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Single-Molecule Study of the Electronic Couplings in a Circular Array of Molecules: Light-Harvesting-2 Complex from *Rhodospirillum Molischianum*

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Applying single-molecule spectroscopic techniques allowed us to determine the mutual angles between the transition-dipole moments associated with optical transitions of the eight bacteriochlorophyll *a* molecules which form the so-called B800 ring of the light-harvesting-2 complex from *Rhodospirillum molischianum*. The orientation of the transition-dipole moment is a sensitive probe for the strength of the local intermolecular interactions because of the well-defined arrangement of the individual molecules within the B800 ring. Our data reveal that the strength of the electronic coupling between individual molecules in the ring is subjected to spatial as well as temporal variations.

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Molecular aggregates have attracted considerable attention both experimentally and theoretically during the last years [1–8]. Such assemblies consist of repeating noncovalently bound molecular units and exhibit interesting physical properties that have stimulated research, because they are directly related to technologically challenging and fundamental aspects of molecular physics [9–12]. Important parameters that determine the character of the electronically excited states of a molecular aggregate are the transition-dipole-dipole interaction V between neighboring molecules and the variations in site energies of these molecules. The latter can usually be characterized by the width Δ of a Gaussian distribution of site energies. In the limit $V/\Delta \ll 1$ the description of the excited states of the aggregate in terms of excitations localized on the individual molecules is a good approximation. For $V/\Delta \gg 1$ the electronically excited states of the aggregate are described more appropriately as excitations that are coherently delocalized over the molecules. Accordingly, the optical properties of such systems are determined not only by the intrinsic properties of the individual molecular building blocks but also by the intermolecular interactions between those units. These interactions depend strongly on the relative orientations and distances of the molecules, and consequently the geometrical arrangement of the molecules has a crucial influence on the electronic excitations of the aggregate. Prominent examples of molecular assemblies are *J* aggregates which appear as linear arrays of pseudoisocyanine molecules [11] and light-harvesting complexes that feature a circular conformation of bacteriochlorophyll (BChl) *a* pigments [3,13–15].

Until now the regime of weak to intermediate coupling where the ratio of V/Δ is less than one has been utterly elusive to experimental studies, because it is principally impossible to separate effects of intermolecular interactions from intrinsic disorder in ensembles of molecular aggregates.

Here we present a single-molecule study on the circular "aggregate" of BChl *a* molecules formed by the B800 ring in the light-harvesting-2 (LH2) complex from the photosynthetic bacterium *Rhodospirillum (Rs.) molischianum*. The LH2 complex, a transmembrane protein involved in the primary steps of photosynthesis in this species, consists of two BChl *a* pigment pools labeled B800 and B850. The B850 ring consists of eight repeating pairs of BChl *a* pigments which are tightly organized as the blades of a turbine, whereas the B800 ring consists of eight well-separated pigments arranged in C_8 symmetry that have their molecular planes perpendicular to the symmetry axis (see Fig. 1(a)), as determined from the x-ray structure [16]. Important characteristics of the B800 system are the equivalence of the eight binding sites, the relatively narrow absorption linewidths, and the nearest-neighbor interaction strength V of about 20 cm^{-1} [17]. We focus on the polarization dependence of the B800 absorptions from individual LH2 complexes, which is sensitive to variations in the electronic coupling between individual BChl *a* molecules. The objective is to establish a relationship between spectroscopic observations, the molecular structure, and the statistical variations of V which may cause the character of the electronic states to vary between localized, partly localized, and delocalized.

The LH2 complexes of *Rs. molischianum* were isolated as described previously [18]. The samples were prepared by spin coating a highly diluted LH2-detergent solution on a Li-fluoride substrate, and then cooled in a liquid He bath cryostat to 1.4 K. The Ti-sapphire-laser-excited fluorescence (spectral window: 20 nm; bandwidth of detection: 1 cm^{-1}) around 880 nm was detected with an avalanche photodiode (SPCM-AQR-16, EG&G). To

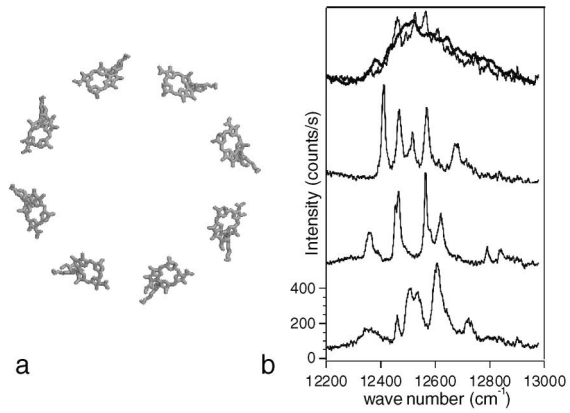

FIG. 1. (a) Top view of the arrangement of the bacteriochlorophyll *a* molecules in the B800 ring of *Rs. molischianum*. The protein backbone and all other chromophores have been omitted for clarity. (b) Fluorescence-excitation spectra of the B800 band from LH2 complexes of *Rs. molischianum*. The top traces show the comparison between an ensemble spectrum (bold line) and the sum of 24 spectra recorded from individual complexes (solid line). The lower three traces display spectra from single LH2 complexes. Each spectrum has been averaged over all possible excitation polarizations. The vertical scale is valid for the lowest trace, all other traces were offset for clarity. All spectra were recorded at 1.4 K with an excitation intensity of 10 W/cm^2.

examine the polarization dependence of the spectra, a $\frac{1}{2}\lambda$ plate was put in the confocal excitation path. For more experimental details see [19].

Figure 1(b) shows the B800 fluorescence-excitation spectra for three individual LH2 complexes together with an ensemble spectrum. The spectra of the individual complexes show several narrow absorption lines which vary from complex to complex with respect to both the number of lines and their spectral positions. In order to investigate the excitation spectra in more detail we have performed a systematic study of their polarization dependence. To this end we have swept the laser through the B800 spectral region and recorded a sequence of spectra in rapid succession while rotating the polarization of the incident radiation by 1.8° between successive scans.

Figure 2(a) shows a part of the spectrum from an individual LH2 complex in a two-dimensional representation, where the horizontal axis gives the wave number and the vertical axis the angle of polarization of the incident light. In Fig. 2(b) the fluorescence-excitation spectrum is given that results from the summation of the traces in Fig. 2(a). In Fig. 2(c) the fluorescence intensity is displayed as a function of the polarization of the incident radiation for the two absorption lines indicated by the boxed regions in Fig. 2(a). The observed variation in intensity is fitted by a $\cos^2$ dependence (full line and dashed line, respectively). From the difference of the phases in the two traces we determined that the mutual angle between the transition-dipole moments related to

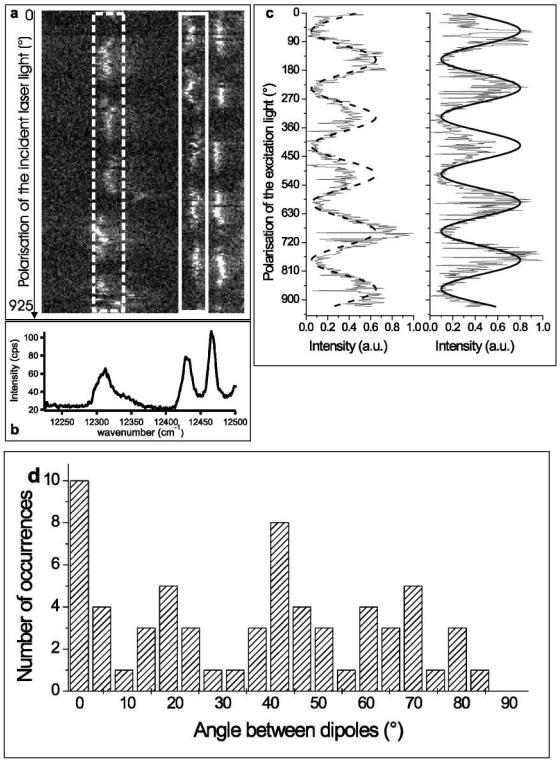

FIG. 2. (a) Two-dimensional representation of 513 fluorescence-excitation spectra from a part of the B800 band of an individual LH2 complex recorded consecutively at a scan speed of 3 nm/s and an excitation intensity of 10 W/cm^2. The gray scale gives the fluorescence intensity. Between two successive scans the polarization of the incident radiation has been turned by 1.8°. The horizontal axis corresponds to wave number and the vertical axis to polarization. (b) Average of all 513 spectra. (c) Intensity of the fluorescence for the two absorptions indicated by the boxes in (a) as a function of the polarization of the excitation. The full and dashed lines correspond to $\cos^2[\alpha(t) + \alpha]$-type functions fitted to the experimental data with phase angles, α, of 148° and 57°, respectively. (d) Histogram of mutual orientations of the transition-dipole moments from 88 absorption lines from 24 individual complexes.

these two absorption lines amounts to 91°. This procedure has been applied to 88 absorption lines from 24 individual LH2 complexes in order to find the distribution of the mutual orientations of the transition-dipole moments within each complex. The values have been obtained either directly from the phase difference $|\alpha_1 - \alpha_2|$ if the result was less than 90° and otherwise from $|\alpha_1 - \alpha_2 - 180°|$ to restrict the scale to the interval 0°–90°. The histogram in Fig. 2(d) shows the result of this study. The distribution covers nearly the whole range between 0° and 90° with slight preferences for the values around 0°, 20°, 45°, and 70°.

For some complexes we observed changes in the spectral position of the individual absorptions as well as in the orientation of the transition-dipole moments during the experiment. An example for such a behavior is shown in

Fig. 3. The spectral pattern can be grouped into three distinct sequences labeled A, B, and C as indicated by the boxes. In sequence A we observe four absorptions, labeled 1–4 with spectral positions 12 525.2 cm^{-1} (1), 12 489.1 cm^{-1} (2), 12 417.9 cm^{-1} (3), and 12 367.7 cm^{-1} (4). The respective phase angles were determined to be 70° (1), 4° (2), 30° (3), and −5° (4) and are visualized by the arrows. During the sequence A absorption "1" creeps continuously in spectral position towards lower energy. None of the absorptions show changes of the phase angle. The creeping of absorption 1 is continued at an increased rate in sequence B, again without any change of the phase angle. This absorption as well as absorption "2" disappears at the end of this sequence. No changes are observed for absorptions "3" and "4." With the beginning of sequence C new absorptions, "2a" and "2b" appear at spectral positions (phase angles) of 12 491.9 cm^{-1} ($-26°$) and 12 481.4 cm^{-1} (41°), respectively. As before, absorptions 3 and 4 do not show any changes.

The question is how the observed polarization of the optical transitions in an individual LH2 complex relates to the structure. The geometrical structure of the B800 ring in the LH2 complex of *Rs. molischianum* yields an interchromophore distance of 22 Å. If we assume that the electronic excitation of the B800 ring is strictly localized on a single BChl *a* chromophore we expect that the mutual angles between the transition-dipole moments of the individual BChl *a* chromophores will be equal to multiples of 45°. However, the coupling between two adjacent molecules will lead to eigenstates different from those of the uncoupled chromophores and consequently to a change in the orientation of the transition-dipole moments. The intracomplex variation in site energy has been measured as described previously [20] and yields a broad distribution ranging from 70–200 cm^{-1} (data not shown). From these values we estimate that the average difference in site energy of two adjacent molecules, δ, is in the order of 10–40 cm^{-1} and that the ratio V/δ between neighboring molecules varies from about 0.3–2.5. For the sake of brevity we restrict the following discussion to two adjacent BChl *a* molecules with excitation energies E_1 and E_2 ($E_1 > E_2$). For the resulting energies and eigenstates of the coupled system one finds

$$E_{\pm} = \frac{1}{2}(E_1 + E_2) \pm \frac{1}{2}\sqrt{\delta^2 + 4V^2}, \tag{1}$$

$$|\Psi_+\rangle = \cos\frac{\theta}{2}|1\rangle + \sin\frac{\theta}{2}|2\rangle, \tag{2}$$

$$|\Psi_-\rangle = -\sin\frac{\theta}{2}|1\rangle + \cos\frac{\theta}{2}|2\rangle,$$

where $\tan\theta = \frac{2V}{\delta}$, $\delta = E_1 - E_2$, and $|i\rangle$ denotes the excited state localized on molecule i. From the two eigenstates one finds for the transition-dipole moments

$$\vec{\mu}_+ = \vec{\mu}_1 \cos\frac{\theta}{2} + \vec{\mu}_2 \sin\frac{\theta}{2}, \tag{3}$$

$$\vec{\mu}_- = -\vec{\mu}_1 \sin\frac{\theta}{2} + \vec{\mu}_2 \cos\frac{\theta}{2},$$

where $\vec{\mu}_i$ denotes the transition-dipole moment of an individual BChl *a* molecule in the B800 ring. From (3) we have calculated the orientations of the transition-dipole moments of the B800 BChl *a* molecules as a function of V/δ; see Fig. 4. The orientations of the initial transition moments, corresponding to $V/\delta = 0$, were set to 0° and 45°. For increasing V/δ the orientations of the transition moments $\vec{\mu}_+$ and $\vec{\mu}_-$ change gradually with respect to the initial orientations and level off at angles of 22.5° and 112.5° for values of V/δ larger than about 6.

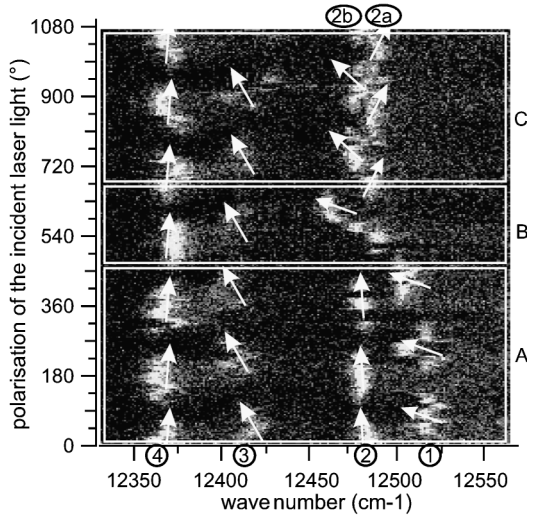

FIG. 3. Two-dimensional representation of a part of 614 fluorescence-excitation spectra from an individual B800 ring. Between two successive scans the polarization has been turned by 1.8°. The arrows show the orientation of the transition-dipole moments of the individual absorptions with respect to a laboratory frame. The aquisition times for intervals A, B, and C are 73, 31, and 65 min, respectively. Experimental conditions as in Fig. 2. For more details see text.

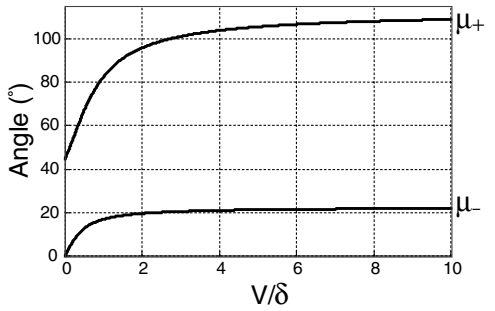

FIG. 4. Dependence of the orientation of the transition-dipole moments $\vec{\mu}_+$ (upper curve) and $\vec{\mu}_-$ (lower curve) on the ratio V/δ. The orientations of the initial transition moments $\vec{\mu}_1$ and $\vec{\mu}_2$ were set to 0° and 45° and provide the reference frame.

From the range of values estimated for V/δ a distribution of mutual orientations with preferences at 22.5° and 112.5° is expected. Taking into account BChl *a* molecules that are weakly coupled, one expects, in addition, to observe differences between the angular orientations of 45° modulo 45°. In total this results for the mutual orientations of the transition-dipole moments in a distribution with preferential values around 0°, 22.5°, 45°, 67.5°, and 90°. Consequently, any observation of mutual orientations of transition-dipole moments different from 0°, 45°, and 90° provides direct evidence for an electronic coupling in the weak to intermediate range between the individual BChl *a* molecules in the B800 assembly. The actual strength of the coupling is subjected to a distribution as a result of the difference in site energies of adjacent molecules.

In addition to spatial fluctuations of the coupling we do observe temporal variations as well, corroborated by the analysis of Fig. 3. From the behavior of absorptions 3 and 4 we can exclude an orientational change of the whole LH2 complex during the experiment. Two other absorptions are visible only either during the interval A (absorptions 1 and 2) or during interval C (absorptions 2a and 2b). The two intervals are separated by a period B where absorption 1 shows a drastic change in spectral position and where absorption 2 vanishes. Our conjecture is that the absorptions labeled 2a and 2b result from two molecules that become electronically coupled during interval B, one of which has initial absorption 2 for the uncoupled situation. This conclusion is based on the following arguments. If we suppose that the transition-dipole moments of the two uncoupled chromophores have initially a mutual orientation of 45°, which is valid for nearest neighbors, we can calculate from (3) the local ratio V/δ from the difference of the polarization angles of absorptions 2a and 2b. For the observed $\Delta\alpha = 67°$ we obtain $V/\delta = 1.07 \pm 0.15$. Another way to calculate this ratio follows from (1) if E_+, E_-, and one of the unperturbed energies are known. Assigning E_1 to absorption 2 and E_+ and E_- to absorptions 2a and 2b yields $V/\delta = 0.95 \pm 0.1$, in agreement with the value obtained from the polarizations. A further independent piece of information is provided by the difference in angle of the transition-dipole moments $\vec{\mu}_1$ and $\vec{\mu}_+$ of the absorptions corresponding to E_1 and E_+. From Fig. 4 a difference of $37° \pm 5°$ is predicted, in reasonable agreement with the observed value of $30° \pm 5°$. So far, our interpretation yields a consistent description for the absorptions 2, 2a, and 2b. However, for the absorption strength of the transitions 2a and 2b one finds within this approach 1.6 and 0.4 monomer units while the experiment yields about equal intensities for the two transitions. This discrepancy may reflect differences in the energy transfer efficiency to the B850 pigment pool, which are not taken into account by the simple dimer approximation.

Possible explanations for the fluctuations in the electronic coupling are changes in the protein backbone or rearrangements of the BChl *a* molecules in their binding pockets, for example, a rotation of the acetyl group or the breakage of hydrogen bonds. Such changes alter the electrostatic environment of the individual chromophores and result in a shift of their absorption energies and consequently in changes of V/δ. Given the low fluorescence quantum yield [21], it is most likely that the observed conformational changes are light induced at low temperature.

In conclusion, we have studied the electronic coupling in a model aggregate of a circular array of molecules by means of single-molecule spectroscopy. The chromophores act as sensitive probes to monitor the local interaction between the individual pigments within the B800 assembly from *Rhodospirillum molischianum*.

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