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Interactions between chromophores in reaction centers of purple bacteria. A reinterpretation of the triplet-minus-singlet spectra of *Rhodobacter sphaeroides* R26 and *Rhodopseudomonas viridis*

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Abstract

The orientations of the optical transition moments contributing to the Q_Y-region of the triplet-minus-singlet absorbance-difference spectrum of the reaction center of *Rhodobacter sphaeroides* R26 have been determined with respect to the triplet axes of the primary donor with linear-dichroic absorbance-detected magnetic resonance. The orientation of the $S_1 \leftarrow S_0$ transition moment of the reaction center relative to the triplet x- and y-axis was found to be $85 \pm 5°$ and $18 \pm 3°$, respectively. A comparison of the (linear-dichroic) triplet-minus-singlet absorbance-difference spectra is made with those obtained previously for the reaction center of *Rhodopseudomonas viridis* (E.J. Lous and A.J. Hoff (1987) Proc. Natl. Acad. Sci. USA 84, 6147–6151). For both types of reaction centers the transitions in the absorption region of the accessory bacteriochlorophylls appear to shift to lower energies upon triplet formation on the primary donor. The orientations of the transition moments, however, do not change upon excitation of the primary donor to its triplet state, from which we conclude that the band shifts in the triplet-minus-singlet spectrum cannot be explained by a change of dipolar interactions between the transitions of the dimer and the adjacent bacteriochlorophylls.

Keywords: Absorbance-detected magnetic resonance; Triplet state; Photosynthesis; Bacterial reaction center

1. Introduction

Since the crystal structures of the reaction centers (RCs) of the photosynthetic purple bacteria *Rhodopseudomonas (Rps.) viridis* and *Rhodobacter (Rb.) sphaeroides* R26 have become available [1–3], attempts have been made to explain the spectroscopic properties of the RCs in terms of pigment structure and configuration. Two bacteriochlorophylls (BChl) in the RC are closely spaced, forming a BChl-dimer, P, with macrocycles that are approximately parallel, and whose pyrrole rings I overlap. The intradimer distance is much smaller than the other interchromophore distances, which are at least ~ 10 Å center-to-center; hence, the interaction between the two dimer-halves, denoted D_L and D_M, is thought to be considerably stronger than other interchromophore interactions. (The L and M subscripts refer to the poplypeptides to which the chromophores are bound.)

A correct understanding of the optical properties of the RC is a prerequisite for the interpretation of the results of time-resolved optical spectroscopy. Recently measured (ultra-) fast kinetics of laser flash-induced absorbance differences have been interpreted in conflicting ways [4–6]. Part of the problem is incomplete understanding of the interactions between the RC chromophores, and of their influence on the optical characteristics.

In quantum-chemical calculations of the BChl dimer, its optical properties are described in terms of charge-transfer and localized $\pi\pi^*$-excitations. Two linear combinations of the latter are commonly referred to as 'exciton' states [7]; if D_L and D_M were strictly equivalent then these states would correspond to the symmetric and antisymmetric linear combinations of the localized excitations. Detailed quantum-chemical calculations for the *entire* chromophore complement of the RC are not yet feasible, so that the spectral properties of the RC, including the singlet

Abbreviations: ADMR, absorbance-detected magnetic resonance; RC, reaction center; P, primary donor; T−S, triplet-minus-singlet; LD, linear dichroism; BChl, bacteriochlorophyll; BPh, bacteriopheophytin.

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ground-state absorption and Triplet-minus-Singlet (T − S) spectra, are usually calculated by merely considering the dipolar interactions between the excitations of the dimer and the two accessory BChls, B_L and B_M, located close to the dimer, and the two bacteriopheophytins, BPh_L and BPh_M, which are located next to B_L and B_M, respectively [8–10].

One way to gain insight in the interchromophore interactions is the study of T − S absorbance-difference spectra. In calculations of the T − S spectrum taking into account dipolar coupling between the Q_Y- ($S_1 \leftarrow S_0$) transition moments of the chromophores, characteristics of the triplet state such as the orientation of the triplet axes, the degree of triplet delocalization, the orientation of the triplet-triplet transition moments, and the interaction between the triplet-triplet transitions and the Q_Y-transitions, were put in as fit parameters [8–10]. From these calculations, it was concluded that for *Rps. viridis* the triplet is localized on one-half of the dimer, D_L [8], whereas for *Rb. sphaeroides* the triplet state was thought to be delocalized symmetrically over the two dimer-halves [9]. Not surprisingly, the use of so many fit parameters has led to different conclusions derived from fits of the same spectra with similar models [8,10].

In this communication we report on an investigation of the singlet and triplet absorption spectra in the visible spectral range of the RC of *Rb. sphaeroides* R26 with the aid of (linear-dichroic) absorbance-detected magnetic resonance ((LD)ADMR) for recording T − S and LD-(T − S) spectra. The results are compared with those obtained previously for the RC of *Rps. viridis* [8]. We also compare our results with recent work of Hartwich et al. [16], a preprint of which was transmitted to us during the preparation of this ms. We discuss the interactions between the dimer and the accessory BChls, and confront current theoretical interpretations of the T − S spectrum with our experimental data. We conclude from the polarizations obtained with LD-ADMR that current theories, which represent the interactions between the primary donor dimer and the accessory BChls solely by dipolar interactions between the Q_Y-transition moments, do not lead to an acceptable description of the T − S and LD-(T − S) spectrum. A more sophisticated treatment of the interchromophore interactions is needed for correctly describing the optical properties of the RC.

2. Experimental section

Reaction centers of *Rb. sphaeroides* R26, isolated as described [11], were dissolved in Tris buffer (pH = 8) containing 0.025% LDAO. Reduction of the first acceptor quinone was carried out by addition of 10 mM sodium ascorbate and illumination of the samples during cooling. Glycerol was added to the reaction center solutions to a concentration of 66% v/v to prevent cracking upon freezing. The samples had an optical density of ~0.35 in a 2-mm perspex cuvette at 800 nm at room temperature. All measurements were carried out at approx. 1.5 K.

The ADMR set-up was basically the same as described in Ref. [12]. Continuous white light from a tungsten-iodine lamp was used for excitation and for probing the transmittance. The light transmitted by the sample was detected by a spectrometer (resolution set at 3 nm) equipped with a Peltier-cooled photodiode. A tunable split-ring cavity (Q ≈ 100), connected to a microwave source, was placed around the sample cuvette, such that the direction of the microwave magnetic field was perpendicular to the light beam. The modulated change in transmitted light intensity ΔI, due to amplitude modulation of the microwaves at a frequency of 312 Hz, was phase-sensitively detected with a lock-in amplifier. The absorbance-difference (ΔA) signal

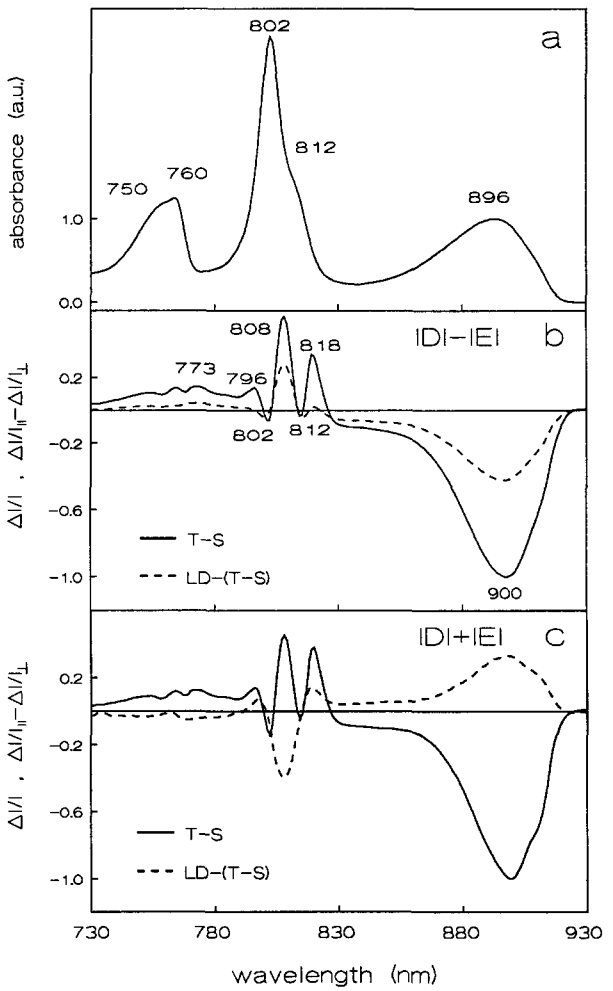

Fig. 1. The Q_Y-region of the absorption spectrum (a) and the microwave-induced T − S and LD-(T − S) absorbance-difference spectra (b,c) of *Rb. sphaeroides* R26. For the T − S and LD-(T − S) spectra, the microwave frequency was set at 467 MHz (|*D*| − |*E*|) (b) and 658 MHz (|*D*| + |*E*|) (c), and was amplitude-modulated at 312 Hz. The optical resolution was set at 3 nm. The subscripts ∥ and ⊥ refer to parallel and perpendicular to the direction of the microwave magnetic field. The extrema (in nm) in the T − S spectra are indicated in (b), and hold also for (c).

is to a good approximation proportional to the difference in transmitted intensity divided by the total transmitted light intensity I [12], which was simultaneously recorded. A photoelastic modulator (PEM) and a Glan-Thompson polarizer were placed between the sample and the monochromator for analyzing the change in transmittance of the sample for light polarized parallel and perpendicular to the direction of the microwave magnetic field. The linear-dichroic ΔA signal was recorded by lock-in detection of the transmittance using two lock-in detectors in series. The first one was locked at twice the modulation frequency of the PEM (100 kHz), while the second one was locked at the modulation frequency of the microwaves.

ΔA spectra were recorded at a fixed microwave frequency by slowly scanning the wavelength of absorption with the polarizer parallel and perpendicular to the microwave magnetic field, and averaged afterwards. The anisotropy ratio of an absorption band in the ΔA spectrum is defined as [13,14]:

$$R_i = \frac{LD - (T-S)}{T-S} = \frac{\Delta A_{\parallel} - A_{\perp}}{A_{\parallel} + A_{\perp}} = \frac{(3\cos^2\alpha_i - 1)}{(3 + \cos^2\alpha_i)}, i = x, y, z. \quad (1)$$

where α_i is the angle between the triplet i-axis, $\vec{i}_T$, and the optical transition moment. The R-value was obtained by extrapolating the T − S and LD-(T − S) signals to zero microwave power, as saturation effects (similar to those in photoselection measurements) occur at finite microwave powers.

The R-values obtained for two (or three) different microwave transitions, which are mutually perpendicular, yield eight possibilities for the orientation of an optical transition moment in the $\vec{x}_T,\vec{y}_T,\vec{z}_T$ triplet axes frame. This leads to four possible relative orientations of two different optical transition moments (within 90°). When both optical transition moments are polarized in the triplet $\vec{x}_T,\vec{y}_T$-plane, the number of relative orientations reduces to two.

3. Results

The T − S and LD-(T − S) spectra of isolated RCs of *Rb. sphaeroides* R26 and the simultaneously recorded absorption spectrum, are shown in Fig. 1 for the Q_Y-($S_1 \leftarrow S_0$) absorption region of the BChls and BPhs. The T − S and LD-(T − S) spectra were recorded at microwave frequencies of 467 MHz and 658 MHz, corresponding with the frequencies of the $|D| - |E|$ and $|D| + |E|$ transitions of the triplet state of P, ^{3}P, respectively, where $|D| - |E|$ ($|D| + |E|$) represents the splitting between the x- (y-) and z-sublevels of ^{3}P (level ordering for D, $E > 0$).

The long-wavelength side of the absorption spectrum is characterized by a broad $S_1 \leftarrow S_0$ absorption of the RC, which is mainly composed of the Q_Y-absorptions of the dimer-BChls. The absorption in the 790–820 nm region is generally believed to be composed of the absorptions of the accessory BChls, B_L and B_M, with a contribution of the high-energy exciton-absorption of the dimer [7,9,15–17]. The absorption in the 750–760 nm region consists of almost pure Q_Y-absorptions of the BPhs [18].

The T − S spectra are characterized by a negative absorbance-difference signal between 820–930 nm, reflecting the increase in ground-state absorbance of P upon applying resonant microwaves. In the region of the accessory BChls (790–820 nm), there are two relatively strong positive bands, centered at 808 and 818 nm, which overlap with negative bands at 802 and 812 nm, the latter corresponding with the absorption bands in the ground-state

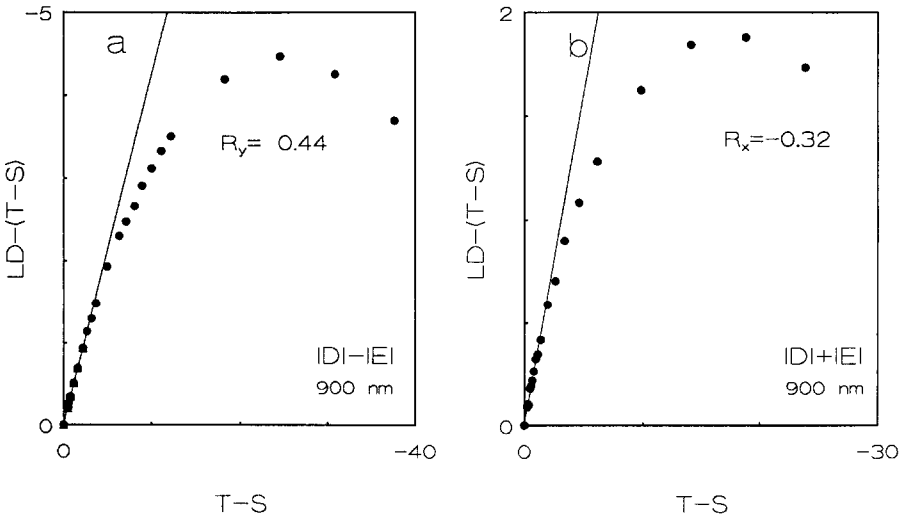

Fig. 2. The amplitude of the LD-(T − S) signal plotted against that of the T − S signal at a detection wavelength of 900 nm, recorded as a function of the microwave power (~ 0.01–50 mW at the microwave source). The microwave frequency was set at 467 MHz ($|D| - |E|$) (a), and at 658 MHz ($|D| + |E|$) (b). The R-value is given by the slope of the straight line obtained with a linear least-square fit through the data points for low microwave powers.

absorption spectrum. These features may be due to band shifts and/or intensity changes of the 802 and 812 nm absorptions, arising from a change in interaction between P and the accessory BChls upon ^{3}P formation.

To obtain more information about the origin of the T − S signals in the 790–820 nm region, the orientations of the various transition moments contributing to the T − S spectrum were determined relative to the $\vec{x}_T,\vec{y}_T,\vec{z}_T$ triplet-axes frame of ^{3}P. First the R-values were determined for the long-wavelength band at the $|D| \pm |E|$ zero-field transitions by recording the T − S and LD-(T − S) signals at 900 nm as a function of microwave power and plotting the LD-(T − S) signal amplitude against the T − S signal amplitude (Fig. 2). The data points at low microwave powers (~ 0.01–0.1 mW at the microwave source), for which the R-value, within the signal-to-noise, is independent of the microwave power, yield the values of α_i. In Fig. 2 this is reflected by the asymptotically linear dependence of the LD-(T − S) signals on the T − S signal at low powers. The slope of the asymptote was fitted with a linear least-square method taking only the points at low microwave power. The number of points participating in the fit was determined by increasing the number of points until the standard deviation of the fit increased significantly. The resulting angle between the Q_Y-transition moment at 900 nm and $\vec{y}_T$ and $\vec{x}_T$ is 18 ± 3° and 85 ± 5°, respectively, where the error corresponds to the least-square deviation for the slope determined above.

For the positive 818 nm band, which is relatively isolated, the values of α_y and α_x are both equal to 45 ± 5°. For other wavelengths, the positive and negative absorptions in the T − S spectrum overlap strongly, and therefore values of the $\alpha_{x,y}$'s could not be obtained directly. They were obtained from a deconvolution of the LD-(T − S) spectra, recorded at relatively high microwave power (~ 1 mW) and normalized on the corresponding T − S spectra using the R-values determined at 900 nm. As a first step in this procedure, the absorption spectrum of Fig. 1 was deconvoluted with four Gaussian bands in the 790–900 nm region, centered at ~ 802, ~ 812, ~ 800-820 and ~ 900 nm. The latter two Gaussian bands were used for a fit of the long-wavelength absorption band. Then, keeping fixed the fit parameters of these bands, which correspond to the negative bands of the T − S spectrum, the positive part of the T − S spectrum was deconvoluted.

Because the shape of the T − S spectrum depends somewhat on the microwave frequency [12], we varied the widths and the positions of the negative Gaussians in the T − S spectrum over a range of a few nm, leaving the positions of the positive Gaussians as free parameters. We then allowed the width of the positive Gaussians to deviate a few nm from that of the negative bands for optimizing the fit. In this way, we find three positive Gaussian bands in the T − S spectrum, centered at 818 ± 1, 808 ± 1 and 801–798 nm, the position and intensity depending on the widths chosen. Because the inhomogeneous linewidths

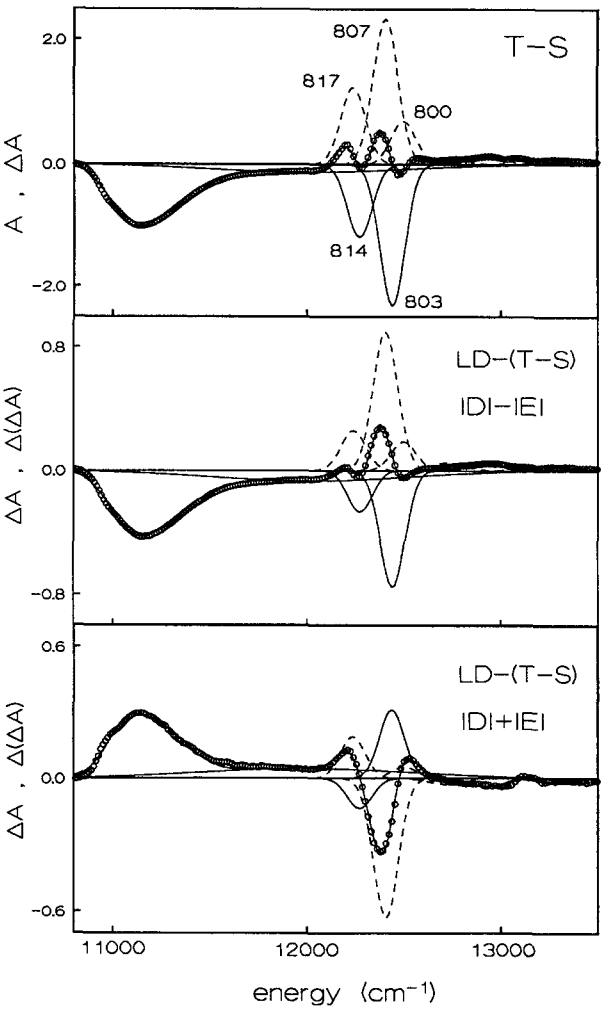

Fig. 3. Gaussian deconvolution of the microwave-induced T − S (a) and LD-(T − S) (b,c) spectra. The T − S spectrum was normalized on the intensity of the long-wavelength band of the corresponding absorption spectrum. The dots and the solid line through the dots represent the experimental spectra and the Gaussian fits, respectively. Gaussians with solid lines represent the deconvolution of the ground-state absorption spectrum, which corresponds with the negative, 'singlet' part of the T − S spectrum. The Gaussian bands with positive amplitude in the T − S spectrum, the 'triplet' part, are represented by dashed lines.

probably do not change upon ^{3}P formation, we assumed that the widths of the 808 and 818 nm Gaussian bands are similar to those of the negative 802 and 812 nm bands. One of the possible deconvolutions is depicted in Fig. 3. We stress that, for an acceptable fit of the 790–820 nm region, we always need two negative and three positive Gaussians, and that the allowed variation in position and width of these Gaussians is only 2 nm and 10%, respectively.

The R-values of the positive and negative bands in the Gaussian deconvolution of the T − S spectrum were obtained subsequently by deconvoluting the LD-(T − S) spectra, while keeping the band positions and widths equal to those of their corresponding T − S spectra. The polar-

Table 1
Positions (λ_{max} in nm) of the absorption bands in the T − S spectrum and the angles (in degrees) between the Q_Y-transition moments and the in-plane triplet axes of ^3P (α_x, α_y), or the LD-orientation axis (α_{LD})

Rb. sphaeroides				*Rps. viridis* [b]			
λ_{max} [c]	$\lvert\alpha_y\rvert$	$\lvert\alpha_x\rvert$	$\lvert\alpha_{LD}\rvert$ [a]	λ_{max}	$\lvert\alpha_y\rvert$	$\lvert\alpha_x\rvert$	$\lvert\alpha_{LD}\rvert$ [a]
900 (−)	18 ± 3	85 ± 5	90	1000 (−)	15 ± 5	72 ± 10	90
818 (+)	45 ± 5	45 ± 5		870 (+)	80 ± 10	10 ± 10	
812 (−)	41 ± 5	48 ± 5	50	850 (−)	80 ± 10	10 ± 10	25
808 (+)	25 ± 5	75 ± 5		840 (+)	41 ± 5	50 ± 3	
802 (−)	30 ± 10	65 ± 10	70	835 (−)	25 ± 10	65 ± 10	70
800 (+)	30 ± 10	65 ± 10		820 (+)	5 ± 10	85 ± 10	
				820 (−)	−	−	70

[a] The values of $\lvert\alpha_{LD}\rvert$ are taken from the LD-absorption data from Breton [15,22].
[b] The values of $\lvert\alpha_{x,y}\rvert$ for *Rps. viridis* are extracted from the experimental LD-ADMR data of Lous and Hoff [8].
[c] Average of acceptable fits as in Fig. 3 (see text).
The sign between brackets, + (or −), corresponds with a positive (or negative) signal in the T − S spectrum.

izations of the 818 and 808 nm bands did not vary more than 5° and 10°, respectively, when varying the positions or the widths of the positive and negative absorption bands in the 790–820 nm region within the limits for acceptable spectral fits (see above). The polarization of the negative 812 nm band, which overlaps with the 818 and 808 nm bands, varied only 5° with the various deconvolutions. For all deconvolutions, we found that the negative 812 nm band has a polarization about equal to that of the 818 nm band, corresponding with $\lvert\alpha_y\rvert$ of 41 ± 5° and $\lvert\alpha_x\rvert$ of 48 ± 5°. The results are presented in Table 1, together with results of Breton et al. from LD-absorption experiments [15]. In the latter work the long-wavelength band of P was assumed to be polarized perpendicular to the C_2 axis of the RC, the latter taken to be the orientation axis in the LD-absorption measurements [15]. Inspection of Table 1 shows that the R-values of the 802(−) and 808(+) nm bands, and of the 812(−) and 818(+) nm bands, are the same within experimental error, whereas those of the negative 802 and 812 nm bands (and of the positive 808 and 818 nm bands) differ from each other (see also Fig. 1). The 808 and 812 nm features, therefore, constitute two separate positive bands, which we interpret as parts of band shifts of the 802 and 812 nm bands (see also below).

The numerical results from Table 1 are visualized in a polar diagram in Fig. 4. In Fig. 4a, the direction of the N_I-N_{III} and N_{II}-N_{IV} axes of the individual chromophores are shown with respect to the dimer axes, $\vec{x}_D$ and $\vec{y}_D$, using the X-ray data for the RC of *Rb. sphaeroides* R26 [2]. The dimer axes $\vec{y}_D$ and $\vec{x}_D$ are taken parallel to the vector sums of the N_I-N_{III} and N_{II}-N_{IV} axes of the two dimer-halves, respectively. $\vec{x}_D$ is approximately parallel to the C_2-symmetry axis of the RC. The Q_Y-transition moments of BChl and BPh are assumed to be parallel to the axis running through the nitrogen atoms N_I-N_{III}, and the Q_X-transition parallel to the N_{II}-N_{IV} axis. In Fig. 4b the transition moments resulting from the LD-ADMR experiments are drawn with respect to $\vec{x}_T$ and $\vec{y}_T$, as determined from single-crystal EPR experiments by Norris et al. [19]. From Table 1 we see that for each transition the sum $\lvert\alpha_x\rvert + \lvert\alpha_y\rvert$ is approx. 90°. Therefore, all transitions are oriented approximately in the $\vec{x}_T\vec{y}_T$-plane. This result,

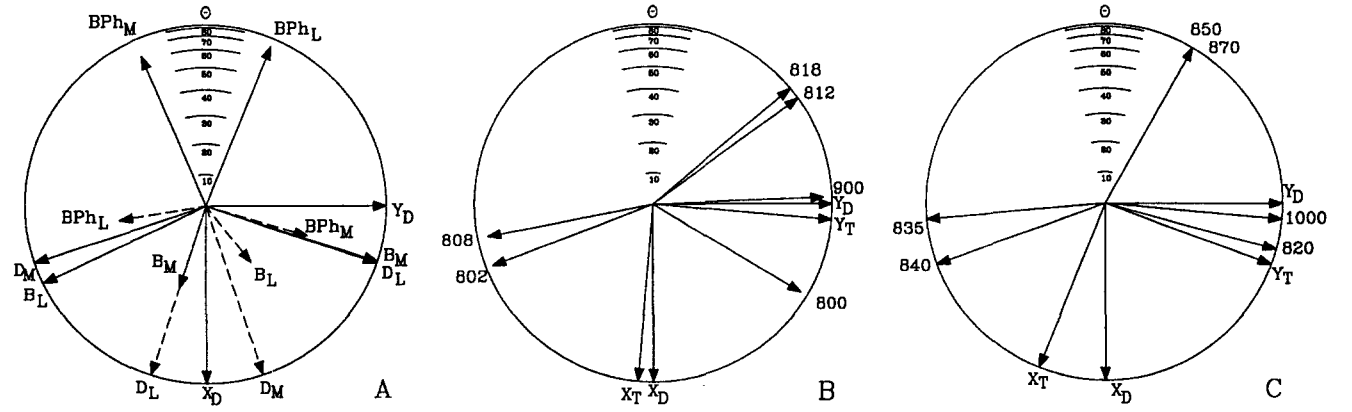

Fig. 4. Polar plot of the reaction center transition moments. (A) Projection of the directions of the N_I-N_{III} (solid lines) and the N_{II}-N_{IV} axes (dashed lines) on the $\vec{x}_D\vec{y}_D$-plane of the dimer derived from the crystal structure. Note that these axes are bidirectional vecs. (B,C) The orientations of the Q_Y-transition moments with respect to $\vec{x}_T$ and $\vec{y}_T$ are the orientations of the triplet axes in the RC coordinate frame as found from single-crystal EPR experiments. The C_2-symmetry axis of the reaction center is assumed to be parallel to $\vec{x}_D$.

which is independent of the deconvolution within the limits discussed above, leads to two possible orientations ($\pm\alpha_i$) of the transitions in the $\vec{x}_T\vec{y}_T$-plane. For clarity, only one of the two possible is shown in Fig. 4b.

Using the orientations of the triplet axes found by Norris et al. [19], we find that the angle between the 812 nm transition and the C_2 axis is 48°. This value agrees well with the angle of approx. 50° [15], found with linear-dichroic absorption spectroscopy when assuming that the long-wavelength band is polarized perpendicular to the C_2 axis. Note that with the orientation of the triplet axes of Ref. [19] the long-wavelength band according to our analysis indeed is polarized almost perpendicularly to the C_2 axis ($|\alpha_x| = 85 \pm 5°$). The agreement between the two independent experiments gives us confidence to take for the polarization of the negative 802 nm band, for which we did not obtain accurate values of $\alpha_{x,y}$, the linear-dichroic absorption data [15], which yields an angle of about 70° with respect to $\vec{x}_T$.

Although the positive bands at 808 and 818 nm in the T − S spectrum are both oriented in the $\vec{x}_T\vec{y}_T$-plane, they have a rather different polarization in this plane. The polarization of the 818 nm band is very close to that of the 812 nm band, from which we conclude that the 818 nm band represents a shift to lower energies of the 812 nm transition. The positive 808 nm band has a polarization similar to that of the negative 802 nm band, and quite different from that of the 812 and 818 nm bands. Therefore, most likely the 808 nm band represents a shift to lower energy of the 802 nm transition upon ^{3}P formation. These conclusions are supported by the T − S spectra of chemically modified RCs involving the modification of the accessory BChls $B_{L,M}$ [16,20,21]. For these RCs a loss of dipolar strength at 812 and 802 nm in the absorption spectrum is accompanied by a loss of dipolar strength at 818 and 808 nm in the T − S spectrum.

In the T − S spectrum of *Rps. viridis*, a positive band has been observed at 870 nm, which is, just as the positive 818 nm in the T − S spectrum of *Rb. sphaeroides*, located at the low-energy side of the absorption of the accessory BChls [8], whereas a negative band at 850 nm corresponds with the 812 nm feature of *Rb. sphaeroides*. For *Rps. viridis* the peak positions in the absorption and T − S spectra, the values for $\alpha_{x,y}$ [8], and the orientation with respect to the approximate C_2 axis from LD-absorption spectroscopy [22], are included in Table 1. We have plotted the experimental results of Lous and Hoff [8] for *Rps. viridis* in Fig. 4c with the orientations of the triplet axes found by Norris et al. [19]. For *Rps. viridis*, the polarizations of the positive 870 and 840 nm bands in the T − S spectrum are close to those of the negative 850 and 835 nm bands, respectively. By analogy with the 818 and 808 nm bands in the T − S spectrum of *Rb. sphaeroides*, we ascribe the positive 870 and 840 nm bands to a shift to lower energy of the 850 and 835 nm bands in the absorption spectrum, respectively.

In both the absorption and the T − S spectrum of *Rps. viridis* an absorption band at ~ 820 nm has been observed [8,22]. The deconvolution of the T − S spectrum of *Rb. sphaeroides* yields a clear, positive band at ~ 800 nm. By analogy with the 820 nm band in the T − S spectrum of *Rps. viridis*, the 800 nm band may represent a band shift and/or an increase of absorption of a band in the ground-state absorption spectrum, which for *Rb. sphaeroides* is not resolved. The 800 nm band disappears in the T − S spectrum of $B_{L,M}$-modified RCs [16,20], and therefore its contribution should be largely that of an accessory BChl. Thus, all three positive bands, at 800, 808, and 818 nm, should contain a large contribution of the Q_Y-transitions of the two accessory BChls.

4. Discussion

4.1. The orientation of the triplet axes of ^3P

From EPR measurements on single crystals of RCs of *Rb. sphaeroides* R26 and *Rps. viridis*, Norris et al. [19] have concluded that the triplet state of *Rb. sphaeroides* R26 is largely delocalized over the whole dimer, whereas for *Rps. viridis* the triplet state was thought to be localized on D_L. Delocalization of both the triplet (coherently or due to fast hopping) and singlet excitations for a system with strict C_2 symmetry implies that $\vec{x}_T$ is parallel to the C_2-symmetry axis and that the low-energy transition of P is oriented perpendicular to the C_2 axis and therefore perpendicular to $\vec{x}_T$. The polarization of the long-wavelength band of *Rb. sphaeroides* R26, which is well isolated from the $B_{L,M}$ transitions, is $18 \pm 3°$ and $85 \pm 5°$ with respect to $\vec{y}_T$ and $\vec{x}_T$, respectively. The latter angle is close to that expected for a delocalized triplet excitation. Because the sum of the angles with $\vec{x}_T$ and $\vec{y}_T$ exceeds 90°, the low-energy transition appears to be oriented somewhat out of the $\vec{x}_T\vec{y}_T$-plane, and, when taking the direction of the triplet axes found by Norris et al. [19], out of the dimer $\vec{x}_D\vec{y}_D$-plane. This out-of-plane orientation, and the sizeable angle between the singlet transition and $\vec{y}_T$ presumably reflects different electronic structures of the singlet and triplet excited states of the dimer, connected to deviations from strict C_2 symmetry.

4.2. The 790–820 nm region of the T − S spectrum

The composition of the absorption bands in the 790–820 nm region is subject to discussion. The 812 nm absorption has been ascribed (1) to an accessory BChl [4], (2) to an almost pure high-energy exciton transition of the dimer [22], (3) to the same transition overlapping with the Q_Y-transition of an accessory BChl [15], and (4) to the high-energy exciton transition of the dimer with *admixture* of the Q_Y-transitions of the accessory BChls [9]. In the latter three interpretations the $\pi\pi^*$-excitations (the Q_Y-transi-

tions) of D_L and D_M are coupled, giving rise to a low- and high-energy 'exciton' transition, P(−) and P(+), located at 900 and 812 nm, respectively [7,9]. For a symmetric dimer the transition moment of P(−) (or P(+)) is aligned along the bisectrix of the Q_Y-transition moments of D_L and D_M (or their complement). We will briefly discuss the four competing interpretations, and show that only the last one is compatible with the experimental evidence.

(1) Assuming that the Q_Y-transition dipole of BChl *a* is oriented parallel to its N_I-N_{III} axis, the polarization of the 812 nm transition as observed from LD-ADMR and LD-absorption spectroscopy [15] does not correspond to that of the Q_Y-transition of any uncoupled BChl in the RC (compare Fig. 4a and Fig. 4b). Even if the Q_Y-transition moment is not parallel to the N_I-N_{III} axis, but is oriented in the BChl macrocycle making a (small) angle with the N_I-N_{III} axis, then, with the known orientation of the triplet axes and the LD-orientation axis ($\sim C_2$ axis), the 812 nm transition cannot be a 'rotated' B_L or B_M-transition. (Although the Q_Y-transitions of $B_{L,M}$ are largely located in the $\vec{x}_T\vec{y}_T$-plane (Fig. 4a), the macrocycle planes of $B_{L,M}$ are not parallel to those of the dimer-BChls, as demonstrated by the orientation of the N_{II}-N_{IV} axes.) Therefore, the interpretation that the 812 nm band is a pure $B_{L,M}$ Q_Y-transition [4] can be excluded. (2) If the 812 nm band is a pure P(+) band, then ^3P formation would lead to the disappearance of the 812 nm transition rather than to a shift to lower energy. Therefore, we can also exclude this interpretation.

The two remaining interpretations, admixture or overlap of P(+) with the Q_Y-transitions of the accessory BChls, are consistent with the observation that the 900 and 812 nm transition moments are not perpendicular, as one would expect for 'pure' exciton components of a symmetric dimer. However, if the P(+) transition overlaps with the Q_Y-transition of an accessory BChl at 812 nm (3), then the T − S spectrum would be expected to show the disappearance of P(+) and a band shift of the Q_Y-transition of the accessory BChl upon ^3P formation. The positive band in the T − S spectrum, being part of the purported band shift, would then have a polarization close to that of the Q_Y-transition of B_L or B_M, and quite different from the average polarization of the two overlapping bands at 812 nm (Fig. 4a). In contrast, we find for the positive 818 nm band the same polarization as for the 812 nm band in the absorption spectrum. We therefore reject also interpretation (3).

Having excluded the first three interpretations, we must conclude that the 812 (and 818) nm transition has contributions of both the Q_Y-transitions of B_M and/or B_L and P. For the 850 and 870 nm transitions in the absorption and T − S spectrum of *Rps. viridis* the same reasoning can be applied. Interaction between the electronic states of B_L and B_M with one or more state(s) of the dimer must lead to at least three mixed transitions in the Q_Y-region of the accessory BChls. Although in the singlet absorption spectrum only two strong aborptions (at 802 and 812 nm) are observable, the third transition may be weak and/or overlap with the 802 nm band, by analogy with the 820 nm band in the absorption spectrum of *Rps. viridis* (see Section 3). In the positive part of the T − S spectrum, the 818, 808 and 800 nm bands may reflect these three transitions. The possible origin of the bands in the 790–820 nm region is discussed below.

4.3. The dipolar model of the interactions between P and $B_{L,M}$

Up to now, attempts to interpret theoretically the optical spectrum of the RC have employed some kind of dipolar coupling model (using point-dipoles [8], so-called extended dipoles [9,10] or point-monopoles [17]), sometimes combined with quantum-chemical calculations of the electronic transitions of the dimer [23]. None of these treatments, however, succeed in explaining all known aspects of the optical spectrum, including the various difference spectra. In this section we enumerate some of the most important discrepancies.

We first discuss the characteristic properties of the exciton model in which the dipolar interactions between the Q_Y-transitions of the six RC pigments is represented by the point-dipole (or extended-dipole) approximation [8–10]. Because the mutual coupling of the components of P is much stronger than their couplings to the other chromophores, the dimer transitions are commonly discussed in terms of the low- and high-energy exciton transitions, P(−) and P(+), respectively. P(−), absorbing at about 900 nm (1000 nm for *Rps. viridis*), is quite well isolated from other transitions. P(+), however, is thought to be located close to the Q_Y-transitions of $B_{L,M}$, and accordingly will couple to them.

4.3.1. Band shifts

Upon excitation to the triplet state ^3P – as opposed to the corresponding excited singlet state – there is no longer an exciton state that is near-degenerate with the excited states of $B_{L,M}$. Accordingly, one would expect that the $B_{L,M}$ Q_Y-transitions revert to pure $B_{L,M}$ transitions at energies close to that for an uncoupled BChl. Experimentally this is seen as band shifts accompanied with some loss/gain of oscillator strength. The extent and direction of the shifts depend on the transition energies chosen for the uncoupled chromophores, which in all calculations are taken as 'free' input parameters. For example, if the transition energy of P(+) is higher than the energies of the Q_Y-transitions of $B_{L,M}$, the $B_{L,M}$-transitions will 'shift' both to higher energies upon ^3P formation [9]. On the other hand, if the P(+) band is located at an energy equal or lower than those of the $B_{L,M}$ transitions, for example around 810 nm, then the 802 nm band, mixed with P(+), may shift to lower energy (808 nm) upon ^3P formation. The 812 nm transition, which in this example has a large

contribution of P(+), will collapse to 808 nm on excitation to ^{3}P. The latter situation is exemplified by the T − S spectrum of *Rps. viridis* calculated by Lous and Hoff [8], where the 835 nm band, the analogue of 802 nm transition of *Rb. sphaeroides*, shifts to lower energy (to 840 nm). Thus, the experimentally observed shifts to lower energies of *both* the 802 and 812 nm bands upon ^{3}P formation cannot be explained by the exciton model, irrespective of the choice of the energies of the uncoupled Q_Y-transitions. The same applies for the 835 and 850 nm bands in the T − S spectrum of *Rps. viridis*.

4.3.2. Polarizations

In any exciton calculation, the polarizations of the bands in the positive part of the T − S spectrum are found to change with respect to those in the negative part because of a change in coupling between the transitions of the accessory BChls and those of the dimer upon ^{3}P formation. However, a change in polarization of the transitions in the 790–820 nm region is not observed experimentally. For example, when assuming that the interaction between P and the accessory BChls decreases upon ^{3}P formation, the orientation of the transition moments contributing to the Q_Y-absorption region of $B_{L,M}$ will change into the orientation of the uncoupled Q_Y-transition moments of $B_{L,M}$. Then, the calculated change of the polarization of the 808 and 818 nm bands relative to the 802 and 812 nm bands (10–20° and 30–35°, respectively) disagrees with the experimentally determined polarizations, which differ by a few degrees at most.

4.3.3. Band intensities

The intensities of the transitions in the positive part depend on the degree of localization of the triplet states over the dimer-halves and on the mechanism of triplet delocalization (see also Section 4.4). If the triplet is localized on D_L (D_M), the appearance of a D_M (D_L) Q_Y-transition is expected upon ^{3}P formation. Admitting only a point dipole-dipole interaction between the Q_Y-transitions of the RC chromophores, the intensity of the transition having most B_L (B_M) character will then change upon ^{3}P formation. This is due to the almost (anti-) parallel alignment (relatively large dipolar interaction) of the Q_Y-transition dipoles of the two pairs B_M-D_L and B_L-D_M. (The B_M-D_M and B_L-D_L dipole-dipole interactions are negligible because of their mutual orientation and relatively large distance.) The relatively strong dipolar coupling of D_M (D_L) with B_L (B_M) will lead to a shift and to 'intensity-borrowing' of D_M (D_L) from B_L (B_M) or vice versa. Irrespective of the degree of triplet delocalization over the two dimer-halves, the total intensity in the 790–820 nm region should increase with about 50% (the dipolar strength of one extra BChl transition, neglecting possible hypochromic effects for the coupled dimer), whereas experimentally the total increase of dipolar strength in the 790–820 nm region is ~ 20%.

A calculation of the linear- and circular-dichroic absorption spectrum of the RC of *Rps. viridis*, using a point-monopole approximation and including intradimer charge-transfer states, has led to results that are very similar to those obtained using the point-dipole approximation [17]. This is not surprising because the interaction between P and $B_{L,M}$ is always so small that the transition energies of the mixed $B_{L,M}$ transitions are close to the energies of the uncoupled $B_{L,M}$ transitions. Therefore, in any approximation the values chosen for the Q_Y-transition energies of the uncoupled chromophores have to be similar, in order to obtain a good fit of the various spectra.

The optical absorption of the RC has also been calculated by first determining the dimer transitions with a quantum-chemical calculation, and then coupling them excitonically to the accessory BChls [23]. This calculation led to basically the same description for the 812 nm band (850 nm for *Rps. viridis*) as that in which just the dipolar couplings between the individual transition moments of all six chromophores were taken into account [8,9].

Because the analysis of the ground-state absorption spectrum in Refs. [17] and [23] lead to comparable electronic composition of the excited states as the point-dipole approximation [8], they lead to the same difficulties in the description of the (LD-) T − S spectra. We therefore conclude that, without further assumptions, *none* of the so-called exciton treatments can explain a shift to lower energies of both the 812 and 802 nm transitions, and the fact that the polarizations of the 812 and 802 nm bands remain equal, upon ^{3}P formation.

4.4. Absorption of the triplet state

4.4.1. Localized triplet states

One way to account for the positive 870 nm transition in the T − S spectrum of *Rps. viridis*, the analogue of the 818 nm transition for *Rb. sphaeroides*, is to abandon the interpretation that it represents a shift of the 850/812 nm transition, and to ascribe it to a new transition, for example a triplet-triplet absorption from the lowest triplet state to a higher excited triplet state, both located on D_L [8]. This particular interpretation, which presumes a localized triplet state, raises several difficulties. First, the absence of the 818 nm transition in the T − S spectrum of *Chloroflexus aurantiacus*, for which ^{3}P is also assumed to be localized [9], is hard to explain. Secondly, comparing the T − S spectrum of the RC with that of monomeric BChl *a* in glasses [24], the 818 and 870 nm bands seem much too narrow for a triplet-triplet absorption. Furthermore, the orientation of the near-infrared triplet-triplet transition moment of ^{3}BChl *a* was found to be parallel to the Q_Y-transition moment of ^{1}BChl *a*, in contrast to the polarization of the 870 (and 818) nm band, which is oriented at a large angle with the Q_Y-transition of D_L. Finally, we note that, as for monomeric BChl *a* in glasses, the T − S spectrum of the RCs shows a broad triplet-triplet absorption at shorter wavelengths, between 500–800 nm [12]. We con-

clude that neither the 870 nm transition (*Rps. viridis*) nor the 818 nm transition (*Rb. sphaeroides*) can be attributed to a localized triplet-triplet transition.

4.4.2. Delocalized triplet states

Exciton description. For *Rb. sphaeroides* R26, the triplet state has been proposed to be delocalized over the BChl-dimer [9,19]. The lowest triplet state of P is, in terms of the standard exciton model, described by the linear antisymmetric ('minus') combination of the two excitations $^3D_L^1D_M$ and $^1D_L^3D_M$, $^3P(-)$ (similar to the description of the lowest singlet excitation of P), possibly with contributions of the intradimer triplet charge-transfer (CT) states $^3(D_L^+D_M^-)$ and $^3(D_L^-D_M^+)$ [9,10,19]. The exchange interaction and on the site splitting between the $^3D_L^1D_M$ and $^1D_L^3D_M$ states determine the degree of delocalization of the triplet state over the dimer-halves. When neglecting the CT states, excitation of the $^3P(-)$ state to higher excited $^3P^*$ states, which are (linear combinations of) the higher excited states $^3D_L^1D_M^*$ and $^1D_L^{*3}D_M$, leads to two transitions, which are linear combinations of the Q_Y-transitions of D_L and D_M [10]. We will denote these linear combinations T(+) and T(−), although they do not necessarily have to correspond with the pure (anti-) symmetric linear combinations of a symmetric dimer. The splitting between T(+) and T(−), their polarizations and their intensities are determined by the exchange interaction and the site splitting between $^3D_L^1D_M$ and $^1D_L^3D_M$ (in ^3P), and that between $^3D_L^1D_M^*$ and $^1D_L^{*3}D_M$ (in $^3P^*$).

The positive part of the T − S spectrum was calculated by Scherer et al. [9] using this description of the triplet state, and assuming that the T(±) transitions and Q_Y-transitions of $B_{L,M}$ are coupled by a dipolar interaction. The CT contribution to the triplet state was neglected for the calculation of the T − S spectrum, because an estimated admixture of ~ 10–20% based on the *D*-value of ^3P was thought to weaken and broaden the T(+) and T(−) transitions somewhat, not to shift them. In the interpretation of Scherer et al. [9] the 818 and 773 nm bands are the T(+) and T(−) transitions, respectively, coupled to the Q_Y-transitions of $B_{L,M}$. In a similar interpretation by Lous et al. [25] and Greis [20], the positive 818 and 808 nm bands have been attributed to transitions resulting from a coupling of the T(+) and T(−), respectively, to the Q_Y-transitions of the accessory BChls.

The above interpretation can only explain the polarization of the 818 nm band if the T(+) transition is coupled to the Q_Y-transitions of $B_{L,M}$ in precisely the same way as P(+), because only then there is no change in polarization upon triplet formation. With regard to the T(−) transition: its oscillator strength is, depending on the exchange coupling between the dimer-halves and the site splitting, calculated to be between those of a monomer and the P(−) band. If it is located at 808 nm [20,25], it will couple strongly to the Q_Y-transitions of both accessory BChls, because of the small energy difference, and because its orientation is close to the Q_Y-transition moments of $B_{L,M}$ (that is, in between the Q_Y-transition moments of D_L and D_M (Fig. 4), depending on the degree of triplet delocalization), and to yield therefore more dipolar strength in the positive part of the T − S spectrum than experimentally observed. The latter problem is solved, of course, if T(−) would be located at 773 nm [9], that is, isolated from the $B_{L,M}$-transitions, but that would entail an unreasonably large exchange interaction between the dimer-halves. Summarizing, the treatment of Scherer et al. [9,10] necessitates that the dipole strength of the T(−) transition is negligible and that the transition energy of T(+) and its dipolar interaction with $B_{L,M}$ are equal to those of P(+) in the ground-state absorption spectrum. The first condition conflicts with the large T(−) dipole strength that follows from exciton calculations, whereas the latter would entail no changes in the electronic composition of the states upon ^3P formation, that is, no band shifts in the T − S spectrum.

Triplet hopping. An additional difficulty with the above interpretation is that, even for a T(−) transition located in the 800 nm region, it necessitates a splitting between T(+) and T(−) of at least 150–200 cm^{-1}, which would require an unusually large exchange interaction in ^3P and/or $^3P^*$. Therefore, in another attempt to explain the positive part of the T − S spectrum, the triplet was assumed to hop between the dimer-halves D_L and D_M rather than being a coherent exciton [20,25], i.e., that coherence in the triplet state is rapidly lost after formation. To first approximation, neglecting the $D_{L,M}$-$B_{L,M}$ interactions, a triplet state that hops between the two dimer-halves would lead to pure D_L and/or D_M Q_Y-transitions in the positive part of the T − S spectrum if the hopping frequency of the triplet state is lower than the difference in the frequencies of the optical transitions, and not to T(+) and T(−) transitions. When approximating the interactions between the dimer and the accessory chromophores by dipolar interactions, only the B_L-D_M and B_M-D_L interactions are important, because the polarizations of the Q_Y-transitions (the N_I-N_{III} axes) of $_L$ and B_M, and of D_M and B_L, are practically parallel (Fig. 4a). Therefore, a hopping triplet would lead to positive bands (i.e., the 808 and 818 nm bands [20,25]) with polarizations close to that of B_L (being parallel to D_M) and B_M (being parallel to D_L), that is, for *all* transitions in the 790–820 nm region to a polarization of ~ 20° and ~ 70° with respect to $\vec{y}_T$ and $\vec{x}_T$, respectively. Experimentally, we find quite different polarizations for the 818 nm band (45° with respect to $\vec{y}_T$ and $\vec{x}_T$ see Table 1). It follows that the hopping model, incorporating dipolar interactions between $D_{L,M}$ and $B_{L,M}$, cannot explain the LD-(T − S) spectra.

Recently, the positive bands at 808 and 818 nm in the T − S spectrum of *Rb. sphaeroides* have been partly ascribed to transitions of the dimer as discussed above [16]. This assignment was based on RCs with chemically modified accessory BChls, whose T − S spectrum shows two relatively weak positive bands at 805 and 815 nm. The

possibility that these bands are due to residual native RCs was rejected because the absorption maxima, 805 and 815 nm, were not exactly the same as those found in native RCs, 808 and 818 nm, respectively. In contrast, the LD-(T − S) spectra of the chemically modified RCs show exactly the same polarizations for the positive 805 and 815 nm bands as those for the 808 and 818 nm bands [16], strongly suggesting that the former bands do have the same origin as those in native RCs. As argued above, these polarizations deviate considerably from those expected for a hopping triplet, or for an excitonic delocalized triplet state. We therefore believe that the 805 and 815 nm bands of the chemically modified RCs represent bands of residual non-modified RCs that are shifted by about 3 nm because of a slightly altered environment, leading to smaller bandshifts upon ^{3}P formation, or simply because the low-intensity 808 and 818 nm bands of residual non-modified RCs are superimposed on the bands of the modified RCs. This interpretation is supported by the fact that the decrease of the 808 and 818 nm bands follows the decrease of the 802 and 812 nm bands when comparing different batches of modified RCs, in which the accessory BChls are substituted to a different extent [16]. Also, the band shifts in the 790–820 nm region of the T − S spectrum of borohydride-treated RCs [21], in which only B_M is replaced by a BChl-derivative [26], are somewhat smaller than the corresponding shifts for native RCs, which may be related to an altered coupling between P and one (or both) of the accessory BChls compared to native RCs.

Summarizing, in the T − S spectra of both *Rps. viridis* and *Rb. sphaeroides* there is no evidence for absorption of the dimer in the positive 'triplet' part of the T − S spectra, and merely band shifts occur upon ^{3}P formation. If these shifts were solely due to changes in pure dipolar coupling of transition moments, then it follows from the small amplitude of those shifts and the equality of the polarizations of the positive and negative bands in the 790–820 nm region that the dipolar couplings between the transition moments of the accessory BChls and the dimer do not change appreciably when the latter is excited to the triplet state. Because in any dipolar couplings scheme based on the RC structure and accepted values of transition energies and polarizations, the generation of ^{3}P does in fact lead to a substantial change of the dipolar couplings, and thus to important amplitudes of the difference bands in the T − S spectrum and to appreciable changes in their polarizations, it follows that the description of the optical transitions of the RC based on a dipolar coupling scheme cannot be correct. As we have shown above, this conclusion holds irrespective of the degree and mode (triplet exciton, incoherent triplet excitation hopping) of delocalization of the triplet state on the dimer-halves.

If dipolar couplings cannot be responsible for band shifts, then which other coupling could explain the observations? A possible candidate is vibronic coupling between the dimer BChls and the accessory BChls upon ^{3}P formation. Recently, it was reported by Vos et al. [27] that the transient absorption spectrum due to P* formation in the 790–820 nm region evolves on a femtosecond time scale, that is, within the vibrational relaxation time. This suggests that vibronic coupling may be important for the interaction between the RC chromophores. A change in vibronic coupling upon ^{3}P formation might shift the energies of the electronic transitions of the accessory BChls slightly without changing the polarizations.

5. Conclusions

With LD-ADMR we have determined the orientations of the optical transition moments contributing to the T − S spectrum of RCs of *Rb. sphaeroides* R26. These results link the results of single-crystal EPR and LD-absorption spectroscopy, thereby fixing the orientations of the optical transition moments in the RC-coordinate frame. The sizeable angle between the $S_1 \leftarrow S_0$ transition moment of P and its triplet *y*-axis presumably reflects different electronic structures of the singlet and triplet excited states of P, connected to deviations from strict C_2 symmetry.

The Q_Y-absorptions of the accessory BChls of RCs of *Rps. viridis* and *Rb. sphaeroides* shift upon ^{3}P formation to lower energies, without a change in polarization. The small shifts and the unaltered polarizations indicate that the coupling between P and the accessory BChls is relatively weak. There is no evidence for the appearance of absorption of the dimer BChls in the positive part of the T − S spectrum upon ^{3}P formation, which implies that the exciton description of the triplet state fails to explain the positive part of the T − S spectra.

We conclude that a description of the electronic transitions solely based on dipole-dipole coupling between P and the accessory BChls (in a point-dipole, extended-dipole or point-monopole approximation) cannot explain the T − S and LD-(T − S) spectra, irrespective of the degree of triplet (de)localization over the dimer-halves. All theoretical interpretations of the absorption spectrum reported in the literature fail to predict a shift to lower energies of the bands in the Q_Y-absorption region of the accessory BChls upon triplet formation, and cannot explain the observed polarizations. The similarity of the T − S spectra of two types of RCs, *Rb. sphaeroides* and *Rps. viridis*, suggests that this failure is not limited to one particular RC, and that a more complete description of the electronic (singlet and triplet) states of the RC chromophores is necessary. Such a description of the optical spectra would require extensive quantum chemical calculations including, for example, vibronic coupling between all RC chromophores, and, likely, their protein environment.

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