Spectroscopy of Individual Light-Harvesting 2 Complexes of *Rhodopseudomonas acidophila*: Diagonal Disorder, Intercomplex Heterogeneity, Spectral Diffusion, and Energy Transfer in the B800 Band

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ABSTRACT This paper reports a detailed spectroscopic study of the B800 absorption band of individual light-harvesting 2 (LH2) complexes of the photosynthetic purple bacterium *Rhodopseudomonas acidophila* at 1.2 K. By applying single-molecule detection techniques to this system, details and properties can be revealed that remain obscured in conventional ensemble experiments. For instance, from fluorescence-excitation spectra of the individual complexes a more direct measure of the diagonal disorder could be obtained. Further spectral diffusion phenomena and homogeneous linewidths of individual bacteriochlorophyll a (BChl a) molecules are observed, revealing valuable information on excited-state dynamics. This work demonstrates that it is possible to obtain detailed spectral information on individual pigment-protein complexes, providing direct insight into their electronic structure and into the mechanisms underlying the highly efficient energy transfer processes in these systems.

INTRODUCTION

The development of techniques to optically study single molecules in the condensed phase (Moerner and Orrit, 1999) opened the way for the investigation of molecular interactions on a truly microscopic scale. These single-molecule measurements reveal the distribution of molecular properties in inhomogeneous systems, properties that are normally obscured by ensemble averaging. In the study presented here single-molecule techniques are used to investigate the electronic structure of antenna complexes of photosynthetic purple bacteria. The initial event in bacterial photosynthesis is the absorption of a photon by a light-harvesting antenna system, which is followed by a rapid and highly efficient transfer of the energy to the reaction center (RC), where a charge separation takes place and the energy becomes available as chemical energy. In most purple bacteria, the photosynthetic membranes contain two types of light-harvesting (LH) complexes, the LH1 and LH2 complexes. LH1 is known to directly surround the RC, whereas LH2 is not in direct contact with the RC but transfers the energy to the RC via the LH1 complex (Papiz et al., 1996). The high-resolution x-ray structure of the LH2 complex of *Rhodopseudomonas (Rps.) acidophila*, along with the lower resolution structural information for LH1, showed a remarkable symmetry in the arrangement of the light-absorbing pigments in their protein matrix (McDermott et al., 1995). This LH2 complex consists of nine copies of a pair of proteins (α and β) arranged in a ring structure with C_9 symmetry, where each $\alpha\beta$ unit binds three BChl *a* and (presumably) two carotenoid molecules. A striking feature of the organization of the 27 BChl *a* molecules is their separation into two parallel rings. One ring consists of a group of 18 closely interacting BChl *a* molecules with their bacteriochlorin rings parallel to the symmetry axis, absorbing at 850 nm (B850). The other ring comprises nine well-separated BChl *a* molecules absorbing at 800 nm. The molecules in this B800 ring have their bacteriochlorin rings perpendicular to the symmetry axis of the complex. Upon excitation, energy is transferred from B800 to B850 molecules in less than 1 ps at room temperature (Hess et al., 1993; Kennis et al., 1996; Monshouwer et al., 1995; Shreve et al., 1991). At cryogenic temperature this process slows down to 2 ps, as determined by spectral hole burning (Caro et al., 1994; Reddy et al., 1991, 1992; Wu et al., 1996) and time-resolved measurements (Hess et al., 1993; Monshouwer et al., 1995; Wu et al., 1996). Energy transfer among the B850 molecules is an order of magnitude faster (Chachisvilis et al., 1997; Jimenez et al., 1996; Vulto et al., 1999b). The transfer of energy from LH2 to LH1 and subsequently to the RC occurs in vivo on a time scale of 30–40 ps (Pullerits and Sundström, 1996b; Visscher et al., 1989), i.e., very fast compared to the decay of B850 in isolated LH2, which has a time constant of ~1 ns.

From intermolecular distances as determined from the x-ray structure it can be concluded that the dipolar interaction between neighboring BChl *a* molecules in the B800 band will be significantly weaker than between the B850 molecules. The size of the transition dipole-dipole interaction strength between neighboring molecules compared to the variations in the site energy of the same molecules governs the extent of delocalization of the excited states of the LH2 complex. For the B800 BChl *a* molecules, the

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dipole-dipole interaction strength is estimated to be about -24 cm^{-1} (Sauer et al., 1996), i.e., much smaller than the variation in site energy of 125 cm^{-1}. In contrast, for the B850 band the dipolar coupling strength is estimated to be $\sim$300 cm^{-1} (Sauer et al., 1996). These values suggest that in the case of B800 the excitation energy is largely localized on individual BChl *a* molecules (Alden et al., 1997), whereas for B850 one expects that the excitation is coherently distributed at least over a part of the ring (Alden et al., 1997; Jimenez et al., 1996; Kennis et al., 1996, 1997a; Monshouwer et al., 1997; Novoderezhkin and Razjivin, 1995; Pullerits et al., 1996a; Sauer et al., 1996; Shreve et al., 1991).

Detailed knowledge of the electronic structure of the excited states of the light-harvesting 2 complex combined with the known geometric structure will contribute to an understanding of the highly efficient energy transfer process in photosynthetic pigment-proteins. In ensemble spectroscopic studies, subtle spectral details are often obscured by statistical averaging over a heterogeneous ensemble. Measurements of a single molecule can reveal the distribution of molecular properties in inhomogeneous systems. Recently, a number of groups have reported spectroscopic experiments on individual LH2 complexes (Bopp et al., 1997; van Oijen et al., 1998; Tietz et al., 1999). By performing polarization-dependent fluorescence-excitation spectroscopy on single complexes at cryogenic temperature, it could be demonstrated that excitations in the B800 band are localized on two or three pigments (van Oijen et al., 1999a) and are completely delocalized in the B850 band (Oijen et al., 1999b). Moreover, these experiments unambiguously showed that the complexes are structurally deformed in their isolated form (Bopp et al., 1999; van Oijen et al., 1999b), an effect that is masked in ensemble-averaged experiments.

Here we report a series of single-molecule experiments at low temperature on the B800 absorption band of LH2. In particular, the results reported here provide a direct discrimination of the two types of spectral heterogeneities, inter- and intracomplex, that amount to the inhomogeneous broadening of the B800 absorption observed in ensemble experiments. The determination of the exact value of the intracomplex heterogeneity, also called diagonal disorder, is of particular importance for modeling energy transfer dynamics in pigment protein complexes. Furthermore, we focus on the spectral diffusion of individual BChl *a* molecules, made visible by a time-resolved recording of fluorescence-excitation spectra of individual LH2 complexes. From the homogeneous linewidths extracted from these data, valuable information on the excited-state dynamics can be obtained.

MATERIALS AND METHODS

The LH2 complexes of *Rps. acidophila* (strain 10050) where prepared as described elsewhere (Kennis et al., 1997a). Poly(vinyl alcohol) (PVA) (BDH, hydrolyzed, MW = 125,000) was purified over a mixed resin to remove ionic impurities (Lösche et al., 1987). Thin polymer films were prepared by adding 1% (w/w) purified PVA to a solution of 5×10^{-11} M LH2 in buffer (0.1% lauryl dimethylamine-*N*-oxide, 10 mM Tris, 1mM EDTA, pH 8.0), which was then spin coated on a LiF substrate. By dropping 10 μl of solution on the substrate and spinning it at 500 rpm for 15 s followed by 2000 rpm for 60 s, high-quality films could be produced with an estimated thickness of less than 1 μm. The sample was mounted in a helium bath cryostat and cooled down to 1.2 K. To perform fluorescence microscopy and fluorescence-excitation spectroscopy the sample was illuminated with the light from a cw tunable Ti:sapphire laser featuring a spectral bandwidth of 1 cm^{-1}. Because we work under liquid helium conditions only a single aspheric lens (numerical aperture 0.55, working distance 850 μm), mounted close to the sample, served as the microscope objective.

To obtain wide-field images of parts of the sample, an area of 100×100 μm^2 was illuminated through a rear window of the cryostat. The fluorescence emitted by the LH2 complexes, with a wavelength of 890 nm, was collected by the aspheric lens and imaged on a CCD camera after passing through appropriate filters to block residual laser light. The field of view was 50×50 μm^2, with a lateral spatial resolution of 0.9 μm. The concentration of the LH2 complexes in the film, 50 pM, was chosen such that the average separation of individual complexes was much larger than 0.9 μm, allowing for spatial selection of a single complex. The substrate that supported the film could be moved in situ in the lateral direction with respect to the wide-field illumination and the aspheric lens, to allow probing of different regions of the film.

To perform detailed experiments on a specific LH2 complex, the microscope was switched to the confocal mode. In this mode the sample was illuminated through the aspheric lens, resulting in an excitation volume of $\sim$1 μm^3. By ensuring that this volume coincided with the position of one of the complexes observed with the CCD camera, the fluorescence of a single LH2 complex, collected by the same lens, was focused confocally on an avalanche photodiode. For all experiments described in this report, the detection occurred in a spectral window of 20 nm (FWHM) centered around 890 nm. By scanning the laser frequency, we could obtain fluorescence-excitation spectra of single LH2 complexes with high signal-to-background ratios. Further experimental details can be found elsewhere (Oijen et al., 1999a).

RESULTS AND DISCUSSION

The confocally detected fluorescence-excitation spectrum of the B800 band of a single LH2 complex, marked by the circle in the wide-field image in Fig. 1 *A*, is shown in Fig. 1 *C*. For comparison, a fluorescence-excitation spectrum of the B800 band for an ensemble of LH2 complexes of *Rps. acidophila* is shown (Fig. 1 *B*). For the single complexes (Fig. 1, *C* and *D*) a collection of narrow lines with a spread of several nanometers around the ensemble peak absorption at 800 nm is observed. As mentioned above, the dipolar coupling between the BChl *a* molecules in the B800 ring is predicted to be small with respect to the variation in site energy, and it is expected that the excitation energy is mainly localized on individual B800 BChl *a* molecules (van Oijen et al., 1999a). We therefore attribute the pattern of spectral lines to absorptions of individual pigments that are separated in their spectral positions because of differences in their local environment. Based on the kinetic properties of the triplet and singlet states of the BChl *a* pigments in the LH2 complex, we estimate the maximum emission rate to be $\sim$200,000 photons/s, similar to the value obtained by

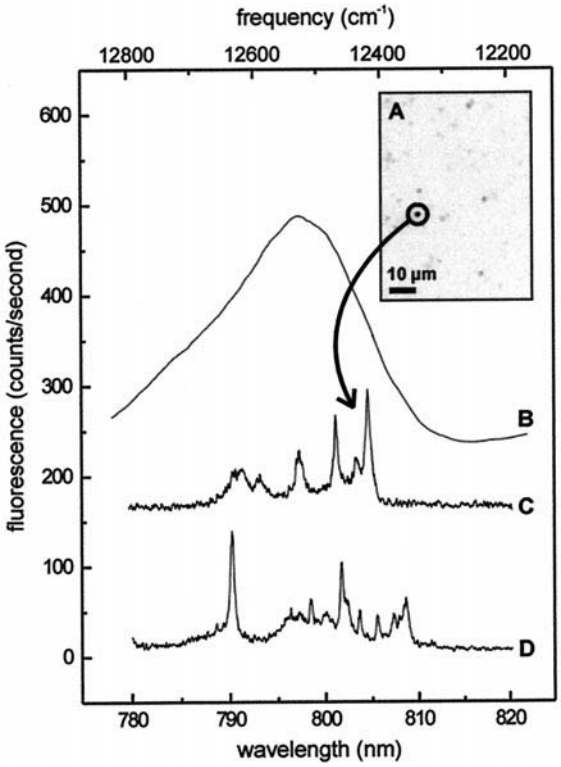

FIGURE 1 (*A*) Wide-field image of a sample region of ~40 × ~60 μm^2 taken at an illumination intensity of 125 W/cm^2. The black dots correspond to diffraction-limited images of the fluorescence of single LH2 complexes of *Rps. acidophila*. (*B*) For comparison, the fluorescence-excitation spectrum of the B800 band of an ensemble of LH2. (*C* and *D*) Comparison of B800 fluorescence-excitation spectra for two different LH2 complexes taken at an illumination intensity of 20 W/cm^2. The spectrum shown in *C* stems from the complex marked in the wide-field image in *A*. For each complex two fluorescence-excitation spectra, recorded with mutual orthogonal polarization of the incident light, were summed. The vertical scale is valid for the lower trace; all other traces are offset for clarity. All experiments were performed at 1.2 K.

Bopp et al. (1997). The collection efficiency of our microscope is ~0.05%, which yields a fluorescence countrate of ~50–100 counts/s for the emission of a single BChl *a* molecule, in agreement with our observations.

Intra- and intercomplex heterogeneity

Fig. 1, *C* and *D*, shows the fluorescence-excitation spectra of the B800 band for two individual LH2 complexes. The spectra reveal significant variations in the spectral distribution of the resonances between different LH2 complexes as well as for the absolute position of the whole line pattern. For pigments embedded in a glass-like protein one expects strong variations in the electrostatic interaction of the local surrounding with each pigment, resulting in a distribution of absorption frequencies of the chromophores. In general we have to consider two independent contributions to the spectral distribution. One is the variation in site energies of BChl *a* molecules within the same LH2 complex, which is referred to as intracomplex heterogeneity or diagonal disorder. The other is changes, for different complexes, in the spectral position of the center of mass of the whole spectrum, which is called intercomplex heterogeneity or sample inhomogeneity. Obviously, the study of individual LH2 complexes allows researchers to discriminate between these two contributions and to investigate them separately.

To find a measure for the intercomplex heterogeneity we have defined the spectral mean value, $\bar{\nu}$, of the fluorescence-excitation spectrum of a single LH2 complex by

$$\bar{\nu} = \frac{\sum_i I(i) \cdot \nu(i)}{\sum_i I(i)}, \tag{1}$$

where $I(i)$ denotes the fluorescence intensity at data point i, $\nu(i)$ is the spectral position corresponding to data point i, and the sum runs over all data points of the spectrum. The respective histogram for $\bar{\nu}$, obtained from the spectra of 46 complexes, is depicted in Fig. 2 *A* and has a width of ~120 cm^{-1}.

The intracomplex heterogeneity or diagonal disorder is extracted from the data by calculating the standard deviations σ_ν of the intensity distributions in the individual spectra:

$$\sigma_\nu = [\overline{\nu^2} - \bar{\nu}^2]^{1/2}, \tag{2}$$

where $\overline{\nu^2}$ is given by

$$\overline{\nu^2} = \frac{\sum_i I(i) \cdot [\nu(i)]^2}{\sum_i I(i)}. \tag{3}$$

The result is shown in Fig. 2 *B*. The distribution for σ_ν is centered at a value of ~55 cm^{-1}. Because the diagonal

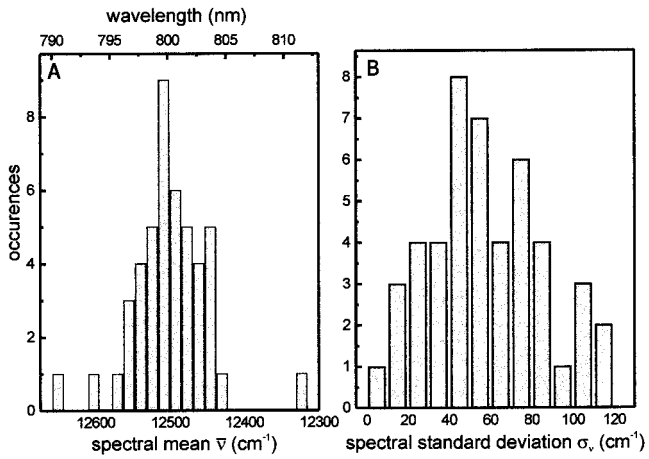

FIGURE 2 (*A*) Distribution of the spectral mean for 46 LH2 complexes, featuring the amount of intercomplex heterogeneity. (*B*) Distribution of standard deviations for the spread of absorption lines in the individual fluorescence-excitation spectra for the same 46 LH2 complexes.

disorder is commonly defined as the full width at half-maximum of the distribution of site energies, this value has to be multiplied by a factor of 2.36 to obtain a value of 130 cm^{-1} for the diagonal disorder. Clearly, an ensemble spectrum reflects the convolution of both contributions to the heterogeneity. From our data we expect for the B800 band a total inhomogeneous linewidth of ~180 cm^{-1}, in excellent agreement with the results from bulk spectra of LH2 of *Rps. acidophila* taken at 1.2 K.

Photostability and light-induced spectral diffusion

In Fig. 3 a sequence of fluorescence-excitation spectra of a single LH2 complex is shown. The first four spectra were recorded consecutively at intervals of 10 min, and it is seen that only minor changes in the excitation spectrum occur. In contrast to the work of Bopp et al. (1997), who observed photobleaching under ambient conditions after a few tens of seconds for similar excitation intensities, our data demonstrate that such effects are very small at cryogenic temperatures. This is illustrated by the last spectrum of the sequence in Fig. 3, which was recorded 20 h after the beginning of the experiment, which covered 10 h of continuous illumination at 80 W/cm^2 and 10 h of darkness. None of the 46 complexes studied showed photobleaching on a time scale of hours.

However, when the laser was tuned into resonance with one of the B800 absorptions of a single LH2 complex and the total emitted fluorescence was recorded as a function of time, strong fluctuations on a time scale of seconds were observed (see Fig. 4). The fluorescence time trace becomes more erratic when the excitation intensity is increased, indicating that the "blinking" behavior is light induced. In ensemble experiments this phenomenon is manifested as nonphotochemical hole burning (Caro et al., 1994; Wu et al., 1996). To study these effects in more detail, fluorescence-excitation spectra were recorded with scan speeds of the laser wavelength of 3 nm/s. This yields a temporal resolution of 10 ms/data point, corresponding to a spectral separation between two data points of 0.5 cm^{-1}. The actual spectral resolution of the scans is then determined by the spectral bandwidth of the Ti:sapphire laser. The upper panel on the left-hand side of Fig. 5 shows a sequence of 200 of such fast scans stacked upon each other. The spectra were recorded with an excitation intensity of 20 W/cm^2, and the fluorescence intensity is given by the color code. The lower panel on the same side of the figure displays the fluorescence-excitation spectrum that results when all 200 of the independent scans are averaged in the computer memory. Apparently, the spectral movements of the absorptions are restricted to a very small spectral range for this illumination condition. The situation changes drastically when the excitation intensity is increased to 80 W/cm^2, as illustrated on the right-hand side of the figure. As is evident from the collection of fast scans, sudden spectral jumps of the absorptions occur, and the averaged fluorescence-excitation spectrum shows significant broadenings of the spectral lines due to the spectral diffusion effects. Interestingly, it is possible to switch between these two situations reversibly by changing the excitation intensity. We think that the spectral diffusion is caused by internal conversion, B800-

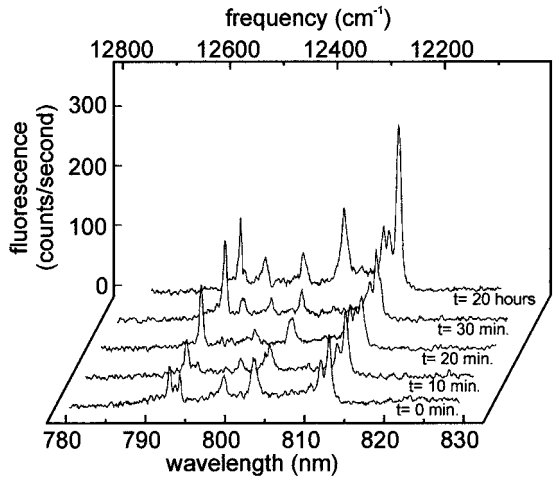

FIGURE 3 Sequence of fluorescence-excitation spectra of an individual LH2 complex. The first four spectra were obtained subsequently at intervals of 10 min, while the last spectrum was recorded 20 h later, including a period of 10 h of continuous illumination at 80 W/cm^2. Some of the data are taken from van Oijen et al. (1998).

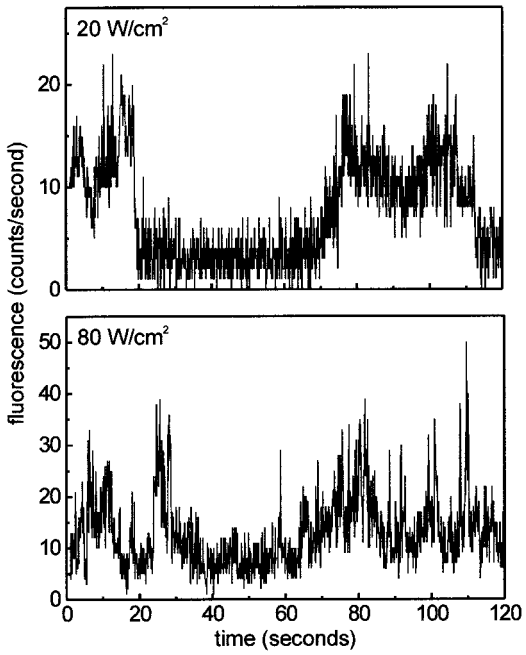

FIGURE 4 Time dependence of the fluorescence intensity when the excitation frequency is tuned into resonance with a particular absorption of a single complex in the B800 spectrum for excitation intensities of 20 W/cm^2 (*top*) and 80 W/cm^2 (*bottom*).

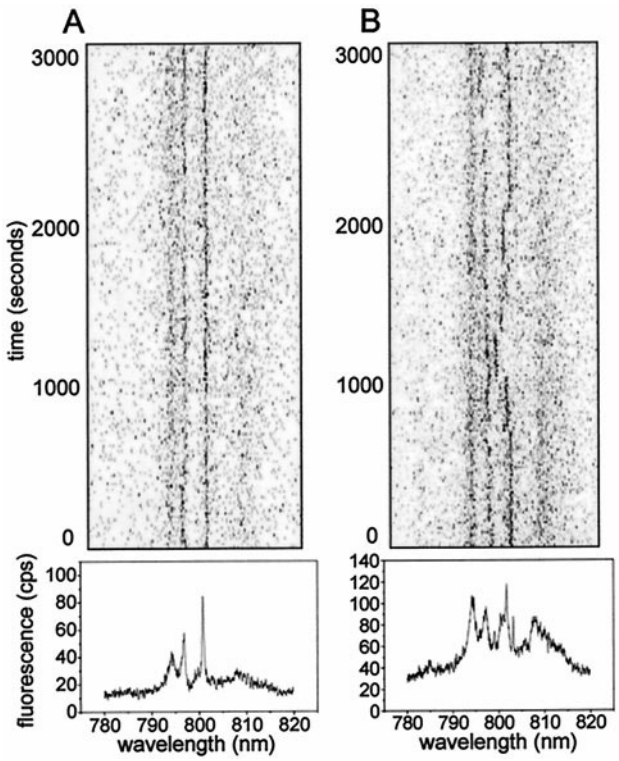

FIGURE 5 (*A*) Stack of 200 fluorescence-excitation scans recorded at a scan speed of 3 nm/s and an excitation intensity of 20 W/cm^2 (*top*). The fluorescence intensity is indicated by the color code. The average of the 200 individual spectra is displayed in the bottom panel. (*B*) Stack of 200 fluorescence-excitation scans recorded at a scan speed of 3 nm/s and an excitation intensity of 80 W/cm^2 (*top*). The fluorescence intensity is indicated by the color code. The average of the 200 individual spectra is displayed in the bottom panel.

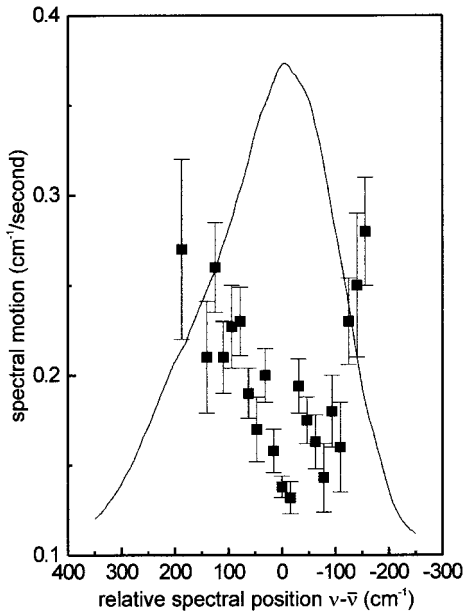

FIGURE 6 Amount of spectral motion of the absorption lines of the fluorescence-excitation spectra from single LH2 complexes with respect to the separation from the spectral mean. All data are obtained using an excitation intensity of 20 W/cm^2.

B850 energy transfer and intersystem crossing, and subsequent dissipation of vibrational energy in the complex. This energy is dumped as heat in the protein surrounding of the pigments, inducing conformational changes, which in turn give rise to changes in the absorption frequencies of the chromophores.

Statistics of the spectral jumps of the B800 transitions

The procedure of data acquisition as described above offers the opportunity to analyze the statistics of the spectral jumps in great detail. This was quantified by fitting every transition in every single sweep with a Lorentzian. From the fits the spectral position of each transition was determined as a function of time. This yields the absolute spectral distance covered per unit time for each particular transition (spectral motion in cm^{-1}/s). Fig. 6 shows the amount of spectral motion for the absorption lines of single LH2 complexes as a function of the spectral position in the B800 band. To exclude intercomplex heterogeneity and to be able to compare the data from different LH2 complexes, the spectral position is given with respect to the spectral mean, $\bar{\nu}$, of the complex under study. Remarkably, the spectral motion of a particular absorption is correlated with its spectral position within the B800 band. The spectral diffusion increases toward the wings of the spectral distribution of absorptions, as can be seen from the ensemble spectrum, which is included in Fig. 6 for illustration. Apparently, the probability of conformational changes in the immediate environment is larger for pigments that show absorption frequencies with large deviations from the spectral mean.

Linewidths and intracomplex energy transfer

The homogeneous linewidth of the individual absorption lines in the B800 band could not be obtained directly because of the spectral-diffusion effects. However, the previously described method of data acquisition allowed us to diminish the influence of the spectral motions on the observed linewidths in the following way. First every single transition in each fast data scan was fitted by a Lorentzian from which the peak position for each absorption was obtained. Second, for a particular transition the separate scans were shifted in their spectral positions such that the fitted peak positions for that transition coincided. In a third step the shifted raw spectra were averaged and the width of the absorption line under study could be determined after deconvolution with the laser linewidth, devoid of artificial line broadening caused by spectral diffusion. This procedure was repeated for all absorption lines in a single com-

plex spectrum. Given the scan speed of the laser, all light-induced spectral movements on a time scale slower than 50 ms could be suppressed. Fig. 7 shows the dependence of the linewidth, Γ, and the emission rate, R, for a particular B800 transition on the excitation power. The data could be fitted by the well-known expressions for the saturation behavior of two-level systems (Ambrose et al., 1991):

$$R(I) = R_\infty \frac{I/I_s}{(1 + I/I_s)},$$

$$\Gamma(I) = \Gamma(0)\sqrt{1 + I/I_s},$$

where R_∞ is the fully saturated emission rate, I_S is the saturation intensity, and $\Gamma(0)$ is the homogeneous linewidth. For the data shown we obtain $R_\infty = 280$ detected photons/s, which corresponds to ~600,000 emitted photons/s. The homogeneous linewidth is determined to be $\Gamma(0) = 1.7 \pm 0.2$ cm^{-1}, resulting in an excited-state lifetime of 3.2 ps.

The homogeneous linewidth of a particular absorption shows a strong dependence on its spectral position within the B800 band, as shown in Fig. 8. To exclude intercomplex heterogeneity we have plotted the inhomogeneous linewidth as a function of the spectral separation from the spectral mean rather than as a function of the absolute spectral position. The homogeneous linewidth decreases from ~10 cm^{-1} on the blue side of the B800 band to less than 2 cm^{-1} in the center and to the red side of this band.

A similar dependence of the homogeneous linewidth on the absolute spectral position has been found by hole-burning studies of other light-harvesting pigment protein complexes of purple bacteria (Caro et al., 1994; Wu et al., 1996). Based on these (ensemble) data it was concluded that for all B800 states B800 → B850 interband excitation energy transfer is effective, whereas for the energetically higher B800 states energy transfer to lower lying B800 molecules also occurs, driven by a Förster-like process (Joo et al., 1996; Kennis et al., 1997b). The different pathways result in variations in the lifetimes of the respective states over the region of the inhomogeneous linewidth. The justification for applying first-order perturbation theory, upon which the Förster model is based, lies in the assumption that the dipole-dipole interaction between neighboring pigments is very small compared to their difference in transition energy. The energy transfer rates in the Förster picture are then determined by the spectral overlap between donor and acceptor molecules. However, pronounced phonon sidebands are absent from our spectra, and the zero-phonon lines are spectrally distributed over a region ~25 times their homogeneous linewidth; both observations imply very small spectral overlap between donor and acceptor. Wu and co-workers have shown that under such conditions Förster transfer would occur with a transfer time of tens of picoseconds (Wu et al., 1996), i.e., much more slowly than observed in hole-burning experiments. This leads us to the conclusion that Förster processes cannot explain the observed B800 intraband energy transfer. Moreover, a comparison of the value of 130 cm^{-1} for the diagonal disorder with the interaction strength, −24 cm^{-1}, in a point-dipole approximation shows that there is no question of a weak coupling between neighboring BChls *a*.

From experiments and modeling on the FMO antenna complex from green sulfur bacteria, it is known that similar ratios of the diagonal disorder and the interaction strength lead to a slight delocalization of the excitation over two or three pigments (Vulto et al., 1999a). In this situation it has been shown that the dynamical properties of the excited states are governed by exciton-phonon interactions and that

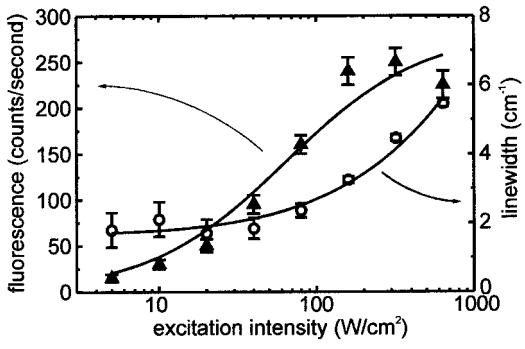

FIGURE 7 The fluorescence emission rate in units of detected photons per second (▲, *left vertical scale*) and the homogeneous linewidth (○, *right vertical scale*) versus the excitation intensity.

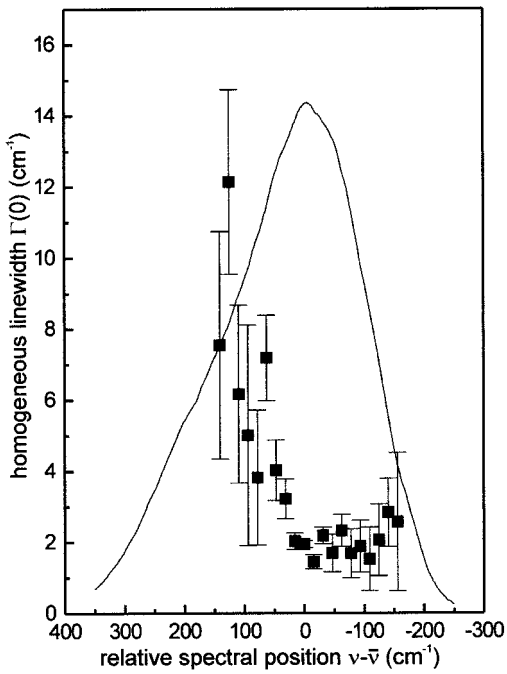

FIGURE 8 Dependence of the homogeneous linewidth of the observed absorption lines on the spectral position in the B800 band with respect to the spectral mean.

the lifetimes of individual levels are determined by vibronic relaxation to the lower states in the exciton manifold. We believe that the same description applies to the dynamic properties of the levels in the B800 band of the LH2, i.e., that the observed increase in the homogeneous linewidth toward the high-energy side of the B800 absorption band is caused by vibronic relaxation rather than a Förster-type energy transfer (van Oijen et al., 1999a). Another possible explanation could be provided by fast energy transfer to the B850 molecules via coupling of the B800 states with upper exciton levels of the B850 ring (Koolhaas et al., 1998; Ma et al., 1997; Wu et al., 1996).

CONCLUSIONS

This study demonstrates that low-temperature single-molecule spectroscopy is a powerful tool for obtaining information on the electronic structure of photosynthetic antenna complexes, information that is obscured in conventional experiments by ensemble averaging. Fluorescence-excitation spectroscopy was performed on the B800 band of individual light-harvesting 2 complexes from the photosynthetic purple bacteria *Rps. acidophila* at 1.2 K, and information was obtained on diagonal disorder, spectral diffusion, and excited-state lifetimes. All complexes showed a remarkably high photostability, enabling us to obtain fluorescence-excitation spectra with high signal-to-noise ratios, despite the low fluorescence signal. In this way the sample heterogeneity and diagonal disorder, both contributing to the ensemble linewidth, could be observed separately and quantified. When the excitation intensity was increased, the individual BChl *a* showed pronounced spectral diffusion, an effect that becomes more significant as the site energy of the BChl *a* deviates from the average site energies of the pigments.

A decrease in the excited-state lifetime of the BChl *a* molecules located in the blue wing of the B800 absorption line was detected by measuring the homogeneous linewidths of the B800 single molecule absorptions. In contrast to earlier ensemble experiments, our single-molecule spectra rule out a Förster-like type of energy transfer as the mechanism responsible for this B800-B800 intraband energy transfer. Rather we propose that vibronic relaxation between the excited states dominates this process.

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REFERENCES

Alden, R. G., E. Johnson, V. Nagarajan, W. W. Parson, C. J. Law, and R. J. Cogdell. 1997. Calculations of spectroscopic properties of the LH2 bacteriochlorophyll-protein antenna complex from *Rhodopseudomonas acidophila. J. Phys. Chem. B.* 101:4667–4680.

Ambrose, W. P., Th. Basché, and W. E. Moerner. 1991. Detection and spectroscopy of single pentacene molecules in a *p*-terphenyl crystal by means of fluorescence excitation. *J. Chem. Phys.* 95:7150–7162.

Bopp, M. A., Y. Jia, L. Li, R. J. Cogdell, and R. M. Hochstrasser. 1997. Fluorescence and photobleaching dynamics of single light-harvesting complexes. *Proc. Natl. Acad. Sci. USA.* 94:10630–10635.

Bopp, M. A., A. Sytnik, T. D. Howard, R. J. Cogdell, and R. M. Hochstrasser. 1999. The dynamics of structural deformations of immobilized single light-harvesting complexes. *Proc. Natl. Acad. Sci. USA.* 96: 11271–11276.

Caro, C. de, R. W. Visschers, R. van Grondelle, and S. Völker. 1994. Inter- and intraband energy transfer in LH2-antenna complexes of purple bacteria. A fluorescence line-narrowing and hole-burning study. *J. Phys. Chem.* 98:10584–10590.

Chachisvilis, M., O. Kühn, T. Pullerits, and V. Sundström. 1997. Excitons in photosynthetic purple bacteria: wavelike motion or incoherent hopping? *J. Phys. Chem. B.* 101:7275–7283.

Hess, S., F. Feldchtein, A. Babin, I. Nurgaleev, T. Pullerits, A. Sergeev, and V. Sundström. 1993. Femtosecond energy transfer within the LH2 peripheral antenna of the photosynthetic purple bacteria *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris* LL. *Chem. Phys. Lett.* 216:247–257.

Jimenez, R., S. R. Dikshit, S. E. Bradforth, and G. R. Fleming. 1996. Electronic excitation transfer in the LH2 complex of *Rhodobacter sphaeroides. J. Phys. Chem.* 100:6825–6834.

Joo, T., Y. Jia, J.-Y. Yu, D. M. Jonas, and G. R. Fleming. 1996. Dynamics in isolated bacterial light harvesting antenna (LH2) of *Rhodobacter sphaeroides* at room temperature. *J. Phys. Chem.* 100:2399–2409.

Kennis, J. T. M., A. M. Streltov, T. J. Aartsma, T. Nozawa, and J. Amesz. 1996. Energy transfer and exciton coupling in isolated B800–850 complexes of the photosynthetic purple sulfur bacterium *Chromatium tepidum*. The effect of structural symmetry on bacteriochlorophyll excited states. *J. Phys. Chem.* 100:2438–2442.

Kennis, J. T. M., A. M. Streltsov, H. Permentier, T. J. Aartsma, and J. Amesz. 1997a. Exciton coherence and energy transfer in the LH2 antenna complex of *Rhodopseudomonas acidophila* at low temperature. *J. Phys. Chem. B.* 101:8369–8374.

Kennis, J. T. M., A. M. Streltsov, S. I. E. Vulto, T. J. Aartsma, T. Nozawa, and J. Amesz. 1997b. Femtosecond dynamics in isolated LH2 complexes of various species of purple bacteria. *J. Phys. Chem. B.* 101:7827–7834.

Koolhaas, M. H. C., R. N. Frese, G. J. S. Fowler, T. S. Bibby, S. Georgakopoulou, G.v.d. Zwan, C. N. Hunter, and R.v. Grondelle. 1998. Identification of the upper exciton component of the B850 bacteriochlorophylls of the LH2 antenna complex, using a B800-free mutant of *Rhodobacter sphaeroides. Biochemistry.* 37:4693–4698.

Lösche, M., G. Feher, and M. Y. Okamura. 1987. The Stark effect in reaction centers from *Rhodobacter sphaeroides* R-26 and *Rhodopseudomonas viridis. Proc. Natl. Acad. Sci. USA.* 84:7537–7541.

Ma, Y. Z., R. J. Cogdell, and T. Gillbro. 1997. Energy transfer and exciton annihilation in the B800–850 antenna complex of the photosynthetic purple bacterium *Rhodopseudomonas acidophila* (strain 10050). A femtosecond transient absorption study. *J. Phys. Chem. B.* 101:1087–1095.

McDermott, G., S. M. Prince, A. A. Freer, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell, and N. W. Isaacs. 1995. Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria. *Nature.* 374:517–521.

Moerner, W. E., and M. Orrit. 1999. Illuminating single molecules in condensed matter. *Science.* 283:1670–1676.

Monshouwer, R., M. Abrahamsson, F. van Mourik, and R. van Grondelle. 1997. Superradiance and exciton delocalization in bacterial photosynthetic light-harvesting systems. *J. Phys. Chem. B.* 101:7241–7248.

Monshouwer, R., I. Ortiz de Zarate, F. van Mourik, and R. van Grondelle. 1995. Low-intensity pump-probe spectroscopy on the B800 to B850 transfer in the light harvesting 2 complex of *Rhodobacter sphaeroides. Chem. Phys. Lett.* 246:341–346.

Novoderezhkin, V. I., and A. P. Razjivin. 1995. Exciton dynamics in circular aggregates: application to antenna of photosynthetic purple bacteria. *Biophys. J.* 68:1089–1100.

Papiz, M. Z., S. M. Prince, A. M. Hawthornthwaite-Lawless, G. McDermott, A. A. Freer, N. W. Isaacs, and R. J. Cogdell. 1996. A model for the photosynthetic apparatus of purple bacteria. *Trends Plant Sci.* 1:198–206.

Pullerits, T., M. Chachisvilis, and V. Sundström. 1996a. Exciton delocalization length in the B850 antenna of *Rhodobacter sphaeroides. J. Phys. Chem.* 100:10787–10792.

Pullerits, T., and V. Sundström. 1996b. Photosynthetic light-harvesting pigment-protein complexes: toward understanding how and why. *Acc. Chem. Res.* 29:381–389.

Reddy, N. R. S., R. J. Cogdell, L. Zhao, and G. J. Small. 1992. Nonphotochemical hole burning of the B800–B850 antenna complex of *Rhodopseudomonas acidophila. Photochem. Photobiol.* 57:35–39.

Reddy, N. R. S., G. J. Small, M. Seibert, and R. Picorel. 1991. Energy transfer dynamics of the B800–B850 antenna complex of *Rhodobacter sphaeroides*: a hole burning study. *Chem. Phys. Lett.* 181:391–399.

Sauer, K., R. J. Cogdell, S. M. Prince, A. A. Freer, N. W. Isaacs, and H. Scheer. 1996. Structure-based calculations of the optical spectra of the LH2 bacteriochlorophyll-protein complex from *Rhodopseudomonas acidophila. Photochem. Photobiol.* 64:564–576.

Shreve, A. P., J. K. Trautman, H. A. Frank, T. G. Owens, and A. C. Albrecht. 1991. Femtosecond energy-transfer processes in the B800–850 light-harvesting complex of *Rhodobacter sphaeroides* 2.4.1. *Biochim. Biophys. Acta.* 1058:280–288.

Tietz, C., O. Chekhlov, A. Dräbenstedt, J. Schuster, and J. Wrachtrup. 1999. Spectroscopy on single light-harvesting complexes at low temperature. *J. Phys. Chem. B.* 103:6328–6333.

van Oijen, A. M., M. Ketelaars, J. Köhler, T. J. Aartsma, and J. Schmidt. 1998. Spectroscopy of single light-harvesting complexes from purple photosynthetic bacteria at 1.2 K. *J. Phys. Chem. B.* 102:9363–9366.

van Oijen, A. M., M. Ketelaars, J. Köhler, T. J. Aartsma, and J. Schmidt. 1999a. Spectroscopy of individual LH2 complexes of *Rhodopseudomonas acidophila*: localized excitations in the B800 band. *Chem. Phys.* 247:53–60.

van Oijen, A. M., M. Ketelaars, J. Köhler, T. J. Aartsma, and J. Schmidt. 1999b. Unraveling the electronic structure of individual photosynthetic pigment-protein complexes. *Science.* 285:400–402.

Visscher, K. J., H. Bergström, V. Sundström, C. N. Hunter, and R. van Grondelle. 1989. Temperature dependence of energy transfer from the long wavelength antenna BChl-896 to the reaction center in *Rhodospirillum rubrum, Rhodobacter sphaeroides* (w.t. and M21 mutant) from 77 to 177 K, studied by picosecond absorption spectroscopy. *Photosynth. Res.* 22:211–217.

Vulto, S. I. E., M. A. de Baat, S. Neerken, F. Nowak, H. van Amerongen, J. Amesz, and T. J. Aartsma. 1999a. Excited state dynamics in FMO antenna complexes from photosynthetic green sulfur bacteria: a kinetic model. *J. Phys. Chem. B.* 103:8153–8161.

Vulto, S. I. E., J. T. M. Kennis, A. M. Streltsov, J. Amesz, and T. J. Aartsma. 1999b. Energy relaxation within the B850 absorption band of the isolated light-harvesting complex LH2 from *Rhodopseudomonas acidophila* at low temperature. *J. Phys. Chem. B.* 103:878–883.

Wu, H.-M., S. Savikhin, N. R. S. Reddy, R. J. Jankowiak, R. J. Cogdell, W. S. Struve, and G. J. Small. 1996. Femtosecond and hole-burning studies of B800's excitation energy relaxation dynamics in the LH2 antenna complex of *Rhodopseudomonas acidophila* (strain 10050). *J. Phys. Chem.* 100:12022–12033.