

Size Enhancement of Transition Dipoles to One- and Two-Exciton Bands in a Photosynthetic Antenna

D. Leupold,¹ H. Stiel,¹ K. Teuchner,¹ F. Nowak,¹ W. Sandner,¹ B. Ücker,² and H. Scheer²

¹Max-Born-Institute for Nonlinear Optics and Short Pulse Spectroscopy, D-12474 Berlin, Germany

²Institute of Botany, University of Munich, D-80638 Munich, Germany

(Received 13 June 1996)

Measurement of nonlinear absorption and differential optical density spectrum as well as fluorescence behavior of the light harvesting antenna LH2 from purple bacterium *Rhodobacter sphaeroides* indicates exciton delocalization over 16 ± 4 bacteriochlorophyll-a molecules, corresponding to probably the full physical length of the circular aggregate responsible for the 850 nm absorption band. The giant dipole moments for the consecutive transitions ground state \rightarrow one-exciton band \rightarrow two-exciton band are 25.5 ± 2.5 D and 21.5 ± 3.5 D, respectively. [S0031-9007(96)01676-6]

PACS numbers: 87.15.Rn, 42.65.-k, 71.35.-y

Systems of organic molecules with strong electronic coupling have been the focus of basic research for decades. A representative example is the threadlike (so-called J-) aggregate of pseudoisocyanine (PIC) [1]. More recently, under the more general rubric of geometrically constrained systems, investigations of the optical properties of electronically coupled systems have been motivated by the following theoretical expectations [2–4].

(i) For confinement dimensions smaller than the optical wavelength, the transition dipole moment from the ground state to the one-exciton band should scale with $N^{1/2}$, where N is the number of molecules in the aggregate. Such a size enhanced transition dipole may be expected also between the one- and two-exciton band.

(ii) At suitable resonance frequencies the third order susceptibility $\chi^{(3)}$ may scale with N^2 .

(iii) The superradiant decay rate of the aggregate's cooperative emission should scale with N .

In real systems, the magnitude of the size enhancement may differ from theory for several reasons [3,5,6]. First, exciton-phonon coupling may cause the oscillator strength to be no longer concentrated in the transition to only the bottom of the exciton band. Second, energetic disorder may result in restricted delocalization of the electronic excitation. However, in the case of strong intermolecular coupling this inhomogeneous broadening may be reduced by exchange narrowing, which in turn may be counteracted by site correlations in the energetic disorder, possibly caused by low frequency phonons [6].

This interplay of processes seems to be far from being completely understood in any real organic aggregate. Much recent work has been concentrated on the PIC J aggregates [1,7–9]. There is agreement in the literature that at room temperature the exciton delocalization is restricted to only a few molecules (10–12 at PIC chloride [9,10]), viz., numbers which are orders of magnitude below the physical size of the aggregates [1].

In this Letter we report on collective optical properties of a natural molecular aggregate. Here the size enhance-

ment of the transition dipole moment between the ground state and the one-exciton band indicates exciton delocalization over 16 ± 4 molecules already at room temperature, which corresponds to the full physical length of the complex. Moreover, about the same enhancement has been found for the one- to two-exciton band transition.

The object investigated is the light harvesting complex LH2 from the purple photosynthetic bacterium *Rhodobacter (Rb.) sphaeroides* [11]. Its two functions are the light absorption in the near infrared (NIR) region—where it has two absorption bands with maxima at about 800 and 850 nm—and the efficient energy transfer to the core complex. The latter consists of the reaction center surrounded by the light harvesting complex 1 (LH1). LH1 absorbs spectrally close to LH2 (at about 875 nm) and is also believed to be spatially close. The antenna LH2 can be extracted from the photosynthetic membrane and is then called according to its NIR-absorption maxima the bulk pigment protein complex B800-850. This complex, extracted from *Rb. sphaeroides* and solubilized in LDAO (*N,N*-dimethyldodecylamine-*N*-oxide) buffer [12] is the subject of this Letter. There is considerable spectroscopic as well as biochemical evidence that its two NIR absorption bands (Fig. 1, inset) belong to two different, weakly interacting pigment complements. The recent x-ray structures of B800-850 complexes from two different purple bacteria suggest a common organizational principle: a ring of closely spaced (<10 Å) bacteriochlorophyll-a (Bchl_a) molecules gives rise to the 850 nm absorption, and a ring of more distant (~ 20 Å from each other and from the Bchl_a in B850) “monomeric” Bchl_a gives rise to the 800 nm absorption [13]. In this Letter we will concentrate on the 850 nm band, which in the two complexes belongs to a waterwheel-like arrangement of 16 or 18 Bchl_a. A similar arrangement is assumed for B800-850 from *Rb. sphaeroides*. Because of the small center-to-center distance of the Bchl_a's and their nearly parallel 0-1 transition dipoles, strong excitonic coupling is expected [14].

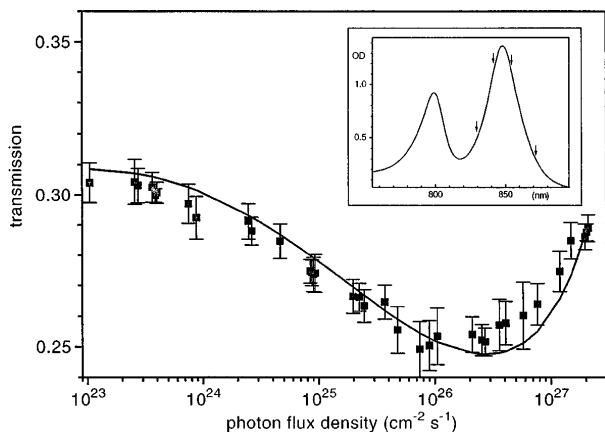


FIG. 1. Experimental nonlinear absorption (intensity dependent transmission) of B800-850 (squares) and standard deviations, measured in a cuvette of $d = 0.1$ cm at $\lambda = 833$ nm. The continuous line represents the result of simulation (cf. text) with the following set of parameters: absorption cross sections $\sigma_{0-1} = (3.5 \pm 0.7) \times 10^{-15}$ cm², $\sigma_{1-2} = (4.9 \pm 0.9) \times 10^{-15}$ cm²; emission cross section $\sigma_E = (1.6 \pm 0.3) \times 10^{-16}$ cm²; interband relaxations $k_{2-1} = 10^{13}$ s⁻¹, $k_{1-0} = \tau_F^{-1} = (1.0 \pm 0.2) \times 10^9$ s⁻¹; intraband relaxation $k_{1,i} = 6.6 \times 10^{12}$ s⁻¹. For completeness, the phenomenon of exciton annihilation, as it is reflected in the intensity dependence of fluorescence yields [19], has also been included in the simulation [20], realized by an additional term $\Gamma \mathbf{v} n_i^2$ in (1). Here $n_i(x, t)$ is the normalized population density of the initial level of annihilation, and the vector \mathbf{v} describes the related level involvement [17]. It results in an annihilations constant $\Gamma = 10^{-4}$ cm² s⁻¹. Inset: NIR-absorption spectrum of the pigment protein complex B800-850, extracted from *Rb. sphaeroides* and solubilized in LDAO buffer. Arrows indicate the wavelengths of nonlinear absorption and of transient absorption (DOD) measurements: 833, 843, 855, and 872 nm.

We wish to present results from nonlinear spectroscopic methods supporting the model of excitation delocalization over the circular aggregate.

The first experimental evidence of this strong coupling is provided by the enhancement of the 850 nm transition dipole moment (electronic ground state \rightarrow first excited state) in comparison to the dipole moment of the corresponding $S_0 \rightarrow S_1$ transition in the Bchla monomer. This result was obtained from nonlinear absorption: In linear absorption, only the product σN (σ , absorption cross section, N , particle density) is available (as the optical density $-\ln T_0 \propto \sigma N = \alpha_0$). σ acts separately, however, with the intensity-dependent beginning of nonlinearity in absorption and can be obtained from adequate modeling [15]. The latter has to be based on the equation of motion for the density matrix of the interacting system's Hamiltonian [16] and reduces under the conditions of the experiment to the rate equation/photon transport equation approach [16,17]. Having obtained in this way the dispersion of the absorption cross section in the spectral region of the transition of interest, the transition dipole moment can be calculated. To this end the nonlin-

ear absorption of B800-850 has been measured at several wavelengths in the 850 nm region (indicated in the inset in Fig. 1) with dye laser pulses (temporal FWHM = 400 ps, spectral FWHM < 1 nm). The equipment is described elsewhere [18]. One experimental data set ($\lambda_{\text{ex}} = 833$ nm) is shown in Fig. 1 together with the simulated curve (see below). As is immediately obvious from the reverse saturable absorption ("darkening"), the nonlinear absorption shows the presence of a second electronic transition with an absorption cross section $\sigma_{1-2} > \sigma_{0-1}$.

In order to obtain the spectral shape of this second transition, differential optical density (DOD) spectra of B800-850 have been measured. The excitation within the 850 nm band has been realized at the same wavelengths as indicated in Fig. 1 and with the dye laser characterized above, at a minimum photon flux density of 5×10^{24} cm⁻² s⁻¹, corresponding to a photon density of 1.7×10^{15} cm⁻² per pulse. The simultaneously interacting probe light was realized by broadband dye laser pulses. Details of this experimental arrangement are described elsewhere [21]. A typical DOD spectrum is shown in Fig. 2(a). It is noteworthy that the characteristic features of the DOD shape—the induced absorption and bleaching behavior as well as the zero crossing point at 843 nm—are independent of the excitation wavelength (when measuring at the lowest experimentally possible excitation intensity). Furthermore, the zero crossing point shifts only little to the blue (≈ 1 nm) when the excitation density is increased by 2 orders of magnitude. This indicates that a limited B850 level scheme is involved in the nonlinear response. It can be modeled by the ground state, the one- and the two-exciton bands, the

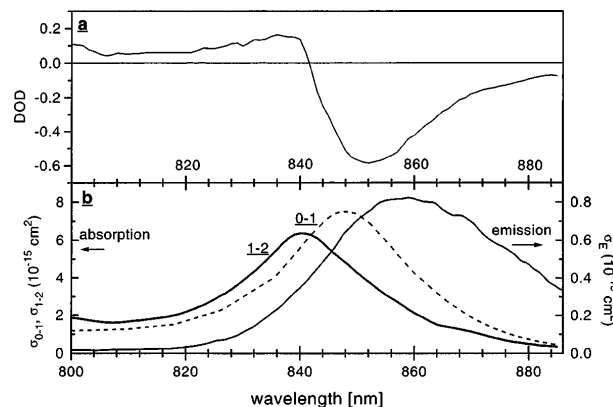


FIG. 2. (a) Transient absorption spectrum of B800-850 ($d = 0.1$ cm), measured simultaneously with the excitation at wavelength $\lambda_{\text{ex}} = 833$ nm (pulse duration 400 ps, photon flux density 5×10^{24} cm⁻² s⁻¹). (b) Cross sections of the transitions from ground state to the one-exciton state (0-1, dashed) and from the latter to the two-exciton state (1-2) (solid line) of B800-850. Both curves belong to the left ordinate. Belonging to the right ordinate: emission cross sections of B800-850. Conditions of emission measurement: room temperature, $d = 1$ cm, $OD \leq 0.01$ at 850 nm, $\lambda_{\text{ex}} = 700$ nm.

induced transitions between them, and intraband as well as interband relaxations. The determination of parameters of this model concerning emission is described further below; the parameters for intraband [22] as well as $2 \rightarrow 1$ interband relaxation [10] were taken from the literature. The only unknown parameters, the absorption cross sections of the two transitions, could then be obtained from the simultaneous fit [17] of the nonlinear absorption functions and the DOD spectra based on a rate equation approach:

$$\frac{\partial \mathbf{n}}{\partial t} = (\hat{A}I + \hat{B})\mathbf{n} \quad (1)$$

where $\mathbf{n}(x, t)$ is the vector of the normalized population densities of the energy levels, the matrices of \hat{A} and \hat{B} contain the cross sections σ and the relaxation constants k of the transitions, respectively. $I(x, t)$ is the intensity of the laser pulse propagating along x according to the photon transport equation:

$$\left(\frac{1}{c} \frac{\partial}{\partial t} + \frac{\partial}{\partial x} \right) I = -N_0 I (\mathbf{g}^T \mathbf{n}) \quad (2)$$

where N_0 is the particle density in the medium and \mathbf{g} is a vector containing elements of \hat{A} [17].

A full set of parameters is given in the legend to Fig. 1, and the absorption cross sections of the two transitions in question ($0 \rightarrow 1, 1 \rightarrow 2$) are shown in Fig. 2(b). While the shape and the spectral displacement of these absorption bands are similar to those of Bchla in solution [23,24], their intensities expressed as cross sections [cf. Fig. 2(b)], dipole moments, and oscillator strengths are of extraordinary size [25]:

$$\mu_{0-1} = 25.5 \pm 2.5D, \quad f_{0-1} = 3.3 \pm 0.6;$$

$$\mu_{1-2} = 21.5 \pm 3.5D, \quad f_{1-2} = 2.4 \pm 0.8.$$

For comparison, the maximum absorption cross section of Bchla in monomeric solution (ether) is $\sigma(772 \text{ nm}) = 3.5 \times 10^{-16} \text{ cm}^2$, the transition dipole moment is $\mu_{s_0-s_1} = 6.3 D$, and the oscillator strength is $f = 0.22$. Very similar values were obtained when the measured absorbance of the B800-850 complex was related to the corresponding Bchla content, i.e., the values were calculated per single Bchla in the 850 nm band [27].

This comparison with the Bchla single-molecules data shows that there is a $\sqrt{16} \pm 4$ -fold enhancement of the 850 nm 0-1 transition dipole moment of the extracted LH2 antenna, i.e., the excitation is delocalized over 16 ± 4 Bchla, which comprises much if not all of the circular aggregate. To our knowledge, this is the largest exciton size found so far in an organic material at room temperature. The corresponding values for the J-aggregates of pseudoisocyanines and polydiacetylene are 4–12 [9,10,28] and about 5 molecules [28], respectively. Remarkably this circular aggregate with the large exciton size is a product of nature.

The second prediction for strongly coupled aggregates concerned the size-enhanced scaling of $\chi^{(3)}$. Values were

determined for the relevant parameter for nonlinearity under resonant conditions, viz., α_2/α_0 . They can be obtained as the slope of the intensity-dependent nonlinear absorption functions [28]. These values for B800-850 are comparable to those of quantum-confined CdS clusters [29], e.g., $\alpha_2/\alpha_0(855 \text{ nm}) = -(5.0 \pm 1.0) \times 10^{-7} \text{ cm}^2/\text{W}$.

In the derivation of the absorption cross sections for the $0 \rightarrow 1$ and $1 \rightarrow 2$ transitions, the influence of the induced emission had to be known. Based on the determined (For experimental details, see Ref. [24] and references therein.) fluorescence decay time $\tau_F = 1.0 \pm 0.1 \text{ ns}$ and quantum yield $\phi_F = 0.10 \pm 0.02$ (values, which are in good agreement with data from the literature [30,31] and which result in $k_r = (1.0 \pm 0.3) \times 10^8 \text{ s}^{-1}$ for the radiative decay rate) the emission cross sections shown in Fig. 2(b) have been obtained. The maximum value is $\sigma_E(860 \text{ nm}) = 8.2 \times 10^{-16} \text{ cm}^2$, the data for the emission dipole moment and oscillator strengths are $\mu_E = (9.5 \pm 1.0)D$ and $f_E = (0.45 \pm 0.10)$. For comparison, the respective values for monomeric Bchla in ether are $\sigma_E(783 \text{ nm}) = 4.5 \times 10^{-16} \text{ cm}^2$, $\mu_E = 6.3D$, $f_E = 0.22$ and $k_r = 6.3 \times 10^7 \text{ s}^{-1}$. From these data we conclude that the excitonic coupling in emission at room temperature is restricted to fewer molecules, probably to a Bchla-dimer within the circular B850 aggregate. (A small exciton size of 4 ± 2 has been recently determined also for the absorption transition [32], in contrast to our results). After lowering the temperature to 4.2 K, the radiative decay rate increases by about a factor of 4 [30,31]. This may indicate a corresponding increase of the number of collectively emitting Bchla molecules in the circular aggregate.

Last but not least, it should be mentioned that there are some indications that the excitonic transition $0 \rightarrow 1$, which we have handled here as one homogeneous band, may be composed of one dominating (sub-) band with one or few weaker subbands: Such heterogeneity at room temperature is indicated by the fact that the width of the B800-850 fluorescence band clearly exceeds that of the corresponding 850 nm absorption band ($\text{FWHM}_E = 550 \text{ cm}^{-1}$, $\text{FWHM}_A = 420 \text{ cm}^{-1}$), and is further substantiated by results from nonlinear polarization spectroscopy in the frequency domain (NLPF) [33]. The additional oscillator strength for these minor band(s) may arise at the expense of the higher-energy bands (hyperchromism) [34].

Further details on such a substructure of the B850 absorption band are expected from present temperature dependent NLPF investigations. They should bridge the gap to the 4.2 K-hole burning data which showed only a single, homogeneous B850 band [35]. Structure-based calculations of the spectrum of *Rps. acidophila* [36] also indicate one dominating band in B850 for one set of 18 strongly interacting molecules.

In conclusion, and irrespective of this possible fine structure, strong excitonic coupling between the 16 ± 4 Bchl_a molecules of the presumably circular aggregate—responsible for the 850 nm absorption band of B800-850—has been characterized, as well as superradiance and a transition from one- to two-exciton state, slightly blue-shifted with respect to the 0-1 excitonic transition. To our knowledge the circular aggregate B850 of the antenna LH2 from *Rb. sphaeroides* is the first natural supramolecular candidate for a giant $\chi^{(3)}$.

This work has been supported by a grant from Deutsche Forschungsgemeinschaft, Bonn (Le 729/1-3 and Sche 140/13-2), and a stipend to B. Ücker from the Hans-Fischer-Gesellschaft, München. Skillful technical assistance by Mrs. R. Lendt (Berlin) and C. Bubenzer-Hange (München) is gratefully acknowledged.

-
- [1] Special issue on 50 years Scheibe Aggregates, *Adv. Mater.* **7**, No. 5 (1995).
- [2] (a) F. C. Spano and S. Mukamel, *Phys. Rev. A* **40**, 5783 (1989); (b) F. C. Spano and S. Mukamel, *Phys. Rev. Lett.* **66**, 1197 (1991).
- [3] F. C. Spano, J. R. Kuklinski, and S. Mukamel, *Phys. Rev. Lett.* **65**, 211 (1990).
- [4] (a) J. Knoester, *Phys. Rev. A* **47**, 2083 (1993); (b) J. Knoester, *Adv. Mater.* **7**, 500 (1995).
- [5] J. Knoester, *Chem. Phys. Lett.* **203**, 371 (1993).
- [6] E. W. Knapp, *Chem. Phys.* **85**, 73 (1984).
- [7] (a) H. Fidder, J. Knoester, and D. A. Wiersma, *Chem. Phys. Lett.* **171**, 529 (1990); (b) H. Fidder, J. Knoester, and D. A. Wiersma, *J. Chem. Phys.* **98**, 6564 (1993).
- [8] J. R. Durrant, J. Knoester, and D. A. Wiersma, *Chem. Phys. Lett.* **222**, 450 (1994).
- [9] S. Kobayashi and F. Sasaki, *Nonlinear Opt.* **4**, 305 (1993).
- [10] H. Stiel, B. Voigt, J. Hirsch, K. Teuchner, and D. Leupold, *Adv. Mater.* **7**, 445 (1995).
- [11] For recent reviews of optical, structural, and functional properties of LH2, cf. (a) D. Leupold, *Photochem. Photobiol.* **62**, 984 (1995); (b) R. van Grondelle, J. P. Dekker, T. Gillbro, and V. Sundström, *Biochim. Biophys. Acta* **1187**, 1 (1994).
- [12] D. Leupold, B. Voigt, M. Pfeiffer, M. Bandilla, and H. Scheer, *Photochem. Photobiol.* **57**, 24 (1993).
- [13] (a) G. McDermott, S. M. Prince, A. A. Freer, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell, and N. W. Isaacs, *Nature (London)* **374**, 517 (1995); (b) J. Koepke, X. Hu, C. Muenke, K. Schulten, and H. Michel, *Structure* (to be published).
- [14] T. V. Dracheva, V. I. Novoderezhkin, and A. P. Razjivin, *Chem. Phys.* **194**, 223 (1995).
- [15] B. Kopański and W. Kaiser, *Chem. Phys. Lett.* **88**, 357 (1982).
- [16] Ch. Li, J. Si, M. Yang, R. Wang, and L. Zhang, *Phys. Rev. A* **51**, 569 (1995).
- [17] H. Stiel, K. Teuchner, D. Leupold, S. Oberländer, J. Ehlert, and R. Jahnke, *Intell. Instrum. Comput.* **9**, 79 (1992).
- [18] H. Stiel, K. Teuchner, and W. Becker, *Exp. Tech. Phys.* **39**, 115 (1991).
- [19] R. van Grondelle, C. N. Hunter, J. G. C. Bakker, and H. J. M. Kramer, *Biochim. Biophys. Acta* **723**, 30 (1983).
- [20] H. Stiel, S. Dähne, and K. Teuchner, *J. Lumin.* **39**, 351 (1988).
- [21] W. Freyer, H. Stiel, K. Teuchner, and D. Leupold, *J. Photochem. Photobiol. A* **80**, 161 (1994).
- [22] T. Pullerits, M. Chachisvilis, M. R. Jones, C. N. Hunter, and V. Sundström, *Chem. Phys. Lett.* **224**, 355 (1994).
- [23] M. Becker, V. Nagarjan, D. Meddendorf, M. A. Shield, and W. W. Parson, in *Current Research in Photosynthesis*, edited by M. Baltscheffsky (Kluwer, Dordrecht, 1990), Vol. I, p. 101.
- [24] K. Teuchner, H. Stiel, D. Leupold, I. Katheder, and H. Scheer, *J. Lumin.* **60 & 61**, 520 (1994).
- [25] Although the mentioned excited state absorption of monomeric Bchl_a is not a supposition for the second excitonic transition ($1 \rightarrow 2$) in the 850 nm region of B800-850, strong excitonic coupling of three-level systems with two sequential absorptions is of recent theoretical interest with respect to the interference of collective and individual effects in nonlinear spectra [26]. B800-850 may then be a suitable model system for more detailed experimental investigations along this line.
- [26] J. Knoester and F. Spano, *Phys. Rev. Lett.* **74**, 2780 (1995).
- [27] R. K. Clayton and B. J. Clayton, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5583 (1981).
- [28] Y. Wang, *J. Opt. Soc. Am. B* **8**, 981 (1991).
- [29] Y. Wang, A. Suna, J. McHugh, E. Hilinski, P. Lucas, and R. D. Johnson, *J. Chem. Phys.* **92**, 6927 (1990).
- [30] R. van Grondelle, H. J. M. Kramer, and C. P. Rijgersberg, *Biochim. Biophys. Acta* **682**, 208 (1982).
- [31] H. Bergström, V. Sundström, R. van Grondelle, E. Åkesson, and T. Gillbro, *Biochim. Biophys. Acta* **852**, 279 (1986).
- [32] T. Pullerits, M. Chachisvilis, and W. Sundström, in *Photosynthesis: from Light to Biosphere*, edited by P. Mathis (Kluwer, Dordrecht, 1995), Vol. I, p. 107.
- [33] D. Leupold, B. Voigt, F. Nowak, J. Ehlert, J. Hirsch, E. Neef, M. Bandilla, and H. Scheer, *Liet. Fizik. Zurnal* **34**, 339 (1994).
- [34] A. Scherz and W. W. Parson, *Biochim. Biophys. Acta* **766**, 666 (1984).
- [35] R. Jankowski, J. M. Hayes, and G. J. Small, *Chem. Rev.* **93**, 1471 (1993).
- [36] H. K. Sauer, R. J. Cogdell, S. M. Prince, A. Freer, N. W. Isaacs, and H. Scheer, *Photochem. Photobiol.* **64**, 564 (1996).