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Fluorescence detected magnetic resonance (FDMR) of green sulfur photosynthetic bacteria *Chlorobium* sp.

Jakub Psencík,[1] Geoffrey F.W. Searle[2,*], Jan Hála[1] and Tjeerd J. Schaafsma[2]
[1]*Department of Chemical Physics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 3, 121 16 Prague 2, Czech Republic*; [2]*Department of Molecular Physics, Wageningen Agricultural University, Dreijenlaan 3, 6703 HA, Wageningen, The Netherlands*; **Author for correspondence*

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Abstract

Fluorescence Detected Magnetic Resonance (FDMR) spectra have been measured for whole cells and isolated chlorosomal fractions for the green photosyntheic bacteria *Chlorobium phaeobacteroides* (containing bacteriochlorophyll *e*, and isorenieratene as major carotenoid) and *Chlorobium limicola* (containing bacteriochlorophyll *c*, and chlorobactene as major carotenoid). The observed transitions at 237 MHz (identical in both bacteria) and > 1100 MHz can be assigned, by analogy with published data on other carotenoids, to the 2E and D + E transitions, respectively, of *Chlorobium* carotenoids. Their zero field splitting (ZFS) parameters are estimated to be: $|D| = 0.0332\ cm^{-1}$ and $|E| = 0.0039\ cm^{-1}$ (chlorobactene), and $|D| = 0.0355\ cm^{-1}$ and $|E| = 0.0039\ cm^{-1}$ (isorenieratene). In the intermediate frequency range 300–1000 MHz the observed transitions can be assigned to chlorosomal bacteriochlorophylls *c* and *e*, and to bacteriochlorophyll *a* located in the chlorosome envelope and water-soluble protein. The bacteriochlorophyll *e* triplet state measured in 750 nm fluorescence (aggregated chlorosomal BChl *e*) is characterised by the ZFS parameters: $|D| = 0.0251\ cm^{-1}$ and $|E| = 0.0050\ cm^{-1}$.

Abbreviations: BChl – bacteriochlorophyll; BPh – bacteriopheophytin; *Chl.* – *Chlorobium*; F(A)(O)DMR – fluorescence (absorption) (optical) detected magnetic resonance; FF – fluorescence fading; ISC – intramolecular intersystem crossing; RC – reaction center; ZFS – zero field splitting

Introduction

Green sulfur photosynthetic bacteria can be divided into two subgroups depending on the pigment composition and colour of their light-harvesting antenna systems: green-coloured, containing bacteriochlorophyll (BChl) *c* or BChl *d* as main light-harvesting pigments together with a small amount of carotenoid, or brownish coloured containing BChl *e* together with a large amount of carotenoid (Gorlenko 1988). *Chlorobium* (*Chl.*) *limicola* is an example of the first group, containing BChl *c*, together with the carotenoids chlorobactene (70–90%) and some γ-carotene (Schmidt 1978). *Chl. phaeobacterioides* is an example of the second group, containing BChl *e*, together with the carotenoids isorenieratene and β-isorenieratene, and traces of chlorobactene and β-carotene (Schmidt 1978). The structures of the major carotenoids, chlorobactene and isorenieratene, are shown in Fig. 1. The light-harvesting antenna complexes in these photosynthetic bacteria, called chlorosomes, are oblong bodies 100–150 nm long and

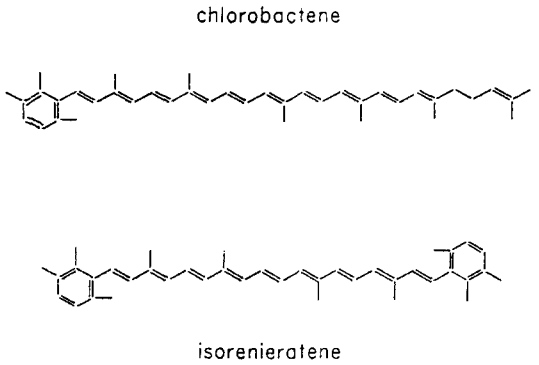

Fig. 1. The structures of the major carotenoids found in *Chlorobium* sp.

30–40 nm in diameter, composed of several rod-like elements (Olson 1980). Each chlorosome contains approximately 10 000 BChl *c* (*d* or *e*) molecules (Wang et al. 1990), and also contains a small amount of BChl *a* in a 'baseplate' (Gerola and Olson 1986).

Light energy absorbed by BChl *c*/*e* or carotenoids in the chlorosome is transferred via singlet excitation energy transfer through the baseplate and a water-soluble BChl *a* protein complex (Gerola et al. 1988), which connects the chlorosome with the membrane, to the cytoplasmic-membrane-bound antenna core complexes and finally to the reaction center (RC) itself.

BChl *c* and BChl *e* in the chlorosomal rod-like elements occurs most likely in the form of aggregates (Brune et al. 1987, Matsuura and Olson 1990, Uehara and Olson 1992). The majority of these pigments are not bound to proteins as in other photosynthetic organisms. In these systems pigment–pigment interactions are very important in determining their photophysical properties. See Zuber and Brunisholz (1991) for a recent review.

Using the fluorescence detected magnetic resonance (FDMR) technique (see e.g. Searle and Schaafsma 1992) we have investigated whole cells of the green-coloured *Chl. limicola*, and whole cells and isolated chlorosomes of the brown-coloured *Chl. phaeobacteroides*, in order to characterise the pigment triplet states, generated by steady-state illumination at low temperature. These triplet states could be used in future work to probe energy transfer within the antenna system in a way similar to that described for a pheophorbide *a*/oligopeptide model system (Hála et al. 1986), and for Photosystem I of higher plants (Searle and Schaafsma 1992).

Materials and methods

Chl. limicola was grown anaerobically at room temperature in SL10 growth medium (Ormerod 1992) at a light intensity of 500 lux from an incandescent lamp. *Chl. phaeobacteroides* was grown under the same conditions, in a mixed culture with *Chl. limicola*. As can be seen in absorption (Fig. 2) and in fluorescence emission spectra (Fig. 3) the proportion of *Chl. limicola* in the mixed culture is low. As the fluorescence emission bands of BChl *c* and BChl *e* are well separated, the presence of *Chl. limicola* does not complicate interpretation of the FDMR spectra of *Chl. phaeobacteriodes* detected in BChl *e* fluorescence. When FDMR is detected in BChl *a* fluorescence the contribution due to *Chl. limicola*, which can be measured in separate experiments, can be subtracted to reveal that due to *Chl. phaeobacteriodes* alone. Experiments were performed on whole cells in their SL10

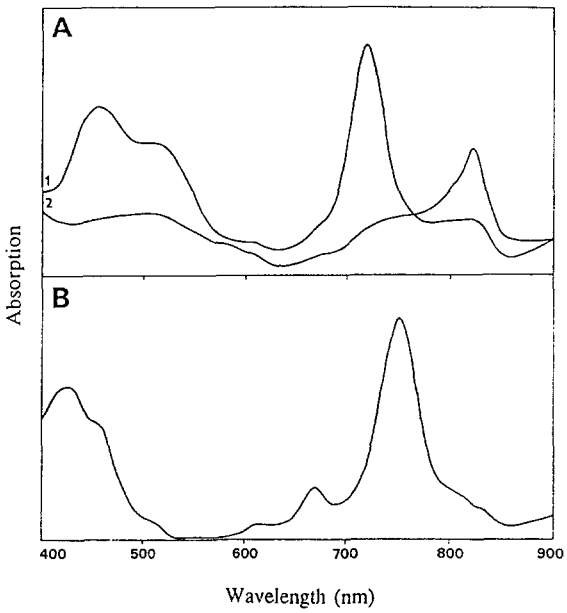

Fig. 2. Absorption spectra of *Chl. phaeobacteroides* chlorosomes (A1), membrane fraction (A2), and of *Chl. limicola* (B) at 300 K.

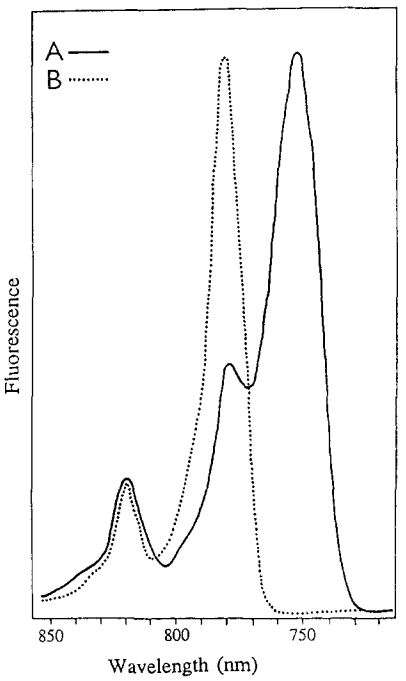

Fig. 3. Fluorescence spectra of *Chl. phaeobacteroides* (A) and *Chl. limicola* (B) at 4.2 K. Excitation wavelength 488 nm. Detection bandwidth 5 nm. Note that the wavelength scale runs from longer to shorter wavelength.

growth medium, and on chlorosomes and membrane fractions prepared from *Chl. phaeobacteroides*, following procedures given in Gerola and Olson (1986). A Branson sonifier B 15 was used for breaking *Chlorobium* sp. cells as described in Griebenow and Holzwarth (1989). Whole cells were stored at 4 °C under strictly anaerobic conditions. The chlorosomal and membrane fractions taken from the sucrose gradient (Gerola and Olson 1986) were stored at −20 °C without further purification. Suspensions of whole cells were diluted with potassium phosphate buffer pH 7.7 (sample: buffer ratio 3:1) before FDMR measurements. Absorption spectra were used to characterise the FDMR samples and were measured on a Uvikon 810 spectrophotometer at room temperature.

To obtain a good FDMR signal-to-noise ratio it was necessary to add sodium dithionite at a final concentration of 20 mM, and also to treat with 10 mM ascorbate and/or 1 min illumination by white light while freezing to 77 K. Subsequently, the samples were quickly frozen to 4.2 K. All samples formed an ice at low temperature, and fluorescence was detected from the laser-excited front surface.

FDMR experiments were carried out using the set-up described in detail in Searle et al. (1983). The sample is contained in a Teflon cup, attached to the lower end of a quartz rod (Suprasil, 5 mm diameter), which is placed in an insulated vessel containing liquid helium. The sample was excited through the quartz rod using a CW Ar ion laser (Coherent Innova 70), operated in single wavelength mode (458 or 476.5 or 488 or 514 nm) at a total intensity, which could be varied using grey filters, between 50 and 500 mW. Fluorescence emitted by the sample was collected from the upper end of the quartz rod and passed through a red glass cut-off filter and a monochromator (Spex Minimate 0.25 m, slit width 5 nm), and finally detected by a Peltier-cooled extended-S20 photomultiplier (RCA 31034A). The photomultiplier output was amplified by an Ithaco 1201 preamplifier and stored using a signal averager (Princeton Applied Research PAR 4203). A microwave field of varying frequency (10–1300 MHz) was generated by a sweep oscillator (Hewlett-Packard 8620C), and RF Plug-in (Hewlett-Packard 86220A, with output setting 0 dB) together with a 800 mW Minicircuits ZHL 2–12 microwave amplifier. Measured microwave-induced changes of the fluorescence intensity were typically of the order of 0.05%. The microwave frequency sweep was repeated typically $\approx 5 \times 10^3$ times in order to obtain an acceptable S/N ratio of the FDMR spectrum. The spectra were found to be independent of the sweep period within the region 0.5–5 s (dwell time per data point of 0.5–5 ms). The frequency of the resonance transitions were determined by calibrating the frequency scale using CW frequency markers generated by the oscillator sweeper. The frequency of these markers were in turn determined to within an error of ≈1 MHz using a Philips PM6676 frequency counter.

The relative total triplet populations of BChls and carotenoids under the conditions of the FDMR experiments were estimated by measuring the response of the fluorescence to a stepwise increase of the excitation light intensity, from zero to a constant value, the so-called fluorescence fading (FF) technique (Van Dorp et

al. 1973). Fluorescence emission spectra (at 4.2 K) and FF curves were measured using the same set-up as described for FDMR, but in the absence of a microwave field. In the FF experiments the CW laser beam was amplitude modulated at a frequency of 7.5 Hz using a mechanical chopper. From the FF curves a total triplet population of ≈1% could be calculated for all samples.

Results

Figure 2 shows absorption spectra of chlorosomal and membrane fractions, after separation on a sucrose gradient, of *Chl. phaeobacteroides* (A), and of intact cells of *Chl. limicola* (B). The main absorption bands in the near infra red are the Q_y bands of BChl *e* and BChl *c*, at 720 nm and 750 nm, respectively, reflecting the different major Bchl pigment present in the antenna systems of these species. Absorption bands at ≈455 nm and ≈515 nm can be attributed to carotenoids (Van Dorssen et al. 1986, Otte et al. 1991). The bands at 425 nm and 670 nm (Fig. 2B) are probably due to non-oligomeric BChl *c* (Brune et al. 1987, Olsen and Pedersen 1988, Uehara and Olson 1992) or BPh *c* (Gerola and Olson 1986, Van Dorssen et al. 1986). The absorption at 610 nm is due to the Q_x band of BChl *a*, whereas BChl *a* Q_y bands of the various pigment protein complexes are seen at $\lambda >$ 790 nm (Kramer et al. 1982). The main absorbing pigment at the wavelengths of excitation in the FDMR experiments (458–514 nm) is expected to be carotenoid.

The fluorescence emission spectra of *Chl. phaeobacteroides* and *Chl. limicola* at 4.2 K (Fig. 3) show strong bands centered at 750 nm and 780 nm, due to BChl *e* and BChl *c*, respectively. A weaker band is observed at 825 nm due to BChl *a*, present in the water-soluble BChl *a*-protein complex. The separation of chlorosomes from the membrane fraction in *Chl. phaeobacteroides* gives rise to new fluorescence bands (spectra not shown) at 680 nm from chlorosomes (probably non-oligomeric BChl *e* (Olson and Pedersen 1988)), and at 840 nm for the membrane fraction, which has been attributed to emission from the RC core complex (Kramer et al. 1982).

The results of our FDMR experiments on whole cells and isolated chlorosomes are presented in Figs. 4–6 and summarised in Table 1. FDMR spectra observed by monitoring the fluorescence emission at 750, 825 and 840 nm of membrane fractions from *Chl. phaeobacteroides* showed no transitions or only very weak ones. Those that could be detected out of the noise (spectra not shown) had similar frequencies to those seen in either whole cells or chlorosomes, and are probably due to slight contamination by chlorosomes.

Our FDMR spectra are dominated by a strong transition at <300 MHz together with one at >1100 MHz. These occur at the same frequencies in chlorosomes and in whole cells, and can be detected in fluorescence from all three BChl's

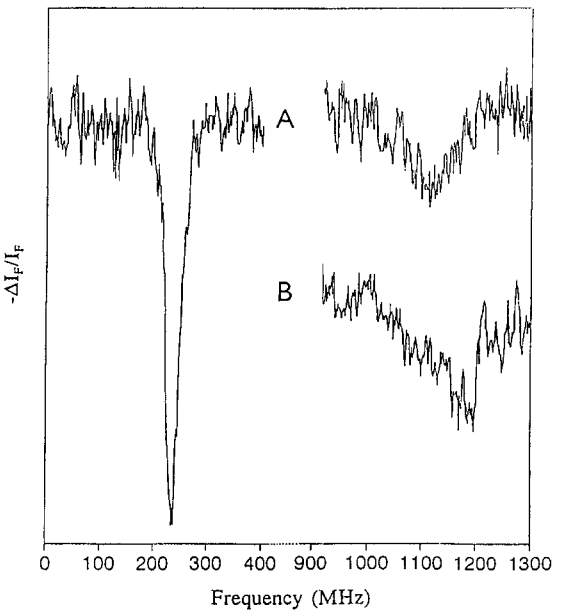

Fig. 4. FDMR spectra at 4.2 K of carotenoids of *Chl. limicola* (A) and *Chl. phaeobacteroides* (B), in whole cells treated as described in 'Materials and methods' (addition of 20 mM dithionite and 10 mM ascorbate, and illuminated whilst freezing in). (A) light excitation wavelength 488 nm, fluorescence detection wavelength 822 nm, 4750 microwave frequency sweeps, dwell time 1.0 ms per data point. (B) as for (A), except 3000 sweeps, and dwell time 0.5 ms. Note that the microwave frequency scale is in two parts separated by 500 MHz. The spectrum in the low frequency region is the same for both (A) and (B). The FDMR spectra are presented as a microwave-induced change ($-\Delta I_f$) in fluorescence intensity (I_f).

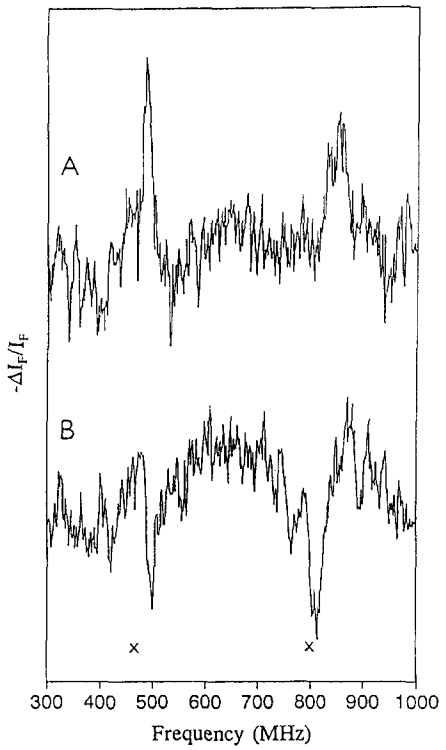

Fig. 5. FDMR spectra of BChl *a* in chlorosomes (A) and whole cells (B) of *Chl. phaeobacteroides* at 4.2 K (X denotes frequencies of resonances found by Vasmel et al. (1984)). (A) light excitation wavelength 488 nm, fluorescence detection wavelength 822 nm, 3500 microwave frequency sweeps, dwell time 0.5 ms per data point. (B) as for A, except 4000 sweeps, and dwell time 1.0 ms. The FDMR spectra are presented as a microwave-induced change ($-\Delta I_f$) in fluorescence intensity (I_f).

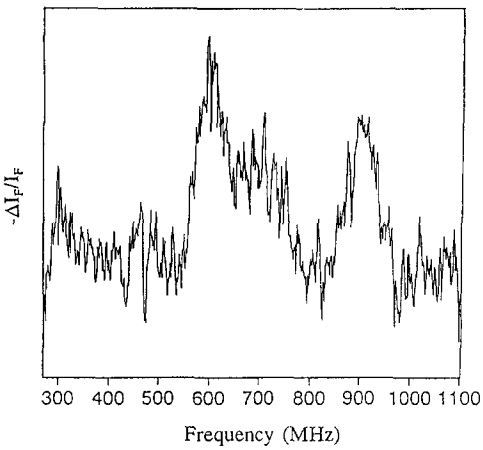

Fig. 6. FDMR spectrum detected in the BChl *e* fluorescence of *Chl. phaeobacteroides* at 4.2 K. Light excitation wavelength 488 nm, fluorescence detection wavelength 752 nm, 6500 microwave frequency sweeps, dwell time 0.5 ms per data point. The FDMR spectrum is presented as a microwave-induced change ($-\Delta I_f$) in fluorescence intensity (I_f).

a, *c* and *e*. Relative intensities and widths of the transitions are found to be independent, within experimental error, of the excitation wavelength in the range 458–514 nm, and of detection wavelength within a given fluorescence emission band. Both resonances represent an increase of fluorescence on application of a resonant microwave field, as was seen in an Absorption-ODMR study of LHC II where a strong 2E transition could definitely be assigned to carotenoids (Carbonera et al. 1992). If by analogy we also assign these transitions in *Chlorobium* sp. to the 2E and D + E transitions of triplet states of carotenoids, then the resulting |D| and |E| values (see Table 1) are similar to those for carotenoids in other photosynthetic systems: lutein ($|D| = 0.0385\,cm^{-1}$, $|E| = 0.0038\,cm^{-1}$) and neoxanthin ($|D| = 0.0400\,cm^{-1}$, $|E| = 0.0038\,cm^{-1}$), Van Der Vos et al. (1991); β-carotene ($|D| = 0.0331\,cm^{-1}$, $|E| = 0.0040\,cm^{-1}$), Frick et al. (1990); neurosporene ($|D| = 0.0365\,cm^{-1}$, $|E| = 0.0035\,cm^{-1}$), spheroidene ($|D| = 0.0324\,cm^{-1}$, $|E| = 0.0036\,cm^{-1}$), and spheroidenone ($|D| = 0.0318\,cm^{-1}$, $|E| = 0.0032\,cm^{-1}$), Frank (1992). We therefore believe that the transitions at <300 MHz together with those at >1100 MHz are due to carotenoids, and an assignment to BChl (monomeric or aggregated) is very unlikely.

The FDMR spectra of whole cells of *Chl. phaeobacteroides* and *Chl. limicola* (Fig. 4) both show a 2E transition at 237 MHz, but the frequencies of the carotenoid D + E transitions are different. The D + E transition of chlorobactene (*Chl. limicola*) is found at 1115 MHz, and that of isorenieratene (*Chl. phaeobacteroides*) at 1185 MHz. It can be calculated that the D − E transitions would therefore be at 878 and 948 MHz, respectively, within the region of the frequencies of the D + E transitions of the BChl's. In both bacteria the intensity of the 2E transition is considerably larger than that of the D + E transition, and its linewidth (30–40 MHz) is significantly smaller (70–90 MHz). In the mixed culture samples containing both *Chl. phaeobacteroides* and *Chl. limicola* the width of

Table 1. The FDMR transition frequencies (2E, D − E, D + E), and zero field splitting parameters (D, E) of triplet states observed in *Chlorobium* sp.

Pigment observed	2E	D − E	D + E	|D|	|E|
Chlorobactene	237	–[a]	1115	0.0332	0.0039
Isorenieratene	237	–[a]	1185	0.0355	0.0039
BChl *a* (chlorosome)	–[a]	483	854	0.0223	0.0062
BChl *a* (water soluble complex)	–[a]	495	811	0.0217[b]	0.0054[b]
BChl *e*	297	598	904	0.0251	0.0050

Frequencies of the 2E D − E and D + E transitions are given in MHz, and the experimental error is estimated to be ± 5–10 MHz. The values of |D| and |E| are given in cm^{-1}, and are the absolute values – for carotenoids D has a negative value whilst E is positive (Van Der Vos et al. 1991).

[a]Transition frequency could not be determined sufficiently accurately.

[b]The values were reported by Vasmel et al. (1984) to be: 0.0210 and 0.0055 cm^{-1}. See text for the fluorescence detection wavelengths used.

the 2E transition is identical to that of *Chl. limicola* alone (Fig. 4), however the broader D + E transitions of the two carotenoids are well separated. The amplitudes of the carotenoid FDMR transitions increase markedly as the excitation light intensity is increased in the range 50–500 mW, and when measured in BChl *e* fluorescence the amplitude of the carotenoid 2E transition shows an approximately linear relationship with the intensity of the laser (476.5 nm, 60–250 mW). Under the same conditions the BChl *e* FDMR transitions (Fig. 6) also increase linearly in amplitude, but with approximately half the slope, so that carotenoid FDMR transitions dominate at high excitation intensities.

BChl *a* resonances can be found in the frequency region 450–900 MHz, but only when the BChl *a* fluorescence (825 nm band) itself was monitored. In whole cells a complicated FDMR spectrum is expected and observed (Fig. 5B). FDMR spectra of BChl *a* in chlorosomes might be expected to be much simpler, and indeed the spectrum is dominated by only two transitions (Fig. 5A). Major transitions at 483 and 854 MHz, which correspond to a decrease of fluorescence, may be attributed to the D − E and D + E transitions of the BChl *a* present in the envelope layer of the chlorosomes (Fig. 5A). The major transitions in whole cells, which are at other frequencies, and whose amplitude is opposite in sign corresponding to an increase in fluorescence (Fig. 5B), are probably due to the BChl *a* in the water soluble protein complex, because the transition frequencies for this complex are close to those reported using ADMR (Vasmel et al. 1984). However, their exact determination is difficult because of the overlap of the FDMR spectrum with that of chlorosomal BChl *a*.

FDMR spectra detected in the 750 nm fluorescence of BChl *e* in *Chl. phaeobacteroides* are the same in whole cells and in chlorosomes (Fig. 6). At low light excitation intensity a weak transition at 297 MHz can be observed, and most likely this transition, together with those at 598 and 904 MHz all originate from one and the same triplet, being the 2E, D − E and D + E resonances of aggregated BChl *e*, respectively. Another broad transition, which cannot originate from the same triplet, is observed as a shoulder at 681 MHz (Fig. 6).

FDMR spectra detected in BChl *c* fluorescence in *Chl. limicola* are more difficult to interpret as the observed resonances, under our measuring conditions, are very weak. Frequencies of some transitions (data not shown) are similar to those of *Chl. phaeobacteroides* (Fig. 6), but the signs of the amplitudes were opposite.

Discussion

All triplet states observed in this work on *Chlorobium* sp. have their origin in intramolecular intersystem crossing (ISC) and T-T energy transfer, as their |D| and |E| values are different to those reported for the RC triplet (Vasmel et al. 1984) and they can all be observed in the isolated chlorosome fraction. No spin polarised triplet states generated via the radical pair mechanism in RCs were observed even under conditions

which are known to favour their observation in EPR (Nitschke et al. 1990). This is in contrast to FDMR results on purple bacteria, where only RC triplets were observed in whole cells (Angerhofer et al. 1984). In *Chlorobium* sp. only weak FDMR spectra were detected in the fluorescence of the isolated membrane fraction, which still contains the RC, and this appeared to be due to some contamination by chlorosomes. The fluorescence intensity from the membrane fraction is very weak because the content of carotenoids, which are the main absorbing pigments in the wavelength region of the excitation light used in our experiments (458–514 nm), is significantly lower than in the chlorosome fraction, although some direct excitation of BChl's cannot be ignored.

In order to detect FDMR spectra, it was necessary to pretreat the samples (including addition of dithionite) as described under 'Materials and methods', a procedure similar to that used by Nitschke et al. (1990) for reducing the primary acceptors in RCs of green bacteria. In green sulfur bacteria redox conditions can also, however, directly affect the energy transfer efficiency (Wang et al. 1990). Blankenship et al. (1993) found that energy transfer from BChl *c* to BChl *a* could only be observed under reducing conditions, as otherwise it was prevented by an unknown quenching mechanism operating at least two distinct sites (oligomeric pigments, BChl *a* protein). Also, recent hole burning experiments (J. Psencík, M. Vácha, F. Adamec, M. Ambroz, J. Dian, J. Bocek and J. Hála, submitted for publication) have indicated that the excited state lifetime of Bchl *c* in the presence of dithionite is longer than that under aerobic conditions, thus favouring the population of the ISC triplet state in the antenna system. The quenching mechanism has still to be identified.

Carotenoid FDMR Transitions

FDMR spectra of the carotenoids (only the 2E and D + E transitions were clearly observed) were the same in whole cells and in isolated chlorosomes, demonstrating that the carotenoids observed in whole cells are indeed located in the chlorosomes, as expected. Carotenoid FDMR resonances were observed in both BChl *a* and BChl *c*/*e* fluorescence bands, which could mean that singlet excitation energy is transferred from carotenoids with high efficiency to both BChl species, either in a parallel or sequential fashion (see e.g. Searle and Schaafsma 1992). It certainly means that there is transfer of triplet excitation energy from BChl to carotenoid, as ^{3}Car can only be generated by T-T energy transfer and not by ISC directly from ^{1}Car (Hoff 1986, Aust et al. 1991). We can therefore conclude that a proportion at least of the BChl interacts closely with carotenoid in the chlorosomes. Carotenoids in photosynthetic systems protect against photooxidative damage by quenching BChl triplet states in this way. However, if the T-T energy transfer is not 100% efficient, or if a proportion of the BChl is not closely associated with carotenoid, then both carotenoid and BChl triplet states would be observed, as seen here in *Chlorobium* sp.

Two features of the FDMR spectra of carotenoids observed in *Chlorobium* sp. give information on the carotenoids *in situ* in the chlorosome: the |D| value is different whereas the |E| value is identical for both chlorobactene and isorenieratene (Table 1); and for both carotenoids the 2E is considerably narrower than the D + E transition (Fig. 4).

The |D| value is given by $3/4\ g^2\beta^2\langle(r^2-3z^2)/r^5\rangle$, where x, y, z are the projections onto the three principal dipolar axes of the vector **r** connecting the two unpaired electrons of the triplet state, g is the electronic g-factor, β is the electronic Bohr magneton and $\langle\ \rangle$ denotes the average over space (Hoff 1986). The |E| value is given by $3/4\ g^2\ \beta^2\langle(y^2-x^2)/r^5\rangle$. Since for carotenoids the triplet z-axis roughly corresponds with the polyenal axis (Frick et al. 1990, Van Der Vos et al. 1991), it can be calculated that the observed |D| values correspond to an effective distance between the unpaired electrons along the polyenal axis of about 4.8 Å for isorenieratene and 4.9 Å for chlorobactene – approximately the length of the constituent isoprene subunits. Both Ros and Groenen (1991) and Aust et al. (1991) have reported an empirical, approximately linear relationship between both |D| and |E| and the reciprocal of the number of conjugated double bonds in all-trans

carotenoids. Chlorobactene and isorenieratene differ in the number of such bonds, chlorobactene having 10 and isorenieratene 9, ignoring the ring systems – see Fig. 1, as suggested by Frick et al. (1990) for the case of β-carotene. The values of |D| found (Table 1) are indeed different and are moreover in very good agreement with the value predicted for |D| by this relationship. The values of |E| would also be expected to be different, however they are found to be identical, with that for isorenieratene agreeing with the predicted value for the all-trans conformation. Aust et al. (1991) have shown that a large change is to be expected in the |E| value on the introduction of a cis conformation. The larger than predicted value of |E| for chlorobactene could be due to the presence of some cis double bond conformation in chlorobactene in *Chl. limicola*.

The narrowness of the 2E compared to that of the D + E transition must reflect a greater homogeneity of the $|x\rangle$ and $|y\rangle$ triplet spin sublevels compared to that of the $|z\rangle$ sublevel (see Van Der Vos et al. 1991). This could be explained by a smaller variation in |E| and thus $(y^2 - x^2)$ compared to that in |D| and thus $(r^2 - 3z^2)$. It appears therefore that the distance between the unpaired electrons of the carotenoid triplet state shows less variation in the *xy*-plane (perpendicular to the long axis of the molecule) than along the length of the molecule.

Assignment of bacteriochlorophyll FDMR transitions

On monitoring BChl *a* fluorescence the carotenoid FDMR transitions were accompanied by two BChl *a* FDMR spectra of triplets, which are probably located in the chlorosomes and in the water-soluble protein complex respectively. Both types of BChl *a* triplets were detected in *Chl. limicola* as well as in *Chl. phaeobacteroides*. |D| and |E| values of chlorosomal BChl *a* (Fig. 5A, Table 1) are similar to those of BChl *a* in vitro, for example in toluene containing 10–15% pyridine (Thurnauer and Norris 1977), although their absorption (Fig. 2) and fluorescence (Fig. 3) spectra are different. The second BChl *a* triplet is only observed in whole cells, and is probably due to the water soluble BChl *a* protein complex (Vasmel et al. 1984). As the spectral line shape of the FDMR transitions were distorted by overlap of the two FDMR spectra (Fig. 5B), the |D| and |E| values given in Table 1 are only approximate, nevertheless, the FDMR transition frequencies are in good agreement with those observed for this well-characterised complex isolated from *Prostecochloris aestuarii* (Vasmel et al. 1984). The three transitions at 297, 599 and 904 MHz, detected by monitoring the 750 nm BChl *e* fluorescence of *Chl. phaeobacteroides* (Fig. 6 and Table 1) can be assigned to the 2E, D − E and D + E transitions of a triplet state of aggregated BChl *e* bound to protein in the chlorosome, which would be to our knowledge the first report of ZFS parameters of BChl *e*.

The origin of other FDMR transitions, which we have observed in *Chlorobium* sp. is less clear. After isolation of chlorosomes from *Chl. phaeobacteroides* a new fluorescence band appeared at 680 nm, which can be attributed to non-oligomeric chlorosomal BChl *e*, in contrast to aggregated BChl *e* fluorescing at 750 nm. The FDMR resonances detected in this new fluorescence band (562, 710 and 1077 MHz) must arise from more than one triplet state. Similarly, in BChl *c* fluorescence from *Chl. limicola* at 780 nm three FDMR transitions arising from more than one triplet state were detected (329, 611 and 859 MHz).

Other observed transitions may be due to artefacts. The weak transition at 681 MHz seen in BChl *e* fluorescence at 750 nm from *Chl. phaeobacteroides* (Fig. 6) was found to increase in intensity when samples were not stored at low temperature, so that this transition is most likely due to a degradation product, such as the pheophytin BPh *e*. An FDMR transition at 780 MHz could be observed in 700 nm fluorescence from *Chl. limicola*, after complete denaturation by heating for 5 min at 70 °C, so that the origin of this fluorescence is again likely to be a degradation product such as BPh *c*.

We plan to carry out more FDMR studies using direct light excitation into the BChl *c*/*e* and BChl *a* Q_y absorption bands, and to compare the results to similar measurements on isolated pigments in vitro in order to confirm the assignments of the observed FDMR transitions,

in particular to assign transitions to oligomeric or non-oligomeric BChl *c/e*. Further, it would be interesting to use our knowledge of the FDMR assignments to investigate singlet and triplet energy transfer pathways between carotenoids and BChls in *Chlorobium* sp. This should include a more detailed study of the dependence on light excitation wavelength and intensity of the relative amplitudes of the carotenoid (2E, D + E) and BChl (D − E, D + E) FDMR transitions detected in both BChl *a* and BChl *c/e* fluorescence. Some interesting observations have already been made, such as that the amplitude of both carotenoid and BChl FDMR resonance transitions are linearly dependent on the intensity of the excitation light, but with a different slope. Also, the possible mechanism of inhibition of singlet energy transfer within the antenna system, and its relief on addition of dithionite, should be clarified further.

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