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ARTICLE *in* BIOCHEMISTRY · SEPTEMBER 2003

Impact Factor: 3.02 · DOI: 10.1021/bi034969m · Source: PubMed

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Circular Symmetry of the Light-Harvesting 1 Complex from *Rhodospirillum rubrum* Is Not Perturbed by Interaction with the Reaction Center†

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Received June 5, 2003; Revised Manuscript Received July 30, 2003

ABSTRACT: The effect of the interaction of the reaction center (RC) upon the geometrical arrangement of the bacteriochlorophyll *a* (BChla) pigments in the light-harvesting 1 complex (LH1) from *Rhodospirillum rubrum* has been examined using single molecule spectroscopy. Fluorescence excitation spectra at 1.8 K obtained from single detergent-solubilized as well as single membrane-reconstituted LH1-RC complexes showed predominantly (>70%) a single broad absorption maximum at 880−900 nm corresponding to the Q_y transition of the LH1 complex. This absorption band was independent of the polarization direction of the excitation light. The remaining complexes showed two mutually orthogonal absorption bands in the same wavelength region with moderate splittings in the range of $\Delta E = 30-85$ cm^{-1}. Our observations are in agreement with simulated spectra of an array of 32 strongly coupled BChla dipoles arranged in perfect circular symmetry possessing only a diagonal disorder of ≤ 150 cm^{-1}. However, in contrast to LH1 complexes alone, excitation spectra that consist of a single absorption band were observed more frequently in the presence of the reaction center. Our results show that the interaction of the RC with the LH1 complex stabilizes the circular symmetric arrangement of the bacteriochlorophyll pigments and are in contradiction to recent studies by other groups using single molecule spectroscopy as well as cryoelectronmicroscopy and atomic force microscopy indicating that the RC induces an elliptical distortion of the LH1 complex. Possible reasons for this discrepancy are discussed.

The high efficiency of light-energy capture in photosynthetic purple bacteria is mediated by the membrane-bound light-harvesting (LH)[1] complexes, designated LH1 and LH2. Both types of complexes are composed of two small (approximately 50 amino acids) nonidentical polypeptides, α and *β*, which show significant homologies between species and bind the pigments bacterichlorophyll *a* (BChla) or BChlb and carotenoids (*1*). The absorption spectrum of the complexes is suited to their functional role *in vivo*. Thus, LH2 complexes show two strong absorption maxima in the near-IR region at 850 and 800 nm, respectively, and the LH1 complexes generally show a single absorption near-IR absorption maxima at 875−882 nm. As shown by spectroscopic studies (*2*, *3*) light energy can be passed preferentially from the LH2 to LH1 by radiationless energy transfer. The latter are then able to transfer energy to the reaction center (RC), which catalyzes the primary process. In recent years, the structure of the LH2 complexes for *Rhodopseudomonas acidophila* (*4*) and *Rhodospirillum molischianum* (*5*) have been elucidated at atomic resolution by X-ray crystallography. Both complexes are ring-like assemblies of the basic building block, the α*β* heterodimer, arranged in a ring containing nine and eight α*β* heterodimers for *Rps. acidophila* and *R. molischianum*, respectively. Each α and *β* polypeptide binds one BChla at a conserved histidine within the membrane bilayer, and the interaction between these pigments, together with the hydrogen bonds between the BChl 2-acetylcarbonyls and adjacent aromatic residues of the apoproteins (*6*) leads to the characteristic absorption maximum at 850 nm. A third BChla coordinated to the carboxyl group of an amino acid ligand present in the crystal structure and located close to the membrane surface exhibits the absorption maximum at 800 nm (*6*).

The structure of the LH1 complex is not known at high resolution at the present time. However, medium-resolution projections at 0.85 nm have been obtained for 2-D crystals of the LH1 complex from *Rhodospirillum rubrum* in the absence (*7*) and presence of the reaction center (*8*), both of which clearly show a ring-like symmetry of 32 transmembrane α helices, assigned to α and *β* polypeptides, by analogy to the structural organization of the LH2 complexes. A membrane-bound conserved histidine on both polypeptide chains (homologous to those binding the B850-BChls of the LH2 complexes (*1*)) has been shown by both Raman spectroscopy (*9*) and site-directed mutagenesis (*10*) to bind BChla. Biochemical analysis has shown that each α*β* dimer of the LH1 complex binds two molecules of BChla and one

† Financial support was provided by the Volkswagen Foundation within the Priority Area "Physics, Chemistry and Biology with Single Molecules" and the Landesgraduiertenförderung Baden-Württemberg.

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[1] Abbreviations: BChla, bacteriochlorophyll *a*; SM, single molecule; cryoEM, cryoelectronmicroscopy; LH, light-harvesting; RC, reaction center; PSU, photosynthetic unit; LDAO, lauryldimethylamine-*N*-oxide; DHPC, 1,2-diheptanoyl-*sn*-phosphatidylcholine; DOPC, 1,2-dioleoyl-*rac*-phosphatidylcholine; CEO, collective electronic oscillator; ZPL, zero phonon line; PSB, phonon sideband; AFM, atomic force microscopy.

10.1021/bi034969m CCC: $25.00
Published on Web 08/14/2003

molecule of carotenoid (spirilloxanthin in the wild-type *R. rubrum* S1) (*11*, *12*). In addition, detailed molecular modeling of the LH1 complexes of *Rhodobacter sphaeroides* (*13*) and *R. rubrum* (*14*) using only the experimental pigment/$\alpha\beta$ polypeptide stoichiometry and the assumption that $\alpha\beta$-$(BChla)_2$ units are arranged in a ring with 16-fold symmetry showed rapid convergence to unique structures after energy minimization.

The observation of circular symmetry for both LH1 and LH2 complexes has been recently subjected to critical analysis by means of single molecule (SM) spectroscopy as well as by structural analysis using both cryoelectronmicroscopy (cryoEM) and atomic force microscopy (AFM). Single molecules of isolated LH2 complexes solubilized in detergent and immobilized in poly(vinyl alcohol) (PVA) spin-coated films have indicated that they are not perfectly symmetrical but elliptically disordered (*15*, *16*). These results are of significance as they imply that the bulk near-IR spectra, for which a large body of spectral data exists (see ref *17*), are the sum of a large number of spectra arising from single LH complexes, each of which possesses shifted absorption maxima, which can be shifted by up to 116 cm^{-1} from the bulk absorption maxima. The shift of the near-IR absorption maxima is suggested to be due to static disorder of the pigments. These data also imply that the position of the pigments as obtained by X-ray crystallography (*4*, *5*) or modeling (*13*, *14*) is also the average of a number of statically disordered structures.

Recently, we have reexamined this question using SM spectroscopy of isolated LH1 complexes of the purple non-sulfur bacterium *R. rubrum* (*18*). Although our results also showed that detergent-solubilized LH1 complexes do indeed show a distribution of near-IR maxima about the bulk absorption maximum at 880 nm (at 1.8 K), the width of the distribution was significantly less than that observed for detergent-solubilized LH2 complexes (*18*). After reconstitution of the LH1 complexes into bilayer membranes, the fluorescence emission spectra show no preferential polarization, thus indicating unambiguously that the membrane environment maintains the LH1 complex in a circularly symmetric state, corresponding to the projection structure deduced from 2-D crystals by cryoelectron microscopy (*7*). Very recently, Ketelaars et al. (*19*) have reported a SM study of detergent-solubilized LH1-RC complexes isolated from *Rps. acidophila* in which they show that a number of states with noncircular symmetry are present. In particular, they ordered the low temperature LH1 spectra into four classes (types I−IV), where type I (30% of the total) and type II (17% of the total) were assigned to a circular symmetric and elliptically distorted exciton, respectively, type III (17% of the total) was assigned to an open partial ring, and type IV (36% of the total) was assigned to a dimeric LH1-RC aggregate. In this previous study, the detergent lauryldimethylamine-*N*-oxide (LDAO) was employed as a solubilizing agent. They have argued that the interaction of the RC with the LH1 complex may stabilize a distortion of the LH1 ring, thus facilitating energy transfer to the RC in vivo. The results of SM spectroscopy in ref *19* are strikingly consistent with very recent structural studies of LH1-RC complexes using either cryoEM of 2-D crystals (*8*) or AFM of natural membranes (*20*). In both of these studies (discussed in detail later), the LH1 rings showed an elliptical distortion with the major and minor axes differing by about 11 and 5−10%, respectively. In the AFM study, it could also be shown that the removal of the RC in situ caused the LH1 ring to revert to circular symmetry.

The present study, employing LH1-RC complexes purified with the detergent 1,2-diheptanoyl-*sn*-phosphatidylcholine (DHPC) as detergent, which below a critical concentration does not promote further dissociation of the LH1-RC complex to small subunits (*21*), contradicts these latter data. We show below that, in contrast to the conclusions drawn from the SM and structural studies mentioned previously, even when solubilized in detergent (DHPC), the interaction of the RC with the LH1 stabilizes the circular symmetry of the latter complex, and when the LH1-RC complex is incorporated into lipid bilayers, these perfect circularly symmetric complexes are present almost exclusively. A rationale for this apparent contradiction will be presented.

MATERIALS AND METHODS

Preparation of Phospholipids. 1,2-Dioleoyl-*rac*-phosphatidylcholine (DOPC) was obtained from Avanti Polar Lipids (Alabaster, AL) or BACHEM (Switzerland). A total of 100 mg of DOPC dissolved in $CHCl_3$ was dried under N_2 as a thin film onto the surface of a round-bottomed flask. The film was then dried for 12 h under high vacuum ($\leq 10^{-3}$ Torr) to remove all traces of solvent and then suspended in 2 mL of H_2O by gentle shaking with glass beads. The suspension was then sonicated to translucency using a microtip probe (Branson), aliquots were taken from this homogeneous preparation for the phosphate determination, and the rest was aliquoted into smaller volumes in Eppendorf tubes and frozen using liquid nitrogen and then stored at −80 °C. The phosphate determination, used for the calculation of the molar ratio below, was performed by the method of Ames and Dubin (*22*). The purity of the lipids was also routinely checked by thin-layer chromatography using silica gel plates and $CHCl_3$/MeOH/H_2O (80:30:4 (v/v/v)) as a running solvent.

Isolation of the LH1-RC Complexes. LH1-RC complexes were purified from isolated photosynthetic membranes of *R. rubrum* S1 (wild type) with the exclusive use of the detergent DHPC (Avanti Polar Lipids, Alabaster, AL) as a solubilizing agent as described previously (*21*). Reconstitution of the LH1-RC complexes into membranes comprised of DOPC at an LH1-$\alpha\beta$/DOPC molar ratio of 1:4000 were also performed as described (*21*), assuming that the LH1 and RC are present in a single complex at a fixed molar ratio (1 LH1 ring/1 RC).

Functional State of the RC in the LH1-RC Complexes. The activity of the RC was confirmed by following the bleaching of the RC P870 band due to the special pair, either by chemical oxidation with $K_3Fe(CN)_6$ or by photobleaching with actinic light as described in ref *23*.

Sample Preparation for SM Spectroscopy. The detergent-solubilized complexes as well as the vesicles containing the LH1-RC complexes were immobilized (by spontaneous ionic interaction) onto a freshly cleaved mica (Goodfellow, Berwyn, PA) surface. After preparation, the samples were immediately transferred into the cryostat and cooled to 77 K within milliseconds and subsequently to 1.8 K.

Fluorescence Spectroscopy. All measurements were performed at 1.8 K with a home-built beam-scanning confocal

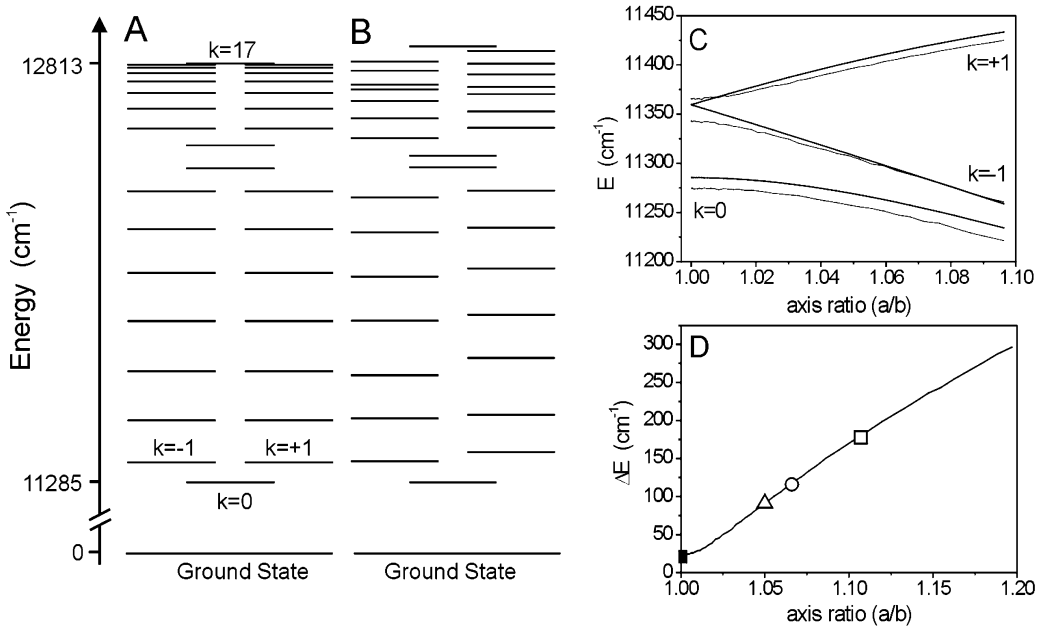

FIGURE 1: (A) Energy level diagram of the B880 ring simulated without any kind of disorder and (B) simulated with a diagonal disorder of $\sigma = 175$ cm^{-1}. (C) Effect of elliptical deformation, expressed as the axis ratio of the ellipse *a*/*b*, upon the three lowest energy states simulated without diagonal disorder (black lines) and with an additional diagonal disorder of $\sigma = 150$ cm^{-1} (gray lines, the energies are averaged over an ensemble of 1000 complexes). (D) The splitting (ΔE) of the $|k = \pm 1\rangle$ states taken from panel C ($\sigma = 150$ cm^{-1}) and plotted against the axis ratio *a*/*b* (the axis ratio *a*/*b* is the inverse of the ratio used in eq 3). The symbols show data taken from the SM study (*19*) (○, $\Delta E = 116$ cm^{-1}), from the cryoEM study (*8*) (□, $a/b = 1.12$), and from the AFM study (*20*) (△, $a/b = 1.05$). ■ depicts the mean ΔE value (17 cm^{-1}) for the membrane-reconstituted complexes measured here.

microscope. Fluorescence excitation spectra from single LH1-RC complexes were obtained by positioning the focus of the microscope objective (Melles Griot) over an LH1-RC complex and subsequently stepwise scanning the linearly polarized, continuous wave excitation laser (Ti:Sa, Spectra Physics 3900-S) over the region of the $|k = \pm 1\rangle$ absorption bands. The fluorescence was detected with an acquisition time of 150 ms at each step with an avalanche photodiode (EG&G, SPCM) at wavelengths >905 nm. To determine a possible splitting of the excitonic states $|k = \pm 1\rangle$, a series of spectra in the range of 835−905 nm was recorded for each single complex, where the polarization direction of the excitation laser was turned by 30° between consecutive scans with a $\lambda/2$-waveplate. To improve the signal-to-noise ratio, each scan was done twice and averaged afterward. For measuring fluorescence emission spectra, the complexes were excited with the laser at 865 nm in their B880 absorption band. The fluorescence was recorded using a 30 cm spectrograph (Acton Research SP-257) with a highly sensitive, liquid nitrogen-cooled back-illuminated CCD camera (Princeton Instruments) as a detector.

Theory: *Exciton Model of the B880 Ring*. The exciton model of the B880 ring used here has been described in detail previously (*18*, *24*) and was used without modification. However, we provide a shortened summary of the analysis used here. The energy level diagram of the B880 ring consisting of 32 interacting BChla molecules is calculated using the effective Hamiltonian for Frenkel excitons:

$$H = \sum_{n=1}^{32} E_0 |n\rangle\langle n| + \sum_{\substack{m,n=1 \\ m \neq n}}^{32} W_{mn} |m\rangle\langle n| \qquad (1)$$

where $|n\rangle$ is the product state of 32 two-level molecules where only one is in the excited state with energy E_0, and W_{mn} is the coupling strength among the states $|m\rangle$ and $\langle n|$. As the distances between all nonneighboring BChla molecules are large enough to use the dipole−dipole approximation, the coupling strengths are given by

$$W_{mn} = C_3 \left(\frac{\vec{\mathbf{d}}_m \cdot \vec{\mathbf{d}}_n}{r_{mn}^{\,3}} - \frac{3(\vec{\mathbf{r}}_{mn} \cdot \vec{\mathbf{d}}_m)(\vec{\mathbf{r}}_{mn} \cdot \vec{\mathbf{d}}_n)}{r_{mn}^{\,5}} \right) \qquad (2)$$

where $\vec{\mathbf{d}}_n$ are the unit vectors of the BChla dipole moments, and $\vec{\mathbf{r}}_{mn}$ are the vectors connecting the Mg atoms of the BChla molecules. The unit vector $\vec{\mathbf{d}}_n$ corresponds to the direction from the nitrogen atoms N_{21} to N_{23} (IUPAC nomenclature (*25*)) of the BChla tetrapyrrole ring, corresponding to the BChla Q_y transition dipole. The atomic positions were taken from the structural model for the LH1 complex of *R. rubrum* by Autenrieth et al. (*14*). The coupling strengths of the next but one pigments W_{13} were set to $W_{13} = -102$ cm^{-1} leading to $C_3 = 415\,500$ Å^3 cm^{-1} . This value for W_{13} was obtained previously (*18*) for the BChla molecules in the LH2 complex of *R. molischianum* using the collective electronic oscillator (CEO) approach (*26*). For the coupling strengths among the intra- and interdimeric pigments used here, the CEO calculations led to values of $\nu_1 = 363$ cm^{-1} and $\nu_2 = 320$ cm^{-1}, respectively. Because of the close sequence and structural homology of the apoproteins of LH2 and LH1, it is reasonable to suppose that the coupling strengths are comparable. We note, however, that these values are somewhat higher than those used in ref *27* for subunit oligomerization in detergent (lithium dodecyl sulfate)-solubilized complexes. The energy level diagram calculated from the above model is shown in Figure 1A. All states except the lowest and the highest of both bands are 2-fold degenerate due to the circular arrangement of the pigments.

For the case of perfect symmetry, the states $|k = \pm 1\rangle$ carry almost all of the oscillator strength with orthogonal dipole moments, whereas the lowest state $|k = 0\rangle$ carries no oscillator strength. Any disorder within the ring leads to a shift of the oscillator strength from $|k = \pm 1\rangle$ to the other states, corresponding to a shift of the energy levels and thereby to a lifting of the degeneracies (Figure 1B). Thus, the introduction of ring disorder leads to a splitting (ΔE) of the fluorescence excitation maxima in the near-IR region. The splitting ΔE is also enhanced by a reduction of symmetry of the ring of BChla pigments, which in its simplest form could correspond to an elliptical deformation of the ring, thereby enhancing C_2 symmetry of the pigments (*15*). An elliptical deformation is usually characterized by the eccentricity ϵ

$$\epsilon = \sqrt{1 - \left(\frac{b}{a}\right)^2} \tag{3}$$

where a and b are the major and minor axes of the ellipse, respectively. The effect of diagonal disorder and ellipticity upon the energy states are shown for the three lowest energy levels ($|k = 0\rangle$ and $|k = \pm 1\rangle$, respectively) in Figure 1C.

RESULTS

General Considerations. For all SM experiments, the LH1-RC complexes were reconstituted into DOPC, which has a phase transition temperature (T_m) at −20 °C. The low T_m ensures that the bilayer membranes are maintained in the liquid-crystalline phase for all biochemical procedures, particularly reconstitution and the subsequent isolation of membrane complexes by means of sucrose gradient centrifugation, performed at 4 °C. In our studies, we make the assumption that rapid freezing in liquid nitrogen prior to measurement, which occurs within milliseconds, ensures the transition to the solid state without transition through the gel ($L_{\beta'}$) phase. Instead, we believe that the membrane is frozen in a disordered state corresponding to a snapshot of the liquid-crystalline phase.

Fluorescence Emission Spectra of Single LH1-RC Complexes. Figure 2 shows four representative fluorescence emission spectra of single LH1-RC complexes membrane-reconstituted (Figure 2A,B; 26 LH1-RC complexes measured) and detergent-solubilized (Figure 2C,D; 32 LH1-RC complexes measured). In fact, for both types of samples, all spectra measured showed the same characteristics. The spectra in Figure 2A,B,D show a sharp spectral line on the short wavelength edge (zero phonon line (ZPL)) followed by a broad phonon sideband (PSB). In the other spectra (e.g., Figure 2C), the ZPL could not be clearly resolved because it is merged with the PSB. However, in general, we found more spectra with a resolved ZPL for membrane-reconstituted LH1-RC complexes than for the detergent-solubilized complexes. The spectral features of the emission spectra from the LH1-RC complexes described above are identical with the previously published emission spectra from the LH1 complex of *R. rubrum* lacking the RC in detergent or reconstituted in membrane bilayers (*18*). Despite the fact that we performed excitation at 865 nm, which corresponds to the absorption maximum of the special pair, no spectral features of the emission spectra are visible that differ from those obtained for LH1 complexes alone. In principle,

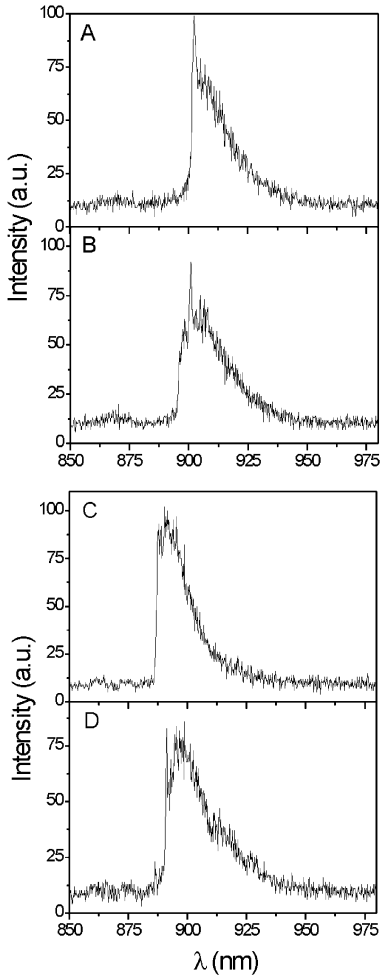

FIGURE 2: Typical fluorescence emission spectra of membrane-reconstituted (A and B) and detergent-solubilized (C and D) single LH1-RC complexes (λ_{exc} = 865 nm, acquisition time 5 s).

fluorescence might be observable for the relaxation processes following excitation of the special pair. The experimental lack of fluorescence because of the RC is probably due to charge separation in the RC resulting in the trapping of the electron by the long-lived Q_A^- state at low temperatures (*28*).

Fluorescence Excitation Spectra of Single LH1-RC Complexes. Typical fluorescence excitation spectra of the $|k = \pm 1\rangle$ states for membrane-reconstituted as well as for detergent-solubilized LH1-RC complexes are shown in Figure 3A−D. The spectra measured with orthogonal polarization of the exciting light are shown in blue and red, respectively. Independent of the environment of the complexes, their excitation spectra showed a similar behavior. Approximately 70% (from 15 complexes investigated) of all membrane-reconstituted and 90% (from 10 complexes investigated) of all detergent-solubilized complexes exhibited fluorescence excitation spectra consisting of a single broad absorption between 860 and 900 nm with a peak maximum around 880 nm that did not depend on the polarization direction of the excitation laser. The widths of these bands were in the range of 250−300 cm^{-1}. This value corresponds to a lifetime of the absorbing states of some tens of femtoseconds. All other complexes showed a single band at each polarization direction. The observed splittings of these bands were in the range of ΔE = 30−85 cm^{-1}. The mean ΔE values for the population of membrane-reconstituted and

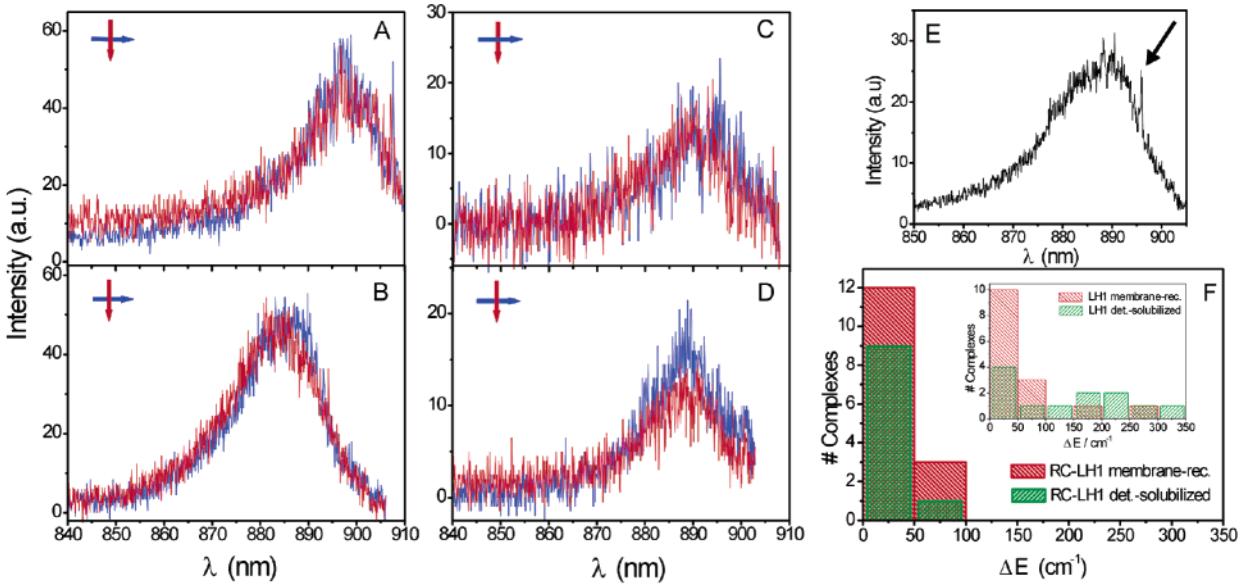

FIGURE 3: Typical fluorescence excitation spectra of membrane-reconstituted (A and B) and detergent-solubilized (C and D) isolated LH1-RC complexes. The red and blue spectra were measured at orthogonal polarization directions (arrows, inset) of the exciting light. (E) The fluorescence excitation spectra of a membrane-reconstituted isolated complex showing a pronounced narrow absorption line on the red wing (arrow). (F) Histogram of the splitting ΔE of the $|k = \pm 1\rangle$ states for the membrane-reconstituted (red) and detergent-solubilized (green) LH1-RC complexes. To emphasize how the presence of the RC influences the energetic positions of the $|k = \pm 1\rangle$ states, the corresponding histogram obtained for single LH1 complexes from *R. rubrum* (without RC, taken from Gerken et al. (*18*)) is shown in the inset.

detergent-solubilized complexes were 19 and 7 cm^{-1}, respectively. In general, the fluorescence of the detergent-solubilized complexes was weaker than for the membrane-reconstituted complexes under comparable conditions, resulting in a lower signal-to-noise ratio in their excitation spectra. In seven excitation spectra of membrane-reconstituted complexes, we observed a narrow absorption line on the red wing of the excitation spectrum as shown in Figure 3E, which varied in intensity from complex to complex. A few spectra showed two and more narrow lines on their red wings, probably due to spectral diffusion. The widths of these narrow lines were ≤ 1 cm^{-1} and are therefore determined by the width of the excitation laser. For the detergent-solubilized complexes, only two excitation spectra showed a clear narrow absorption line on the red wing due to the low signal-to-noise ratio. These narrow absorption lines most probably represent the $|k = 0\rangle$ state from the Frenkel exciton picture used above. The mean energetic distance of this line from the $|k = \pm 1\rangle$ absorption maxima is 84 cm^{-1}, which is in good agreement with the calculated value of 75 cm^{-1} (as shown in Figure 1).

DISCUSSION

The stunning three-dimensional representations of the LH2 complexes from *Rps. acidophila* and *R. molischianum*, respectively, obtained by X-ray crystallography, have revealed arrays of $\alpha\beta(BChla)_3$ dimers arranged in a ring-like structure with perfect circular symmetry (*4*, *5*). The subsequent medium-resolution projection structures of the LH1 complexes from *R. rubrum* (*7*) and *Rb. sphaeroides* (*29*) obtained by cryoEM also showed larger arrays of 32 $\alpha\beta$ heterodimers with flawless circular symmetry (*7*). An exception to this rule has been reported (*30*) for the photosynthetic unit (PSU) of the *Rb. sphaeroides* strain RCLH10. In this case, cryoEM analysis of intact membranes indicated an open LH1 ring structure partially surrounding the RC. The intact PSU appeared to consist of a dimer of these units arranged with 2-fold symmetry and with the RC accessible to the lipid bilayer. However, so far this organization seems to be exceptional, and it is not clear whether it is attributable to the relatively low resolution of the images obtained after negative staining or the highly unusual tubular morphology of the intracellular photosynthetic membranes in this strain that lacks LH2. Certainly, it is now well-established that the more commonly found strains of *Rb. sphaeroides* (with spheroidal intracellular membranes) contain closed LH1 rings with 16-fold symmetry (*29*). However, we note that the *Rb. sphaeroides* LH1 employed in the latter study lacked PufX. We will therefore limit ourselves to the 16-fold symmetric LH1 organization shown here to be present exclusively in *R. rubrum*. Recent analysis of the geometry of the BChla pigments in LH2 rings using SM spectroscopy, which is exquisitely sensitive to small geometric deformations, seemed to indicate that the centrosymmetric view of the LH2 complexes may be severely biased by the averaging techniques employed by X-ray crystallography and cryoEM. Thus, the fluorescence excitation spectra obtained at low temperature for the ring of BChla pigments contributing to the B850 band of the LH2 complexes from *Rps. acidophila* show two or more absorption bands in the 850 nm region with a characteristic energy splitting between the $|k = \pm 1\rangle$ states of approximately 110 cm^{-1} (*15*). In this previous work, it has been argued that the characteristic splitting of the B850 absorption band at low temperature is not a matrix effect but is due to a correlated disorder of the pigments, which could be explained by an elliptical deformation with an eccentricity of $\epsilon = 0.52$ ($b/a = 0.85$). More recent experiments (*19*) on detergent (LDAO)-solubilized LH1-RC complexes have indicated that also these exhibit a variety of splittings in the fluorescence excitation spectra, with only

about 30% of the LH1-RC complexes showing a clear signature of a circular symmetric ring-like arrangement of their pigments. The remaining 70% of the complexes (from a population of 24 complexes) show excitation spectra that are only compatible with a gross deviation from circular symmetry (type II spectra in ref *19*) and have been attributed either to an open ring or a partial ring of BChla pigments (type III spectra in ref *19*) or a dimeric LH1-RC aggregate (type IV spectra in ref *19*). The assignment of elliptical distortion with C_2 symmetry to the BChla pigments of the LH1 ring in LH1-RC complexes has been suggested to be compatible with the 2-fold symmetry of the RC, which might act as a mold for the LH1. To long-term workers in the field, the distortion of the LH1 ring, due to a site-specific interaction with the RC or even the presence of partial LH1 rings, is attractive for a number of reasons. First, a distortion of the LH1 ring stabilized by an interaction with the RC would allow a localization of excitation energy at a fixed point of the ring and thus facilitate energy transfer to the special pair. Second, considerable controversy exists concerning the molecular homogeneity of the LH1 complex. In several cases, additional polypeptides, such as PufX in *Rb. sphaeroides* (*31*) and *Rhodobacter capsulatus* (*32*), the γ-polypeptide in *Rps. viridis* (*33*), and the Ω-polypeptide in *R. rubrum* (*34*), have been found to be associated with the LH1 complex. At least in one case, PufX, this additional polypeptide component seems to be essential for functional electron transfer from the RC to the cytochrome bc_1 complex, presumably by forming a pore with the closed LH1 ring (*35*). An excellent summary of these data is given in Ketelaars et al. (*19*). Third, evidence from structural analyses of LH1 or LH1-RC complexes has also repeatedly indicated that the LH1 ring may be susceptible to elliptical distortion. Thus, although the medium-resolution projection of the *R. rubrum* LH1 ring was calculated by 16-fold averaging, necessarily generating circular symmetry, it was noted that unit cells were also present showing a noticeable ellipticity (*7*). More recent images of the *R. rubrum* LH1-RC complexes in 2-D crystals showed an elliptic deformation, with the major and minor axes differing by about 11% (*8*), corresponding to an a/b ratio of 1.12. In addition, modeling studies that dock the RC into the LH1 ring show that the distances between the special pair and the accessory BChla pigments to those of the LH1 ring are rather large (approximately 42 and 36 Å for the Mg−Mg distances, respectively (*36*, *37*)). At these distances, theory indicates that only minor energetic interactions are expected (*38*, *39*). In our calculations, we have not included possible influences of the RC special pair or accessory BChls upon the energy levels of the LH1 BChls as they are not observed experimentally.

The exciton model for a ringlike aggregate with perfect symmetry predicts that the lowest state of the exciton manifold $|k = 0\rangle$ carries no oscillator strength, thus preventing the appearance of fluorescence at low temperature, when only the lowest state is occupied. Nevertheless, strong low temperature fluorescence emissions (Figure 2) are experimentally observed in bulk (*40*) and SM measurements (ref *19* and this paper) of single LH1-RC complexes, indicating that a minimal distortion of the ring must be present. This disagreement between the experimental results and the theory is overcome by assuming that the BChla molecules in the ring are distributed with a diagonal disorder σ, which corresponds to the full width half-maximum of the Gaussian distribution of excitation energies about E_o. In fact, bulk measurements have also indicated that a diagonal disorder of about 200 cm^{-1} of the BChls must be present (*40*, *41*). The diagonal disorder not only leads to a shift of oscillator strength to the state $|k = 0\rangle$ but also causes a splitting of the degenerated excitonic states. The fact that the excitation spectra mostly showed only a single broad line independent of the polarization suggests that the B880 ring is only weakly perturbed so that the states $|k = \pm 1\rangle$ remain nearly degenerate. Our experimentaly observed distributions of splittings ΔE of the states $|k = \pm 1\rangle$ (Figure 3F) are compatible with a moderate diagonal disorder of $\sigma \leq 150$ cm^{-1}. In an impressive study of intact photosynthetic membranes from *Rps. viridis* using AFM, Scheuring et al. (*20*) have very recently presented data that indicates that the LH1 ring is elliptical in shape when present as a complex with the RC. In this latter study, intact LH1 rings containing no RC (which had been extracted in some cases as a consequence of the AFM measurement) showed a reversion to circular symmetry. Thus, both AFM and cryoEM studies appear to lead to the same conclusion, namely, that the LH1 rings of the LH1-RC complexes present in bilayer membranes possess elliptical geometry. The (a/b) ratios obtained in these two experiments were similar ($a/b = 1.12$ and 1.05−1.11 for the cryoEM and AFM studies, respectively (see Figure 1D)). By contrast, our own data show unambiguously that not only LH1 rings in LH1-RC complexes exhibit a minimal distortion from perfect circular symmetry when reconstituted into lipid bilayers, but also, even more strikingly, that the circular symmetry is *stabilized* by the interaction with the RC, even when the LH1-RC particles are present in the detergent-solubilized state. How can the discrepancy between our study here and the structural and spectroscopic studies be rationalized? On one hand, we note that both the cryoEM (*8*) and the AFM (*20*) studies have been performed using 2-D crystalline arrays, where the LH1-RC complexes are closely packed. Thus, it seems likely that the observed ellipticity is a consequence of a packing artifact and is not attributable to a fundamental property of the LH1-RC interaction. The study of Scheuring et al. (*20*) may indicate that an elliptical ring geometry is physiologically meaningful only for a photosynthetic membrane where the complexes are packed in an almost perfect 2-D crystal, such as those from *Rps. viridis* (*42*) or *Ectothiorhodospira halochloris* (*43*). However, this perfect packing is exceptional. In most photosynthetic membranes from bacteria such as *R. rubrum*, *Rb. sphaeroides*, or *Rb. capsulatus*, both kinetic and structural evidence exists that most LH1-RC complexes exist in domains (varying from approximately 100 LH1-RC complexes at high light intensities to as little as four LH1-RC complexes at low light intensities (*3*)), which are significantly less well-ordered than in *Rps. viridis* or *E. halochloris*. Although a preliminary AFM study of one such example (from *Rps. acidophila*) has been performed, the resolution obtained in this earlier study was not sufficient to obtain information concerning the ellipticity of the LH1 rings (*44*). We believe that the apparent correspondence of the SM data of Ketelaars et al. (*19*) with the cryoEM (*8*) and AFM (*20*) data refers to a different molecular phenomenon than a packing effect. We have observed repeatedly that the formation of regular 2-D crystalline arrays for

isolated LH1 (Karrasch et al. (*7*)) or LH1-RC complexes (*21*) requires the presence of 1−2 mol of phospholipid/$\alpha\beta$ domain (*11*, *45*) to be present. We speculate that the large number of distorted or open LH1 complexes observed by Ketelaars et al. (*19*) may be due to their use of LDAO as a detergent. In our hands, as judged by Gaussian analysis of the absorption spectra obtained from LH1-RC complexes in this latter detergent (R. Ghosh, unpublished data), it has not been possible to obtain perfect absorption spectra of either isolated LH1 or LH1-RC rings without evidence of non-native spectral contributions not observed for LH1 rings in vivo or for reassembled complexes obtained after extensive dialysis. This is not true when the detergent DHPC (a short-chain phospholipid) is employed as a solubilizing agent, provided that a critical detergent concentration (usually between 0.07 and 0.09% (w/v)) is not exceeded (see Materials and Methods). The phosphocholine headgroup may stabilize interactions between $\alpha\beta$ chains, similiar to that observed in 2-D crystals and probably in vivo as well. Under these conditions, native (which we interpret as corresponding to LH1 rings showing no detergent-induced destabilization or dissociation) absorption spectra (and near-IR CD spectra, data not shown) are obtained.

In conclusion, our data demonstrate unambiguously that the LH1 ring present in a homogeneous 2-D potential of the lipid bilayer exhibits near-perfect circular symmetry, even when interacting with the reaction center. The stabilization of a circularly symmetric LH1 by interaction with an intrinsically asymmetrical RC points to an unusual and subtle structural/thermodynamic phenomenon that we will pursue in future studies.

ACKNOWLEDGMENT

We thank Ms. Gerasimoula Gerasimidou for expert technical assistance. We thank Dr. Fedor Jelezko for fruitful discussion and critical reading of the manuscript.

REFERENCES

1. Zuber, H. (1986) *Encycl. Plant Physiol. 19*, 238.
2. Monger, T. G., and Parson, W. W. (1977) *Biochim. Biophys. Acta 460*, 393.
3. Vos, M., van Grondelle, R., and Duysens, L. N. M. (1986) *Biochim. Biophys. Acta 850*, 501.
4. McDermott, G., Prince, S. M., Friend, R. H., Hawthornthwaite-Lawless, A. M., Papiz, A. M., Cogdell, R. J., and Isaacs, N. W. (1995) *Nature 374*, 517.
5. Koepke, J., Hu, X., Muenke, C., Schulten, K., and Michel, H. (1996) *Structure 4*, 581.
6. Fowler, G. J. S., Sockalingum, G. D., Robert, B., and Hunter, C. N. (1994) *Biochim. Biochem. J. 299*, 695.
7. Karrasch, S., Bullough, P. A., and Ghosh, R. (2000) *EMBO J. 14*, 631.
8. Jamieson, S. J., Wang, P., Qian, P., Kirkland, J. Y., Conroy, M. J., Hunter, C. N., and Bullough, P. A. (2002) *EMBO J. 21*, 3927.
9. Robert, B., and Lutz, M. (1985) *Biochim. Biophys. Acta 807*, 10.
10. Olsen, J. D., Sturgis, J. N., Westerhuis, W. H. J., Hunter, C. N., S., and Robert, B. (1997) *Biochemistry 36*, 12625.
11. Picorel, R., Belanger, G., and Gingras, G. (1983) *Biochemistry 22*, 2491.
12. Cogdell, R. J., Lindsay, J. G., Valentine, J., and Durant, I. (1982) *FEBS Lett. 150*, 151.
13. Hu, X., and Schulten, K. (1998) *Biophys. J. 75*, 683.
14. Autenrieth, F., Ritz, T., Schulten, K., and Ghosh, R. (2002), submitted.
15. van Oijen, A. M., Ketelaars, M., Köhler, J., Aartsma, T. J., and Schmidt, J. (1999) *Science 285*, 400.
16. Bopp, M. A., Jia, Y., Li, L., Cogdell, R., and Hochstrasser, R. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 10630.
17. Dekker, J. P., Gilbro, T., and Sundström, V. (1994) *Biochim. Biophys. Acta 1187*, 1.
18. Gerken, U., Jelezko, F., Götze, B., Branschädel, M., Tietz, C., Ghosh, R., and Wrachtrup, J. (2003) *J. Phys. Chem. B 107*, 338.
19. Ketelaars, M., Hoffmann, C., Köhler, J., Howard, T. D., Cogdell, R. J., Schmidt, J., and Aartsma, T. J. (2002) *Biophys J. 83*, 1701.
20. Scheuring, S., Seguin, J., Marco, S., Levy, D., Robert, B., and Rigaud, J.-L. (2003) *Proc. Natl. Acad. Sci. U.S.A. 18*, 1690.
21. Stahlberg, H., Dubochet, J., Vogel, H., and Ghosh R. (1998) *J. Mol. Biol. 282*, 819.
22. Ames, B. N., and Dubin, D. T. (1960) *J. Biol. Chem. 235*, 769.
23. Noël, H., van der Rest, M., and Gingras, G. (1972) *Biochim. Biophys. Acta 275*, 219.
24. Hu, X., Ritz, Th., Damjanovic, A., and Schulten, K. (1998) *J. Phys. Chem. B 101*, 3854.
25. Moss, G. P. (1987) *Pure Appl. Chem. 59*, 779.
26. Tretiak, S., Middleton, C., Chernyak, V., and Mukamel, S. (2000) *J. Phys. Chem. B 104*, 9540.
27. Westerhuis, W. H. J., Hunter, C. N., van Grondelle, R., and Niedermann, R. A. (1999) *J. Phys. Chem. B 103*, 7333.
28. Paschenko, V. V., Gorokhov, V. V., Grishanova, N. P., Goryacheva, E. A., Korratovsky, B. N., Knox, P. P., Zakharova, N. I., and Rubin, A. B. (1998) *Biochim. Biophys. Acta 1364*, 361.
29. Walz, T., Jamieson, S. J., Bowers, C. M., Bullough, P. A., and Hunter, C. N. (1998) *J. Mol. Biol. 282*, 833.
30. Jungas, C., Ranck, J. L., Joliot, P., Rigaud, J.-L, and Vermeglio, A. (1999) *EMBO J. 18*, 524.
31. Farchaus, J. W., Barz, W. P., Grünberg, H., and Oesterhelt, D. (1992) *EMBO J. 11*, 2779.
32. Youvan, D. C., Bylina, E. J., Alberti, M., Begusch, H., and Hearst, J. E. (1984) *Cell 37*, 949.
33. Jay, F., Lambillote, M., Stark, W., and Mühlenthaler, K. (1984) *EMBO J. 3*, 773.
34. Ghosh, R., Ghosh-Eicher, S., Di Berardino, M., and Bachofen, R. (1994) *Biochim. Biophys. Acta 1148*, 28.
35. Barz, W. P., Vermeglio, A., Francia, F., Venturoli, G., Melandri, B. A., and Oesterhelt, D. (1995) *Biochemistry*, *34*, 15248.
36. Papiz, M. Z., Prince, S. M., Hawthornthwaite-Lawless, A. M., McDermott, G., Freer, A. A., Isaacs, N. W., and Cogdell, R. J. (1996) *Trends Plant Sci. 1*, 198.
37. Hu, X., Damjanovic, A., Ritz, Th., and Schulten, K. (1998) *Proc. Natl. Acad. Sci. U.S.A., 95*, 5935.
38. Novoderezkhin, V. I., and Razjivin, A. P. (1994) *Photosynth. Res. 42*, 9.
39. Owen, G. B., Hoff, J. A., and Jones, M. R. (1997) *J. Phys. Chem. B 101*, 7197.
40. Monshouwer, R., Abrahamsson, M., van Mourik, F., and van Grondelle, R. (1997) *J. Phys. Chem. B 101*, 7241.
41. Visser, H. M., Somsen, O. J. G., van Mourik, F., and van Grondelle, R. (1996) *J. Phys. Chem. 100*, 18859.
42. Miller, R. M. (1982) *Nature 300*, 53.
43. Engelhardt, H., Engel, A., and Baumeister, W. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 8972.
44. Scheuring, S., Reiss-Husson, F., Engel, A., Rigaud, J.-L., and Ranck, J.-L. (2001) *EMBO J. 12*, 3029.
45. Ghosh, R., Hauser, H., and Bachofen, R. (1988) *Biochemistry 27*, 1004.

BI034969M