Red Antenna States of PS I of Cyanobacteria: Stark Effect and Interstate Energy Transfer†

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Previously, Stark hole-burning spectroscopy and effects of pressure at low temperature were used to determine the number of red antenna states of the cyanobacteria *Synechococcus elongatus* and *Synechocystis PCC6803* (Hayes, J. M.; Matsuzaki, S.; Rätsep, M.; Small, G. J. *J. Phys. Chem. B* **2000** *104*, 5625. Zazubovich, V.; Matsuzaki, S.; Johnson, T. W.; Hayes, J. M.; Chitnis, P. R.; Small *Chem. Phys.* **2002**, *275*, 47). Distinct differences in linear pressure shift rates, the magnitude of the permanent dipole moment change, $f\Delta\mu$, and electron−phonon coupling strength clearly show that in *Synechococcus* there are three red states (C708, C715, and C719), whereas in *Synechocystis*, there are two red states (C708 and C714). In the Stark hole-burning spectra of the lowest states of these two systems, hole splitting was not observed, only hole broadening, for excitation polarization both parallel and perpendicular to the Stark field direction. The theories of Stark hole burning predict that splitting should occur for one of the polarizations unless there is a large, random component to the induced dipole moment change, $\Delta\boldsymbol{\mu}_{\mathbf{ind}}$, which is not expected to be the case for pigment−protein complexes in which the orientations of pigments relative to the protein matrix are nonrandom. In this paper, Stark hole burning at higher resolution is used to reinvestigate the absence of splitting. However, even at higher resolution, no splitting is detected. This is explained by invoking large variations of the inherent dipole moment change $\Delta\boldsymbol{\mu}_{\mathbf{0}}$ of the dimer (the origin of the red-state absorption) rather than of the induced dipole moment change. These arise from a distribution of the relative orientations and separations between the components of the dimer. This distribution also results in a random component of the polarizability change tensor, $\Delta\boldsymbol{\alpha}$. The random components of $\Delta\boldsymbol{\mu}_{\mathbf{0}}$ and $\Delta\boldsymbol{\alpha}$ not only obscure the Stark splitting but also cause the large inhomogeneous broadening observed for these lowest-energy red states. Temperature-dependent hole widths were also measured for C708 and C714 of *Synechocystis*. For C714, a $T^{1.3}$ temperature dependence was observed, consistent with dephasing by the disordered protein matrix. At 708 nm, however, much higher fluences were required to saturate the absorption of the blue edge of the C714 band and then begin to burn C708. The contribution of the C708 component to the broadening was weakly temperature dependent over the range measured, 2 to 14 K. This contribution is due to energy transfer from C708 to C714, and the width measured corresponds to an energy-transfer time of 6 ps. This observation provides further proof of the existence of two red antenna states, C708 and C714.

1. Introduction

Elimination of the effects of static inhomogeneous broadening by spectral hole burning at low temperatures[1] has proven to be an invaluable technique for the study of complex molecular systems such as protein−pigment complexes. When such complexes contain significant protein−pigment and/or pigment−pigment interactions, the power of the spectral hole-burning technique can be further enhanced by including the effects of external fields on the hole spectra. In particular, studies of photosynthetic pigment−protein complexes where excitonic coupling of the chlorophyll pigments and strong electron−phonon (pigment−protein) coupling are important for electron transfer and energy transfer have benefited greatly from including the effects of pressure[3−6] and electric fields (the Stark effect).[3,4,7,8]

For coupled pigments, the rate of linear pressure shifts to the red of spectral holes can be correlated to the coupling strength. In the case of weak coupling, linear shift rates $\leq|0.15$ cm^{-1} $MPa^{-1}|$ are typical. (Shift rates of -0.05 to -0.15 cm^{-1} MPa^{-1} are typical for the $\pi\pi^*$ transitions of isolated chromophores in polymers and glasses.[9,10]) However, for strongly coupled pigments, linear shift rates $\geq|0.3$ cm^{-1} $MPa^{-1}|$ are found. Such large shift rates have been argued to indicate that electron exchange contributes significantly to the coupling.

In the case of strongly coupled pigments, the electronic transition is often associated with a large change in the permanent dipole moment of the molecule, which can arise, for example, when the excited state possesses significant charge-transfer character. The magnitude of the change in the dipole moment, $\Delta\mu$, can be measured by Stark spectroscopy. (Here and below, bold variables are vectors, and regular variables are their magnitudes.) For example, the bacteriochlorophyll *a* special pair (P870) of the *Rhodobacter sphaeroides* reaction center has $f\Delta\mu = 5.2$ D,[11] where f is the local-field correction factor. (Although a value of $f \approx 1.5$ is sometimes used, we prefer to report values as $f\Delta\mu$ because the dielectric constant of the protein medium is not known.) The large value of $f\Delta\mu$ for P870 can be contrasted with the values typical of monomeric chlorophyll *a* (Chl *a*), 0.5−0.6 D.[3,8]

The above points regarding pressure and Stark effects on hole spectra of Chl *a* in pigment−protein complexes are well

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illustrated in several papers on the red antenna states of PS I of cyanobacteria.[3,4] These states, absorbing light at energies lower than that of the primary electron donor, P700, have attracted considerable interest and remain incompletely understood.[12] Although these states can undergo upward energy transfer to P700 at physiological temperatures, it is not known if this is their primary function or if photoprotection is more important.[13]

Interest in the red antenna states was further stimulated by the publication of the 2.5-Å-resolution crystal structure of PS I from *Synechcoccus elongatus*.[14] In the structure, three dimers and a trimer of Chl *a* with Mg···Mg separations of ~8 Å were identified. It was speculated that some of these might be the source of the red absorbing states, and papers proposing possible assignments of particular multimers to individual red absorptions have appeared.[15−17]

Pressure and electric field effects on the hole spectra of both *Synechococcus elongatus* and *Synechocystis PCC6803* were used by Small and co-workers to resolve the red-state absorptions better.[3,4] In *Synechocystis*, where the red absorption consists of a single broad band at 708 nm even at 4 K, zero-phonon holes (ZPH) burned to the red side of the band show much stronger electron−phonon coupling than holes burned on the high-energy side, suggesting that there are (at least) two states underlying the absorption. This conclusion was reinforced by both Stark and pressure effects on the ZPHs, which had larger values of both $f\Delta\mu$ (2.3 D at 714 nm) and linear pressure shifts (-0.45 cm^{-1} MPa^{-1} at 713 nm) than on the blue side of the band ($f\Delta\mu = 1.0$ D at 708 nm, pressure shift rate $= -0.17$ cm^{-1} MPa^{-1} at 706 nm). From these results and from fitting the hole spectra as a function of the burn wavelength, λ_B, it was concluded that there are two red absorbing states at 714 nm (C714) and 708 nm (C708). Although these states exhibit considerable spectral overlap, C714 was definitely associated with a strongly coupled Chl *a* dimer possessing significant charge-transfer character.

Similar analysis and results were also obtained for *Synechococcus* where there are two red absorption bands (C708 and C719) resolvable in the low-temperature absorption spectrum.[4,18] From the combination of hole burning with Stark fields and high pressure, an additional absorption or state (C715) was identified.[4] The lowest state C719 has properties that are very similar to those of C714 of *Synechocystis*, whereas the other two states have smaller values for $f\Delta\mu$ and the linear shift rate, but these values are still larger than those typically measured for monomeric antenna Chl *a*.

A puzzling feature of the Stark hole spectra of the cyanobacteria is the absence of splitting of the ZPH burned into the lowest-energy (red) antenna band.[3,4] In an electric field, the transition frequency of the molecule shifts as

$$\Delta\omega = \hbar^{-1}\left(f\,\Delta\boldsymbol{\mu}\cdot\mathbf{E}_S + \frac{1}{2}f^2\,\mathbf{E}_S\cdot\Delta\alpha\cdot\mathbf{E}_S\right) \qquad (1)$$

where $\Delta\boldsymbol{\mu}$ is the permanent dipole moment change between excited and ground electronic states, $\mathbf{E}_S$ is the Stark field, $\Delta\boldsymbol{\alpha}$ is the polarizability difference tensor, and f is the local-field correction factor, as mentioned above. The first term in eq 1 describes the linear Stark effect, and the second term is the quadratic Stark effect. In what follows, only the linear term will be of interest (i.e., it is relevant to the field strengths $\mathbf{E}_S$ used in the experiments). The dipole moment difference, $\Delta\boldsymbol{\mu}$, can be described as a sum of the free-molecule dipole moment difference, $\Delta\boldsymbol{\mu}_0$, and the dipole moment difference induced by the protein lattice, $\Delta\boldsymbol{\mu}_{ind}$. According to refs 19−21, when $\Delta\boldsymbol{\mu}_0$ is dominant over $\Delta\boldsymbol{\mu}_{ind}$ a splitting of the ZPH should be observed for one of the two orthogonal polarizations of the exciting laser relative to the Stark field. However, if $\Delta\boldsymbol{\mu}_{ind}$ is dominant and has a large random component, such as in a polymer or glass matrix, only hole broadening will be observed independent of laser polarization. In view of this, the absence of the aforementioned splitting for the 714- and 719-nm states of *Synechocystis* and *Synechococcus* is surprising because the large $f\Delta\mu$ values of ~2.4 D are most reasonably attributed mainly to $f\Delta\boldsymbol{\mu}_0$ (of the dimer), not $f\Delta\boldsymbol{\mu}_{ind}$.

In this paper, the previous Stark results for PS I are reviewed, and new nonphotochemical hole-burning (NPHB) results obtained at higher resolution (50 MHz) are reported. The intent of the higher-resolution experiments is to determine if there is truly no ZPH splitting for the red-most antenna state of the cyanobacterial PS I or if the splitting was not observed because of limitations of the previous experiments. These limitations are not due to only moderate (~0.5−1 cm^{-1}) resolution per se. (The resolution in refs 3 and 4 was determined by the resolution of the Fourier transform spectrometer. The laser bandwidth was ~2 GHz.) Note that the ZPH splitting can be observed when the polarized laser light creates anisotropy in the sample by burning only molecules (or states) with transition dipole moments approximately parallel to the laser light polarization. The latter condition was quite likely not satisfied in the experiments of Rätsep et al.[3] and Zazubovich et al.[4] because the holes used for the Stark effect measurements were close to saturated and/or fluence-broadened (in order to see holes of reasonable fractional depth despite the low resolution), and as a result, the polarization selection of the transition dipole moments of the burned states could be lost. Because electron−phonon coupling for the C714 state is strong (Huang−Rhys factor $S \approx 2$ due to 18- and 70 cm^{-1} phonons), the maximal fractional ZPH depth that can be achieved for this state is 0.13 ($\exp(-S)$). Thus, 10%-deep holes may be already saturated enough to prevent the observation of the splitting. In the experiments by Rätsep at al.[3] and Zazubovich et al.,[4] the noise level was ~1% of the signal, which resulted in a hole depth-to-noise ratio of not more than 10 for 10%-deep (and probably saturated) holes at zero applied field. Broadening of the hole in the electric field resulted in a further reduction of the hole-to-noise ratio, making those holes barely detectable.

A second objective of this work is to find out if excitation-energy transfer occurs from higher-energy red states to lower-energy ones and to determine the rate of such a transfer. The formation of a lower-energy (714-nm) satellite hole upon higher-energy (706-nm) excitation was observed for *Synechocystis*.[3] However, whether this effect is due to energy transfer or to structural changes that accompany nonphotochemical hole burning of the states directly excited was not clarified. The shape of the nonline-narrowed emission spectrum (peaked at 722 nm)[3] suggests that the C708 state does not fluoresce. Thus, experimental observations indicate that C708−C714 energy transfer most likely does occur. This issue will be clarified by the precise determination of the homogeneous widths of the ZPH burned into the C708 state; these widths are inversely proportional to the energy-transfer time.

2. Experimental Section

The research was focused mainly on photosystem I of *Synechocystis*. Because it has only two red antenna states, the results can be interpreted more easily. As we will see, the results for *Synechococcus* (less-detailed studies performed) were similar. *Synechocystis* and *Synechococcus* wild-type trimer PS I samples were prepared as described in refs 3 and 4,

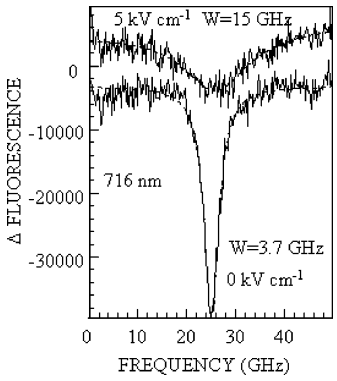

Figure 1. Experimental hole spectra (—) and Lorentzian fits (- - -) for $\lambda_B = 716$ nm, laser polarization parallel to the direction of the Stark field, and field strengths of 0 and 5 kV cm^{-1}. Burn fluence was ~0.01 J cm^{-2}. For ZPH widths for intermediate field strengths, see Figure 2. $T = 2$ K.

respectively. To improve the resolution, holes were probed in the fluorescence excitation mode as opposed to the absorption detection used in refs 3 and 4. Hole burning was carried out, and the spectra were scanned using the Coherent 699-29 (Autoscan) laser at 50-MHz resolution. Laser intensity was stabilized with a CRI power stabilizer and attenuated with neutral density filters. The intensity when scanning was on the order of 2 μW cm^{-2}; the intensities used for burning were about 100 times higher. The total burning doses were in the range of 0.001 to 0.27 J cm^{-2} depending on the burn wavelength (i.e., at least 100 times smaller than those used in refs 3 and 4). Hole spectra were scanned within a 50-GHz window precisely centered at the burn wavelength. Fluorescence was detected at 90° (in relation to excitation) by a cooled photomultiplier (Hamamatsu) through an AELP730 interference filter (Omega optical). The Stark hole-burning apparatus is described elsewhere.[8] Briefly, the sample in a gelatin capsule (Torpak) was squeezed between the electrodes of the Stark cell (distance between electrodes = 4 mm). Voltages of up to 3.5 kV were obtained from a Trek model 610C high-voltage power supply, which resulted in electric field strengths of up to 8750 V cm^{-1}. Experiments were performed for laser polarization both parallel and perpendicular to the Stark field. A polarization plane rotator (Thorlabs) was used to rotate the laser polarization by 90°. The Stark cell was immersed in liquid helium in a Janis 8 DT cryostat with temperature control and stabilization at 2 K by a Lakeshore Cryotronic model 330 temperature controller.

To burn deep holes into the C708 band, it was necessary to use higher fluences. These holes were measured with a resolution of 6 GHz using no etalons in the burn laser (laser bandwidth = 2 GHz). Holes were burned for several fluences (up to 100 J cm^{-2}), and hole widths were extrapolated to zero fluence.

Some spectra exhibited a sinusoidal modulation. To account for that, the preburn spectrum was fit to a sinusoid, and that sinusoid was subtracted from the hole spectra before fitting to a Lorentzian shape. Alternatively, if the signal-to-noise ratio allowed, preburn spectra were subtracted from postburn spectra before fitting.

3. Results

Figure 1 shows an example of a 6%-deep hole at 716 nm in the PS I of *Synechocystis* in zero field and the same hole in an applied field of 5.0 kV cm^{-1} with $\mathbf{E}_S||\mathbf{E}_{laser}$. No splitting was observed in either polarization even for very shallow holes (3% deep), which were used to ensure that any splitting was not obscured by the saturation of the hole. To determine $f\Delta\mu$, ~6%-deep holes were used because a more reliable value could be obtained as a result of the higher signal-to-noise ratio of the deeper holes. Values for $f\Delta\mu$ were determined for several wavelengths in both parallel and perpendicular polarizations (relative to the Stark field direction) using the theory of Kador et al.[22] The results are given in Table 1, and a representative plot of the hole width, Γ_{hole}, versus the applied field is shown in Figure 2. For *Synechococcus*, measurements were made at 710 and 723 nm using parallel polarization only. Values of $f\Delta\mu$ were similar to those for *Synechocystis* (i.e., 2.6 and 1.6 D at 723 and 710 nm, respectively).

Table 1 also includes the values of $f\Delta\mu$ reported in ref 3. In that work, it was reported that within experimental error there was no difference in the values measured for the two orthogonal polarizations. Although this might be true for $\lambda_B = 707.5$ nm in the present work, at longer wavelengths the effect for parallel

TABLE 1: Changes in the Permanent Dipole Moment for the Red Antenna States

		$f\Delta\mu$ (D)		
	λ (nm)	$\perp$ and $\|\|^a$	$\|\|^b$	$\perp^b$
Synechocystis	690	0.5		
	692	0.6		
	695	0.5		
	698.5	0.7		
	702	0.6		
	707.5	0.8	1.9	1.7
	708	1.0	2.5	
	709		2.4	1.9
	710	2.0		
	712	1.8	2.5	1.8
	714	2.3		
	716	2.4	2.6	2.0
Synechococcus	692	0.5		
	694	0.7		
	696	0.8		
	698	0.5		
	701	0.6		
	704	0.8		
	706	0.6		
	708	0.7		
	710	1.0	1.6	
	712	1.0		
	714	1.3		
	716	2.2		
	718	2.2		
	720	2.3		
	723		2.6	

[a] *Synechocystis* values are from ref 3; *Synechococcus* values are from ref 4. [b] All values are from this work. The error is ±0.2 D.

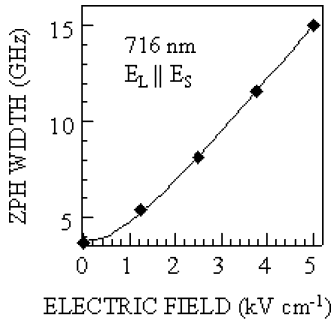

Figure 2. Dependence of the ZPH width on the external electric field for the ~6%-deep hole (Figure 1) burned at 716 nm (♦) and the best fit based on the model[22] (—). Laser polarization was parallel to the direction of the Stark field, and the burn fluence was ~0.01 J cm^{-2}. $f\Delta\mu = 2.7$ D.

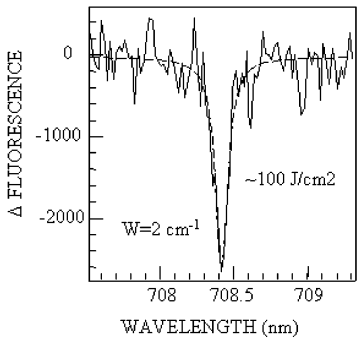

Figure 3. Experimental hole spectrum (—) and Lorentzian fit (- - -) for $\lambda_B = 708.4$ nm. Burn fluence was ~100 J cm^{-2}; fractional ZPH depth is 7−8%; $T = 10$ K.

polarization is significantly larger than for perpendicular. Also note that in earlier work the dipole moment change clearly decreased below 708 nm (to ≤1 D), whereas in the present work, the values are nearly constant over the range of the experiment.

Turning to the hole widths, there is a weak wavelength dependence and a fluence dependence over the range from 716 to 707.5 nm. For example, at 716 nm the width was 2−3 GHz for a 3%-deep hole, increasing to 6−7 GHz for a 10%-deep hole. At 709 nm, however, the width was 4−5 GHz at the lowest fluence used, with a depth of 3%. Increasing the fluence to that used to burn the 10%-deep hole at 716 nm caused a slight increase in depth to 4% while increasing the width to 8−9 GHz. Further increases in fluence did not increase the hole depth but rather caused broadening. Our inability to burn deep, narrow holes at ~708 nm and the results of Stark experiments (see above) indicate consistently that shallow holes burned in this wavelength range belong to the higher-energy side of the broad C714 band. This is the reason that $f\Delta\mu$ values obtained at ~708 nm in this work differ from those reported in ref 3. Because of the much higher burn fluence used in ref 3, the C708 state was preferentially probed in that work for burn wavelengths around 708 nm. A small contribution from the C714 state to spectral holes burned in that region was not resolved in ref 3. In the present work, the slight decrease in the $f\Delta\mu$ values with the decrease in the burn wavelength may be explained by the C708 state just starting to contribute to the spectral holes for the burn fluences used. The significant (at least an order of magnitude) difference in hole-burning efficiencies for the C714 and C708 states is most easily explainable assuming different excited-state lifetimes. That the $f\Delta\mu$ value for 15-GHz (in zero field) 4%-deep ZPH at 708 nm was still close to 2 D indicates that the lifetime of the C708 state is shorter than 20 ps. Because a reliable determination of the ZPH widths larger than 15 GHz in high-resolution mode is somewhat problematic, we switched to a lower-resolution (6 GHz) mode to determine the lifetime of the C708 state precisely. Using a broader burn laser (2 GHz) and fluences such as those used in refs 3 and 4 (up to 100 J cm^{-2}), a 10%-deep hole with a width of 1.7 cm^{-1} was obtained at 707 nm. (The widths given so far were all obtained at 2 K.) The broad holes burned in the C708 band exhibited a slow increase of the homogeneous ZPH width with temperature. This indicates that the ZPH width is, in the case of the C708 band, determined mainly by a temperature-independent process (most likely energy transfer, see Discussion), with some contribution from dephasing. An example of a 2.0-cm^{-1}-wide hole burned at 708.4 nm at 10 K is shown in Figure 3. For the C714 band ($\lambda_B = 715-716$ nm), the ZPHs broadened according to a $T^{1.3}$ power law over the range from 2 to 14 K (results not shown). Thus, the width of the ZPHs burned into the C714 band is determined by TLS-assisted dephasing only.

4. Discussion

A. Stark Hole-Burning Results. Under high resolution and optimum conditions, Stark splitting of the ZPH burned into the red-most C714 and C719 absorption bands of *Synechocystis* and *Synechococcus* was not observed. The only photosynthetic pigment−protein complex in which splitting has been observed in the Stark hole-burning spectrum is the Fenna−Matthews−Olsen (FMO) protein.[8] FMO is a trimeric antenna complex from green sulfur bacteria. Each of the three proteins that make up the intact complex contain seven bacteriochlorophyll *a* molecules. Coupling between BChl *a* belonging to different proteins is weak, and the lowest-energy absorption band (825 nm) of the complex is due to a single BChl *a* in each protein. Because coupling between these three BChl *a* molecules is weak, the 825-nm band is the sum of three transitions that would be degenerate except for structural disorder. The properties of this state are nearly ideal for the observation of splitting in the Stark hole spectra from the viewpoint of the relevant theories,[19−21] according to which hole splitting is best resolved when the angle, γ, between the transition dipole moment, **D**, and $\Delta\boldsymbol{\mu}$ is ~0° and the laser polarization, $\mathbf{E}_L$, is parallel to $\mathbf{E}_S$, the Stark field. Such was the case for the FMO complex. In ref 8, it was estimated that $\gamma < 15°$. Furthermore, the Stark broadening that occurs along with splitting was small, so the splitting was more easily resolved. Also, as is often the case when excitonic coupling is weak, the electron−phonon coupling is also weak for the FMO system, so a ZPH could be burned with a high signal-to-noise ratio. Finally, inhomogeneous broadening is relatively small in FMO (~70 cm^{-1}), which correlates with smaller contributions from the random components of $\Delta\boldsymbol{\mu}$(vide infra).

Turning to the lack of ZPH splitting in this work for the red-most antenna states of *Synechocystis* and *Synechococcus*, let us consider first the source of the hole broadening induced by the Stark field. The only broadening mechanism included in the theories in their simplest form (see Figure 6 by Kohler et al. or Figure 2 by Schatz and Meier) is broadening due to $\cos^2 \upsilon$ distribution of the transition dipoles of the burned molecules (where υ is the angle between the transition dipole **D** and the laser polarization $\mathbf{E}_L$). This broadening is always present, even if $\Delta\boldsymbol{\mu}_{ind} = 0$. According to this simplified theory, splitting should be observed for one laser polarization or the other, although it should be less resolvable for $\gamma \approx 90°$ than for $\gamma \approx 0°$. Following Schätz and Maier[20] (a less detailed discussion, without the calculation of sample hole spectra, is given also in refs 19 and 21), we write the matrix-induced term as

$$\Delta\boldsymbol{\mu}_{ind} = \Delta\boldsymbol{\alpha}\cdot\mathbf{E}_{int} \tag{2}$$

where $\mathbf{E}_{int}$ is the field induced at the position of the chromophore by the surrounding lattice. $\Delta\boldsymbol{\mu}_{ind}$ can be represented as a sum of fixed (in the frame of chromophore molecules) and random contributions:

$$\Delta\boldsymbol{\mu}_{ind} = \Delta\boldsymbol{\mu}_{ind,fixed} + \Delta\boldsymbol{\mu}_{ind,random} \tag{3}$$

The angle γ between **D** and $\Delta\boldsymbol{\mu}_0$ is considered to be well defined. It is obvious from eqs 1−3 that if $\Delta\boldsymbol{\mu}_{ind,random}$ is dominant over $\Delta\boldsymbol{\mu}_{fixed} = \Delta\boldsymbol{\mu}_{ind,\,fixed} + \Delta\boldsymbol{\mu}_0$ then no hole splitting is expected for any polarization, only broadening. (This is the case treated by Kador et al.[22] for small variance in the magnitude of the randomly oriented dipole moment change.) A less ob-

vious result follows from the detailed simulations by Schätz and Maier:[20] a lack of splitting can also occur even if $\Delta\boldsymbol{\mu}_{\text{ind,random}}$ is not dominant. For example, for **D** and $\Delta\boldsymbol{\mu}_{\text{fixed}}$ approximately perpendicular, splitting is not observable for $|\Delta\boldsymbol{\mu}_{\text{fixed}}|/|\Delta\boldsymbol{\mu}_{\text{ind,random}}| = 2$. (See Figure 8C in ref 20.) As mentioned above, in photosynthetic complexes the orientation of chlorophylls in relation to the protein is approximately fixed, and $\Delta\boldsymbol{\mu}_{\text{ind,random}}$ is not expected to be large. However, because of structural disorder, it is not expected to be zero either. (The rough analysis given below indicates that random and fixed contributions may be of comparable magnitude for C714 of *Synechocystis*, even if the results are interpreted so as to maximize $\Delta\boldsymbol{\mu}_{\text{fixed}}$.) Significant static inhomogeneous broadening ($\sim$50−200 cm^{-1}) of Chl $S_1(Q_y) \leftarrow S_0$ absorption bands is generally observed in photosynthetic complexes. This broadening may be treated in terms of the solvent shift and expressed through the same variables as used above:[19]

$$\Delta\omega_{\text{solvent shift}} = -\hbar^{-1}\left(\Delta\boldsymbol{\mu}_0\cdot\mathbf{E}_{\text{int}} + \frac{1}{2}\mathbf{E}_{\text{int}}\cdot\Delta\boldsymbol{\alpha}\cdot\mathbf{E}_{\text{int}}\right) \quad (4)$$

The solvent shift depends only on the parameters of the chromophore ($\Delta\boldsymbol{\mu}_0$ and $\Delta\boldsymbol{\alpha}$) and the "pocket field" $\mathbf{E}_{\text{int}}$. It is not dependent on the external field. On the basis of eq 4, the inhomogeneous broadening is positively correlated with the degree of orientational and translational disorder and with the magnitude of the random contribution to $\mathbf{E}_{\text{int}}$, resulting in a large $\Delta\mu_{\text{ind,random}}$ value. We emphasize that the inhomogeneous broadening for the C714 state of PS I of *Synechococystis* is unusually large, $\sim$300 cm^{-1},[23] whereas the lowest-energy band of the FMO complex consists of three quasidegenerate bands, each with a width of only $\sim$70 cm^{-1}.

Next, taking into account that C714 is the lowest exciton component of a dimer, the frequency of the electronic transition for the C714 state is determined not only by diagonal disorder (described in the previous paragraph as differences in solvent shifts) but also by off-diagonal disorder, which is due to differences in the interpigment couplings from complex to complex. These differences result from the variations in distance and mutual orientation of the chlorophylls of the dimer. The distribution of orientations of the pigments in relation to each other results in the distribution of the angle γ between **D** and $\Delta\boldsymbol{\mu}_0$ as well as distributions of the magnitudes of both vectors. (Here **D** and $\Delta\boldsymbol{\mu}_0$ are redefined as the transition dipole moment of the lowest state and the permanent dipole moment change, respectively, for a "free" dimer.) In other words, in the case of a dimer in a glasslike protein both $\Delta\boldsymbol{\mu}_0$ and $\Delta\boldsymbol{\mu}_{\text{ind}}$ have a random component.

$$\Delta\boldsymbol{\mu}_0 = \Delta\boldsymbol{\mu}_{0,\text{fixed}} + \Delta\boldsymbol{\mu}_{0,\text{random}} \quad (5)$$

It is quite likely that the components of the polarizability difference tensor $\Delta\boldsymbol{\alpha}$ for the lowest state of the dimer are also subject to a distribution for the same reasons that there is a distribution of $\Delta\boldsymbol{\mu}_0$. We stress that whereas for a monomeric chromophore the origin for a distribution of $\Delta\boldsymbol{\mu}_{\text{ind}}$ is a distribution of $\mathbf{E}_{\text{int}}$ only, for the dimer there are distributions of both $\mathbf{E}_{\text{int}}$ and $\Delta\boldsymbol{\alpha}$.

The quantity observable in Stark hole-burning experiments is the magnitude of

$$\Delta\boldsymbol{\mu} = \Delta\boldsymbol{\mu}_{0,\text{fixed}} + \Delta\boldsymbol{\mu}_{0,\text{random}} + \Delta\boldsymbol{\mu}_{\text{ind,fixed}} + \Delta\boldsymbol{\mu}_{\text{ind,random}} \quad (6)$$

The right side of eq 6 is a vector sum, so fixed and random contributions can be grouped together. If the fixed components fully canceled each other, then splitting would be unobservable for any angle γ, and the values of $f\Delta\mu$ would be equal for both polarizations. Although hole splitting is not resolved in our experiments, the observations indirectly suggest that splitting may be present but hidden by the broadening. First, the difference in $f\Delta\mu$ values for different polarizations observed here but not in ref 3 or 4 may be due to splitting for parallel polarization. It has also been observed that the $f\Delta\mu$ values obtained from the shallowest holes (less reliable) are systematically larger than those obtained from more saturated holes. For example, values of 2.9 and 2.2 D were obtained for parallel and perpendicular polarizations, respectively, for a 3%-deep hole burned at 716 nm. If these values are correct, then they may indicate the presence of a fixed contribution to $f\Delta\boldsymbol{\mu}$ that gradually becomes unobservable with the loss of ensemble anisotropy by hole saturation. To be more specific, we present an alternative analysis of the hole spectra from Figure 1. The bottom of the 5 kV cm^{-1} hole shown is slightly flattened, which may indicate the onset of hole splitting. Although a good fit is obtained by fitting this hole to a single Lorentzian, a similarly good fit can also be obtained by using two 10-GHZ-wide Lorentzians separated by 6 GHz. From such a shape, a rough estimate of $f\Delta\mu_{\text{fixed}}$ is 1.2 D for zero angle between $f\Delta\boldsymbol{\mu}_{\text{fixed}}$ and **D** based on $\Delta\omega_{\text{split}} = f\Delta\mu E_S \cos\varphi$, with φ being the angle between $\Delta\mu$ and $\mathbf{E}_S$. If $\mathbf{E}_S||\mathbf{E}_{\text{laser}}||\mathbf{D}$, then $\cos\varphi \approx 1$, and an estimate of $f\Delta\mu_{\text{random}}$ is $\sim$1.8 D, according to the theory of Kador et al.[22] This value agrees well with those measured for the perpendicular polarizations. Note, however, that because $\Delta\boldsymbol{\mu}_{\text{random}} = \Delta\boldsymbol{\mu}_{0,\text{random}} + \Delta\boldsymbol{\mu}_{\text{ind,random}}$ the condition of small variance in the magnitude of $\Delta\boldsymbol{\mu}_{\text{random}}$ may not be satisfied. There is also no reason to believe that the angle between $\Delta\boldsymbol{\mu}_{\text{fixed}}$ and the transition dipole moment **D** is indeed zero. An $\sim$25° angle was initially estimated by Lockhart and Boxer using Stark modulation spectroscopy for the lowest state of the special pair of the bacterial RC.[24] This angle increased to $\sim$38 and $\sim$45° with subsequent refinements of the analysis.[11,25]

To explain the "real" lack of splitting, one may assume, in addition to large fluctuations in $\Delta\boldsymbol{\mu}_0$, $\Delta\boldsymbol{\alpha}$, and $\mathbf{E}_{\text{int}}$, that the angle γ' (between **D** and $\Delta\boldsymbol{\mu}_{\text{fixed}}$) is closer to 90 than to 0°. As mentioned above, in the latter case splitting is less pronounced even for $\Delta\boldsymbol{\mu}_{\text{random}} = 0$. However, $\gamma' \approx 90°$ would be in contradiction to the broadening of the hole being faster for parallel laser polarization and an external field direction.

Some discussion on recent Stark modulation spectroscopy results by Frese et al.[26] is also in order. Frese et al. performed their experiments at 77 K in magic-angle configuration, which eliminates any dependence on γ and on the precise shape of $\Delta\boldsymbol{\alpha}$. They observed that Tr($\Delta\boldsymbol{\alpha}$) = 600 Å^3 f^{-2} for C719 of *Synechococcus* and that Tr($\Delta\boldsymbol{\alpha}$) = 275 Å^3 f^{-2} for the red antenna band of *Synechocystis.* (The two different states were not resolved.) First, because Frese et al. did not resolve the C708 and C714 bands of *Synechocystis*, the value of 275 Å^3 f^{-2} is most likely an average value for the two states, and the value for C714 alone should be closer to 600 Å^3 f^{-2} as measured for *Synechococcus*. Previous hole-burning spectroscopy results have shown that properties of C714 of *Synechocystis* and C719 of *Synechococcus* are very similar.[3,4] The inability to resolve the C708 and C714 band is probably why a very small (compared to that of C719) dipole moment change ($f\Delta\mu = 0.4$ D) was observed for the red band of *Synechocystis*. We assume that the "correct" value for $f\Delta\mu$ for C714 obtained by modulation Stark spectroscopy would be close to that for C719 of *Synechococcus* (i.e., 3.6 D ± 15%). Random components were not considered in refs 11 and 26. Note that for $f\Delta\mu_{\text{fixed}} = 3.6$ D the hole splitting would be observed much better than it

really is. Thus, this is another example of the $f\Delta\mu$ values obtained by modulation spectroscopy exceeding those obtained with hole burning. For a pocket field on the order of 10^6 V/cm, the polarizability difference values reported by Frese et al. would yield ~2D for $f\Delta\mu_{ind}$.

A meaningful independent estimation of different components of $\Delta\boldsymbol{\mu}$ requires detailed knowledge of the nature of the charge-transfer state as well as the structure of the protein pocket. This, in turn, requires assigning the C714 state to some chlorophyll aggregate known from structure data. Such data is not available for *Synechocystis*. However, recent observations that the structure of plant PS I[27] is very similar to that of *Synechococcus* suggests that the latter structure could be used for *Synechocystis* without producing significant errors. One can argue that the dimer with the smallest Mg···Mg distance of 7.6 Å (B37−B38)[14] is the most likely candidate for the origin of the charge-transfer state. This dimer is the one for which high interpigment couplings were obtained in various approximations.[15,16] Site energies of individual chlorophylls, as estimated in ref 16, are also reasonably low. Although this dimer was rejected[15,16] as being a red state based on its lower excitonic state being less intense than the higher state, we believe that it is not necessarily a problem. Quite the opposite, it is in agreement with the total absorption strength of the red antenna states of *Synechocystis* being approximately equal to that of ~3 Chl *a* molecules and with the relative weakness of the C719 band in *Synechococcus* when the presence of the C715 state is taken into account. Recently, Gobets et al.[28] proposed that the C719 state of *Synechococcus* originates from the B31/32/33 trimer. However, such an interpretation ignores the fact that several properties of C719 of *Synechococcus* are essentially identical to those of C714 of *Synechocystis*,[3,4] which does not contain a trimer. These properties are $f\Delta\mu$, the linear pressure shift rate, electron−phonon coupling, and static inhomogeneous broadening. Although agreement in assigning the red-most state to a particular dimer has definitely not been reached, it is widely accepted that the red antenna states are the lowest exciton states of strongly coupled chlorophylls.

B. Energy Transfer between the Red Antenna States. Next, we consider the hole widths. The $T^{1.3}$ dependence of the ZPH width Γ_{ZPH} is commonly seen for the dephasing of chromophores in disordered solvents.[29,30]

$$\Gamma_{ZPH}(T) = \Gamma_{ZPH}(T = 0) + aT^{1.3} \qquad (7)$$

It is not surprising to see this dependence for the C714 state because the holes are burned by a nonphotochemical mechanism, which also depends on the protein matrix being disordered. Similar behavior was observed for the lowest-energy states of several photosynthetic complexes. The values of the prefactor *a* were 0.2 GHz/K$^{1.3}$ for FMO,[31] 0.2 GHz/K$^{1.3}$ for CP-43,[7] 0.35 GHz/K$^{1.3}$ for PS-II-RC,[32,33] ~3 GHz/K$^{1.3}$ for the B870 band of LH-2,[34] and ~1 GHz/K$^{1.3}$ for LHC-II.[35] (The latter two values were, however, obtained with low spectral resolution.) $a = 0.9$ GHz/K$^{1.3}$ observed for the C714 band of the *Synechocystis* PS-I lies within the same range.

Of more interest is the behavior of the holes near 708 nm. First, there are two possible explanations of why burning deep (ZPHs with a fractional depth of ~15% and a width of ~2 cm^{-1} were burned at 706−708 nm by Rätsep et al.[3]), narrow holes at ~708 nm is impossible in fluorescence excitation mode. According to the first scenario, the C708 state does not transfer energy to C714 state. In this case, the C708 state must contribute much less to the fluorescence excitation spectrum than to the absorption spectrum. (Note that the electron−phonon coupling is smaller for C708 than for the C714 state. In the absence of energy transfer, the emission from the C708 state would peak at ~710 nm and be detected much less efficiently than that from the C714 state with our experimental setup, including a >730-nm cutoff filter.) Figure 4 represents the broad-range fluorescence excitation spectrum of *Synechocystis* PS I. It is obvious from Figure 4 that at wavelengths longer than ~695 nm the fluorescence excitation spectrum (dashed line) is a perfect match for the absorption spectrum (solid line). Thus, the C708 does contribute to the fluorescence excitation spectrum. (The discrepancies at shorter wavelengths may be real and reflect the fact that part of the energy harvested by antenna pigments is not transferred to the C714 state. Alternatively, these discrepancies may be an experimental artifact due to the optical density of the sample that is too high at these wavelengths, causing reabsorption effects.) Note also that the nonline-narrowed emission spectrum of PS I of *Synechocystis* peaks at ~722 nm[3] and does not contain any noticeable shoulder at ~710 nm, where the emission from the C708 state would be expected. Finally, the observation that it is possible to burn ~10%-deep ZPHs, though broad, at 708 nm in fluorescence excitation mode also argues against the C708 state not contributing to the fluorescence excitation spectrum.

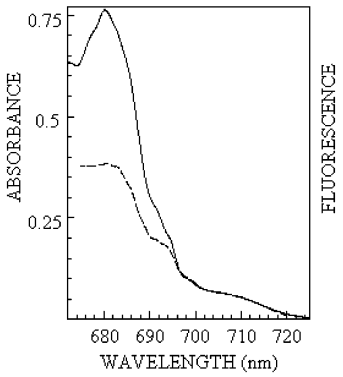

Figure 4. Absorption (—, from ref 3) and fluorescence excitation (- - -, this work) spectra of the PS I from *Synechocystis*. $T = 5$ K.

Consequently, we reject the above scenario and conclude that the C708 → C714 energy transfer does occur and that it is fast, which results in hole-burning efficiency for the C708 state being orders of magnitude lower than for the C714 state. Thus, the failure to burn deep holes at ~708 nm at low fluence is explained, attributing the observed shallow, narrow holes to the blue edge of the C714 band rather than to the C708 band. According to ref 23, about 20% of the absorption at 708 nm is due to C714, which is consistent with being able to burn only ~4% ZPH, given the Huang−Rhys factor for the C714 state. For higher fluence and a 2-GHz laser bandwidth, the observed ~10% holes, with widths of ~1.7 cm^{-1} at 2 K, are no doubt due to burning into the C708 band, followed by energy transfer to the C714 state. The hole width is determined by the energy-transfer time, ~6 ps. The presence of such an energy transfer is additional proof of the existence of two distinct red antenna states in *Synechocystis* PS I.

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