

Energy Transfer and Charge Separation in Photosystem I: P700 Oxidation Upon Selective Excitation of the Long-Wavelength Antenna Chlorophylls of *Synechococcus elongatus*

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ABSTRACT Photosystem I of the cyanobacterium *Synechococcus elongatus* contains two spectral pools of chlorophylls called C-708 and C-719 that absorb at longer wavelengths than the primary electron donor P700. We investigated the relative quantum yields of photochemical charge separation and fluorescence as a function of excitation wavelength and temperature in trimeric and monomeric photosystem I complexes of this cyanobacterium. The monomeric complexes are characterized by a reduced content of the C-719 spectral form. At room temperature, an analysis of the wavelength dependence of P700 oxidation indicated that all absorbed light, even of wavelengths of up to 750 nm, has the same probability of resulting in a stable P700 photooxidation. Upon cooling from 295 K to 5 K, the nonselectively excited steady-state emission increased by 11- and 16-fold in the trimeric and monomeric complexes, respectively, whereas the quantum yield of P700 oxidation decreased 2.2- and 1.7-fold. Fluorescence excitation spectra at 5 K indicate that the fluorescence quantum yield further increases upon scanning of the excitation wavelength from 690 nm to 710 nm, whereas the quantum yield of P700 oxidation decreases significantly upon excitation at wavelengths longer than 700 nm. Based on these findings, we conclude that at 5 K the excited state is not equilibrated over the antenna before charge separation occurs, and that ~50% of the excitations reach P700 before they become irreversibly trapped on one of the long-wavelength antenna pigments. Possible spatial organizations of the long-wavelength antenna pigments in the three-dimensional structure of photosystem I are discussed.

INTRODUCTION

Photosystem I (PS I) of green plants, algae, and cyanobacteria is a membrane-bound pigment-protein complex that uses light energy to transport electrons from reduced plastocyanin or cytochrome c_6 to soluble ferredoxin (Brettel, 1997). After absorption of light by an antenna pigment, the excitation energy is efficiently transferred to the primary electron donor, a chlorophyll a (Chl a) dimer called P700. P700 in the lowest electronically excited singlet state donates an electron to the primary electron acceptor, a Chl a monomer referred to as A_0 . The charge separation is stabilized by subsequent electron transfer to secondary acceptors, i.e., the phylloquinone A_1 and the three [4Fe-4S] clusters F_X , F_A , and F_B .

In green plants and algae, the PS I complex can be divided into two parts: the core complex and the light-harvesting complex I (LHC-I). The core complex contains

all redox cofactors and an antenna system of ~100 chlorophyll a and 20 β -carotene molecules. The core proteins are surrounded by a number of LHC-I antenna proteins (Boekema et al., 1990), which together bind roughly another 100 chlorophyll molecules (Chl a and Chl b at a ratio of ~3.5:1), as well as ~20 xanthophyll molecules. Cyanobacteria lack the peripheral LHC-I antenna system. In these organisms, however, the PS I core complex can be isolated in a monomeric and a trimeric form (Boekema et al., 1987; Rögner et al., 1990). The trimer is proposed to be the native form, at least under certain physiological conditions (Kruip et al., 1994).

The structure of the trimeric core complex of the cyanobacterium *Synechococcus elongatus* has recently been determined to a resolution of 4 Å (Krauss et al., 1996; Schubert et al., 1997). The structure reveals a central part in which six chlorophyll molecules and three iron-sulfur clusters have been observed. Two branches can be recognized, which are approximately related by a twofold symmetry axis extending from F_X near the stromal side to a pair of chlorophylls near the luminal side that presumably constitute P700. Two other chlorophylls have been observed at positions similar to those of the monomeric bacteriochlorophylls in purple bacterial reaction centers, whereas one of the remaining chlorophylls (denoted eC3 and eC3') at positions similar to those of the bacteriopheophytins in bacterial reaction centers was assumed to be A_0 (Schubert et al., 1997). The position of the phylloquinone could not positively be identified in the electron density.

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The central part is surrounded by the core antenna system in which 83 of the ~ 90 Chl *a* molecules have been recognized (Schubert et al., 1997). The nearest center-to-center distances between these chlorophylls range between 7 and 16 Å. The distances to the central chlorophylls are larger than 19 Å for all chlorophylls, except for two symmetrically located chlorophylls designated cC and cC', which each have center-to-center distances to the central chlorophylls eC3 and eC3' of ~ 14 Å. It was suggested that these Chl molecules play an important role in the energy transfer from the antenna system to the reaction center (Krauss et al., 1996).

The short center-to-center distances between the chlorophylls suggest very fast energy transfer and charge separation kinetics. Indeed, the average rate constant of energy transfer from chlorophyll to chlorophyll has been estimated to be about $(200 \text{ fs})^{-1}$ (Owens et al., 1987; Du et al., 1993). In isolated PS I particles from *Synechococcus elongatus*, the trapping of excitation energy via charge separation has been estimated to occur in ~ 35 ps (Holzwarth et al., 1993). In PS I of the cyanobacterium *Synechocystis* PCC 6803 and in the PS I core complex of green plants and algae, the trapping time is probably somewhat shorter (~ 20 – 30 ps) (Hecks et al., 1994; Hastings et al., 1994, 1995a), whereas in native plant complexes (PS I-200 preparations), longer trapping times were observed (see for a review, e.g., van Grondelle et al., 1994). A number of groups have suggested that the charge separation kinetics are basically "trap-limited," which means that the equilibration of the excitation energy over the complete antenna proceeds faster than charge separation and thus that the intrinsic rate of charge separation determines the kinetics (Holzwarth et al., 1993; Hastings et al., 1994). An alternative view has recently been proposed by a number of authors (van Grondelle et al., 1994; Valkunas et al., 1995; Trissl, 1997), who suggested that not the intrinsic rate of charge separation, but the rate at which the "equilibrated" excitation energy migrates to P700 determines the overall kinetics, and that the kinetics can best be described as "transfer-to-the-trap-limited." This view was based on a putative larger distance scaling parameter between the bulk antenna chlorophylls and P700 rather than among the bulk antenna chlorophylls, and seems justified at least in part by the most recent three-dimensional structural model (Krauss et al., 1996; Schubert et al., 1997). Within the context of this description, the excitation energy will be spectrally equilibrated among all bulk antenna chlorophylls before transfer to the central reaction center chlorophylls occurs.

An intriguing aspect of the antenna system of photosystem I (and of the antenna systems of many other photosystems) is the pronounced absorption at wavelengths longer than those of the primary electron donor. In PS I, some of the different spectral forms are observed at wavelengths as long as 720–735 nm. At room temperature, the thermal energy will in most cases be sufficient to allow uphill energy transfer from these long-wavelength antenna pigments to P700, but the overall trapping kinetics will prob-

ably be slower. Trissl (1993) argued that a moderate decrease in the trapping time does not significantly affect the efficiency of PS I, and that, on the other hand, the larger absorption cross section will be physiologically quite advantageous. The numbers and energies of long-wavelength chlorophylls vary considerably in the PS I of different organisms. A relatively simple arrangement was found in the core antenna of *Synechocystis* PCC 6803 (Gobets et al., 1994), in which two chlorophylls were found to absorb maximally at 708 nm, with their Q_y transitions almost in the plane of the membrane (van der Lee et al., 1993). It was suggested that these C-708 chlorophylls are excitonically coupled and carry a much broader phonon sideband than other antenna chlorophylls. This suggestion implies that pigment-pigment interactions largely determine the red shift, although specific pigment-protein interactions may contribute as well. The homogeneous and inhomogeneous linewidths at 4 K were shown to be ~ 170 and 215 cm^{-1} , respectively, and the Stokes' shift is ~ 10 nm (Gobets et al., 1994). The peripheral LHC-I antenna of green plants shows even broader absorption bands peaking around 716 nm (C-716). Trimeric PS I complexes of *Synechococcus elongatus* bind more C-708 pigments per complex (about four to six) and, in addition, bind four to six C-719 chlorophylls (Pålsson et al., 1996), which suggests that *Synechococcus elongatus* contains substantially more long-wavelength antenna chlorophylls than *Synechocystis* PCC 6803. The most red-shifted chlorophyll has been found in PS I of *Spirulina platensis*, in which chlorophylls with an absorption maximum near 735 nm give rise to fluorescence peaking at 760 nm at 77 K (Shubin et al., 1995).

At room temperature, the energy transfer from the long-wavelength antenna pigments to P700 is probably very efficient (see, e.g., Trissl et al., 1993; Shubin et al., 1995; Hastings et al., 1995b; Croce et al., 1996), in line with the low fluorescence yield, which is largely determined by the fast trapping of excitation energy in ~ 30 ps. At cryogenic temperatures, however, the yield of the emission from the long-wavelength pigments increases dramatically, suggesting that a significant part of the excitations no longer reach P700.

In this report, we analyze the energy transfer to and from the long-wavelength antenna pigments and the charge separation efficiency in PS I complexes of *Synechococcus elongatus* in much more detail. In particular, we report relative yields of P700 oxidation as a function of excitation wavelength and as a function of temperature. The results suggest that at room temperature, the probability that an absorbed photon of a certain wavelength results in the oxidation of P700 is the same for every wavelength, even for excitation wavelengths as long as 750 nm. As the temperature is lowered to 4 K, the yield of P700 photooxidation decreases to $\sim 50\%$ with nonselective excitation. At 4 K the yield further decreases as the excitation wavelength is shifted from ~ 700 nm to ~ 720 nm. The results are discussed in the context of the recently determined three-dimensional structure of photosystem I.

MATERIALS AND METHODS

Trimeric PS I complexes containing ~ 100 Chl/P700 were prepared from the thermophilic cyanobacterium *Synechococcus elongatus* as described by Witt et al. (1987), except that PS I was extracted with 0.6% (w/w) *n*-dodecyl- β ,*D*-maltoside (β -DM) and purified by anion exchange chromatography on a Q-Sepharose High-Performance column (Pharmacia), as described by Jekow et al. (1995). Monomeric PS I complexes containing ~ 95 Chl/P700 were obtained by applying an additional osmotic shock before the purification (Jekow et al., 1995). The purified PS I complexes were suspended in a buffer containing 20 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) (pH 6.4), 0.02% (w/w) β -DM, and ~ 40 mM MgSO_4 (in the case of PS I monomers) or 100 mM MgSO_4 (in the case of PS I trimers) and stored at -30°C . For the measurements, the PS I complexes were diluted with buffer (either buffer A, containing 20 mM tricine, pH 7.5, 25 mM MgCl_2 , and 0.02% (w/w) β -DM, or buffer B, containing 20 mM MES, pH 6.5, 20 mM CaCl_2 , 10 mM MgCl_2 , and 0.02% (w/w) β -DM) and glycerol to reach a final concentration of 65% (v/v) glycerol, to obtain a transparent glass at low temperatures. Na-ascorbate was added to a final concentration of 5 mM. Where indicated, phenazine methosulfate (PMS) was added to a final concentration given in the figure legends. The low-temperature measurements were performed in an Oxford liquid helium flow cryostat (CF1204) or in a Utreks liquid helium flow cryostat.

Absorption spectra and light-minus-dark absorbance difference spectra at low temperature were measured with a spectral resolution of 0.5 or 1 nm with different spectrophotometers (Cary 219, Cary 1E, or Cary 5 from Varian). Flash-induced absorption changes were measured as described before by Lüneberg et al. (1994). The sample was excited with a xenon flashlamp (pulse duration 15 μs) whose emission was filtered by a colored glass (CS96-4 from Corning). The amplitude of the flash-induced absorption change at 826 nm was measured as a function of the excitation flash energy at various temperatures between 5 K and 295 K. The measured saturation curves could be fitted satisfactorily with the function

$$\Delta A = \Delta A_{\text{max}} \left\{ 1 - \exp\left(-\frac{\ln 2 \cdot E}{E_{1/2}}\right) \right\} \quad (1)$$

which yielded the half-saturating flash energy $E_{1/2}$ as a function of temperature. The excitation energy was varied with neutral density filters.

Selectively excited absorbance difference spectra were recorded with the double modulation set-up described before by Kwa et al. (1994). A cw Ti-sapphire laser (Coherent 890) pumped by a cw Ar ion laser (Coherent Innova 310) was used as the excitation source. The spectral bandwidth of the laser excitation was ~ 1 cm^{-1} (FWHM). Probe light was provided by a 150-W halogen lamp in combination with a 1/4 m monochromator (Oriel 77200) with a spectral resolution of 1 nm (2 nm in the measurements at 5 K). The wavelength was scanned in steps of 1 nm, stepping once every 2 s. The intensity of the probe beam, modulated at 100 kHz with a photoelastic modulator (Hinds PEM 80), was measured with a photodiode (Centronics OSD 5-3T). The signal from the photodiode was fed into a first lock-in amplifier locked at 100 kHz, yielding the probe light intensity I . The laser excitation was chopped at 20 Hz, which modulates the absorbance of the sample and thus the intensity I . To obtain the difference in I with the laser excitation on and off ($= \Delta I$), the output of the first lock-in was fed into a second lock-in amplifier locked at 20 Hz.

This modulation frequency was selected by taking into consideration the lifetime of the charge separated state. At room temperature, the decay kinetics of $\text{P700}^+\text{F}_{\text{A/B}}^-$ depend on the concentrations of the added electron acceptors and donors. In the reaction medium containing 5 mM Na-ascorbate and PMS, both the fully oxidized and reduced forms of PMS are present. The reduced terminal iron-sulfur cluster is oxidized in less than 1 ms. Therefore, the rate for the recovery of the ground state $\text{P700F}_{\text{A/B}}^- (= k_d)$ is determined by the reduction of P700^+ by reduced PMS ($t_{1/2} \approx 3$ ms in the presence of 60 μM PMS at pH 6.5). The rate for the formation of $\text{P700}^+\text{F}_{\text{A/B}}^-$ is given by $k_{\text{cs}} = \sigma I \Phi$, where $\sigma(\lambda)$ is the absorption cross section of the PS I complexes, I is the photon flux per cm^2 , and Φ is the quantum yield of charge separation. For the absorbance difference as a

function of I we obtain

$$\Delta A = \Delta A_{\text{max}} \left(\frac{\sigma I \Phi}{k_d + \sigma I \Phi} \right) \quad (2)$$

This equation describes rather well the measured dependence of ΔA at 703 nm on the intensity of the excitation light (not shown). The half-maximum absorbance difference was obtained at ~ 10 mW/cm^2 upon excitation at 700 nm. The dependence of the absorbance difference on the excitation wavelength was measured under conditions in which ΔA is, to a good approximation, proportional to I , which means that $k_{\text{cs}} \ll k_d$. Because of the particular settings of the modulation frequency and the intensity of the excitation light, the experimental set-up selects for processes decaying in the time range of ~ 1 –10 ms. Shorter lived species will not be photoaccumulated, whereas longer lived processes will not decay and therefore will not be recorded.

At low temperature ($T < 100$ K), reversible absorbance changes decay mainly with a half-life of ~ 170 μs , because of the charge recombination of the radical pair $\text{P700}^+\text{A}_1^-$. In this case, higher intensities of the excitation light are required to photoaccumulate the shorter-lived secondary radical pair.

Fluorescence spectra at 295 K were recorded in an Aminco SLM8000C photon counting spectrometer. The temperature dependence of the fluorescence spectra between room temperature and 4 K was recorded in a home-built set-up with a CCD camera, as described before by Pålsson et al. (1996). Fluorescence excitation spectra at 5 K were recorded on a home-built fluorimeter with excitation and emission beams at right angles, using trimeric PS I preparations at an optical density of 0.32 at 680 nm at 5 K, which corresponds to an optical density of 0.25 at 680 nm at room temperature. The excitation source was a 150-W tungsten halogen lamp, equipped with a 1/2-m Chromex 500 SM monochromator with a bandwidth of 1.6 nm, a sheet polarizer, and a 400-nm high-pass filter. The emission light was passed through a second sheet polarizer and a 1/8-m Oriel 77250 monochromator, fixed at 750 nm with 18-nm bandwidth, and detected with a S-20 photomultiplier. The influence of background light was minimized by using chopped excitation light and lock-in detection. The measurements were performed with vertically polarized excitation light and with both horizontally and vertically polarized emission. The measurements were corrected for the difference in sensitivity for horizontally and vertically polarized light, and for the spectral sensitivity of the set-up. The presented spectra are the constructed isotropic spectra, i.e., $I_{\text{vertical}} + 2I_{\text{horizontal}}$. Transmission was measured on the same set-up using a photodiode placed behind the sample. A baseline was recorded with the sample lifted up in the cryostat and subtracted.

RESULTS AND DISCUSSION

Absorption spectra at 5 K

Fig. 1 shows the 5 K absorption spectra of monomeric (*solid line*) and trimeric (*dotted line*) PS I complexes from *Synechococcus elongatus* between 650 nm and 750 nm. The spectra clearly exhibit various bands in the Q_y absorption region of the chlorophylls, which can be assigned to specific pools of pigments. The spectrum of the trimeric complexes is slightly better resolved, which could indicate a more ordered structure. Nevertheless, the second derivatives of the spectra of both types of PS I complexes indicate completely corresponding absorption bands peaking at about 665, 672, 679, 685, 692, 698, 708, and 719 nm (not shown). The most remarkable difference is observed around 720 nm, where the monomeric complexes show less absorption.

We deconvoluted the 5 K absorption spectrum of the trimeric complexes into Gaussian bands and found that

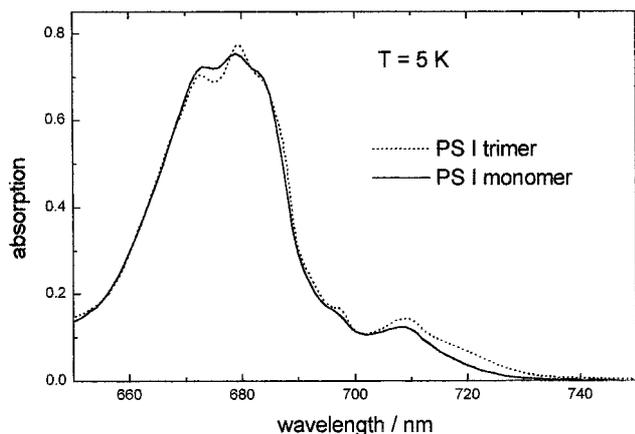


FIGURE 1 Absorption spectra at 5 K of monomeric (—) and trimeric (.....) PS I complexes of *Synechococcus elongatus*. The spectra were normalized at 700 nm.

these complexes most likely contain five or six C-708 pigments and four or five C-719 pigments per P700, in accordance with our previous estimate (Pålsson et al., 1996). The deconvolution of the 5 K absorption spectrum of the monomeric complexes into Gaussian bands (not shown) yielded five or six C-708 pigments and about two C-719 pigments per P700. Thus the main difference in the contents of long-wavelength chlorophylls in trimeric and monomeric PS I complexes from *Synechococcus elongatus* is the lower number of C-719 pigments in the monomers.

It should be pointed out that monomeric PS I complexes prepared from a *psaL*⁻ strain of *Synechococcus elongatus* have virtually the same content of long-wavelength pigments as the monomers obtained by trimer dissociation (U. Mühlenhoff, C. Flemming, and E. Schlodder, unpublished results). From this we conclude that the lower content of C-719 pigments in the monomers is not an artefact of the procedure for trimer dissociation. Deletion mutant studies indicate that the *psaL* subunit, which is assumed to be part of the connecting domain, is required for trimerization (Chitnis and Chitnis, 1993; Mühlenhoff and Chauvat, 1996). Therefore, we consider it likely that the binding of the C-719 pigments is associated in part with the connection domain of the monomeric PS I complexes within the trimer.

Excitation equilibration at room temperature

Fig. 2 shows the absorption and nonselectively excited emission spectra of trimeric PS I complexes from *Synechococcus elongatus* at 295 K. The absorption spectrum (dashed line) peaks at 680 nm and shows much intensity at wavelengths longer than 700 nm, which is primarily caused by P700 and the pools of the long-wavelength antenna pigments. The absorption extends to ~760 nm. The fluorescence spectrum (solid line) peaks at 721 nm and shows a shoulder at ~691 nm.

We noted that the particular shape of the emission spectrum at room temperature depends heavily on the concen-

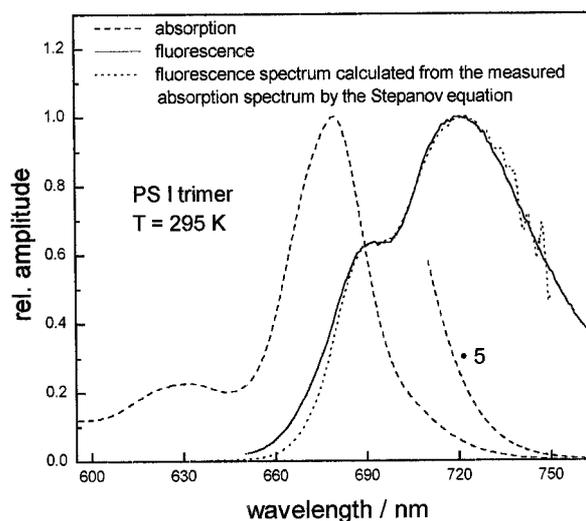


FIGURE 2 ---, Absorption spectrum of trimeric PS I complexes at 295 K, normalized to 1 at 680 nm. —, Emission spectrum excited at 430 nm of trimeric PS I complexes at 295 K in a glycerol/buffer (pH 6.5) mixture (65:35, v/v) with 5 μ M Chl, 5 mM ascorbate, and 0.007% (w/v) β -DM. The spectrum was normalized to 1 at 721 nm., Emission spectrum calculated from the absorption spectrum (---) by the Stepanov equation (see text for details).

tration of the detergent (β -DM). When the concentration of this detergent was raised to the critical micelle concentration (CMC) (~0.009% for β -DM), a pronounced peak appeared at 685 nm, and a further increase in the detergent concentration resulted in a further increase in the amplitude of this peak and in an additional blue shift (not shown). The spectrum also appeared to be affected by the presence of glycerol, which might be due to an increase in the CMC of the detergent with increasing glycerol concentration. An almost identical dependence of the shape of the fluorescence spectrum on the detergent concentration was observed by Croce et al. (1996) for PS I-200 preparations from *Zea mays* in the presence of octyl- β ,D-pyranoside (OGP). This effect therefore seems to be a general phenomenon of isolated PS I complexes, has also been reported for PS II membrane fragments from spinach (Irrgang et al., 1988), and may be caused by a less efficient energy transfer from a small amount of antenna pigments to the reaction center when the detergent concentration is raised above the CMC. The emission spectrum shown in Fig. 2 is more or less similar to the spectrum reported by Holzwarth et al. (1993) of PS I particles from *Synechococcus elongatus* in the presence of 0.05% Triton X-100. Their spectrum, however, peaks at ~710 nm (at 308 K), which could be the result of the loss of some of the long-wavelength pigments during the purification of the particles. The increased fluorescence around 687 nm observed in their emission spectrum may also be due to a detergent effect. We note here that the steady-state emission spectrum was used as a major fitting parameter in the modeling work of Trinkunas and Holzwarth (1994, 1996).

We calculated the emission spectrum $F(\nu)$ from the absorption spectrum $A(\nu)$ of the trimeric PS I particles from *Synechococcus elongatus* by the Stepanov relation (Stepanov, 1957):

$$F(\nu) = C \cdot \nu^3 \cdot \exp\left(\frac{-h\nu}{kT}\right) \cdot A(\nu) \quad (3)$$

A good correlation between the calculated and experimentally determined emission spectra has been interpreted by Jennings and co-workers to indicate a complete distribution according to Boltzmann of the excited state over all different spectral chlorophyll forms before the charge separation occurs (see, e.g., Zucchelli et al., 1995; Croce et al., 1996). Fig. 2 shows that, indeed, a good correlation between the experimental and calculated spectra can be achieved (*solid* and *dotted lines*, respectively). The somewhat larger amplitude around 670 nm in the experimental spectrum could be due to a very small amount of uncoupled Chl (Zucchelli et al., 1995).

We would like to point out that a good correlation between the calculated and experimentally determined emission spectra according to Stepanov does not necessarily mean that the excited states in the antenna are fully distributed according to Boltzmann over all spectral chlorophyll forms before charge separation occurs. It is possible, for instance, that the “real” (unperturbed) fluorescence spectrum of the PS I particles is even below the calculated spectrum between 660 and 690 nm (i.e., it is possible that the contribution of free chlorophyll is larger than suggested from the difference between the two curves in Fig. 2), in which case one could conclude that some of the excitations in the bulk antenna arrive at P700 before complete equilibration with the red spectral forms occurs. The emission spectrum is very sensitive to the presence of uncoupled chlorophylls and to aggregation effects, which depend heavily on parameters like the concentration of detergent and the amount of glycerol. Artefacts can be due to uncoupled Chl if the detergent concentration is too high, and to aggregation if the detergent concentration is too low.

Based on a decomposition of the fluorescence spectrum at 295 K (Fig. 2, *solid line*) with Gaussian components, we estimate that ~80% of the fluorescence is emitted from the long-wavelength antenna chlorophylls. This is roughly in agreement with the conclusion of Croce et al. (1996) for PS I-200 preparations from *Zea mays*, that ~90% of the excited states are associated with the red chlorophyll spectral forms at room temperature.

Around 700-nm deviations between the experimental and calculated fluorescence spectra can be expected if the energy transfer and trapping processes in PS I are described by a “transfer-to-the-trap-limited” model (Valkunas et al., 1995; Jennings et al., 1997). In this model, singlet-excited P700 will not easily give its excitation energy back to the bulk antenna because of a relatively large distance to the bulk antenna. This situation is not unrealistic, in view of the location of the PS I reaction center and antenna chlorophylls

in *Synechococcus elongatus* (Krauss et al., 1996). However, we did not observe a significant deviation around 700 nm (also not if the curves were normalized at, for instance, 715 nm), but in view of the uncertainties with the Stepanov analysis and the small number of chlorophylls in the central part of the reaction center, we do not consider this as evidence against the transfer-to-the-trap model. We also did not observe the deviation around 695 nm reported by Croce et al. (1996) in PS I-200 complexes from *Zea mays*. Therefore, we do not see the need to propose three or four antenna chlorophylls strongly coupled to P700 and absorbing around 695 nm.

There is a very remarkable coincidence between room temperature emission spectra of PS I-200 from higher plants and trimeric PS I from *Synechococcus*, which indicates a very similar way of equilibration in both systems, despite the fact that most of the red pigments in PS I-200 are located on the Chl *a/b* binding peripheral antenna LHC-I (see, e.g., van Grondelle et al., 1994; Schmid et al., 1997) and that all red pigments in PS I of *Synechococcus* are located on the core antenna. It is possible that both systems have evolved in the same way to achieve an optimal situation of absorption, energy transfer, and trapping. In other cyanobacteria, however, this situation may be very different. In *Synechocystis* PCC 6803 there are far fewer special long-wavelength pigments per complex than in *Synechococcus elongatus* (Gobets et al., 1994), and consequently, in this system the excitation energy will be localized to a much smaller extent on the red pigments. Indeed, the 295 K emission spectrum of (detergent-free) thylakoids of a PS II-less mutant of *Synechocystis* PCC 6803 shows a much smaller contribution at longer wavelengths (Woolf et al., 1994).

Selective excitation at room temperature

To obtain more direct information on the extent to which excitation energy is transferred from the long-wavelength antenna chlorophylls to P700 in PS I complexes of *Synechococcus elongatus*, we recorded light-induced oxidized-minus-reduced absorbance difference spectra of P700, using a tunable CW laser (690–770 nm) as the excitation source. We used the double-modulation set-up described in detail before by Kwa et al. (1994), which allowed us to record fully reversible absorption changes with a tunable light-on, light-off frequency. In this particular case, we modulated the excitation light at a frequency of 20 Hz, which ensured an optimal signal in view of the ~3-ms decay of P700⁺ in the presence of 5 mM ascorbate and 60 μM PMS at pH 6.5 (see Materials and Methods). This means that with this technique, it is possible to photoaccumulate a significant steady-state level of P700⁺ and to record the absorbance difference spectrum of P700 oxidation as a function of excitation wavelength.

Fig. 3 (*solid line*) shows the absorbance difference spectrum obtained with selective laser excitation at 720 nm. The spectrum shows bleachings at 703 and 685 nm, local max-

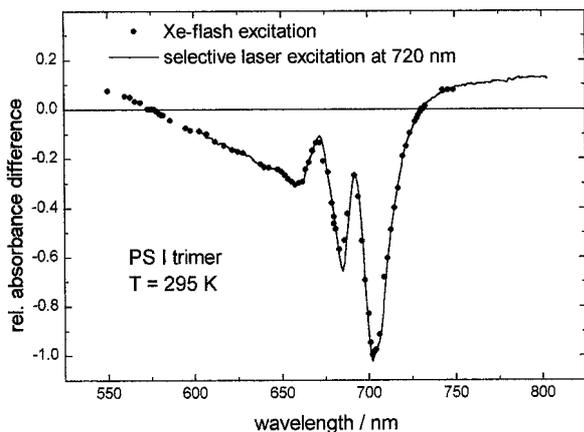


FIGURE 3 —, Absorbance difference spectrum of trimeric PS I complexes at 295 K, excited with a laser at 720 nm, measured with the double-modulation technique at pH 6.5 in the presence of 5 mM sodium ascorbate and 60 μM PMS. ●, Xe-flash-induced P700^+ -minus- P700 absorbance difference spectrum of trimeric PS I complexes at 295 K, recorded at pH 7.5 with 5 mM sodium ascorbate and 30 μM PMS.

ima at 692 and 672 nm, and a zero crossing at 731 nm. The spectrum is identical to the flash-induced P700^+ / P700 absorbance difference spectrum recorded with the same preparation of trimeric PS I complexes (Fig. 3, dots). In the absence of PMS and ascorbate, no reversible light-induced absorbance changes could be observed (not shown), because P700^+ does not decay within 50 ms under these conditions. From these observations we conclude that the solid line in Fig. 3 is not contaminated by a triplet or other signals, and represents the pure oxidized-minus-reduced absorbance difference spectrum of P700.

To analyze the quantum efficiency of P700 oxidation as a function of excitation wavelength, we measured the action spectrum. In Fig. 4 we show P700 absorbance difference spectra obtained at different excitation wavelengths from trimeric PS I complexes, normalized to the incident photon flux. Very similar difference spectra have been obtained

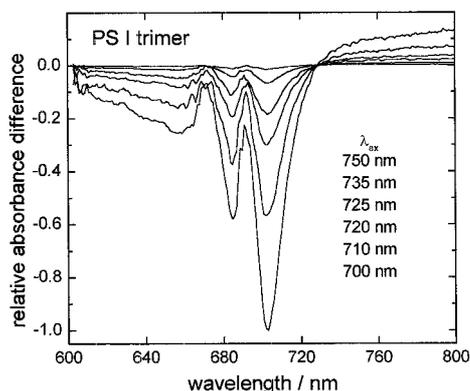


FIGURE 4 P700^+ -minus- P700 absorbance difference spectra of trimeric PS I complexes, recorded at pH 6.5 and $T = 295$ K in the presence of 5 mM sodium ascorbate and 60 μM PMS upon selective excitation between 700 nm and 750 nm. The spectra were normalized to the same number of incident photons at every excitation wavelength.

with monomeric complexes (not shown). For both preparations, there is a very good match between the relative absorption changes at 703 nm (Fig. 5, A and B, squares and circles) and the (1-transmission) spectrum of the sample (Fig. 5, A and B, solid lines). This indicates that the quantum efficiency is constant at room temperature, and thus that there is efficient uphill excitation energy transfer from the long-wavelength antenna chlorophylls to P700, even at wavelengths of up to 760 nm.

A remarkable feature of the spectra presented in Fig. 4 is that the shape of the P700 difference spectrum is almost independent of the excitation wavelength. In Fig. 6 we replotted the difference spectrum obtained from trimeric PS I complexes upon excitation at 700 nm and compared it with that obtained upon excitation at 750 nm after normalization of the signal amplitudes at 703 nm. All positions of the peaks and zero-crossings are the same within the error limits, which means that energetically identical fractions of P700 are oxidized, even upon excitation in the far red edge of the spectrum. The reason for the slightly larger bleaching around 685 nm upon excitation at 750 nm is not clear.

The main result of the experiments presented in Figs. 4–6 is that every photon absorbed in the far red edge of the absorbance band (e.g., 755 nm) has virtually the same probability of causing P700 oxidation as every absorbed photon of higher energy. Thus excitation in the far red edge of the absorption spectrum results in an uphill energy transfer by using thermal energy kT from the surroundings. We note that kT equals 205 cm^{-1} at 295 K, which corresponds to ~ 11 nm at 750 nm. To explain the obvious decrease in

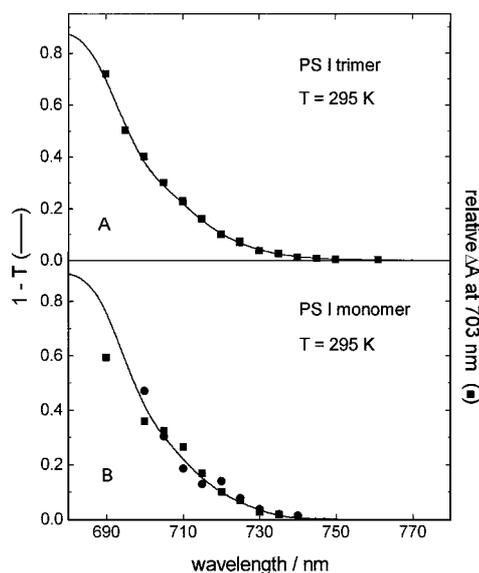


FIGURE 5 Comparison of the (1-transmission) spectrum of trimeric (upper curve) and monomeric (lower curve) PS I complexes (—) with the relative absorption changes at 703 nm (■, ●) as a function of excitation wavelength, normalized to the same incident photon flux. $T = 295$ K. At each wavelength, measurements with different excitation intensities have been performed (e.g., between 0.5 and 3 mW/cm^2 at 690 nm and between 30 and 70 mW/cm^2 at 750 nm).

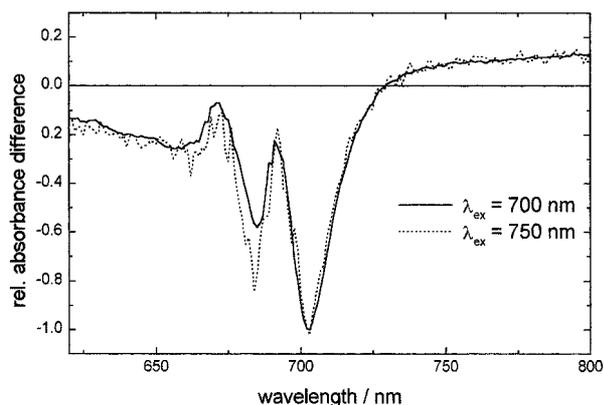


FIGURE 6 Comparison of the shape of the P700⁺-minus-P700 absorbance difference spectrum in trimeric PS I complexes at 295 K, measured with the double-modulation technique upon excitation at 700 nm (—) and at 750 nm (---), normalized to the peak amplitude at 703 nm.

the free energy upon equilibration, not only the energy differences between the various pools of pigments should be taken into account, but also the number of pigments in each pool (the entropy term).

In relation to this, two points should be mentioned. First, the quantum yield of the primary charge separation reaction depends only moderately on the trapping rate. The quantum yield is given by

$$\Phi = \frac{k_p}{k_p + k_{\text{loss}}} \quad (4)$$

where k_p is the rate constant for the decay of the excited state due to trapping by photochemistry, and k_{loss} is the sum of the rate constants for all other decay processes of the excited state. For instance, if we assume that $k_{\text{loss}} = (2 \text{ ns})^{-1}$ and $k_p = (30 \text{ ps})^{-1}$, then the quantum yield would be 98.5%. Accordingly, a slowing down of the trapping rate by a factor of 10 would only result in a slight decrease in the quantum yield to 87%. In addition, in the “transfer-to-the-trap-limited” model, the kinetics of reaching the trap limit the overall trapping time, not the primary charge separation reaction itself.

Second, P700 possibly absorbs up to at least 750 nm at room temperature. Indications that the low-energy excitonic band of P700 could be extremely broad at room temperature can be found in the shape of the oxidized-minus-reduced difference spectrum, which starts to decrease around 750 nm when the spectrum is scanned toward shorter wavelengths (see, e.g., Fig. 3). If the positive P700⁺ spectrum is featureless in this part of the spectrum, then the lowering must be explained by the bleaching of the absorption of P700. We estimate a FWHM of $\sim 20 \text{ nm}$, or 400 cm^{-1} , if a Lorentzian shape is assumed. At low temperature (1.6 K), a linewidth of 350 cm^{-1} was determined by spectral hole burning (Gillie et al., 1989). This feature of P700 could facilitate the trapping of excitations localized on the long-wavelength antenna pigments.

Temperature dependence of steady-state emission

In Fig. 7 we show the yield of the nonselectively excited steady-state emission as a function of temperature between 295 K and 4 K. The spectra were recorded in the same buffer as in Fig. 2, so they are not distorted because of contributions of uncoupled Chl. Upon cooling from 295 K to 175 K, the peak wavelength shifted from 721 nm for both preparations to $\sim 730 \text{ nm}$ (monomers) or 732 nm (trimers), whereas upon further cooling the peak wavelengths stayed constant within 1 nm (not shown). In the trimeric complexes the yield increases ~ 11 -fold as the temperature is decreased from 295 K to 4 K, whereas in the monomeric complexes the yield increases by ~ 16 -fold (see the next section for a discussion of this result). Half-maximum values are observed at $\sim 140 \text{ K}$ (trimers) and 120 K (monomers). The curves differ considerably from those obtained for thylakoids from PS II-less *Synechocystis* PCC 6803 (Wittmershaus et al., 1992), where the half-maximum fluorescence occurs at $\sim 75 \text{ K}$. This suggests that in *Synechocystis* less thermal energy is required to transfer excitation energy from the long-wavelength pigments to the photochemical trap, and thus that the long-wavelength pigments are closer in energy to P700 than in *Synechococcus*, consistent with the absence of C-719 in this organism. In chloroplasts from higher plants, however, the half-maximum fluorescence occurs at $\sim 125 \text{ K}$ (Tusov et al., 1980), further supporting the idea suggested above that the antenna systems of intact PS I of plants and *Synechococcus* have qualitatively similar amounts and transition energies of long-wavelength antenna pigments.

Attempts to fit the temperature dependencies with an Arrhenius type of curve with a single activation energy, as in Wittmershaus et al. (1992) and in Tusov et al. (1980), failed at temperatures below 100 K (not shown). Above 100 K the curves could be fitted well by assuming activation energies of 45 and 38 meV for trimers and monomers,

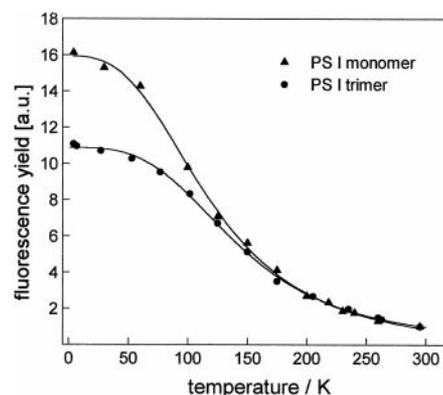


FIGURE 7 Fluorescence quantum yield as a function of temperature in trimeric (●) and monomeric (▲) PS I complexes. Excitation wavelength 430 nm. The measurements were performed in a glycerol/buffer (pH 7.5) mixture (65:35, v/v) containing 5 mM sodium ascorbate. All data points were normalized to 1 at 295 K.

respectively. These values correspond to 363 and 306 cm^{-1} , respectively, or to 17 and 15 nm at 700 nm . It is possible that lower activation energies caused by the C-708 pigments are responsible for the further increase in the fluorescence yield below 100 K .

Temperature dependence of the quantum yield of P700 oxidation

The results of Fig. 7 suggest that at low temperatures a considerable part of the (nonselective) excitation energy becomes trapped on the long-wavelength antenna pigments, and thus that a considerable part of the excitation energy will not result in P700 photooxidation. To estimate the extent to which excitation energy is lost for charge separation, we measured the quantum yield of charge separation as a function of temperature. We recorded light-induced absorption changes at 826 nm due to the oxidation of P700 upon illumination with blue Xe flashes as a function of the excitation flash energy. Fig. 8 shows that for both complexes, the half-saturation values gradually increase as the temperature is lowered, to reach values at 4 K of 2.17 and 1.73 times the values at 295 K for trimeric and monomeric complexes, respectively. This suggests that the relative yields of charge separation at 4 K are 46% and 58% for trimeric and monomeric complexes, respectively. The somewhat higher yield at 4 K in the monomeric complexes can be rationalized by the lower number of long-wavelength chlorophylls. In these particles the probability is smaller

that an excitation becomes trapped on the long-wavelength pigments. We note that the values in Figs. 7 and 8 add up to roughly the same value at all temperatures (not shown), indicating a complementary relationship between charge separation and fluorescence yield.

The results of Fig. 8 suggest that the larger increase of the steady-state emission in monomers upon cooling to 4 K (Fig. 7) is not due to a larger decrease in P700 oxidation. On the contrary, the relative yield of P700 oxidation is larger in monomers than in trimers at 4 K . The fluorescence yields presented in Fig. 8 are normalized to 1 at 295 K . It is therefore not clear whether the steady-state fluorescence at 4 K is higher in monomers than in trimers, and/or whether the steady-state fluorescence at room temperature is lower in monomers than in trimers. The latter possibility could point to a decreased trapping time in monomers at room temperature, perhaps because the excitation has a somewhat higher probability of reaching P700, which is, in fact, expected if a considerable fraction of the C-719 pigments are missing. These pigments no longer take part in the total equilibrated pool of excitation energy, because of which the probability of finding the excited state on P700 is increased, and the fluorescence yield will be lower. However, measurements at 295 K under identical conditions using the same chlorophyll concentration revealed that the fluorescence yield of trimers is only $\sim 15\text{--}20\%$ higher than that of monomers (not shown). This difference accounts for only a part of the larger increase in the steady-state emission in monomers, which suggests that the steady-state emission at 4 K is higher in monomers than in trimers. Similar to what has been found for artificial chlorophyll aggregates, it is possible that the larger number of C-719 chlorophylls in the trimers increases the probability that the excited state decays via radiationless decay pathways.

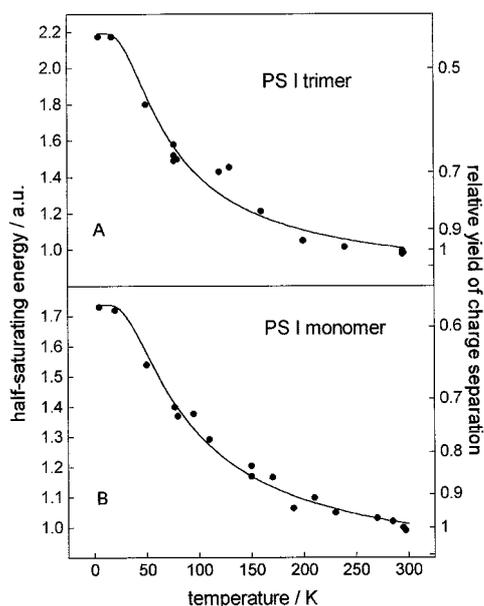


FIGURE 8 Half-saturating energies of the excitation flashes normalized to 1 at 295 K as a function of temperature for trimeric (A) and monomeric (B) PS I complexes, obtained by measurements of the flash-induced absorbance changes at 826 nm due to the oxidation of P700 as a function of the flash energy. The measurements were performed in a glycerol/buffer (pH 7.5) mixture (65:35, v/v) containing 5 mM sodium ascorbate and $20\text{ }\mu\text{M}$ PMS.

Fluorescence excitation spectra at 4 K

The results described above show that nonselective excitation at 4 K leads to P700 oxidation in about half of the reaction centers, and to excitation of the long-wavelength pigments in the other half. To get an idea of the extent to which this bipartite behavior modifies if selective excitation is employed, we recorded at 4 K the excitation spectrum of the fluorescence at 750 nm of trimeric complexes, and compare this with the (1-transmission) spectrum (Fig. 9, *solid line*). If the excitation spectrum is divided by the (1-transmission) spectrum, a horizontal line is expected if all pigments contribute to the same extent to the emitting species. Fig. 9 (*dashed line*) shows that a horizontal line is observed at excitation wavelengths shorter than $\sim 690\text{ nm}$, but at wavelengths longer than 690 nm , the ratio increases to reach a factor of ~ 2 at wavelengths longer than 710 nm .

Thus, at excitation wavelengths shorter than 690 nm , about half of the excitation energy is lost for fluorescence at 4 K , in agreement with the results on P700 oxidation obtained with nonselective excitation in the previous sections.

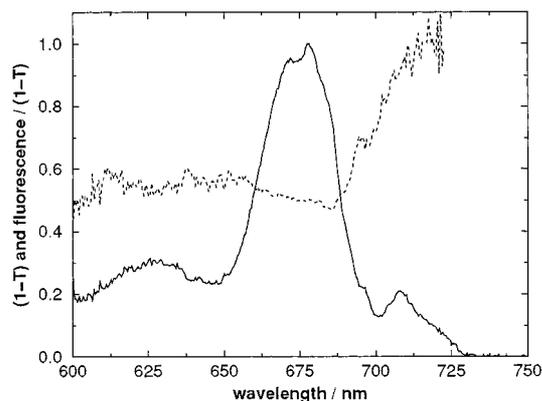


FIGURE 9 —, 1-transmission spectrum of trimeric PS I particles at 4 K. ---, Fluorescence excitation spectrum at 4 K (excitation wavelength 750 nm) divided by the solid line, normalized to 1 at 718 nm.

On the other hand, excitation at 4 K at wavelengths longer than ~ 710 nm results in the selective excitation of the long-wavelength pigments, and probably does not give rise to P700 oxidation at all.

Selective excitation at 4 K

To obtain more direct information on the yield of P700 photooxidation at 4 K upon selective excitation at wavelengths longer than 690 nm, we recorded reversible light-on-minus-light-off absorbance changes as described above for 295 K. The samples were frozen in the presence of 5 mM ascorbate in the dark, so that P700 was reduced before illumination at 5 K. Fig. 10 shows spectra obtained with trimeric PS I complexes at different excitation wavelengths, normalized to the incident photon flux. At low temperature ($T < 100$ K), a stable charge separation $P700^+F_{A/B}^-$ is induced in $\sim 45\%$ of the PS I complexes in which charge separation occurs at all (Schlodder et al., 1995; Brettel,

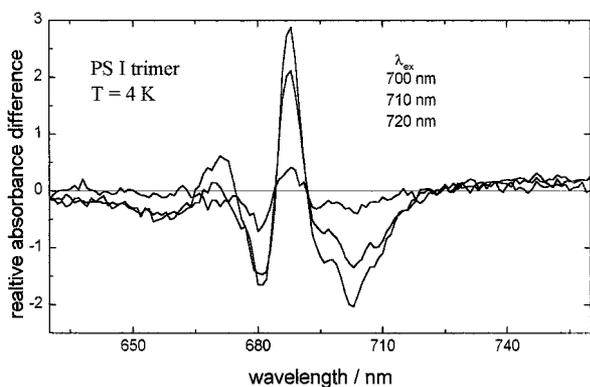


FIGURE 10 $P700^+$ -minus- $P700$ absorbance difference spectra of trimeric PS I complexes at 5 K, recorded with the double-modulation technique in a glycerol/buffer (pH 7.5) mixture (65:35, v/v) containing 5 mM sodium ascorbate upon selective excitation between 700 nm and 720 nm. The spectra were normalized to the same number of incident photons at every excitation wavelength.

1997), whereas in the remaining complexes reversible absorbance changes occur. In ~ 80 – 90% of these remaining complexes, the absorbance changes decay with a half-time of ~ 170 μ s due to the recombination of the radical pair $P700^+A_1^-$. A smaller decay phase with $t_{1/2} > 10$ ms (10–20%) could be attributed to the charge recombination of $P700^+F_X^-$ (Lüneberg et al., 1994). Furthermore, the quantum yield of charge separation at 5 K is only $\sim 50\%$ compared to 295 K (see above). Therefore, the signal-to-noise ratio is significantly lower than at room temperature. A rough estimate reveals that the light-on-light-off absorbance difference is about a factor of 50 smaller at 5 K than at 295 K, provided that the intensity of the excitation light is the same.

Nevertheless, the spectra in Fig. 10 can largely be attributed to the oxidation of P700, in view of the resemblance to the 5 K spectrum of the irreversible absorbance changes recorded with trimeric PS I complexes from *Synechococcus* (Fig. 11). To obtain this spectrum, the absorbance spectrum at 5 K before illumination (see Fig. 1) was subtracted from that measured after illumination with 30 Xe flashes and subsequent dark adaptation. Fig. 11 shows that the main features (a broad bleaching at 703 nm, a strong absorbance increase at 691 nm, a further bleaching at 685 nm, and a zero crossing at 718 nm) are the same as those observed in Fig. 10, although the spectrum presented in Fig. 11 exhibits additional narrow bands due to the higher spectral resolution. A similar spectrum, except for the sharp spectral features, was reported for the low-temperature light-minus-dark absorbance difference of PS I particles from spinach (Sétif et al., 1984). The bleaching at 703 nm is probably caused by the disappearance of the ground state of P700 dimer, whereas the up and down going features between 670 and 700 nm are most likely dominated by electrochromic shifts of nearby chlorophylls.

To estimate the quantum yield of P700 oxidation as a function of excitation wavelength, we compared the absor-

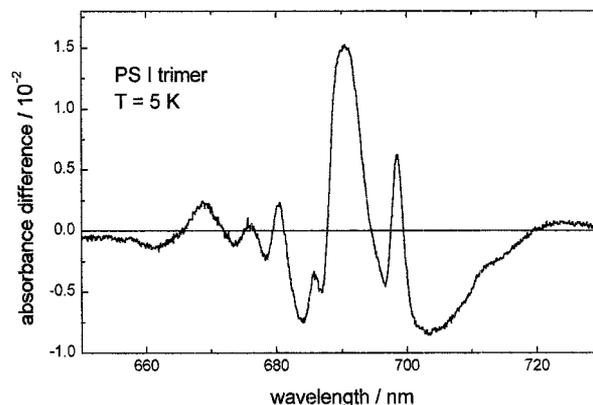


FIGURE 11 Light-minus-dark absorbance difference spectrum of trimeric PS I complexes at 5 K measured in a glycerol/buffer (pH 7.5) mixture (65:35, v/v) containing 5 mM sodium ascorbate. The absorbance spectrum at 5 K before illumination was subtracted from that measured after illumination with 30 Xe flashes.

bance changes at 703 nm (Fig. 12, A and B, squares) with the (1-transmission) spectrum of the samples (Fig. 12, A and B, solid lines). Fig. 12 shows that with excitation at wavelengths longer than 700 nm, a large part of the excitation energy does not reach P700, both in trimers (Fig. 12 A) and in monomers (Fig. 12 B), in agreement with the fluorescence excitation spectra shown in Fig. 9. The relative yield is $\sim 50\%$ at 710 nm and almost zero at 720 nm. Fluorescence, however, could easily be recorded at excitation wavelengths longer than 730 nm (Pålsson et al., 1996).

CONCLUSIONS AND IMPLICATIONS FOR THE FUNCTION OF PS I

In this report we show that in trimeric and monomeric PS I complexes of *Synechococcus elongatus* at room temperature, all absorbed light, even of wavelengths of longer than 750 nm, has the same very high probability of resulting in a stable P700 photooxidation.

At 5 K, however, the long-wavelength antenna chlorophylls form a deep trap of excitation energy and therefore give rise to a strong increase in the fluorescence yield (Fig. 7). The analysis of the yield of charge separation (Fig. 12) and of the fluorescence (Fig. 9) upon selective excitation provides evidence that uphill excitation energy transfer from the long-wavelength antenna pigments to P700 does not occur at 5 K. This means that excitations that reach one of the red pigments are lost for photochemical energy conversion. This is, in fact, expected, because $kT \approx 3.5 \text{ cm}^{-1}$ at 5 K, which is too small to bridge the energy gap between the C-719 and C-708 pigments and P700. Additionally, the

entropy contribution to the free energy change can be neglected at low temperatures. Nevertheless, the quantum yield of charge separation decreases only by a factor of ~ 2 as the temperature is decreased from 295 K to 5 K (Fig. 8). The reduction in yield is stronger in trimeric PS I complexes than in monomeric complexes, indicating a positive correlation of the reduction in yield with the number of C-719 pigments.

Based on these findings, we conclude that at 5 K, thermal equilibration of the excited state in the antenna is not established before charge separation. After equilibration the excited state should be totally localized on the red-most pigments, because according to the Boltzmann distribution, the probability of finding the excitation on P700 is $< 10^{-5}$ for $T < 40 \text{ K}$ in the trimeric complexes. Thus photochemical charge separation would not occur to a significant extent in the case of complete equilibration. Assuming that the molecular rate constant of primary charge separation does not depend very strongly on the temperature, the trapping time due to charge separation would be $> 100 \text{ ns}$, i.e., much longer than the excited-state lifetime. It should be noted that the Stepanov analysis of the low temperature absorption and emission spectrum (not shown) supports this conclusion. At cryogenic temperatures, the calculated emission spectrum does not match the experimentally determined spectrum (see also Croce et al., 1996).

Our results show that at 5 K there is a probability of $\sim 50\%$ of reaching P700 before the excitation becomes irreversibly trapped on one of the long-wavelength antenna pigments. The specific location of P700 (Krauss et al., 1996) suggests that the rate of primary charge separation is most likely much faster than the back-transfer of the excitation energy from P700* to neighboring antenna pigments, so this part of the excitation energy will give rise to primary charge separation. The situation at low temperatures indicates a kinetic competition between two possibilities: the excitation is trapped either by P700, resulting in charge separation, or by one of the long-wavelength antenna pigments (see also van Grondelle et al., 1994). In the latter case, the excited state decays by fluorescence or radiationless processes, but not by photochemical charge separation.

It is interesting to note that recently, Liebl et al. (1997) have drawn similar conclusions based on wavelength-selective femtosecond transient absorption measurements at 20 K, using membranes of the heliobacterium *Heliobacillus mobilis*, which contain a photosystem of the same type as photosystem I of oxygen-evolving organisms. These authors found very different rates of P798⁺ formation upon excitation of spectrally different pools of pigments, and suggested that there is a competition of energy transfer from the singlet-excited bulk-antenna spectral form Bchl g 793 to either the primary electron donor P798 or the long-wavelength spectral form Bchl g 808, in line with our results on PS I. On the other hand, Chiou et al. (1997) concluded from similar types of experiments on *Heliobacillus mobilis* that at 20 K basically all excitations are transferred to Bchl g 808, followed by a transfer to P798 and charge separation with a

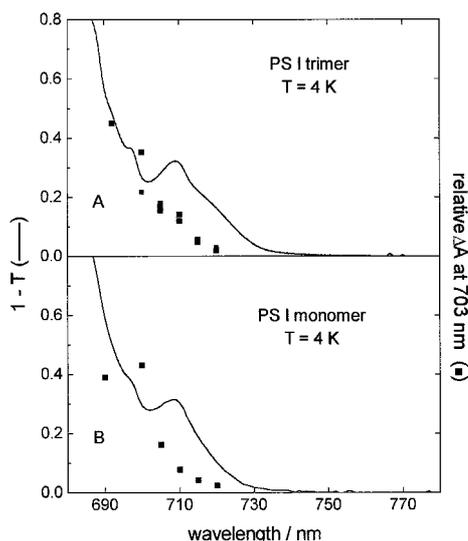


FIGURE 12 Comparison of the (1-transmission) spectrum of trimeric (upper curve) and monomeric (lower curve) PS I complexes (—) with the relative absorption changes at 703 nm (■) normalized to the incident photon flux as a function of the excitation wavelength. The measurements were performed with the double-modulation technique upon selective excitation between 690 and 720 nm. $T = 5 \text{ K}$. The excitation intensity has been varied between 30 mW/cm^2 at 690 nm and 130 mW/cm^2 at 720 nm.

time constant of 55–70 ps in ~85% of the centers. Kleinherenbrink et al. (1992) reported that there is no excitation wavelength dependence of P oxidation at 6 K in *Heliobacterium chlorum*. Our results on the quantum yield of P700 oxidation as a function of excitation wavelength indicate, however, that in PS I the uphill energy transfer from the long-wavelength antenna pigments to P700 is negligible at 5 K, and that charge separation basically takes place without the involvement of the long-wavelength pigments at this temperature.

We can only speculate on the mechanisms and structures that can explain our findings. A specific spatial arrangement of the spectrally different antenna pigments is probably required to model the low-temperature data. A completely random organization of the long-wavelength chlorophylls in the ring-shaped antenna can most likely be excluded, because in this case the probability would be too large that one of the long-wavelength antenna chlorophylls would be excited before P700 can be reached. Furthermore, a “funnel” model in which all antenna chlorophylls close to P700 (or one of the four other central chlorophylls-Krauss et al., 1996) are responsible for the long-wavelength absorption would lead to too much excitation on the red chlorophylls at cryogenic temperatures. If the antenna of PS I in *Synechococcus elongatus* consists of four large clusters of chlorophylls, each mutually connected by only one chlorophyll (Krauss et al., 1996; Fromme, 1996; Schubert et al., 1997), then it is perhaps most likely that the long-wavelength antenna chlorophylls occur in only one or two of these clusters. Excitation of one of the chlorophylls in a cluster without red pigments would then be preferably led to P700 oxidation at very low temperatures, whereas excitation of one of the chlorophylls in a cluster with long-wavelength antenna pigments would preferably lead to trapping by the red chlorophylls.

Our experiments do not provide conclusive evidence for the extent of spectral equilibration before charge separation at room temperature. If the situation were similar to that at 4 K, then ~50% of the excitations would flow directly to P700, and the other 50% would flow via the long-wavelength antenna pigments. It is possible, however, that the spectral overlap between the bulk antenna chlorophylls and the long-wavelength pigments is rather poor at 4 K, and that this overlap increases as the temperature is increased, because of a broadening of the various spectral bands. This effect may be stronger for the red antenna pigments than for P700, because the former group of pigments absorbs more to the red. Therefore, energy transfer via the Förster mechanism to the red chlorophylls may increase significantly, leading to a larger energy flow via the red chlorophylls. For the quantum efficiency of charge separation, however, the question of complete versus incomplete equilibration before charge separation is irrelevant, because the “slow” energy flow via the red antenna chlorophylls (~35 ps at room temperature) is more than fast enough to ensure a high quantum efficiency of charge separation.

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