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Fluorescence-Excitation and Emission Spectra from LH2 Antenna Complexes of *Rhodopseudomonas acidophila* as a Function of the Sample Preparation Conditions

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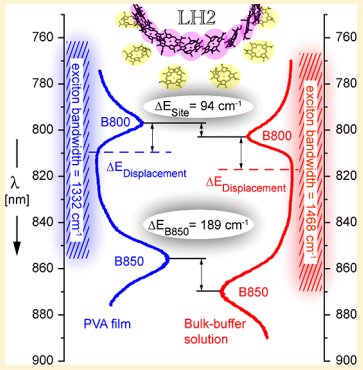
ABSTRACT: The high sensitivity of optical spectra of pigment–protein complexes to temperature and pressure is well known. In the present study, we have demonstrated the significant influence of the environments commonly used in bulk and single-molecule spectroscopic studies at low temperatures on the LH2 photosynthetic antenna complex from *Rhodopseudomonas acidophila*. A transfer of this LH2 complex from a bulk-buffer solution into a spin-coated polymer film results in a 189 cm^{-1} blue shift of the B850 excitonic absorption band at 5 K. Within the molecular exciton model, the origin of this shift could be disentangled into three parts, namely to an increase of the local site energies, a contraction of the exciton band, and a decrease of the displacement energy.



1. INTRODUCTION

The light-harvesting (LH) systems of photosynthetic organisms, plants, algae, and bacteria, are highly optimized for efficient collection and delivery of solar energy to the photochemical reaction centers (RC).[1,2] The mechanisms of this optimization, as well as its regulation by variable environmental conditions, have been of considerable long-time interest. The spatial arrangement of the LH complexes within the photosynthetic membranes is very random, though sometimes this is not immediately obvious. In many cases, some order is followed so that the chromophores closer to the RC absorb progressively more red-shifted light and an energetic funnel toward the RC is formed.[3,4] Wonderful examples of this funneling principle are seen in purple photosynthetic bacteria, where two types of LH pigment–protein complexes, the core RC-LH1 and the peripheral LH2 complexes, are found working in unison.[5−7] The LH1 complex with its intense long-wavelength absorption band at about 875 nm, in close resonance with the respective RC band, directly encircles the RCs, whereas the usually more numerous LH2 complexes, featuring strong absorption bands around 800 and 850 nm, are located in the periphery.[8,9] Bacteriochlorophyll *a* (Bchl *a*) acts as the main LH molecule in both of these complexes.

The high-resolution crystal structure of the LH2 complex from *Rhodopseudomonas (Rps.) acidophila* reveals that it is a cyclic array of nine transmembrane α,β-polypeptide pairs.[10] Each polypeptide pair binds 2 BChl *a* molecules at the outer membrane surface and one molecule on its intracytoplasmic side, forming two concentric pigment circles with C_9-symmetry. The inner ring consists of 18 strongly excitonically coupled BChl *a* molecules that feature intermolecular distances of less than 1 nm. These slightly dimerized molecules give rise to the intense absorption band around 850–870 nm in the various species of purple bacteria, traditionally named the B850 band.[11] The 9 largely monomeric BChl *a* molecules in the other ring, being separated by about 2 nm from each other, absorb light at around 800 nm (B800 band).

The B850 and B800 bands are related to the lowest-energy Q_y singlet electronic transitions in BChl *a* molecules. The exact spectral position of these bands, however, depends in a complex manner on the binding of the BChl *a* chromophores to the surrounding protein as well as on the distance and mutual orientation of the chromophores with respect to each other.[11] For simplicity, it is commonly assumed that the transition energies for the individual chromophores (site energies for short) are determined by the chromophore–protein interactions. The ability to flexibly change both the site energies and the exciton coupling energies is an ecological advantage that photosynthetic bacteria use to adapt to varying environmental conditions. For example, the light-harvesting apparatuses of

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several species of purple bacteria feature additional peripheral LH complexes with the exciton absorbance at 820 nm when grown under low-light conditions.[12−17] Subtle changes in the surroundings of chromophores are thus sensitively probed by the exciton spectra of LH complexes.

Many of the spectroscopic investigations of photosynthetic LH complexes are carried out on the systems that are grown under artificial, laboratory-based illumination conditions involving solubilization of these complexes out of their native membranes and assembly into non-natural environments. The present study is motivated by the observation of significant variations in the published optical absorption, fluorescence-excitation, and fluorescence spectra of LH pigment−protein complexes in principle assumed to be similar.[18−23] This problem is far from being new, having been raised and studied in multiple contexts in the past (e.g., see refs 23−26 and references therein). In this work, rather than trying to solve the general problem, we specifically investigate possible influences of the surrounding medium on the low temperature fluorescence and fluorescence-excitation/absorption spectra of the LH2 complexes from *Rps. acidophila* (strain 10050), a paradigmatic photosynthetic light-harvesting complex. Experiments were designed in order to assess to what extent the single-molecule spectroscopy data[14,19,20,27,28] are comparable with the spectral data obtained by selective spectroscopy methods on bulk samples.[18,23,26,29,30] Because in the bulk studies the detergent solubilized complexes are usually embedded in a glassy buffer−detergent−glycerol matrix, whereas in single-molecule experiments they are immobilized in thin polymer films, this issue directly addresses the question of whether and how the spectral observations correlate with the preparation conditions of the samples. The optical spectra of LH2 antenna complexes from *Rps. acidophila* are compared when the samples are embedded in buffer−detergent and buffer−detergent−glycerol solutions, a thin polymer film, or in a native membrane.

2. EXPERIMENTAL SECTION

Isolation and purification of the LH2 complexes were carried out following a standard protocol.[31] The highly concentrated stock solution, stored at −80 °C, was diluted with a 20 mM Tris−HCl buffer (pH 8.0) containing 0.1% of the detergent lauryldimethyl amine oxide (LDAO) to yield the desired optical density of the bulk-buffer sample. For the preparation with glycerol, the stock solution was diluted with a buffer−glycerol mixture (1:2 volume ratio) and the concentration of LDAO was increased to 1% to maintain well-isolated complexes.[32] For the thin film samples, 2% polyvinyl alcohol (PVA) was added to a buffer that was diluted to an LH2 concentration of 10^{-6} M. A small drop of this solution was spin-coated onto a quartz substrate with a 10 mm diameter, giving a polymer film sample of about 100 nm thickness.[33] Native membranes from *Rps. acidophila* (strain 10050), stored at −20 °C, were diluted with a 20 mM Tris−HCl buffer (pH 8.0) without detergent.

In optical experiments, a continuous wave Ti:Sapphire laser (Model 3900S, Spectra Physics) with a spectral bandwidth of ∼1 cm^{-1} served as an excitation source. For the fluorescence-excitation spectroscopy, the fluorescence of the sample was detected with an avalanche photodiode (SPCM-AQR-16, Perkin-Elmer) after passing through the set of band-pass (BP) and long-pass (LP) filters (BP 890/20, BP 900/50, BP 935/40 and LP 885, AHF Analysetechnik). Emission spectroscopy was carried out using a spectrometer and a CCD camera, either SpectraPro 150 (Acton Research) combined with iKon-M DU934N-BR-DD (Andor Technology) or Shamrock SR-303i (Andor Technology) and iDus DV420A-OE (Andor Technology). For fluorescence-anisotropy measurements, two high-contrast polarizers placed in front of the entrance slit of the spectrograph have been used. The first one, which was set parallel or perpendicular to the vertically polarized excitation light, served as an analyzer; the second one, which was set at 45° with respect to the polarization axis of the excitation light, served to minimize the deformation of the polarization due to the spectrograph. The detected emission was restricted to different spectral windows; that is, 880 nm−915 nm for the bulk-buffer solution, 875 nm−910 nm for the thin film sample, and 890 nm−950 nm for the natural membrane sample. The low temperature measurements were performed at 5 K or at 1.2 K using a liquid helium cryostat. For bulk-buffer samples, the quartz cuvettes with optical path lengths of 1.5 or 10 mm were used in different measurements; the PVA film samples were measured either under vacuum conditions (at room temperature) or in an inert helium environment (at low temperature). In all cases, care was taken that the optical density of the sample at the peak of the B850 absorption band did not exceed 0.1 to avoid fluorescence reabsorption effects.

The measured spectra were evaluated with standard software (OriginPro8). In both modes, fluorescence-excitation and emission spectroscopy, the standard deviation from the mean value of the spectral position of the bands did not exceed more than 0.7 nm in any series of the measurements. Similarly, the variations of the spectral bandwidths from the mean value were smaller than ±18 cm^{-1}.

3. RESULTS AND DISCUSSION

Fluorescence−Excitation and Emission Spectra. The room temperature fluorescence-excitation and emission spectra of the LH2 complexes from *Rps. acidophila* that either dissolved in bulk-buffer solution or bulk-buffer−glycerol solution or embedded in a thin PVA film are compared in Figure 1 with the fluorescence-excitation and emission spectra of a membrane sample from *Rps. acidophila*. The spectra from the isolated LH2 complexes show small variations with respect to each other. Though the fluorescence-excitation spectra of the two bulk-buffer samples (with and without glycerol) practically coincide, the spectrum for the PVA film sample is slightly shifted to the higher energies. The B850 band shift is approximately 1 nm and the B800 band shift is circa 3 nm with respect to the two bulk-buffer samples. The full width at half-maximum (fwhm) of the B850 band shows similar values for the bulk-buffer and the PVA film samples, whereas the B800 bandwidth is approximately 10% broader for the PVA film sample. Broadening of the B800 bandwidth of the PVA film sample is also reflected in the ratio of the B850 to B800 peak intensities, which are 1.4 for the bulk-buffer samples and 1.5 for the PVA film samples. For precise band position and bandwidth values, refer to Figure 1 and Table 1.

For reference, Figure 1 also shows the fluorescence-excitation spectrum from native membranes, complete with both LH1 and LH2 light-harvesting complexes. The absorption bands from the LH2 complexes are located at 803.2 nm (B800) and 862.8 nm (B850) and feature a peak intensity ratio of 1.4. The widths of the bands amount to 307 cm^{-1} (B800) and 413 cm^{-1} (B850). The refined parameters of the B850 band are evaluated via the deconvolution of the spectrum of the native membranes with the LH2 and LH1 subspectra in the spectral region from

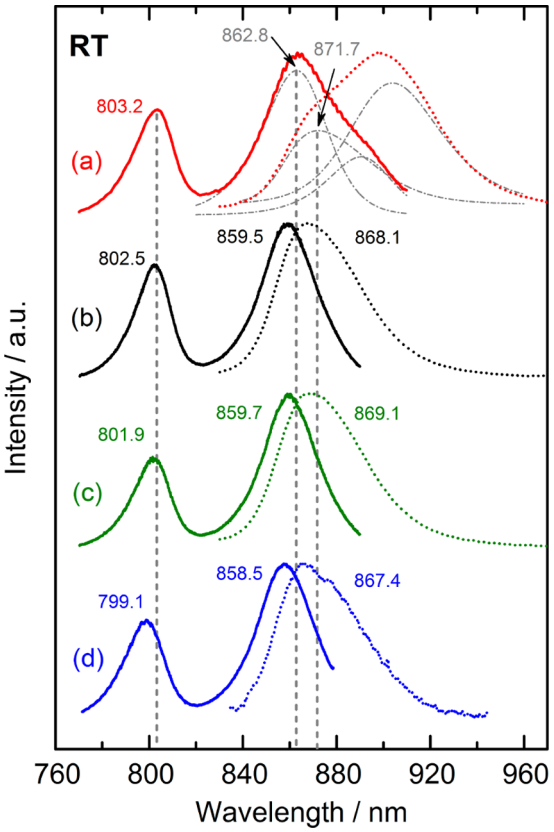

Figure 1. Comparison of the fluorescence-excitation (continuous line) and emission (dotted) spectra of the samples from *Rps. acidophila* taken at room temperature. (a) Fluorescence-excitation and emission spectra of native membranes in plain buffer solution, the gray dash-dotted spectra present deconvoluted LH2 and LH1 fluorescence-excitation and emission subspectra. (b) Fluorescence-excitation and emission spectra of LH2 complexes in bulk-buffer solution. (c) Fluorescence-excitation and emission spectra of LH2 complexes in bulk-buffer solution with glycerol. (d) Fluorescence-excitation and emission spectra of LH2 complexes in the PVA film. The spectra are normalized to the peak of the B850 band. The two gray vertical lines at 803.2 and 862.8 nm denote the peak positions of the fluorescence-excitation spectrum of the native membrane sample due to LH2 absorption; the third gray vertical line at 871.7 nm denotes the peak position of the evaluated LH2 emission in the native membrane sample.

820 to 910 nm. The bands of the isolated LH2 complexes are all blue-shifted (i.e., at higher energy) with respect to the spectra from the native membranes. Similar features have been observed for absorption spectra of the isolated LH2 complexes from *Rb. sphaeroides* in bulk-buffer solution in previous work.[24,29,34] The band shifts in the PVA film and bulk-buffer sample with (and without) glycerol relative to the native membranes are, 64 cm^{-1} and 20 cm^{-1} (11 cm^{-1}) for the B800 band and 58 cm^{-1} and 42 cm^{-1} (44 cm^{-1}) for the B850 band, respectively.

The emission spectra of isolated LH2 complexes at room temperature also show small variations as a function of the preparation method. The emission band positions deviate by less than 2 nm from each other, featuring the shortest wavelength value of 867.4 nm for the PVA film sample. The Stokes shift (i.e., the spectral interval between the peak of the B850 fluorescence-excitation band and the peak of the emission band) agrees within the experimental accuracy for all three LH2 samples and amounts to 120 ± 10 cm^{-1}. The width of the emission band of the PVA film sample (576 cm^{-1}) is slightly broader with respect to the bulk-buffer samples (560 cm^{-1} and 546 cm^{-1} for the samples with and without glycerol, respectively). Comparison of the emission spectral parameters of isolated LH2 complexes with the corresponding parameters from the emission spectrum of the native membranes is more complex due to the dominant emission of the LH1 antenna. Still, a deconvolution with the LH2 and LH1 subspectra (see Figure 1) allows facilitating this comparison. The deconvoluted LH2 emission component from the native membranes appears a few nanometers toward the red compared to the isolated LH2 emission spectra, which is in accordance with the respective B850 fluorescence-excitation band red shift (see Table 1). As a consequence, the Stokes shift for the native membrane samples (118 ± 16 cm^{-1}) practically coincides with the Stokes shift for the isolated LH2 samples in different environments.

The differences between the LH2 spectra as a function of the matrix material become more apparent upon cooling the samples to cryogenic temperatures. In Figure 2, the low temperature fluorescence-excitation and emission spectra of the LH2 complexes from *Rps. acidophila* dissolved in different matrixes and of native membranes are presented. The experiments were conducted at two different temperatures, 1.2 and 5 K, and yielded consistent results. Therefore, in the following, these data are commonly denoted as the low temperature data. Like at room temperature, the fluorescence-excitation spectra of LH2 complexes in the bulk-buffer sample with and without glycerol are rather similar to each other. They are also similar to the spectrum of the native membrane sample. In contrast to the band positions that vary only less than 1 nm with respect to each other, the fwhm of the bulk-buffer LH2 sample bands appear clearly narrower compared with the native membrane sample values (see Table 2). At the same time, the fluorescence-excitation spectrum of the PVA film sample differs from the spectra of the three other samples significantly. The positions (linewidths) of the B800 and the B850 bands in the spectrum of the PVA film sample are 796.9 nm (229 cm^{-1}) and 855.6 nm (276 cm^{-1}), respectively, equivalent to a blue shift of the B800 band by 94 cm^{-1} and the B850 band by 189 cm^{-1} with respect to the bands from the native membranes. The variations in the B850/B800 intensity ratio are also detectable depending on the sample environment. The peak intensity ratios are 1.3 for both bulk-buffer LH2 samples, 1.6 for the PVA film sample, and 1.5 for the native membrane sample.

The low temperature emission spectra of LH2 complexes in the different matrixes behave similarly to the fluorescence-excitation spectra. The emission band position of the bulk-buffer sample with glycerol is located at 889.8 nm, and for the bulk-buffer sample without glycerol, it lies at 889.0 nm. The bandwidths for both samples practically coincide at about 240 cm^{-1}. In contrast, the emission spectrum of the PVA film sample is significantly shifted to higher energies and peaks at 875.6 nm. This corresponds to the spectral blue shift by 182 cm^{-1} with respect to the bulk-buffer sample with glycerol. The fwhm of the PVA film sample emission band is 311 cm^{-1}, showing an ~30% broadening compared to the bulk-buffer sample values. Despite these shifts of the excitation and emission spectra of the samples in different matrixes, the Stokes shifts observed are similar and vary only from 261 cm^{-1} for the bulk-buffer–glycerol sample to 267 cm^{-1} for the PVA film sample. We note that the Stokes shifts in the low temperature

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Table 1. Spectral Positions, Linewidths, and Shifts (Mean Value ± Standard Deviation) of the B800 Band, B850 Band, and the Emission of Native Membranes and Isolated LH2 from *Rps. acidophila* Embedded in Various Matrix Materials and Studied at Room Temperature[a]

sample		native membranes	LH2:bulk-buffer solution	LH2:bulk-buffer solution with glycerol	LH2:PVA film
fluorescence-excitation B800	spectral position (nm)	803.2 ± 0.2	802.5 ± 0.1	801.9 ± 0.1	799.1 ± 0.7
	relative spectral shift (cm^{-1})	0	11 ± 5	20 ± 5	64 ± 14
	width (cm^{-1})	307 ± 6	283 ± 5	306 ± 5	329 ± 10
fluorescence-excitation B850	spectral position (nm)	863.2 ± 0.5 862.8 ± 0.5[b]	859.5 ± 0.6	859.7 ± 0.1	858.5 ± 0.6
	relative spectral shift (cm^{-1})	0	44 ± 15	42 ± 8	58 ± 15
	width (cm^{-1})	535 ± 13 413 ± 18[b]	380 ± 5	380 ± 5	386 ± 13
B800−B850 energy gap (cm^{-1})		860 ± 10	826 ± 10	838 ± 3	866 ± 19
emission	spectral position (nm)	898.5 ± 0.3 871.7 ± 0.7[b]	868.1 ± 0.1	869.1 ± 0.3	867.4 ± 0.4
	relative spectral shift (cm^{-1})	0	48 ± 18	34 ± 13	57 ± 15
	width (cm^{-1})	554 ± 16[b]	546 ± 7	560 ± 4	576 ± 9
Stokes shift (cm^{-1})		118 ± 16	115 ± 9	126 ± 5	120 ± 13

[a]The spectral shifts of the bands are given relative to the spectral position of the respective band in the native membrane. In the lowest line, the Stokes shift is given. [b]Evaluated spectral parameters for LH2 antenna.

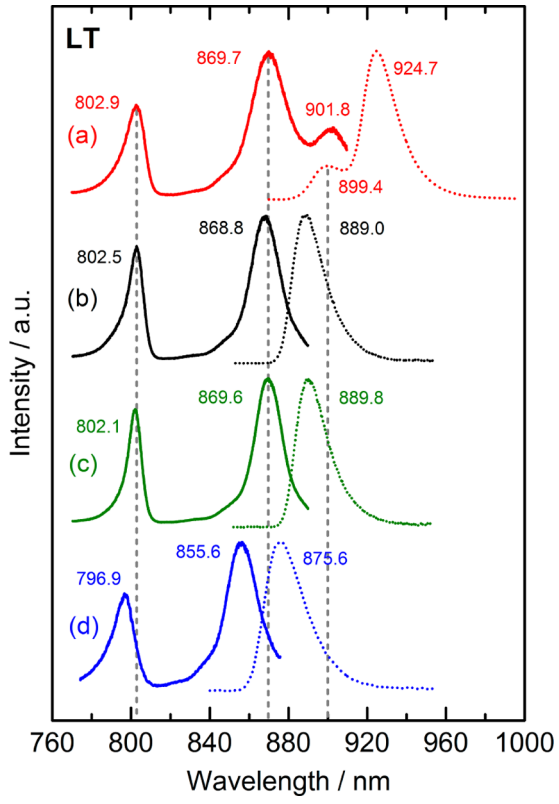

Figure 2. Comparison of the fluorescence-excitation (continuous line) and emission (dotted) spectra of the samples from *Rps. acidophila* taken at low temperature. (a) Fluorescence-excitation and emission spectra of native membranes in plain buffer solution. (b) Fluorescence-excitation and emission spectra of LH2 complexes in bulk-buffer solution. (c) Fluorescence-excitation and emission spectra of LH2 complexes in bulk-buffer solution with glycerol. (d) Fluorescence-excitation and emission spectra of LH2 complexes in PVA film. The spectra are normalized to the peak of the B850 band. The gray vertical lines denote the peak positions in the native membrane sample corresponding to the absorption in the B800 and B850 rings of LH2 (at 802.9 and 869.7 nm, respectively) and to the B850 fluorescence emission (899.4 nm).

spectra have increased by more than a factor of 2 with respect to the room temperature spectra.

At low temperatures, the comparison of the emission characteristics of isolated LH2 samples with those of the native membranes is not useful because, due to the energy transfer in the membranes, the fluorescence is mostly emitted from the lowest-energy LH1 antenna complexes, effectively shifting the emission band circa 30−35 nm to the longer wavelengths. The well-detected signal peaking at 899.4 nm in the emission spectrum of the native membranes (see Figure 2 as well as the inset of Figure 3b) is obviously related to the LH2 antenna pools that are energetically poorly contacted with the core antennas.[29,35−39]

The general findings of these measurements for the LH2 samples in different environments can be summarized as follows. At room temperature, the B850 band in the fluorescence-excitation spectrum from the bulk-buffer and PVA film samples feature about the same peak positions. Upon cooling to cryogenic temperatures, one observes a strong red shift of the B850 band for the bulk-buffer samples (for samples with glycerol, from 859.7 to 869.6 nm, corresponding to −132 cm^{-1}) and a slight blue shift of this band for PVA film sample (from 858.5 to 855.6 nm, corresponding to +39 cm^{-1}). Qualitatively similar behavior is found for the B800 band, yet the spectral red shift for the bulk-buffer samples is only a few wavenumbers, whereas the band for the PVA film sample blue shifts by 35 cm^{-1}, similar to the spectral shift of the B850 band for the same sample. Peaks of the B850 and B800 bands in bulk-buffer samples almost coincide with those in native membranes at room and low temperatures. The spectral positions of the emission peaks of the LH2 samples in different matrixes at room temperature have rather similar values. At low temperature, all the emission spectra feature an essential red shift; for the bulk-buffer samples, it amounts to −268 cm^{-1} (peaking at 889.8 nm), and for PVA samples, it is −108 cm^{-1} (peaking at 875.6 nm). Despite these different shifts of the emission spectra for the bulk-buffer and PVA samples, the Stokes shifts between their B850 excitation and emission spectra are similar, being 261 cm^{-1} for the bulk-buffer sample and 267 cm^{-1} for the PVA sample.

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Table 2. Spectral positions, linewidths, and shifts (mean value ± standard deviation) of the B800 band, B850 band and the emission of native membranes and isolated LH2 from *Rps. acidophila* embedded in various matrix materials and studied at low temperature.[a]

sample		native membranes	LH2:bulk-buffer solution	LH2:bulk-buffer solution with glycerol	LH2:PVA film
fluorescence-excitation B800	spectral position (nm)	802.9 ± 0.4	802.5 ± 0.3	802.1 ± 0.1	796.9 ± 0.2
	relative spectral shift (cm^{-1})	0	6 ± 11	12 ± 8	94 ± 9
	width (cm^{-1})	197 ± 10	156 ± 10	134 ± 6	229 ± 13
fluorescence-excitation B850	spectral position (nm)	869.7 ± 0.5	868.8 ± 0.6	869.6 ± 0.1	855.6 ± 0.4
	relative spectral shift (cm^{-1})	0	12 ± 15	1 ± 8	189 ± 12
	width (cm^{-1})	310 ± 15[b]	248 ± 4	226 ± 5	276 ± 9
B800–B850 energy gap (cm^{-1})		957 ± 13	936 ± 6	968 ± 3	861 ± 9
emission	spectral position (nm)	924.7 ± 0.4 899.4 ± 0.4[c]	889.0 ± 0.4	889.8 ± 0.3	875.6 ± 0.7
	relative spectral shift (cm^{-1})	0	130 ± 10	119 ± 9	302 ± 14
	width (cm^{-1})	250 ± 9 232 ± 18[c]	246 ± 8	242 ± 7	311 ± 11
Stokes shift (cm^{-1})		380 ± 12	262 ± 13	261 ± 5	267 ± 15

[a]The spectral shifts of the bands are given relative to the spectral position of the respective band in the native membrane. In the lowest line, the Stokes shift is given. [b]Additionally broadened due to contribution from LH1 absorption. [c]Evaluated spectral parameters for LH2 antenna.

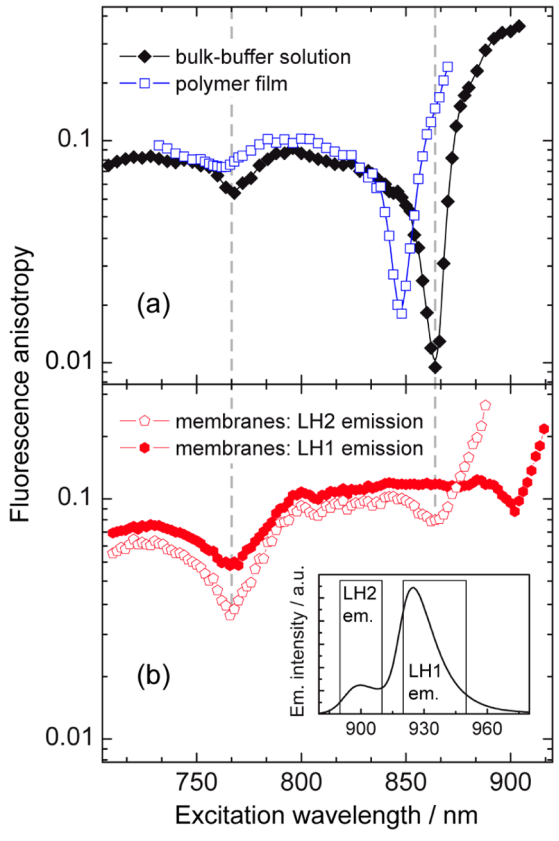

Figure 3. Fluorescence-anisotropy excitation spectra from *Rps. acidophila* recorded at 5 K. (a) Isolated LH2 complexes in bulk-buffer–glycerol solution (black filled diamonds) and isolated LH2 complexes in a PVA film (open blue squares). (b) Native membranes in bulk-buffer solution. Emission was recorded between 890 and 910 nm (the LH2 emission range, open red dots) or between 920 and 950 nm (the LH1 emission range, filled red dots), as shown in the inset. The gray vertical lines at 766.7 and 863.9 nm denote the minima found in the native membrane sample when recording in the LH2 emission range.

Fluorescence-Anisotropy Excitation Spectra. The main differences in the absorption band positions of the BChl *a* molecules that are organized in the B800 ring and those organized in the B850 ring stem from the strong (~300–400 cm^{-1}) resonant coupling between the transition-dipole moments of the closely packed B850 BChl *a* molecules.[5,18,40] For the B800 molecules, the pigment–pigment interactions are in the order of 20–30 cm^{-1}.[5,27,40–42] Disregarding these relatively weak interactions, the spectral position of the B800 band is mainly determined by the site energies of the pigments. In contrast, in the B850 assembly, the much stronger intermolecular interactions lead to the formation of exciton states.[6,11,43–47] In the simplest approximation, neglecting both the dimerization in the B850 ring and diagonal/off-diagonal disorder, the excited-state manifold for the C_{18}-symmetric assembly of the pigments features two nondegenerate (denoted as $k = 0$ and $k = 9$) and eight pairwise degenerate ($k = \pm 1$, $k = \pm 2$, ..., $k = \pm 8$) exciton states. Due to the circular arrangement of the pigments, only the exciton states $k = 0$, $k = \pm 1$, $k = \pm 8$, and $k = 9$ can be optically excited. Because the transition-dipole moments of the individual B850 pigments are oriented mainly in the plane of the ring, only a little of the total oscillator strength is associated with the nondegenerate exciton states $k = 0$ and $k = 9$. Given the head-to-tail arrangement of the transition–dipole moments of adjacent B850 BChl *a* molecules, nearly all of the oscillator strength is concentrated in the $k = \pm 1$ exciton states, which is reflected in a strong electronic transition seen as the main absorption band at about 850 nm. The upper exciton components, $k = \pm 8$, carry less than 3% of the total oscillator strength[5,48] and give rise to very weak absorptions in the spectral range around 750–780 nm,[18,49,50] depending on the bacterial species.

It is worth noting that in the systems with strong excitonic coupling, an additional contribution that determines the exact spectral position of an absorption band is given by the displacement energy. This accounts for the difference of the Coulomb interaction between neighboring pigments in the electronically excited state and in the ground state.[51] An ab initio estimation of the displacement energy for multi-chromophore systems is still a demanding theoretical task. From these considerations, it is clear that changes of the spectral position of the B800 band reflect, in the first approximation, only interactions that affect the chromophore

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site energies, whereas the origin for changes of the spectral position of the B850 band is more complex and reflects contributions from interactions that affect both the site energies and their excitonic interactions.

In order to separate the various contributions to the spectral changes in the B850 band, it would be very helpful to have information about the B850 exciton bandwidth as a function of the sample. Generally, this could be obtained from the spectral signatures of the $k = \pm 1$ and $k = \pm 8$ exciton states, which can be considered as markers for the width of the exciton band. However, as pointed out above, the total oscillator strength of the $k = \pm 8$ states is low and their weak absorption is buried under the much stronger absorption tail of the B800 molecules. As shown in ref 49, this obstruction can be overcome by exploiting polarization-resolved fluorescence-excitation spectroscopy. The fluorescence-anisotropy, r, is defined as $r = (I_{VV} - I_{Vh})/(I_{VV} + 2I_{Vh})$, where I_{vv} and I_{vh} are the emission intensities that are polarized parallel (vv) and perpendicular (vh) with respect to the orientation of the electric field vector of the linearly polarized excitation light, respectively. Because the transition-dipole moments within the degenerate pairs $k = \pm 1$ and $k = \pm 8$ are mutually orthogonal, there exists an energy for each pair of states where the exciton states with mutually perpendicular oriented transition-dipole moments can be excited with equal probability. Hence, the fluorescence-anisotropy will feature a minimum at those energies, effectively designating the spectral position of the respective pair of exciton states.[49,52]

The fluorescence-anisotropy excitation spectra of the LH2 complexes from *Rps. acidophila* in PVA film and bulk-buffer environments with glycerol are compared in Figure 3a. The spectrum of the bulk-buffer sample without glycerol practically overlaps with the latter spectrum, and for clarity, it is not presented in the figure. For both PVA and bulk-buffer samples, the anisotropy is low, between 0.06 and 0.10, at short wavelengths, rising toward the theoretical limiting value of 0.4 across the B850 band. The most striking features of the anisotropy curves are the two distinct minima at low and high energies that are assigned to the edges of the B850 exciton state manifold.[18,49,50] The separations of the anisotropy minima are 1450 cm^{-1} (1461 cm^{-1}) for the bulk-buffer sample with (and without) glycerol and 1332 cm^{-1} for the PVA film sample (see Table 3). The data for the bulk-buffer samples coincide within the experimental uncertainty with the respective values determined.[18,50] The exciton bandwidth in the PVA film sample is thus significantly narrowed (by 120−130 cm^{-1}) compared with the width in bulk-buffer samples. Moreover, the whole fluorescence-anisotropy excitation spectrum of the PVA film is shifted to the blue with respect to the bulk-buffer spectra. The shift is asymmetric, being circa 16 nm (~220 cm^{-1}) at low energies and circa 6 nm (~98 cm^{-1}) at high energies.

Table 3. Bandwidths (Mean Value ± Standard Deviation) in Wavenumbers of the B850 Excitons in the LH2 Complexes of *Rps. acidophila* Embedded into Various Matrix Materials and Studied at 5 K.[a]

sample	native membranes	LH2:bulk-buffer solution	LH2:bulk-buffer-glycerol solution	LH2:PVA film
bandwidth (cm^{-1})	1468 ± 18	1461 ± 24	1450 ± 24	1332 ± 24

[a]Evaluated as the energy gap between the two minima in the fluorescence-anisotropy excitation spectrum (see ref 55).

In the one-dimensional molecular arrays, the width of the exciton band, δE, can be approximated by the nearest-neighbor interaction energy, V, as $\delta E \approx 4V$.[51] Hence, taking the separation of the minima in the anisotropy spectra as a measure of δE, average intermolecular coupling energies between the B850 BChl *a* molecules in the bulk-buffer sample and in the PVA film can be deduced as 365 cm^{-1} and 333 cm^{-1}, respectively. A more thorough modeling based on crystallographic structural data and taking into account both static and dynamic disorders in the bulk-buffer sample with added glycerol has resulted in values of V between 370 cm^{-1} and 390 cm^{-1}.[18,50]

Figure 3b displays the fluorescence-anisotropy excitation spectra for the native membrane samples. In order to distinguish the contributions from LH2 and LH1, the detection was restricted either to the spectral range from 890 to 910 nm or to the range from 920 to 950 nm. As indicated in the inset of Figure 3b, these ranges correspond to the spectral regions of predominant emission from the LH2 or LH1 complexes. The separation of the anisotropy minima recorded in the LH2 emission range is 1468 cm^{-1}, which is consistent with the values measured for LH2 in bulk-buffer samples (see Table 3). In the LH1 emission range the separation of the anisotropy minima is much larger and amounts to 1951 cm^{-1}, which is in close agreement with the value of 1969 cm^{-1} obtained in ref 39. The exciton bandwidths of core complexes from different photosynthetic bacterial species appear to be similar. For example, the bandwidth 1978 cm^{-1} has been found for the RC-LH1 complexes in bulk-buffer−glycerol solution of mutant membranes of *Rhodobacter (Rb.) sphaeroides*[53] that only contain core complexes. Also, in both *Rps. acidophila* and *Rb. sphaeroides* species, the high energy minima for the B850 and B875 exciton state manifolds overlap almost perfectly, meaning that the broader B875 exciton band totally embraces the narrower B850 one. Presumably, the good resonance between the electronically excited states is advantageous for efficient B850→B875 energy transfer.[39]

Stokes Shift between the Fluorescence-Excitation and Emission Spectra. Above, it was shown that at low temperature, the exciton bandwidth of the LH2 complexes in PVA film is circa 9% narrower than in the bulk-buffer environment. It is reasonable to assume that energetic distances between different exciton levels are as well compressed in PVA sample compared to the bulk-buffer sample. The energy gap between the $k = 0$ and $k = \pm 1$ exciton states, which largely determines the Stokes shift of the spectra, is estimated to be 203 cm^{-1} in the bulk-buffer sample of isolated LH2 complexes.[23] Accordingly, in the PVA environment this value should be ~185 cm^{-1}. At the same time, the measured Stokes shift for the PVA sample (267 cm^{-1}) is a few cm^{-1} larger than that for the bulk-buffer sample (262 cm^{-1}). From the first glance, these data are inconsistent with each other, and one option to explain this discrepancy might be that the electron−phonon coupling strength in the PVA matrix is larger with respect to the bulk-buffer samples. In refs 29 and 30, the homogeneous lineshapes and the exciton−phonon coupling strengths as defined by the Huang−Rhys factors were determined for the isolated LH2 complexes from *Rb. sphaeroides* in bulk-buffer environment. It was shown that not only the homogeneous line shape and the exciton−phonon coupling strength but also the emission oscillator strength

depend on the spectral position.[54] Particularly, one of the important parameters of the homogeneous spectrum, the fwhm of the phonon sideband, changes from 160 cm^{-1} at the center of the inhomogeneous distribution function (IDF) to the 260 cm^{-1} at the red tail of the IDF. Hence, the Huang−Rhys factor increases from 2.0 to 3.1. For the LH2 complexes of *Rps. acidophila* in the PVA environment, comparable data are available from single molecule experiments.[28] In the single molecule spectra, the fwhm values range from 160 cm^{-1} to 280 cm^{-1} in the same IDF region. These numbers are close to the phonon sideband fwhm data from *Rb. sphaeroides* in bulk-buffer environment, practically excluding the option of a significantly larger electron−phonon coupling in the PVA samples.

In looking for another possibility to explain the above disagreement, it is instructive to note from Table 2 that at low temperatures, the B800 and B850 bands in the fluorescence-excitation spectra are much broader in the PVA matrix with respect to the spectra in bulk-buffer samples; the emission spectrum follows a similar trend. In Gaussian approximation for the band shapes, one can estimate an extra inhomogeneous broadening of the B800 absorption band in PVA relative to that in bulk-buffer solution as: $(229^2 - 156^2)^{1/2}$ $cm^{-1} \approx 168$ cm^{-1}. This is a considerable broadening, which, along with the rising oscillator strength toward the red edge of the IDF, readily explains the apparent increase of the Stokes shift between the fluorescence-excitation and emission spectra.

Comparison of the B850 Excitons in the LH2 Complexes Embedded into Native Membrane or into Spin-Coated Polymer Film at Low Temperatures. As we have shown, the fluorescence-excitation spectra of LH2 complexes in bulk-buffer solution and in native membranes are conserved within the experimental uncertainty. In contrast, the spectrum of the PVA film manifest significant blue shift, its B800 component shifting by 94 cm^{-1} and the B850 component by 189 cm^{-1}. The shifts are accompanied by narrowing of the B850 exciton state manifold as shown in Table 3. We have applied a simple model to evaluate the energetic parameters that are responsible for these spectral modifications.

As implied already above, the exciton Hamiltonian written down in matrix form includes diagonal and nondiagonal elements. The diagonal terms of the matrix correspond to the energies of uncoupled molecules, termed above as site energies. The site energies contains not only the electronic transition energy of the free molecule in vacuum but also the solvent shift caused by the interactions of the molecule with its local surroundings, which has a random component due to the static or dynamic fluctuations of the environment.[45] Ignoring for the moment differences in the exposure to the solvent and the different ligation of the B800 and B850 pigments, as well as the weak excitonic couplings between the B800 molecules, we use the peak position of the B800 fluorescence-excitation band in a specific solvent as the mean site energy for all the 27 pigment molecules in the LH2 protein dissolved in that solvent. Similarly, the width of the B800 band is a measure for the random fluctuations thereof. Under these approximations, the 94 cm^{-1} relative shift found for the B800 absorption band between the native membranes and the PVA film samples is assigned to the relative shift between the site energies of the pigments in the B850 assembly. This implies that about half the overall 189 cm^{-1} relative shift between the B850 fluorescence-excitation bands is attributed to interactions that affect site energies of all the pigments in LH2. Another component that contributes to the relative shift is the exciton bandwidth, which is narrower in the PVA film. Once again, roughly half of the experimentally determined difference between the exciton bandwidths in native membranes and PVA matrix (i.e., $1/2 \times (1468 - 1332)$ $cm^{-1} = 68$ cm^{-1}) contributes into the relative shift of the B850 bands. The fraction is left over from the overall relative shift amounts $(189 - 94 - 68)$ $cm^{-1} \approx 27$ cm^{-1} and can be associated with the change in the displacement energy for the B850 molecules.

On the basis of the simplified model given above, it is possible to give an estimate for the absolute values of the displacement energies. The energy gap between the spectral positions of the B800 and B850 absorption bands can be regarded as the sum of two components: the half value of the B850 exciton bandwidth and the displacement energy value of the B850 excitons. An evaluation of the absolute values of the displacement energies results in ∼223 cm^{-1} and ∼195 cm^{-1} for the native membranes and the PVA samples, respectively. These numbers are fairly small, less than the exciton coupling energies.

A blue shift of the B850 excitonic absorption band that follows a transfer of the LH2 complex from a native membrane into a spin-coated polymer film at low temperatures can thus be disentangled into three parts: 50% due to an increase of the local site energies, 36% due to a contraction of the exciton band, and 14% due to a decrease of the displacement energy. The provided interpretation, although rather speculative in terms of specific numbers due to multiple assumptions involved, for the first time allows a hint toward the significance of various energetic components (and related physical mechanisms), including the displacement energy, in spectral tuning of the photosynthetic LH complexes with strong exciton coupling between the chromophores.

The systematic decrease observed for the nearest neighbor exciton coupling energy, $V \approx \delta E/4$, in the B850 system from ∼367 cm^{-1} in the native membrane over ∼363 cm^{-1} in the bulk-buffer−glycerol solution to ∼333 cm^{-1} in the polymer film as calculated from the data in Table 3 (see also ref 18), points toward a looser packing (and thus weaker coupling) of the B850 BChl *a* molecules in the detergent-isolated LH2 complexes in comparison with the situation where the proteins are spontaneously assembled into the native membrane. It also appears that the specific bulk solvent where the detergent-isolated LH2 complexes are hosted plays some role in stabilizing the light-harvesting proteins, despite the detergent micelle tightly covering the hydrophobic parts of the membrane protein. From all the studied environments the LH2 spectra are most changed (in terms of the extent of the blue shift and broadening of the bands) in the PVA matrix.

An extra red shift of the exciton absorption bands (such as the B850 band in LH2 and the B875 band in LH1 complexes) relative to the absorption bands of localized excitations (B800/monomeric BChl *a*) upon application of hydrostatic external pressure due to increased exciton coupling has been observed.[55−60] Pressure-induced red shifts of the B850 band from *Rps. acidophila* (strain 10050) of about −760 cm^{-1}/GPa have been recorded at room temperature up to the pressures of 0.6 GPa.[58,59] The corresponding shift rate at low temperature is approximately half the size: −398 cm^{-1}/GPa.[56] If we associate the blue shift observed in the PVA matrix with a decrease of the local pressure within the protein, a blue shift of 189 cm^{-1} observed at low temperature corresponds to a pressure change of almost 0.5 GPa. From the compressibility data as a function of temperature,[57,61−64] it can be estimated that pressures of 0.5

GPa may result in the B850 interpigment distance changes in the order of 0.1 Å. Changes on this length scale are small with respect to the overall structure of the assembly of the B850 molecules yet large enough to influence the molecular interactions to show up in the optical spectra. A negative thermal expansion observed in polymer films upon cooling below the glass transition temperature as reported in ref 65 supports the assumption made above, that the LH2 complexes embedded in the PVA film might sense a higher conformational freedom, which in turn points toward a looser packing of the B850 pigments. Furthermore, this might also explain the broadening of the spectral lineshapes of the LH2 complexes embedded in the PVA film compared to those observed in the bulk-buffer environment.

4. SUMMARY

This work was set out to unify a body of conflicting data obtained by single-molecule spectroscopy and by more traditional spectroscopic methods. The data presented here show how (see Table 2) and why the details of the optical spectra of LH2 complexes (i.e., the exact spectral positions and widths of the absorption and emission bands) vary as a function of the environment in which the sample is housed. As an example, in the LH2 complexes from *Rps. acidophila* (strain 10050), changing the matrix from bulk-buffer solution to thin films of PVA leads to a 189 cm^{-1} blue shift of the B850 exciton absorption band at low temperature. Taking into account a simultaneous increase of the B800 site energies by 94 cm^{-1}, the blue shift of the B850 band could be disentangled into three parts: because of the increase of the B850 local site energies, the contraction of the B850 exciton band, and the decrease of the respective displacement energy. The differences of the LH2 exciton due to different sample preparations discovered in this study might help to ease the comparison of results stemming from the single-molecule research area with respect to those coming from the ensemble domain.

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Notes

The authors declare no competing financial interest.

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ABBREVATIONS

BChl: bacteriochlorophyll; LH1: light-harvesting 1 (complex); LH2: light-harvesting 2 (complex); IDF: inhomogeneous distribution function; fwhm: full width at half-maximum.

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