments probably serve as a deep trap at low temperatures. Another explanation for the C-714 band is that the state represents some admixture of the charge transfer state, which might be present in the dimer (oligomer) complexes in the antenna.

The presence of the two distinct pools of the longest-wavelength absorbing pigments in the PSI from *Synechocystis sp.* could explain the kinetic heterogeneity in this spectral region observed recently (Savikhin et al., 1999, 2000). An additional ~6 ps component corresponding to the energy transfer from the core antenna to the red-most pigments in the experiments with initially oxidized RCs (Savikhin et al., 1999, 2000) suggests that in the heterogeneous C-708 + C-714 absorption band some of the red pigments are close enough to feel the redox state of the RC. Heterogeneity of the red pigment band may also be determined by a heterogeneous distribution of the red pigments between many PSI complexes. This means that in some complexes the excitation trapped on the longest-wavelength absorbing pigments are never transferred to P_{700} at low temperatures.

The appearance of the bleaching bands at 683 and 675 nm when exciting into the red side of the absorption spectrum (>690 nm) at the very initial times after excitation (Fig. 6 *A*, see also Melkozernov et al., 2000b) allowed us to conclude that the long wavelength band is related to the excitonically coupled dimers and, thus, these blue bands may be attributed to their higher energy transitions. This is supported by recent anisotropy decay data of Savikhin et al. (2000), who concluded that the higher energy spectral component around 680 nm is redox state-independent and is related to the red pigments.

It should be noted that for an excitonically coupled dimer the intensity of excited-state absorption (ESA) equal to the buildup of the SE has to be present in the transient spectra, in addition to PB bands of lower and higher energy transitions (Valkunas et al., 1999; van Amerongen et al., 2000). However, no pronounced ESA of Chl *a*, both at 77 K and room temperature, was observed in the transient spectra with excitations at 710 and 720 nm. Due to the selectivity of the excitation used in the experiments, this absence of the ESA cannot be explained as canceling of this spectrum by the ground-state absorption by other pigments at the same wavelength. Therefore, the appearance of the transient bleaching band at 683 nm resonant with the excitation

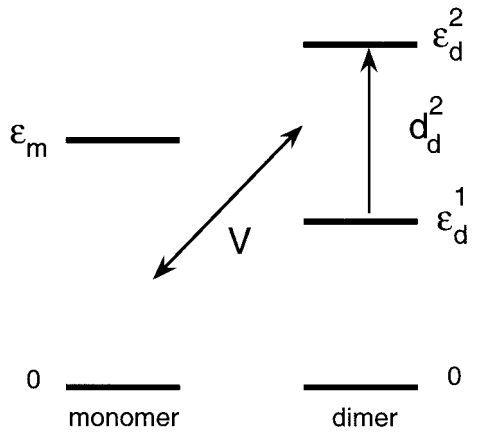

FIGURE 9 Energy scheme demonstrating the mixing between the ground state of the monomer and the excited state of the dimer. ϵ_m determines the excited state of the monomer; ϵ_d^1 and ϵ_d^2 are the first and higher excited states of the dimer, which are close to the resonance of the ground state absorption of the monomer. d_d^{21} is the corresponding dipole strength of the dimer and V is the resonance interaction between monomer and dimer transitions under consideration.

wavelength at 710 or 720 nm has to be caused by another effect.

The fact that the positions of the transient bands at 683 and 678 nm (Fig. 6 *A*) do not change with the shift of the excitation in the red region of the spectrum suggests that some Chl monomers of the bulk antenna located in the vicinity of the dimeric red pigments are sensitive to the excitation of red pigments. Such a sensitivity can result from mixing of these states with the ESA of a nearby situated dimer, i.e., hetero-dimer mixing (a dimer and a monomer, see Fig. 9). Then the bleaching in the blue part of the absorption band can result from mixing of the excited states in the heterodimer. For instance, it can be attributed to the borrowing of the dipole strength by the ground-state absorption of the monomer from the ESA of the dimeric red pigments according to the following estimation using perturbation theory (Valkunas et al., 1999; van Amerongen et al., 2000):

$$B_{683} \cong 2\frac{2(\epsilon_d^2 - \epsilon_d^1)V}{(\epsilon_m)^2 - (\epsilon_d^2 - \epsilon_d^1)^2}\, d_d^{21} \qquad (3)$$

where B_{683} is photobleaching at 683 nm, ϵ_m and $\epsilon_d^{1,2}$ are the excited states of the monomer and the first and second excited states of the dimer, respectively; V is a resonance interaction between the monomer and the excited state of the dimer; and d_d^{21} is the dipole strength of the dimer's ESA. The bleaching at 678 nm can be attributed to the mixing with other monomers or with the vibrational sub-band of the same monomer. Moreover, due to the suggestion of the presence of the charge-transfer character in the excited state of the dimer (C-714 state), its ESA spectrum can be broad and diffusive, which is in line with the experimental observations. In this case, the difference in the kinetics of both photobleaching bands might be due to a dephasing of the excitonic coupling between monomers and dimers. The presence of some charge transfer character in the excited state of the longest-wavelength absorbing spectral forms could also explain a fast broadening of the photobleaching C-714 band in the transient spectra after direct excitation (see Fig. 6).

This work was supported by National Science Foundation Grant MCB-9727607 (to R.E.B.). This is publication 448 of the Center for the Study of Early Events in Photosynthesis at Arizona State University.

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