Fluorescence Spectroscopy of the Longwave Chlorophylls in Trimeric and Monomeric Photosystem I Core Complexes from the Cyanobacterium *Spirulina platensis*[†]

Navassard V. Karapetyan,[‡,§] Dieter Dorra,[‡] Gerd Schweitzer,[‡,||] Irina N. Bezsmertnaya,[§] and Alfred R. Holzwarth*[,‡]

Max-Planck-Institut für Strahlenchemie, Stiftstrasse 34-36, D-45470 Mülheim a.d. Ruhr, Germany, and A. N. Bakh Institute of Biochemistry, Russian Academy of Sciences, Leninsky pr. 33, 117071 Moscow, Russia

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ABSTRACT: The organization and interaction of chlorophylls (Chl) and the kinetics of the energy transfer in the core antenna of photosystem I (PSI) trimeric and monomeric complexes, isolated from *Spirulina platensis* with Triton X-100 have been studied by stationary and time-resolved fluorescence. At 295 K both complexes show an unusually intense long-wavelength emission band with prominent peaks at 730 nm (trimers) or 715 nm (monomers), whose intensity is independent of the redox state of P700. A broad band extending from 710 to 740 nm in the absorption and fluorescence excitation spectra of trimers also indicates the existence of the longwave Chls at 295 K. The 77 K fluorescence emission of PSI trimers frozen after addition of dithionite under illumination (P700 and the PSI acceptor side reduced) shows an intense band at 760 (F760) and a smaller one at 725 nm (F725); when P700 is oxidized, the intensity of F760 decreases about 15 times. In the 77 K spectrum of monomers only F725 is present in the longwave region, and its intensity does not depend on the redox state of P700. Bands of Chls with maxima near 680, 710, and 738 nm were found in the 77 K excitation spectrum of trimers, and bands near 680 and 710 nm were seen in the spectrum of monomers. Five spectrally different red Chl forms in PSI trimers and three red Chl in monomers have been resolved by deconvolution of their 77 K absorption spectra. The difference absorption spectrum, trimers-minus-monomers, shows that the appearance of the 735 nm band in trimers is accompanied by a decrease of 708, 698, and 688 nm bands present in monomers. The reversible changes of F760 intensity of *Spirulina* membranes as a result of their salt treatment confirm the idea that the most longwave Chl form originates from an interaction of Chls bound to different monomeric PSI subunits forming the trimer. The time-resolved fluorescence spectra of PSI trimers and monomers, measured at 287 K in the region 680−770 nm, are substantially different, although a set of similar lifetimes (9, ∼30, ∼66, and 1400−2200 ps) was necessary for a good fit. No effect of P700 redox state was observed on the fluorescence kinetics of both complexes at 287 K.

Photosystem I (PSI)[1] complexes of cyanobacteria, in contrast to PSI complexes of higher plants, are organized as trimers and monomers (Boekema et al., 1987; Hladik & Sofrova, 1991; Shubin et al., 1992, 1993; Kruip et al., 1994; Tsiotis et al., 1995). One of the main lines of evidence for the existence of PSI trimers *in vivo* derives from the presence of a fluorescence band at 760 nm (F760) in the 77 K emission spectra of isolated trimeric PSI complexes and of intact membranes from the cyanobacterium *Spirulina platensis* (Shubin et al., 1991, 1993), while that band is missing in monomeric complexes. Notably, in PSI crystals the trimers form a tight cylindrical unit (Fromme et al., 1996). Cyanobacterial PSI contains the reaction center core complex but lacks any peripheral light-harvesting complex. The major part of the chlorophylls (Chl) is located on a heterodimeric apoprotein of 82−83 kDa molecular mass, forming the core antenna−reaction center complex (Golbeck & Bryant, 1991). The antenna size (Chl/P700) was determined to be about 100 for both trimeric and monomeric complexes, indicating that each monomeric subunit in the trimer contains a P700 electron donor (Rögner et al., 1990a; Shubin et al., 1992). Cyanobacterial PSI trimeric and monomeric complexes are very stable and are independent of the detergents used for isolation. The equilibrium between PSI trimeric and monomeric complexes in isolated detergent-free membranes could be regulated by changing their electrostatic interaction at various salt conditions (Kruip et al., 1994).

The molecular organization of the trimeric PSI reaction center complex from cyanobacteria was determined using X-ray structure analysis with a resolution of 6 Å (Krauss et al., 1993) and later with 4.5 Å resolution (Fromme et al., 1996), but the exact location and orientation of Chls and carotenoids in the PSI core antenna is not yet clear. The PSI core antenna is spectrally highly heterogeneous and the Chls can be classified as belonging to two main pools (about 680 nm and >700 nm) differing spectrally and by the lifetime of the energy migration (Holzwarth et al., 1993; Turconi et

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* Author to whom correspondence should be addressed.
[‡] Max-Planck-Institut für Strahlenchemie.
[§] A. N. Bakh Institute of Biochemistry.
[||] Current address: Department of Chemistry, Institute for Molecular Dynamics and Spectroscopy, University of Leuven, 3001 Heverlee, Belgium.

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[1] Abbreviations: Chl, chlorophyll; Chl735 (Chl710, Chl742, ...), chlorophylls absorbing at 735 (710, 742, ...) nm; DAS, decay-associated spectrum; F760 (F730, ...), fluorescence band peaked at 760 (730, ...) nm; PMS, phenazine methosulfate; PS, photosystem; P700, primary electron donor of photosystem I.

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al., 1993; van Grondelle et al., 1994; Hastings et al., 1995). One of these pools includes the main part of Chls (bulk Chl), and the absorbed energy migrates downhill to P700 with high efficiency. The other main pool contains the longwave (or red, low-energy) Chls, absorbing at longer wavelength than P700 and showing at 77 K the main emission band at 720−725 nm or longer. The role of these Chls and the molecular interaction responsible for this extreme red shift are unclear. It was suggested that the red Chls may focus the excitation to the reaction center (van der Lee et al., 1993; van Grondelle et al., 1994; Shubin et al., 1995), be an intermediate trap (Wittmershaus, 1987; Woolf et al., 1994), increase the absorption cross section (Trissl, 1993), or protect the reaction center against excess excitation (Mukerji & Sauer, 1989).

PSI trimeric and monomeric complexes isolated from the unicellular thermophilic cyanobacterium *Synechocystis* sp. do not differ in their stationary and time-resolved fluorescence spectra (Turconi et al., 1996), nor in their steady-state polarized spectroscopic properties (van der Lee et al., 1993), and show an identical charge recombination time (Kruip et al., 1993). In contrast, the trimeric complexes from *Spirulina* contain the most extreme long-wavelength Chls, emitting at 760 nm at 77 K (F760), which are absent in the corresponding PSI monomers (Shubin et al., 1992). The trimeric and monomeric complexes from *Spirulina* show very similar dimensions as the corresponding complexes from *Synechocystis* sp. (Böttcher et al., 1992; Shubin et al., 1993). Another peculiar and unique feature of PSI trimers of *Spirulina* is the fact that the intensity of F760 of membranes and isolated PSI trimers at 77 K depends strongly on the redox state of P700 (Shubin et al., 1991, 1992). Illumination of trimers, frozen with dithionite or ascorbate in the dark, quenches F760 with kinetics similar to that of P700 photooxidation. A similar effect has been observed for PSI particles isolated from *Pseudoanabaena* sp. M2 (Duval et al., 1986), although it is not so pronounced in that system. It was proposed that light-induced F760 quenching is a result of the energy migration from the longwave Chl absorbing at ∼735 nm (Chl735) to the cation radical of P700 ($P700^+$) formed during illumination, since the overlap integral of F760 with the absorption band of $P700^+$ at 77 K is much higher than that with the band of reduced P700 (Shubin et al., 1995).

Concomitant with the observed quenching, the lifetime of F760 of *Spirulina* trimers at 77 K was found to be highly dependent on the redox state of P700: it was found to be 800 ps with P700 reduced and less than 100 ps with P700 oxidized (Karapetyan et al., 1992). The 77 K lifetime of F730 in PSI monomers of *Spirulina* was about 500 ps, independent of the redox state of P700. No dependence of the fluorescence yield or lifetime of the red Chls on the redox state of P700 at 77 or 295 K was found for PSI complexes isolated from other cyanobacteria (Sparrow et al., 1990; Turconi et al., 1993), which do not show the extreme red fluorescence, nor for PSI particles from higher plants (Karapetyan et al., 1973; Butler et al., 1979). A doubling of the short fluorescence decay of PSI particles in the presence of dithionite as compared with ferricyanide was reported (Owens et al., 1988) but could not be confirmed by other groups.

To understand the role of longwave Chls in the energy migration in PSI antenna of cyanobacteria, we studied the PSI trimeric and monomeric complexes isolated from *Spirulina*. The stationary fluorescence emission and excitation spectra of PSI trimers and monomers at 295 K give direct evidence that at least part of the longwave Chls exist at physiological conditions (a Chl absorbing around 735 nm). It is proposed that the longwave Chl form in *Spirulina* PSI trimers, emitting at 760 nm at low temperatures, originates from the interaction of Chls located on different monomeric subunits forming the PSI trimer. Deconvolution of the 77 K absorption spectra of both PSI complexes allowed us to resolve the longwave Chls and to estimate their relative amounts. The fluorescence decays of the complexes at 287 K show differences in the energy migration pathways for PSI trimers and monomers, but no effect of the redox state of P700 on decay kinetics was found at this temperature.

MATERIALS AND METHODS

Preparation of PSI Complexes. PSI trimeric and monomeric complexes were isolated from membranes of the cyanobacterium *Spirulina platensis* with Triton X-100 (detergent:Chl ∼ 30) using DEAE-Toyopearl column chromatography as described earlier (Shubin et al., 1992). Complexes in 0.05 M Tris-HCl buffer (pH = 7.8) containing 0.07% Triton X-100 have been stored at −42 °C without any additions. The amount of P700 was determined by measuring of light-induced (Shubin et al., 1991) or chemically induced absorption changes (Perkin-Elmer Hitachi 340 spectrophotometer). For reduction of P700, 10 mM dithionite was added to the dissolved sample in 0.2 M Tris-HCl buffer (pH 8.5); 0.5−1 mM ferricyanide was added to oxidize P700. The Chl/P700 ratio determined in light-induced measurements (Shubin et al., 1992) was 95 ± 10, in accordance with Rögner et al. (1990b).

Stationary Fluorescence and Absorption Spectra. Steady-state fluorescence spectra at 295 K, corrected for the wavelength sensitivity of the detection system, were measured in a 1 mm quartz cuvette on a spectrofluorometer (Spex Fluorolog with photomultiplier RCA C31034) in front face regime according to Roelofs et al. (1991); the Chl concentration was adjusted to 18 μg/mL to have a high signal/noise ratio in the region 720−780 nm. The emission spectra were registered for samples with P700 reduced (20 mM ascorbate or 10 mM dithionite) or oxidized (0.5−1 mM ferricyanide). Under these oxidizing conditions no changes in fluorescence kinetics due to possible Chl oxidation were observed in agreement with (Turconi et al., 1993). Fluorescence excitation spectra in the region 710−750 nm at 295 K were measured with higher Chl concentration (80 μg/mL). Corrected 77 K fluorescence spectra of samples in tubes (inner diameter 4 mm) were measured on the same instrument. The samples in 0.2 M Tris buffer (pH 10.6) were adjusted to have P700 and PSI acceptor side reduced (10 mM dithionite) or P700 oxidized (0.5−1 mM ferricyanide) and then were diluted by glycerol (60% v/v) and frozen under illumination; the final Chl concentration was 5 μg/mL.

Absorption spectra of PSI complexes at 295 and 77 K were measured on a fast-scanning Omega spectrophotometer (Bruins Instruments, München); an Oxford Instruments cryostat was employed for measurements at 77 K. Samples were frozen in a 1 cm plastic cuvette with 60% glycerol. The 77 K absorption spectra were deconvoluted into Chl components according to Trinkunas and Holzwarth (1994).

Time-Resolved Fluorescence Measurements. To measure the fluorescence kinetics at 287 K, the sample (10 mL) was

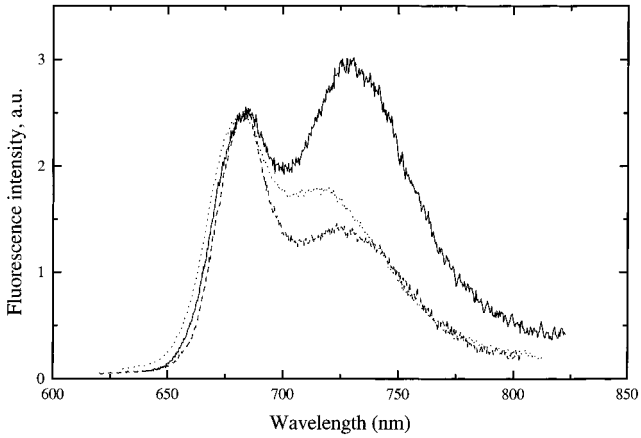

FIGURE 1: Corrected stationary fluorescence emission spectra of membranes (dashed line) and PSI trimeric (solid line) and monomeric (dotted line) complexes from *Spirulina* at 295 K. The spectra are normalized at their shortwave band. The sample was excited at 440 nm. The Chl concentration was 18 μg/mL.

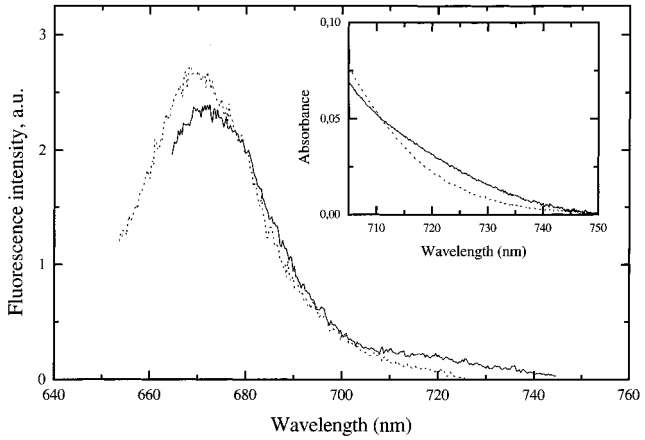

FIGURE 2: Corrected 295 K fluorescence excitation spectra of *Spirulina* PSI trimers (solid line), measured for the emission at 760 nm, and PSI monomers (dotted line), measured for the emission at 750 nm; the spectral curves are normalized at 680 nm. Inset: Absorption spectra of PSI trimers and monomers in the region 700−750 nm. Optical density of sample 0.45 at 678 nm (1 mm cuvette), Chl concentration 80 μg/mL. Here (and in Figure 4) the excitation spectra are distorted around the maximum near 680 nm because a high Chl concentration was chosen in order to obtain the longwave bands.

pumped through the measuring cuvette (1.5 × 1.5 mm cross-section) at a flow rate of 20 mL/min. To avoid photooxidative destruction of PSI complexes, oxygen was removed by adding 0.4 μM catalase and 2 μM glucose oxidase with glucose to the sample, which was bubbled with nitrogen during the measurement. To check for possible sample degradation during lifetime measurements, stationary fluorescence spectra were monitored *in situ* before and after the lifetime measurements. The spectra and maxima were unaffected, indicating the absence of any significant sample changes during experiments. Fluorescence decays were measured with both types of PSI complexes, in which P700 was prereduced (20 mM ascorbate + 20 μM PMS) or preoxidized (1 mM ferricyanide). Time-resolved fluorescence was measured by a single-photon-timing technique with time resolution of 5 ps (Turconi et al., 1996). The emission decay was recorded with excitation at 670 nm and detection between 680 and 770 nm in steps of 10 nm and was analyzed by single-decay and global analysis methods (Wendler & Holzwarth, 1987; Holzwarth, 1996).

RESULTS

Steady-State Spectra. Figure 1 shows the steady-state fluorescence emission spectra (excitation wavelength 440 nm) of the isolated membranes and PSI trimers and monomers from *Spirulina* at room temperature. Surprisingly, the membranes and both types of complexes, in contrast to those from *Synechocystis* sp. (Turconi et al., 1996), show very intense longwave emission bands: 725 nm (F725) in membranes, 730 nm (F730) in trimers, or 715 nm (F715) in monomers, in addition to the band at 680−685 nm. Excitation of the sample in the carotenoid region (470 nm) yields an emission band at 685 nm; in the case of direct Chl excitation (440 or 660 nm), this emission band is shifted to 680−682 nm. This slight blue shift of the fluorescence band in monomeric complexes is due to emission of some minor amount of uncoupled Chls formed during the isolation, which can be clearly identified in the time-resolved spectra (see below). For both PSI complexes the ratio of the emission bands at 715−730 nm to the bands at 683−685 nm was higher than that for the membranes (Figure 1). This is expected since emission at 685 nm in the membranes is partly due to fluorescence from PSII. Excitation of carotenoids also increases the ratio F715−725/F685 in PSI complexes in favor of the longwave bands (not shown), since carotenoids transfer their energy presumably only to coupled Chls (Shubin et al., 1993).

No dependence of the longwave emission bands on the redox conditions (P700 prereduced with 20 mM ascorbate + 10 μM PMS or preoxidized with 1 mM ferricyanide) was found at 295 K for PSI trimers and monomers from *Spirulina*, in accordance with data from other cyanobacterial complexes (Turconi et al., 1993; Sparrow et al., 1990). Only higher amounts of ferricyanide (3−5 mM) changed the spectra, presumably as a result of Chl oxidation and subsequent fluorescence quenching.

Fluorescence excitation spectra of the longwave bands were measured at 295 K to reveal the Chl forms responsible for the emission bands. A broad band in the region 700−740 nm was found in the excitation spectrum of trimers measured for the emission band at 760 nm, and a band in the region 700−720 nm was found in the spectrum of monomers measured for the band at 750 nm (Figure 2). These longwave bands are difficult to distinguish in the absorption spectra of both complexes, measured at room temperature. Since the bands of Chls responsible for the longwave fluorescence are difficult to determine at 295 K (Figure 2, inset), the fluorescence emission and excitation spectra of PSI monomers and trimers have been measured at 77 K.

The long-wavelength emission predominates in the fluorescence spectra of PSI trimers and monomers frozen with 10 mM dithionite under illumination, in accordance with data reported by Shubin et al. (1992). In the 77 K fluorescence spectrum of trimers the maximum is located at ∼760 nm. In the spectrum of monomers the long-wavelength emission is located at 725 nm (Figure 3B). When P700 in trimers is preoxidized by a small amount of ferricyanide (0.5−1.0 mM) or only by freezing the sample in the light, the maximum at 760 nm disappears and is seen as a shoulder to the main 725 nm emission. From these data we can estimate that the F760 intensity decreases about 15 times when P700 is

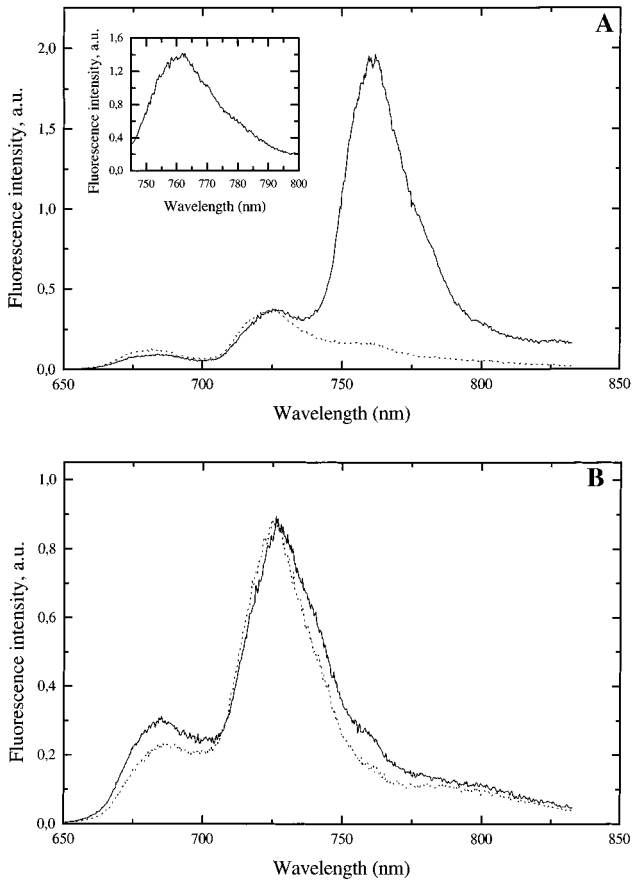

FIGURE 3: Corrected 77 K stationary fluorescence emission spectra of *Spirulina* PSI trimeric (A) and monomeric complexes (B) for P700 in the reduced (solid line) and oxidized (dotted line) state. The spectral curves are normalized at 725 nm. Inset: Emission spectrum (77 K) of trimers under excitation at 730 nm. Samples were frozen after addition of 10 mM dithionite under illumination or 0.5 mM ferricyanide. The excitation wavelength was 440 nm; Chl concentration was 5 μg/mL.

oxidized (Figure 3A). The intensity of F725 in PSI monomers was independent of the redox state of P700; only a 1−2 nm red shift was observed for that band in the presence of dithionite (Figure 3B), which could be caused by reduction of some quenchers formed during the isolation of momomers or produced by ferricyanide (Karapetyan et al., 1980). Note that in the case of carotenoid excitation the F685 band was even less pronounced than with excitation of Chls (not shown). The amount of uncoupled Chls in complexes is rather low, according to the ratio of the long-wavelength bands to the 685 nm band. Excitation at 730 nm leads to a single emission band of PSI trimers at 760 nm (Figure 3A, inset).

In the fluorescence excitation spectrum of *Spirulina* PSI trimers frozen with dithionite under illumination, bands at 680, 710, and 738 nm were observed for detection at 780 nm (Figure 4). In the 77 K excitation spectrum of PSI monomers detected at 770 nm, only bands at 680 and 710 nm were found (Figure 4). The 77 K absorption spectra of PSI complexes show longwave bands in the region 700−740 nm for monomers and 700−760 nm for trimers (Figure 5). The efficiency of the energy transfer from bulk antenna Chls to Chl735 estimated by comparison of the 77 K absorption and fluorescence excitation spectra of PSI trimers (Figures 4 and 5) was found to be about 0.3.

We performed deconvolution of the red part of 77 K absorption spectra (645−760 nm) of both PSI complexes.

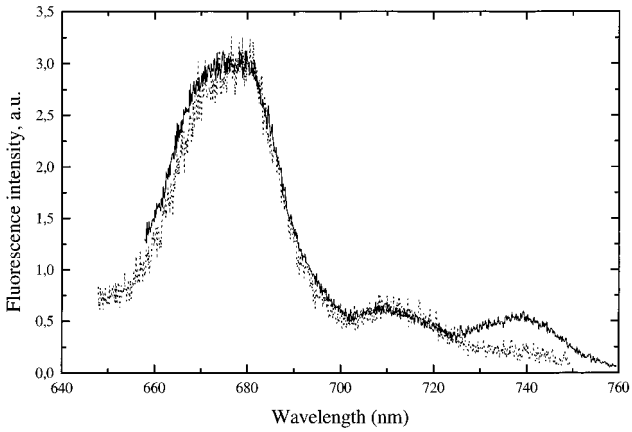

FIGURE 4: Fluorescence excitation spectra (77 K) of PSI trimeric (solid line) complexes, measured for the longwave emission at 780 nm, and PSI monomeric (dotted line) complexes, measured for emission at 770 nm; spectral curves are normalized at 725 nm. Samples were frozen with 10 mM dithionite under illumination; Chl concentration was 5 μg/mL.

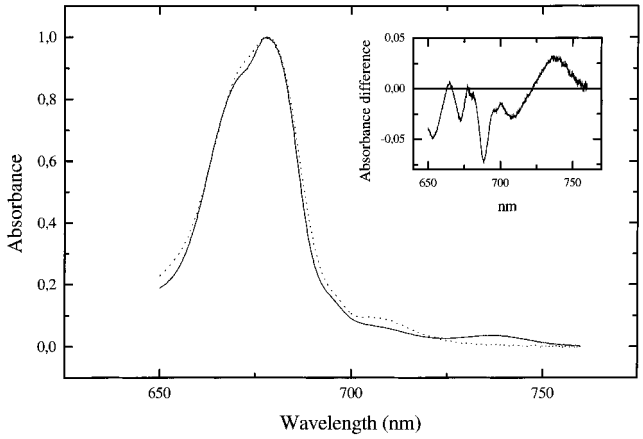

FIGURE 5: Absorption spectra (77 K) of *Spirulina* PSI trimeric (solid line) and monomeric (dotted line) complexes normalized at 677 nm, and difference between the spectral curves "trimers minus monomers" (inset). There is a—though small—subjective factor for the scaling and substraction of these curves and thus the positioning of the zero line in the difference spectrum. However, clearly the ~735 nm band will always remain positive and the ~680 and also the ~708 nm bands will remain negative, whatever the assumptions are within the possible limits.

In addition to bulk Chls absorbing at 650, 657, 664, 670, 677, 683, and 694 nm (Table 1), five longwave Chl forms were revealed absorbing at 705, 714, 726, 737, and 746 nm with an area ratio of 2.1:1.5:0.8:1.3:0.8 (per monomeric subunit) for trimers. Deconvolution of the absorption spectrum of monomers resolved three red Chl forms absorbing at 707, 715, and 725 nm with an area ratio of 2.9:1.7:0.6. The very low intensity component at 739 nm in PSI monomers (substantially less than 1 Chl equiv) is presumably due to some remaining trimers as a result of partial reassembly of isolated monomers. The total amount of red Chls (based on the area of the spectrum) was estimated to be ~6.4% of all antenna Chls in PSI trimers and ~5.2% in monomers.

The comparison of the 77 K fluorescence excitation and absorption spectra and data of Table 1 indicates that the most longwave Chls absorb at 737−746 nm in PSI trimers and at 715−725 nm in monomers. The 77 K difference absorption spectrum trimers-minus-monomers shows that the appearance of the long-wavelength band at 735 nm in PSI trimers is

Table 1: Spectral Characteristics of Chl Forms Resolved under the Deconvolution of 77 K Absorption Spectra of PSI Trimers and Monomers from *Spirulina* on the Chl Bands[a]

PSI trimers		PSI monomers	
position (nm)	rel area under curve (%)	position (nm)	rel area under curve (%)
650	2.9	651	3.8
657	3.6	657	4.7
664	11.3	664	11.2
670	19.4	670	19.5
677	24.9	677	22.3
683	26.5	683	22.8
		687	6.4
694	5.1	696	3.8
705	2.1	707	2.9
714	1.5	715	1.7
726	0.8	725	0.6
737	1.3	(739)	*(0.2)*[b]
746	0.8		

[a] The same full width at half maximum (200 cm^{-1}) has been used for all bands, according to Trinkunas and Holzwarth (1994). [b] We assign this small band to the absorption of trimer as a result of partial reassembly of monomers and it was not used for calculation of the area.

accompanied by a decrease of bands at 708, 698, and 688 nm which belong to monomers (Figure 5, inset). The broad positive band at 735 nm in the difference spectrum can be deconvoluted to 734 and 742 nm components, in agreement with the decomposition of the trimer spectrum**.** The short-wave negative bands (< 680 nm) are presumably due to some uncoupled Chls in the monomers (Figure 5).

According to Table 1, PSI trimers, in addition to the longwave Chl forms at 737 and 746 nm, contain also the same set of Chl forms as monomers. We suggest that the most longwave Chl forms originate from the assembly of monomeric subunits into trimers, which makes possible the interaction of Chls bound at the surface of different monomeric subunits. To check this suggestion, the effect of salt treatment of intact cyanobacterial membranes (Kruip et al., 1994) was studied, using the fluorescence band at 760 nm as an internal marker of PSI trimers in *Spirulina* membranes. The membranes were incubated with 5 mM (low salt) or 150 mM $MgSO_4$ (high salt) for 10−12 s at 15 °C, and then 10 mM dithionite was added and samples were frozen in the presence of 60% glycerol. Figure 6 shows the decrease of F760 intensity in the high salt incubated membranes. This finding is interpreted as a deaggregation of PSI trimers into monomers. Double washing of high salt treated membranes with 0.2 M Tris buffer + 5 mM $MgSO_4$, followed by centrifugation, restored up to 50% of the initial intensity of F760, indicating the re-formation of PSI trimers in the membranes at low salt conditions. Thus, the most longwave Chl band in absorption and emission seems to arise indeed from the Chl−Chl interaction of pigments, bound to different monomeric subunits of the PSI trimer. We cannot exclude, however, the possibility that this longwave band may arise also from a conformational change upon trimerization, although we consider this to be less likely for reasons discussed below.

The experiments described above also support the idea that the ratio between PSI trimeric and monomeric complexes in membranes can be regulated by salt conditions, as proposed by Kruip et al. (1994), although our high salt conditions use nonphysiological salt concentrations. The

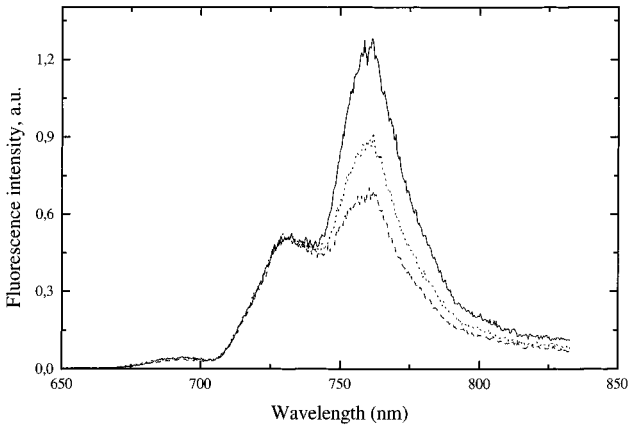

FIGURE 6: Corrected 77 K fluorescence emission spectra of *Spirulina* membranes incubated with 5 mM $MgSO_4$ (low salt, solid line) or 150 mM $MgSO_4$ (low salt → high salt, dashed line) or washed from salts (high salt → low salt, dotted line). The spectral curves are normalized at 730 nm; excitation wavelength was 440 nm; Chl concentration was 5 μg/mL.

effect of electrostatic interactions on cyanobacterial PSI trimeric complexes in the membranes are in agreement with the fact that PSI trimers can be isolated from the membranes using nonionic detergents, e.g., Triton X-100 or dodecyl β-D-maltoside.

Time-Resolved Spectra. Picosecond time-resolved fluorescence measurements have been performed using 670 nm excitation on both PSI trimeric and monomeric complexes from *Spirulina* with P700 prereduced (20 mM ascorbate + 20 μm PMS) or preoxidized (1 mM ferricyanide). However, no effect of the P700 redox state was observed on the fluorescence decays at room temperature, in agreement with the steady-state spectra. Figure 7 thus only shows the decay-associated fluorescence spectra (DAS) of PSI complexes measured in the presence of ascorbate + PMS. Four lifetimes (9, ∼30, ∼65, and a small amplitude of 1400−2200 ps) were necessary for a good fit of the decays. While the lifetimes are nearly the same in both complexes, however, the time-resolved spectra differ significantly, reflecting additional contributions of the extreme longwave Chls in PSI trimers. The spectrum of the fastest component shows positive and negative amplitudes with a zero-crossing point at ∼710 nm. The 30 ps component and the 65 ps component are all-positive. In the case of monomers, their spectra are less different and display peaks at ∼720 and ∼730 nm (Figure 7B), respectively, and the faster component dominates the slower one in amplitude. For trimers (Figure 7A) the spectrum of the 30 ps component shows a peak at ∼720 nm and the peak of the 65 ps spectrum is shifted to ∼740 nm. The long-lived component has a quite small amplitude and no pronounced spectral features. Only in the monomer decay does it show a pronounced amplitude at ∼680 nm, which is due to an emission of some energetically uncoupled Chls.

DISCUSSION

Evidence for the Red Pigments in Cyanobacterial PSI Antenna at Room Temperature. The data presented here confirm the existence of at least some of the longwave Chls in PSI complexes of cyanobacterial membranes at physiological temperatures (Figure 1). The very broad band in the fluorescence excitation spectra of PSI complexes at 295

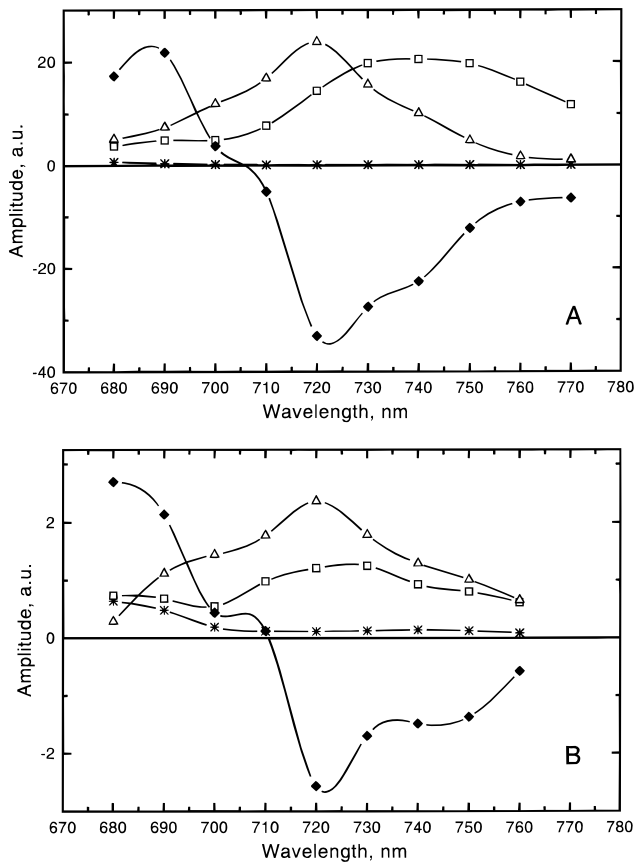

FIGURE 7: Decay-associated fluorescence spectra (287 K) of *Spirulina* PSI trimeric (A) and monomeric complexes (B) measured in the presence of ascorbate + PMS and oxygen consumption conditions (see Materials and Methods); excitation wavelength 670 nm. The fitted lifetimes are 9 ps (◆), 28 ps (△), 69 ps (□), and 1.4 ns (*) for trimers and 9 ps (◆), 31 ps (△), 65 ps (□), and 2.2 ns (*) for monomers. The optical density at 678 nm was 0.69 for PSI trimers and 0.72 for monomers.

K (Figure 2) did not allow us to determine exactly the absorption band position of Chls responsible for the broad emission bands at >730 nm in trimers and ~715 nm in monomers at 295 K. If the Chls (or states) responsible for F760 were present already at room temperature, the longwave band should be much stronger in emission of trimers and most of the emission should result from that state. This is not the case and we thus conclude that the pigments (states) responsible for F760 are only formed at low temperature. Since F760 is detected at low temperatures only, no evidence is available for the existence at room temperature of the Chl (presumably Chl 746) responsible for F760. We believe that the DAS (Figure 7A) of the 69 ps component in trimers supports the presence of Chl737 at room temperature. Its emission becomes much stronger at temperatures 233 K or lower (not shown). F760 is an internal marker of PSI trimers in *Spirulina*. Though PSI trimers exist in other cyanobacteria too, they do not show such an extreme longwave Chl form.

Energy Transfer in Antenna of PSI Trimers and Monomers Containing Red Pigments. Significant differences are present in the DAS of PSI trimers and monomers from *Spirulina,* in contrast to analogous PSI complexes from unicellular cyanobacteria such as *Synechocystis* sp. (Turconi et al., 1996), which hardly showed any differences between trimers and monomers. We will limit our discussion to data obtained with cyanobacterial PSI core complexes in order to avoid the complexities of energy exchange and coupling between the core and light-harvesting complex I, which is present in addition in PSI complexes of green plants (Palsson et al., 1995).

The spectra of the fastest (9 ps) component, which is ascribed to the slowest component of energy transfer from the bulk antenna to the red pigments[2] (Holzwarth et al., 1990, 1993; Turconi et al., 1993, 1994; Hastings et al., 1994, 1995; Trinkunas & Holzwarth, 1994, 1996), are quite comparable for both types of PSI complexes from *Synechocystis* sp. (Turconi et al., 1996), suggesting similar pathways of energy transfer from Chls emitting at ~690 nm to longwave Chls in both complexes.

The spectra of the ~30 ps component, which we attribute mostly to overall charge separation (Holzwarth et al., 1993; Turconi et al., 1993, 1996), are quite comparable for both *Spirulina* PSI complexes. This component shows only one maximum at about 720 nm in the spectra of trimers and monomers. The low level of F690 vs F725 (Figure 7B) and the absence of significant long-lived components indicate a rather small amount of disconnected Chls in PSI monomers from *Spirulina*. A 25 ps component was found for PSI from *Synechocystis* sp. mutants deficient in PSII (Hecks et al., 1994; Dimagno et al., 1995) and a 31 ps component reflecting energy trapping by P700 was observed for the spinach PSI core complex at 298 K (Pålsson et al., 1995). The lack of the fastest equilibration component in these data may be due to insufficient time resolution.

The main difference between the DAS of *Spirulina* PSI monomers and trimers is manifested in the spectral shape of the additional all-positive ~66 ps component. For trimers this band extends much more to the red tail of the spectrum than does the corresponding component of monomers. Also there is a >10 nm difference in the maximum position. The spectra of the additional ~66 ps component have also been observed in *Synechocystis* sp. PSI complexes but are characterized by a quite low amplitude, as in the spectrum of PSI monomers from *Spirulina*.

The origin of the ~66 ps component is not yet clear. In a simple homogeneous system that can be described by a set of first-order differential equations, only one all-positive component (corresponding to an all-positive eigenvector) is possible [see, e.g., Turconi et al. (1994)]. This consideration is, however, idealized since small negative amplitudes may either remain unresolved in the analysis or lie outside the detected wavelength range. In that previous work we tried to explain the presence of several all-positive components in the DAS by an antenna heterogeneity, i.e., the partial lack of the PSI light-harvesting complex antenna. While this explanation may still hold—at least in part—for PSI from higher plants, it is quite unlikely for cyanobacterial PSI cores, lacking any light-harvesting complex. A new explanation based on protein dynamics has been proposed recently (Trinkunas & Holzwarth, 1996) but has not been fully explored experimentally so far. For the time being we consider the occurrence of two all-positive DAS components in the time-resolved spectra of *Spirulina* as an open question. A detailed modeling study of the measured fluorescence

[2] Much faster additional energy transfer components occur in the antenna but are not resolved in our measurements [see, e.g., Hastings et al. (1995) and Trinkunas and Holzwarth (1994, 1996)]. We resolve here only the slowest energy transfer component that can be unequivocally, by positive/negative amplitudes, assigned to such a process.

kinetics considering the various possibilities will be the subject of a forthcoming publication.

Origin of the Most Red-Shifted Pigment(s) and Their Location in the Antenna of Cyanobacterial PSI Trimers. The spectral investigation of PSI trimers and monomers from *Spirulina* clearly indicates the appearance of extreme long-wave Chls in trimers. These seem to be the most red-shifted Chls observed in any PSI complex or any other Chl *a*-containing antenna, both at room temperature and even more so at low temperature. We suggest that cyanobacterial PSI trimers, in addition to the longwave Chls, contain the whole set of Chl forms as monomers (Table 1) since no Chl seems to be lost or set free when dissociating into monomers, and the deconvolution gave a very similar set within the error limits. Most likely the close interaction of two or more Chls, bound in different monomeric subunits, causes the appearance of a new additional longwave Chl form Chl737 in trimers at room temperature and an even longer-wave Chl (Chl 746) at low temperature (Figure 5, inset). The red shift of interacting Chls in aggregates is well-known (Katz et al., 1991). Recent structural data (Krauss et al., 1996) show that Chls are present at the surface of PSI at a closest distance to P700 of about 33−35 Å that would seem to be suitable for such an interaction. Also, our data on reversible changes of the F760 intensity of *Spirulina* membranes at different salt conditions support the idea that the most longwave Chls of PSI trimers (F760) originate from the interaction of Chls bound in different monomeric subunits. Alternatively, the interaction between monomeric subunits may also modify the protein conformation as seen, e.g., by a change of the circular dichroism spectrum in the carotenoid region (512 nm): the rotational strength of carotenoids in trimers (and in membranes) is higher than that in monomers (Shubin et al., 1993). We consider the latter possibility as unlikely, however, since such a strong shift would probably only be caused by a nearby charge interaction with a Chl (Gudowska-Nowak et al., 1991). Given the fact that F760 appears only at low temperatures speaks more in favor of a small distance change and thus increased excitonic couplings at low temperatures rather than a gross conformational change that would probably be required for drastically increasing a charge interaction.

Since Chl with emission at 760 nm senses in some way the redox state of P700, it has to be proposed to be located in the vicinity of P700. However P700 is located in the center of the 82−83 kDa apoprotein (Krauss et al., 1993, 1996; Fromme et al., 1996), and thus any changes of interaction of pigments that surround the reaction center in the trimer vs monomer would seem to be difficult. However, we note that a Chl at the surface of a monomer has a closest distance of 33−35 Å to P700. Thus a Förster radius of about 50 Å for the respective donor/acceptor pair would be sufficient for the observed quenching. The required Förster overlap of F760 with P700 would be negligible for P700 but might be sufficient for $P700^+$, due to overlap with the longwave absorption band of the Chl^+. It is difficult to get any reliable quantitative measures on that overlap. Thus this question cannot be finally answered. As an alternative explanation for the observed quenching of F760 we also consider the possibility that an antenna Chl closer to F760 might be oxidized by $P700^+$ at low temperature, which would then act as a quencher, rather than P700 itself. Kinetic modeling in an irregular two- and three-dimensional model lattice has resulted in a placement of red Chls somewhere in the vicinity of, but not in immediate contact with, P700 (Trinkunas & Holzwarth, 1994, 1996). Such arrangement also seems possible for the *Spirulina* monomers based on the time-resolved data (Figure 7B). It remains to be seen whether incorporation of the more detailed recent structural data into the modeling of the kinetics influences these conclusions. Neither the data (Figure 7A) on the trimers nor the previous results from simulations (Trinkunas & Holzwarth, 1994, 1996) gives a clear answer at present on the origin and location of the extreme F760 pigments, however.

Typical Stokes shifts for monomeric Chls do not exceed 5 nm and are typically less (Croce et al., 1996). Estimation of the Stokes shift for Chl735 (Shubin et al., 1991, 1995) as well as recent site-selection work (Pålsson et al., 1996) have shown that some of the red Chls in PSI have large Stokes shifts, which may point to an excitonic origin, thus supporting the idea of Chl−Chl interaction as a cause for the longwave emission. Such a large Stokes shift also seems to characterize in particular the F760-emitting pigment(s) in trimers of *Spirulina* (Figures 3A, 4, and 5 and Table 1), which again would support the interaction hypothesis. We presently believe that modeling of the fluorescence kinetics of trimers taking into account the specific spectral properties (Trinkunas & Holzwarth, 1994, 1996) and the structural details (Krauss et al., 1996) may be a suitable way to clarify the location and origin of these extreme red pigments.

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