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Linear-dichroic triplet-minus-singlet absorbance difference spectra of reaction centers of the photosynthetic bacteria *Chromatium vinosum*, *Rhodopseudomonas sphaeroides* R-26 and *Rhodospirillum rubrum* S1

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For the first time, linear-dichroic triplet-minus-singlet (LD-(T − S)) spectra of reaction centers of the photosynthetic bacteria *Chromatium vinosum*, *Rhodopseudomonas sphaeroides* R-26 and *Rhodospirillum rubrum* S1 have been measured using an extension of the technique of absorbance-detected magnetic resonance (ADMR) of the triplet state. For all bacteria studied the LD-(T − S) spectra exhibit a bleaching of the long-wavelength absorbance band that is either split or has a clear shoulder to longer wavelengths. The components are approximately parallel-polarized, indicating that they do not form an exciton pair. Around 800 nm a band appears with a width of about 7 nm, which does not form part of a band shift and that may be attributed to an appearing monomer band. Small features in the LD-(T − S) spectra at both sides of this band are well explained by band shifts of the two components of the 800 nm reaction center absorption band. The transition moment of the component at about 818 nm in reaction centers of *Rps. sphaeroides* R-26 is at an angle larger than 55° with both the x and the y triplet spin axes. In none of the bacteria do we find evidence for the bleaching of an exciton component of P-860 near 810 nm.

Introduction

Recently, we have introduced the technique of absorbance-detected magnetic resonance (ADMR) of the triplet state for the measurement of triplet-minus-singlet absorbance difference (T − S) spectra [1]. The technique has been applied to reaction centers of the photosynthetic bacteria *Rhodopseudomonas viridis* and *Rhodopseudomonas sphaeroides* R-26 [1–3] and to subchloroplast particles of Photosystems I and II of plants [4,5]. It has proved possible to extend the technique for the measurement of polarized triplet-minus-singlet absorbance difference (LD-(T − S)) spectra, using linear-dichroic absorbance-detected magnetic resonance (LD-ADMR) [6].

In this communication we report on the T − S and LD-(T − S) absorbance difference spectra of reaction centers of the photosynthetic bacteria *Chromatium vinosum*, *Rhodospirillum rubrum* S1 and on the LD-(T − S) spectrum of *Rps. sphaeroides* R-26. It is shown that in *Rps. sphaeroides* R-26 and *C. vinosum* the long-wavelength absorption band is made up of two components that have approximately the same polarization. For all bacteria there is a band appearing at about 800 nm (807 nm for *Rps. sphaeroides* R-26 and *R. rubrum*, 804 nm for *C. vinosum*) which

Abbreviations: ADMR, absorbance-detected magnetic resonance; P-860, generic label for the primary donor; T−S, triplet-minus-singlet; BChl *a*, bacteriochlorophyll *a*.

cannot form part of a band shift. The LD-(T − S) spectra give additional evidence that there is no bleaching of an exciton component of P-860 (absorbing at 890 nm at cryogenic temperatures) in this wavelength region. Small features at both sides of the appearing bands are well interpreted as band shifts of the two components of the 800 nm absorption band. The LD-(T − S) spectra reinforce our earlier interpretation of the 807 nm band in the T − S spectrum of *Rps. sphaeroides* R-26 and by analogy of the corresponding bands in *R. rubrum* and *C. vinosum,* that it is an absorption band of a monomeric BChl that appears because of the formation of the triplet excited state of the primary donor. Furthermore, they confirm our conclusion that the two accessory B800 pigments are equivalent with respect to the strength of their interaction with P-860.

Materials and Methods

ADMR was performed as in Ref. 7, ADMR-monitored T − S spectra were recorded as in Ref. 1. The technique of LD-ADMR is described in Ref. 6. Briefly, triplet states are photoinduced by a beam of unpolarized white light with in principle an axially symmetric spatial distribution. In practice, because of energy transfer among reaction center pigments, the distribution of excited triplet states is approximately isotropic. Transitions between the triplet sublevels are induced by linearly polarized microwaves. It can be shown [8] that any particular microwave transition in zero magnetic field is polarized along one of the spin axes of the triplet state. Hence, the microwave transition probability is proportional to $|B_1|^2 \cos^2\beta$, where β is the angle between the direction of the microwave field B_1 and one of the spin axes, which is selected by the particular ADMR transition used. The resonant microwaves, therefore, select an oriented slice out of the isotropic distribution of the triplet states, for which an oriented change in the concentration of triplet states is produced [6,7]. This means that, at those wavelengths where the creation of the triplet-excited state of the primary donor results in a change in the absorbance of the sample, the transmitted light becomes partially polarized. The ellipticity is detected via a Morvue Hinds FS4 photoelastic modulator and an analyzing polarizer and the difference spectrum $((T - S)_{\parallel} - (T - S)_{\perp})$ recorded, where $\parallel$ and $\perp$ refer to the directions of the electric light vector parallel and perpendicular to the magnetic vector of the microwave field, respectively.

Reaction centers of *Rps. sphaeroides* R-26, *R. rubrum* S1 and *C. vinosum* were prepared as described in Refs. 9–11, respectively. Reduction was carried out as described in Ref. 1; for *C. vinosum* the same method was followed as for *Rps. viridis* to prevent accumulation of the reduced intermediate acceptor by illumination at low temperature. The samples were diluted to 70% (v/v) ethyleneglycol and slowly frozen to form a clear glass. The experiments were performed at 1.5 K in order to slow spin-lattice relaxation between the triplet sublevels.

Results and Discussion

Zero-field parameters

In zero magnetic field the degeneracy of the three energy levels of the triplet state is lifted by the dipole–dipole interaction between the two unpaired electrons. The energies of the three sublevels are the principal values of the dipole–dipole interaction tensor, i.e., the diagonal elements $-X$, $-Y$ and $-Z$ of this tensor in its principal axes (x, y, z) system, where for monomeric chlorophylls x and y are in the plane of the macrocycle and z is perpendicular to this plane. As the tensor is traceless, $X + Y + Z = 0$, and the energies of the x, y and z levels are commonly expressed in the two independent so-called zero-field splitting parameters D and E. The relative order of the x, y and z levels depends on the sign of D and E. For *R. rubrum,* and by inference for the other bacterial reaction centers, D is positive [12] and the z level is the lowest. As the sign of E for the primary donor triplet has as yet to be determined, the relative order of the x and y levels is unknown. It is customary to take the x level highest ($E < 0$). Then the energy separation between the z and y levels is $|D| - |E|$, and that between the z and x levels is $|D| + |E|$.

Of the three possible zero-field microwave transitions, in bacterial reaction centers only the $|D| + |E|$ and the $|D| - |E|$ transitions show measurable intensity in a single resonance experiment. (For a

review of zero field resonance in bacterial photosynthesis, see Ref. 13.) From the transition frequencies at resonance the absolute values of D and E can be accurately measured. Since these values are a sensitive probe of the environment of the triplet state, they form a useful check on the intactness of the isolated reaction center compared to that in whole cells and chromatophores.

In Table I the microwave transition frequencies and the $|D|$ and $|E|$ values of the reaction centers of the three organisms studied in this work are collected. They are virtually identical to those earlier obtained with fluorescence-detected zero field resonance on whole cells and chromatophores [14,15], indicating that the isolation procedures leave the structure of the primary donor and its immediate surroundings intact.

Triplet-minus-singlet spectra

The application of microwaves resonant between two triplet sublevels changes the concentration of the triplet state. This allows the measurement of triplet-minus-singlet (T − S) optical absorbance difference spectra [1]. For unpolarized microwaves applied to a random sample, one expects an isotropic change in concentration, regardless of the resonant transition, and consequently one expects the T − S spectrum to be identical for the two ADMR transitions observed in bacterial reaction centers. In an actual experiment small differences in the peak wavelength of the long-wavelength absorbance difference band remain. This can be attributed to so-called site effects [2,3]. The ADMR microwave transitions are inhomogenously broadened [14], and excitation with a sharply defined microwave frequency selects a particular slice out of the distribution of $|D|$ and $|E|$ values that together make up the ADMR lines. It has been demonstrated that especially the peak wavelength of the long-wavelength absorption band of reaction centers is sensitive to the microwave frequency within one ADMR transition [2,3]. As there is no one-to-one correspondence between the distribution of $|D|$ and $|E|$ values selected for excitation within either the $|D|+|E|$ or the $|D|-|E|$ transition, the long-wavelength absorbance difference band will generally be slightly different for these two ADMR transitions. All other bands, however, are virtually identical.

For LD-ADMR where the microwaves are linearly polarized, the situation is totally different. The microwave field now introduces a direction in the random sample in the same way as a polarized light beam does this in a photoselection experiment. Hence, an oriented changes in triplet concentration is produced, and, similar to photoselection, the intensity of a particular optical transition in the T − S difference spectrum becomes proportional to $(1-3\cos^2\theta)$, where θ is the angle between the optical transition moment and the spin axis corresponding to the monitoring microwave frequency (Fig. 1). Recording the T − S spectrum with an analyzer parallel and perpendicular to the direction of the microwave field then yields the $(T-S)_{\parallel}-(T-S)_{\perp}$ or LD-(T − S) spectrum [6].

The direction of the microwave transition moment corresponding to the $|D|+|E|$ microwave transition between the z- and the x-axis ($E<0$) is polarized along the y-spin axis and that of the $|D|-|E|$ transition between the z- and the y-axis ($E<0$) is polarized along the x-spin axis. Because the orientational distribution function $1-3\cos^2\theta$ for a particular optical transition moment will generally be different for the two perpendicular microwave transition moments of the $|D|+|E|$ and the $|D|-|E|$ transitions, the LD-(T − S) spectrum

TABLE I

MICROWAVE TRANSITION FREQUENCIES AND ZERO-FIELD SPLITTING PARAMETERS OF THE TRIPLET STATE OF THE PRIMARY DONOR OF ISOLATED REACTION CENTERS MEASURED WITH THE ADMR METHOD

Species	ν_1 (MHz)	ν_2 (MHz)	$\lvert D\rvert$ ($\times 10^{-4}$ cm^{-1})	$\lvert E\rvert$ ($\times 10^{-4}$ cm^{-1})
Rps. sphaeroides R-26	468	660	188.0 ± 0.4	32.0 ± 0.4
R. rubrum	465	668	188.8 ± 0.4	33.8 ± 0.4
C. vinosum	434	637	178.5 ± 0.4	33.8 ± 0.4

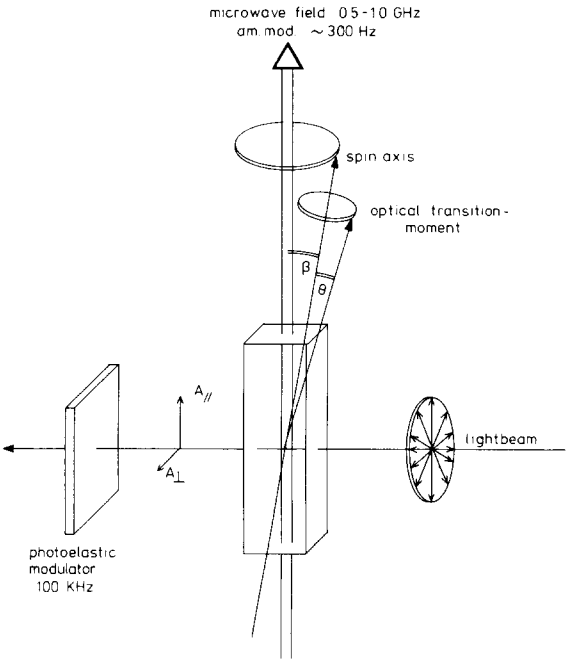

Fig. 1. Configuration of experimental set-up for LD-ADMR spectroscopy.

will generally be different for the two microwave resonances. It is this characteristic of the LD-ADMR technique, viz. that one obtains dichroic spectra with excitation in two mutually perpendicular transition moments, that offers a special advantage over the more common singlet photoselection technique. It makes it easier to discriminate between overlapping optical transitions, and there is less ambiguity in determining the orientation of optical transition moments.

In this communication we wish to demonstrate that the LD-(T − S) spectra recorded at the two ADMR microwave frequencies are of considerable help in unraveling the T − S spectrum. In principle, of course, quantitative LD-ADMR experiments allow one to extract the relative orientation of the various optical transition moments in the T − S spectrum. For the present purpose, however, we will confine ourselves to an analysis of the qualitative aspects of the LD-(T − S) spectra. To this end, it is important to note that the sign of the T − S spectrum determines whether a feature is a bleaching or an appearing band, but that in the LD-(T − S) spectrum the sign of a particular band is determined by the product of the sign of the angular distribution functions and the sign of the band in the T − S spectrum, and cannot be used to discriminate between bleachings and appearing bands. Obviously, absorption bands with mutually parallel transition moments will show the same changes, going from the $|D| - |E|$ to the $|D| + |E|$ monitored LD-(T − S) spectra, whereas for bands with optical transition moments under an appreciable angle this change will be quite different. If two bands of opposite sign form a band shift, then both bands should behave similar (i.e. decrease or increase simultaneously) going from the LD-(T − S) spectrum monitored at the $|D| - |E|$ microwave transition to that of the $|D| + |E|$ transition. Finally, the two exciton components of a dimeric pigment complex will in general show opposite polarization. Using these general notions, we will now discuss the T − S and the LD-(T − S) spectra of the three organisms studied.

Rps. sphaeroides R-26

In Fig. 2 the T − S and the LD-(T − S) spectra of reaction centers of *Rps. sphaeroides* R-26 are displayed. The longest-wavelength band of the T − S spectrum corresponds to the bleaching of P-860, which at 1.5 K has its peak absorbance at 890 nm. It was shown earlier [2], that the bleaching in the T − S spectrum depends on the monitoring microwave frequency, which effect was attributed to site-selection. At 468 MHz (within the $|D| - |E|$ ADMR transition) the strongest bleaching is at 886 nm, at 650 MHz (within the $|D| + |E|$ ADMR transition) the bleaching is at 890 nm [2].

The bleaching of P-860 in the $T - S$ spectrum monitored at 650 MHz shows a clear shoulder at 905 nm. In the $T - S$ spectrum monitored at 468.6 MHz (not shown) and in the 468.6 MHz LD-($T - S$) spectrum the shoulder is not resolved. It was found earlier [2] that the site-selection offered by the precisely defined microwave frequency has a stronger influence (resolving effect) on the $|D| + |E|$ microwave transition than on the $|D| - |E|$ transition. In Ref. 2 it was discussed that the shoulder could have various origins, viz. two distinct distributions of sites, two exciton components of P-860 or charge transfer interaction within the dimer or with an adjacent B-800 pigment [17,18]. The relative intensity at the position of the shoulder and at the main peak is practically the same for both

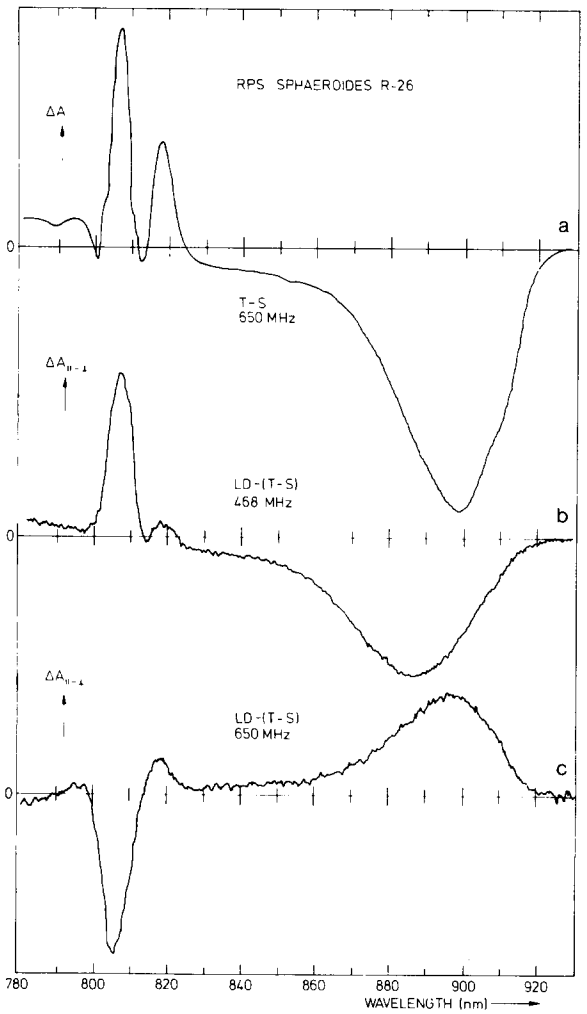

Fig. 2. Triplet-minus-singlet (T − S) (a) and linear dichroic triplet-minus-singlet spectra (LD-(T − S)) (b and c) of reaction centers of the photosynthetic bacterium *Rhodopseudomonas sphaeroides* R-26. The spectra are single scans with a time constant of 1 s and a sweep of 600 s; spectral resolution is 3.2 nm. The microwave field was set resonant within the $|D|+|E|$ transition (650 MHz) (a and c) and the $|D|-|E|$ transition (468 MHz) (b). The two LD-(T − S) spectra (b and c) are drawn at the same vertical scale. Note that the sign of the T − S spectrum is reversed with respect to that in Refs. 2–5.

LD-(T − S) spectra. There exists only one construction that allows two mutually perpendicular optical transitions to be symmetrically placed with respect to the two perpendicular triplet axes. However, in this case the optical transition moments of the 890 nm band make an angle of 60° with both the x- and y-spin axis, and consequently would have negative sign for both the LD-(T − S) spectra, contrary to the observation. Also magnetophotoselection data suggest that the main band of P-860 makes angles of about 5° and 90° with the x- and y-triplet axes, respectively, when the ordering of the zero-field triplet sublevels from lower to higher energy is taken to be z, y, x [19]. We therefore disregard the 'pathological' case and conclude that the shoulder does not represent an exciton component but is due to either site inhomogeneity not resolved by the microwave selection, or to charge transfer effects, with the two transition moments at 905 and 890 nm roughly parallel. These conclusions agree with our earlier work on *Rps. viridis* [3,6] and with the singlet photoselection experiments of Vermeglio et al. [17] and the work of Maslov et al. [18], also on reaction centers of *Rps. viridis*. At present it is not possible to discriminate between the above two possibilities, the relative merits of which are extensively discussed in [3,17,18] for the similar phenomenon in *Rps. viridis* (see also below).

In all T − S spectra, the long-wavelength band sits on a 'pedestal' which extends beyond the 800 nm region. This low intensity wing is typical for a so-called phonon wing which arises from the coupling between low-frequency phonon excitations in the surrounding medium induced by the molecular motions upon electronic excitation and the electronic excitation itself.

Turning to the 800 nm region we note that the T − S spectra are quite similar for the two microwave frequencies (i.e., the two perpendicular microwave transitions). As observed before in Ref. 2, site-selection effects in this wavelength region are apparently much smaller than for the long-wavelength absorption band.

In the two LD-(T − S) spectra the sharp positive band at 807 nm has about equal intensity, but opposite sign, whereas in each of the LD-(T − S) spectra the sign of the 807 nm band is opposite to that of the 890 nm band. If one assumes that the latter band is composed of one, totally allowed exciton transition that is roughly parallel to one of the spin axes, then the Q_y transition of one of the monomers forming P-860 that appears when the electrostatic coupling between the two monomers is decreased due to triplet formation, should be parallel to the (bleached) exciton transition and

appear with a different sign in the two LD-(T − S) spectra. This agrees with observation and supports our earlier assignment of the 807 nm band to the absorption of this uncoupled monomer [1].

The band in the T − S spectrum at about 818 nm keeps its positive sign going from the 650 MHz LD-(T − S) to the 468.6 MHz LD-(T − S) spectrum. This means that the band giving rise to the band shift must make an angle larger than 55° with either of the two spin axes. This agrees nicely with the work of Vermeglio et al. [16] that a band at about 805–810 nm is polarized perpendicular to the P-860 transition. On the basis of a comparison of the T − S spectrum with that of *Rps. viridis* it was argued earlier [1] that this band (a bleaching) forms part of a red shift, the other lobe being largely obscured by the 807 nm band. The present LD results are in harmony with this interpretation, as it is possible to simulate both the LD-(T − S) spectra between 830 and 800 nm with a shift of a band centered at about 813–818 nm, and an absorption band centered at 806.5 nm. If it is assumed that the band at 818 nm is a single band, then it is difficult to simulate T − S and LD-(T − S) spectra without taking recourse to ad hoc changes in the shape of the band. We note that in the low-temperature absorbance spectrum a distinct shoulder of the 803 nm band is observed at about 813 nm [20,21], which shoulder seems to correspond to the shifting band in the T − S spectra.

The features in the T − S spectrum around 798 nm were earlier interpreted as a blue shift of the main absorption band at 803 to 798 nm upon triplet formation in P-860. The band seems to be absent in the two LD-(T − S) spectra, although it is difficult to ascertain the position of the base line. Especially in the 650 MHz LD-(T − S) spectrum a small band seems to be present around 797 nm, which may be interpreted as a remnant of a blue shift of the 803 nm band. The above observations suggest that the transition moment of this band makes an angle of about 55° (the magic angle) with both the x- and y-triplet spin axes.

We note that, because of the smallness of the other features in the 800 nm region, the width of about 7 nm of the 806.5 nm band in the LD-(T − S) spectra is close to the true band width. This width is much smaller than the width of the 800 nm absorption band in the absorption spectra of the reaction centers. As noted, however, this band is made up of at least two components, one centered at about 803 and another at 813 nm [16], with widths of about 12 nm each. Taking into account some additional narrowing going from 77 to 1.5 K, the appearing band at 807 nm has a width comparable to either of the 800 nm components.

The interpretation of the LD-(T − S) spectra of *Rps. sphaeroides* fully support the earlier interpretation of its T − S spectrum [1] and the conclusion that there is no evidence for the bleaching of an exciton component of P-860 at 800 nm as suggested in [21].

R. rubrum

The T − S spectrum of *R. rubrum* (Fig. 3) is almost identical to that of *Rps. sphaeroides* R-26, with the exception that the shoulder of the longest-wavelength bleaching in the 668 MHz spectrum is somewhat less pronounced. Again the T − S spectra monitored at the lower and higher microwave transition are very much alike. Just as for *Rps. sphaeroides* R-26, in the LD-(T − S) spectrum the long-wavelength band behaves uniformly over its entire width for the two microwave transitions, indicating that the transition moments of the shoulder at 910 nm and the main peak at 895 nm are roughly parallel.

As D is positive [12], the ordering of the triplet sublevels is (from lower to higher energy) z, y, x for $E < 0$ or z, x, y for $E > 0$. In other words, the low-frequency microwave transition is polarized along the x-axis for $E < 0$ and along the y-axis for $E > 0$, and vice versa for the high-frequency transition. The LD-(T − S) band at 890 nm which represents $\Delta A_{\parallel} - \Delta A_{\perp}$ is negative when monitored at the low-frequency microwave transition and positive for the high-frequency transition. This means that the transition moment at 890 nm makes an angle of less than 35° with, dependent on the sign of E, either the x- or the y-spin axis. In fact, the ratio of the 890 nm band in the two LD-(T − S) spectra of *R. rubrum* is about 2, close to the theoretical value for alignment along a spin axis. If we take $E > 0$ as found for monomeric BChl *a* in vitro [12], then the 890 nm band is parallel to the y-axis. However, the sign of E is sensitive to exciton interaction within the presumably dimeric P-860, so that this assignment is tentative and valid

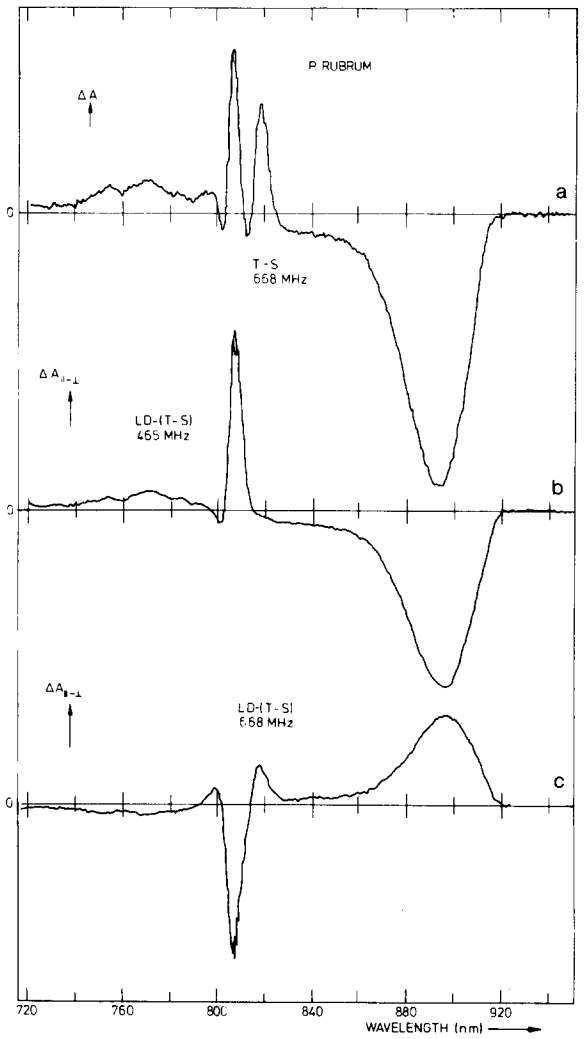

Fig. 3. T − S (a) and LD-(T − S) (b and c) spectra of reaction centers of the photosynthetic bacterium *Rhodospirillum rubrum*. The microwave field was set resonant within the $|D|+|E|$ transition (668 MHz) (a and c) and the $|D|-|E|$ transition (465 MHz) (b). Further conditions as in Fig. 1.

only for a dimer made up of monomers whose spin axes are not too far from parallel. Note that the designations x and y in [19] are arbitrary and correspond to $D > 0$, $E < 0$. Thus, our result is in quantitative agreement with the magnetophotoselection data on *Rps. sphaeroides* R-26 [19].

The 800 nm region of the LD-(T − S) spectra is somewhat different from that of *Rps. sphaeroides* R-26. At 800 nm there is a distinct feature that has the opposite sign relative to the 807 nm band in the spectra for both microwave transitions. A band at 819 nm in the 668 MHz LD-(T − S) spectrum is absent in the 465 MHz LD-(T − S) spectrum, indicating an orientation with respect to the triplet x-axis that is close to the magic angle. As for *Rps. sphaeroides,* the features at 801 and 819 nm may be interpreted as parts of a triplet-induced blue shift and red shift, respectively. Apparently, the main features of the 800 nm region of the LD-(T − S) spectra are similar for both bacteria, but the transition moments of the accessory pigments have a somewhat different orientation with respect to the triplet axes.

C. vinosum

The T − S and LD-(T − S) spectra of *C. vinosum* are displayed in Fig. 4. It is seen that many details deviate considerably from those found for

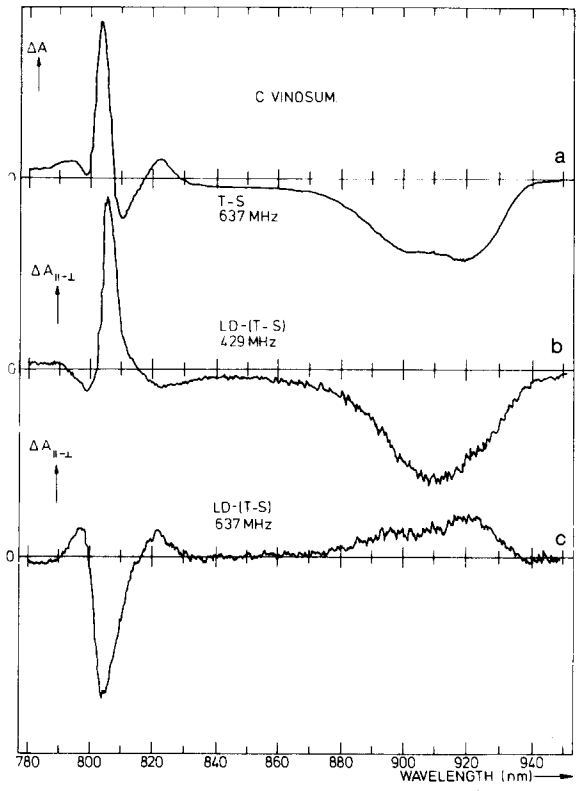

Fig. 4. T − S (a) and LD-(T − S) (b and c) spectra of reaction centers of the photosynthetic bacterium *Chromatium vinosum*. The microwave field was set resonant within the $|D|+|E|$ transition (637 MHz) (a and c) and the $|D|-|E|$ transition (429 MHz) (b). Further conditions as in Fig. 1.

the previous two organisms. The resolution of the long-wavelength absorption band into two components is striking. The transition moments of these components again appear to be roughly parallel.

Fig. 5 displays the long-wavelength bleaching in the T − S spectrum monitored at different positions within the $|D| + |E|$ microwave transition. As we have previously found for *Rps. sphaeroides* R-26 [2], the relative amplitude of the two components of the band is sensitive to the precise frequency at which the T − S spectrum is monitored. This was attributed to the selection offered by the microwave frequency from a distribution of 'sites', i.e. slightly different configurations of the primary donor and its adjacent accessory pigments [2]. The longest-wavelength component, which appears as a shoulder in the other bacterial species investigated, has been attributed to a band arising from charge-transfer interaction between the primary donor dimer and one of the associated BChl molecules that may function as a transient electron acceptor [3,17,18]. If this interpretation is correct, it is surprising that in *C. vinosum* this band acquires so much intensity and even predominates (Fig. 5a and b). This agrees with the almost parallel configuration of the transition moment of the two components only if either the transition dipole moment of the charge-transfer band, which is oriented along the axis through the two separated charges, is parallel to the transition dipole moment of P-860, or if the states have about equal charge-transfer and P*-860 character with small oscillator strength of the charge-transfer band [3,18].

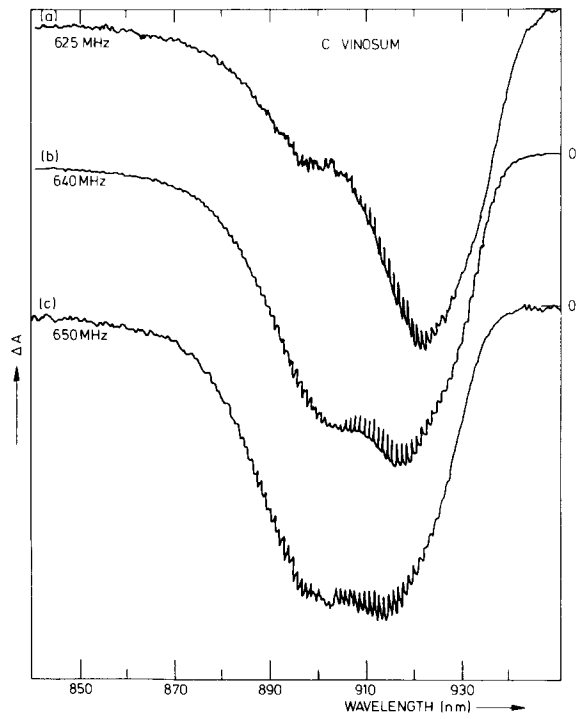

Fig. 5. The triplet-minus-singlet absorbance difference spectrum of the long-wavelength absorbance band of reaction centers of *C. vinosum*. The resonant microwaves were set at various frequencies within the $|D|+|E|$ triplet ESR transition line as indicated. The spectra are single scans with a time constant of 1 s and a sweep of 300 s, normalized at their maximum.

The 800 nm region of the T − S spectrum shows an aspect different from that of *Rps. sphaeroides* R-26 and *R. rubrum*. The appearing band at 804 nm is analogous to the 807 nm band in these bacteria, but the band at 822 nm is much less pronounced, whereas now there is a relatively strong bleaching at 810 nm. We similarly interpret the two bands at 822 and 810 nm as a triplet-induced red shift of a pigment absorbing at about 810 nm, which is better resolved than for the other two species because the appearing monomer band now absorbs at a somewhat shorter wavelength. In agreement, the feature at about 798 nm is less pronounced than in the other bacteria. In general, in the 800 nm region the LD-(T − S) spectra of *C. vinosum* much resemble the corresponding spectra of *R. rubrum*. This indicates that in these two organisms the orientation of the accessory pigments with respect to the triplet spin axis is similar.

In conclusion, we have shown that LD-ADMR is a powerful tool to help unravel complex T − S spectra. The technique is similar to photoselection and a more involved analysis of quantitative LD-(T − S) spectra aiming at the determination of angles of orientation will be the subject of a future publication.

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