Fluorescence and Absorption Detected Magnetic Resonance of Chlorosomes from Green Bacteria *Chlorobium tepidum* and *Chloroflexus aurantiacus*. A Comparative Study†

Donatella Carbonera,* Enrica Bordignon, and Giovanni Giacometti

Department of Physical Chemistry, University of Padova, via Loredan 2, 35131 Padova, Italy

Giancarlo Agostini

Centro Studi Stati Molecolari Radicalici ed Eccitati CNR, via Loredan 2, 35131 Padova, Italy

Alberto Vianelli and Candida Vannini

Department of Structural and Functional Biology, University of Insubria, via J.H. Dunant 3, 21100 Varese, Italy

Received: May 12, 2000; In Final Form: October 4, 2000

A comparative study on the isolated chlorosomes from *Chloroflexus aurantiacus*, a green filamentous photosynthetic bacterium and *Chlorobium tepidum*, a green sulfur photosynthetic bacterium, was done by ODMR (optically detected magnetic resonance). Correlation between the results obtained by fluorescence and absorption detection is shown to be a source of information about the functional interactions among pigments. Analogies and differences are pointed out between the light-harvesting systems of the two species. Triplet states are easily detected in both bacteria at 1.8 K under steady-state illumination and are assigned to BChl *c*, BChl *a*, and carotenoid molecules. Carotenoids are found to be able to quench BChl *a* triplet states, but no evidence of BChl *c* triplet states quenching by this triplet−triplet transfer mechanism is found in both systems. Then from the data it appears that some carotenoids are in close contact with BChl *a* molecules. The relevance of this finding to the localization of carotenoids in the chlorosomes is discussed. In *Cb. tepidum* three different pools of BChl *c* oligomers connected to BChl *a* were found by detection of their triplet state, while only one pool of BChl *c* was evidenced in *Cf. aurantiacus*. The latter appears to be unconnected, at least at 1.8 K, to BChl *a*. On the other hand, heterogeneity in the BChl *a* triplet population was detected in *Cf. aurantiacus*. Even though the two bacteria show common features in the way the light excitation induces triplet formation at low temperature, the detected triplet states show spectroscopic properties that strongly depend on the system. The results clearly indicate that differences in pigment organization exist both in the core and in the baseplate of the chlorosomes from the two different bacteria.

Introduction

From a phylogenetic point of view the two families of green bacteria *Chlorobiaceae* and *Chloroflexaceae* are considered two separate phyla.[1] However, unexpectedly, they contain very similar light harvesting entities, the so-called chlorosomes. The comparative study on the structure−function relationships of the isolated chlorosomes of *Chloroflexus aurantiacus* and *Chlorobium tepidum*, as representative of the two families, is then of some interest.

Chlorosomes are organelles consisting of a core and of an envelope. The core contains preponderantly bacteriochlorophyll (BChl) *c*, *d*, or *e*, depending on the bacterium, assembled into rodlike elements[2−5] as oligomers. The pigment−pigment interactions are important in determining the supramolecular organization in the rods.[6,7] Proteins are present in the lipid envelope, which is formed by a monolayer of galacto-lipids, but possibly not in the rod elements.[5] A baseplate in the envelope connects the chlorosomes to the cytoplasmic membrane. Chlorosomes from green sulfur bacteria are found to be larger compared to the chlorosomes from green filamentous bacteria.[5]

A small number of BChl *a* molecules (up to 1% in *Cb. tepidum* and up to 5% in *Cf. aurantiacus* with respect to the total amount of BChl *c*) are complexed with proteins[8] and likely located in the baseplate[9,10] assembly, although the finding that BChl *a* is correlated to the CsmA protein raises some doubts about the location of BChl *a* in the baseplate.[8] BChl *a* is also found in a membrane-bound antenna complex (B808-866) in green filamentous bacteria and in the well-known FMO-protein in green sulfur bacteria. These latter two antenna complexes are thought to mediate the energy transfer from the chlorosomes to the reaction centers.[5] The main structural difference therefore is found in the contact region between the chlorosomes and the cytoplasmic membrane. In *Cf. aurantiacus* the baseplate is in direct contact with the membrane, while in *Cb. tepidum* the FMO-protein is assumed to mechanically couple the chlorosome and the membrane.

Carotenoids also contribute to the whole pigment composition of chlorosomes. They represent 30% of pigment content in *Cf.*

† Abbreviations: ODMR, optically detected magnetic resonance; ZF-FDMR, zero field fluorescence detected magnetic resonance; ZF-ADMR, zero field absorption detected magnetic resonance; MIA, microwaves induced absorption; T−S, triplet-minus-singlet; ZFS, zero field splitting; BChl, bacteriochlorophyll; *Cb.*, *Chlorobium*; *Cf.*, *Chloroflexus*; RC, reaction center: ISC, Inter System Crossing.

* Corresponding author. Fax: +39(49)8275135. E-mail: D.Carbonera@chfi.unipd.it.

10.1021/jp001778+ CCC: $20.00
Published on Web 11/30/2000

aurantiacus and 10% in *Cb. tepidum*. Chlorobactene is the prevailing species of *Cb. tepidum*, followed by 1′-2′-dihydrochlorobactene while β-carotene and γ-carotene are mainly found in *Cf. aurantiacus*. A 20% of the total carotenoid content in *Cf. aurantiacus* is represented by glucoside and OH− carotenoids, while only a few percent of this B-type carotenoids are found in *Cb. tepidum*. The localization of carotenoids in the chlorosomes is still uncertain. The role of carotenoids in chlorosomes is probably of the same kind as that already recognized in other photosynthetic antenna systems, where they serve as light-harvesting pigments and as photoprotectors from singlet oxygen, although this latter function should not be so relevant in anaerobic organisms. Quinones are also found in chlorosomes and seem to play an important role in affecting the fluorescence and the energy transfer under oxic conditions in green sulfur bacteria[11] while their role in green filamentous bacteria is not clear.[12]

The organization of pigments in the chlorosomes has been under discussion during the last 10 years and is still controversial. Due to molecular aggregation, excitonic states seem to dominate the absorption characteristic of BChl *c* molecules in the chlorosomes; however, the presence of different BChl *c* pools has been suggested by several authors.[10,13] Analogies and differences between the two families of green bacteria have also been pointed out.[5,14]

We use both ZF-FDMR and ZF-ADMR to detect triplet formation at low temperature, under broad band photoexcitation, in isolated chlorosomes from *Cb. tepidum* and from *Cf. aurantiacus* with the aim of throwing light on energy transfer processes and pigment interactions in these two species of green bacteria. Previous work on other photosynthetic systems has shown that these techniques are helpful for structure and funcion analyses on both antenna and reaction center complexes.

FDMR of whole cells and isolated chlorosomes of *Cb. phaeobacteroides* and *Cb. limicola* has been already reported by Psencik et al.[15] Indeed, several triplet species have been detected and assigned to BChl *a*, carotenoids, and BChl *e* (or *c*) molecules of the chlorosomes. In this study we perform ODMR for the first time in the isolated chlorosomes of a green filamentous bacterium and extend the previous work on the chlorosomes of green sulfur bacteria. FDMR is done by detecting the fluorescence changes selectively on the BChl *c* and BChl *a* emission bands at different wavelengths, upon modulation of triplet population by resonant amplitude modulated microwaves. In this way, energy transfer pathways can be proven and the presence of different pigment pools can also be investigated.

Microwave-induced absorption gives the triplet minus singlet (T−S) spectra of the different triplet states observed by FDMR and allows the assignment to specific pigment in the chlorosome absorption spectrum. Since ADMR monitors the bulk population directly, it is possible to evaluate the relative triplet yield of different pigments in a more reliable way as compared to the FDMR. ADMR in isolated chlorosomes from the two families of green bacteria is here used for the first time and the results are compared.

Materials and Methods

Chlorosomes fractions of *Cb. tepidum* were prepared according to the procedure of Gerola et al.[16] (omitting NaSCN) to a final concentration of 1 mg BChl/mL. The absorption spectrum was the same as those reported in the literature for the same kind of preparation. The samples were poised at pH 7.4 with potassium phosphate buffer (10 mM) containing 25% (w/w) sucrose and diluted to 200 μg BChl/mL before the addition of 20 mM dithionite under nitrogen. This reduction procedure improves significantly the signal-to-noise ratio of the triplet spectra, as expected on the basis of the redox potential control of fluorescence and energy transfer yields already reported.[11,17]

Cells of *Cf. aurantiacus*, strain J-10-fl, were grown at 55 °C, under anaerobic conditions and under illumination (9 μE m^{-2} s^{-1}). Chlorosomes were prepared using NaSCN throughout the purification as described by Gerola and Olson,[16] except that Tris 10 mM pH 8 buffer[18] was used. The samples were poised at pH 7.8 with Tris buffer (10 mM) to a final concentration of 200 μg of BChl/mL. The absorption spectrum was the same as those previously reported for the same kind of preparation.[19]

Glycerol was added at 66% vol/vol just before freezing of the samples to avoid matrix cracking, and oxygen was removed using a glucose/glucose oxidase system as described.[20]

The home-built FDMR apparatus was previously described.[21] The detection wavelength was selected by cutoff and band-pass filters in the range 770−850 nm (bandwidths 10 nm). The ADMR apparatus, together with the experimental setup for the detection of triplet minus singlet (T−S) spectra, was also previously described.[22] Different modulation frequencies of the amplitude modulated microwaves have been used depending on the triplet lifetime: 325 Hz for carotenoids and BChl *a* triplet states and 33 Hz for BChl *c* triplet states.

Results

The absorption spectra of isolated chlorosomes are dominated by the bands of BChl *c* in oligomeric form. At room temperature the Q_y band is found at 753 nm in *Cb. tepidum*, while in *Cf. aurantiacus* it is found at 741 nm, both red-shifted with respect to the monomer absorption (670 nm). The BChl *a* molecules, which are present in a small amount, absorb in the long wavelength tail of the Q_y band of BChl *c*, at about 795 nm. A shoulder at 510 nm is indicative of the presence of carotenoids in both systems. The relative amount of BChl *a* and carotenoids is higher in *Cf. aurantiacus* and is clearly observed in the absorption spectra (not shown).

Figure 1 shows the emission spectra of isolated chlorosomes of *Cb. tepidum* and *Cf. aurantiacus*, taken at 1.8 K. The fluorescence spectra show two main bands. The first, centered at 783 nm in *Cb. tepidum* and at 764 in *Cf. aurantiacus*, is due to the emission from BChl *c*; the other, centered at 827 nm in *Cb. tepidum* and at 819 nm in *Cf. aurantiacus*, is due to BChl *a*. The emission band due to BChl *a* is the most intense in *Cf. aurantiacus* while the emission band due to BChl *c* dominates the fluorescence spectrum of *Cb. tepidum*. In the figure the transmittance of the band-pass filters used for the FDMR detection are also shown.

The FDMR results are presented in Figures 2−6. With the fluorescence detection set at different wavelengths, several triplet states have been detected, which can be assigned to BChl *c*, BChl *a*, and carotenoid molecules. The assignments can be done on the basis of the ZFS parameters and, in some cases, on the basis of the microwave-induced triplet-minus-singlet (T−S) spectra (see below).

BChl *c* Triplet States. The changes in the maximum of the BChl *c* emission band due to resonant microwave fields reveal the presence of triplet states under illumination at 1.8 K, in both samples (see Figure 2). In *Cf. aurantiacus* two transitions centered at 648 and 875 MHz have been detected corresponding to the $|D| - |E|$ and $|D| + |E|$ transitions of an oligomeric BChl *c* triplet state[23] having $|D| = 0.0254$ cm^{-1} and $|E| = 0.0038$ cm^{-1}. Similarly, in *Cb. tepidum* two transitions centered at 641

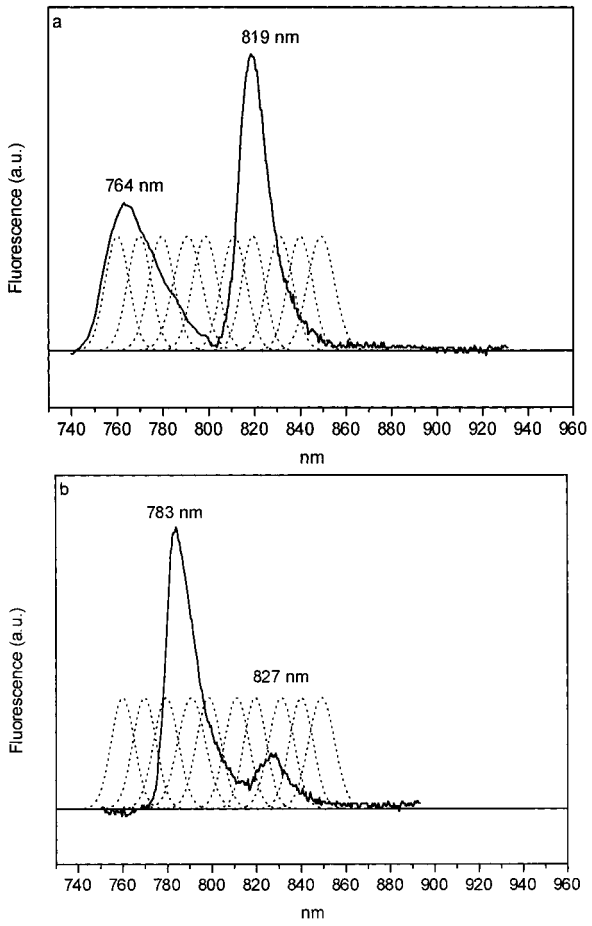

Figure 1. Fluorescence spectra of isolated chlorosomes taken at 1.8 K: (a) *Cf. aurantiacus*; (b) *Cb. tepidum*. Excitation: white light from 250 W tungsten lamp, filtered by $CuSO_4$ solution (1 M, 5 cm). 1.5 nm resolution. Band-pass filters used in the FDMR experiments to select the detection wavelength are also shown (dashed lines).

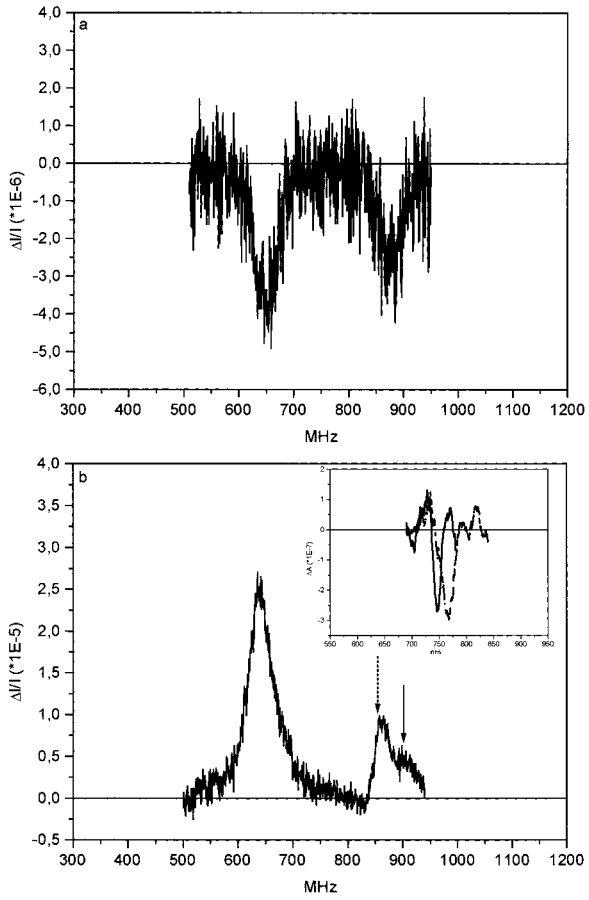

Figure 2. FDMR spectra of isolated chlorosomes: (a) *Cf. aurantiacus* (detection wavelength: 770 nm); (b) *Cb. tepidum* (detection wavelength: 780 nm). Experimental conditions: $T = 1.8$ K; modulation frequency, 33 Hz; microwave power, 500 mW; scan rate, 5 MHz/s. Inset: T−S spectra taken at 901 MHz (solid line) and 856 MHz (dashed line) indicated by arrows in the FDMR spectrum of *Cb. tepidum.* Experimental conditions: $T = 1.8$ K; modulation frequency, 33 Hz; microwave power, 500 mW; scan rate, 0.1 nm/s.

and about 900 MHz have been found, corresponding to a triplet state with $|D| = 0.0257$ cm^{-1} $|E| = 0.0043$ cm^{-1}, as already found in ref 23. BChl *c* triplet states can be populated in both systems by low-temperature illumination, but comparison of the two spectra shows that several differences are present.

The signs of FDMR signals are opposite in the two bacteria. Moreover the $|D| + |E|$ transition splits into two components (at about 856 and 901 MHz) indicated by the arrows in Figure 2, only in *Cb. tepidum*. There are thus two slightly different BChl *c* triplet states in this system. It is interesting to note that the relative intensity of the FDMR signals due to these two different triplet states depends on the growing conditions: at low light intensity the 856 MHz species dominates, while at high light the 901 MHz component prevails (data not shown). The T−S spectra taken at the resonance frequencies of these two triplet states in the Q_y absorption region of BChl *c*, confirm that the triplet states are due to two BChl *c* species in oligomeric form. Indeed, the main singlet−singlet bleachings are found at 740 and 760 nm (Figure 2). The intensities of the T−S signals are quite low, indicating a very small triplet yield of BChl *c* under steady-state illumination at low temperature. The even lower intensity of the signal in *Cf. aurantiacus* prevents the detection of the T−S spectrum of the BChl *c* triplet state detected by FDMR. As shown in Figure 3 for the most intense transitions ($|D| - |E|$), the dependence of the FDMR signals on the detection wavelength is also different in the chlorosomes of the two bacteria. The signals due to BChl *c* triplet states are well detectable up to 850 nm in the case of *Cb. tepidum*, while they disappear at about 800 nm in *Cf. aurantiacus*. The sign of BChl *c* FDMR signals does not change when passing from the emission band of BChl *c* itself to the emission band of BChl *a* while the signal intensity is maintained. Apparently, in *Cf. aurantiacus* the molecules carrying the triplet states belong to a BChl *c* pool that is unconnected to BChl *a* fluorescent molecules, and for this reason the FDMR disappears as soon as the BChl *c* fluorescence component vanishes.

When the $|D| + |E|$ transitions are detected at different wavelengths (range 800−900 MHz), a new transition centered at 828 MHz, with a maximum of intensity at 800 nm, appears only in the spectra of *Cb. tepidum* (Figure 4b); this transition overlaps with the $|D| + |E|$ transition of the BChl *a* triplet state (see below). However, it is distinguishable at detection wavelengths (790−810 nm) at which ^{T}BChl *a* does not contribute. We assign this triplet state to a third different BChl *c* triplet state on the basis of double resonance experiments (data not shown), which indicate a $|D| - |E|$ transition at about 630 MHz. Interestingly, this third pool of BChl *c* (oligomer) shows a different polarization and a selection for long emitting BChl *c* molecules.

BChl *a* Triplet States. By monitoring the microwave-induced fluorescence changes in the emission band of BChl *a*, together with the above-discussed FDMR signals due to BChl *c* in *Cb. tepidum*, new FDMR signals, in a different microwave range, have been detected in both bacteria. The new transitions are

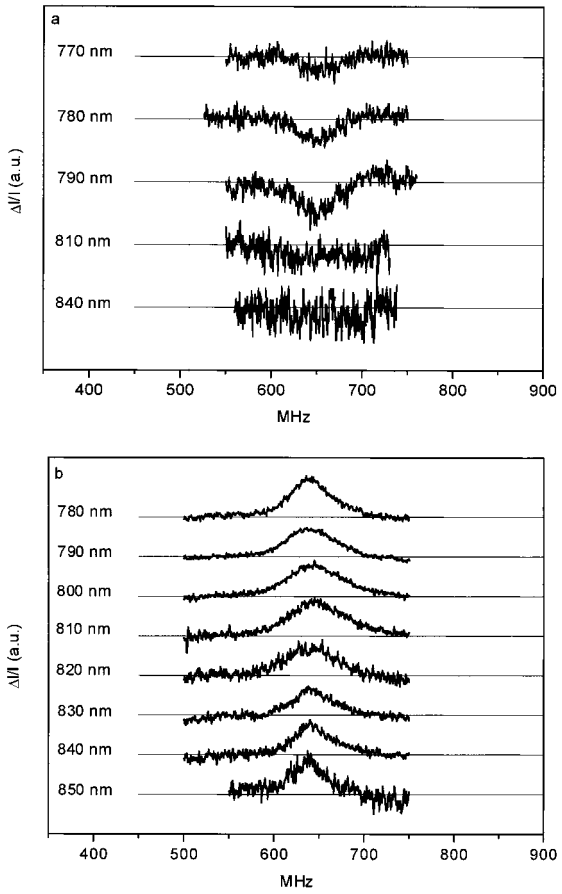

Figure 3. FDMR spectra of the BChl *c*, corresponding to the $|D| - |E|$ transitions, taken at different detection wavelengths, as indicated in the figures: (a) *Cf. aurantiacus*; (b) *Cb. tepidum*.

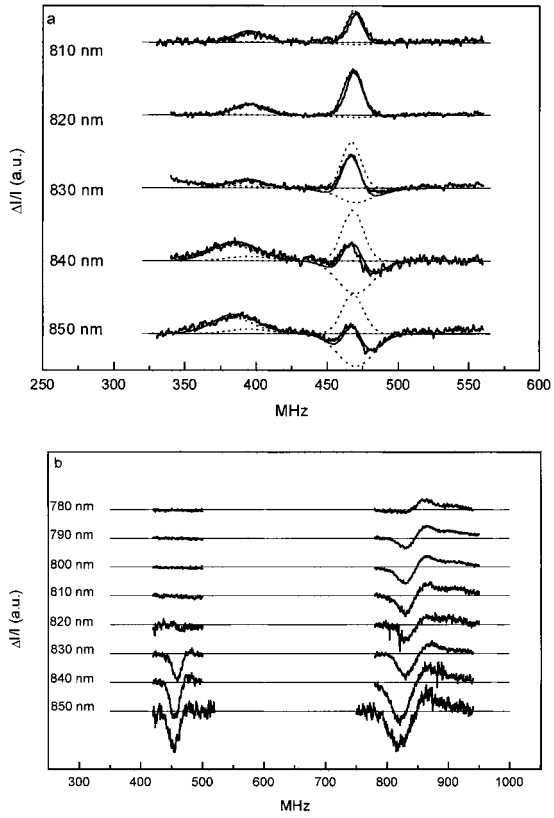

Figure 4. FDMR spectra of isolated chlorosomes taken at different detection wavelengths, as indicated in the figures. Experimental conditions: $T = 1.8$ K, modulation frequency, 320 Hz; microwave power, 500 mW; scan rate, 5 MHz/s. (a) *Cf. aurantiacus* (detection wavelength: from 810 to 850 nm). Dashed lines correspond to components of a fitting function which is the sum of Gaussian curves. In the fitting of the signals at different detection wavelengths, the frequencies, reported in Table 1, of the Gaussian conponents are kept fixed and only their relative contributions are changed. (b) *Cb. tepidum* (detection wavelength: from 780 to 850 nm).

shown in Figure 4 and can be assigned to BChl *a* triplet states by comparing the ZFS parameters of the observed triplet states with the in vivo and in vitro values reported for BChl *a* triplet states.[24]

Also in the case of BChl *a* triplet population, analogies and differences characterize the two type of chlorosomes. In *Cb. tepidum* the resonance frequencies are centered at 457 and 820 MHz, the third transition being too weak to be detected. The FDMR transitions correspond, in this case, to a decrease of emission and can be assigned to BChl *a* present in the baseplate, as previously suggested[9,10] on the basis of the ZFS: $|D| = 0.02213$ cm^{-1}, $|E| = 0.0061$ cm^{-1}. It is worth noting that these BChl *a* transitions are not observed when the BChl *c* emission is detected at 780 nm, possibly due to the lack of back energy transfer at 1.8 K, if this is the main factor influencing the steady-state populations of the BChl *c* emitters when the triplet population of BChl *a* is modulated. Another possibility is that the BChl *a* molecules giving the triplet states are unconnected from the BChl c molecules and absorb light only directly. In *Cf. aurantiacus* the signals have opposite sign and the 2*E* transition is detectable at about 400 MHz. The $|D| + |E|$ transition on the other hand is expected to be found at 875 MHz; however, the overlap with the stronger transitions due to carotenoids is large in this microwave region (see the following section).

These are not the only differences that can be found by comparing the two systems. In fact, changing the detection wavelength in the BChl *a* emission band from 810 to 850 nm, we found that in *Cb. tepidum* the signal shapes and the resonance frequencies do not change, while in *Cf. aurantiacus* the transitions change dramatically (Figure 4). The behavior of the line shape suggests the presence of two different BChl *a* triplet states, correlated to different optical properties. In the figure a fitting with two Gaussian components for each transition is shown. From the fitting we may see that one triplet state shows the maximum of intensity at 820 nm and the other at 840 nm. These components have different polarization, but both show a detectable 2*E* transition.

Carotenoid Triplet States. Together with the signal due to BChl *a* triplet states, other FDMR transitions have been detected in both systems when monitoring the BChl *a* emission, but not when monitoring the BChl *c* emission. These transitions, shown in Figure 5a,b, are easily assigned to carotenoid triplet states on the basis of the ZFS values, which correspond roughly to a number of 9−10 conjugated double bonds[25] ($|D| = 0.0334$ cm^{-1}; $|E| = 0.00347$ cm^{-1}), and on the basis of the characteristic pattern of the T−S spectra, reported in Figure 5a,b, which have been detected in correspondence with the maximum of the low-frequency FDMR transitions (2*E*). The T−S spectra in both samples show positive bands at 475, 510, and 555 nm and negative bands at about 485 and 525 nm, assigned respectively to triplet−triplet and singlet−singlet absorption. Very similar T−S spectra of carotenoids in photosynthetic antenna complexes have been found previously both in higher plants,[26−28] and in purple bacteria.[29] The intensity of the T−S signal is low, indicating small carotenoid triplet population. For this reason the T−S spectra were not detected in the broader $|D| + |E|$ transitions. As already found for all the carotenoid triplet states

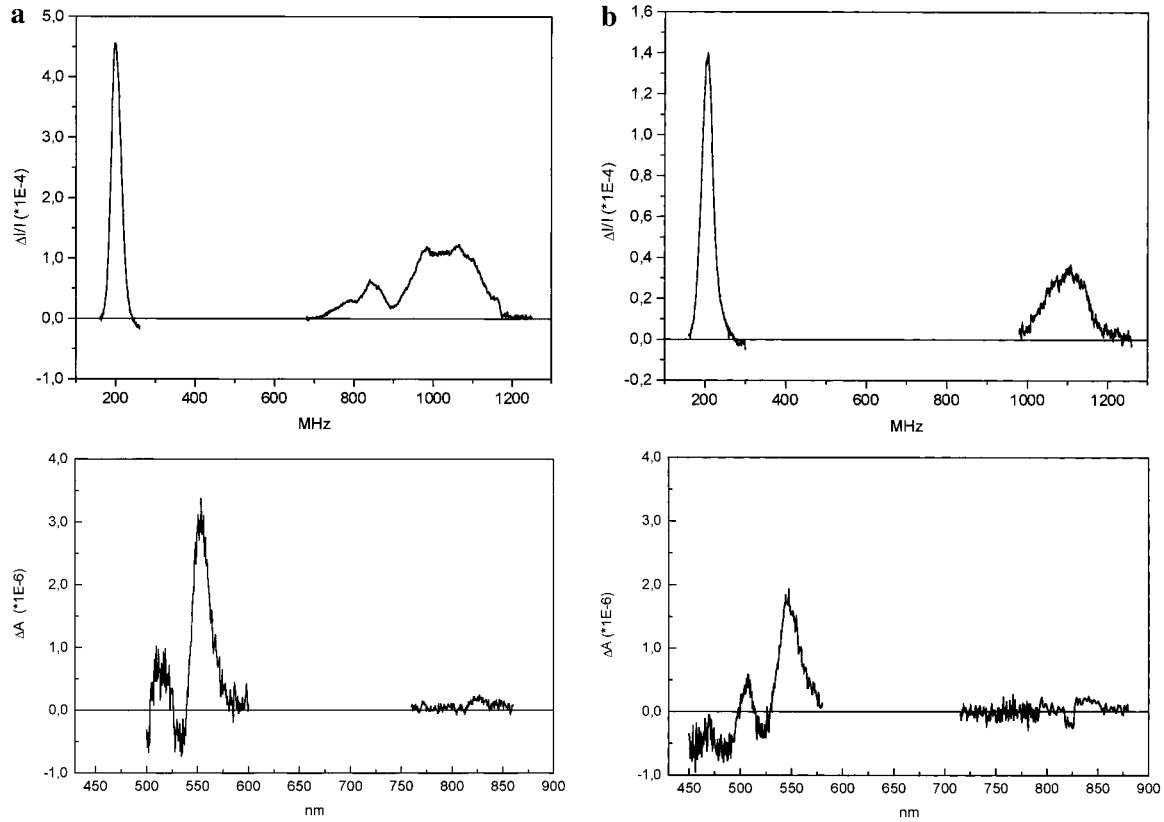

Figure 5. FDMR and T−S spectra of carotenoid triplet states of isolated chlorosomes. Experimental conditions: $T = 1.8$ K; modulation frequency, 320 Hz; microwave power, 500 mW. (a) *Cf. aurantiacus*: (upper traces) FDMR taken at 830 nm, scan rate 5 MHz/s; (bottom trace) T−S spectrum taken at 198 MHz (2*E* transition), resolution 3 nm, scan rate 0.08 nm/s. (b) *Cb. tepidum*: (upper traces) FDMR taken at 830 nm, scan rate 5 MHz/s; (bottom trace) T−S spectrum taken at 208 MHz (2*E* transition), resolution 3 nm, scan rate 0.08 nm/s.

reported in photosynthetic systems, special features are found in the BChls absorption regions of the T−S spectra, due to the change in the electronic state of the nearby carotenoid molecules.[26−29]

The dependence of the FDMR signals ($|D| + |E|$ transitions) on the detection wavelength is shown in Figure 6. An analysis of the line shape shows that several components contribute to the signals. In *Cf. aurantiacus* the relative contribution of the different components changes with the detection wavelength, while no changes, except for the intensity of the resonance, are found in *Cb. tepidum*. The heterogeneity is better seen in the $|D| + |E|$ transitions, which are more structure sensitive than the stronger 2*E* transitions.

Again, together with some analogies, differences between the two bacteria are found. Actually, no $|D| - |E|$ transitions have been detected in *Cb. tepidum* while in *Cf. aurantiacus* the $|D| - |E|$ transition is clearly seen and is inhomogeneously broadened in a similar way with respect to the $|D| + |E|$ transition. In the chlorosomes of *Cb. tepidum* the $|D| - |E|$ transition is expected at about 900 MHz. In this microwave range the transitions due to BChl *c* triplet states are also found. However, we can exclude the presence of an overlapping $|D| - |E|$ transition due to carotenoids since, when the frequency modulation of the amplitude of the microwaves is changed from 33 to 325 Hz, the signal disappears completely (not shown) while the $|D| + |E|$ and the 2*E* transitions of carotenoids are usually found to show a maximum of intensity at the latter frequency, due to the lifetime of carotenoids triplet state, which is close to 10 μs. On the contrary, BChl *c* triplet states are long-lived (milliseconds) and for this reason, the intensity of the signal decreases when the modulation frequency is increased.

Discussion

The energy transfer processes which occur in the chlorosomes are aimed to funnel the energy toward the reaction centers bound to the cytoplasmic membrane. Energy can be transferred in a few tens of picoseconds from BChl *c* to BChl *a* pigments, which are thought to be coordinated to protein in the baseplate.[8,14,16] The efficiency of this process ranges from 30 to 90%,[6,14,30−32] depending on the system and on the experimental conditions. In green sulfur bacteria, quenching of BChl *c* excited states and decrease of energy transfer to BChl *a* is observed under oxidizing conditions.[11,16] This redox-depending quenching, which is not found in *Cf. aurantiacus*, appears to involve some components that are inactive in their reduced state, likely quinone molecules.[12,33]

At physiological temperatures the excitation can move within large domains of the chlorosomes,[34−36] and the lifetimes of BChl *c* excited states are found to be longer[5] in green sulfur bacteria than in *Cf. aurantiacus*. The kinetics of energy transfer within the BChl *c* antenna molecules have remained unclear, but they are assumed to occur in less than 10 ps.[13] It has been suggested[14] that spectral heterogeneity plays a larger role in chlorosomes from *Cb. tepidum* than in chlorosomes from *Cf. aurantiacus*. Moreover, the BChl *c* transition moments seem to be more collinear in *Cf. aurantiacus* than in *Cb. tepidum*,[14] as deduced by the anisotropy of fluorescence decay.

Hole burning experiments at 1.8 K allowed to recognize the lowest excited singlet state of BChl *c* aggregates in the two bacteria at 774 nm in *Cb. tepidum* and at 752 nm in *Cf. aurantiacus*.[37]

Carotenoids are assumed to transfer excitation energy to BChl *c* with a transfer efficiency that in *Cf. aurantiacus* was calculated

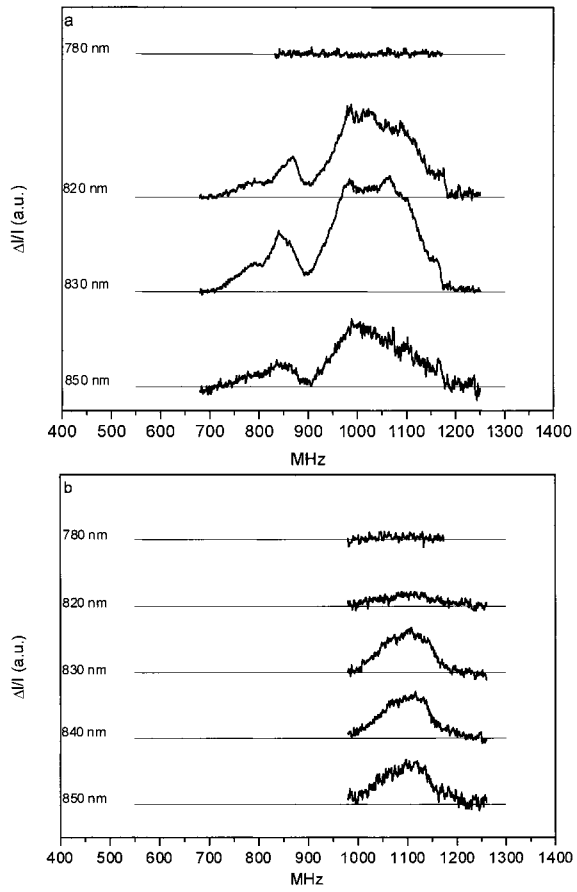

Figure 6. FDMR spectra of carotenoid triplet states of isolated chlorosomes taken at different detection wavelengths, as indicated in the figures, in the microwave range corresponding to the $|D| - |E|$ and $|D| + |E|$ transitions: (a) *Cf. aurantiacus*; (b) *Cb. tepidum*. Other experimental conditions as in Figure 5.

to be 65% at 4 K,[32] and probably most of the energy transfer from the carotenoids to BChl *a* proceeds via BChl *c*. Recently, Psencik et al.[15] have shown that the carotenoid triplet state can be formed under illumination of cells and of isolated chlorosomes suggesting a possible photoprotective role for these pigments, similar to the one they play in purple bacteria and higher plants.[38,39]

In this general description of the excited states and of the energy transfer within chlorosomes, the formation of triplet states under steady-state illumination seems to be a process with very low probability. It is well-known, however, that energetic disorder leads to localization at very low temperature. If the difference in the energy among different oligomer excited states is below the *kT* value at room temperature, it may well become critical at l.8 K, the temperature of ODMR experiments. In such a way localization of triplet state can be possible even in the BChl *c* population and the triplet states can be used as probes of the antenna structure and of the energy transfer processes.

The first result of our experiments is that in isolated chlorosomes, from the two green bacteria studied, triplet states are populated by low-temperature illumination, in all kinds of pigments present: BChl *c*, BChl *a*, and carotenoids. This fact suggests that the general pigment distribution and the general features of energy transfer processes and decay mechanisms are quite similar in the two species. However, despite these similarities, the details of triplet formation and their spectral characteristics point to a different "local" organization within the chlorosomes.

As expected, the triplet yields of all the observed species are very low, as indicated by the microwave induced relative changes of fluorescence ($\Delta I/I$ values of about 10^{-6} in the FDMR spectra) and as indicated by the microwave induced ΔA values detectable in the T−S spectra ($10^{-7}-10^{-6}$).

BChl *c*. The formation of BChl *c* triplet states under illumination is larger in *Cb. tepidum*, and three different BChl *c* pools are observed where the triplet states become localized. As seen in the T−S spectra taken at 856 and 901 MHz, the BChl *c* oligomers that undergo the change in the electronic state absorb respectively at 767 and 747 nm. These values are very close to the absorption wavelengths of the BChl *c* main populations found by optical experiments.[10,13,40]

The third component (828 MHz) gives a very weak T−S spectrum (not shown) with a main bleaching at about 765 nm and seems to be associated with long emitting BChl *c* molecules (790−800 nm). This finding points to a higher heterogeneity of BChl *c* oligomers in green sulfur bacteria compared to green filamentous bacteria. All these pools are, directly and/or indirectly, connected to BChl *a* via energy transfer, as proved by the fact that FDMR of BChl *c* triplet states may be performed not only by detection of BChl *c* emission but also by detection of BChl *a* fluorescence. The sign of FDMR signals is conserved going from 770 to 850 nm detection wavelength, as expected for a triplet state localized in a molecule that is a donor of singlet excitation, as BChl *c* is assumed to be with respect to BChl *a*. A different situation is found for *Cf. aurantiacus*. In this case BChl *c* triplet states become populated at low temperature under continuous illumination, but the triplet state can only be detected when monitoring the BChl *c* emission. This suggests that the triplet trap is not connected, at least at the very low temperature of the experiments, to BChl *a* molecules and that the back-transfer to other BChl *c* molecules is also poor. It can be seen that the FDMR signal has a maximum at 780 nm, while the BChl *c* emission maximum is centered at 765 nm, indicating that the triplet state is likely to be localized in some BChl *c* molecules absorbing at the red tail of the Q_y absorption band. In principle, it is also possible that the observed triplet state is localized in a population that has been damaged during the sample preparation. The different sign of FDMR transitions found for the two triplet states with respect to the third one in *Cb. tepidum* and with respect to the observed transition in *Cf. aurantiacus* is surprising. However, the polarization pattern of the signals is sensitive to the environment and to the pigment organization. The differences among different pools may then be explained on this basis. In any case the chance to populate the triplet state in BChl *c* oligomers that are functional to the energy transfer process is higher in the green sulfur bacterium than in the gliding bacterium. The formation of BChl *c* triplet states under illumination could in principle represent a danger for the photosystems in the presence of oxygen. The higher triplet yield in *Cb. tepidum* would make this species more susceptible to damage. However, it is well-known that in aerobic conditions, quenchers of BChl *c* excited states reduce their lifetimes. Under these conditions even the formation of triplet states becomes unlikely. The quenching mechanism is not present in *Cf. aurantiacus*; however, in this case the triplet yield is much lower and it does not represent a serious problem for the system.

The ZFS parameters of the BChl *c* triplet states are shown in Table 1. The values are in good agreement with those already reported, obtained in whole cells of *Cb. tepidum* and assigned to BChl *c* in oligomeric form.[23] Our T−S results, at least in

TABLE 1: ZFS Parameters of All Assigned Triplet States in Isolated Chlorosomes of *Cf. aurantiacus* and of *Cb. tepidum*[a]

pigment	$2\|E\|$ (MHz)	$\|D\| - \|E\|$ (MHz)	$\|D\| + \|E\|$ (MHz)	$\|D\|$ (cm^{-1})	$\|E\|$ (cm^{-1})
Chloroflexus aurantiacus					
BChl *c*	n.d.	(−) 648	(−) 875	0.0254	0.0038
BChl *a* (I)	(+) 395	(+) 468	n.d.	0.0222	0.0066
BChl *a* (II)	(+) 385	(−) 470	n.d.	0.0221	0.0064
carotenoids	(+) 198	(+) ∼830	(+) ∼1050	0.0314	0.0033
Chlorobium tepidum					
BChl *c* (I)	n.d.	(+) 640	(+) 868	0.0252	0.0038
BChl *c* (II)	n.d.	(+) 640	(+) 905	0.0258	0.0044
BChl *c* (III)	n.d.	(*) 634	(+) 829	0.0246	0.0033
BChl *a*	n.d.	(−) 456	(−) 818	0.0213	0.0060
carotenoids	(+) 206	n.d.	(+) ∼1100	0.0333	0.0034

[a] (+): microwaves induced fluorescence increase. (−): microwaves induced fluorescence decrease. (*): double resonance data. n.d.: not detected.

Cb. tepidum, correlate the triplet formation with the bleachings of oligomers of BChl *c* singlet states.

BChl *a*. BChl *a* molecules are found in the chlorosomes of green bacteria. The organization of these molecules is still uncertain and their location in the baseplate has been proposed on the basis of several experimental evidence. Taking account of the average dimensions of chlorosomes, and the average number of BChls per chlorosome (10^3), van Noort and colleagues[41] calculated, in *Chlorobium vibrioforme*, that there would be about 90 BChl *a* in the envelope, their average distance being 65 Å. This distance is too high to explain the experimental finding by the same group that randomization of the excitation among BChl *a* molecules, measured by time-resolved anisotropy decay, has a time constant of about 20 ps. This would suggests that BChl *a* is organized in clusters.

The role of the proteins present in the envelope is also unclear. Evidence of the role of the CsmA protein in the ligation of BChl *a* has been obtained in *Cf. aurantiacus* on the basis of the effect induced by SDS, 1-hexanol, and Triton on the composition of treated chlorosomes;[8] in view of the sequence homology of CsmA between *Cf. aurantiacus* and *Cb. tepidum*, this might hold true for the latter species as well. The relative amount of BChl *a* with respect to the BChl *c* is higher in the chlorosomes of *Cf. aurantiacus*, 4% vs 1%. In *Cf. aurantiacus* two spectroscopically different BChl *a* species are suggested to exist[42−45] based on the time-resolved fluorescence results obtained in whole cells and to the experiments done by Mimuro et al.[45] on the effects induced by 1-hexanol treatment in the baseplate. The conclusion of that work was that there is a BChl *a* pool, absorbing at 793 nm and fluorescing at 813 nm at room temperature, which is a functional baseplate component for the energy transfer to the B808−866 complex, and a second BChl *a* pool (absorption maximum at 813 nm and fluorescence maximum at 826 nm), which is an additional baseplate form not involved in the energy transfer to the membrane. Differently, in green sulfur bacteria the energy transfer is thought to occur between the BChl *a* molecules in the baseplate and the BChl *a* molecules in the FMO protein before reaching the reaction centers.

The FDMR spectra of BChl *a* triplet states in the chlorosomes clearly show that the pigment organization differs in the two species studied. The ZFS parameters are reported in Table 1. In both systems BChl *a* triplet states can be detected under illumination at very low temperature; however, in *Cf. aurantiacus* two types of BChl *a* pool have been detected corresponding to different optical and magnetic properties, as suggested by the fact that the intensity of different FDMR signals has the maximum at different wavelengths of detection.

These two pools are probably related to those previously reported.[45] In *Cb. tepidum* only one type of BChl *a* triplet state has been detected. A marked difference is also found when considering the polarization of the BChl *a* FDMR signals in the two bacteria. If the assignment is correct, as we believe on the basis of the behavior of the signal intensity with the detection wavelength and on the basis of the deduced ZFS values, the BChl *a* triplet states in *Cf. aurantiacus* show an unusual polarization. Indeed, the 2*E* transitions are well detectable, while in the light harvesting complexes from purple bacteria and in solvent,[46] BChl *a* triplet states have a polarization that resembles the one found in *Cb. tepidum*: two transitions ($|D| - |E|$ and $|D| + |E|$) comparable in intensity and with the same sign. Since the polarization, besides the molecular structure, can be influenced by several factors such as pigment−pitment interactions and/or exposure to lipid or protein environment, the ODMR data strongly suggest a different organization of these molecules in the two types of chlorosomes. *Cf. aurantiacus*, which lacks the FMO proteins, seems to have a more complex distribution of BChl *a* pigments likely in the contact region with the cytoplasmic membrane.

Carotenoids. Carotenoids are nonfluorescent species and have a very low ISC probability, so that their triplet states are not easily populated by photoexcitation from the excited singlet states.

The common function of carotenoids in photosynthetic systems is 2-fold.[39,47−52] First they act as efficient antenna pigments and transfer excitation to (B)Chl molecules, in the pigment−protein light-harvesting complexes. Second, they function as quenchers of (B)Chl triplet states to avoid singlet oxygen formation, both in the antenna complexes and in some reaction centers. Since green sulfur bacteria and *Cf. aurantiacus* are anaerobes (although the latter is a facultative aerobe), it seems surprising to find that, in both bacteria, some carotenoids are able in principle to perform the photoprotective role, as suggested by our finding that carotenoid triplet states are populated. Actually, green sulfur bacteria may encounter oxic conditions in their environment due to vertical mixing of zones in the chemocline in stratified lakes. In fact *Cb. tepidum*[12] under oxidizing conditions activates a mechanism in the chlorosomes that quenches excited BChl *c* singlet states, decreasing the energy transfer to the BChl *a* in the baseplate. This uncoupling mechanism is reversible and prevents the formations of toxic reactive oxygen species from photosynthetically produced reactants in the reaction centers. *Cf. aurantiacus*, on the other hand, does not possess such a quenching mechanism, probably because its PSII-type reaction center does not readily form reactive oxygen. However, *Cf. aurantiacus* is a facultative aerobe and can be harmfully exposed to oxygen during the transition from photosynthetic to respiratory metabolism in aerobic environments. Both systems, then, can be exposed to oxygen and the possibility to form singlet oxygen in the chlorosomes cannot be ruled out if the BChl triplet state can be populated under particular conditions.

By ODMR we found that both BChl *c* and BChl *a* triplet states become populated in isolated chlorosomes at least at low temperature, but the main triplet species present in the chlorosomes under illumination at low temperature are carotenoid triplet states. FDMR of these triplet states, in both bacteria, can only be done by detection of BChl *a* fluorescence.

We found that the carotenoid triplet states are not detected by BChl *c* fluorescence at 780 nm, even though, as is the case

of *Cb. tepidum*, the fluorescence yield is higher at 780 nm than at 820 nm. This suggests a closer contact of carotenoids to BChl *a* species rather than to BChl *c* and then, as a consequence, a location of carotenoids in the region where BChls *a* are present, likely the baseplate of the chlorosomes. The finding seems to be in contrast with the conclusions drawn by Freese et al.[53] based on the Stark effect, LD, and CD in normal and carotenoid-deficient chlorosomes of *Cf. aurantiacus*. In that case, the spectral changes in the Q_y transition of BChl *c* observed in the absence of carotenoids seem to suggest the vicinity of carotenoids and BChl *c* in the chlorosomes, even though the observed effect is quite small. However, with the ODMR technique we select only the carotenoids that are able to quench BChl triplet states. Triplet−triplet transfer requires close distances between the pigments involved in the energy transfer since the Dexter exchange mechanism depends on the overlap of the excited-state molecular orbitals. Singlet−singlet energy transfer may be possible at larger distances since it may proceed via the Förster mechanism, which depends on the strength of the transition dipoles between ground and excited states. Then we may not exclude that some carotenoid population is able to transfer singlet excitation to BChl *c* molecules in the core of the chlorosomes, as is indeed suggested by Takaichi,[54,55] who found the core of chlorosomes, in both *Cb. tepidum and Cf. aurantiacus*, enriched in β and γ carotene.

In several antenna complexes of higher plants and purple bacteria, T−S spectra taken at the resonance frequencies of carotenoid triplet states show characteristic features in the Q_y absorption region of (B)Chls.[26−29] These spectral features have been interpreted as due to electronic interactions between the carotenoid molecule carrying the triplet state and Chl molecules close to it and likely involved in the triplet−triplet transfer process that populates the carotenoid triplet state. At wavelengths corresponding to the Q_y absorption region of BChl *a* (800−830 nm), small negative and positive bands seem indeed to be present in the T−S spectrum of the chlorosomes, recorded at the resonance frequency of carotenoid triplet state.

It has been noted[29] that the intensity of these interaction bands is directly correlated to the rate of triplet transfer. We may then say that in the chlorosomes the efficiency of triplet−triplet transfer is not very high, since the interaction bands are very weak. This is also in agreement with the presence of detectable triplet states of BChl *c* and BChl *a* molecules, which indicate that the carotenoids present in the chlorosomes are not able to quench the BChls with high efficiency. It is possible that the carotenoids which are able to quench BChl *a* triplet states are located in the baseplate where they may have a main structural role in anchoring the chlorosomes to the membrane, as already suggested,[56] and the fact of being able to play a photoprotective function may have been only fortuitous at the beginning of the existence of these ancient photosystems, when the atmosphere contained no oxygen.

The line shape of the $|D| + |E|$ transitions in the FDMR spectra shows that in both samples several components contribute to the whole spectra. In *Cf. aurantiacus* this heterogeneity can also be found in the spread of the $|D| + |E|$ transition, which is also detectable. The strong $2E$ transitions are less broad due to a small spread in the E parameter for the observed triplet states. The D values are determined by the effective distance between the two unpaired electrons along the long polyene chain axis (z axis). The E values depend on the asymmetry of the dipolar tensor perpendicular to the long polyene chain axis, and the variation of the electron distribution in the xy plane is probably less influenced than the variation along the chain axis. According to the empirical relationship given by Ros et al.,[57] the D and E values depend on the inverse of the number of conjugated double bonds. If we consider the pigment composition[55,58] in the chlorosomes, the carotenoids which are present in larger amount are chlorobactene and 1,2-dihydrochlorobactene (10 conjugated double bonds) plus a small amount of γ-carotene (10 conjugated double bonds) and glucoside-γ-chlorobactene in *Cb. tepidum*; γ-carotene, β-carotene (9 conjugated double bonds) and a 20% amount of glucoside-γ-carotene in *Cf. aurantiacus*. On the basis of the number of conjugate double bonds and on the basis of the carotenoids abundance, we would expect to find the same transition frequencies in the two samples except for the β-carotene, which should have somewhat larger ZFS values. On the contrary in *Cf. aurantiacus* the resonant $|D| + |E|$ transitions occur at lower frequencies with respect to the observed transitions in *Cb. tepidum*. Since by ODMR we are selecting only the carotenoid populations that are able to populate the triplet state, these could differ substantially with respect to the chemical composition. Moreover, the environment and the molecular conformation may influence the resonance frequencies as well. Since the polarization pattern of carotenoid triplet states in *Cf. aurantiacus* is different compared to that observed in *Cb. tepidum*, and at least for some components, the resonance frequencies are also different, the exposure of the involved carotenoids to a different environment as well as a difference in their conformation, may play an important role in determining the magnetic characteristic of the triplet states. We then suggest that if BChls *a* are localized in the baseplate, then the latter also contains carotenoids molecules, but the organization, and likely the amount of these pigments in the two bacteria, is different. Possibly, the glucoside-carotenoids in *Cf. aurantiacus* are more involved in the structure of the baseplate. Actually, they are found to be present in the baseplate with a 50% contribution.[55]

Finally, a further difference found between the two systems is that the carotenoid triplet states are formed with some site selection by quenching of BChl *a* molecules: we found that the line shape of the transitions depends on the detection wavelength in the 800−850 nm range only in *Cf. aurantiacus*. This fact, together with the observed heterogeneity in the detected BChl *a* triplet states, points toward an higher complexity of the contact region between the chlorosomes and the membrane, likely due to the absence of the FMO protein.

The results concerning triplet formation in prereduced chlorosomes from *Cb. tepidum* are very similar to the results from *Cb. limicola* reported by Psencik et *al.*;[15] however, we found that the detection of FDMR signals in the emission band of BChl *c* gives prevalent contributions of BChl *c* triplet states. No $|D| + |E|$ FDMR transitions to be assigned to carotenoids have been detected. Very weak signals in the $2E$ microwave region have been detected but the overlap with $2E$ transitions due to BChl *c* triplet states does not allow us to assign them to carotenoids with certainty. In *Cf. aurantiacus* the signals in this region are even less intense. Following these experimental results we may assume that the carotenoid populations that are able to populate the triplet state are mainly interacting with BChl *a* molecules and are then localized close to them.

Conclusions

The comparison of ODMR results obtained from the analysis of the spectra of isolated chlorosomes in the two bacteria chosen as representative of the two phylogenetically distinct species of green bacteria, revealed important analogies and differences between the two systems.

The general energy transfer processes and the formation of triplet states under illumination at low temperature follow the same line. We found that BChl *c*, BChl *a*, and carotenoid triplet states are populated in low yield in the chlorosomes in both systems. The carotenoid triplet states seem to be formed by quenching of BChl *a* triplet states, and a weak interaction between carotenoids and BChl *a* molecules is a common feature in *Cf. aurantiacus* and in *Cb. tepidum*. These results strongly suggest that some carotenoid molecules are present in close contact with BChl *a* molecules, likely in the baseplate, where, in principle, they are able to perform also a photoprotective role.

Several differences, however, appear when considering the characteristics of these triplet states, which reveal as, either in the core or in the baseplate, the local organization of the pigments is characteristic of the system. The chlorosomes of *Cb. tepidum* present distinct BChl *c*-oligomers easily distinguished because of the associated triplet states and of their T−S spectra. The origin of these different forms, which are connected to BChl *a* via energy transfer also at the very low temperature of the ODMR experiments, is not clear; it may, however, depends on the degree of alkylation of the homologues. On the contrary, in the chlorosomes of *Cf. aurantiacus*, we found spectrally distinct forms of BChl *a*, instead of BChl *c*. A homogeneous BChl *c* population in *Cf. aurantiacus* has been also suggested[59] by pump−probe experiments and may be related to the lack of different triplet traps at very low temperature.

The possible role of different pools of BChl *a* in the energy transfer process to the RCs remains uncertain.

Carotenoids quench selectively different BChl *a* forms in *Cf. aurantiacus*, while in *Cb. tepidum* no site selection has been found. Again this can be taken as an indication of the higher level of complexity in the baseplate of *Cf. aurantiacus* which lacks the FMO proteins.

We are planning to perform ODMR experiments on whole cells of the two green bacteria strains, under different redox conditions, to correlate the information we already gained in isolated chlorosomes to the interactions of these antenna systems with the reaction centers.

Acknowledgment. This investigation was supported in part by MURST BIOSTRUTT program and by EC contract NO. ERB FMRX- 0214; both agencies are gratefully acknowledged. We also thank Prof. Shinichi Takaichi (Nippon Medical School, Kawasaki), Prof. Shigeru Itoh (Nagoya University), and Prof. Katsumi Matsuura (Tokyo Metropolitan University) for useful discussions.

References and Notes

(1) Woese, C. R. *Microbiol. Rev.* **1987**, *51*, 221−271.

(2) Brune, D. C.; Nozawa, T.; Blankenship, R. E. *Biochemistry* **1987**, *26*, 8644−8651.

(3) Hirota, M.; Moriyama, T.; Shimada, K.; Miller, M.; Olson, J. M.; Matsuura, K. *Biochim. Biophys. Acta* **1992**, *1099*, 271.

(4) Holzwarth, A. R.; Müller, M. G.; and Girebenow, K. J. *Photochem. Photobiol. B Biol.* **1990**, *5*, 457.

(5) Olson, J. M. *Photochem. and Photobiol.* **1998**, *67* (1), 61−75.

(6) Holtzwarth, A. R. and Schaffner, K. *Photosynth. Res.* **1994**, *41*, 225−233.

(7) Brune, D. C.; King, G. H.; Blankenship, R. E. In *Photosynthetic Light-Harvesting System*; Scheer, H., Schneider, S., Eds.; Walter de Gruyter: Berlin, 1988; pp 141−151.

(8) Sakuragi, Y.; Frigaard, N. U.; Shimada, K. and Matsuura, K. *Biochim. Biophys. Acta* **1999**, *1413*, 172−180.

(9) Sprague, S. G. and Fuller, R. C. In *Structure of Phototrophic Prokaryotes*; Stolz, J. F., Ed.; CRC Press: Boca Raton, FL, 1991; pp 79−103.

(10) Otte, S. C. M.; van der Heiden, J. C.; Pfenning, N. and Amesz, J. *Photosynth. Res.* **1991**, *28*, 77−87.

(11) Frigaard, N. U.; Matsura, K.; Hirota, M.; Miller, M. and Cox, R. P. *Photosynth. Res.* **1998**, *58*, 81−90.

(12) Frigaard, N. U. and Matsura, K. *Biochim. Biophys. Acta* **1999**, *1412*, 108−117.

(13) Psencik, J.; Polivka, T.; Nemec, P.; Dian, J.; Kudrna, J.; Maly, P. and Hala, J. *J. Phys. Chem.* **1998**, *102*, 4392−4398.

(14) Savikin, S.; van Noort, P. I.; Zhu, Y.; Li, S.; Blankenship, R. E. and Struve, W. *Chem. Phys.* **1995**, *194*, 245−258.

(15) Psencik, J.; Searle, G. F. W.; Hala, J. and Schaafsma, T. J. *Photosynth. Res.* **1994**, *40*, 1−10.

(16) Gerola, P. D. and Olson, J. M. *Biochim. Biophys. Acta* **1986**, *848*, 69−76.

(17) Wang, J.; Brune, D. C. and Blankenship, R. E. *Biochim. Biophys. Acta* **1990**, *1015*, 457−463.

(18) Feick, R. G.; Fitzpatrick, M. and Fuller, R. C. *J. Bacteriol.* **1982**, *150*, 905−915.

(19) Van Noort, P. I.; Zhu, Y.; LoBrutto, R.; Blankenship, R. E. *Biophys. J.* **1997**, *72*, 316−325.

(20) Borland, C. F.; Cogdell, R. J.; Land, E. J. and Truscott, T. J. J. *Photochem. Photobiol.* **1989**, *3*, 237−245.

(21) Carbonera, D.; Giacometti, G. and Agostini, G. *Appl. Magn. Reson.* **1992**, *3*, 859−872.

(22) Carbonera, D.; Giacometti, G. and Agostini, G. *FEBS Lett.* **1994**, *343*, 200−204.

(23) Psencik, J.; Schaafsma T. J.; Searle G. F. W.; Hala J. *Photosynth. Res.* **1997**, *52*, 83−92.

(24) Angerhofer, A.; von Schütz, J. U. and Wolf, H. C. *Progress in Photosynthesis Research*; Biggins, J., Ed.; Proceedings of the VIIth International Congress on Photosynthesis Providence, RI, 1986; M. Nijhoff Publishers: Dordrecht, 1987; Vol. 1, pp 4427−4430.

(25) Carbonera, D.; Giacometti, G.; Agostini, G. and Toffoletti, A. *Gazz. Chim. Ital.* **1989**, *119*, 225−228.

(26) Carbonera, D.; Giacometti, G.; Agostini, G.; Angerhofer, A. and Aust, V. *Chem. Phys. Lett.* **1992**, *194*, 275−281.

(27) Carbonera, D.; Giacometti, G. and Segre, U. *J. Chem. Soc., Faraday Trans.* **1996**, *92*, 989−993.

(28) Van der Vos, R.; Carbonera, D. and Hoff, A. J. *Appl. Magn. Reson.* **1991**, *2*, 179−202.

(29) Angerhofer, A.; Bornhaüser, F.; Gall, A. and Cogdell, R. J. *Chem. Phys.* **1995**, *194*, 259−274.

(30) Zhu, Y.; Ramakrishna, B. L.; van Noort, P. I. and Blankenship, R. E. *Biochim. Biophys. Acta* **1995**, *1232*, 197−207.

(31) Savikhin, S.; Buck, D. R.; Struve, S.; Blankenship, R. E.; Taisova, A.; Novoderezhkin, V. I. and Fetisova, Z. G. *FEBS Lett.* **1998**, *430*, 323.326.

(32) Van Dorssen, R. J.; Vasmel, H. and Amesz, J. *Photosynth. Res.* **1986**, *9*, 33−45.

(33) Frigaard, N. U.; Tokita, S. and Matsuura, K. *Biochim. Biophys. Acta* **1999**, *1413*, 108−116.

(34) Schmidt, K. A. and Trissl, H. W. *Photosynth. Res.* **1998**, *58*, 43−55.

(35) Vos, M.; Nuijs, A. M.; van Grondelle, R.; van Dorssen, R. J.; Gerola, P. D. and Amesz, J. *Biochim. Biophys. Acta* **1987**, *891*, 275−285.

(36) Savikin, S.; Buck, D. R.; Struve, W. S.; Blankenship, R. E.; Taisova, A. S.; Novoderezhkin, V. I. and Fetisova, Z. G. *FEBS Lett.* **1998**, *430*, 323−326.

(37) Fetisova, Z. G.; Mauring, K. and Taisova, A. S. *Photosynth. Res.* **1994**, *41*, 205−210.

(38) Cogdell, R. J. and Frank, H. *Biochim. Biophys. Acta* **1987**, *895* (2), 63−79.

(39) Siefermann-Harms, D. *Physiol. Plantarum* **1987**, *69*, 561.

(40) Steensgard, D. B.; Matsuura, K.; Cox, R. P. and Miller, M. *Photochem. Photobiol.* **1997**, *65*, 129−134.

(41) Van Noort, P. I.; Francke, C.; Schoumans, N.; Otte, S. C. M.; Aarstma, T. J. and Amesz, J. *Photosynth. Res.* **1994**, *41*, 193−203.

(42) Brune, D. C.; King, G. H.; Infosio, A.; Steiner, T.; Thewalt, M. L. W. and Blankenship, R. E. *Biochemistry* **1989**, *26*, 8652−8658.

(43) Mimuro, M.; Nozawa, T.; Shimada, K.; Yamazaki, I.; Lin, S.; Knox, R. S.; Wittmershaus, B. P.; Brune, D. C. and Blankenship, R. E. *J. Phys. Chem.* **1989**, *93*, 7503−7509.

(44) Mimuro, M.; Nozawa, T.; Tamai, N.; Nishimura, Y.; and Yamazaki, I. *FEBS Lett.* **1994**, *340*, 167−172.

(45) Mimuro, M.; Nishimura, Y.; Nozawa, T.; Yamazaki, I.; Kobayashi, M.; Yu Wang, Z.; Nozawa, T.; Shimada, K. and Matsuura, K. *Photosynth. Res.* **1996**, *48*, 263−270.

(46) Hoff, A. J. In *Triplet state ODMR spectroscopy*; Clarke, R. H., Ed.; Wiley: New York, 1982; Chapter IX.

(47) Thomas, J. B. *Biochim. Biophys. Acta* **1950**, *5*, 186.

(48) Thomas, J. B. and Goedheer, J. C. *Biochim. Biophys. Acta* **1953**, *10*, 358.
(49) Goedheer, J. C. *Biochim. Biophys. Acta* **1959**, *35*, 1−8.
(50) Siefermann-Harms, D. *Biochim. Biophys. Acta* **1985**, *811*, 325.
(51) Cogdell, R. J. *Pure Appl. Chem.* **1985**, *57*, 723−728.
(52) Cogdell, R. J. and Frank, H. A. *Biochim. Biophys. Acta* **1987**, *895*, 63.
(53) Freese, R.; Oberheide, U.; van Stokkum, I.; van Grondelle, R.; Foidl, M.; Oelze, J. and van Amerongen, H. *Photosynth. Res.* **1997**, *54*, 115−126.
(54) Takaichi, S. and Oh-oka, H. *Plant Cell Physiol.* **1999**, *40* (7), 691−694.
(55) Takaichi, S.; Tsuji, K.; Hanada, S.; Matsuura, K. and Shimada, K. In *Photosynthesis: from Light to Biosphere*; Mathis, P., Ed.; Kluver Academic Publishers: Netherlands, 1995; Vol. IV, pp 127−130.
(56) Foidl, M.; Golecki, J. R. and Oelze, J. *Photosynth. Res.* **1997**, *54*, 219−226.
(57) Ros, M. and Groenen, E. J. J. *J. Chem. Phys.* **1991**, *94*, 7640−7648.
(58) Takaichi, S.; Wang, Z. Y.; Umetsu, M.; Nozawa, T.; Shimada, K. and Madigan, M. T. *Arch. Microbiol.* **1997**, *168*, 270−276.
(59) Savikin, S.; Yinwen, Z.; Blankenship, R. E.; Struve, W. S. *J. Phys. Chem.* **1996**, *100*, 3320−3322.