Exciton Coherence and Energy Transfer in the LH2 Antenna Complex of *Rhodopseudomonas acidophila* at Low Temperature

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Excited-state dynamics and exciton delocalization in the isolated LH2 antenna complex (B800−850) of the photosynthetic bacterium *Rhodopseudomonas acidophila* were studied at low temperature by means of femtosecond transient absorption spectroscopy. By comparing the contribution by stimulated emission to the absorbance difference signal of B850 with the bleaching of the primary donor P of reactions centers of *Rhodobacter sphaeroides* R26, we found that at 5 K the thermalized B850 transition has a 1.85 times larger oscillator strength than P. This corresponds to an oscillator strength equivalent to 3.4 reaction center pigments. With the in vivo extinction coefficients for the reaction center and LH2 from the literature, we arrive at an oscillator strength equivalent to 2.3 antenna pigments. Comparison of the stimulated emission band of B850 with the optical signal of B800 yields an oscillator strength equivalent with that of 2.7 B800 pigments. Our results strongly support a model in which the exciton states are partly localized as a result of static disorder. Energy-selective steady-state fluorescence measurements indeed indicate inhomogeneity of the B850 band. Energy transfer from B800 to B850 takes place with a time constant of 1.8 ps at 10 K.

Introduction

The recent establishment of the three-dimensional structures of the B800−850 (LH2) light-harvesting complexes of *Rhodopseudomonas acidophila* and *Rhodospirillum molischianum*[1,2] has provided an important step toward an understanding of their excited-state dynamics on a molecular level. These complexes, whose function is to absorb light and transfer the excitation energy to the reaction center core complex, were found to form ring structures of α and β polypeptides with a 9 or 8-fold symmetry, which bind the bacteriochlorophyll (BChl) molecules. The complexes show absorption bands near 800 and 850 nm, which are ascribed to two populations of the BChls, B800 and B850, respectively. The three-dimensional structures indicate that the B800 forms a ring of 9 or 8 essentially monomeric BChls, whereas B850 forms a circular aggregate of 18 or 16 closely spaced BChl molecules.

Time-resolved studies on the LH2 complex of a number of species have shown that energy transfer from B800 to B850 occurs in less than 1 ps at room temperature.[3−6] At cryogenic temperatures, the transfer slows down to values of about 2 ps.[4,5,7−10]

There is general agreement that excitonic interactions to a great extent determine the character of the excited state of B850. The number of strongly coupled molecules with coherent excitation, however, is a matter of debate. Estimates of delocalization length range from two,[11−13] four,[14] or more[6] pigments to the entire aggregate.[15−19] From theoretical investigations on the properties of J-aggregates it is known that energy disorder reduces the exciton coherence length.[20] Coupling to phonons causes localization as well,[21,22] as was recently discussed for light-harvesting systems.[23] The ratios between intermolecular coupling and inhomogeneous and homogeneous line widths, determine the delocalization length. However, to date there are no precise numbers available for either of these quantities. The actual value of the delocalization length has important implications for the energy transfer mechanism that applies. In the case of exciton delocalization over the entire aggregate, energy transfer is purely coherent. With a decreasing number of coherent molecules, energy transfer over the B850 ring obtains an incoherent, Förster-like character.

The most sensitive measure for the number of coherent molecules on the aggregate is the oscillator strength of the B850 transition. This quantity can be determined by measuring the superradiance.[24] Another possibility is to measure the optical response of the B850 transition with respect to that of a reference system, for instance the monomeric B800 molecules present in the complex[6] or the bacterial reaction center.[25] In this paper we present the results of such a study on the LH2 complex of *Rps. acidophila* at low temperature. We will show that the thermalized B850 transition has an oscillator strength of 2.3−3.4 monomeric pigments. Our results strongly support a model in which the exciton wave functions are partly localized as a result of energy disorder.

Experimental Section

Reaction centers of *Rhodobacter sphaeroides* R26 were prepared according to standard protocols.[26] Cells of *Rps. acidophila* strain 10050 were grown as described in ref 27. The B800−850 complex was isolated by the method of Cogdell.[27] In brief, chromatophores were incubated for 2 h in a 2% LDAO solution and subsequently ultracentrifuged overnight on a discontinuous sucrose gradient. The samples were diluted in a 30 mM Tris/EDTA buffer (pH = 8.0) containing 0.3% LDAO. After the addition of glycerol (66% v/v) the sample was transferred to a 0.5 mm cuvette, which was placed in a helium flow cryostat (Oxford Instruments). The low-temperature absorbance of the sample was 1.0 at the maximum of the B850 band.

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For measurements with reaction centers as a reference sample, a layer of reaction centers in 60% glycerol was put on top of a layer of LH2 sample in a 0.5 mm cuvette. As a result of the slightly different densities, these two layers remained separated. The low-temperature absorbance of the two samples at 800 nm was 0.8 and the same within 5%. The sample was cooled in a helium flow cryostat (Utreks, Estonia), in which the cuvette could be vertically translated with respect to the laser beams, ensuring that overlap between pump and probe was maintained for measurements with either the reaction center layer or the LH2 layer. The latter point was verified by filling the whole cuvette with a solution of IR140 dye in ethanol and recording difference spectra upon excitation at 820 nm with the cuvette set in either of the two positions.

Magic angle pump−probe measurements with a time resolution of 300 fs and optical multichannel analyzer detection were performed with a home-built amplified dye laser system operating at 10 Hz as described earlier.[6] The system had been modified by inserting a Bethune cell of 6 cm path length for the last amplification stage. The design for this cell was kindly provided by F. van der Woude of the van der Waals Zeeman Laboratory of the University of Amsterdam. Both probe and reference beams passed through the sample. Group velocity dispersion in the white light continuum was recorded by measuring the optical Kerr effect in CS_2.[28] The time-resolved absorbance difference spectra presented in Figure 2 were corrected accordingly. The spectra of Figure 5 were not corrected, but they were recorded at sufficiently long delay (the B850 and reaction center signals in Figure 5A,B), or the signal was sufficiently narrow (the B800 signal in Figure 5A) to ignore the effects of group velocity dispersion. The pump intensity was tuned with a circular variable neutral density filter (Edmund Scientific).

Steady-state absorption and fluorescence measurements were performed with a single-beam spectrophotometer.[29]

Results

Steady-State Spectroscopy. Figure 1A shows the steady-state absorption spectrum of the *Rps. acidophila* LH2 complex at 10 K (solid line). The B800 and B850 bands have maxima at 802 and 869 nm, respectively. The absorption of the R26 reaction center preparation used in this study is shown as well (short dashes), along with the spectrum of the pump pulse applied in the experiments (long dashes). Figure 1B shows fluorescence spectra of the LH2 complex at 10 K at various excitation wavelengths. Upon excitation at 800 nm the spectrum shows a maximum at 890 nm, which corresponds to a Stokes shift of 21 nm with respect to the B850 absorption maximum. Figure 1B also shows energy-selective fluorescence spectra upon excitation in the B850 band. We observe that, upon scanning the excitation light over the red edge of the absorption band, the fluorescence shifts to the red by up to 7 nm. These observations are very similar to those reported for LH1[30] and the LH2 complex of *Rhodospirillum molischianum*[31] and prove that B850 is inhomogeneous. The fact that, upon long-wavelength excitation (885−890 nm), no fluorescence is emitted at wavelengths shorter than of the excitation source indicates that the homogeneous line width is much smaller than the inhomogeneous line width at those wavelengths. Therefore, the absorption maximum of the selected states will not lie at wavelengths shorter than the excitation wavelength. The Stokes shift of these states then is at most 8 nm, which indicates that the electron−phonon coupling is weak, as is generally found for antenna complexes.[7,32,33] A Stokes shift of 8 nm would be identical with that of the B820 subunit, for which a temperature-dependent fluorescence study was modeled using a Huang−Rhys factor of 0.5.[34]

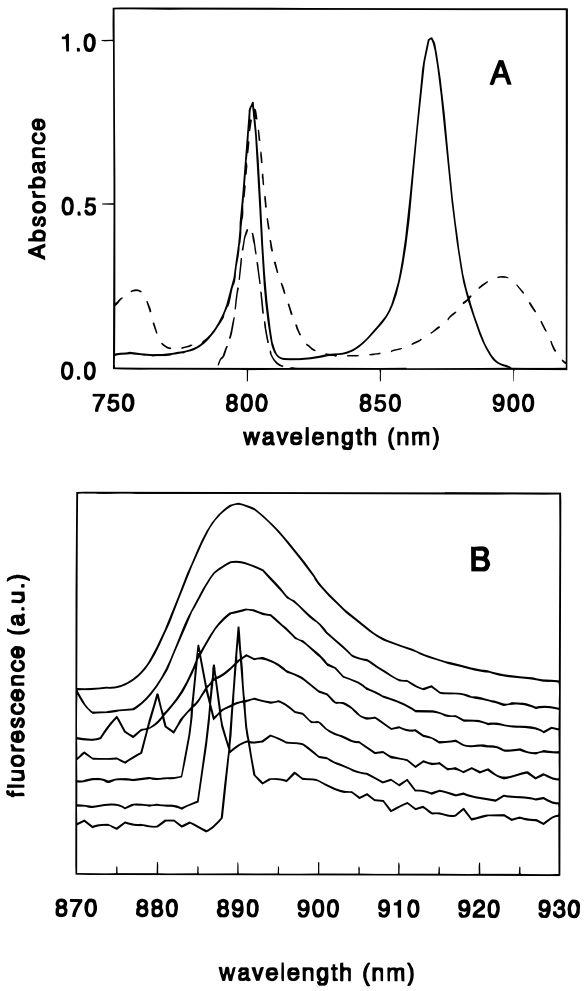

Figure 1. (A) Absorption (solid line) of the isolated LH2 complex of *Rps. acidophila* at 10 K. The spectra of the *Rhodobacter sphaeroides* R26 reaction center preparation (short dashes) and the pump pulse (long dashes) used in the time-resolved experiments are shown as well. (B) Fluorescence spectra of the LH2 complex at 10 K upon excitation at (from top to bottom): 800, 870, 875, 880, 885, 887, and 890 nm. The spectra are vertically offset with respect to each other for clarity. The triangular peaks are due to scattered excitation light. The excitation bandwidth was 2 nm.

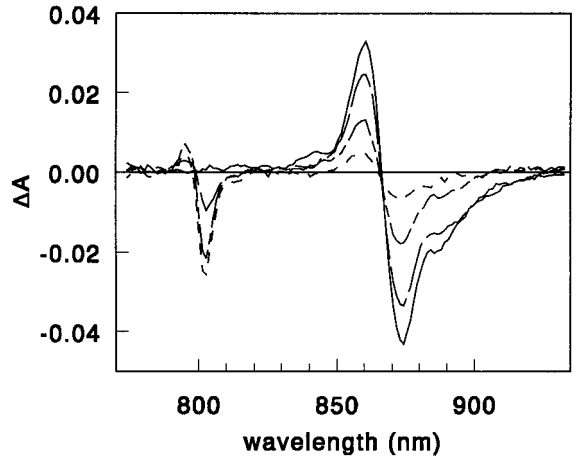

Figure 2. Absorbance difference spectra of the *Rps. acidophila* LH2 complex at 10 K upon excitation at 801 nm at the delays 0.3 (short dashes), 0.7 (short and long dashes), 2.2 (long dashes), and 9 ps (solid line).

Time-Resolved Measurements. Figure 2 shows the time-

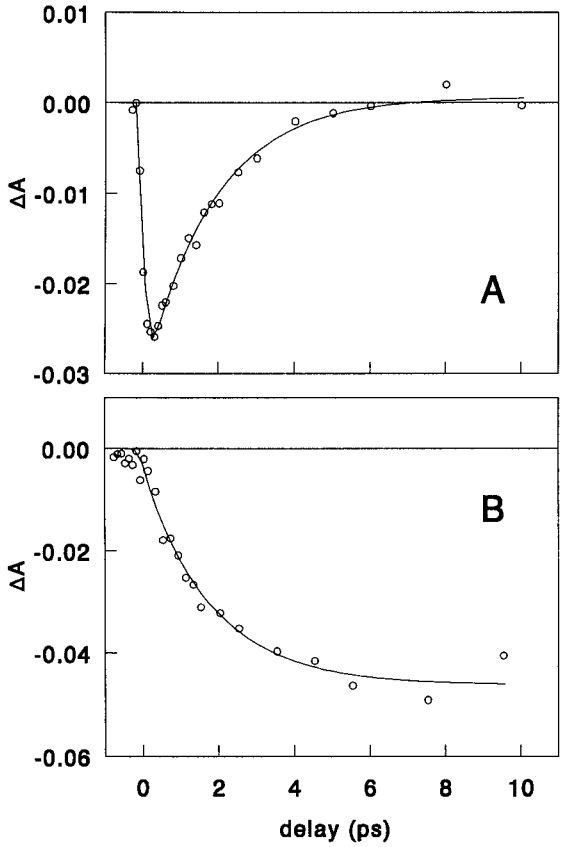

Figure 3. Kinetics of absorbance changes of the *Rps. acidophila* LH2 complex at 10 K upon excitation at 801 nm at (A) 803 and (B) 874 nm. The solid lines represent a monoexponential decay and risetime with a time constant of 1.8 ps for the signals at 803 and 874 nm, respectively. A convolution with a 300 fs wide $(sech)^2$ function was used for both fits.

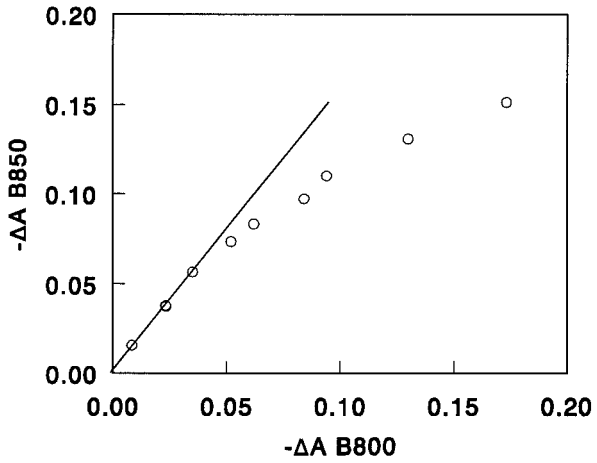

Figure 4. The B850 signal at 873 nm at a delay of 10 ps as a function of the B800 signal at 803 nm at a delay of 0.3 ps (circles). Up to a Δ*A* signal of about 0.04 for B800, the relation is linear (solid line).

resolved Δ*A* spectra of the LH2 complex of *Rps. acidophila* upon excitation with a 300 fs pulse at 801 nm at 10 K. At a delay of 0.3 ps (short dashes), we observe a bleaching around 803 nm, which we attribute to excited B800. Around 870 nm a small signal is observed, which is partly due to a small amount of directly excited B850. At increasing delays the bleaching around 803 nm diminished, concomitant with an increase of the absorbance difference signal around 870 nm, indicating energy transfer to B850. At a delay of 9 ps (solid line) energy transfer was complete, and the Δ*A* spectrum was entirely due to excited B850. It shows a maximum bleaching at 873 nm,

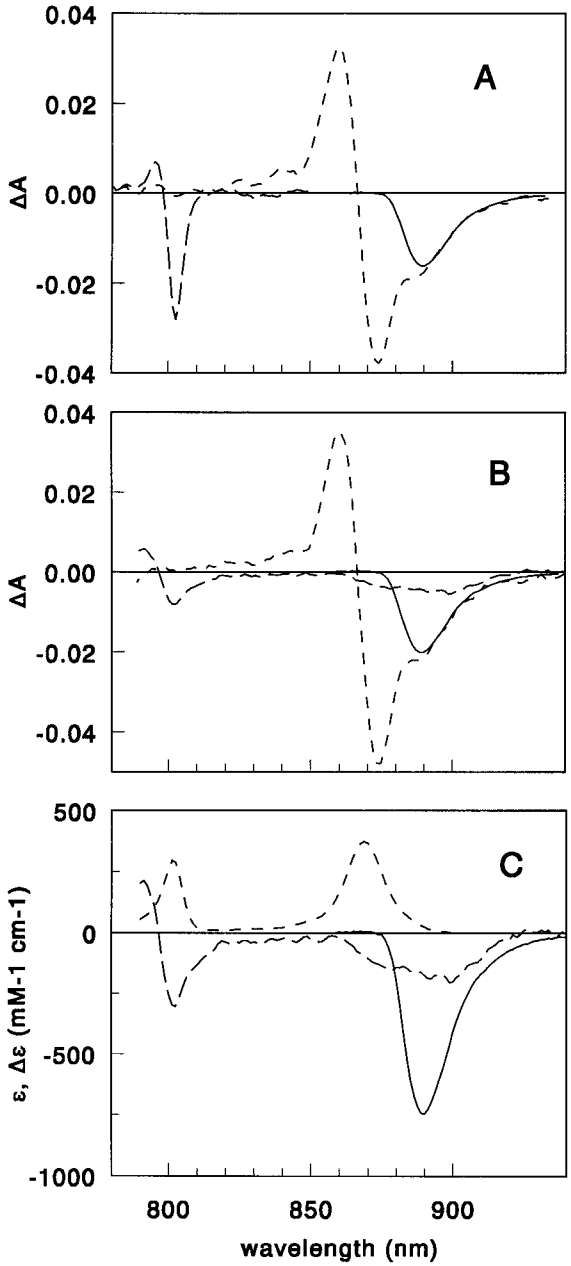

Figure 5. (A) Long dashes: Δ*A* spectrum of B800 extrapolated to zero delay upon excitation at 801 nm in the LH2 complex of *Rps. acidophila* at 10 K. Short dashes: Δ*A* spectrum for B850 at a delay of 10 ps. Solid line: simulation of the red edge of the B850 Δ*A* spectrum with the steady-state emission spectrum of the LH2 complex upon excitation at 800 nm taken from Figure 1B. (B) Long dashes: Δ*A* spectrum of reaction centers of *Rb. sphaeroides* R26 at 5 K upon excitation at 801 nm at a delay of 20 ps. Short dashes: Δ*A* spectrum of LH2 of *Rps. acidophila* at 10 ps under the same conditions. Solid line: simulation of the stimulated emission shoulder of B850 as described for (A). (C) The Δ*A* spectrum of R26 reaction centers (long dashes) and the stimulated emission contribution of LH2 (solid line) of panel B expressed on an extinction coefficient scale, using a differential extinction coefficient of 187 $mM^{-1}cm^{-1}$ for the reaction center (see text). Also shown is the absorption spectrum of the LH2 complex expressed in extinction coefficient units (short dashes).

an isosbestic point at 867 nm, and an absorbance increase with a maximum at 860 nm. The bleaching around 873 nm may be assigned to ground-state depletion of B850. At 886 nm, the spectrum shows a pronounced shoulder, which is close to the fluorescence maximum of the complex (see Figure 1B) and may thus be assigned to stimulated emission from the excited state to the ground state. The absorbance increase at the blue side

of the B850 band is partly assigned to absorption from the singly excited level to the doubly excited level of B850. The B850 difference spectrum is very symmetric around the isosbestic point and resembles a band shift. In fact, such a shift seems to make a significant contribution to the difference spectrum, because the spectral positions of the ground-state depletion maximum, 873 nm, and stimulated emission, 890 nm, do not correspond to the Stokes shift determined in the energy-selective fluorescence spectra presented in Figure 1B. Interestingly, low-temperature time-resolved spectra of LH1−RC core complexes of *Rb. sphaeroides*[35] and *Rps. acidophila* (Kennis, J., unpublished results) do not show such a prominent band shift feature.

The spectrum at a delay of 0.3 ps of Figure 2A also demonstrates that excited B800 shows some excited-state absorption. This does not mean that exciton coupling plays a role between the B800 molecules, as monomeric BChl *a* in solution exhibits this phenomenon as well.[36,37] In fact, the inhomogeneous broadening of B800, about 200 cm^{-1},[7,9] is much larger than the intermolecular coupling, which can be estimated to be about 30 cm^{-1}, and this will cause strong localization on single B800 molecules.

Figure 3 shows the kinetics at 803 and 873 nm, derived from the time-resolved spectra. At 803 nm, the signal decayed with a time constant of 1.8 ps. The rise of the signal at 873 nm exactly matches this decay, from which we conclude that energy transfer from B800 to B850 takes place with a time constant of 1.8 ps at 10 K. This number is in close agreement with the results of hole burning and of a recent transient absorption study on the same complex.[8,10]

With the excitation densities used in this study, singlet−singlet annihilation, which arises from the appearance of multiple excitations in a single complex, might take place. To check this, we performed intensity-dependent measurements with difference spectra taken at delays of 0.3 and 10 ps. Figure 4 shows the B850 signal at 873 nm at a delay of 10 ps as a function of the B800 signal at 803 nm at a delay of 0.3 ps. We observe that the B850 signal starts to saturate at ΔA values of B800 larger than 0.04. This is likely the result of singlet−singlet annihilation. Singlet−triplet annihilation can be ruled out because of the low repetition frequency of the laser system. This experiment shows that the time-resolved data presented in Figure 2 are taken below the limit of the appearance of annihilation. Annihilation is expected to take place predominantly on the B850 aggregate, because the lifetime of excited B800 is only 1.8 ps, and B800−B850 and B850−B850 energy transfer are much faster than B800−B800 transfer.[9,13] A time-resolved study on LH1 at room temperature has shown that annihilation occurs with a typical time constant of 1 ps,[12] and we expect about the same rate for B850 in our case.

As mentioned in the Introduction, the optical response of B850 with respect to that of B800 is a measure for the exciton delocalization on the B850 aggregate. The spectrum at 0.3 ps in Figure 2 (short dashes) shows the signal observed for B800 at 10 K, whereas the spectrum at 9 ps (solid line) shows the B850 signal after energy transfer to B850 had been completed. Clearly, the ΔA signal of B850 is much larger than that of B800. It thus seems that the B850 transition has a considerably larger oscillator strength than B800. Similar observations have already been made for the LH2 complexes of *Chromatium tepidum*[6] and *Rb. sphaeroides* and *Rps. acidophila*[38] at room temperature and have been taken as evidence for the delocalized nature of excited B850. However, in general, the time-resolved spectra are a superposition of contributions from ground-state depletion, excited-state absorption, stimulated emission, and band shifts, which severely complicates their interpretation. Yet, in the specific case of B850 the separation of the spectra associated with ground-state depletion and stimulated emission is large enough to distinguish the stimulated emission part in the difference spectrum. We simulated the red region of the ΔA spectrum with the steady-state fluorescence spectrum of the *Rps. acidophila* LH2 complex, assuming that in this region the contribution by the transition to the doubly excited B850 level is negligible. This is shown in Figure 5A, where data are shown that were obtained with a better signal-to-noise ratio than in Figure 2 by extended data averaging. Comparison of this stimulated emission signal (solid line) with the ΔA spectrum of B800 (long dashes) shows that it is several times larger in magnitude. It does, however, not directly yield the oscillator strength of B850, because B800 has an excited-state absorption that is in principle unknown. Becker et al.[36] have determined the cross section for excited-state absorption of monomeric BChl *a* dissolved in methanol at room temperature, and they concluded that it had a total cross section in the Q_y region comparable to that of the ground-state absorption. To make a quantitative estimate, we digitized the excited-state absorption and ground-state absorption spectra in Becker's paper, and we found that the integrated cross section for excited-state absorption exceeds that of ground-state absorption by a factor of 1.17. We applied this result to the ΔA spectrum of B800, which is a superposition of excited-state absorption, ground-state depletion, and stimulated emission. Assuming that ground-state absorption and stimulated emission have equal (wavelength-integrated) cross sections, which is reasonable since the Einstein coefficients for these processes are equal, it follows that the wavelength-integrated ΔA spectrum of B800 equals the ground-state bleaching of 0.83 monomeric pigments. The relation between the extinction coefficient $\epsilon(\nu)$ and the oscillator strength $|\mu|^2$ of a transition is given by[39]

$$|\mu|^2 = A \int \frac{\epsilon(\nu)\, d\nu}{\nu} \qquad (1)$$

where A is a constant that incorporates the dielectric constant and ν is the frequency of the transition. If we then integrate the areas of the B800 ΔA signal and the stimulated emission of B850, we arrive at an oscillator strength of 2.7 B800 molecules for the lowest transition of B850.

The excited-state absorption of a BChl molecule bound to a protein at low temperature may, however, be different from that of monomeric BChl in a solvent at room temperature. To circumvent this problem, we performed measurements with reaction centers from *Rb. sphaeroides* R26 as a reference sample. This reference sample was contained in the same cuvette as the LH2 sample, and the low-temperature absorbances at 800 nm of these two samples were adjusted to the same value within 5%, as depicted in Figure 1A. Both samples were excited at 800 nm. Figure 5B shows the ΔA spectrum at 5 K measured in reaction centers at a delay of 20 ps (long dashes) and the ΔA spectrum of LH2 at a delay of 10 ps (short dashes). The reaction centers show a bleaching of the primary donor band at 890 nm and a band shift at 800 nm, which is typical for the charge-separated state.[40] Because energy transfer from the accessory BChls to the primary donor P and electron transfer from P to the active bacteriopheophytin takes place on time scales of 140 fs[41] and 1.2 ps,[42] respectively, it can safely be assumed that after 20 ps the bleaching at 890 nm is solely due to oxidation of P. The shape of the ΔA spectrum of LH2 is identical with that of Figure 5A. Like in Figure 5A, the stimulated emission shoulder is fitted with a steady-state fluorescence spectrum (solid line).

At room temperature, the extinction coefficient at 865 nm of P equals 128 mM^{-1} cm^{-1}, and the change in extinction coefficient associated with P oxidation is known to be 112 mM^{-1} cm^{-1} at the top of the band.[43] At low temperature, the absorption maximum shifts to 890 nm and becomes a factor of 1.67 times higher. Thus, at 5 K the extinction coefficient of P at 890 nm equals 1.67×128 mM^{-1} $cm^{-1} = 213$ mM^{-1} cm^{-1}, and it is reasonable to assume that the change in extinction coefficient at 890 nm for P oxidation amounts to 1.67×112 mM^{-1} $cm^{-1} = 187$ mM^{-1} cm^{-1}. To compare the oscillator strengths of the ground-state absorption of P and the stimulated emission transition of LH2, we have to integrate the area of the stimulated emission of B850 and that of the bleaching of P in Figure 5B according to eq 1. We then find that the area of stimulated emission from LH2 is 2.1 times larger than the bleaching of P. Correcting for the slight difference in the extinction coefficient of the ground-state absorption of P and of its oxidation, if follows that the thermalized B850 transition has an oscillator strength that is 1.85 times larger than P. Considering that P is a BChl dimer that has 90% of its oscillator strength in the lowest exciton state, this value corresponds to 3.4 BChl pigments.

We can also relate the changes in extinction coefficient that result from oxidation of P and stimulated emission from B850 to the in vivo extinction coefficient of LH2 BChl. At room temperature this latter quantity has been determined to be 184 mM^{-1} cm^{-1},[44] and at 5 K it becomes exactly twice as large, 370 mM^{-1} cm^{-1}. Figure 5C shows the bleaching of P (long dashes) and the stimulated emission signal of LH2 (solid line) expressed in the same extinction units. For the latter a (difference) extinction coefficient of 748 mM^{-1} cm^{-1} is obtained. The spectrum of the LH2 complex (short dashes) expressed in extinction coefficient units is depicted in Figure 5C as well. Integration of the B850 absorption and stimulated emission bands according to eq 1 then yields an oscillator strength of 2.3 pigments for the stimulated emission transition from B850 to the ground state.

This oscillator strength value of 2.3 pigments significantly deviates from the one we have derived directly from the primary donor bleaching, which was 3.4 pigments, and this would imply that the oscillator strength per pigment in the antenna is larger than in the reaction center. To check whether this is reasonable, we have examined the low-temperature absorption spectra of membranes of the LH1−RC only containing species *R. rubrum*, *R. sodomense*, and *Rps. marina*. We compared the height of the B880 peak of LH1 with that of the 800 nm peak of the accessory BChls in the reaction center (data not shown). Under the assumption that there are 28 BChls in the LH1 antenna per reaction center,[45−49] the reported in vivo extinction coefficients of the LH1 antenna[45] and the reaction center of R26[43] were consistent with the absorption spectra in the species *R. sodomense* and *Rps. marina*. However, for *R. rubrum* we found a discrepancy between these numbers, indicating that the extinction coefficient of the reaction center would be underestimated with respect to the LH1 antenna by 40%. This result would also affect LH2, because its in vivo extinction coefficient has been determined in relation to that of LH1[44] and leads to an oscillator strength of 2.9 pigments for the lowest state in B850.

Discussion

When comparing the stimulated emission contribution to the absorbance difference spectrum of B850 with the bleaching of the primary donor band in reaction centers of *Rhodobacter sphaeroides* R26, we find that at 5 K the relaxed B850 state has a 1.85 times larger oscillator strength than the primary donor. This corresponds to an oscillator strength equivalent to 3.4 reaction center pigments. Taking the in vivo extinction coefficient of LH2 as a reference, we arrive at an oscillator strength of 2.3 antenna pigments. However, as we discussed above, this number may be too low because of an uncertainty concerning the relative extinction coefficients of antenna and reaction center. Taking the monomeric B800 molecules as a reference, we arrive at an oscillator strength equivalent to 2.7 pigments.

The lowest exciton state of a homogeneous circular aggregate, $k = 0$, is optically forbidden and does not show spontaneous or stimulated fluorescence. Our measurements thus give direct evidence that after thermalization the exciton wave functions are not delocalized over the entire aggregate. It is well-known from theoretical studies on J-aggregates that site disorder causes localization of exciton wave functions.[20] In the case of circular aggregates such as LH2, site disorder causes the oscillator strength to be redistributed over the exciton manifold by mixing of the $k = 0$ level with the optically allowed $k = \pm 1$ levels. This implies that with increasing disorder the exciton wave functions become partly localized, and the lowest exciton state gains oscillator strength at the expense of the $k = \pm 1$ states higher up the exciton manifold. It was recently shown that the site disorder must have about the same value as the intermolecular coupling for the lowest exciton state to obtain the oscillator strength of 2.3−3.4 pigments determined in this study.[50] In this regime, the pure exciton levels have to a considerable extent lost their character, and the lowest-energy exciton wave function is extended over 2−3 pigments.[50]

The presence of static disorder in the LH2 complex is indicated by the energy-selective fluorescence spectra of Figure 1B. At 5 K, the thermal energy available is very low, and considering the weak electron−phonon coupling in B850 (see above), it seems likely that phonon scattering contributes only slightly to localization of the lowest exciton state.

Our data agree very well with recent superradiance measurements, which have indicated an oscillator strength of the emitting state of the LH2 complex of *Rb. sphaeroides* at 4.2 K equivalent to 2.8 monomeric BChl *a* pigments in solution.[50] Pullerits et al. have simulated the entire difference spectrum of B850 to estimate the exciton delocalization length on the B850 aggregate.[14] Our approach is quite different, in that we determine the absolute cross section of the absorbance difference spectrum, and we directly extract the oscillator strength of the lowest state of B850 by considering the emission region of the difference spectrum only. Pullerits and colleagues found a coherence size of 3 pigments in the LH2 complex of *Rb. sphaeroides* at 4.2 K,[51] in nice agreement with our results.

Our measurements thus strongly support a model in which the excitons are partly localized as a result of disorder, as opposed to the situation in which the exciton wave functions are fully delocalized.[15−19] On the basis of our data, a weakly coupled dimer model as proposed by Jimenez et al.[13] and Visser et al.[11] cannot be completely ruled out. However, such a model is unlikely in view of recent structure-based calculations which demonstrate that the interactions within the dimer and between adjacent dimers are only slightly different.[17]

With our present data at hand we must refine the views we expressed in our previous paper on the LH2 complex of *Chr. tepidum* at room temperature.[6] In the latter work we found that the total bleaching part of the B850 difference spectrum was 4 times larger than the bleaching of B800, which is actually very similar to what we observe now at low temperature (see Figure 5A) and what we measured recently for the LH2 complexes of *Rps. acidophila* and *Rb. sphaeroides* at room temperature.[38] On the basis of the factor of 4 difference between the total

bleachings of B800 and B850, we came to the conclusion that the excitations on the B850 aggregate would be strongly delocalized and suggested exciton delocalization possibly over the entire aggregate. The latter suggestion is untenable considering our present low-temperature measurements. These low-temperature data give more insight in the problem of exciton delocalization than our room-temperature data; the entire ground-state bleaching/stimulated emission part of the B850 ΔA spectrum is not as good a quantitative measure of exciton delocalization as the separated stimulated emission band we utilize in this work, because the ground-state bleaching overlaps significantly with the excited-state absorption at the blue side of the absorption band. This situation is furthermore complicated by the possible occurrence of electrochromic band shifts in the B850 band, which may contribute to the pump−probe signals as a result of a charge-transfer character mixed in the lowest exciton state.[52] Unfortunately, at room temperature, it is not possible to assess the stimulated emission contribution in the B850 difference signal, because the stimulated emission and absorption bands overlap significantly. In their superradiance measurements on the LH2 complex of *Rb. sphaeroides*, Monshouwer et al. have found a nearly temperature-independent enhancement of the fluorescence,[50] whereas Pullerits and colleagues found no temperature dependence of the coherence size in the LH2 complex.[51] These observations are consistent with the present low-temperature and our earlier room-temperature pump−probe spectra,[6,38] which show similar enhancement of B850 bleaching with respect to that of B800.

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