Photosynthetic Light-Harvesting Pigment−Protein Complexes: Toward Understanding How and Why

TÕNU PULLERITS AND VILLY SUNDSTRÖM*

Department of Chemical Physics, Lund University, P.O. Box 124, 22100 Lund, Sweden

Received February 2, 1996

Introduction

Two Coupled Pigment Systems for Energy Storage. The light-driven reactions of photosynthesis are the means by which nature converts energy of light into a stable electrochemical potential, which eventually is stored as chemical energy through a series of dark reactions. The light reactions occur in two closely coupled pigment systems; light energy is absorbed by a network of so-called antenna pigment−proteins and very efficiently transported through energy transfer to the photochemical reaction center (RC) where the energy is converted through a sequence of electron transfer reactions. Early time-resolved picosecond measurements[1,2] showed that this conversion process is typically finished within ∼100 ps, explaining the high, ∼95%, overall quantum yield of the process, known already in the early days of photosynthesis research. More recent studies have revealed that the individual energy transfer steps in the antenna are in the subpicosecond time range.[3−6] At the same time the transfer from the antenna into the RC is a relatively slow process.[7,8]

In this Account we will be concerned with the light-harvesting processes and examine in detail how light is absorbed by antenna pigments and transferred to the RC. The recent progress in structural determination of light-harvesting pigment−proteins of photosynthetic purple bacteria[9] combined with the previously known RC structure[10] makes these organisms very useful for such a study, since a detailed knowledge of the arrangement of the system provides the framework where time-resolved spectroscopy can be used to reveal structure−function relationships and reaction mechanisms. But before we start to examine the light-harvesting processes in the purple bacterial antennae, we will give a brief summary of what has been learned from almost 30 years of studies of antenna protein structure and spectroscopy.

Toward Understanding Structure−Function Relationships. For more than 20 years the three-dimensional structure of the water soluble bacteriochlorophyll (Bchl) *a* pigment−protein complex of the green sulfur bacterium *Prostechochloris aestuarii*, the so-called Fenna−Mathews−Olson (FMO) protein, has been known.[11] During this period a number of spectroscopic measurements and calculations of spectral properties have been performed.[12] However, despite this detailed structural knowledge with precise information on chromophore positions, it has turned out to be very difficult to in detail theoretically reproduce steady state absorption and linear and circular dichroism spectra simultaneously.[13] The high pigment density within the complex and the observed spectral features have nevertheless been interpreted to suggest that there is strong interaction between the Bchl *a* molecules, which leads to the formation of so-called exciton states which are delocalized over several Bchl *a* molecules. Singlet minus triplet spectroscopy has given the same conclusion.[14] More recently, time-resolved[15,16] and hole-burning[17] experiments have been performed to probe the excitation dynamics within the FMO complex. Again, it has been difficult to obtain a detailed understanding of the kinetics, despite the fact that this is the structurally best characterized photosynthetic antenna pigment−protein.

Accessory antenna pigment−protein complexes of blue-green bacteria C-phycocyanin (C-PC) and phycoerythrocyanin (PEC) are with known structures.[18,19] Förster transfer calculations[20] based on the structure of C-PC appear to be in reasonable agreement with

Tõnu Pullerits graduated from Tartu University, Estonia, as a solid state physicist in 1986 and obtained his Ph.D. in 1991 with Arvi Freiberg. He then joined the Department of Biophysics at the Free University of Amsterdam as an EMBO post-doctoral fellow with Rienk van Grondelle. After that he was a postdoctoral fellow with Villy Sundström at Umeå University. He is presently a research assistant of the Department of Chemical Physics at Lund University. His research interests focus on the primary energy transfer and trapping in photosynthesis.

Villy Sundström received his Ph.D. from the University of Umeå, Sweden, in 1977, but a major part of his training he received with Peter M. Rentzepis at Bell Labs, Murray Hill, NJ (1975–76). Subsequently he founded the first picosecond spectroscopy laboratory in Scandinavia in Umeå. In 1994 Sundström moved to Lund University, Sweden, for a newly created chair in Chemical Dynamics. His current scientific interests are the application of ultrafast laser techniques in chemistry and biology.

(1) Borisov, A. Yu.; Freiberg, A. M.; Godik, V. I.; Rebane, K. K.; Timpmann, K. K. *Biochim. Biophys. Acta* **1985**, *807*, 221.
(2) Sundström, V.; Van Grondelle, R.; Bergström, H; Åkesson, E; Gillbro, T. *Biochim. Biophys. Acta* **1986**, *851*, 431.
(3) Du, M.; Xie, X., Jia, Y.; Mets, L.; Fleming, G. R. *Chem. Phys. Lett.* **1993**, *210*, 535.
(4) Hess, S.; Åkesson, E.; Cogdell, R.; Pullerits, T.; Sundström, V. *Biophys. J.* **1995**, *69*, 2211.
(5) Visser, H. M.; Somsen, O. J. G.; Van Mourik, F.; Lin, S.; Van Stokkum, I.; Van Grondelle, R. *Biophys. J.* **1995**, *69*, 1083.
(6) Shreve, A. P.; Trautman, J. K.; Frank, H. A.; Owens, T. G.; Albrecht, A. C. *Biochim. Biophys. Acta* **1991,** *1051*, 280.
(7) Timpmann, K.; Zhang, F. G.; Freiberg, A.; Sundström, V. *Biochim. Biophys. Acta* **1993**, *1183*, 185.
(8) Kleinherenbrink, A. M.; Deinum, G.; Otte, S. C. M.; Hoff, A. J.; Amesz, J. *Biochim. Biophys. Acta* **1992**, *1099*, 175.
(9) McDermott, G.; Prince, S. M.; Freer, A. A.; Hawthornthwaite-Lawless, A. M.; Papiz, M. Z.; Cogdell, R. J.; Isaacs, N. W. *Nature* **1995,** *374*, 517.
(10) Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *Nature* **1985**, *318*, 618.
(11) Matthews, B. W. Fenna, R. E. *Acc. Chem. Res.* **1980**, *13*, 309.
(12) Pearlstein, R. M.; Hemenger, R. P. Proc. *Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4920.
(13) Pearlstein, R. In *Chlorophylls*; Scheer, H., Ed.; CRC Press: Boca Raton, FL, 1991; p 1047.
(14) Van Mourik, F.; Verwijst, R. R.; Mulder, J. M.; Van Grondelle, R. *J. Lumin.* **1992**, *53*, 499.
(15) Savikhin, S.; Zhou, W.; Blankenship, R. E.; Struve, W. S. *Biophys. J.* **1994**, *66*, 110.
(16) Louwe, R. J. W.; Aartsma, T. J. *J. Lumin.* **1994**, *58*, 154.
(17) Johnson, S. G.; Small, G. J. *J. Phys. Chem.* **1991**, *95*, 471.
(18) Schirmer, T.; Bode, W.; Huber, R.; Sidler, W.; Zuber, H. *J. Mol. Biol.* **1985**, *184*, 257.
(19) Duerring, M.; Huber, R.; Bode, W.; Miller, M.; Hackert, M. L. *J. Mol. Biol.* **1990**, *211*, 633.

S0001-4842(95)00110-5 CCC: $12.00 © 1996 American Chemical Society

the results of recent subpicosecond experiments.[21] However, the exciton model has also been used for the very similar allophycocyanin.[22,23] It was also suggested that this antenna may belong to the intermediate coupling range.[24] The structures of the LHCII antenna protein[25] and of photosystem I (PSI)[26] are today also known to nearly atomic resolution.

Many more photosynthetic light-harvesting pigment−proteins and membrane systems of bacteria and green plants have been studied with steady state and time-resolved spectroscopy. However, the lack of atomic-scale resolution of most other pigment−proteins necessarily implied difficulties in interpreting spectroscopic data in detail, and the picosecond time resolution available in early time-resolved studies often resulted in integrated information where the elementary steps could not be distinguished. This point will be evident from the following discussion which shows that the time scale of photosynthetic energy transfer is ∼100 fs, correlating to the shortest pigment−pigment distances of ∼10 Å found in light-harvesting proteins. Alternatively expressed, in order to interpret spectroscopic and dynamic data in terms of a detailed molecular level reaction mechanism, atomic-scale structural information is required. However, even in the systems where structural information is available, it is still difficult to assess the significance of structural features from the point of view of overall performance and function of photosynthetic light-harvesting, because the detailed assembly of the whole photosynthetic unit (PSU) frequently consisting of several antenna complexes and a RC is not known.

An important step forward in photosynthesis research was taken when the structure of the purple bacterial reaction center was determined as the first membrane bound protein complex.[10] The corresponding step for membrane bound antenna proteins was achieved very recently when the LH2 antenna complex of *Rhodopseudomonas acidophila* was crystallized and its structure determined to 2.5 Å resolution.[9] Methods for site selective mutagenesis of individual amino acid residues already have been developed and applied to these proteins.[27] Also ultrafast spectroscopy is well developed for bacterial antenna systems.[4,28,29] All this has contributed to make these pigment systems among the most studied and best characterized molecular assemblies, and they can be expected to advance our conceptual understanding of energy transfer.

The fact that there now exist high-resolution structures of the RC and the LH2 peripheral antenna complex as well as a lower resolution structure of the LH1 core antenna complex,[30] all from purple bacteria, makes the purple bacterial photosynthetic unit (PSU) the structurally best characterized complete photosynthetic system. Although the full three-dimensional structure of the whole PSU has not yet been obtained, it is possible to construct a feasible model of the PSU based on the known structures of the RC, LH2, and LH1 building blocks. This provides us with a powerful basis for analyzing spectroscopic and dynamic properties of these systems, ranging from the most elementary steps on the ∼100 fs time scale up to the complete process of light-harvesting and trapping involving the full PSU and occurring on the picosecond to ∼100 ps time scale. In general the excitation dynamics in these systems can be described as a sequence of transfer steps between distinct spectral bands, B800 → B850 → B880 → RC. We will see that the elementary processes are in fact much more complicated than this simple scheme shows.

Elements of Energy Transfer Theory. From the LH2 structure (see Figure 1) we can see that there are basically two different sets of nearest neighbor pigment−pigment distances within the complex, one short, ∼9 Å, for the B850−B850 contacts, and one longer, ∼18 Å, for the B800−B800 and B800−B850 distances. It can be estimated by using a simple point−dipole interaction description[13] that the larger distance corresponds to a quite weak interaction (∼10 cm^{-1}) whereas the shorter distance will lead to approximately an order of magnitude stronger coupling, ∼100 cm^{-1}. With regard to the mechanism of energy transfer, the interaction strength (V) relative to the spectral bandwidth (Δ) (or diagonal energy disorder, or inhomogeneous spectral broadening) is an important measure. If V/Δ is much less than unity, we are talking about very weak interaction and energy transfer in the incoherent Förster limit, where the energy transfer is thought of as a hopping motion from molecule to molecule with the energy localized to only one molecule at each instant. In the limit of $V/\Delta \gg 1$ the interaction is very strong and the excited state of the individual molecule is no longer a good description; the excited state energy is now delocalized over the interacting molecules, and the excited states of the individual molecules are replaced by an exciton band structure. For a strongly interacting dimer there will be two exciton states and for an aggregate of N molecules, N states. The energies of these states and the selection rules for optical transitions are determined by the interaction strength and the structure of the aggregate. In addition to the first excited exciton states (one-exciton manifold), there is a corresponding set of higher excited exciton states (two-exciton manifold), and optical transitions between the two manifolds are also governed by the interaction strengths and organization of the molecular aggregate. Below, we will use this fact to characterize the excited state of B850. In the following sections we will examine the various energy transfer steps within LH2 in more detail. In addition we will combine the structural data of LH2, LH1, and the reaction center with results of ultrafast measurements relevant to energy transfer between complexes to construct a model for their interaction.

(20) Debreczeny, M. P.; Sauer, K.; Zhou, J.; Bryant, D. A. *J. Phys. Chem.* **1995**, *99*, 8412.
(21) Sharkov, A. V.; Kryukov, I. V.; Khoroshilov, E. V.; Kryukov, P. G.; Fischer, R.; Scheer, H.; Gillbro, T. *Chem. Phys. Lett.* **1992**, *191*, 633.
(22) Holzwarth, A. R.; Bittersmann, E.; Reuter, W.; Wehrmeyer, W. *Biophys. J.* **1990**, *57*, 133.
(23) Beck, W. F.; Sauer, K. *J. Phys. Chem.* **1992**, *96*, 4658.
(24) Xie, X.; Du, M.; Mets, L.; Fleming, G. R. *Time-Resolved Laser Spectroscopy in Biochemistry III*; SPIE: New York, 1992; Vol. 640, p 690.
(25) Kühlbrandt, W.; Wang, D. N.; Fujiyoshi, Y. *Nature* **1994**, *367*, 614.
(26) Krauss, N.; Hinrichs, W.; Witt, I.; Fromme, P.; Pritzkow, W.; Dauter, Z.; Betzel, C.; Wilson, K. S.; Witt, H. T.; Saenger, W. *Nature* **1993**, *361*, 326.
(27) Fowler, G. J. S.; Visschers, R. W.; Grief, G. G.; van Grondelle, R.; Hunter, C. N. *Nature* **1992**, *355*, 848.
(28) Van Grondelle, R.; Dekker, J. P.; Gillbro, T.; Sundström, V. *Biochim. Biophys. Acta* **1994**, *1187*, 1.
(29) Sundström, V.; Van Grondelle, R. In *Anoxygenic Photosynthetic Bacteria*; Blankenship, R. E., Madigan, M. T., Bauer, C. E., Eds.; Kluwer Academic: Dordrecht, Boston, London, 1995; p 349.
(30) Karrasch, S.; Bullough, P. A.; Ghosh, R. *EMBO J.* **1995**, *14*, 631.

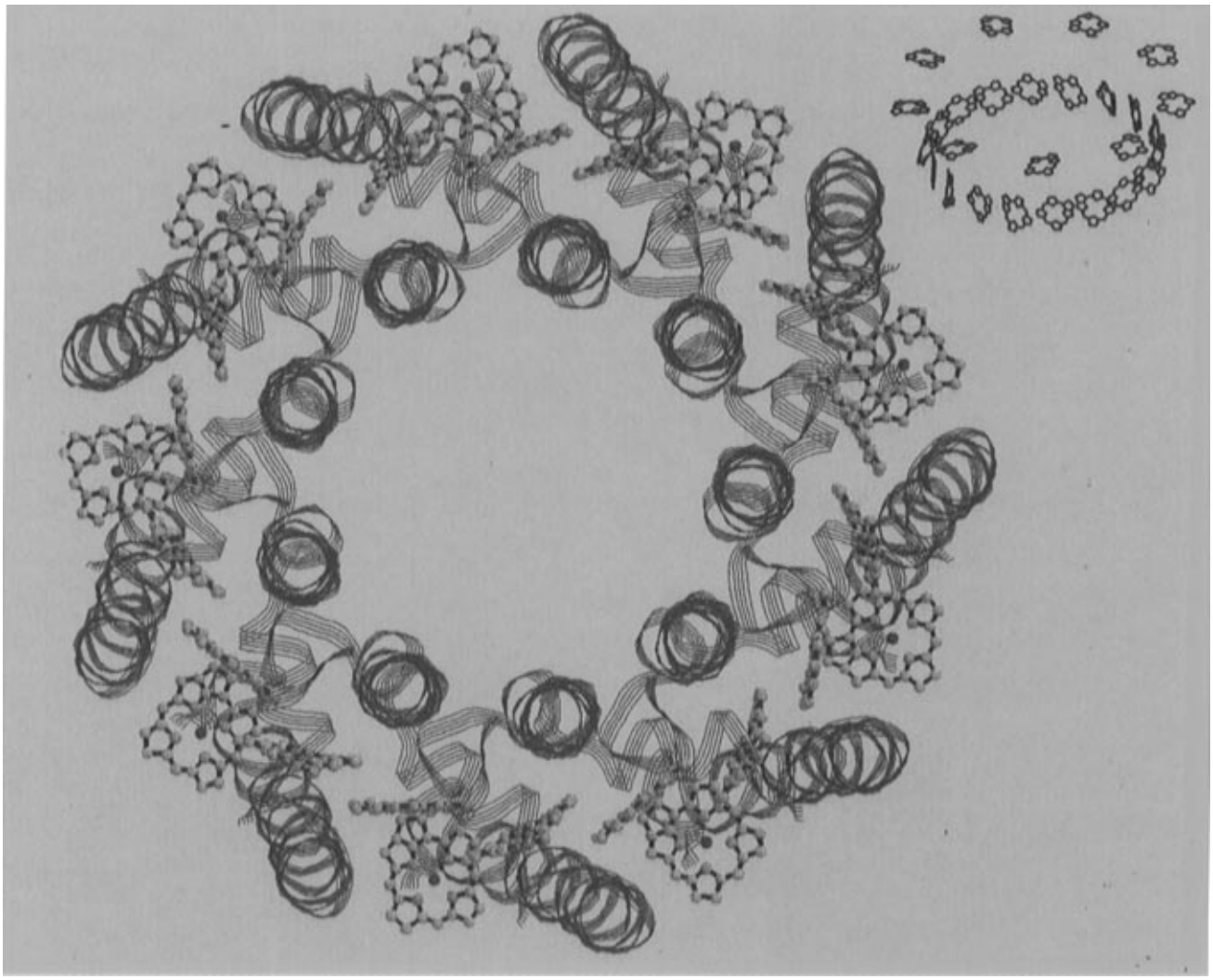

Figure 1. Structure of the LH2 antenna from *Rps. acidophila*.[9] Green and red strands are α- and β-helices, respectively. For clarity we show only the porphyrin macrocycles of the Bchl *a* molecules. The B800 Bchl *a* molecules (blue bonds) are parallel to the membrane plane, whereas B850 molecules (red bonds) are perpendicular to the membrane plane. In the top right corner one can see that there are two different sets of distances in LH2. The B850 molecules are quite densely packed, having neighbor to neighbor distances of 9−10 Å. Distances between B800 molecules and from B800 to B850 are about a factor of 2 larger (18−21 Å).

B800 to B850 Energy Transfer

In the aggregation model of the photosynthetic unit illustrated below, all pigments (B850, B875, and P of the reaction center) except B800 are facing the same side of the membrane. When energy is deposited in B800 of the peripheral LH2 antenna, the first step contributing to the downhill energy transfer toward the reaction center is B800 → B850 transfer across the membrane. As we mentioned above the B800−B850 distance of ∼18 Å suggests that the transfer should be reasonably well characterized by Förster transfer. In order to examine this process in some detail, we measured the B800 → B850 transfer in a series of B850 mutants with gradually blue-shifted B850 absorption bands.[31] A single or double mutation of the tyrosine 44-tyrosine 45 motif of the α-polypeptide of LH2 of *Rhodobacter sphaeroides* results in a shift of B850 to B839 and B826,[27] respectively (see Figure 2). If the B800 → B850 transfer is controlled by the Förster mechanism, it is expected that the rate is directly proportional to the spectral overlap between the B800 fluorescence and B850 absorption spectra.[31]

The B800 → B850 transfer rate was obtained by measuring the B800 excited state lifetime at 77 K of the three complexes, and these rates were compared with the calculated spectral overlaps, assuming that the mutations did not induce any structural changes. Since the fluorescence of B800 is very weak (due to the fast energy transfer to B850) and difficult to detect, we used calculated[32] absorption and fluorescence spectra in order to estimate spectral overlap. The measured and calculated B800 → B850 energy transfer times at 77 K (see Figure 3) are in quite good agreement. Only for the fastest rate (the B826 mutant) is the discrepancy larger than the experimental error and the uncertainties of the simulations. The results of the spectral overlap calculations show that there are two essential features to account for the variation of the energy transfer rate through the mutant series and as a function of temperature, a 750 cm^{-1} vibrational mode[33] and the phonon side-wing of the Bchl molecules.[31] At very low temperature when the phonon side-wing is narrow and low in amplitude,

(31) Hess, S.; Visscher, K. J.; Pullerits, T.; Sundström, V.; Fowler, G. J. S.; Hunter, C. N. *Biochemistry* **1994**, *33*, 8300.

(32) Pullerits, T.; Monshouwer, R.; van Mourik, F.; van Grondelle, F. *Chem. Phys.* **1995,** *194*, 395.

(33) Reddy, N. R. S.; Picorel, R.; Small, G. J. *J. Phys. Chem.* **1992,** *96*, 6458.

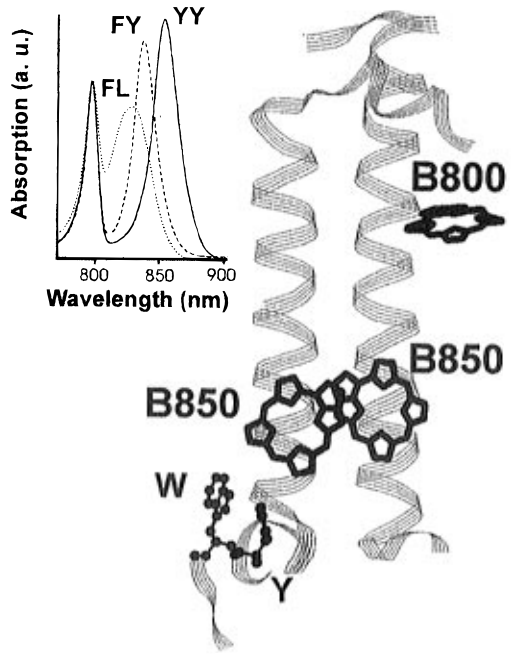

Figure 2. Structure of one α,*β*-subunit of LH2 of *Rps. acidophila*.[9] The corresponding structure of *Rb. sphaeroides* is believed to be very similar. Two residues, Trp and Tyr, which have been mutated are drawn out as balls and sticks and are marked as W and Y, respectively. At the top left are absorption spectra of wild type *Rb. sphaeroides* (YY) and of two blue-shifted B850 mutants (YY → FY and YY → FL; F is phenylalanine, and L is leucine).[31]

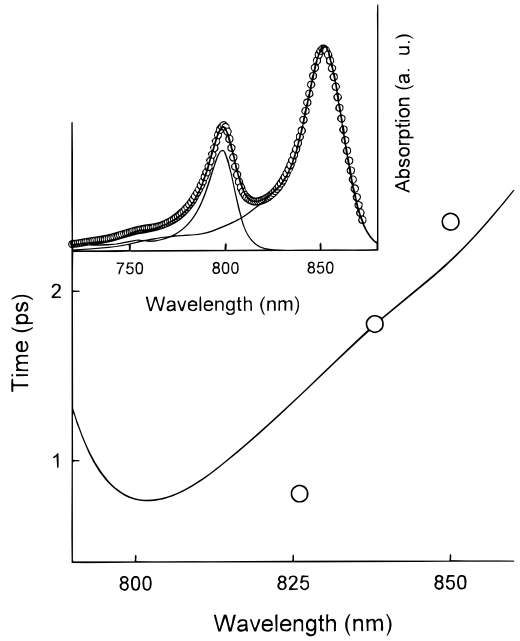

Figure 3. Dependence of the B800 → B850 energy transfer time at 77 K on the position of the B850 band. Circles represent the experimentally measured time constants, and the solid line represents the calculated transfer times. The inset gives the absorption spectrum of the *Rb. sphaeroides* WT B800−850 complexes at 150 K (circles) together with the simulated B800 and B850 spectra (solid lines). The latter were used to calculate the rate of B800 → B850 transfer (see the text).

the 750 cm^{-1} vibrational structure accounts for most of the spectral overlap and results in a much smaller variation of the B800 → B850 energy transfer rate in different mutants than what would be expected on the basis of overlap without that mode.[34] This also explains the relatively small change of the B800 → B850 transfer time between 4 K (2.4 ps[33,34]) and 77 K (1.8 ps[4]). At temperatures above 77 K, the phonon wing starts to dominate the spectral overlap which leads to a transfer time of 0.7 ps at room temperature.[4] From these results it appears that energy transfer from B800 to B850 over a distance of ∼18 Å and at a speed of ∼1 ps is basically controlled by the incoherent Förster mechanism. However, the discrepancy between measured and simulated rates for the mutant with the highest energy transfer rate (B826) may indicate that transfer from vibrationally nonrelaxed donor excited states may contribute. Also the excitonic character of the B850 band (see below) may lead to additional contributions to the overlap integral (overlap with a weak upper excitonic component of B850, e.g.) not included here.

Energy Transfer within B800

The LH2 structure shows that there is a relatively large center-to-center distance between the B800 molecules, which may suggest that this transfer is incoherent. The B800 is represented by a single absorption band, which may suggest that measurements of transient absorption or fluorescence anisotropy decay would be the only means of monitoring energy transfer within the band. However, it has turned out from steady state[33−35] and time-resolved[4] measurements that the spectrum of B800 is inhomogeneously broadened as a result of site variations in pigment−protein interactions. This implies that energy transfer within the inhomogeneously broadened B800 band will also be visible through absorption or fluorescence spectral changes.

Figure 4 A shows the one-color transient absorption kinetics of B800 at 790, 800, and 810 nm at 77 K, measured with ∼80 fs pulses. The B800 excited state is seen to decay in a nonexponential fashion, characterized by two time constants, 0.35 ± 0.05 and 1.8 ± 0.2 ps. From the discussion of the previous section we now know that the slower time constant is related to the B800 → B850 transfer whereas the 0.35 ps time is assigned to the energy transfer within the spectrally inhomogeneous B800 band. This assignment is supported by the results of a measurement of the absorption anisotropy within the B800 band (Figure 4B) which yields a similar decay time of 0.3 ps as well as a slower decay (1.5−5 ps) of lower amplitude. In order to correlate these results to the structure of LH2, we performed computer simulations of the excitation transfer described as incoherent hopping in a system of spectrally inhomogeneous antenna complexes, using a master equation approach.[36,37] The simulations show that the measured B800 dynamics is well described as energy transfer with a characteristic average nearest neighbor pairwise transfer time of 0.35 ps among ∼10 Bchl molecules in a circular arrangement,[4] in good agreement with the LH2 structure. The anisotropy kinetics of Figure 4B shows another interesting result; the slow decay component of the anisotropy has a much higher amplitude at 790

(34) Van Der Laan, H.; Schmidt, Th.; Visschers, R. W., Visscher, K. J.; Van Grondelle, R.; Völker, S. *Chem. Phys. Lett.* **1990**, *170*, 231.
(35) De Caro, C.; Visschers, R. W.; Van Grondelle, R.; Völker, S. *J. Phys. Chem.* **1994**, *98*, 10584.
(36) Pullerits, T.; Freiberg, A. *Biophys. J.* **1992**, *63*, 879.
(37) Pullerits, T.; Visscher, K. V.; Hess, S.; Sundström, V.; Freiberg, A.; Timpmann, K.; Van Grondelle, R. *Biophys. J* **1994**, *66*, 236.

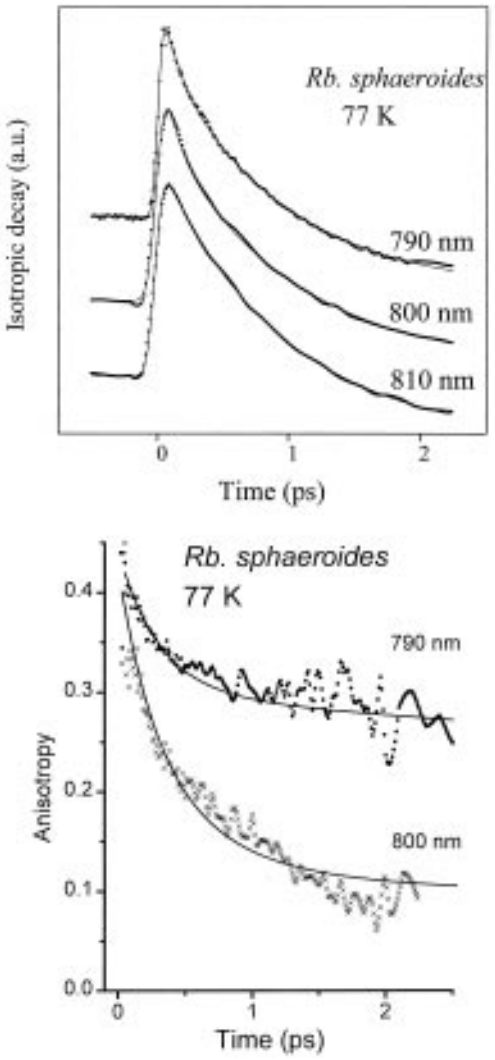

Figure 4. (A, top) Isotropic transient absorption decay of B800 measured at 790, 800, and 810 nm at 77 K. (B, bottom) Anisotropy decay of B800 at 790 and 800 nm at 77 K.

nm in the blue wing of the absorption band than at 800 nm in the center of the band. A similar behavior is observed for anisotropy measured in the red wing of the B800 band. The simulations show that this is a direct consequence of the mainly inhomogeneous spectral broadening of B800 at low temperature, which results in trapping of the energy on molecules in the wings of the spectral inhomogeneous distribution function (IDF). Molecules with absorption at these positions in the spectrum have few neighbors with a good spectral overlap and suitable for efficient energy transfer. The phenomenon is a direct analogy to the well-known Anderson localization[38] in molecular crystals.

Energy Transfer within B850

Excitation transfer in photosynthetic antenna systems is in most cases described as incoherent Förster hopping. In the previous two sections we saw that also B800 → B850 and B800 ⇔ B800 energy transfer can be reasonably well described by the incoherent Förster mechanism. On the other hand also the exciton concept has sometimes been applied for photosynthetic antenna systems. An exciton is delocalized

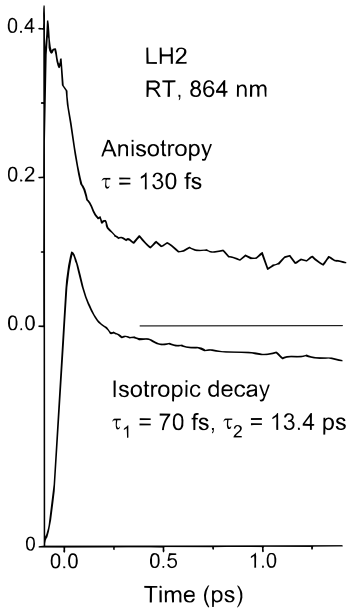

Figure 5. Anisotropic and isotropic decays of B850 measured at 864 nm at room temperature. From these and other similar kinetic measurements the anisotropy decay time was found to be less than 1.8 times the isotropic decay time.

over a number of pigment molecules, and the dynamics occurs through relaxation between different exciton states. However, often it is not so straightforward to unambiguously distinguish in experiment the two qualitatively different kinetic processes incoherent hopping and exciton relaxation. The actual dynamics can in addition be a combination of these limiting cases. For example, smaller sections of the full system might behave as a small exciton whereas the dynamics on a larger scale may correspond to the hopping-like transfer of this small exciton. An argument against the pure incoherent Förster mechanism is the recent observation of coherent nuclear motions in the antenna complexes of photosynthetic bacteria.[39,40] The vibrational coherence is preserved for approximately the same time as the estimated single step transfer time in these systems. It means that one of the main assumptions of Förster theory, that the vibronic relaxation occurs much faster than the excitation transfer, is not fulfilled. Furthermore, the structural data show a densely packed Bchl *a* system, where electronic interactions are quite strong.

In order to characterize the B850 excited state, we measured the polarized femtosecond absorption decays and transient absorption spectra of the LH2 complex of *Rb. sphaeroides*. With 40 fs time resolution we measured the isotropic and anisotropic pump−probe signal in the B850 band. In Figure 5 the anisotropic and isotropic decays at room temperature at 864 nm are presented. Both traces have a fast subpicosecond component of 130 and 70 fs for anisotropic and isotropic kinetics, respectively. Qualitatively it is understandable that for a ringlike structure of spectrally inhomogeneous Bchl *a* molecules it takes a few jumps before anisotropy drops considerably, because the transition dipole moments of the adjacent B850 molecules are nearly parallel. At the same time the

(38) Anderson, P. W. *Phys. Rev.* **1958**, *109*, 1492.

(39) Chachisvilis, M.; Pullerits, T.; Jones, M. R.; Hunter, C. N.; Sundström, V. *Chem. Phys. Lett.* **1994**, *224*, 345.

(40) Chachisvilis, M.; Fidder, H.; Pullerits, T; Sundström, V. *J. Raman Spectrosc.* **1995,** *26*, 513.

spectral equilibration inside the inhomogeneous distribution function (IDF) is faster. However, direct numerical simulations show that for the B850 ring of 18 Bchl *a* molecules at room temperature in the case of excitation into the red side of the absorption band the fast component of the anisotropy decay is 3 times longer than the corresponding component of the isotropic decay.[41] We have found similar contradictions also for other temperatures and wavelengths. This result strongly suggests that the entity which acts as an elementary excitation is not a single excited Bchl *a* molecule. The question to be answered is, How big is this elementary excitation? Is it a dimer, is the full ring acting as an exciton, or is the reality something in between?

Exciton Delocalization Length in B850. The nonlinear optical properties of aggregates are sensitive to the delocalization length of the electronically excited states. For example, in a linear aggregate of two-level molecules, one can calculate the exciton delocalization length from the approximate expression $\Delta\omega \approx 3\pi^2 V/(N+1)^2$, where $\Delta\omega$ is the difference between pump−pulse induced bleach (ground state to one-exciton transition) and pump−pulse induced absorption (one-exciton to two-exciton transition) maxima, V is the dipole−dipole interaction between nearest neighbors, and N is the exciton delocalization length.[42] However, monomeric Bchl *a* has a strong excited state absorption in the region of the Q_y transition, and therefore B850 is much more realistically described as an aggregate of multilevel (not two-level) molecules where the one- to two-exciton transition has a significant intramolecular component where two photons double-excite a single molecule.

Unfortunately, the strength and energy of the monomeric induced absorption for Bchl *a* are not well-defined quantities. According to the available experimental studies of Bchl *a* in solutions the monomeric induced absorption (transition from the singly to doubly excited monomeric state) is located about 100 cm^{-1} to the blue as compared with the Q_y transition. However, the induced absorption is very broad, and it is possible that there is actually more than one state involved, but we do not consider this possibility here. From the available data we also conclude that the induced absorption is about half of the strength of the Q_y transition and its dipole moment is nearly parallel to the Q_y transition dipole moment. We have calculated the interactions between B850 molecules by using published structural information.[9] According to our estimates the nearest neighbor interaction in an α,β-polypeptide is $\sim$450 cm^{-1}, for adjacent polypeptides $\sim$ 320 cm^{-1}.[41] However, it has to be pointed out that despite the known LH2 structure there are a few not-so-well-determined parameters in this calculation. Consequently, at this stage the calculated interaction should be viewed as a rough estimate even with the atomic coordinates in hand (see ref 41). The precise value has to be obtained from experiment. In the case of the elementary building block of LH1, a so-called B820 Bchl *a* dimer,[43] the experimentally estimated interaction is 230 cm^{-1}. However, the interaction in the B850 ring need not be the same as in LH1, and the biochemical treatment which leads to the formation of B820 may, furthermore, result in structural alterations with subsequent changes of the interaction energy.

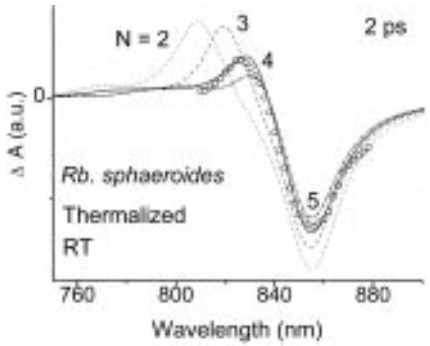

Figure 6. Experimental (circles) and calculated (lines) absorption difference spectra of B850 2 ps after excitation of the B800 band. Calculated lines correspond to exciton delocalization lengths of 2 (dotted line), 3 (dashed line), 4 (solid line), and 5 (dashed−dotted line).

In Figure 6 we have made a comparison of the calculated spectra for the thermalized population with the experimental absorption difference spectrum recorded 2 ps after excitation with a femtosecond laser pulse into the B800 band. The calculated spectra are shifted in a way that they have the bleaching maximum at 856 nm in accordance with the measurements. The best correspondence between calculated and measured spectra is for $N = 4$. However, it has to be pointed out that this result is sensitive to the assumed parameters for the monomeric induced absorption. For example, if we assume that the strength of the monomeric induced absorption is equal to the strength of the Q_y transition, then we would get a reasonable agreement for $N = 2$ or 3. Therefore, for a more definite value of the delocalization length very accurate information about the excited state absorption of the Bchl *a* monomer is needed. At this stage we can nevertheless conclude that our results do not support the idea that the exciton in B850 is delocalized over the full ring (at least 2 ps after the excitation of B800), but rather that the exciton delocalization length is 4 ± 2 Bchl *a* molecules. The wide error limit recognizes the fact that the monomeric Bchl *a* excited state absorption is poorly defined.

Energy Transfer over the Antenna Network. LH2 → LH1 Transfer

So far we have discussed the most elementary energy transfer steps involving energy transfer between individual pigment molecules of a single antenna complex. The antenna network of a photosynthetic organism as a rule contains more than one complex, and the different complexes are closely interfaced to each other to give an efficient overall energy transfer toward the reaction center. It is therefore of special interest to examine how different antenna complexes interact and how energy is transferred between them. We have done this for the LH2 and LH1 complexes of purple bacteria.

Up to now there was little information available on the rate of energy transfer between the outer (LH2) and inner (LH1) antenna systems. Measurements

(41) Pullerits, T.; Chachisvilis, M.; Sundström, V. *J. Phys. Chem.* **1996**, *100*, 10787.
(42) Van Burgel, M.; Wiersma, D. A.; Duppen, K. *J. Chem. Phys.* **1995**, *102*, 20.
(43) Visschers, R. W.; Chang, M. C.; van Mourik, F.; Parkes-Loach, P. S.; Heller, B. A.; Loach, P. A.; Van Grondelle, R. *Biochemistry* **1991**, *30*, 2951.

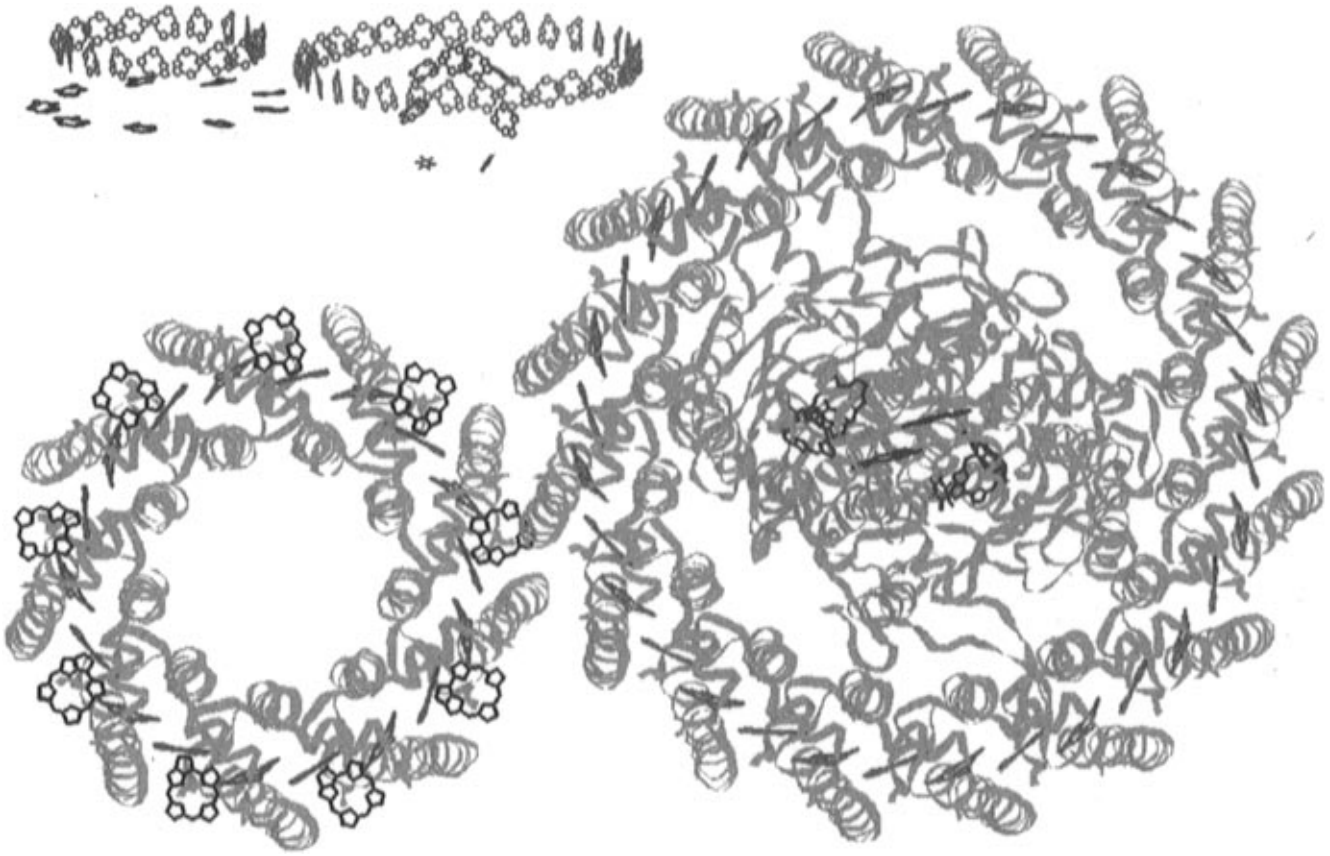

Figure 7. Model of the purple bacterial photosynthetic unit based on the structural data in refs 9, 10, and 30. Colors of the different pigment molecules: B800, blue; B850, green; B880, orange; RC, red.

with picosecond time resolution[44] and indirect information obtained from the overall antenna energy equilibration time suggest that this transfer step occurs on the few picosecond time scale. In a femtosecond two-color measurement[45] we obtained direct information on the LH2 → LH1 transfer step. The buildup of B875 excitations in *Rb. sphaeroides* at room temperature and 77 K was measured following excitation of B800, and the B850 → LH1 transfer step was found to occur with a time constant of 3 ps at room temperature and 5 ps at 77 K. From these results we can construct a model for the LH2−LH1 interaction.

A Model for the Association of LH2 and LH1 Complexes. Assuming that LH2 to LH1 transfer arises from a Förster type hopping mechanism, we can use the 5 ps transfer time at 77 K (3.3 ps at room temperature) to estimate the distance between B850 molecules and LH1. The model in Figure 7 is based on the recent crystallographic data of LH2[9] and LH1.[30] The overall arrangement of the LH1 model is based on the 16α/16β ring structure determined for the LH1 complex of *Rhodospirillum rubrum*,[30] but the arrangement of the Bchls is essentially that described for the B850 molecules of *Rps. acidophila*.[9] As a reference energy transfer time we have used the LH1 to RC transfer time of ∼35 ps.[46,47] Assuming that the RC is positioned inside the LH1 circle, as illustrated in Figure 7, leads to a distance of ∼ 45 Å between the LH1 Bchl *a* molecules and the center of the special pair in the RC.[30] If we further assume that the relative pigment orientations and spectral overlaps are similar for the LH2 → LH1 and LH1 → RC processes, we can estimate a distance between the B850 molecules of LH2 and the Bchl *a* molecules of LH1. The time of ∼5 ps at 77 K for B850 → LH1 transfer should therefore correspond to 30 Å, given the R^6 dependence on distance of the Förster energy transfer rate. Since the high-resolution data of McDermott et al.[9] for LH2 show that the B850 Bchls are, at most, 15 Å from the outer edge of the complex, then the 30 Å BChl−BChl distance between LH2 and LH1 rings calculated from our measurements is consistent with the structural data (see Figure 7).

Energy Transfer from the LH1 Core Antenna to the Reaction Center

How the light-harvesting antenna makes contact with the reaction center and how energy is transferred into the reaction center (trapped) are important aspects of the function of the whole photosynthetic unit. Information about this can be obtained by measuring the rate of energy transfer from LH1 to the special pair electron donor in the reaction center, and from it estimating the distance between the LH1 molecules and the special pair. The description of the LH1 excited state is of crucial importance for the result; whether the excitation energy is delocalized over the whole LH1 ring or localized to only a few Bchl molecules will obviously be important for how we

(44) Zhang, F. G.; Van Grondelle, R.; Sundström, V. *Biophys. J.* **1992**, *61*, 911.
(45) Hess, S.; Chachisvilis, M.; Timpmann, K.; Jones, M. R.; Hunter, C. N.; Sunström, V. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 12333.
(46) Visscher, K. J.; Bergström, H; Sundström, V.; Hunter, C. N.; Van Grondelle, R. *Photosynth. Res.* **1989**, *22*, 211.
(47) Bergström, H.; Van Grondelle, R.; Sundström, V. *FEBS Lett.* **1989**, *250*, 503.

interpret the measurements. The exciton delocalization length of LH1 is not yet determined (as it was for B850 (see above)), but in view of the structural similarity between the two complexes it is reasonable to assume that also for LH1 the exciton delocalization is relatively small and extends over ~4 Bchl molecules. This would imply that energy is transferred from approximately 8 LH1 antenna sites to the reaction center (assuming 1 RC/32 Bchl LH1 rings).

A transfer time of ~35 ps was obtained for this process by measuring the decay of LH1 excitations in the temperature range 77−177 K.[46,47] In these measurements LH1 was excited by a picosecond pulse in the red wing of the absorption spectrum in order to avoid kinetics associated with energy equilibration over the antenna; judging from the high anisotropy (r = 0.20−0.25), at this temperature and under these excitation conditions, only limited energy transfer occurs in the antenna, and consequently the measured antenna to RC transfer time should approximately correspond to the pairwise transfer time from one LH1 site to the special pair of the RC. The slow 35 ps transfer time shows that the transfer of energy from the antenna to the RC is the slowest step in the overall trapping of light energy by the reaction center. While equilibration of energy within individual antenna complexes and transfer between different such complexes occur on the subpicosecond to few picosecond time scale, the final step to get it into the RC is at least 10 times slower. This slow rate is a direct consequence of the size of the reaction center protein, which prevents a shorter antenna−RC distance. We have termed this situation "transfer-to-trap-limited" excitation dynamics.[48]

Discussion

Above we have characterized the sequence of basic excitation transfer steps in the antenna system of purple photosynthetic bacteria and linked it to the known structural information. Despite this general understanding there are still several open questions. Here we try to address in a very qualitative way some possible structure−function relationships in these pigment−protein complexes. For example, what is the function of organizing this system in the way structural investigations have revealed? Does it make excitation trapping more efficient? What is the possible role of the excitons?

We first consider the B800 to B850 transfer. The most simplistic description of this process is a transfer between a monomeric B800 molecule and an aggregate of four B850 molecules. The dipole moment of the aggregate of N molecules is $N^{1/2}$ times the monomeric dipole moment. The Förster transfer rate is proportional to the square of the dipole−dipole interaction which eventually results in N times faster transfer for that step than between two monomers. On the other hand from the LH2 structure we can see that there are two Bchl *a* molecules in B850 which could efficiently accept excitation from B800. This would increase the overall transfer rate for monomer-to-monomer transfer by a factor of 2. Thus, we have a rough estimate that the exciton in B850 increases the rate of B800 to B850 transfer by approximately a factor of 2. Following the same logic we can conclude that due to the excitons the LH2 to LH1 transfer rate is about 4 times higher than it would be for monomers. We can estimate that the worst possible transfer time for LH2 → LH1 without excitons would be about 15 ps. This is still fast compared to the overall trapping time and leads us to conclude that this gain is not a significant optimization factor and most likely has not driven evolution.

The final step, trapping by the RC, deserves a little more attention. The trapping by the RC in the antenna lattice can be expressed as a sum of the so-called first passage time and the revisiting time.[49] The ringlike configuration of LH1-RC implies that the first passage time is equal to the transfer time from a LH1 aggregate to the RC special pair. For an aggregate of four Bchl *a* molecules this process is 4 times faster than it would have been in the case of monomers. The revisiting time is proportional to the number of antenna sites, implying that if one site corresponds to an aggregate of four, then for the aggregate we again have 4 times shorter time compared to the monomer case. The situation is actually not that simple because in fact we can form the same number of different aggregates of four neighboring molecules in the ring as the number of monomers. However, the different aggregates with overlapping areas are not independent. Without giving a rigorous proof, it appears that the aggregation does reduce the revisiting time. We can therefore conclude that excitons significantly improve the trapping efficiency in the LH1−RC system and might play a significant role in the overall performance of primary photosynthesis.

We did not specifically discuss the dynamics of excitons in B850. This dynamics may also play an important role in the overall performance of the LH2 to LH1 transfer as the excitation first has to be transferred to the site where LH2 makes contact with LH1. From the point of view of the general description of the dynamics in excitonic dimers,[50] it appears that the densely packed Bchl *a* system is an important factor to make the diffusion of the excitation fast, because the rate constants which correspond in this general description to Förster transfer depend strongly on the interpigment distance. However, it is not so clear whether the existence of excitons as a correlation between monomeric excited states plays any important role in making light-harvesting more efficient in B850.

Excitons may also be important in tuning the spectra of pigment−protein complexes, making the transfer toward the RC energetically more favorable. Besides well-understood excitonic shifts of the absorption bands, it may also work through the enhanced dispersive solvent shift in the case of excitons where some states may have a significant charge transfer character.

We conclude that excitons are present and may have a significant role in making primary energy transfer and trapping in photosynthesis more efficient. The most significant improvement appears to be in the slow final transfer stage between the core antenna and RC. As discussed above, the overall reduction of the

(48) Timpmann, K.; Freiberg, A.; Sundström, V. *Chem. Phys.* **1995**, *194*, 275.

(49) Pearlstein, R. M. *Photochem. Photobiol.* **1982**, *35*, 835.

(50) Rahman, T. S.; Knox, R. S.; Kenkre, V. D. *Chem. Phys.* **1979**, *44*, 197.

energy trapping time with the fundamental antenna excitation being an exciton extending over four Bchl *a* molecules as compared to the Förster limit could be as much as approximately a factor of 5. This could be a sufficiently large difference for being a significant driving force in the evolution of the antenna organization. In order to more precisely access the functional role of excitons in the faster intra- and intercomplex energy transfer process, more knowledge has to be obtained about aspects of the energy transfer such as exciton diffusion, exciton−phonon interactions, interplay between localized and delocalized excitations, etc.

We thank our co-workers of the Department of Chemical Physics, Lund University. We also thank Professors R. J. Cogdell and N. W. Isaacs for making the atomic coordinates of LH2 available to us. This work was supported by the Swedish Natural Science Research Council and EC Grants SCI-CT92-0796 and ERBCHBGCT 930361.

AR950110O