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LETTERS

Spectroscopy of Single Light-Harvesting Complexes from Purple Photosynthetic Bacteria at 1.2 K

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In this Letter we present the first observation of the fluorescence-excitation spectra of individual light-harvesting complexes (LH2) from purple photosynthetic bacteria at 1.2 K. The spectra reveal the electronic transitions to the individual excitonic states of the assembly of absorbing bacteriochlorophyll *a* (BChl *a*) molecules. From the experiment we can extract information about site energies of the individual pigments, degree of disorder, dipolar coupling, excited-state dynamics, and spectral diffusion.

Photosynthesis is initiated by electronic excitation of an aggregate of light-harvesting pigment−protein complexes, followed by energy transfer to the reaction center where the charge separation takes place. Many studies have been performed to unravel the electronic and geometrical structure of these aggregates and to explain their efficiency in the light-harvesting and energy-transfer process. A system that has attracted considerable interest is the light-harvesting 2 complex (LH2) of purple bacteria, in particular after the elucidation of its three-dimensional structure by X-ray diffraction.[1] As yet, our insight in the electronic structure of the aggregate of absorbing bacteriochlorophyll *a* (BChl *a*) molecules of this LH2 complex is limited seriously by the inhomogeneous broadening of the optical absorption lines, resulting from variations in the local environment of the pigments. To circumvent this problem we have performed excitation spectroscopy on a single LH2 complex of this bacterium. This method opens the way for a precise study of the factors determining the electronic structure of the assembly of BChl *a* molecules forming the absorbing aggregate of the complex.

The photosynthetic purple bacteria contain two types of antenna complexes LH1 and LH2, which both have a ringlike structure. The reaction center is presumably associated with LH1, while the LH2 complexes are arranged around the perimeter of the LH1 ring in a two-dimensional structure.[2] The LH2 complex consists of 27 BChl *a* molecules arranged in two distinct rings, each with a 9-fold symmetry axis that coincides with that of the cylindrical structure of the complex.[1] One ring consists of a group of nine well-separated BChl *a* molecules absorbing at 850 nm (B800). The other ring consists of 18 closely interacting BChl *a* molecules absorbing at 850 nm (B850). The 18 B850 molecules are oriented with the plane of the molecules nearly parallel, and the B800 molecules perpendicular, to the symmetry axis. Upon excitation, energy transfer at low temperature occurs from B800 to B850 molecules in up to 2 ps,[3−5] while among B850 molecules it is an order of magnitude faster.[6] The transfer of energy from LH2 to LH1 and subsequently to the reaction center occurs in vivo on a time scale of 5−10 ps, i.e., very fast compared to the decay of B850 in isolated LH2, which has a time constant of 1 ns.

LH2 provides a key to understand the details of the highly efficient energy-transfer process in the light-harvesting complexes. It represents one of the rare cases of pigment−protein complexes of this size, of which the geometric structure is

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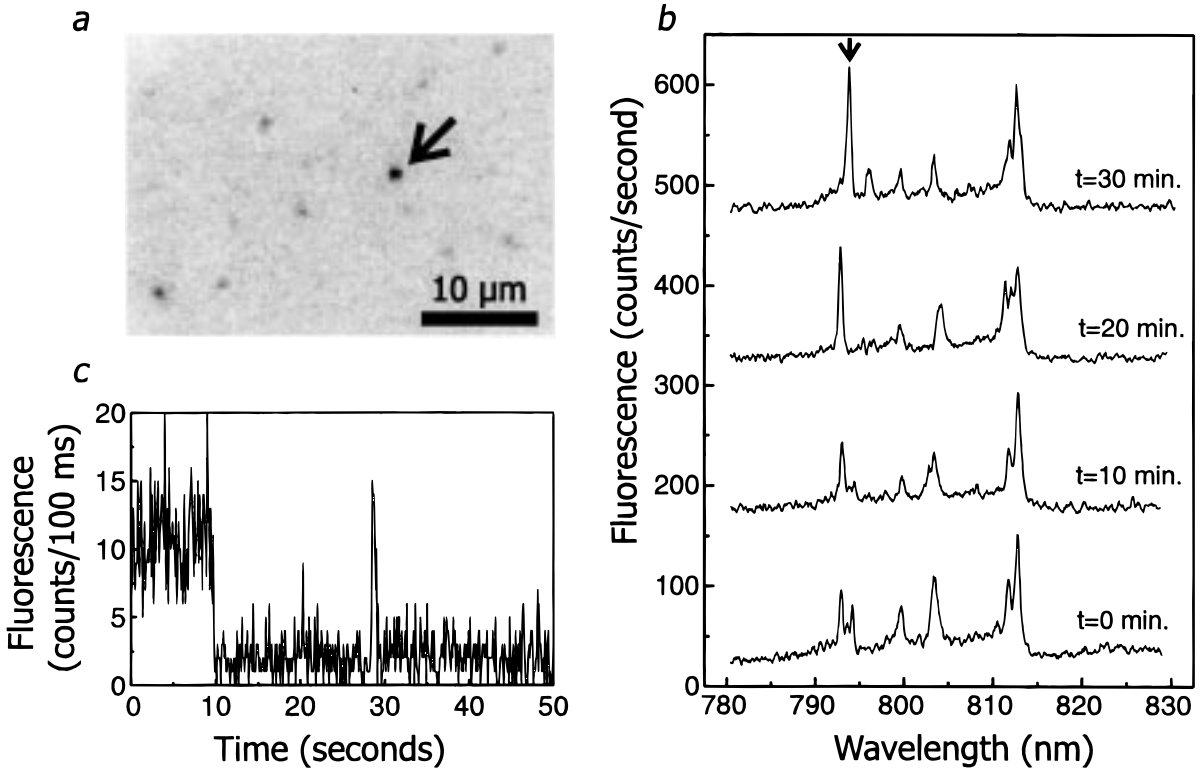

Figure 1. (a) Wide-field fluorescence image of a 35 × 25 μm^2 region of a polymer film doped with LH2. The films were prepared by adding 1% poly(vinyl alcohol) (PVA; M_w 12 500) to a solution of 50 pM LH2 from *Rps. acidophila* in buffer (0.1% LDAO/10 mM Tris/1 mM EDTA/pH 8.0), which was then spin-coated on a LiF substrate. The illumination wavelength is 798 nm at an intensity of 125 W/cm^2. The fluorescence at $\lambda = 890 \pm 10$ nm is detected with an intensified CCD camera after passing suitable filters. The black dots represent single LH2 complexes in resonance with the excitation. (b) Sequence of consecutively recorded fluorescence-excitation spectra of the single LH2 complex marked with an arrow in part a of the figure. The spectra have been obtained in the confocal mode of the microscope with an illumination intensity of 80 W/cm^2 and detecting the resulting fluorescence at $\lambda = 890 \pm 10$ nm with an avalanche photodiode. The spectral resolution is 0.06 nm (1 cm^{-1}). The vertical scale is valid for the lowest trace; other traces are offset for clarity. (c) Time dependence of the fluorescence at 890 nm if the excitation is kept in resonance with the absorption at 792 nm of the complex studied in part b indicated by the arrow. The temporal resolution of the trace is 100 ms. All experiments have been performed at 1.2 K.

known in detail. As yet, the problem in spectroscopic studies of this complex is the difficulty to determine unambiguously the various parameters that govern the energy-transfer process, such as the transition dipole−dipole interaction J between adjacent BChl *a* molecules, the actual site energy of the individual pigments, the spread in energy and in dipolar interactions, and the strength of the electron−phonon interaction. For the B800 BChl *a* molecules, J is estimated to be about 20 cm^{-1},[7] i.e., much smaller than the inhomogeneous width of 125 cm^{-1} of the absorption line. The latter is caused by a spread in site energies of the individual BChl *a* molecules (intracomplex disorder) and by sample inhomogeneity (intercomplex disorder). In contrast, for the 18 B850 BChl *a* molecules J is estimated to be about 300 cm^{-1}.[7] These estimates suggest that in the case of B800 the excitation energy is largely localized on individual BChl *a* molecules, whereas for B850 one expects that the excitation is coherently distributed at least over a part of the ring. Theoretical and experimental estimates[3,7−13] of the number of B850 molecules, over which the excitation is coherently delocalized, range from 2 to essentially the whole ring.

Here we present the results of a study of isolated LH2 complexes of *Rhodopseudomonas acidophila* (strain 10050) by single-molecule fluorescence-detected excitation spectroscopy. This method has been successfully applied in recent years to detect single guest molecules in crystalline and amorphous matrices.[14] To be able to select a single LH2 complex, polymer films were prepared by adding 1% poly(vinyl alcohol) (PVA) to a 50 pM solution of LH2, which was then spin-coated on a LiF substrate to a thickness of less than 1 μm. The samples were mounted in a bath cryostat, cooled to 1.2 K, and illuminated with the output of a cw, tunable Ti−sapphire laser. The excitation light was directed toward the sample either directly through a home built microscope (confocal mode) or through a lens for wide-field illumination (wide-field mode). In both modes the fluorescence of the LH2 complexes at 890 nm was detected through the same microscope with suitable band-pass filtering. Note that this selective fluorescence detection also strongly reduces the probability of observing features due to impurities. The fluorescence intensity was recorded with an avalanche photodiode (confocal mode), while the sample could be imaged simultaneously with an intensified CCD camera (wide-field mode). The objective of the microscope consisted of an aspheric singlet lens (NA = 0.55, $f = 1.4$ mm) yielding a lateral resolution of 0.9 μm when immersed in liquid helium. This defines an excitation volume of less than 1 μm^3. The concentration of the LH2 complexes, typically 50 pM, was adjusted such that the average separation of individual LH2 complexes was much larger than 1 μm allowing for spatial selection of a single complex.

The selection of a single complex took place in two steps. First a wide-field image of a 35 × 25 μm^2 region of the sample was taken with the CCD camera to locate individual LH2 complexes (Figure 1a). Subsequently the microscope was switched to the confocal mode such that the excitation volume coincided with one of the complexes observed with the CCD camera. In this mode the superior background suppression

allowed recording of a fluorescence-excitation spectrum of the B800 band in less than 10 min. In Figure 1b a sequence of four consecutively recorded fluorescence-excitation spectra of a single LH2 complex is shown. The spectra show a high degree of reproducibility, although some small variations occur presumably owing to spectral diffusion. Figure 1c shows the time dependence of the fluorescence of the single LH2 complex when the laser is tuned into resonance with the feature at 792 nm of Figure 1b. After 10 s the count rate drops suddenly to the background level, and after about 30 s the complex reappears shortly into resonance. The time-dependent behavior is clearly light-induced. From our observations we conclude that we are dealing with two types of processes. On a short time scale of several seconds reversible changes in the line intensities occur that depend on the number of photons absorbed by the complex per unit time, while on a long time scale of about 1 h irreversible spectral changes take place. This relatively high photostability allows us to record the excitation spectra. The light-induced behavior differs from that observed by Bopp et al.[15] at room temperature where real photobleaching occurs on a much shorter time scale.

Evidence that we are dealing with a single LH2 complex stems from the following observations. First, from the photophysical parameters of a single BChl *a* molecule as given in ref 15, we estimate the fully saturated fluorescence emission rate to be about 200 000 photons/s. From previous single-molecule experiments we know that the collection efficiency of our microscope is about 0.05%, which yields a fluorescence count rate of about 50−100 counts/s for the emission of a single BChl *a* molecule, in agreement with our observations. Second, the number of dots observed in an image scales linearly with the LH2 concentration of the sample. Third, for 50 pM concentration and a sample thickness of 1 μm the probability for finding two complexes is already less than 0.1%. Fourth, upon prolonged irradiation the fluorescence behaves in an on-and-off (blinking) fashion typical for single quantum systems[14,16] (Figure 1c).

In Figure 2 the fluorescence-excitation spectra of the B800 band of three individual LH2 complexes are compared. For each complex two spectra with mutually orthogonal polarization of the excitation light are presented. For two complexes (Figure 2a,b) we observe a discrete pattern of several fluorescence-excitation lines, typically 4−6, with a spread of several nanometers around the peak absorption of the ensemble value at 802 nm. The fluorescence count rates vary between 50 and 150 counts per second (cps). As mentioned, the intermolecular interaction of the BChl *a* molecules in the B800 band is predicted to be small compared to the variation in site energy, and the excitation is expected to be largely localized on individual BChl *a* molecules in the ring. The pattern of excitation lines in Figure 2a,b may be attributed to the absorption of individual molecules in the ring, and the strong variation in the relative intensities with the polarization of the exciting laser beam may be explained by the different direction of their transition dipole moments. However, if the transition energies of nearest neighbor differ by less than the interaction strength, significant effects of dipolar interactions are expected, in particular, a delocalization of the electronic excitation and a redistribution of oscillator strength. As a matter of fact, for one complex (Figure 2c) we have been able to record an excitation spectrum consisting of a single line at 801 nm featuring a fluorescence count rate of about 600 cps. We believe that this spectrum corresponds to a LH2 complex where the variation in site energy is very small, at least much smaller than

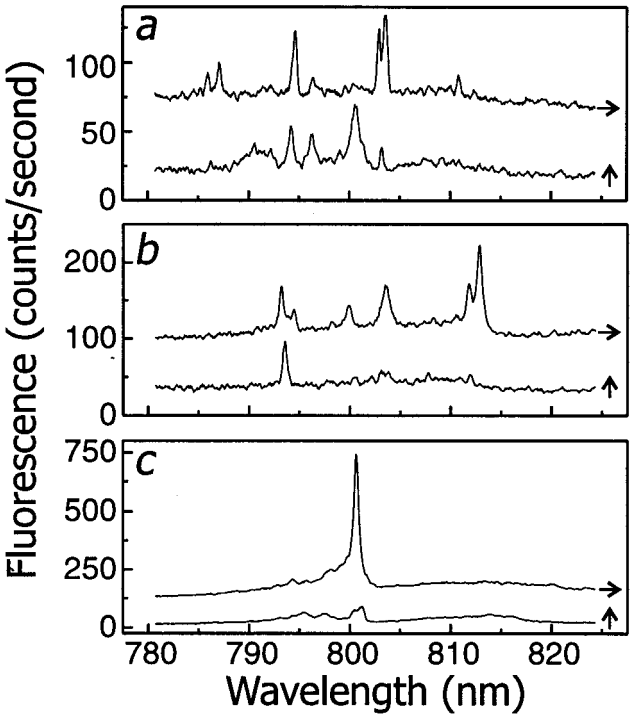

Figure 2. Comparison of fluorescence-excitation spectra of three individual LH2 complexes of *Rps. acidophila.* For each complex, two spectra with mutually orthogonal polarization of the excitation light are shown as indicated by the arrows. The laser intensity is 80 W/cm^2. The vertical scales apply for the lowest traces; the other traces are offset for clarity. Note the different intensity scales for the three complexes.

the dipolar interaction strength. For this particular complex we assume that the excitation is coherently delocalized over a substantial number, possibly all, of the BChl *a* molecules of the B800 ring. The high count rate observed experimentally is an indication of the enhanced oscillator strength expected for such a delocalized state. Thus the fluorescence-excitation spectrum of an ensemble represents a mixture of highly localized and delocalized excitations. We have performed model calculations in which the site energy is varied at random within the inhomogeneous line width of the B800 band, and the results are in agreement with our observations.

The line widths of the absorptions are distributed around an average value of (0.5 ± 0.1) nm. This line width would correspond to an upper limit of the excited-state lifetime of about 700 fs, slightly less than that obtained from time-resolved experiments.[3−5] The observed line width is probably influenced by spectral diffusion effects during the scans, and as yet we have not been able to find a dependence of the line width on the wavelength of absorption.[17] Further studies are necessary to solve this problem and to draw conclusions about the energy-transfer mechanism among the B800 pigments and between the B800 and B850 BChl *a* molecules.

The results presented in this report illustrate the advantage of single-molecule spectroscopy. Highly resolved excitation spectra of individual LH2 complexes are obtained devoid of inhomogeneous line-broadening effects caused by variations in the local environment of the individual BChl *a* molecules within the complex. The present results pertain in particular to LH2 complex from *Rps. acidophila*, but we expect that, in view of the photostability of this complex, the method will be applicable to other pigment−protein complexes as well. Thus a detailed study of the electronic structure of such complexes and of the

interactions responsible for the efficient energy-transfer processes in the primary steps of photosynthesis will become possible.

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