Red Pool Chlorophylls of Photosystem I of the Cyanobacterium *Thermosynechococcus elongatus*: A Single-Molecule Study†

Alexandra F. Elli,*,‡ Fedor Jelezko,‡ Carsten Tietz,‡ Hauke Studier,§ Marc Brecht,§ Robert Bittl,§ and Jörg Wrachtrup‡

Physikalisches Institut, Universität Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany, and Fachbereich Physik, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany

Received October 24, 2005; Revised Manuscript Received December 9, 2005

ABSTRACT: Photosystem I reaction centers of the cyanobacterium *Thermosynechococcus elongatus* have been investigated using single-molecule spectroscopy. Single-molecule fluorescence emission spectra reveal a new fluorescence band located at 745 nm. Fluorescence polarization spectroscopy and fluorescence autocorrelation analysis show that only a few chlorophylls are responsible for the photoemission from the Photosystem I trimer at low temperature. Intersystem crossing parameters of the red pool chlorophylls have been determined via fluorescence autocorrelation measurements. The triplet yield of the red chlorophylls is strongly reduced in comparison to chlorophyll *a* in solution. Strong quenching of the triplet state indicates that the red chlorophylls are located in close contact to carotenoids.

Photosystem I (PSI), a pigment−protein complex, is one of two photosystems that are responsible for the conversion of light energy into chemical energy in oxygenic photosynthesis. Under illumination, PSI transfers electrons from plastocyanin or cytochrome *c*6 to ferredoxin or flavodoxin, respectively. In 2001, the structure of the PSI complex was resolved with a resolution of 2.5 Å (*1*). The knowledge of the PSI structure with atomic resolution provides detailed information on the positions and orientation of the 96 chlorophyll (Chl) molecules responsible for light absorption, energy transfer, and charge separation. Structural data by themselves do not provide exact information on the energy transfer within the PSI complex, because not only the protein environment determines absorption bands of chlorophylls, but also dipole−dipole interactions between pigments influence the spectral behavior of the chromophores. Hence, additional theoretical and experimental work is required for a detailed understanding of the connection between photophysical properties of the PSI complex and structural data.

One of the most intriguing spectral features of the PSI complex is the presence of so-called red chlorophylls or red pool chlorophylls. This group of pigments shows an absorption band, which is red-shifted with respect to the absorption of the primary donor P700. Although the number of those pigments is small, they strongly affect the energy transfer and trapping in PSI reaction centers. At first glance, the energetic position of the red chlorophylls seems to be incompatible with an efficient energy trapping by the primary donor. Recent studies indicate that red pigments may play an important role in maintaining an efficient energy equilibration within PSI complexes (*2*). It was suggested that those pigments act as intermediate reservoirs, which funnel energy toward the reaction center and increase the efficiency (*3*). Having red pool chlorophylls additional to the bulk chlorophylls increases the cross section for the absorption to larger wavelengths (*4*), giving a biological advantage to the organism under shadowy conditions (*5*). It is also possible that the existence of the red chlorophylls is only the consequence of the packing density of the Photosystem I antenna (*6*).

The number and spatial location of these red pigments has remained controversial. Studies based on absorption spectroscopy indicate that four to five pigments contribute to the 708 nm and five to six pigments to the 719 nm pool (numbers are given per monomer), which make up the red absorption bands of PSI from cyanobacterium *Thermosynechococcus elongatus* (*7*). Hole-burning studies show a third low-energy antenna state at 715 nm in photosystem I of *T. elongatus* (*8*). These red chlorophylls are strongly coupled chlorophylls with a significant charge-transfer character (*8*−*11*). It has been concluded that a trimer of chlorophylls is responsible for the band at 708 nm and chlorophyll dimers are responsible for the absorption bands at 715 and 719 nm (*8*, *10*, *11*). Besides giving information about the number of red pool chlorophylls, results of the hole-burning experiments roughly indicate the location of the red chlorophylls. The red chlorophylls are bound to PsaA or PsaB and are located near the interface of the heterodimer PsaA and PsaB and the subunits PsaL and PsaM (*8*, *10*). Jordan et al. (*1*) suggested a localization of some red chlorophylls in the trimerisation region. But a clear assignment of the red pool chlorophylls to the 90 antenna chlorophylls based on experimental data is still not possible yet.

Quantum chemical calculations have been performed to identify the red chlorophylls. Calculations based on excitonic coupling alone (without taking into account the difference of chlorophyll site energies) are not able to describe the low-

† This work was supported by Volkswagenstiftung in the framework of the program "Physics, chemistry and biology with single molecules".

* To whom correspondence should be addressed. E-mail, A.Elli@physik.uni-stuttgart.de; phone, ++49-711-685-4868; fax, ++49-711-685-5281.

‡ Universität Stuttgart.

§ Freie Universität Berlin.

10.1021/bi0521700 CCC: $33.50
Published on Web 01/13/2006

temperature spectra of the red pool adequately (*12*). Calculations of chlorophyll site energies based on structural data have been performed recently (*13*, *14*). Although these calculations reproduce the main features of the bulk chlorophyll absorption spectrum well, they are not able to assign the red chlorophylls. Hence, experimental studies providing detailed information on site energies, number of these chlorophylls, and coupling between red chlorophylls are of considerable importance. Detailed experimental investigations using standard fluorescence spectroscopy are difficult because of the heterogeneity of PSI complexes resulting in featureless spectra of bulk PSI. This obstacle can be avoided by using site-selective spectroscopic techniques such as spectral hole burning (*11*) and single-molecule spectroscopy (*15*).

Single-molecule detection is the unique method that allows prevention of the ensemble averaging completely. Hence, this approach gives access to an accurate determination of photophysical parameters instead of getting the ensemble average of these values. This is especially important for photosynthetic proteins where the heterogeneity of pigments can be attributed to the interaction within the PSI complex as well as to the heterogeneity between different PSI complexes. Corresponding investigations are also done on other pigment protein complexes such as LH1 (*16*, *17*), LH2 (*18*−*20*), and LHCII (*21*). Single-molecule spectroscopy of PSI complexes has been already proven to be a powerful technique resolving fluorescence bands of individual chlorophyll pools. The two chlorophyll pools responsible for the low-temperature emission of PSI trimers have been investigated in a previous paper (*22*). The present paper addresses the question of the number of chlorophylls accountable for the red pool fluorescence and their photophysical properties.

EXPERIMENTAL PROCEDURES

The main part of the setup consists of a low-temperature confocal microscope with a high numerical aperture objective lens (NA = 0.85, Mikrotek) placed in a liquid helium bath cryostat operating at $T = 2$ K. The detection yield of the setup is about 0.5%. Individual PSI complexes have been excited using a CW dye laser (Coherent 699, line width 1 cm^{-1}) operating at 672 nm. The sample was scanned in a two-dimensional plane by the laser beam using a beam scanner (General Scanning, Inc.); the fluorescence light is refocused via the same microscope objective, filtered from the excitation light by a holographic Notch Filter (675 Notch Plus, Kaiser) and an interference long pass filter (LP 679, Omega Filters), and directed to two avalanche photodiodes (APD-SPCM-14, Perkin-Elmer). The two-detectors photon-counting scheme allows the analysis of the fluorescence autocorrelation function in a time scale of nanoseconds. For this purpose, a multiscaler photon counter card (B&H, MSA 300) was employed. Fluorescence emission spectra were recorded using a 30 cm imaging spectrograph (Acton Research, Inc.) equipped with a back-illuminated CCD camera (Rooper Scientific; model 100EB).

Trimeric PSI complexes containing 96 Chl/P700 were isolated from the thermophilic cyanobacterium *T. elongatus* as described by Witt et al. (*23*). The purified PSI complexes were suspended in a buffer containing 100 mM glycine, pH 10, and 0.02% β-DM (*n*-dodecyl-β-maltoside). To avoid an accumulation of oxidized P700, sodium dithionite and phenazine methosulfate (PMS) were added to a final concentration of 50 mM and 10 μM, respectively. The sample preparation was carried out at room temperature and under green light. The sample was incubated in darkness for at least 15 min before glycerol was added as a cryoprotectant to a final concentration of about 50% (v/v). Finally, the sample was frozen in darkness. Under these conditions, the terminal iron−sulfur clusters F_A and F_B should be prereduced and a cyclic electron transfer in the electron-transfer chain of PSI should be guaranteed. A careful control of the pH and the redox conditions is necessary for time-resolved measurements, since the fluorescence dynamics of PSI depends on its charge state. A typical excitation intensity used in the experiments was 10 μW yielding a fluorescence signal of 10^4 counts/s. Taking into account the detection efficiency of the setup ($\sim$ 0.5%), we expect excitation rates of $\approx 2 \times 10^6$ s^{-1}. Under these conditions, the excitation rate is much larger than the recombination rate of the phylloquinone acceptor A_1^-. This means that the electron occupies the A_1^- acceptor most of the time. Excitation intensity up to 600 μW was used in the fluorescence autocorrelation experiments. The integrity of PSI complexes under high illumination conditions was verified by measuring the fluorescence emission spectra. The fluorescence spectra were found in agreement with those measured under significance lower excitation intensities (as, e.g., in ref *22*).

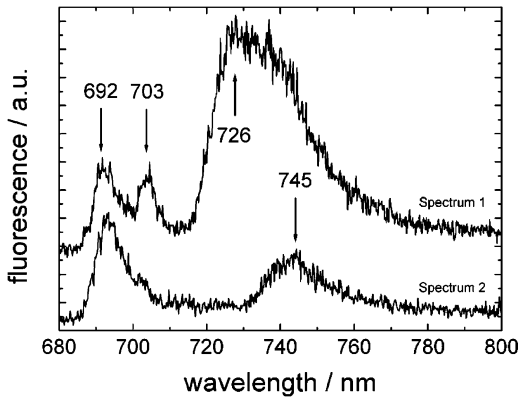

FIGURE 1: Evolution of single PSI fluorescence spectra during photobleaching. Two consecutive low-temperature spectra (1 and 2) are vertically shifted for clarity. Spectrum 1 was recorded immediately after positioning the laser excitation spot on the PSI complex. Spectrum 2 was recorded after 300 s of intense PSI illumination. Acquisition time was 10 s. Note stepwise-photobleaching of several red-pigment pools in spectrum 2. The chlorophyll pools are marked with arrows.

RESULTS AND DISCUSSION

Single PSI complexes can be detected via fluorescence emission from the long-wavelength chlorophylls at cryogenic temperatures because of an increased fluorescence quantum yield related to trapping of excitation by the red chlorophylls (*22*). At low temperatures, a significant part of the excitation energy does not reach the primary donor (*24*), whereas at room temperature, the energy transfer from long-wavelength antenna pigments to P700 is reportedly very efficient (*4*, *25*). Spectrum 1 in Figure 1 shows the fluorescence spectrum of a single PSI complex at $T = 2$ K; spectrum 2 in Figure 1 shows the fluorescence spectrum of the same single PSI complex recorded after about 300 s of continuous illumina-

tion. In spectrum 1, three fluorescence emission bands are visible: 692, 703, and 726 nm. Note that the red-most band contains a pronounced wing and extends up to 760 nm. Single-molecule PSI spectra are highly heterogeneous, and the position of the spectral pools varies about 5 nm from one complex to another. Hence, the previously reported ensemble analysis indicates either the presence of two Chl pools at 708 and 719 nm (*7*) or three Chl pools at 708, 715, and 719 nm (*8*), respectively, which is the result of the averaging over an inhomogeneously broadened absorption spectra. The line width of the spectral bands corresponding to different Chl pools varies strongly. The two red-most bands in our spectra are broad, whereas the two bands of higher energy (692 and 703 nm) show rather narrow lines (spectrograph resolution limited, smaller than 3 cm^{-1}) for short acquisition time (data not shown). These narrow spectral lines exhibit a pronounced spectral diffusion resulting in a bandwidth around 3−5 nm for acquisition times ≥ 10 s.

Unlike the results of an earlier work carried out with proteins treated at pH = 7 (*22*), prereduced proteins fixed in a glycerol/buffer glass show changes in the fluorescence emission spectrum after illumination. Selective bleaching of some Chl pools is visible in spectrum 2: the group of chlorophylls at 692 nm is unaffected by strong laser illumination, whereas the pools located at around 703 and 726 nm are bleached. A new, previously unreported Chl pool emitting at 745 nm becomes visible. Probably this group of chlorophylls also contributes to the spectrum before photobleaching (Figure 1; spectrum 1), but it becomes obvious only after bleaching of the intense 726 nm Chl pool. This band is broad, indicating a strongly coupled state (*8*−*11*). The fact that this spectral site was not identified in the deconvolution of an ensemble absorption spectrum can be related to the weak oscillator strength of the corresponding transition.

To obtain more detailed information about the number of chlorophylls responsible for the red pool emission, a fluorescence polarization analysis for the red chlorophylls of PSI trimers was carried out. The degree of polarization of the emitted light characterizes the number and the relative orientation of emitters in the complex. For a single emitter, the fluorescence light is linearly polarized. When several uncoupled, emitting chromophores are present, the degree of polarization is reduced compared to a single chromophore, unless all emitters are collinear. The degree of polarization of the distinct Chl pools was determined by using a rotating polarizer in front of the spectrometer. Figure 2 shows the dependence of the detected fluorescence emission spectrum as a function of the polarizer orientation. The data indicate a high degree of polarization of the emitted light (visible as deep modulation of fluorescence intensity when the polarizer is rotated). Note that previous ensemble studies indicate that 4−5 chlorophylls per monomer contribute to the 708 nm band (we can tentatively assign the 710 nm band of this complex to the previously reported 708 nm band). A strong polarization of the fluorescence emission shows that either the fluorescence is emitted by a number of chlorophylls with parallel transition dipole moments or the emission is originating from a single emitting state. The latter requires an efficient (faster than the fluorescence decay time) energy transfer between the 708 nm chlorophylls of different monomeric subunits to the lowest trap state of the PSI trimer.

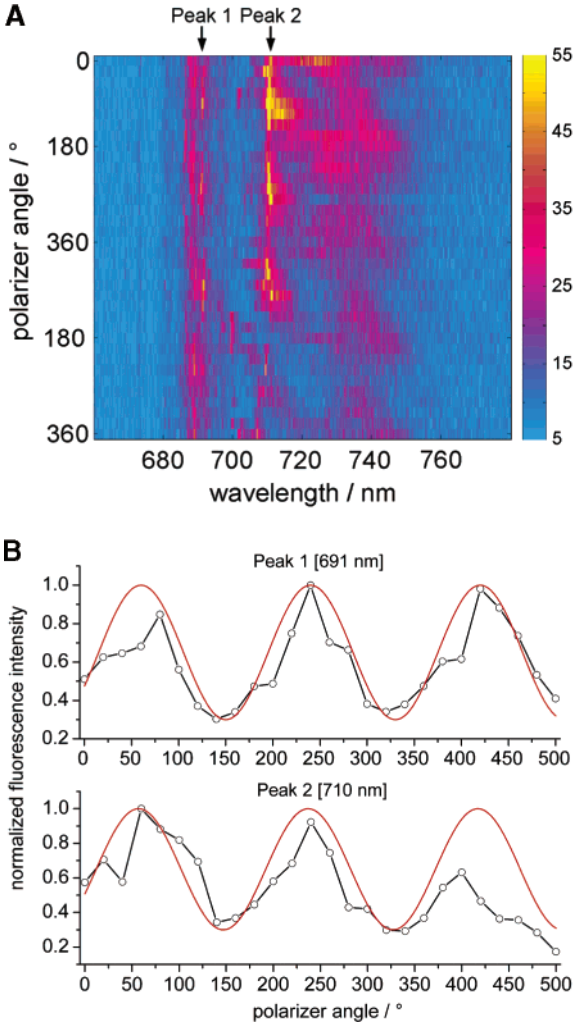

FIGURE 2: (A) Trail of fluorescence emission spectra of a single PSI complex as a function of the orientation of the polarizer. The acquisition time was 10 s for each spectrum. The angle of the polarizer is shown on the left side. The fluorescence intensity is encoded in the color scale. (B) Integrated intensity of the 691 and 710 nm pools as a function of the orientation of the polarizer.

Narrow spectral lines in the bulk antenna spectral region (691 nm band) are visible in the majority of complexes. This indicates the presence of weakly coupled chlorophylls, which are not able to transfer excitation energy to the red pool chlorophylls. Note that those lines are linearly polarized (see Figure 2). This indicates that the fluorescence emission comes from few strongly emitting, uncoupled or weakly coupled molecules rather than from a bulk of chlorophylls. Those weakly coupled chlorophylls may still efficiently funnel energy toward P700 at physiological conditions. At room temperature, the emission bands become broader which may possibly lead to a better spectral overlap between the fluorescence band of these antenna chlorophylls and the absorption band of P700. This improves the energy transfer efficiency.

To obtain more details on the number and the photophysical properties of the fluorescent chlorophylls, the statistics of the photon emission from single PSI complexes were investigated using fluorescence correlation spectroscopy (FCS). FCS is an experimental technique which is able to unravel dynamic molecular events based on statistical analysis of the fluctuations of the fluorescence originated

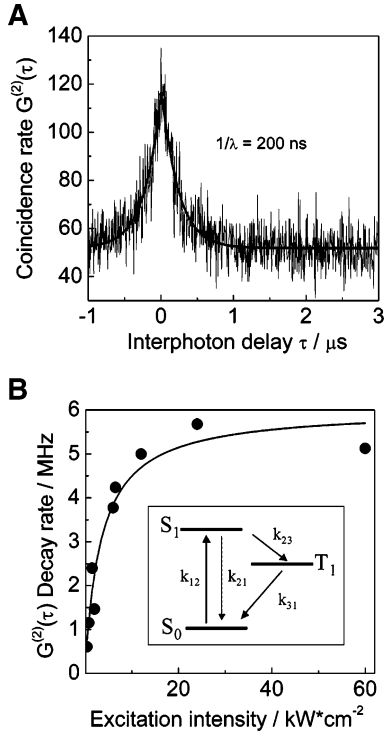

FIGURE 3: (A) Fluorescence intensity autocorrelation function of a single PSI complex. The autocorrelation function was recorded for the whole fluorescence band of wavelengths longer than 690 nm. The fit function represents a single-exponential decay. Although three different rates corresponding to transitions toward *X*, *Y*, and *Z* are expected, experimental data can be adequately fitted with a single-exponential law. This is possibly related to a low ISC rate toward the *X* and *Y* triplet sublevels. (B) Excitation power dependence of the decay rate of the correlation function. Data has been fitted with the following fit-function: $\lambda = k_{31} + k_{31}\cdot I/I_S\cdot(1/(1 + (2k_{31}/k_{23})\cdot I/I_S))$ (*28*), where I is the excitation intensity and I_S the saturation intensity. The fit gives the value of $k_{23}^{-1} = 170$ ns. The inset shows the relevant energy level scheme of a chlorophyll molecule.

by chromophores. When this technique is applied to immobilized chromophores, FCS gives access to internal dynamics of dye molecules (such as intersystem crossing yield and triplet lifetime). Usually, a dye molecule can be described in terms of a three-level system including ground and excited singlet states and a metastable triplet state. Under optical excitation, the fluorescence emission of single molecules shows interruptions in the fluorescence signal related to intersystem crossing events. Such fluctuations of the fluorescence intensity can be analyzed using a second-order fluorescence intensity correlation function, $G^{(2)}(\tau) = \langle I(t)I(t+\tau)\rangle$, where *I(t)* is the fluorescence intensity and the averaging is over time.

The fluorescence correlation function is defined as the conditional probability density $G^{(2)}(\tau)$, which corresponds to the probability to detect a photon at time $t + \tau$ when another photon was detected at time t. For a light source, which exhibits a Poissonian photon statistics, the photons are homogeneously distributed in time and the correlation function is flat. Deviations from a Poissonian distribution of photons can be considered as a characteristic feature of single-molecule fluorescence (*26*, *27*). Under continuous illumination, single chlorophylls can be excited from the ground singlet state to the first excited singlet level (see energy level scheme in Figure 3). A radiative decay of this state results in the emission of fluorescence photons (this corresponds to the "on" state in the detected photon stream). If a metastable triplet state is present, the molecule may escape to this state. When trapped in an excited triplet state, the chlorophyll cannot be excited (that corresponds to the "off" state in the detected photon flow) before the molecule relaxes back to the ground singlet state. This process leads to a "bunching" of fluorescence photons resulting in an exponential decay of the correlation function. The decay rate λ approaches $k_{23} + k_{31}$ for high excitation intensities and k_{31} for low intensity (*28*). k_{23} is the population rate (intersystem crossing rate), and k_{31} is the depopulation rate of the triplet state.

Figure 3A shows a correlation function of a single PSI complex at $T = 2$ K. The superpoissonian (bunching) term displays a characteristic decay time τ of 200 ns. The decay time decreases with an increasing laser power (see Figure 3B), approaching the value of 170 ns at full saturation. Usually, the population rate of the triplet state k_{23} is much larger than the depopulation rate of the triplet state k_{31} in organic molecules; hence, the measured saturated decay rate λ corresponds to the intersystem crossing rate k_{23} of the pigments responsible for the fluorescence emission. Taking into account the fluorescence lifetime of Chl *a* (~1.5 ns) (*29*), an intersystem crossing yield $\phi_{ISC} = 0.01$ can be estimated. This value is lower than the reported intersystem crossing yield $\phi_{ISC} = 0.64$ for monomeric chlorophyll *a* in solution (*30*). Note, the triplet quantum yield can be strongly affected by the pigment environment and can be as low as 5×10^{-3} as has been reported recently (*31*). The bunching decay time τ at low laser power tends to the microsecond range, which at zero power limit corresponds to the triplet state lifetime. This decay time is at least 2 orders of magnitude shorter than the triplet decay of chlorophyll *a* in solution (0.6 ms) (*31*), indicating an efficient quenching by carotenoids. Such quenching involves triplet energy transfer and occurs via Dexter mechanism. Exchange coupling requires electron tunneling, and its efficiency depends exponentially on the donor−acceptor distance. Hence, our data indicate that the red chlorophylls are located in close contact to carotenoids. Modification of triplet state parameters (both, decrease of intersystem crossing rate and increase of triplet decay rate) for red chlorophylls leads to a reduced population of the triplet state. This has a photoprotective effect for the PSI complex, since a significant population of the triplet state would have a negative impact on the photostability of the PSI complex. This quenching is especially important for the red pool, because those pigments are possibly located in the focus of the energy pathway towards the reaction center. The bunching of the fluorescence photons in the nanosecond time scale was detected for prereduced and preoxidized samples (data not shown). This indicates that the bunching effect is not a result of quenching by the special pair P700.

Note that if the number of emitting states responsible for the emission is rather large, the contrast of the correlation decreases proportionally to the number of states. Hence, the observation of bunching in the fluorescence emission of single PSI complexes indicates that a limited number of emitting states contribute to the fluorescence. This, together with the linear polarization of emission of distinct pigment pools, is a sign of an efficient energy transfer between monomeric subunits of the PSI trimer. This is in good

agreement with the structure-based description of excitation transfer in PSI, which predicts 40% of intermonomer excitation transfer efficiency (*32*).

CONCLUSION

Spectroscopic properties of the red pool chlorophylls of cyanobacterial Photosystem I trimers have been investigated. Fluorescence emission spectra of prereduced Photosystem I complexes unravel a previously unknown 745 nm emission band. Polarization studies and the analysis of the photon statistics indicate that a limited number of chlorophylls are responsible for the photoemission of Photosystem I. This implies an efficient energy transfer between the monomeric subunits of the PSI trimer. Fluorescence autocorrelation data show an efficient triplet state quenching of the red chlorophylls.

ACKNOWLEDGMENT

The authors thank P. Fromme (Arizona State University) for the PSI isolation. Helpful discussions with E. Schlodder (TU Berlin) are gratefully acknowledged.

REFERENCES

1. Jordan, P., Fromme, P., Witt, H. T., Klukas, O., Saenger, W., and Krauss, N. (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 angstrom resolution, *Nature 411*, 909−917.
2. Karapetyan, N. V., Holzwarth, A. R., and Rögner, M. (1999) The photosystem I trimer of cyanobacteria: molecular organization, excitation dynamics and physiological significance, *FEBS Lett. 460*, 395−400.
3. Werst, M., Jia, Y. W., Mets, L., and Fleming, G. R. (1992) Energy-transfer and trapping in the Photosystem-I core antenna—a temperature study, *Biophys. J. 61*, 868−878.
4. Trissl, H. W. (1993) Long-wavelength absorbing antenna pigments and heterogeneous absorption-bands concentrate excitons and increase absorption cross-section, *Photosynth. Res. 35*, 247−263.
5. Rivadossi, A., Zucchelli, G., Garlaschi, F. M., and Jennings, R. C. (1999) The importance of PSI chlorophyll red forms in light-harvesting by leaves, *Photosynth. Res. 60*, 209−215.
6. Gobets, B., and van Grondelle, R. (2001) Energy transfer and trapping in photosystem I, *Biochim. Biophys. Acta 1507*, 80−99.
7. Pålsson, L. O., Dekker, J. P., Schlodder, E., Monshouwer, R., and van Grondelle, R. (1996) Polarized site-selective fluorescence spectroscopy of the long-wavelength emitting chlorophylls in isolated Photosystem I particles of *Synechococcus elongatus*, *Photosynth. Res. 48*, 239−246.
8. Zazubovich, V., Matsuzaki, S., Johnson, T. W., Hayes, J. M., Chitnis, P. R., and Small, G. J. (2002) Red antenna states of photosystem I from cyanobacterium *Synechococcus elongatus*: a spectral hole burning study, *Chem. Phys. 275*, 47−59.
9. Ihalainen, J. A., Rätsep, M., Jensen, P. E., Scheller, H. V., Croce, R., Bassi, R., Korppi-Tommola, J. E. I., and Freiberg, A. (2003) Red spectral forms of chlorophylls in green plant PSI—a site-selective and high-pressure spectroscopy study, *J. Phys. Chem. B 107*, 9086−9093.
10. Rätsep, M., Johnson, T. W., Chitnis, P. R., and Small, G. J. (2000) The red-absorbing chlorophyll a antenna states of photosystem I: a hole-burning study of *Synechocystis* sp. PCC 6803 and its mutants, *J. Phys. Chem. B 104*, 836−847.
11. Reinot, T., Zazubovich, V., Hayes, J. M., and Small, G. J. (2001) New insights on persistent nonphotochemical hole burning and its application to photosynthetic complexes, *J. Phys. Chem. B 105*, 5083−5098.
12. Sener, M. K., Lu, D. Y., Ritz, T., Park, S., Fromme, P., and Schulten, K. (2002) Robustness and optimality of light harvesting in cyanobacterial photosystem I, *J. Phys. Chem. B 106*, 7948−7960.
13. Byrdin, M., Jordan, P., Krauss, N., Fromme, P., Stehlik, D., and Schlodder, E. (2002) Light harvesting in photosystem I: modeling based on the 2.5-angstrom structure of photosystem I from *Synechococcus elongatus*, *Biophys. J. 83*, 433−457.
14. Damjanovic, A., Vaswani, H. M., Fromme, P., and Fleming, G. R. (2002) Chlorophyll excitations in photosystem I of *Synechococcus elongatus*, *J. Phys. Chem. B 106*, 10251−10262.
15. Moerner, W. E., and Orrit, M. (1999) Illuminating single molecules in condensed matter, *Science 283*, 1670−1676.
16. Gerken, U., Lupo, D., Tietz, C., Wrachtrup, J., and Ghosh, R. (2003) Circular symmetry of the light-harvesting 1 complex from *Rhodospirillum rubrum* is not perturbed by interaction with the reaction center, *Biochemistry 42*, 10354−10360.
17. Ketelaars, M., Hofmann, C., Köhler, J., Howard, T. D., Cogdell, R. J., Schmidt, J., and Aartsma, T. J. (2002) Spectroscopy on individual light-harvesting 1 complexes of *Rhodopseudomonas acidophila*, *Biophys. J. 83*, 1701−1715.
18. Tietz, C., Chekhlov, O., Dräbenstedt, A., Schuster, J., and Wrachtrup, J. (1999) Spectroscopy on single light-harvesting complexes at low temperature, *J. Phys. Chem. B 103*, 6328−6333.
19. Bopp, M. A., Sytnik, A., Howard, T. D., Cogdell, R. J., and Hochstrasser, R. M. (1999) The dynamics of structural deformations of immobilized single light-harvesting complexes, *Proc. Natl. Acad. Sci. U.S.A. 96*, 11271−11276.
20. van Oijen, A. M., Ketelaars, M., Köhler, J., Aartsma, T. J., and Schmidt, J. (1999) Unraveling the electronic structure of individual photosynthetic pigment−protein complexes, *Science 285*, 400−402.
21. Tietz, C., Jelezko, F., Gerken, U., Schuler, S., Schubert, A., Rogl, H., and Wrachtrup, J. (2001) Single molecule spectroscopy on the light-harvesting complex II of higher plants, *Biophys. J. 81*, 556−562.
22. Jelezko, F., Tietz, C., Gerken, U., Wrachtrup, J., and Bittl, R. (2000) Single-molecule spectroscopy on photosystem I pigment−protein complexes, *J. Phys. Chem. B 104*, 8093−8096.
23. Witt, I., Witt, H. T., Gerken, S., Saenger, W., Dekker, J. P., and Rögner, M. (1987) Crystallization of reaction center-I of photosynthesis—low-concentration crystallization of photoactive protein complexes from the cyanobacterium *Synechococcus.sp*, *FEBS Lett. 221*, 260−264.
24. Pålsson, L. O., Flemming, C., Gobets, B., van Grondelle, R., Dekker, J. P., and Schlodder, E. (1998) Energy transfer and charge separation in photosystem I: P700 oxidation upon selective excitation of the long-wavelength antenna chlorophylls of *Synechococcus elongatus*, *Biophys. J. 74*, 2611−2622.
25. Shubin, V. V., Bezsmertnaya, I. N., and Karapetyan, N. V. (1995) Efficient energy-transfer from the long-wavelength antenna chlorophylls to P700 in Photosystem-I complexes from Spirulina-platensis, *J. Photochem. Photobiol., B 30*, 153−160.
26. Moerner, W. E. (1997) Polymer luminescence—those blinking single molecules, *Science 277*, 1059−1060.
27. Orrit, M., and Bernard, J. (1990) Single pentacene molecules detected by fluorescence excitation in a para-terphenyl crystal, *Phys. Rev. Lett. 65*, 2716−2719.
28. Basché, T., Kummer, S., and Bräuchle, C. (1996) Excitation and emission spectroscopy and quantum optical measurements, in *Single-Molecule Optical Detection, Imaging and Spectroscopy* (Basché, T., Moerner, W. E., Orrit, M., and Wild, U. P., Eds.) pp 31−67, VCH Verlagsgesellschaft mbH, Weinheim, Germany.
29. Byrdin, M., Rimke, I., Schlodder, E., Stehlik, D., and Roelofs, T. A. (2000) Decay kinetics and quantum yields of fluorescence in photosystem I from *Synechococcus elongatus* with P700 in the reduced and oxidized state: are the kinetics of excited-state decay trap-limited or transfer-limited?, *Biophys. J. 79*, 992−1007.
30. Bowers, P. G., and Porter, G. (1967) Quantum yields of triplet formation in solutions of chlorophyll, *Proc. R. Soc. London, Ser. A 296*, 435−441.
31. Chibisov, A. K., Slavnova, T. D., and Gorner, H. (2003) Effect of macromolecules and Triton X-100 on the triplet of aggregated chlorophyll in aqueous solution, *J. Photochem. Photobiol., B 72*, 11−16.
32. Sener, M. K., Park, S., Lu, D. Y., Damjanovic, A., Ritz, T., Fromme, P., and Schulten, K. (2004) Excitation migration in trimeric cyanobacterial photosystem I, *J. Chem. Phys. 120*, 11183−11195.

BI0521700