Biochemistry

© Copyright 1998 by the American Chemical Society Volume 37, Number 20 May 19, 1998

Accelerated Publications

Ultrafast Carotenoid Band Shifts Probe Structure and Dynamics in Photosynthetic Antenna Complexes[†]

J. L. Herek,[‡] T. Polívka,[‡] T. Pullerits,[‡] G. J. S. Fowler,[§] C. N. Hunter,[§] and V. Sundström*,[‡]

Department of Chemical Physics, Lund University, P.O. Box 124, S-22100 Lund, Sweden, and Krebs Institute and Robert Hill Institute for Photosynthesis, Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2UH, U.K.

Received January 15, 1998; Revised Manuscript Received March 12, 1998

ABSTRACT: We report observations of ultrafast carotenoid band shifts correlated with energy transfer dynamics between bacteriochlorophyll (BChl) molecules within the peripheral light-harvesting complex (LH2) from the photosynthetic bacterium *Rhodobacter sphaeroides*. Direct excitation of the bacteriochlorophyll Q_y bands yielded distinct changes in the carotenoid S_2 absorption from 430 to 530 nm. Transient absorption spectra and kinetics were measured in a femtosecond pump−probe experiment, revealing the ultrafast carotenoid response to excited BChl pigments. These data are an indication of a new property of carotenoids that is manifested as a unique ability to detect and report changes in their immediate environment, thereby serving as sensitive probes of local structure and dynamics.

The diverse roles played by carotenoids in photosynthesis, including light harvesting, photoprotection, and structure stabilization, are directly related to the efficiency and viability of the photosynthetic organism (*1*). Understanding the mechanisms of the functions of carotenoids is a major goal in photosynthesis research and relies on information from a variety of techniques that probe the structural, spectroscopic, and ultrafast temporal dynamics of carotenoids. The recent determinations of the structures of light-harvesting (LH)[1] complexes in purple bacteria (*2*, *3*) have extended the possibilities for direct correlation of function and design within a relatively simple photosynthetic system.

The LH complexes capture sunlight and pass it on to reaction centers where it fuels the charge separation and is converted into chemical energy. The remarkable efficiency of this process reflects the unique assembly of pigment molecules (*4*). The organization of bacteriochlorphyll (BChl) and carotenoid pigments within the peripheral LH complex of purple bacteria, LH2, has been partially visualized using the 2.5 Å resolution crystal structure obtained for *Rhodopseudomonas acidophila* (*2*) and is illustrated in Figure 1. Two types of BChl molecules, distinguished by their binding sites and separated by ~20 Å, are labeled according to their peak absorption wavelengths as B800 and B850. Two carotenoids are observed to "snake" between the BChl molecules. The first, fully resolved in the crystallographic structure, spans the membrane on the inside of the ring with the B800 BChl located at approximately the center of the

---

[†] This work was supported by the Swedish Natural Science Research Council, the Knut and Alice Wallenberg Foundation, and the European Union (Grant ERBFMGECT950020). J.L.H. was supported by a Wenner Gren Foundation postdoctoral fellowship. G.J.S.F. and C.N.H. were supported by grants from the BBSRC (U.K.).

[‡] Lund University.

[§] University of Sheffield.

[1] Abbreviations: BChl, bacteriochlorophyll *a*; LH, light-harvesting; LH1, core light-harvesting complex; LH2, peripheral light-harvesting complex; fwhm, full width half-maximum; ESA, excited state absorption.

S0006-2960(98)00118-4 CCC: $15.00

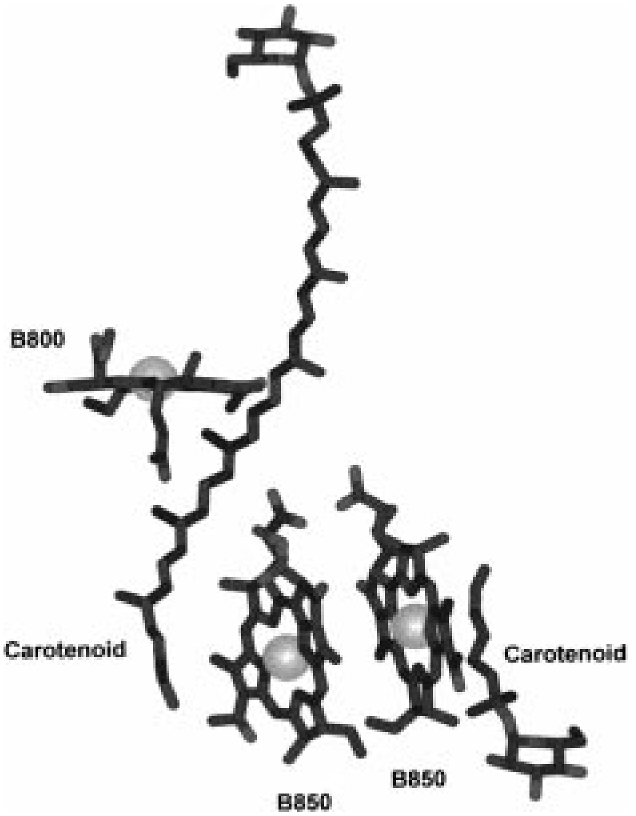

FIGURE 1: Organization of pigments in the protamer unit of LH2 of *Rps. acidophila* (*2*). This unit is repeated nine times to form a highly symmetric ring structure. Note that the carotenoids shown here for *Rps. acidophila* are rhodopin glucoside, while samples used in this study contained neurosporene.

conjugated region. The head of this carotenoid lies near the N terminus of the protein, where several polar residues can be found. The second carotenoid has been only partially resolved, with the head located on the opposite end of the membrane below the B850 BChls.

Downward energetics between pigment molecules allows for funneling of light energy within LH2 and further onto the core LH complex (LH1) and the reaction center. Energy absorbed by carotenoids is quickly transferred to both B800 and B850 molecules (*5*, *6*). From B800, energy migrates efficiently to B850 with a time constant of 0.7 ps at room temperature and 1.2 ps at 77 K (*7*−*9*). Both of these processes have been modeled by Förster theory, which assumes a dipole−dipole interaction between donor and acceptor species. For B800 → B850 energy transfer, however, the predicted rates appear to be too slow to describe the measured dynamics, implying that a different mechanism—perhaps one involving the carotenoid—must also be operating (*9*, *10*).

The experiments reported here show that the carotenoid is sensitive to the B800 → B850 energy transfer process. We excite the sample selectively within the B800 (or B850) absorption band and then monitor changes in the spectral region corresponding to the carotenoid with femtosecond resolution. The resulting ultrafast carotenoid band shifts are observed to correlate directly with the energy transfer dynamics of the BChl pigments.

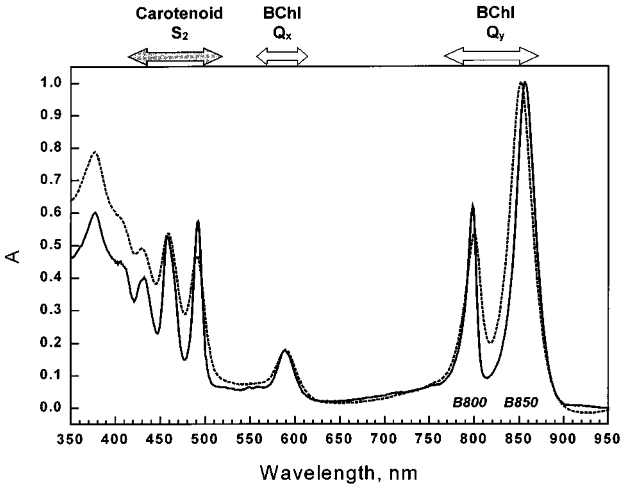

FIGURE 2: Steady state absorption spectra of *Rb. sphaeroides* LH2 complexes at room temperature (dashed line) and 77 K (solid line). The strain was G1W2:DG2, a mutant containing the carotenoid neurosporene (and methoxy and hydroxy derivatives).

MATERIALS AND METHODS

Membranes of mutants of *Rhodobacter sphaeroides* containing only the LH2 complex were employed in these studies; specifically, we used a "green" strain (DD13/G1) containing neurosporene as the predominant carotenoid (details of the sample preparation are found in ref *11*). The samples were dissolved in buffer solution (50 mM Tris at pH 8) and diluted with ~60% glycerol for low-temperature studies, such that the absorption at the excitation wavelength was ~0.5. The absorption spectra recorded at room temperature and 77 K are shown in Figure 2. Note that the carotenoid bands are clearly resolved in this mutant strain; wild-type *Rb. sphaeroides*, containing a mixture of spheroidene and spheroidenone, exhibits a broad absorption spectrum in this region (not shown).

The femtosecond spectrometer employed in these studies is based on an amplified titanium−sapphire laser system (*12*). The oscillator (Spectra Physics, Tsunami) provided <100 fs pulses at 795 nm with a repetition rate of 82 MHz; these were amplified 10^6 times (Spectra Physics, Merlin/Spitfire) to an intensity of ~200 μJ/pulse and a repetition rate of 5 kHz. The output of the amplifier was split into pump and probe beams which underwent frequency conversion as required. Specifically, to generate pump pulses at 865 nm, we employed an optical parametric generator/amplifier (Light Conversion, TOPAS), and to produce probe pulses spanning the visible spectral region, we focused the beam into a 5 mm sapphire plate which yielded a white-light continuum. The pump and probe pulses traveled different optical paths to the sample; the length of the former was determined by a computer-controlled optical delay line. The probe pulse was further divided by a 50/50 beam splitter to produce an additional beam which was used as a reference in the pump−probe experiment. This beam also passed through the sample but did not overlap with the pump and probe beams. The probe and reference beams were detected by photodiodes following dispersion through a single-grating monochromator (Oriel, 1/8 m). In all experiments, the pump beam was attenuated to ~20 nJ/pulse and focused to ~500 μm

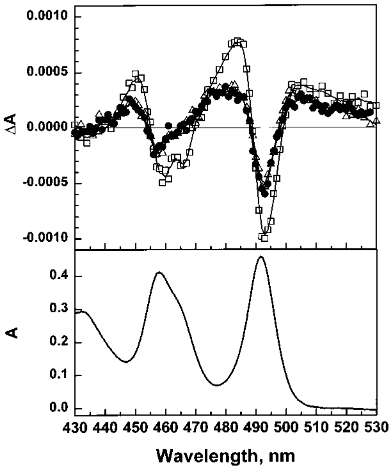

FIGURE 3: Transient absorption spectra of the carotenoid region at 77 K. For comparison, the steady state absorption spectrum is shown in the bottom panel. The spectra with open symbols were recorded 500 fs (squares) and 10 ps (triangles) after excitation in the B800 band at 795 nm. The spectrum with filled circles was recorded ~2 ps after excitation in the B850 band at 865 nm. The samples were prepared to have the same concentration ($OD_{795} = OD_{865} = 0.5$), and the same excitation density was used for all measurements (5×10^{13} photons/cm^2). The curves through data points in the transient absorption spectra were obtained by a smoothing function.

[corresponding to an intensity of $\sim 5 \times 10^{13}$ photons/(cm^2 pulse) at 800 nm]. The polarization of the pump and probe beams was set to magic angle (54.7°).

Following excitation in the Q_y regions of the BChls with a 100 fs pulse, the carotenoid region (430−530 nm) was probed directly by two techniques. (a) For a fixed pump−probe delay time, the full transient absorption spectrum spanning the visible to near-infrared region was obtained by scanning the detection monochromator. We compensated for group velocity dispersion by properly shifting the delay line for each wavelength. (b) At a fixed detection wavelength, we scanned the delay line to record the transient absorption kinetics of a given species (identified by its characteristic absorption properties). For these experiments, the instrument response function was measured to be the cross-correlation of the pump and probe pulses at the sample position, fitted to a Gaussian function. This measurement was made immediately prior to recording the kinetics and yielded a fwhm of ~140 fs.

RESULTS

As shown in Figure 3, significant changes in the transient absorption spectrum throughout the carotenoid region are observed. The data shown were recorded at 77 K; similar spectra are also observed at room temperature. Strong negative signals approximately 3 nm to the red of the steady state absorption maxima (A = 491, 463 and/or 457, and 431 nm; ΔA = 494, 467 and/or 460, and 434 nm) are superimposed upon the broad positive signal due to excited state absorption (ESA) from the B800, B850, and/or carotenoid pigments. The signals are strongest approximately 0.5 ps after excitation in the B800 band, though considerable intensity remains at later times after energy transfer to B850 is complete. In fact, the transient absorption spectrum recorded following excitation in the B850 band (at 865 nm) is identical to that observed 10 ps after excitation at 795 nm. The transient absorption spectrum measured for wild-type LH2 is dominated by the ESA signal with only a hint of negative structure appearing at 525 nm.

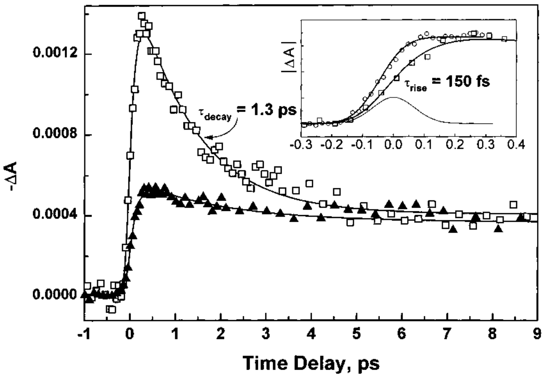

FIGURE 4: Transient absorption kinetics recorded at 494 nm and 77 K: (□) excitation at 795 nm and (▲) excitation at 865 nm. The same excitation intensity was used for both measurements. The fits were obtained by convoluting an exponential function with the measured response function (140 fs fwhm). Shown in the inset is a comparison of rises of signals measured at 494 nm (squares) and 530 nm (circles), following excitation at 795 nm. The signal recorded at 530 nm corresponds mainly to excited state absorption of B800 and is characterized by an instantaneous (pulse-limited) rise. Note that the data plotted here are the absolute magnitudes of the absorbance changes, which have opposite signs at the two wavelengths. Also shown in the inset is a typical pump−probe response function (Gaussian fit to measured signal).

The kinetics recorded at the most pronounced carotenoid band (λ_{det} = 494 nm) following excitation at 795 nm show a fast rise component (150 ± 50 fs) followed by a biexponential decay (see Figure 4). The data were fitted to an exponential function (three components) convoluted with the Gaussian response function. The time constant extracted for the major decay component matches that of the B800 → B850 energy transfer process: 0.9 ± 0.2 ps at room temperature and 1.3 ± 0.2 ps at 77 K (*9*). The minor, slow decay component persists for hundreds of picoseconds and is shown to be directly associated with the B850 dynamics; following excitation at 865 nm, the carotenoid signal shows a fast rise (~150 fs) followed by only a slow decay that is identical in both lifetime and amplitude to the slow decay component observed following 795 nm excitation.

As further confirmation of the rise component in the signal measured in the carotenoid region, we compared the shape of the kinetics obtained at 494 nm to those measured in a spectral region which is not associated with ground state absorption from any of the pigments in LH2 (see Figure 2). The signal at 530 nm is attributed solely to ESA from the B800 and B850 molecules, and the kinetics measured there reflect the corresponding dynamics of both species. Direct comparison of the kinetics measured at 494 and 530 nm following excitation at 795 nm (see the inset of Figure 4) reveals the instantaneous rise of the signal at 530 nm (due to B800 ESA) and the slower buildup of the signal at 494 nm.

In all experiments, the excitation intensity was kept sufficiently low to preclude the possibility of direct (two-photon) excitation of the carotenoids. The measured signals, in both the carotenoid and bacteriochlorophyll regions, were scaled linearly with the intensity, and no indications of excitation annihilation were observed.

DISCUSSION

The experiments reported here clearly demonstrate the presence of an interaction between the carotenoid and BChl pigments. But what, precisely, is the nature of this interaction, and what is the basis of our observation that the carotenoid response reflects the B800 → B850 energy transfer dynamics? A mechanism of resonant energy transfer by way of a carotenoid intermediate [B800 → carotenoid (S_1) → B850] is unlikely given energetic considerations; for neurosporene, the lowest-lying singlet excited state (S_1) is predicted to be $\sim$4000 cm^{-1} above the B800 level (*5*). In addition, the carotenoid signal observed following excitation in the B850 band cannot be explained by this scheme, assuming unidirectional energy flow. The contribution of back transfer (i.e. B850 → B800) is negligible at 77 K. Alternatively, the shifts in the carotenoid absorption bands may be explained by local field effects induced by the excited pigments, with the dynamics reflecting the overall transfer and trapping of energy in LH2.

The carotenoids in LH2 have previously been shown to be sensitive probes of electric fields. Specifically, one pool of carotenoid pigments, marked by a red-shifted absorption spectrum and associated with the B800 BChls, has been assigned as being electrochromically active (*13*, *14*). The idea of two pools of carotenoids in LH2 is supported by the structure (*2*), in which two distinct binding sites are shown (see Figure 1). These two pools of carotenoids are indeed distinguished spectrally, as the steady state absorption spectrum reveals a slight splitting of the carotenoid peaks at low temperatures (see Figure 3), and as previously concluded from linear dichroism measurements (*15*).

Early experiments of electrochromic effects in LH2 employed light to initiate the electron transfer dynamics in the reaction centers; the resulting charge separation spanned the entire membrane, and the corresponding potential induced spectral changes in the LH2 carotenoids (*16*−*18*). Other studies showed that ionic gradients (*13*, *14*, *19*−*21*) and applied electric fields (*22*, *23*) can also generate carotenoid band shifts. In addition, these studies revealed that the carotenoid response is linear with respect to the transmembrane potential, reflecting the highly organized environment within the protein complex and the presence of point charges (*22*, *24*).

Common to all of the previous observations of carotenoid band shifts in LH2 is the presence of a transmembrane potential. To our knowledge, this is the first observation of a carotenoid electrochromic response to an induced local field within a pigment−protein complex. Such a field is created upon excitation of the BChl pigments. The change in permanent dipole moment ($\Delta\mu_{B800} \sim 1$ D, $\Delta\mu_{B850} \sim 3$ D; *25*) generates an electric field with a magnitude on the order of 10^8 V/m at a distance of 5 Å, according to a simple point−dipole approximation.[2] While the field strength falls off with a $1/r^3$ dependence, even 20 Å from the excited dipole, the magnitude of the local field is comparable to that of the uniform transmembrane potential used in Stark and ion gradient experiments (*19*−*23*).

The transient absorption spectra of Figure 3 show that both pools of carotenoids respond to the initially excited B800 molecules, as both spectral sub-bands (i.e. 457 and 463 nm) are affected. After the energy is transferred to B850, however, only the blue-shifted pool (457 nm) of carotenoid pigments senses the excitation and manifests a band shift. It is worth noting that this effect is also evident in the carotenoid band at 491 nm, where after excitation transfer is complete the remaining carotenoid signal is on the blue edge of the band. Consistent with this interpretation, when the B850 BChls are excited directly, again only the blue-shifted pool of carotenoid pigments contributes to the transient absorption signal. The specificity of the carotenoid response to the location of the excitation in LH2 implies that such signals may provide structural information.

The response of the carotenoids to the excited BChl pigments is not instantaneous, as shown by the 100−200 fs rise time of the transient absorption signal. This observation is especially curious and poses an interesting challenge to theorists. If the carotenoids respond to local electric fields from the excited BChls, then the onset of the signal should presumably be defined by the excitation (i.e. pulse-limited). Time-dependent solvation by the protein and/or neighboring pigments may affect the field felt by the carotenoids, though part of the rise still should occur immediately. It is possible that biphasic rise kinetics are also consistent with our data. Resolution of a delayed response by the carotenoid makes it unlikely that the band shifts could be caused by perturbation of the coupling of BChl and carotenoid electronic transitions, as such an effect should manifest an instantaneous response. Vibrational relaxation, exciton dynamics, and exciton scattering between the B800 and B850 manifolds could perhaps also lead to a finite rise time of the carotenoid response. These and other possible explanations for the observed BChl−carotenoid interaction will be discussed more thoroughly in a future publication.

The shifts of carotenoid absorption spectra in response to local fields generated by nearby excited pigments may serve as a general tool in elucidating structural and dynamical information in a variety of systems. While in studies involving transmembrane potentials in LH2 only one pool of carotenoids was observed to be electrochromically active (perhaps due to preferential alignment with respect to the applied field), here both pools of carotenoids responded to local fields, albeit with distinct specificity to the excited pigments. The transient absorption kinetics provide direct information about energy transfer in the neighboring BChl pigments. We are currently extending our studies to other systems to further explore this unique ability of carotenoids to serve as sensitive probes of energy transfer dynamics.

ACKNOWLEDGMENT

We thank A. Yartsev for technical assistance and helpful discussions. We also thank Prof. W. W. Parson for helpful suggestions.

REFERENCES

1. Frank, H. A., and Cogdell, R. J. (1996) *Photochem. Photobiol. 63*, 257−264.

---

[2] The calculation of the electric field is based on $\Delta\mu_{B800}$, in the absence of dielectric effects (i.e. the "vacuum" field). The estimates of $\Delta\mu$ (*25*) were obtained from Stark measurements, in which a local field correction due to the effect of the protein environment was not included. Note that in B850 such a calculation may not be valid, as the excitation is delocalized over several neighboring pigments.

2. Freer, A., Prince, S., Sauer, K., Papiz, M., Hawthornthwaite-Lawless, A., McDermott, G., Cogdell, R., and Isaacs, N. W. (1996) *Structure 4*, 449−462.
3. Koepke, J., Hu, X., Muenke, C., Schulten, K., and Michel, H. (1996) *Structure 4*, 581−597.
4. Hu, X., and Schulten, K. (1997) *Phys. Today*, 28−34.
5. Koyama, Y., Kuki, M., Andersson, P. O., and Gillbro, T. (1996) *Photochem. Photobiol. 63*, 243−255.
6. Ricci, M., Bradforth, S. E., Jimenez, R., and Fleming, G. R. (1996) *Chem. Phys. Lett. 259*, 381−390.
7. Shreve, A. P., Trautman, J. K., Frank, H. A., Owens, T. G., and Albrecht, A. C. (1991) *Biochim. Biophys. Acta 1058*, 280−288.
8. Monshouwer, R., Ortiz de Zarate, I., van Mourik, F., and van Grondelle, R. (1995) *Chem. Phys. Lett. 246*, 341−346.
9. Pullerits, T., Hess, S., Herek, J. L., and Sundström, V. (1997) *J. Phys. Chem. B 101*, 10560−10567.
10. Scholes, G. D., Harcourt, R. D., and Fleming, G. R. (1997) *J. Phys. Chem. B 101*, 7302−7312.
11. Jones, M. R., Fowler, G. J. S., Gibson, L. C. D., Grief, G. G., Olsen, J. D., Crielaard, W., and Hunter, C. N. (1992) *Mol. Microbiol. 9*, 1173−1184.
12. Chachisvilis, M. (1996) Electronic and Vibrational Coherence in Photosynthetic and Model Systems, Ph.D. Thesis, Lund University, Lund, Sweden.
13. Crielaard, W., van Mourik, F., van Grondelle, R., Konings, W. N., and Hellingwerf, K. J. (1992) *Biochim. Biophys. Acta 1100*, 9−14.
14. Goodwin, M. G., and Jackson, J. B. (1993) *Biochim. Biophys. Acta 1144*, 191−198.
15. Kramer, H. J. M., van Grondelle, R., Hunter, C. N., Westerhuis, W. H. J., and Amesz, J. (1984) *Biochim. Biophys. Acta 765*, 156−165.
16. Fleischman, D. E., and Clayton, R. K. (1968) *Photochem. Photobiol. 8*, 287−298.
17. Holmes, N. G., and Crofts, A. R. (1977) *Biochim. Biophys. Acta 459*, 492−505.
18. Holmes, N. G., Hunter, C. N., Niederman, R. A., and Crofts, A. R. (1980) *FEBS Lett. 115*, 43−47.
19. Jackson, J. B., and Crofts, A. R. (1969) *FEBS Lett. 4*, 185−189.
20. Webster, G. D., Cogdell, R. J., and Lindsay, J. G. (1980) *Biochim. Biophys. Acta 591*, 321−330.
21. Crielaard, W., Visschers, R. W., Fowler, G. J. S., van Grondelle, R., Hellingwerf, K. J., and Hunter, C. N. (1994) *Biochim. Biophys. Acta 1183*, 473−482.
22. Gottfried, D. S., Steffen, M. A., and Boxer, S. G. (1991) *Science 251*, 662−665.
23. Gottfried, D. S., Steffen, M. A., and Boxer, S. G. (1991) *Biochim. Biophys. Acta 1059*, 76−90.
24. Kakitani, T., Honig, B., and Crofts, A. R. (1982) *Biophys. J. 39*, 57−63.
25. Beekman, L. M. P., Frese, R. N., Fowler, G. J. S., Picorel, R., Cogdell, R. J., van Stokkum, I. H. M., Hunter, C. N., and van Grondelle, R. (1997) *J. Phys. Chem. B 101*, 7293−7301.

BI980118G