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Low-temperature absorption and site-selected fluorescence of the light-harvesting antenna of *Rhodopseudomonas viridis*. Evidence for heterogeneity

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Abstract

The main infrared absorption band of the antenna of the purple bacterium *Rps. viridis* is investigated using low-temperature absorption and site-selected fluorescence spectroscopy. Low-temperature absorption spectra show that at least three bands are present in the near-infrared. Gaussian fits indicate that the maxima are located around 1015, 1030 and 1045 nm. Fluorescence measurements using narrow-band laser excitation in the red flank of the absorption band show that the emission is highly polarized ($p > 0.3$). This was found for membranes and (isolated) core complexes. In isolated core complexes, the polarization of the emission increases smoothly as a function of the excitation wavelength, starting in the center of the absorption band, whereas in membranes the increase is abrupt and occurs in the extreme red edge. These results differ significantly from similar measurements performed on core light-harvesting complexes from *Rhodobacter sphaeroides* [Van Mourik, F., Visschers, R.W., Van Grondelle, R. (1992) Energy transfer and aggregate size effect in the inhomogeneously broadened core light-harvesting complex of *Rhodobacter sphaeroides*, Chem. Phys. Lett. 193, 1–7] and cannot be interpreted by assuming a single inhomogeneously broadened pool of antenna pigments. Moreover, the low-temperature absorption spectrum gives clear evidence for a long-wavelength spectral form. Therefore, it is concluded that the antenna is heterogeneous, with a minor long-wavelength spectral form (B1045). The fluorescence measurements show that this B1045 band is inhomogeneously broadened.

Keywords: Photosynthesis; Inhomogeneous broadening; Light harvesting antenna; Quantasome; Bacteriochlorophyll *b*; (*Rps. viridis*)

1. Introduction

The photosynthetic apparatus of purple bacteria is composed of reaction centers and a light-harvesting (LH) or antenna system. The latter consists of pigments (bacteriochlorophyll (BChl), carotenoid) bound to specific proteins that assemble in light-harvesting or antenna complexes whose function it is to absorb the incident light and transport the excited-state energy to the reaction center (RC), where a charge separation takes place. In purple non sulphur bacteria the pigment-binding proteins consist of a pair of small transmembrane polypeptides, α and β, that each bind at least one BChl to a central histidine residue, while in some species a third polypeptide γ was identified that probably has a structural role [1]. The antenna complex is organized in some aggregate $(\alpha\beta)_n$. The absorption properties of the pigments associated with the antenna complexes are dependent on the kind of BChl and the electronic coupling between them (exciton interactions), but also on the local environment provided by the protein (which is different for each bacterium), and the different binding sites of the proteins. For instance, the antenna of *Rb. sphaeroides* contains BChl *a*, and has absorption bands at 800, 850 and 870 nm. The bands at 800 and 850 belong to a peripheral antenna, LH2, while the band at 870

Abbreviations: BChl, bacteriochlorophyll; LH, light-harvesting; *A*, optical absorbance; RC, reaction center; ZPL, zero phonon line; P, primary electron donor.

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nm is due to the core antenna, LH1, that directly surrounds the RC. The species studied in this work, *Rps. viridis*, contains only a core antenna with BChl *b* as the essential pigment and has a main absorption band around 1000 nm (see for general reviews: [2–4]).

In the past, evidence has accumulated that the LH1 antenna of BChl-*a*- containing purple bacteria like *Rs. rubrum* and *Rb. sphaeroides* is not homogeneous. In fluorescence [5,6], singlet-singlet annihilation [7], hole burning [8] and time-resolved absorption measurements [9–12] the signals were found to depend on the wavelength of excitation within the band. Time-resolved fluorescence measurements on the LH1 antenna of *Rs. rubrum* at 4.2 K by Timpmann et al. [13] have shown that at least 2 or 3 components were present in the decay, with fast components dominating the 'blue' part of the emission spectrum. To explain this observation, at least three spectrally different components in the *Rs. rubrum* antenna have to be assumed. Subsequently, on the basis of energy-selected fluorescence measurements on isolated LH1 complexes of BChl-*a*-containing purple bacteria, Van Mourik et al. [5] suggested that at 4.2 K the absorption spectrum of the LH1 antenna is inhomogeneously broadened, i.e., the antenna consists of identical chromophores, which have, however, a statistical distribution of their absorption maxima, probably due to small perturbations in the surroundings of the pigment. Note that this is different from spectral heterogeneity, where the differences are due to structurally or chemically different sites in the protein. A statistical model based on these assumptions was found to be qualitatively and quantitatively in good agreement with measurements on the LH1 antenna of *Rb. sphaeroides* [5] and time-resolved and steady state spectroscopy on the LH 1 antenna [14,15] and the isolated B820 subunit [16,17] of *Rsp. rubrum*.

Even though there seems to be a high degree of homology between the BChl-*a*-containing bacteria mentioned above and *Rps. viridis* [1], there are some significant structural and spectral differences. Electron microscopy studies on *Rps. viridis* have shown that the LH1 antenna forms well-defined ringlike structures around the RC, and that these 'rings' are arranged in a tightly-packed two dimensional lattice in the photosynthetic membrane [18,19]. Such a strong ordering has not been reported for other bacteria. Striking spectral features of *Rps. viridis* are the extremely red shifted Q_y absorption band, around 1000 nm, and the remarkable location of the absorption maximum of the primary donor, apparently about 35 nm to the blue compared to the absorbance maximum of the surrounding antenna pigments [20]. Despite this apparently unfavorable energy gap between antenna and RC, trapping of antenna excitations by the RC still occurs with a high quantum-yield, even at low temperatures [20]. Another clear difference from the BChl-*a*-containing purple bacteria is that, unlike *Rb. sphaeroides* and *Rs. rubrum*, measurements at low temperature so far have given no indication of inhomogeneity or heterogeneity of the antenna absorption band [21–23].

In our study of the spectral nature of the photosynthetic antenna, which is crucial for our understanding of its functioning as a light-energy collecting device, we have performed site-selected fluorescence measurements on membranes and isolated core complexes of *Rps. viridis*. Absorption spectra recorded at 4.2 K show that the absorption band of both preparations is not homogeneous, at least three distinct near-infrared absorption-bands can be distinguished. Also the excitation spectra measured at 4.2 K indicate that the band is not homogeneous, since excitation in the red part of the band results in highly-polarized fluorescence. For *Rb. sphaeroides* and *Rs. rubrum*, this feature was attributed solely to inhomogeneous broadening. However, in the case of *Rps. viridis* the situation is more complicated, since clearly distinct bands can be distinguished in the long-wavelength absorption profile. Therefore, the measurements have to be interpreted with contributions due both to heterogeneity and to inhomogeneous broadening.

2. Materials and methods

Membrane fragments of *Rhodopseudomonas viridis* were obtained by suspending the cells in a 20 mM Tris-HCl buffer (pH 8.0) and sonicating for about 3 min in ice. Core complexes were made following the method described by Jay et al. [24]. In brief: membranes were incubated in a 50 mM Tris-HCl (pH 8.0) buffer with 1% of deoxycholate (DOC) and 1% of lauryl dimethylamine oxide (LDAO). The suspension was centrifuged to remove the cell debris and subsequently applied onto a sucrose-gradient and centrifuged overnight at $100\,000 \times g$. The core complexes were stored at $-20°$ C until further use. For low-temperature measurements the samples were contained in acrylic cuvettes (Starstedt, pathlength 1 cm) and cooled in a liquid-helium flow cryostat (Oxford Instruments, CF1204). To obtain glassy samples, glycerol was added to the sample to a concentration of 70% (v/v). In the case of core complexes DOC was added to the glycerol to sustain the 1% concentration. To increase the fluorescence intensity (especially when exciting in the extreme red edge of the absorbance spectrum) high absorbance samples (about 1 cm^{-1} at the absorption maximum) were used. This does not distort the shape of the emission band since the absorption spectra hardly overlap with the fluorescence emission spectra. The absorbance measurements were made on a home-built single-beam spectrophotometer, with a bandwidth of 1 nm. Polarized-fluorescence spectra were measured as described before [5]. In brief: the sample was excited using a Ti:Sapphire laser (coherent 890) pumped by an argon-ion laser (Coherent Innova 310). Fluorescence was detected at 90° through a double 1/8 m monochromator (bandwidth 4 nm) using a photodiode (HUV 4000B)

or a cooled S1-photomultiplier (EMI 9684B). The spectra were not corrected for the wavelength-dependent sensitivity of the detector. The polarization of the emission was measured by placing a Glann-Thompson polariser in the excitation as well as the detection path and calculating the polarization following Ref. [25].

3. Results

The measurements described here have been performed on both the isolated photosynthetic unit, the quantasome, and whole membranes of *Rps. viridis*. Shown in Fig. 1 are the absorption spectra and the inverted second derivatives of the absorption spectra of the core complexes and membranes of *Rps. viridis* at 4.2 K. Cooling down from room temperature to 4.2 K results in a large red-shift of the main infrared absorption-bands for both preparations (not shown). For the core complexes the maximum shifts from around 1000 nm at room temperature to 1030 nm at 4.2 K. As has been observed before [24], the absorption maximum of the core complex preparation is slightly (about 10 nm at room temperature) blue-shifted compared to the membranes. The second derivative of the membrane absorbance spectrum has clear maxima at 1049, 1042 and 1030 nm. We have further performed a gaussian fit of the membrane absorbance spectrum using a non linear least square fitting algorithm. The results of this is shown in Table 1. We think that the difference in the number of bands and the location of the maxima illustrates the inaccuracy of the analysis method for these spectra. One reason for the difference is that both methods respond differently to noise and baseline errors of the original spectrum. Moreover determining the underlying structure

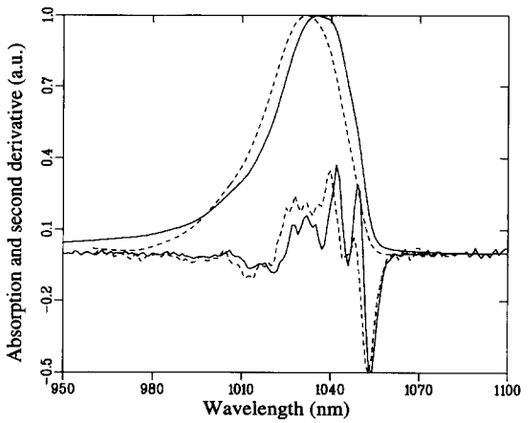

Fig. 1. Absorption spectra and second derivatives of *Rps. viridis* preparations at 4.2 K. Shown are membrane and core complex absorption spectra (solid and dashed respectively) and the inverted second derivatives of these spectra (analogous line-type). The absorption spectra are normalized on the maximum and have a resolution of 1 nm. Especially in the membrane spectrum the bands indicated by the second derivative spectrum are visible as shoulders in the absorption spectrum.

Table 1
Fitting parameters used to fit the membrane absorption spectrum of *Rps. viridis*

	Max. (nm)	FWHM (nm)	Area
Band 1	1044 (2)	12.4	0.37
Band 2	1033	19.8	1.0
Band 3	1018	32	0.61
Band 4	984	67	0.35

The error in the wavelength of the first band was estimated by slightly varying and fixing this value and subsequently re-fitting the spectrum. From this we conclude that the error in this band is approx. 2 nm. The location and width of the fourth band has a very large error. The areas are normalized on the 2nd band.

of an absorption band using these methods has validity only under certain conditions, specifically when the bands are (symmetric) gaussians. Especially the last constitutes a problem in analyzing these spectra, since calculations and hole-burning measurements [26,27] have shown that in general the homogeneous spectra of bacterial antenna pigments are asymmetric. We therefore think that deriving the precise location of these without a priori assumptions about the shape of the bands has limited validity. The prediction of the number of bands by the second derivative method is, however, relatively insensitive for the specific lineshape. We can therefore conclude that the absorption spectra of both membranes and core complexes are highly heterogeneous and constitute at least three absorption bands. Also the spectrum of the core complex shows, apart from a blueshift of about 3 nm, features identical to those of the membrane spectrum. To obtain an idea of the accuracy of the gaussian fit we have carefully compared the results of the second derivative analysis and various fits made with 3 or 4 bands. To estimate the error in especially the B1045 pool, we have shifted and fixed the center of this band and optimized the other parameters to get a good fit. From these results it is clear that satisfactory fits can be obtained only when the center of the red-most band is between is 1042 ± 3 and 1044 ± 2 nm for core complexes and membranes, respectively. The reason that the red-most band is relatively accurate from the fitting analysis is because this band is responsible for the steep flank on the blue side of the absorbance spectrum. All the other fit parameters have a larger uncertainty.

In Fig. 2 two emission spectra of core complexes at 4.2 K are shown. The spectra are normalized to make the red wings overlap. The solid curve is the emission observed upon narrow-band excitation in the blue part of the Q_y absorption band (at 1018 nm). This emission band has a maximum at 1054 nm. The dashed line shows the emission upon excitation in the red flank of the spectrum (at 1047 nm). The large peak on the blue side of this curve is due mainly to scattering of the incident laser light. Fig. 2 clearly demonstrates that, although the shape of the 'blue' side of the emission spectrum depends on the excitation wavelength, the shape of the red flank of the emission

band and the position of the maximum of the emission, are independent of the excitation wavelength. These results are very different from those obtained for isolated LH1 complexes from *Rb. sphaeroides* [5].

In Fig. 3A, a typical example of horizontally and vertically detected fluorescence spectra of core complexes is shown together with the resulting polarization curve (excitation at 1031 nm). Clearly, the polarization is not constant over the band. Apart from spectra excited in the extreme red edge of the absorption band, all emission spectra of the core complexes showed an increased polarization of the emission in the blue region of the spectrum (about 0.3 to 0.4), which drops to a constant value in the center and red part of the band.

To examine the dependence of the fluorescence polarization on the wavelength of excitation, the polarization of the excitation spectrum was measured. This was done by recording polarized emission spectra for several excitation wavelengths and plotting the value of the polarization in the central, horizontal part of the curve versus the excitation wavelength. Fig. 3B shows the result together with the absorption spectrum of core complexes at 4.2 K. The polarization starts to increase smoothly in the center of the band and reaches a maximum value of about 0.3 when exciting in the red part. Excitation in the blue and center of the band yields a polarization of about 0.1, which is close to the values that have been found before in steady state [23], time-resolved polarized fluorescence [22] and transient absorption [21] measurements.

Analogous polarized-fluorescence measurements on whole membranes of *Rps. viridis* are shown in Fig. 4. The individual emission spectra (not shown) indicate, analogous to Fig. 2 for the core complexes, that the emission maximum does not shift upon scanning the excitation wavelength. Also, as is illustrated by Fig. 4B, the polarization increases upon excitation in the red part of the band. There are, however, some pronounced differences. Firstly, for all excitation wavelengths the polarization of the emission is constant over the whole emission band (see for example Fig. 4A). Secondly it is apparent from Figs. 3B and 4B that the increase of the polarization of the excitation spectra is red-shifted and more abrupt in the case of membranes.

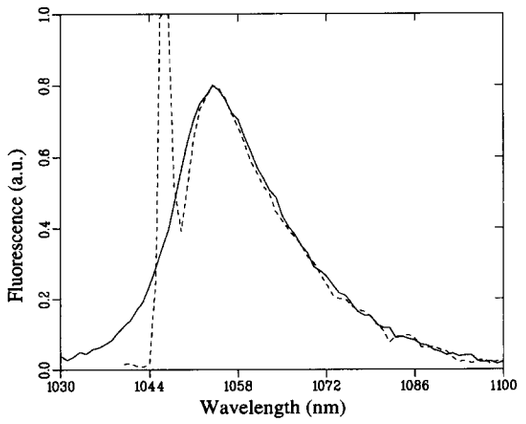

Fig. 2. Emission spectra of core complexes at 4.2 K (plotted is: $I_{vv} + 2I_{vh}$). I_{vv} and I_{vh} denote the vertically and horizontally polarized emission spectra upon excitation of the sample with vertically polarized light. Excitation was at 1018 nm (solid) and 1047 nm (dashed). The resolution was 4 nm. The spectra are normalized on their emission maxima.

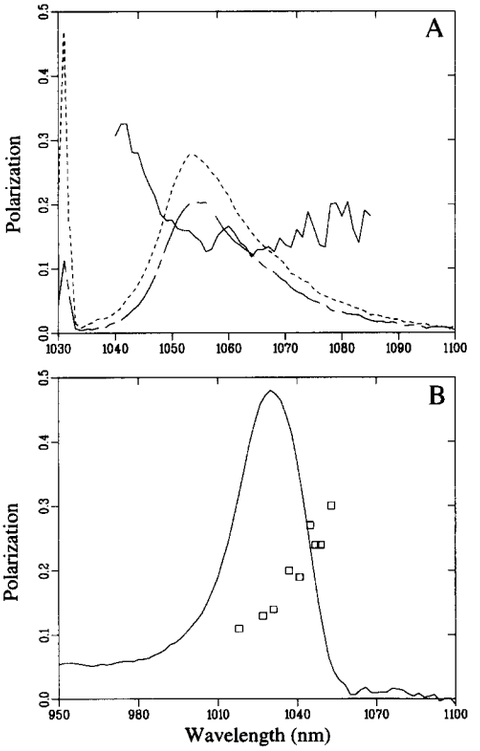

Fig. 3. (A) Typical polarized emission spectra of core complexes at 4.2 K. Shown are the vertically and horizontally detected emission spectra (dashed and chain dashed respectively). The excitation was at 1031 nm and the exciting beam was polarized vertically. The solid curve is the polarization of the emission. (B) The polarization of the emission of core complexes plotted versus the wavelength of excitation. The 4.2 K absorption spectrum of core complexes (solid) is added for clarity.

4. Discussion

4.1. Absorption analysis

Contrary to earlier reports [21–23], we found the near-infrared absorption band of *Rps. viridis* to be heterogeneous at cryogenic temperatures. This is clearly illustrated by the absorbance spectra of both membranes and isolated core complexes as shown in Fig. 1. The inverted second derivatives of the spectra indicate that 3 or 4 bands are present in this part of the spectrum. To examine the band structure, we fitted the absorbance spectra using a non-linear least-squares algorithm with gaussian bands (see Table

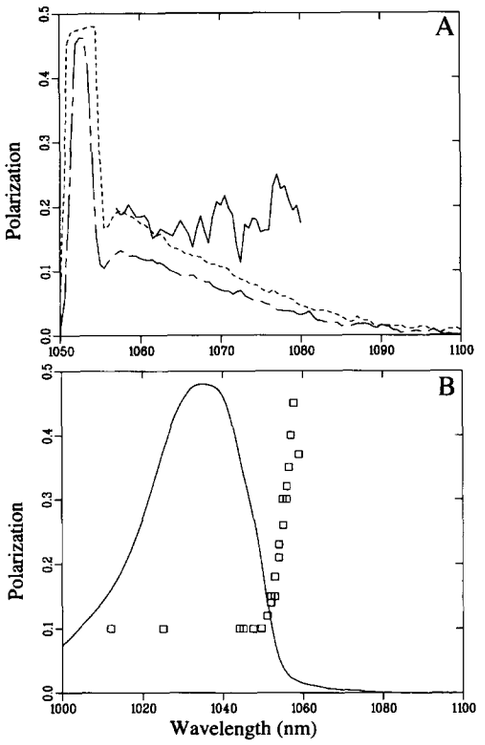

Fig. 4. Typical anisotropic emission spectrum of membranes of *Rps. viridis* recorded at 4.2 K. The excitation wavelength was 1053 nm. Parameters are as in Fig. 3. Shown are vertically (dashed) and horizontally (chain dashed) polarized emission and the polarization (solid). (B) shows the polarization of the emission plotted versus the wavelength of excitation. The solid line is the absorption spectrum of membranes at 4.2 K.

1). As stated previously, one has to be very careful when analyzing the heterogeneity of the absorption band with either the second derivative or a gaussian fit without a priori assumptions about the shape of the bands. However, it is clear that there is a strong heterogeneity in both the membrane and isolated core complexes of *Rps. viridis*. For convenience we shall denote the red-most pigment pool by B1045. The second derivative of the isolated core complex is slightly blue-shifted (about 3 nm) and there seems to be less contribution from the red-most component compared to the membrane spectra. The broad band in the blue is probably due to the accumulated effect of the asymmetric line-shapes (vibrational bands) of the other components and to baseline effects.

The absorption in the spectral region around 1000 nm is due the Q_y transitions of the antenna pigments and the special pair of the reaction center. Kleinherenbrink et al. concluded from difference spectra between the ground state and the charge separated state (P^+Q^-) that in membranes the special pair absorption band is located around 1018 nm at 6 K [20]. This is close to one of the bands that is needed to fit our 4.2 K absorption spectrum (at 1015 nm). However, the band observed in our absorption spectra contains about 25% of the dipole strength of the whole absorption band. This does not agree with the composition of the core complex, which would suggest (assuming a similar extinction coefficient) a stoichiometry of 1 to 12 for the P band relative to the remaining antenna pigments [19]. Therefore, the bands obtained from our gaussian decomposition are predominantly due to the antenna and most likely the spectrum of the special pair of the RC is not resolved.

4.2. Nature of the absorption bands

Several explanations have been given for the spectrally different pigment pools observed in bacterial light-harvesting complexes. Both pigment–protein interactions [28,29] and pigment–pigment interactions [30–32] probably play a prominent role. The biochemical building-block of the core antenna of *Rps. viridis* consists of three small transmembrane polypeptides α, β and γ [19,24,33]. The α and β polypeptides are analogous to the LH1 proteins found in BChl-*a*-containing bacteria. The γ-polypeptide is small and probably does not bind BChl. The structural information that is known so far strongly suggests that the core antenna of *Rps. viridis* consists of one reaction center surrounded by six antenna subunits ($\alpha_2\beta_2\gamma_2BChl_4$) arranged in a structure with 6-fold symmetry. The structural measurements cited do not suggest any special binding sites that could be responsible for the spectral heterogeneity of the antenna band. However, it has been noted that the 6-fold symmetry must be due to averaging effects, since the RC itself is asymmetric [19,22]. Therefore, different BChl sites could exist due to the asymmetry of the core complex. Alternatively the different bands in the antenna could be explained by an exciton band structure [8]. In order to explain their hole-burning data on the LH1 antenna of *Rb. sphaeroides*, Reddy et al. [8] suggested that a red-shifted state (B896) exists in the long-wavelength absorption band that is probed through hole burning. In their view, B896 of *Rb. sphaeroides* corresponds to the lowest exciton level of the core complex. In view of the measurements in this work it is difficult to distinguish between the above models. Both will give rise to separate bands in absorption as well as an increase of the emission-polarization upon excitation in the red part of the band. The CD spectrum of core complexes (Monshouwer, unpublished results) gives no indication for an excitonic origin of the observed antenna bands. However, this is no proof for the absence of exciton interaction since the CD signal depends on the strength of the coupling and on the geometry of the pigments.

A third option we have considered is that the structure found in the absorption spectrum reflects the shape of the underlying homogeneous spectrum of the chromophores. This spectrum consists of a sharp zero phonon line and a much broader phonon side band that is due to internal vibrations of the chromophore and electron-phonon cou-

pling. The B1045 band would then be explained as an inhomogeneously broadened distribution of zero phonon lines and the other bands due to the associated phonon and vibration spectrum. However, in this case the width of the observed emission spectrum (about 18 nm for membranes) sets the maximum width of the phonon side band. This is however much too narrow to explain the broad absorption band (about 30 nm). Thus, an explanation in terms of a single pool with pigments that have a very large homogeneous spectrum due to strong electron phonon coupling does not agree with the observed spectra and we therefore propose that a heterogeneous model is needed to explain the absorption and fluorescence data. However we note that the 1045 nm band could be due to the ZPL contributions of a strongly red-shifted pigment pool.

4.3. Fluorescence data analysis

Indications for heterogeneity of the long-wavelength band are also found in the polarized-fluorescence measurements (Figs. 3 and 4). In both preparations the polarization of the emission was found to increase upon excitation in the red wing of the band, though in the case of membranes the onset of this effect is more abrupt and shifted to the red. The latter is probably the reason why, so far, only low polarization values were observed [22,23], since narrow band (i.e., laser) excitation is needed to observe the increased emission polarization in membranes.

Two mechanisms have been proposed in the past to explain the increase of emission polarization in the red flank of antenna bands of photosynthetic bacteria. It was proposed by Kramer et al. that in the antenna of *Rs. rubrum* and *Rb. sphaeroides* a spectrally distinct band, called B896, was present (i.e., that the band is heterogeneous) [6]. This explains the increase of polarization, since upon excitation of the low energy band and assuming that no further transfer takes place the emission will be highly polarized. The suggestion of Kramer et al. is essentially the same as the model proposed by Reddy et al. [8]. However, Reddy et al. explicitly ascribe B896 to the lowest exciton state within the LH1 complex rather than to different sites within the protein. A different model was proposed by Van Mourik et al. [5]. In this model, site selected polarized fluorescence measurements were explained by assuming that the band is not heterogenous (so no special B896 pigment or excitonic band is present), but that the band is mainly inhomogeneously broadened due to variations in the local environment of the pigments. This model in combination with the idea that in the isolated LH1 unit energy transfer can only take place over a limited number of pigments predicts that, dependent on the number of connected pigments, the polarization and the position of the emission maximum exhibit a specific dependence on the excitation wavelength. The reason for this behavior is that excitation into the red wing of the spectrum corresponds to a pre-selection from the ensemble of core complexes of a subset which contains a relatively red-shifted pigment. Since in *Rb. sphaeroides* LH1 a redshift was found of the emission maximum, something that is not expected in a heterogeneous model, Van Mourik et al. concluded that the LH1 antenna is inhomogeneously broadened [5]. Subsequent measurements on various other species like LH1 of *Rhodospirillum molischianum* [34], LH2 of *Rb. sphaeroides* (R.W. Visschers and R. Monshouwer, unpublished results) and LH2 of *Ectothiorhodospira halophila*. [35] have demonstrated very similar behavior in other BChl-*a*-containing bacteria. In *Rps. viridis*, however, the situation seems to be more complicated. The absorbance spectra show that the antenna is in fact heterogeneous. Therefore, it is not surprising that a model based on purely statistical fluctuations is inappropriate in this case. A combination of both models, as was suggested for the PS I antenna of *Synechocystis* PCC 6803, where the individual pools are inhomogeneously broadened seems more appropriate [36].

To further assess the depolarization and thus energy-transfer properties of the core complex of *Rps. viridis* we have compared the polarization curve in Fig. 3B, with the integral of the red-most band that was used to fit the core complex absorbance spectrum. This band was mandatory to get a satisfactory gaussian fit and the location and the width have a relatively small error in the gaussian fits. First we note that at 4.2 K the thermal excitation energy corresponds to a spectral difference of approximately 0.3 nm. Therefore, uphill transfer of excitations between pigments with energetic differences of the order of more than a few nm is highly unlikely, and we can directly relate the amount of depolarization to the probability that the originally excited pigment is connected to a pigment (with different transition dipole orientation) that is lower in energy. Comparison of the integral of the red-most band with the actual anisotropy curve shows that the wavelength at which the polarization starts to increase roughly coincides with the start of this band. In general, the shapes (integral of absorption and increase of polarization) seem to be similar. From this we can conclude that excitations on pigments that belong to the B1045 pool undergo little depolarization, whereas excitations on other pigments are readily transferred and depolarized.

An explanation for the observed polarization behavior is that the B1045 band of the core complex consists of pigments/states that at 4.2 K can not transfer excitation energy to other antenna pigments. So, either there is in fact only one B1045 state per core, or there are more states, but they are not connected by excitation energy transfer. The stoichiometry of the B1045 band (see Table 1) suggests that the B1045 band corresponds to four pigments per core. The observation that there is no observable shift of the emission maximum upon scanning the excitation wavelength would suggest that there is a minor degree of inhomogeneous broadening within the B1045 band [36]. The fact that in the core complexes the polarization rises

only to 0.3 (instead of the expected value of 0.5) is most likely due to the spectral overlap of the red-most transition (1042 nm) with the transition at 1031 nm.

In membranes the onset of the polarization increase occurs at a much longer wavelength. From this we can conclude that extensive energy-transfer takes place within the B1045 band, which agrees with previous singlet-singlet annihilation measurements by Deinum et al. [23]. The difference between the membranes and the core complexes could entirely be due to the change in the effective size of the antenna. In the isolated core complexes the B1045 species is probably not 'connected' i.e. transfer between different B1045 pigments has to occur via the main B1030 band and therefore has to cross an energetic barrier. The dense packing of the core complexes in the membranes probably results in energy transfer between B1045 'pigments' on different core complexes. As a consequence of the excitation energy transfer among different B1045's, the onset of the polarization increase (1052 nm) is red-shifted relative to the maximum of the red-most state (1045 nm) and is rather steep.

Another feature of the polarized emission spectra is that in core complexes the emission is more polarized close to the excitation wavelength (see Fig. 3A). We think that this reflects the (spectrally resolved) contribution of the initially excited pigment to the emission. In membranes this feature disappears because the distribution of emitting pigments is very narrow.

4.4. Trapping in the reaction center

Contrary to other purple bacteria, the primary donor of *Rps. viridis* is assumed to be at a rather high energy relative to the major infrared antenna: the primary donor of the reaction center (in membranes) appears to absorb around 1010 nm [20] at 6 K, whereas we found that the long-wavelength band of the antenna peaks around 1045 nm. If we were to take the maxima of the absorption spectra as representative for the energy difference between the levels, this would correspond with an energy gap of $35^{*}k_{B}T$ at 6 K. It should be obvious that 'uphill' transfer from the antenna to the reaction center will be strongly suppressed at low temperature. However, measurements of the quantum efficiency of charge separation and the excited-state lifetime of antenna excitations indicate that the trapping is still moderately efficient at cryogenic temperatures. Kleinherenbrink et al. [20] found that at 6 K the yield of charge separation was 45% of the yield at room temperature. Time-resolved absorption difference and fluorescence measurements all indicate that the trapping rate is roughly around $(150\ ps)^{-1}$ at 77 K [21,37,38]. The models that have been used so far to account for this data, like that by Zhang et al. assume that the core antenna is homogeneous [21]. Our observation that the spectral structure of the band is heterogenous significantly influences the results of these transfer calculations. The main reason is that, especially at low temperatures, the excitations present in the antenna, will migrate to the B1045 pigment pool. This will effectively enlarge the energy gap between the special pair and the excited pigments, and is thus expected to result in a decrease of the transfer rate to the reaction center. However, the band positions should not be directly interpreted as the energy levels of the antenna and RC states involved. The spectrum of the special pair of the RC is broadened due to strong electron-phonon coupling, leading to a width of the homogeneous spectrum of about 40 nm [20,39]. The observed 4.2 K absorption spectrum of the special pair is blue-shifted relative to the zero-phonon transitions by about 30 nm due to the width of the homogeneous spectrum and consequently the ZPH-region of P (in the membrane) is located around 1040 nm. The B1045 band is relatively narrow. Therefore, the major absorption band is less blue-shifted relative to the B1045 zero-phonon transitions and the ZPL-region of the B1045 band is probably around 1050 nm. This still leaves us with a minor discrepancy: 1050 vs. 1040 nm. Part of this discrepancy could be due to the experimental difficulty of measuring the exact position of the absorption band of P in the membrane bound reaction center. From our absorption spectra the absorption band of P cannot be resolved. Kleinherenbrink et al. measured the P^{+} spectrum of membranes and observed a bleached band around 1010 nm. However this P^{+} spectrum is clearly distorted at the red side due to an electrochromic bandshift of the antenna. If we assume the contribution of the antenna to the signal to be proportional to the first derivative, then the antenna signal could be responsible for a blue shift of the apparent bleaching band of P.

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