Exciton Delocalization Probed by Excitation Annihilation in the Light-Harvesting Antenna LH2

G. Trinkunas*
Institute of Physics, Vilnius 2600, Lithuania

J. L. Herek,* T. Polívka, V. Sundström, and T. Pullerits†
Department of Chemical Physics, Lund University, P.O. Box 124, S-22100 Lund, Sweden
(Received 28 August 2000)

Singlet-singlet annihilation is used to study exciton delocalization in the light harvesting antenna complex LH2 (B800-B850) from the photosynthetic purple bacterium *Rhodobacter sphaeroides*. The characteristic femtosecond decay constants of the high intensity isotropic and the low intensity anisotropy kinetics of the B850 ring are related to the hopping time τ_h and the coherence length N_{coh} of the exciton. Our analysis yields $N_{\mathrm{coh}} = 2.8 \pm 0.4$ and $\tau_h = 0.27 \pm 0.05$ ps. This approach can be seen as an extension to the concept of the spectroscopic ruler.

DOI: 10.1103/PhysRevLett.86.4167 PACS numbers: 87.15.–v, 31.70.Hq, 71.35.Aa, 78.47.+p

Highly efficient light collection and excitation delivery to the reaction center initiates solar energy conversion in photosynthesis. This process takes place in a so-called light-harvesting antenna network composed of pigment arrays specially arranged by a protein scaffold. Recent emergence of the structures of a number of light-harvesting antenna complexes and the availability of suitable femtosecond light sources has boosted the use of ultrafast spectroscopy in photosynthesis research [1,2]. Particularly, the peripheral light-harvesting antenna (LH2) of purple bacteria has been extensively studied [3].

The structure of LH2 from the purple bacteria *Rhodopseudomonas (Rps.) acidophila* [4] and *Rhodospirillum molischianum* [5] shows a regular organization of bacteriochlorophyll (Bchl) molecules in two concentric rings called B800 and B850 (see Fig. 1). Excitation is transferred from B800 to B850 in 0.7 ps at room temperature [6]. In molecular systems with strong electronic coupling (e.g., J-aggregates), experimental observables such as superradiance [7], motional narrowing [8], and electronic transitions from one- to two-exciton bands [9] have been interpreted in terms of excitons—collective excited states of more than one molecule of the aggregate. It is generally accepted that the excited states of B800 molecules have a monomeric character, whereas in B850 the states are delocalized over a considerable number of Bchls (see [10] and references therein). Currently, there is no common agreement about the extent of exciton delocalization in B850. Estimates of the delocalization length in different studies propose values ranging from a few Bchl molecules [7,11] to the whole B850 ring [12].

Excitation annihilation is conceived as a Förster-type incoherent energy transfer [13] from the excited donor to the excited acceptor molecule with the result of a doubly excited acceptor state. The latter quickly relaxes to the singly excited state. The description of excitation annihilation is well developed for an extended network of light-harvesting antennas [14–18]. An individual LH2 is a finite system, however, and the above results are not directly applicable. Therefore an explicit excitation hopping model has been adapted assuming a migration-limited character of the annihilation. A similar random walk formalism can be used for the anisotropy decay. In this Letter, we present a simultaneous study of these two different experimental observables, excitation annihilation and anisotropy kinetics, in the B850 ring. This approach provides the basis for addressing the issue of exciton delocalization in B850 from an unusual angle, enabling us to obtain new insight into the problem of exciton coherence in photosynthetic light harvesting.

The experiments were performed via conventional femtosecond pump-probe spectroscopy techniques. Briefly, the output of a commercial amplified Ti:sapphire laser consisted of pulses of 100 fs duration with a central wavelength of 800 nm and energy of 200 μJ at a repetition rate of 5 kHz. Most of this light was directed to an optical parametric generator/amplifier to produce excitation (pump) pulses at 850 nm. The remaining light ($\sim$5 μJ) was used to generate spectrally broad pulses with a white light continuum formed in a rotating quartz plate

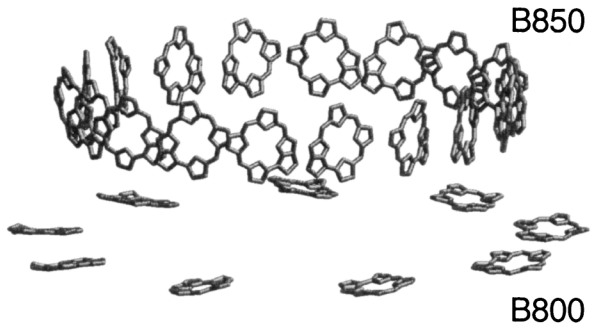

FIG. 1. Pigment arrangement in LH2 from the purple bacterium *Rps. acidophila* [4]. The upper ring of 18 Bchl molecules absorbs at 850 nm and the lower ring of nine absorbs at 800 nm. The rings are therefore called B850 and B800, respectively. Center-to-center distances between the pigments in different rings are about 9 Å (B850) and 21 Å (B800). In the B850 ring, the close pigment packing suggests that coherent excitations may occur.

(5 mm). A monochromator positioned after the sample allowed detection at specific spectral windows, with 3 nm resolution. For the intensity studies, the polarization of pump and probe pulses was set to the magic angle (54.7°) and the intensity of the pump pulses was varied from 10 to 500 μW using a Berek compensator placed before a Glan polarizer. This arrangement provided minimal beam walk-off at the pump-probe overlap region in the sample, especially important given the small beam diameters used in these experiments (pump: 225 μm; probe: 80 μm). For studies of the anisotropy decay, separate measurements were performed with parallel and perpendicular pump-probe polarization, from which the time-dependent anisotropy change $r(t)$ was calculated according to $r(t) = [I_{\parallel}(t) - I_{\perp}(t)]/[I_{\parallel}(t) + 2I_{\perp}(t)]$. Kinetic traces were obtained by scanning the pump-probe delay time and recording the transient absorption signal in a dual-beam (probe and reference) detection system. Care was taken to ensure that changes in the path length of the pump beam (for varying the pump-probe delay time) did not affect the spatial overlap at the sample.

The samples, isolated complexes of LH2 from *Rhodobacter (Rb.) sphaeroides* 2.4.1 (obtained from R. J. Cogdell, Glasgow) were diluted in a 50 mM Tris/HCl (pH 8) buffer with 0.1% lauryldimethylamine oxide added to prevent aggregation. Measurements were performed on samples at room temperature in a rotating cell (2 mm optical path length); The optical density was ~0.3 at the excitation wavelength. Steady-state absorption spectra recorded before and after the measurements showed minimal sample degradation.

Figure 2(a) shows transient absorption decay traces following direct excitation of the B850 ring ($\lambda_{\text{exc}} = 850$ nm) obtained for various excitation intensities. In all curves, after the initial fast decay the signal levels off, reaching a long-lived kinetic component corresponding to the slow decay of a single remaining excitation in the LH2 ring ($\tau \sim 1$ ns [7]). The lowest excitation intensity traces have a negligible amount of annihilation contributions and, at early times, contain only a very small spectral evolution component; the dominant signal is the long-living component due to the overall relaxation of excitation. To analyze the data, the "single-excitation" level (we later refer to this as the residual level) is scaled to match the long-lived kinetic component and subtracted, leaving only the pure excitation annihilation kinetics. The resulting signal is therefore called a reduced absorption difference $\Delta A_{\text{red}}(t) = \Delta A(t) - \Delta A_{\text{res}}$. When plotted on a semilogarithmic scale [see Fig. 2(b)], the tails of the ΔA_{red} kinetics show the asymptotic exponential decay that does not vary with excitation intensity.

The transient absorption anisotropy decay at low excitation intensity is shown in Fig. 3. It is well approximated by a single exponential decay with a time constant of about 100 fs, in agreement with earlier studies [11].

Intuitively, the slowest asymptotic phase of the annihilation-related decay is due to the last two remaining excitations in a ring. Further justification of this point comes from the fact that the normal mode formulation of the incoherent dynamics of multiple excitations in a finite size linear aggregate leads to eigenvalues which do not depend on initial conditions [19]. This simplifies our problem to annihilation of two excitations in a ring. The kinetics of such a system can be represented as a random walk of one excitation with respect to another fixed excitation. In this representation, the transfer rate of the moving excitation is

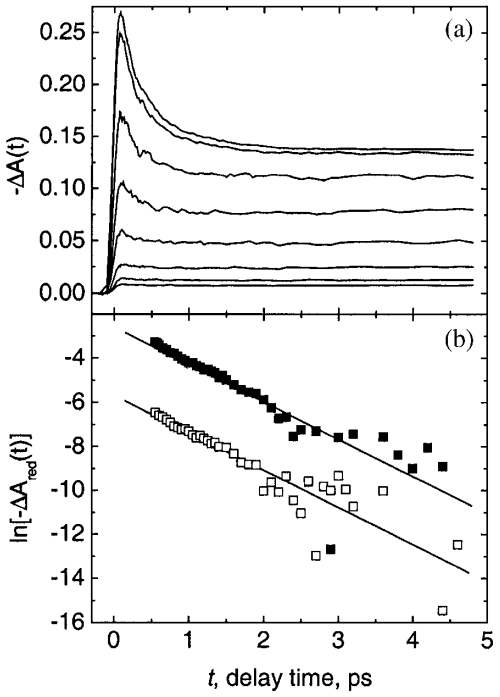

FIG. 2. (a) Transient absorption kinetics obtained by exciting LH2 at 850 nm and probing at 860 nm for eight different excitation intensities P (from top to bottom): 550, 400, 200, 100, 50, 25, 15, and 7 μW. (b) Linear regression analysis of the asymptotic annihilation decay of the reduced absorption difference for the two highest excitation intensities 550 μW (solid squares) and 400 μW (open squares). Both fits give $\tau_a = 0.59 \pm 0.05$ ps.

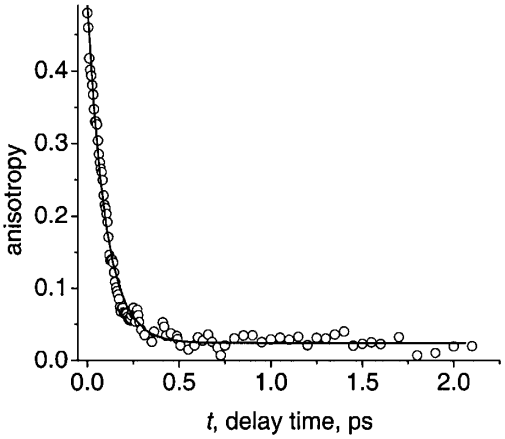

FIG. 3. Low excitation intensity (40 μW) transient absorption anisotropy of B850 excited at 850 nm and probed at 860 nm. The decay is fit by a single exponential with time constant of $\tau_d = 0.1 \pm 0.01$ ps.

twice the ordinary transfer rate [17], and we describe the dynamics in space of the distance between two excitations. Letting the fixed excitation act as a trap includes the annihilation process. The master equation of such a system reads

$$\frac{dp_n}{dt} = \frac{2}{\tau_h}(p_{n-1} - 2p_n + p_{n+1}), \quad n = 1, \ldots, N-1, \tag{1}$$

where p_n is the probability that the distance between excitations is n sites, τ_h is the pairwise transfer time, and N is the overall number of sites in the ring. Note that N in this model is equivalent to a maximum number of excitons fitting together in the ring. Therefore, N_{Bchl}/N, where N_{Bchl} is the number of Bchl molecules in a ring, yields the coherence length of the exciton N_{coh}. The factor 2 on the right-hand side accounts for the doubling of the rate of the mobile excitation with respect to the fixed one. In this simplified description, we have excluded the fine details of the dynamics where the exciton has not yet moved entirely away from the original N_{coh} Bchl molecules. Absorbing boundary conditions,

$$p_N = p_0 = 0, \tag{2}$$

introduce trapping (annihilation) by the fixed excitation if the distance between excitations becomes zero. The probabilities p_0 and p_N are the same because of the ring symmetry.

When expressing p_n via normal modes,

$$p_n = \phi_n e^{-\lambda t}, \tag{3}$$

Eq. (1) transforms into an eigenvalue problem. A straightforward solution (see, e.g, [20]) gives a complete set of eigenfunctions and the corresponding eigenvalues,

$$\lambda_m = \frac{8}{\tau_h}\sin^2\left(\frac{\pi m}{2N}\right), \qquad m = 1, \ldots, N-1. \tag{4}$$

The lowest mode λ_1 here corresponds to the inverse of the asymptotic annihilation decay time τ_a observed in experiments:

$$\lambda_1^{-1} = \tau_a = \frac{\tau_h}{8\sin^2(\pi/2N)}. \tag{5}$$

In Eq. (5) we still have two unknown parameters: N and τ_h. The same two parameters determine the fluorescence and transient absorption anisotropy decay time due to the random walk of excitation on the ring [21]:

$$\tau_d = \frac{\tau_h}{4\sin^2(2\pi/N)}. \tag{6}$$

Elimination of the hopping time from Eqs. (5) and (6) results in a transcendent equation for the number of hopping sites N:

$$\frac{4}{\alpha}\left[\cos^2\left(\frac{\pi}{N}\right) + \cos^3\left(\frac{\pi}{N}\right)\right] = 1, \tag{7}$$

where $\alpha = \tau_a/\tau_d$. The approximate solution of Eq. (7) gives

$$N \cong \sqrt{\frac{29\pi^2}{30 - \sqrt{174\alpha - 492}}}. \tag{8}$$

Thus, two experimentally determined quantities, the time constants of the asymptotic decay of annihilation τ_a and the anisotropy decay τ_d, can be related to the number of hopping sites N. Given the total number of pigment molecules, it is straightforward to estimate the delocalization length of the excitation in the B850 ring.

The experimental data of Figs. 2 and 3 yield an asymptotic annihilation decay time $\tau_a = 0.59 \pm 0.05$ ps and a depolarization time $\tau_d = 0.1 \pm 0.01$ ps, respectively. Substituting the ratio of these time constants into Eq. (8) gives the number of hopping sites $N = 6.6 \pm 0.9$. The hopping time $\tau_h = 0.27 \pm 0.05$ ps then follows from Eq. (5) or (6) straightforwardly. A very similar hopping time has been estimated for the light-harvesting antenna of *Rhodospirillum rubrum* [22].

High-resolution structural information of LH2 from *Rb. sphaeroides* does not exist up to this date. However, the lower resolution electron microscopy studies [23] indicate that the structure of LH2 from *Rb. sphaeroides* is very similar to that of *Rps. acidophila*. Therefore, it is expected that also in *Rb. sphaeroides* B850 consists of 18 Bchl molecules. We conclude that the hopping site consists of $N_{\mathrm{coh}} = 18/N = 2.8 \pm 0.4 \cong 3$ Bchl molecules giving the estimate for the exciton coherence length in thermalized LH2. This value should be taken as a lower estimate of the coherence length since the anisotropy decay may be partly due to the exciton relaxation inside the hopping site, which would make the values of both estimated parameters N_{coh} and τ_h larger. This effect, however, cannot be too strong since the transition dipole moments of 3–4 neighboring Bchls of B850 are nearly parallel. Consequently, the anisotropy change due to the exciton relaxation is rather small. In our model, we do not explicitly take into account the spectral disorder (random distribution of the site energies). At the same time, this disorder is one of the main reasons why the excitation is not delocalized over the full ring. We point out that due to motional narrowing the distribution of exciton energies is considerably smaller than the distribution of molecular site energies [8]. The standard deviation of the excitonic energies is smaller than kT at room temperature. Consequently, the spectral disorder does not affect substantially the dynamics nor the conclusions drawn from kinetic experiments. It is worth noting that N_{coh} is time dependent. Since our method is not sensitive to the fastest stages of the dynamics, we cannot determine the initial coherence length. Our results correspond to the thermalized excitons. Substantial excess energy is dissipated during the excitation annihilation and local heating may have a further localizing effect on the excitons.

The number of photon hits per LH2 ring in our experiment has been estimated by analyzing the excitation

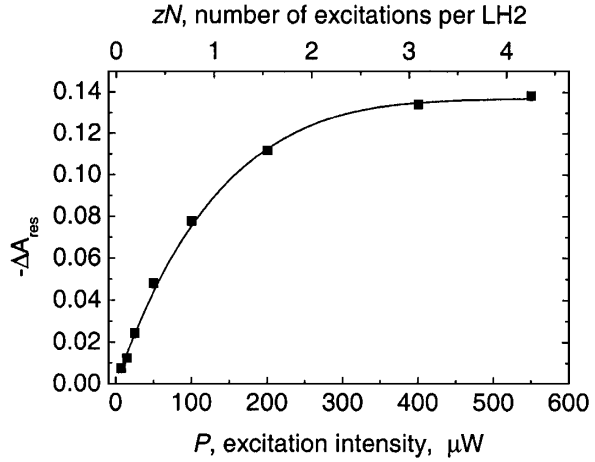

FIG. 4. Excitation intensity dependence of the residual absorption difference ΔA_{res}. Experimental values (solid squares) are taken from Fig. 2. Solid line is the best fit by a binomial distribution [Eq. (10)]. The adjustable parameters are $C = 0.13$ and $f = 0.001\,27$. The latter value enables calculation of the actual number of excitations per LH2 complex at different excitation intensities, which is presented as the top axis of the plot.

intensity dependence of the level of residual absorption difference [$\Delta A(t)$, $t \geq 5$ ps]. The residual absorption is due to the last remaining excitation in a ring and therefore is proportional to the probability that a B850 ring was excited by one or more excitations. Assuming that the initial distribution of excitations is random, the probability that a B850 received i excitations is governed by a binomial distribution:

$$B_i(N, z) = \binom{N}{i}(1 - z)^{N-i} z^i, \tag{9}$$

where $z = fP$ is the average number of excitations created per hopping site, and P and f are the excitation intensity and a scaling factor, respectively. Then the residual steady state levels of transient absorption shown in Fig. 2 can be expressed as

$$\Delta A_{\mathrm{res}}(z) = C[1 - B_0(N, z)] = C[1 - (1 - z)^N]. \tag{10}$$

This expression fits very well the residual absorption difference levels (see Fig. 4), enabling us to estimate the average number of excitations created per LH2 (top axis of Fig. 4). It can be seen that, at very high excitation intensities, a substantial level of excited state absorption may be created (four out of six sites are excited). Consequently, direct excitation of the higher excited states and subsequent annihilation can occur without any exciton migration. This can affect the initial fast components of the annihilation decay. In the present approach, this problem is avoided by considering only the last stage of the annihilation decay, the asymptotic exponential decay.

In this Letter, we have analyzed the excitation annihilation in a photosynthetic pigment ring. The approach is general and can be extended to any molecular aggregate by straightforward modification of the boundary conditions for Eq. (1). Thus, a comparative analysis of annihilation kinetics and anisotropy decay based on Eqs. (6) and (7) provides a way to estimate the properties of the elementary excitations in ordered natural or fabricated molecular systems with known structure. For systems of weakly coupled molecules without exciton delocalization, this approach may provide the means to measure the size of the aggregate. It can also be considered as an extension of the concept of the spectroscopic ruler [24], widely used to measure the donor-acceptor distances via Förster excitation energy transfer.

This work was financially supported by the Swedish Natural Science Research Council (NFR), the Crafoord Foundation, and the Swedish Institute.

*These authors contributed equally to this work.
†Corresponding author.

[1] T. Pullerits and V. Sundström, Acc. Chem. Res. **29**, 381 (1996).
[2] G. R. Fleming and R. van Grondelle, Curr. Opin. Struct. Biol. **7**, 738 (1997).
[3] V. Sundström, T. Pullerits, and R. van Grondelle, J. Phys. Chem. B **103**, 2327 (1999).
[4] G. McDermott *et al.,* Nature (London) **374**, 517 (1995).
[5] J. Koepke *et al.,* Structure **4**, 581 (1996).
[6] A. P. Shreve *et al.,* Biochim. Biophys. Acta **1058**, 280 (1991).
[7] R. Monshouwer, M. Abrahamsson, F. van Mourik, and R. van Grondelle, J. Phys. Chem. B **101**, 7241 (1997).
[8] E. W. Knapp, Chem. Phys. **85**, 73 (1984).
[9] H. Fidder, J. Knoester, and D. A. Wiersma, J. Chem. Phys. **98**, 6564 (1993).
[10] M. Chachisvilis, O. Kühn, T. Pullerits, and V. Sundström, J. Phys. Chem. B **101**, 7275 (1997).
[11] T. Pullerits, M. Chachisvilis, and V. Sundström, J. Phys. Chem. **100**, 10 787 (1996).
[12] D. Leupold *et al.,* Phys. Rev. Lett. **77**, 4675 (1996).
[13] V. M. Agranovich and M. D. Galanin, *Electron Excitation Energy Transfer in Condensed Matter* (North-Holland Elsevier, Amsterdam, 1982).
[14] L. Valkunas, G. Trinkunas, V. Liuolia, and R. van Grondelle, Biophys. J. **69**, 1117 (1995).
[15] L. Valkunas, G. Trinkunas, and V. Liuolia, in *Resonance Energy Transfer,* edited by D. L. Andrews and A. A. Demidov (Wiley, New York, 1999), pp. 244–307.
[16] L. Valkunas, E. Åkesson, T. Pullerits, and V. Sundström, Biophys. J. **70**, 2327 (1996).
[17] W. T. F. den Hollander, J. G. C. Bakker, and R. van Grondelle, Biochim. Biophys. Acta **725**, 492 (1983).
[18] J. G. C. Bakker, R. van Grondelle, W. T. F. den Hollander, Biochim. Biophys. Acta **725**, 508 (1983).
[19] K. Krebs, M. P. Pfannmueller, B. Wehefritz, and H. Hinrichsen, J. Stat. Phys. **78**, 1429 (1995).
[20] N. G. van Kampen, *Stochastic Processes in Physics and Chemistry* (North-Holland Elsevier, Amsterdam, 1992).
[21] S. E. Bradforth *et al.,* J. Phys. Chem. **99**, 16 179 (1995).
[22] H. M. Visser *et al.,* Biophys. J. **69**, 1083 (1995).
[23] T. Walz *et al.,* J. Mol. Biol. **282**, 833 (1998).
[24] L. Stryer and R. P. Haugland, Proc. Natl. Acad. Sci. U.S.A. **58**, 719 (1967).