Biochimica et Biophysica Acta 808 (1985) 363–382
Elsevier

BBA 41796

Magnetic-field effects in photosynthetic bacteria

I. Magnetic-field-induced bacteriochlorophyll emission changes in the reaction center and the antenna of *Rhodospirillum rubrum*, *Rhodopseudomonas sphaeroides* and *Prosthecochloris aestuarii*

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(Received March 6th, 1985)

Key words: Magnetic field effect; Bacteriochlorophyll emission; Singlet fission; Carotenoid; Bacterial photosynthesis; *(R. rubrum, P. aestuarii, Rps. sphaeroides)*

In cells, chromatophores, isolated antenna complexes and isolated reaction centers of a number of photosynthetic bacteria the magnetic-field-induced emission is determined as a function of the magnetic-field strength, redox state of the reaction center, temperature and the emission and excitation wavelength. Two types of magnetic-field-dependent emission were observed. Category I is closely associated with the charge recombination in the reaction center, decreases upon cooling, is found in reaction center-containing preparations in which the first quinone acceptor Q_1 has been reduced, and the relative magnetic-field-dependent emission ($\Delta F/F$) does not depend on the excitation wavelength. Category II is associated with the antenna of the carotenoid-containing bacteria, does not depend upon temperature or the redox state of the reaction center, occurs only upon direct carotenoid excitation and is not found in isolated reaction centers. Upon carotenoid excitation of reduced cells or chromatophores, magnetic-field-dependent emission of both categories is found. The magnetic-field-dependent emission of the first category is discussed in terms of the radical pair mechanism and suggestions are made to explain the differences observed between cells, chromatophores and reaction centers. The magnetic-field-dependent emission of the second category is

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Abbreviations: A, absorbance; ABChl, antenna bacteriochlorophyll; ACar, antenna carotenoid, BChl, bacteriochlorophyll; BPh, bacteriopheophytin; B800, B850 and B880, bacteriochlorophyll with an absorbance maximum near 800, 850 and 880 nm, respectively; Car, carotenoid; F, total emission yield; ΔF, magnetic field-induced emission change; ΔF_{max}, maximum value of ΔF; ϕ_T, total triplet yield; $\Delta\phi_T$, magnetic field-induced triplet yield change; $H_{1/2}$, magnetic field strength at which the magnetic field-dependent triplet or emission yield is half maximum; H_0, magnetic field strength ($\neq 0$) at which no net magnetic field-dependent triplet yield or emission occurs; H_{max}, magnetic field strength at which the magnetic field-dependent triplet yield or emission is maximum; I, intermediate electron acceptor; J, exchange interaction; LDAO, lauryldimethylamine N-oxide; P, reaction center bacteriochlorophyll dimer; P-800, P880, reaction center bacteriochlorophyll with an absorbance maximum near 800 or 880 nm, respectively; Q_1, first reaction center quinone electron acceptor; RBChl, reaction center bacteriochlorophyll; RCar, reaction center carotenoid; superscripts *, S, T, the excited, the first excited singlet or the excited triplet state of the molecule involved, respectively; superscripts +, −, the radical cation or anion of the molecule involved, X, unspecified participant of triplet pair.

0005-2728/85/$03.30

discussed in terms of singlet fission of the first excited singlet state of an antenna carotenoid molecule into a double triplet state of the carotenoid and another nearby molecule. A process of homofission explains our results well, but with some restrictions a process of heterofission might also account for the observed magnetic-field-dependent emission.

Introduction

The study of magnetic field effects on excitation transfer and primary electron transport has been an important aspect of photosynthetic research during the last decade [1]. The phenomena observed involve magnetic-field-induced changes of the (bacteriochlorophyll (BChl)) emission [2–9] and triplet yields (Refs. 7,10 and 11; see also Ref. 12 for this issue) and these results are mainly discussed in terms of the radical pair mechanism [13]. Some years ago, Rademaker et al. [14] discovered in whole cells of the purple bacterium *Rhodospirillum rubrum* a strong magnetic-field dependence of the antenna carotenoid triplet yield upon carotenoid excitation. These authors pointed out that the change in triplet yield could be explained either by the mechanism of charge separation and subsequent recombination (radical pair mechanism) or by a magnetic-field-dependent fission reaction of a carotenoid excited singlet state into two triplet states (see Ref. 15 for a review of the process of fission). The process of energy transfer, charge separation and charge recombination for *R. rubrum* is shown schematically in Fig. 1 [16–19]. Two types of magnetic-field effects occur and will be discussed separately.

Upon excitation of antenna carotenoid or BChl molecules a fraction of the excitation energy is transferred to the BChl species absorbing at the longest wavelength (B880). The transfer efficiencies upon carotenoid excitation vary strongly among various purple bacteria, i.e., 30% for transfer from spirilloxanthin to B880 in *R. rubrum* and close to 90% for transfer from neurosporene or sphaeroidene to B800, B850 or B880 in *Rps. sphaeroides* and *Rps. capsulata* [20]. So far, no satisfactory explanation for these differences has been proposed.

Upon arrival of the excitation in the BChl network the excitation performs a diffusive type of transfer process among a large number of B880 molecules, and visits several reaction centers before trapping takes place. The traps are not perfect, i.e., excitations arriving at a trap have a high probability of escaping back into the surrounding antennae (90% for *R. rubrum*, 70% for *Rps. capsulata* [8,21,22]).

Charge separation and recombination in the reaction center

Upon excitation of the reaction center BChl dimer P-880 or P, a charge separation may take place resulting in the radical pair P^+I^- (I is a bacteriopheophytin (BPh)), which after its formation oscillates between a singlet state $(P^+I^-)^S$ and a triplet state $(P^+I^-)^T$. The mixing of these states depends upon a magnetic field [23]. In a low field the energy difference between the singlet and triplet levels is $2J(3G < J < 8G$ [24,51], while anisotropic electron spin–spin dipole interactions split the triplet levels into three so-called zero-field states. In principle, all three levels are available for S–T conversion of the radical pair. At high magnetic field, Zeeman splitting shifts the $m_s = +1$ and $m_s = -1$ levels of the triplet state far beyond reach of the singlet level, and only the $m_s = 0$ level participates in S–T conversion. The reaction center triplet yield is thus lower in the presence of a magnetic field. When the primary quinone acceptor Q_1 is oxidized, the electron transfer from I^- to Q_1 is so fast that these processes have no chance to occur; only if Q_1 is reduced is the lifetime of P^+I^- long enough (approx. 10 ns [24,25]) to allow S–T conversions. Under these conditions the decay of the state P^+I^- is mainly governed by recombination of $(P^+I^-)^S$ to the ground state (P I) or to the excited state P*I and of $(P^+I^-)^T$ to the reaction center triplet state P^TI, which in carotenoid containing species rapidly decays into the reaction center carotenoid triplet state ($RCar^T$). The back reaction to P* followed by back transfer to the antenna is responsible for the observed magnetic-field-dependent emission in this scheme. These processes and their associated rate constants are summarized in the right part of Fig. 1.

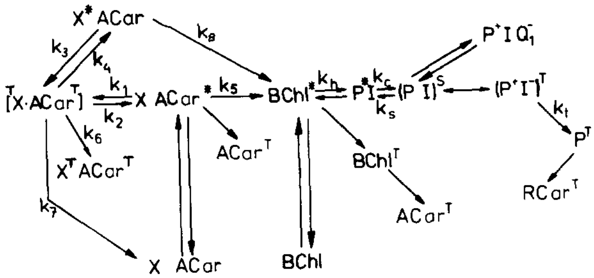


Fig. 1. Scheme of energy and electron transfer in *R. rubrum* S1. For explanation, see text.

In earlier work we showed that in *R. rubrum* the reaction-center-associated magnetic-field dependent emission could be studied by using selective BChl excitation, and we concluded that these effects can quantitatively be explained in terms of a matrix model of energy transfer [8,25].

Singlet fission and fusion in the antenna

Upon excitation of the antenna carotenoid molecule the energy may be transferred to an adjacent BChl molecule or the carotenoid excited state may form two triplet states involving either a second identical carotenoid molecule (homofission) or a different molecule, e.g., BChl, tyrosine or tryptophan (heterofission). The occurrence of these processes with high efficiency in organic crystals has been well documented [15,27–31]. Although the process of singlet-fission is the inverse of triplet-fusion, the explicit details of the process need not be identical. We nevertheless assume that the theory adopted for the fusion process is also valid in the case of fission [15]. The state of the triplet pair thus formed is described by nine spin states, each of which may have some singlet, triplet or quintet character. The probability of triplet or singlet formation depends upon the fraction of the nine states, that contains triplet or singlet character. As fission is a spin-conserving process [15], a pure triplet state can be formed from a singlet excited state only if there exists a substate with a mixed singlet–triplet character. The rate constants of fission k_1, k_3 and fusion k_2, k_4 increase with the number of substates with some singlet character, which in its turn decreases with increasing magnetic field [15,28,30]. If the triplet pair consists of two different molecules or of two identical but randomly oriented molecules, the rate constants of fission and fusion will decrease with increasing magnetic field. In the case of homofission with two identical specially oriented molecules, no triplet will be formed in zero field (pure singlet, triplet and quintet substates), while at moderate field strengths the substates will be mixed singlet–triplet), resulting in triplet formation. At high fields the rate constants of fission and the triplet yield will again decrease due to a lower number of substates with some singlet character, resulting in a decrease of the triplet yield [15,28,30]. The ratio of rate constants for fission and fusion depends on the energy differences between the various excited states involved, and therefore an effect of the temperature upon both emission and triplet yield may occur. The BChl emission will in principle show a magnetic-field dependence more or less complementary to that of the antenna triplet yield, because the transfer of excitations competes with the fission process (see Fig. 1, rate constants k_1 and k_5). If, however, fusion (rate constant k_4) follows fission (rate constant k_1), the effect of a magnetic field on the emission and triplet yield will depend on the value of k_1, k_2, k_3, k_4, k_6 and k_8 (Fig. 1, left side). Recent reports have shown the existence of magnetic-field-induced emission changes, which may result from such fusion and fission processes [9,32–34]. This work is an extensive study on the origin of magnetic-field-induced emission changes in various purple bacteria. A study dealing with the mechanism of triplet formation in the reaction center and the antenna complex will be presented in an accompanying paper [12].

Materials and Methods

R. rubrum (wild type and FR1 VI mutant) and *Rps. sphaeroides* (wild type, G1C and R26 mutant) were grown anaerobically in the light on media described by Cohen-Bazire et al. [35] and Slooten [36], respectively, in a continuous culture described elsewhere [37]. *P. aesturarii*, strain 2 K, was grown anaerobically in a mixed culture known as *Chloropseudomonas ethylica* [38] as described by Holt et al. [39]. The cells were harvested by centrifugation and resuspended in buffer containing 250 mM tricine/5 mM K_2HPO_4/5 mM $MgCl_2$ (pH = 8.0).

Chromatophores of *R. rubrum* and *Rps. sphaeroides* were prepared using a French press or by 10 min sonication at 273 K. After centrifugation for 20 min at 20000 × *g*, the remaining supernatant was centrifuged for 2 h at 110000 × *g*. The pellet was resuspended in buffer containing 60% glycerol and stored in the dark at 240 K until use. Reaction centers of *R. rubrum* S1 and *Rps. sphaeroides* G1C were isolated as described by Slooten [40]. Reaction centers of *Rps. sphaeroides* R26 were isolated as described by Kendall-Tobias and Seibert [41]. The reaction centers showed normal P oxidation and reduction kinetics. The pigment-protein and reaction center pigment-protein complexes were prepared as described by Swarthoff et al. [42]. The B800–850 light-harvesting complexes of *Rps. sphaeroides* 2.4.1 and G1C were prepared using the metfhod of Clayton and Clayton [43]. The B850 complex was prepared from the B800–850 complex by dialysis against 1% LDS [44]. The B880 complex of *R. rubrum* wild type was isolated as described by Cogdell and Thornber [45] but instead of an hydroxyl-apatite column we used a 10–40% continuous sucrose gradient (2–5 h, 200000 × *g*). Samples of the isolated light-harvesting complex and the reaction center preparations were suspended in 250 mM tricine buffer containing 5 mM K_2HPO_4/5 mM $MgCl_2$/0.1% LDAO/0.1% SDS (pH 8.0). Samples were prepared by adding dithionite 1 mg/ml (5 mM, reduced samples) or by adding $K_3Fe(CN)_6$ up to a final concentration of 1–2 mM (oxidized samples). In oxidized samples of whole cells or chromatophores, the oxidized primary donor P^+ was accumulated in the light as was checked by absorbance difference spectroscopy. For measurements at temperatures below 273 K, glycerol (60%, v/v) and 0.5 M sucrose were added to prevent crystallization upon cooling. The B800–850 complex as well as the B880 complex were in some cases (see Results) oriented by pressing a polyacrylamide gel [46]. The magnetic-field-induced emission changes and the total BChl emission were measured in an apparatus described elsewhere [37]. The bacteriochlorophyll emission was detected by a S1-type photomultiplier (Dumont KM 2290) through a Kodak KV 550 and appropriate interference filters. By applying a sinusoidally 50 Hz modulated magnetic field with an amplitude of maximally 1300 gauss, it was possible to compute the saturation curves of the magnetic-field-induced emission changes from half a period after extensive averaging. For most experiments a cuvette was used in which a heating element was mounted and through which cold nitrogen flowed continuously. Temperature was monitored by a Cu-constantan thermocouple extended into the sample and controlled within 1 K by a feedback system. The experiments were performed at 18°C if not indicated otherwise.

Results

Upon excitation of cells or chromatophores of *R. rubrum*, *Rps. sphaeroides* 2.4.1 and *Rps. sphaeroides* G1C, either with Q_1 reduced, with P-880 oxidized or at intermediate redox potentials, small magnetic-field-induced emission changes are observed, typically of the order of a few percent of the total emission or less. Part of these changes are due to reaction center associated phenomena and are therefore also observed in isolated reaction centers, and the observations are described in the next section. A second type of magnetic-field-dependent emission is associated with the light-harvesting antenna of purple bacteria, is also observed in isolated light-harvesting complexes from these species and occurs only upon carotenoid excitation. These phenomena are described in the section dealing with The Antenna-Associated Magnetic-Field-Dependent Emission. The temperature dependence of magnetic-field-dependent emission for both types of phenomena is described in the following section.

To exclude that these small emission changes are caused by orientational effects due to the magnetic field [31], a 50 Hz sinusoidally modulated magnetic field superimposed on a stationary magnetic field was applied, and the magnetic-field-dependent emission was studied by varying the amplitude of both the alternating and the stationary field. In addition, the viscosity of the sample was varied by adding various amounts of glycerol, sucrose or gelatine. For all experiments reported in this work, the observed magnetic-field-dependent emission did not depend on the type of magnetic field applied or on the viscosity of the medium. In this paper, only experiments using the 50 Hz sinusoidally modulated field are shown.

For the experiments reported under reducing or oxidizing conditions, the relative magnetic-field-dependent emission ($\Delta F/F$) did not depend upon the excitation light intensity for $I \leqslant 45$ mW/cm^2 ($\lambda = 603$ nm) or the extinction of the sample (absorbance$_{880}$ ranges from 0.1 to 1.0).

The reaction-center-associated magnetic-field-dependent emission

R. rubrum

Fig. 2A shows the maximum relative magnetic-field-dependent emission increase ($\Delta F_{\mathrm{max}}/F$) in cells of *R. rubrum*, reduced by 5 mM dithionite, as a function of the emission wavelength upon 603 nm excitation. The magnetic field strength, $H_{1/2}$, at which the emission change is half maximum was 240 gauss and was independent of the emission wavelength between 840 and 970 nm. The broken line shows the total emission spectrum.

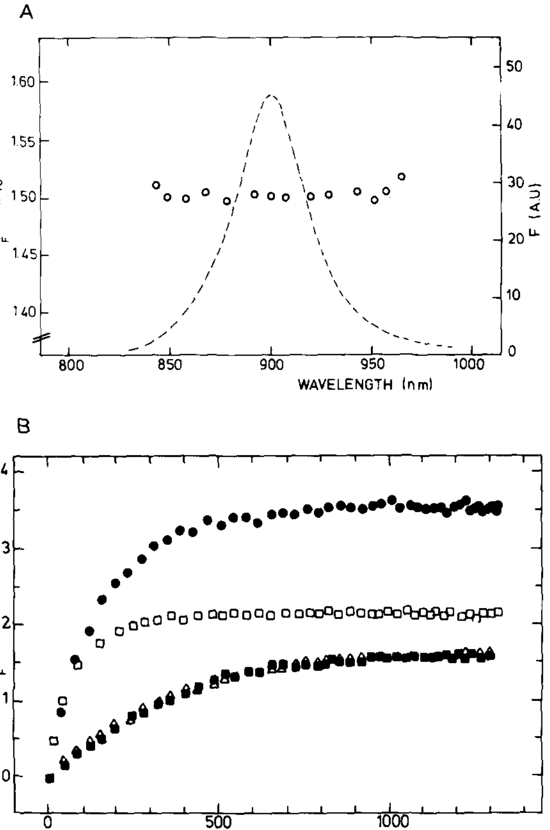


Fig. 2. (A) Spectrum of the ratio $\Delta F_{\mathrm{max}}/F$ (○) and the total emission F (broken line) in reduced cells of *R. rubrum* S1. 5 mM dithionite added. $A_{880-960} = 0.3$; optical pathlength, 1 mm; excitation wavelength, 603 nm. (B) $\Delta F/F$ monitored at 905 nm as a function of the magnetic field strength in the presence of 5 mM dithionite in cells (■), chromatophores (△), cells at pH = 3.0 (□) and isolated reaction centers (●) of *R. rubrum* S1. $A_{880-960} = 0.3$; optical pathlength, 1 mm; excitation wavelength, 603 nm.

Fig. 2B shows $\Delta F/F$ detected at 905 nm as a function of the magnetic field strength in several preparations of *R. rubrum* reduced by 5 mM dithionite. The lower curves show the emission change upon 603 nm excitation in whole cells (■) and in chromatophores (△). However, the saturation curves are dependent on the excitation wavelength (not shown). Upon direct excitation of the carotenoid between 420 and 570 nm, $H_{1/2}$ increased from 240 to maximally 290 gauss and $\Delta F_{\mathrm{max}}/F$ increased from 1.5 to 1.6%. If cells/chromatophores were incubated at low pH (less than 3.5) $\Delta F_{\mathrm{max}}/F$ increased and $H_{1/2}$ decreased (□). A similar change was observed if a suspension of whole cells in growth medium was kept in the dark for at least 6 h. Upon lowering the pH the absorbance spectrum showed a marked decrease, or even disappearance of the P-800 absorbance and a shift to 885 nm of the 880 nm band (not shown).

In isolated reaction centers of *R. rubrum* S1 $\Delta F_{\mathrm{max}}/F$ varied between 2.1 and 3.7% (803 nm excitation, ●) depending on the batch of the reaction center preparation. $H_{1/2}$ was about 100 gauss and did not depend on the excitation wavelength. The magnetic-field -dependent emission saturation curve upon 603 nm excitation of cells of the carotenoid-lacking mutant *R. rubrum* FR1 VI (■) is shown in Fig. 3. Similar results were obtained in chromatophores of FR1 VI (data not shown). The curve did not depend upon the excitation wavelength. No magnetic field-dependent emission was observed in oxidized FR1 VI (Fig. 3, (□)).

Rps. sphaeroides

Fig. 3 shows the magnetic field-dependent emission saturation curve in reduced cells (○) and chromatophores (△) of the carotenoidless mutant *Rps. sphaeroides* R26. The $H_{1/2}$ value was about 220 gauss in both cases. The same figure gives the magnetic-field-dependent emission saturation curve in reduced R26 reaction centers which

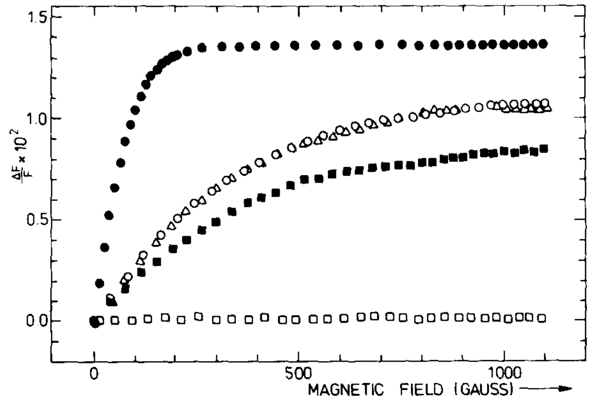


Fig. 3. $\Delta F/F$ as a function of the magnetic field strength in cells (○), chromatophores (△) and reaction centers (●) of *Rps. sphaeroides* R26 in the presence of 5 mM dithionite detected at 863 nm in reduced cells (■) and oxidized (1 mM $K_3Fe(CN)_6$) cells (□) of *R. rubrum* FR1 VI detected at 900 nm. Excitation wavelength, 603 nm. $A_{880-960}$ (*R. rubrum* FR1 VI), 0.3; $A_{850-960}$ (*Rps. sphaeroides* R26), 0.25; optical pathlength, 1 mm.

yielded $H_{1/2} \approx 50$ gauss (●). The curves for R26 were independent of the excitation/emission wavelength. No magnetic-field-dependent emission was observed in oxidized R26.

Fig. 4A shows the ratio $\Delta F_{max}/F$ at various wavelengths in reduced cells of *Rps. sphaeroides* 2.4.1 upon 603 nm excitation. The solid line shows the spectrum of the total emission. $H_{1/2}$ did not depend upon the emission wavelength.

Fig. 4B shows $\Delta F/F$ detected at 890 nm as a function of the magnetic-field strength in reduced cells of *Rps. sphaeroides* 2.4.1 upon 603 nm excitation (●). The shape of the saturation curve was strongly dependent on the excitation wavelength. Excitation in the region of the absorbance spectrum where energy is mainly absorbed by the carotenoid sphaeroidene (430–530 nm) showed an initial decrease of the emission in low magnetic field followed by an emission increase in high magnetic field (○, excitation at 510 nm). H_0, the magnetic field strength at which no net emission change was observed, shifted as a function of the excitation wavelength. Similar results were obtained with chromatophores of *Rps. sphaeroides* 2.4.1.

Fig. 5A shows the ratio of $\Delta F_{max}/F$ as a function of the emission wavelength in reduced cells of *Rps. sphaeroides* G1C (○) and the spectrum of the total emission (solid line) upon 603 nm excitation. $H_{1/2}$ at 603 nm (≈ 260 gauss) excitation did not depend on the emission wavelength.

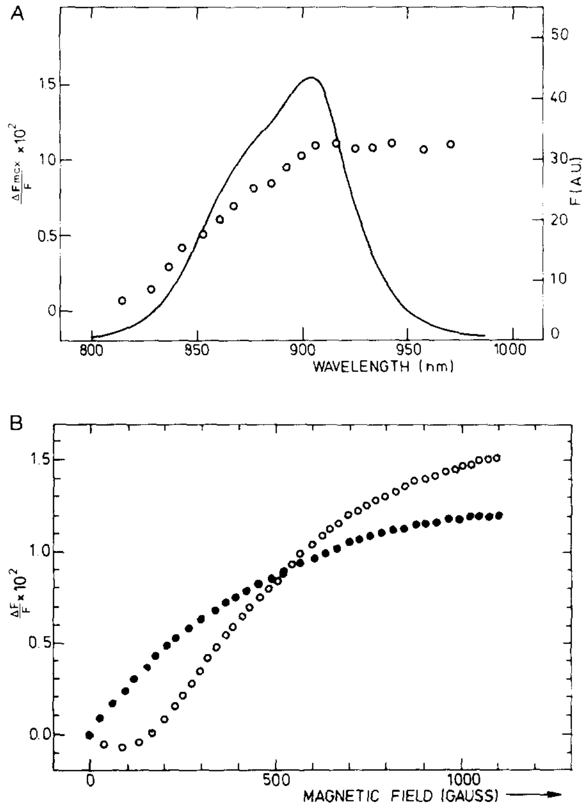


Fig. 4. (A) Spectrum of the ratio $\Delta F_{max}/F$ (○) and the total emission F (solid line) in reduced cells of *Rps. sphaeroides* 2.4.1. 5 mM dithionite added. $A_{850-960}$, 0.3; optical pathlength, 1 mm; excitation wavelength, 603 nm. (B) $\Delta F/F$ detected at 890 nm as a function of the magnetic-field strength in the presence of 5 mM dithionite in cells of *Rps. sphaeroides* 2.4.1 upon 603 nm (○) and 510 nm (○) excitation; $A_{850-960} = 0.3$; optical pathlength, 1 mm.

Fig. 5B shows $\Delta F/F$ detected at 890 nm in reduced cells of *Rps. sphaeroides* G1C as function of the magnetic field strength upon 603 nm excitation (●) and 494 nm excitation (○). In chromatophores of *Rps. sphaeroides* G1C similar results were obtained. The upper curve (×) shows $\Delta F/F$ versus the magnetic field strength in reaction centers of G1C, which did not depend upon the excitation wavelength.

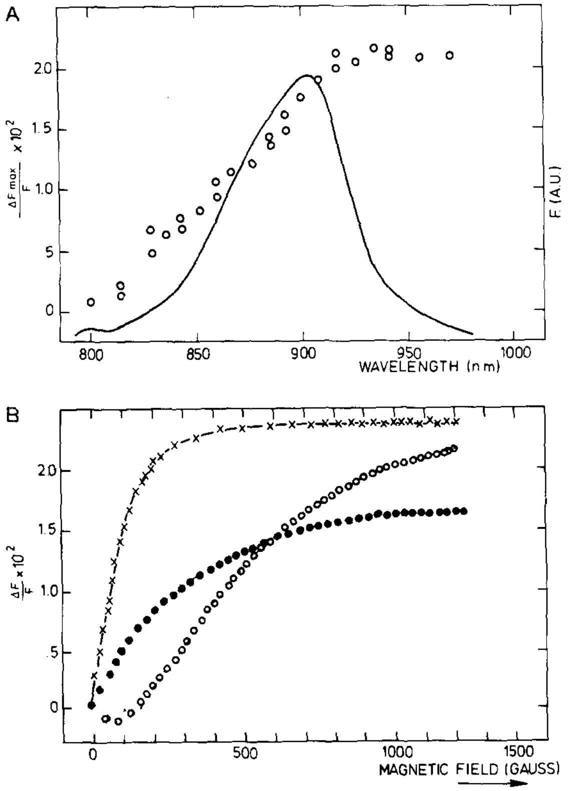


Fig. 5. (A) Spectrum of $\Delta F_{max}/F$ (○) and the total emission F (solid curve) in reduced cells of *Rps. sphaeroides* G1C upon 603 nm excitation. 5 mM dithionite added. $A_{850-960}$, 0.3; optical pathlength, 1 mm. (B) $\Delta F/F$, detected at 890 nm as a function of the magnetic-field strength in the presence of 5 mM dithionite in cells (●, ○) and reaction centers (×) of *Rps. sphaeroides* G1C. (●, ×) excitation at 600 nm; (○) excitation at 494 nm. $A_{850-960}$, 0.3; optical pathlength, 1 mm.

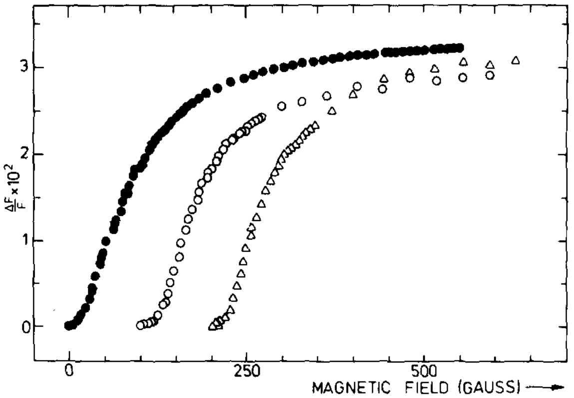


Fig. 6. $\Delta F.F$ detected at 853 nm as a function of the magnetic-field strength in the presence of 5 mM dithionite in cells (●), the pigment-protein complex (○) and the reaction center pigment-protein complex (△) of *P. aestuarii*. (Curves shifted by 100 gauss for clarity). $A_{810-960}$, 0.3; optical pathlength, 2 mm.

Prosthecochloris aestuarii

Fig. 6 shows the magnetic field-induced emission change $\Delta F/F$ in whole cells (●), the pigment-protein (○) and the reaction center pigment-protein (△) complex of the green bacterium *P. aestuarii* under reducing conditions. The shape of the curve was very similar for all three preparations (the curves are shifted by 100 gauss for clarity) and did not depend upon the excitation or the emission wavelength. In cells, magnetic-field-dependent emission was observed only after reduction. In contrast, in the unreduced pigment-protein complex about 30% of the maximum magnetic-field-dependent emission (in the presence of dithionite) was observed. The magnetic-field-dependent emission in the reaction center pigment-protein complex did not depend on the presence of dithionite. The amplitude of the magnetic-field-dependent emission in the pigment-protein and reaction-center pigment-protein complex varied from 2 to 6% dependent on the preparation. In all preparations (cells, pigment-protein and reaction-center pigment-protein complexes) the amplitude of the magnetic-field-dependent emission changes depended on the excitation wavelength (data not shown, see Ref. 47). The magnetic-field-dependent emission was maximum at emission wavelengths above 850 nm.

The antenna-associated magnetic-field-dependent emission

No magnetic-field-dependent emission changes could be detected in oxidized reaction centers of the purple bacteria studied, independent of the wavelength of excitation.

R. rubrum

Fig. 7A shows $\Delta F/F$ detected at 905 nm as a function of the magnetic-field strength in oxidized cells (○) and in the B880 complex (●) of *R. rubrum* upon 510 nm excitation and in oxidized cells upon 603 nm excitation (□). In both prepara-

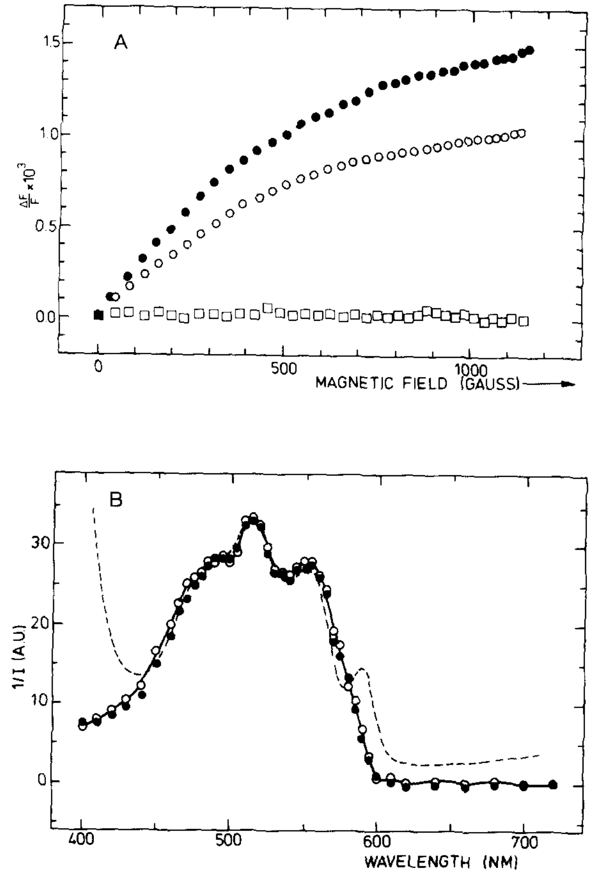
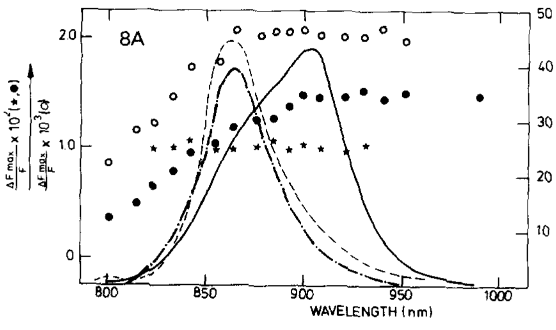


Fig. 7. (A) $\Delta F/F$ detected at 905 nm as a function of the magnetic-field strength in oxidized cells (○, □) and in the B880 antenna complex (●) of *R. rubrum* S1. (○, ●) 510 nm excitation; (□) 603 nm excitation, 1 mM $K_3Fe(CN)_6$ added. $A_{880-960}$, 0.4; optical pathlength, 2 mm. (B) Excitation spectrum of ΔF_{max} detected at 905 nm in oxidized cells (○) and the B880 complex (●) of *R. rubrum* S1, 1 mM $K_3Fe(CN)_6$ added. $A_{880-960}$, 0.4; optical pathlength, 2 mm; *I*, number of incident photons needed to induce a fixed ΔF_{max} value; A.U., arbitrary units.



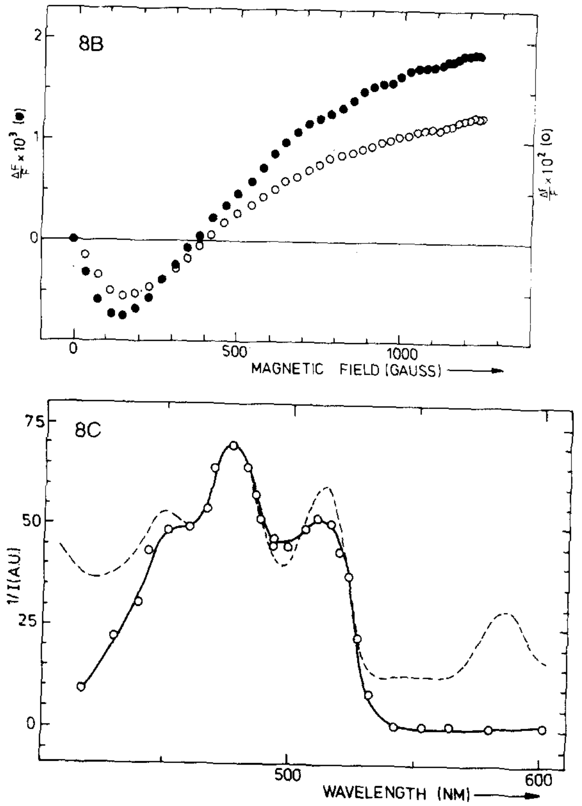


Fig. 8. (A) Spectrum of the ratio $\Delta F_{max}/F$ upon 515 nm excitation in oxidized cells (●), the B850 complex (*) and the B800–850 complex (○) of *Rps. sphaeroides* 2.4.1. The drawn curves show the total emission (F) spectrum of oxidized cells (————), the B850 complex (— — —) and the B800–850 complex (–·–) of *Rps. sphaeroides* 2.4.1, 1 mM $K_3Fe(CN)_6$ added. $A_{850-960}$, 0.35; optical pathlength, 2 mm. (B) $\delta F/F$ detected at 890 nm as a function of the magnetic-field strength upon 515 nm excitation in oxidized cells (○) and the B800–850 complex (●) of *Rps. sphaeroides* 2.4.1., 1 mM $K_3Fe(CN)_6$ added. $A_{850-960}$, 0.35; optical pathlength, 2 mm. Note the different scales. (C) Excitation spectrum of ΔF_{max} detected at 890 nm in oxidized cells (○) of *Rps. sphaeroides* 2.4.1. The broken line indicates the absorbance spectrum of oxidized cells of *Rps. sphaeroides* 2.4.1, 1 mM $K_3Fe(CN)_6$ added. $A_{850-960}$, 0.35; optical pathlength, 2 mm; *I*, number of incident photons needed to induce a fixed ΔF_{max} value; A.U., arbitrary units.

tions $\Delta F/F$ was independent of the emission wavelength.

Fig. 7B shows the excitation spectrum of ΔF_{max} in oxidized whole cells (○) and in the B800 complex (●). The broken line shows the absorption spectrum of whole cells of *R. rubrum* S1. Note that only the carotenoids contribute significantly to the excitation spectrum.

Rps. sphaeroides 2.4.1 and G1C

Fig. 8A gives the wavelength dependence of the ratio $\Delta F_{max}/F$ in oxidized cells (●), the B800–850 complex in LDAO (○) and the B850 complex in LDS (*) of *Rps. sphaeroides* 2.4.1 upon 515 nm excitation. $H_{1/2}$ and H_0 did not depend upon the emission wavelength. The drawn curves show the total emission spectra of the cells and complexes.

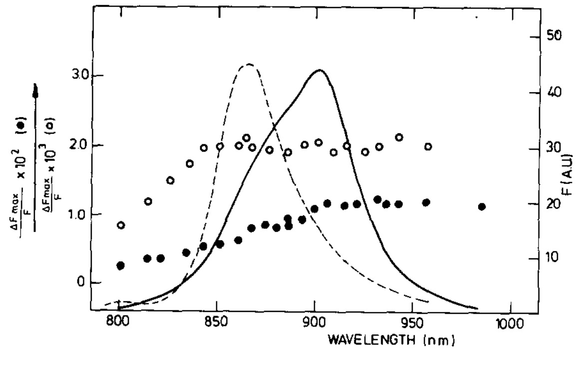
Fig. 8B shows $\Delta F/F$ as a function of the magnetic field strength for both oxidized cells (○) and the B800–850 complex (●) of *Rps. sphaeroides* 2.4.1 upon 515 nm excitation. The shape of these curves did not depend on the excitation wavelength. The excitation spectrum of ΔF_{max} in oxidized whole cells is shown in Fig. 8C. The absorbance spectrum of whole cells of *Rps. sphaeroides* 2.4.1 is indicated by the broken line.

The wavelength dependence of the ratio $\Delta F_{max}/F$ in oxidized cells (●) and in the B800–850 complex (○) of the mutant *Rps. sphaeroides* G1C upon 494 nm excitation is given in Fig. 9A. The saturation curves show a similar shape as those of the wild type (Fig. 9B). In the carotenoid region of the spectrum, the excitation spectrum (Fig. 9C) follows the absorbance spectrum (broken line). $H_{1/2}$ and H_0 did not depend upon the excitation and the emission wavelength.

R. rubrum FRI VI, Rps. sphaeroides R26 and P. aestuarii

In the oxidized cells, chromatophores of *R. rubrum* FR1 VI (Fig. 3,), *Rps. sphaeroides* R26 and in the various preparations of *P. aestuarii*, no magnetic field-dependent emission could be detected.

In light-harvesting complexes of *R. rubrum* and *Rps. sphaeroides* G1C we checked if the bacteriochlorophyll emission yield was quenched by products or intermediates of the process which lead to the magnetic-field-dependent emission. The samples were excited in the carotenoid region of the absorbance spectrum and both ΔF_{max} and F_{max} were detected. If the bacteriochlorophyll emission is quenched by products of the



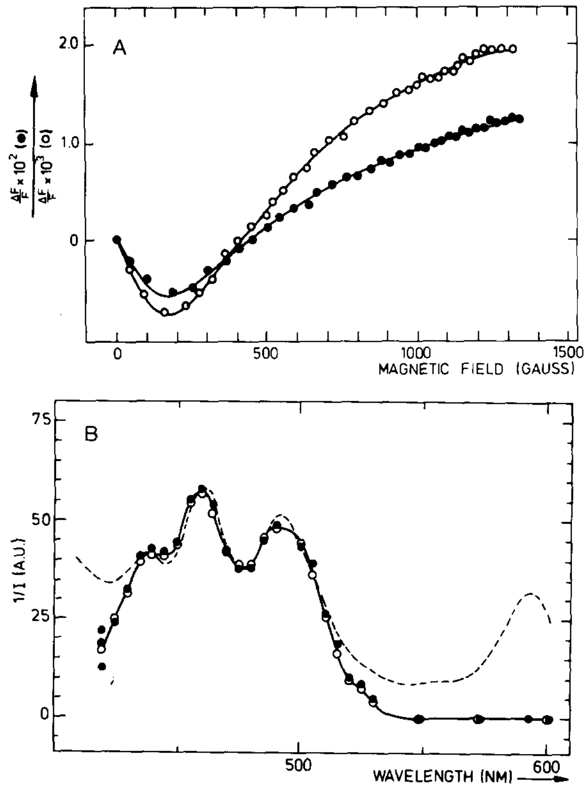


Fig. 9. (A) Spectrum of $\Delta F_{max}/F$ upon 494 nm excitation in oxidized cells (●) and the B800–850 complex (○) of *Rps. sphaeroides* G1C. The drawn curves show the total emission (F) spectrum of cells (————) and the B800–850 complex (— — —) of *Rps. sphaeroides* G1C, 1 mM $K_3Fe(CN)_6$ added. $A_{850-960}$, 0.33; optical pathlength, 2 mm; A.U., arbitrary units. (B) $\Delta F/F$ detected at 890 nm as a function of the magnetic field strength upon 494 nm excitation in oxidized cells (●) and the B800–850 complex (○) of *Rps. sphaeroides* G1C, 1 mM $K_3Fe(CN)_6$ added. $A_{850-960}$, 0.33; optical pathlength, 2 mm. Note the different scales. (C) Excitation spectrum of ΔF_{max} detected at 890 nm in oxidized cells (●) and the B800–850 complex (○) of *Rps. sphaeroides* G1C. The broken line indicates the absorbance spectrum of oxidized cells of *Rps. sphaeroides* G1C, 1 mM $K_3Fe(CN)_6$ added. $A_{850-960}$, 0.33; optical pathlength, 2 mm; I, number of incident photons needed to induce a fixed ΔF_{max} value; A.U., arbitrary units.

magnetic-field-dependent process, additional excitation of the bacteriochlorophyll would result in an increase of ΔF_{max} and F_{max}. In the B880 complex of *R. rubrum* S1 (data not shown) and in the B800–850 complex of *Rps. sphaeroides* G1C the total emission increases but ΔF_{max} remains the same upon additional bacteriochlorophyll excitation (Table I). Upon orientation of the B800–850 complex of *Rps. sphaeroides* G1C in a polyacrylamide gel [46] and/or photoselection by excitation and/or detection through polarization filters, no change in $\Delta F/F$ as a function of the magnetic-field strength was observed.

Magnetic-field-dependent emission temperature dependence

R. rubrum S1

In oxidized samples and in antenna complexes of *R. rubrum* S1 the magnetic-field-dependent emission did not depend on the temperature between 300 and 77 K. In contrast, $\Delta F_{\mathrm{max}}/F$ decreased upon cooling in reduced cells, chromatophores and reaction centers (Fig. 10A and B). In reaction centers and in reduced whole cells, upon bacterioclorophyll excitation, the temperature dependence did not vary with the emission wavelength (840–920 nm) and below 150 K no magnetic-field-dependent emission was detected (open symbols). In addition, $H_{1/2}$ in these samples did not depend on the temperature within 5%. However, upon direct carotenoid excitation (Fig. 10A, closed symbols) in reduced cells and chromatophores magnetic-field-dependent emission was observed below 150 K which remained constant down to 77 K, while $H_{1/2}$ shifted from 240 gauss at room temperature to more than 400 gauss at 77 K. In the reaction centers, the temperature dependence did not vary with the excitation wavelength between 420 and 650 nm (Fig. 10B).

Rps. sphaeroides 2.4.1 and G1C

As in the B880 antenna complex of *R. rubrum*, the relative magnetic-field-dependent emission in the isolated B850 and B800–850 complexes of both strains of *Rps. sphaeroides* did not depend upon the temperature between 300 and 77 K.

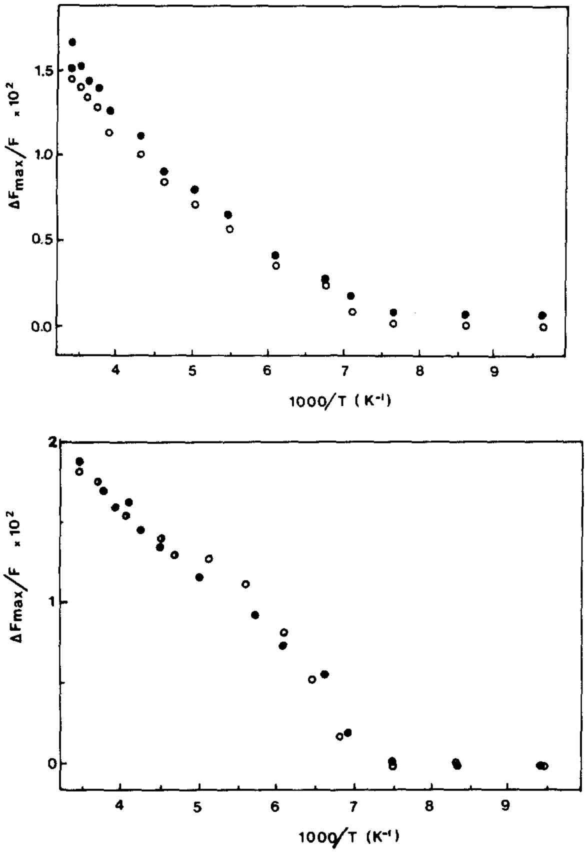


Fig. 10. (A) $\Delta F_{\mathrm{max}}/F$ as a function of the temperature under reducing conditions (5 mM dithionite added) in cells of *R. rubrum* S1 detected at 905 nm upon 603 nm (○) and 510 nm (●) excitation. (B) Same as in (A) but with reaction centers of *R. rubrum* S1 detected at 905 nm upon 603 nm (○) and 510 nm (●) excitation.

In reduced reactions centers, $\Delta F_{\mathrm{max}}/F$ decreased upon cooling, but $H_{1/2}$ remained constant within 5%, independent of the excitation wavelength. In reduced whole cells or chromatophores upon bacteriochlorophyll excitation, $\Delta F_{\mathrm{max}}/F$ decreased upon cooling but again $H_{1/2}$ remained the same. Upon direct carotenoid excitation of reduced cells and chromatophores, the shape of the saturation curve changed drastically upon cooling (data not shown). At room temperature the curve showed only a small initial emission decrease in low field (see Figs. 4B and 5B, open symbols). Below 150 K down to 77 K the magnetic-field dependence remained constant and was similar to the curves found in the oxidized samples and the antenna complexes. In the temperature range be-

TABLE I

ΔF_{max} AND THE TOTAL EMISSION F UPON 494 nm (16 mW/cm^2) OR 603 nm (12 mW/cm^2) AND 494+603 nm EXCITATION IN THE B800–850 COMPLEX OF *RPS. SPHAEROIDES* G1C IN THE PRESENCE OF 1 mM $K_3Fe(CN)_6$ A_{850} = 0.2; A.U., arbitrary units.

Excitation wavelength (nm)	ΔF_{max} (A.U.)	F (A.U.)
494	5.6	2840
603	0	1683
494+603	5.6	4391

tween 300 and 150 K, a continuous change of the shape of the curve was observed [37].

R. rubrum FR1 VI and Rps. sphaeroides R26

The temperature dependence of magnetic field-dependent emission in these carotenoid-lacking mutants was similar to that observed in the strains with carotenoids under reducing conditions and selective bacteriochlorophyll excitation (see Fig. 10A and B). $H_{1/2}$ did not depend on the temperature regardless the excitation wavelength. Below 150 K no magnetic-field-dependent emission could be detected.

Interpretation and Discussion

We propose that the magnetic-field-dependent emission observed in bacterial photosynthetic systems in a number of preparations and under a variety of conditions can be divided into two categories, i.e., reaction center and antenna associated phenomena.

The first category contains the observations of magnetic field-dependent emission increases in reaction centers of *R. rubrum* S1, *R. rubrum* FR1 VI, *Rps. sphaeroides* G1C, *Rps. sphaeroides* R26 and the reaction center pigment-protein complex of *P. aestuarii*. These effects do not depend on the excitation wavelength, are strongly temperature activated (Figs. 2B, 3, 4B, 5B and 6) and are only found if the first quinone acceptor Q_1 is reduced (see also Ref. 8).

The second category consists of the observation of magnetic-field-dependent emission in whole cells and chromatophores, in which the primary donor P-880 is oxidized, and in isolated light-harvesting complexes of *R. rubrum* S1, *Rps. sphaeroides* 2.4.1 and *Rps. sphaeroides* G1C. Magnetic-field-dependent emission is only observed upon direct excitation of the carotenoids (Figs. 7B, 8C and 9C) and does not depend on the temperature.

In reduced, carotenoid-containing cells or chromatophores of purple bacteria, magnetic-field-dependent emission usually shows contributions from both categories. Upon carotenoid excitation the excitation transfer process induces a magnetic-field-dependent emission associated with the second category. After energy transfer to the BChl a charge separation is produced in the reaction center, charge recombination then causes the characteristics of magnetic-field-dependent emission associated withthe first category. In purple bacteria lacking carotenoids and in the green bacterium *P. aestuarii*, only magnetic-field-dependent emission associated with charge recombination in the reaction center is observed.

Magnetic-field-dependent emission associated with the reaction center upon selective BChl excitation in reduced samples

R. rubrum S1

The spectrum of the total emission (prompt plus variable fluorescence) in reduced cells shows a maximum near 905 nm (Fig. 2A) and most likely arises from the antenna bacteriochlorophyll B880, due to a high rate of back transfer from the excited reaction center ot B880 and the abundance of B880 over reaction centers. The ratio $\Delta F_{max}/F$ was independent of the emission wavelength (Fig. 2A), which supports the notion that the antenna of *R. rubrum* is composed of one major BChl species. In isolated reaction centers the emission yield is about 50-times lower than that in cells and chromatophores, but the amplitude of $\Delta F_{max}/F$ for reaction centers (2.1–3.7%) is of the same order of magnitude as that observed in cells/chromatophores (approx. 1.5%). These data show that rapid energy transfer among antenna molecules and reaction centers occurs.

The shape of the saturation curves (Fig. 2B) depends on the preparations, pH and time of dark adaptation. In cells and chromatophores $H_{1/2}$ is about 240 gauss, whereas in isolated reaction centers $H_{1/2}$ is about 100 gauss; this confirms earlier reports showing marked differences between reaction center preparations and more intact systems [2,3,5,8]. In spite of extensive theoretical studies concerning the origin of the magnetic field dependence of the charge recombination process [1,7,13,23,24,49,50], no satisfactory explanation for the differences in $H_{1/2}$ values observed in reaction centers and cells and chromatophores is available. A dramatically different exchange interaction could possibly explain these differences. However, from ESR data a relatively low exchange interaction of 1–5 gauss has been estimated for the electron spins on P-880$^+$ and I$^-$ [51]. It has been argued therefore that, if the reaction center pigment P-800 acts as an intermediate electron carrier between P-880 and I and if the electron is delocalized extensively enough over P-800 and I, a large exchange interaction between P-800 and P-880 would prevent a fast dephasing of the electron spins of the radical pair into a triplet state [52,53]. However, indications for the role of P-800 as an electron carrier between P-880 and I have only been obtained in isolated reaction centers [53]. No evidence for P-800 as an electron carrier could be found [54] in cells and chromatophores, in which even larger $H_{1/2}$ are obtained requiring even higher values for the exchange interaction or more effective delocalization of the electron on P-800.

Recently, we have shown that in several cases a shortening of the P^+I^- average lifetime, either by studying the $P^+I^-Q_I^-$ decay time in the presence of a large number of open traps (P I Q_I) or by applying an electrical field across the membrane, leads to a significant increase in observed $H_{1/2}$ [8,55,56]. Consequently, one may speculate that the increase of the P^+I^- lifetime in reaction centers (10–15 ns [57]) compared to cells/chromatophores (8–10 ns [54]) leads to a decreased $H_{1/2}$ in reaction centers, although the observed decrease seems much too large. However, it is not at all clear whether the lifetime of P^+I^- in cells/chromatophores reflects the same mixture of decay processes as in reaction centers. In cells/chromatophores a significant fraction of the decay of P^+I^- proceeds via loss in the antenna due to rapid back transfer from P-880* to B880 [8,48]. In reaction centers this pathway will be about 40-times slower and may represent only a minor contribution (Ref. 57 and Parson, W.W., personal communication). The fact that the P^+I^- lifetimes in the two systems are almost identical suggests that different decay processes operate in reaction centers compared to cells/chromatophores, and make the assignment of $H_{1/2}$ to a specific rate constant ambiguous.

Upon lowering the pH of the reduced cells/chromatophores to pH = 3.0, $H_{1/2}$ decreases to about 50 gauss. The value of $\Delta F_{max}/F$ increases somewhat, which suggests that the fraction of P^+I^- decaying via P-880* and B880* has increased, for example by an increase of the lifetime of (P^+I^-). In addition it was observed that in cells/chromatophores the P-800 absorption band disappears upon lowering the pH to 3.0, making a correlation between the P-800 and the reaction center associated magnetic-field-dependent emission even more unlikely. These effects of pH will be fully discussed elsewhere. For the moment it suffices to remark that the $H_{1/2}$ value of 100 gauss reported by Rademaker et al. [7] for reaction-center-associated magnetic-field-dependent emission in reduced cells may be ascribed to the rather high concentration of dithionite (100 mM) in poorly buffering growth medium, which rapidly leads to a large decrease in pH (Ref. 58; Kingma, H., unpublished results).

Rps. sphaeroides 2.4.1 and G1C

The spectra of the total emission (Figs. 4A and 5A) show a maximum near 900 nm, a shoulder near 865 nm and a small peak at 800 nm. The antenna of these bacteria is composed of a B800–850 complex and a B880 complex of which the latter is closely associated with the reaction center [20]. The wavelength dependence of the ratio $\Delta F_{max}/F$ (Figs. 4A and 5A) shows that the magnetic-field-dependent emission is closely related to B880. $\Delta F_{max}/F$ decreases at wavelengths where the B850 emission contributes to the total emission. This indicates that at room temperature energy transfer from the reaction center pigment P880 to the antenna mainly proceeds towards B880 and that only a fraction of this excitation energy is transferred to the B800–850 complex. These findings agree with the fact that most of the variable emission observed upon closing the reaction centers (P880$^+$I Q_I^-) is due to B880 and argue against a rapid thermal equilibrium between excitation in B880 and B800–850.

Similar to what is observed for *R. rubrum* S1, $\Delta F_{max}/F$ is of the same order of magnitude in reaction centers and cells/chromatophores (Fig. 5). The low $H_{1/2}$ values in reaction centers of *Rps. sphaeroides* G1C may again be ascribed to the absence of energy transfer to the surrounding antenna or changes in conformation due to the isolation procedure, resulting in, e.g., an increase of the effective lifetime of the radical pair state.

The magnetic field dependence of the emission in both *Rps. sphaeroides* 2.4.1 and G1C does not significantly differ from that observed in *R. rubrum* S1, nor does the temperature dependence of $\Delta F/F$ and $H_{1/2}$. Apparently, the carotenoid composition, sphaeroidene in 2.4.1 (wild type), neurosporene in G1C and spirilloxanthin in *R. rubrum* S1 does not affect magnetic-field-dependent emission associated with the reaction center.

R. rubrum FR1 VI and Rps. sphaeroides R26

In the various preparations of these carotenoid lacking mutants we observed magnetic-field-dependent emission similar to that found in the wild strains (Fig. 3). Only under reducing conditions is magnetic-field-dependent emission observed. Thus the presence of the carotenoid molecules is not required for magnetic-field-dependent emission associated with the primary charge separation and the absence of carotenoids does not significantly affect the $H_{1/2}$ or $\Delta F_{max}/F$ values.

P. aestuarii

As in the carotenoidless purple bacteria discussed above, magnetic field-dependent emission in cells of *P. aesuarii* is observed only after reduction of the secondary electron acceptor. The presence of magnetic-field-dependent emission in the non-reduced pigment-protein and reaction-center pigment-protein complex is presumably caused by charge recombination due to a partially disturbed electron transport to a secondary electron acceptor [47,59]. Upon 600 nm excitation, the energy is transferred to the 815 nm emitting BChl species, probably a water-soluble light-harvesting BChl *a* protein [60] and partly to the 838 nm emitting BChl species closely associated with the reaction center [47]. In contrast, upon 670 nm excitation only the 838 nm emission band is excited and no 815 nm emission is found. The maximum magnetic-field-dependent emission is observed upon 670 nm excitation at emission wavelengths above 850 nm, where the 815 nm emission contribution can be neglected. This indicates that magnetic-field-dependent emission is closely associated with the recombination lumenescence in the reaction center.

In contrast to the purple bacteria studied, the magnetic-field-dependent emission saturation curves are similar for cells and the pigment-protein and reaction center pigment-protein complexes (FIg. 6). The reaction-center pigment-protein complex contains about 40 BChl molecules per reaction center [60], and in this respect resembles the chromatophore of the purple bacteria and not the reaction center. In the reaction-center pigment-protein complex the lifetime of the radical pair is about 30 ns, which is relatively long compared to the lifetime of about 8–10 ns observed in purple bacteria [54]. It seems not unlikely that this long lifetime is the main cause for the low $H_{1/2}$ values observed in the various preparations of *P. aestuarii*. The threshold of 10 gauss found in each preparation, independent of the excitation or emission wavelength, may tentatively be accounted for by assuming an interaction between the electron spins on $P838^+$ and BPh^- [1,23,24].

The magnetic-field-dependent emission associated with the antenna complex

In oxidized reaction centers (state P^+I^-) no magnetic-field-dependent emission could be detected, either on carotenoid nor on bacteriochlorophyll excitation. However, in oxidized cells/chromatophores and in isolated antenna complexes of the purple bacteria studied, magnetic-field-dependent emission is observed upon direct carotenoid excitation, but not upon selective BChl excitation. In the oxidized carotenoid-lacking mutants no magnetic field-dependent emission was detected regardless the excitation wavelength. Therefore, the process described in this section most likely takes place in the antenna and requires the presence of a carotenoid molecule. The shape of the magnetic-field-dependent emission as a function of the magnetic field strength depends strongly upon the species. The antenna-associated magnetic-field-dependent emission is absent in all preparations of *P. aestuarii*.

R. rubrum S1

The relative emission increase (Fig. 7A) caused by a field of about 1200 gauss was about 0.10–0.15% and is one order of magnitude smaller than reaction-center-associated magnetic-field-dependent emission in the same species. This may explain why Rademaker et al. [7] detected no significant magnetic-field-dependent emission in oxidized cells. Because $\Delta F_{\max}/F$ is independent of the emission wavelength (not shown), we conclude that the emission arises predominantly from the B880 antenna. For all preparations $H_{1/2}$ exceeded 400 gauss. These observations agree with earlier reports on magnetic field-dependent emission in reaction center-less mutants and in cells and antenna complexes of *Rps. capsulata* (Refs. 9 and 32; see also Frank, H.A., personal communication). The excitation spectra of $\Delta F_{\max}$ in oxidized cells and chromatophores and in the B880 complex (Fig. 7B) are very similar and closely follow the absorption spectra in the carotenoid region (peaks at 480, 510 and 550 nm). In oxidized cells/chromatophores of the carotenoidless mutant *R. rubrum* FR1 VI no magnetic field-dependent emission is observed. We therefore conclude that in *R. rubrum* S1 magnetic field-dependent emission associated with the antenna exists and that it requires excitation of the only carotenoid present in the antenna, spirilloxanthin. To explain these phenomena either a radical pair mechanism involving a charge separation from the excited carotenoid or a singlet fission mechanism may be proposed. If a radical pair mechanism is operative, the large $H_{1/2}$ value has to be ascribed to extremely high spin dipole–dipole interactions and/or exchange interactions or a short lifetime of the radical pair [23,24,49,50]. However, Frank et al. [61] observed no spin-polarized triplet in the ESR spectrum of reaction-center-less mutants of *Rps. capsulata*, which may indicate that in this antenna at least the radical pair mechanism is not involved. Recently [62] it was reported that the antenna carotenoid triplet in chromatophores of *R. rubrum*, which partly is formed directly from the excited carotenoid, is formed within 100 ps. This also argues against the formation of the triplet via a radical pair mechanism. For these reasons we will assume that the process of singlet fission is responsible for both the magnetic field-dependent antenna carotenoid triplet (magnetic-field-dependent triplet yield [12,14,34] and the observed magnetic-field-dependent emission. The $H_{1/2}$ values for magnetic-field-dependent triplet yield and magnetic-field-dependent emission are similar. The two members of the triplet pair must both be present in the B880 complex, which contains BChl and spirilloxanthin in a 1 : 1 ratio and which probably has a tetramer of B880 [63] as the minimal subunit. One of the members of the triplet pair will be spirilloxanthin; the other would be a second spirilloxanthin (homofission) or, e.g., bacteriochlorophyll (heterofission).

The total BChl emission is only slightly quenched (3%) by products or intermediates of the fission process (Table I). The magnetic-field-dependent emission thus arises either from the competition between fission and the energy transfer of excited antenna carotenoid (ACar*) to BChl; or it may (partly) arise from fusion processes if BChl acts as the second partner of the triplet pair (Fig. 1) in the case of heterofission ($^{\mathrm{T}}\mathrm{ACar} \cdot \mathrm{BChl}^{\mathrm{T}}$). First we will consider the possibility of heterofission, i.e., the triplet pair consists of a spirilloxanthin and a BChl molecule. Most likely aromatic amino acids such as tryptophan or tyrosine present in the antenna protein complex have a triplet energy that is too high for triplet formation through the triplet pair. The triplet energies, however, of all molecules involved are poorly known and only rough estimates are possible (Moore, T., personal communication). Such an estimate results in an energy of the spirilloxanthin-BChl triplet pair of about 1.6–1.7 eV. The first excited singlet states of antenna carotenoid and BChl are about 2.2 and 1.4–1.55 eV, respectively [64–66].

However, it might be that the triplet pair is not directly formed by fission of the state Car*. It has been shown [67] that the state Car* might rapidly decay into a lower energetic state Car*′, which would have an energy of about 1.65 eV. If the triplet pair is generated by fission of the state Car*′, which cannot be populated directly from the ground state [67], fusion from ($^{\mathrm{T}}\mathrm{Car} \cdot \mathrm{BChl}^{\mathrm{T}}$) to Car*′ is probably possible (rate constant k_2,

Fig. 1). In this case a marked temperature dependence might occur [15], which was not observed. This indicates that the pair is either generated by fission of the state Car* directly or that the energy of the pair is less than 1.6–1.7 eV (e.g., homofission, see below).

In addition, fission of BChl* into the triplet pair state cannot be excluded, and the absence of magnetic-field-dependent emission upon specific BChl excitation does not rule out the possibility of heterofission, considering the uncertainty of the energy difference between the triplet pair state and the excited BChl singlet state. However, fission is a rather efficient process in organic crystals [15] and would result, in the case of heterofission, in an efficient fusion of the triplet pair state to BChl* (rate constants k_4, and k_8, Fig. 1). This could lead to a decrease of the bacteriochlorophyll emission in high-magnetic field: the rate constants of both fission and fusion decrease with increasing magnetic field [15,28,30]. An increase of emission is clearly observed, which is expected if the energy transfer from ACar* directly to BChl (rate constant k_5, Fig. 1) is more efficient than through the fission and fusion channel (k_1, k_4, k_8).

In the case of homofission the excitation spectrum (Fig. 7B) indicates that the two molecules involved are both spirilloxanthin. BChl excitation now obviously does not result in singlet fission whereas only the competition between the rate constants k_1 and k_5 accounts for the magnetic-field-dependent emission increase. Due to the large energy difference of about 2.2–1.13 = 0.9 eV between the triplet pair state (^{T}ACar · ACar^{T}) and the state ACar* [64] no temperature dependence is expected, which fully agrees with our results. The molecules present in the antenna complex are part of a more or less rigid structure and have a fixed orientation with respect to the external magnetic field vector. However, orientation of the sample and/or photoselection did not affect the magnetic field dependence if the direction of the magnetic field vector was altered. Therefore, we did not find the low or high resonance as described by Svenberg and Geacintov [15] and Geacintov et al. [31] for both homo- and heterofission. A possible explanation is that, even with perfect orientation of one chromophore with respect to the orientation axis, the orientation of the second chromophore will be circularly degenerate (Geacintov, N.E., personal communication). In *R. rubrum* the yield of ACar^{T} decreases with increasing magnetic field strength and a triplet is presumably formed in zero field by fission upon direct carotenoid excitation, considering the difference of the triplet yield of 0.2 upon BChl excitation (state P880^{+}I Q_1) and 0.3 upon carotenoid excitation [12,14].

Summarizing we conclude that the process of homofission involving two carotenoid molecules explains the pertinent experimental data, but the process of heterofission involving a carotenoid and a BChl molecule, with some restrictions with respect to the energetics also could account for the magnetic-field-dependent emission observed. The presence of a triplet formed by fission in zero field, however, seems to exclude the possibility of homofission with a very specific orientation of the molecules involved [12,15].

Rps. sphaeroides 2.4.1 and G1C

As in *R. rubrum* S1, in both *Rps. sphaeroides* 2.4.1 and G1C a magnetic-field effect originating from a process associated with the antenna is found. Magnetic-field-dependent emission observed in cells/chromatophores in the state P880^{+}I Q_1 or in isolated antenna complexes, is only found upon carotenoid excitation and is absent in oxidized reaction centers and the carotenoidless mutant R26. The magnetic-field-dependent emission changes are larger than in *R. rubrum* which may be related to the differences in energy of the singlet excited states of the carotenoids involved ($E_{\text{neurosporene}}{}^* = 2.6$ eV, $E_{\text{sphaeroidene}}{}^* \simeq 2.4$ eV, $E_{\text{spirilloxanthin}}{}^* = 2.2$ eV) and the triplet pair states ($E_{\text{neurosporene}}{}^{\text{T}} \simeq 1.9$ eV, $E_{\text{sphaeroidene}}{}^{\text{T}} \simeq 1.7$ eV, $E_{\text{spirilloxanthin}}{}^{\text{T}} \simeq 1.65$ eV) (Refs. 64–66; Frank, H.A., personal communication). If fission of Car* into the triplet pair state would occur via the, optically forbidden, lower energetic state Car*′ [67], fusion (rate constant k_2, Fig. 1) would become possible.

The magnetic-field-dependent emission in the antenna complexes does not depend upon the temperature (for the temperature dependencies in whole cells and chromatophores, see the next section). Again the excitation spectra (Figs. 8B and 9C) clearly indicate that at least one molecule of the triplet pair is a carotenoid, sphaeroidene in

Rps. sphaeroides 2.4.1 and neurosporene in *Rps. sphaeroides* G2C. The absence of magnetic field-dependent emission in oxidized preparations of the carotenoidless mutant R26 confirms the conclusion that the carotenoids play a major role in these processes.

The magnetic-field-dependent emission saturation curves (Figs. 8B and 9B) markedly differ from those found in the reduced samples. A significant initial decrease of magnetic-field-dependent emission is apparent at field strengths below 400 gauss. This initial decrease in low field very nicely agrees with the magnetic-field-dependent emission changes observed in organic crystals [15,29]. The complex curve can be explained by a process of homofission, but if the molecules involved have similar D and E values, a process of heterofission could give a similar result. As has been shown recently [12,34] the carotenoid triplet yield in the B800–850 complex of *Rps. sphaeroides* 2.4.1 and of *Rps. sphaeroides* G1C is similar upon BChl and upon carotenoid excitation (approx. 1.4–1.6%). Upon direct carotenoid excitation, a magnetic-field-induced increase of the triplet yields is found in low field, whereas the triplet yields in zero field and high fields are about the same. Upon BChl excitation no magnetic field effect was obtained. These phenomena agree with a process of homofission involving two carotenoid molecules with a special mutual orientation, where no single triplet state can be formed in zero-magnetic field by the fission process (see the Introduction). Because fission is a spin-conserving process no single triplet (e.g. $\mathrm{Car}^{\mathrm{T}}$) can be formed from an excited singlet state (Car*) if no substate with a mixed singlet-triplet character is present. Assuming homofission, at low field the emission decreases and the triplet yield increases due to the mixing-in of singlet and triplet character in the substates of the pair. As the field strength increases the number of substates with singlet character decreases again and so does the rate constant of fission, resulting in a decrease back to zero of the triplet yield formed by fission and an increase of the emission yield as compared to that in zero field.

The spectra of the ratio $\Delta F_{\mathrm{max}}/F$ in several preparation of *Rps. sphaeroides* 2.4.1 and G1C together with the total emission spectra indicate that in intact cells/chromatophores the main contribution to the magnetic-field-dependent emission is from the B880 complex. In chromatophores of *Rps. sphaeroides* WT, which contained a relatively large amount of B880 as compared to B800–850 (a generous gift from Dr. R.A. Niederman), the ratio $\Delta F_{\mathrm{max}}/F$ at 900 nm is about 5-times larger than the ratio at 850 nm. The B880 complex consists of a minimal unit of six BChl molecules [68] and three carotenoids, which on the basis of their CD spectra show relatively strong interactions [69]. The relatively low efficiency of energy transfer (60%) may be in agreement with the competing fission process. A magnetic-field-dependent emission very similar to that found in cells and chromatophores is observed in B800–850 also, but its contribution is apparently smaller in the intact cells/chromatophores compared to the B880 contribution. The experiments suggest a major role for the carotenoid associated to B850. Homofission would require at least two strongly coupled carotenoid molecules in a unit of B800–850. This is not in contradiction with spectroscopic data, which suggest that the minimal functioning unit of B800–850 contains at least three carotenoid molecules, two associated to four BChl 850 molecules and one associated to two B800 molecules [68]. The higher efficiency of energy transfer from carotenoid to BChl (more than 90%) also suggests a much weaker competing fission process in this antenna complex.

The temperature dependence of magnetic-field-dependent emission

Based upon the results in oxidized chromatophores of *R. rubrum* and the isolated B880, B800–850 and B850 complexes we conclude that the fission process in *R. rubrum* S1, *Rps. sphaeroides* 2.4.1 and G1C does not depend on the temperature. A temperature-independent fission may be ascribed to low values of the fussion rate constant k_2 and the fission rate constant k_3 (in the case of heterofission) resulting in only downhill reactions (k_1, k_4, k_6, k_7 and k_9; Fig. 1).

The magnetic-field-dependent emission changes in the reduced samples upon BChl excitation (Figs. 2B, 4B, 5B and 6) which are selectively associated with the charge recombination of the radical pair, decrease upon cooling. Below 150 K no magnetic-field-dependent emission can be detected upon BChl excitation (below 150 K only the fission process might contribute (Figs. 2B, 4B and 5)). In agreement with others (Boxer, S., personal communication) we found that the $H_{1/2}$ values of the reaction-center-associated magnetic-field-dependent emission in all preparations studied do not depend upon the temperature. In chromatophores of *R. rubrum* the decay time of the recombination luminescence (approx. 8 ns [54]) does not depend on the temperature between 77 and 300 K, whereas the amplitude decreases upon cooling. In contrast, recombination of P-880$^+$I$^-$ in reduced samples increases from 10 ns at 300 K to about 25 ns at 77 K [54,56]. These seemingly conflicting data can be explained by assuming at least two substates of P$^+$I$^-$ with slightly different energy (Van Bochove, A.C. et al., unpublished results); this suggestion is analogous to the scheme by Parson et al. [57] to explain the multiphasic decay of the luminescence in reaction centers. The presence of several substates of P$^+$I$^-$ may eventually lead to an explanation of the discrepancies between the observed $H_{1/2}$ values for magnetic-field-dependent emission and the measured P-880$^+$I$^-$ lifetime. It seems likely that $H_{1/2}$ is associated with the lifetime of the lumenscence, both being constant as a function of the temperature.

The decrease of $\Delta F_{max}/F$ seems to be correlated with the decrease of the amplitude of the recombination luminescence upon cooling and should therefore reflect the energy difference between the states P-800*I and (P800$^+$I$^-$)^S.

A detailed quantitative analysis of the magnetic-field-dependent emission and magnetic-field-dependent triplet yield temperature dependence will be presented elsewhere. It will be concluded that the temperature dependence of the magnetic-field-dependent emission, the magnetic-field-dependent triplet yield, the emission and the triplet yield cannot be explained by a simple model with the charge recombination in the reaction center as the only temperature-dependent reaction, in contrast to earlier conclusions [48,70].

Magnetic-field-dependent emission in reduced samples upon carotenoid excitation

If magnetic-field-dependent emission is induced by direct carotenoid excitation in reduced cells or chromatophores of *R. rubrum* S1, *Rps. sphaeroides* 2.4.1 or G1C, complex saturation curves are found (FIgs. 4B and 5B). In *R.* rubrum $H_{1.2}$ shifts from 240 to 290 causs and $\Delta F_{max}/F$ slightly increases depending on the excitation wavelength (420 -570 nm). Under these circumstances both the singlet fission process in the antenna and the charge recombination in the reaction center contribute to $\Delta F/F$. The relative contribution depends on the ratio of the quanta absorbed by the carotenoid and BChl. Because of the different saturation curves for the two processes in *Rps. sphaeroides* (Figs. 4B, 5B, 8B and 9B) these phenomena are more clearly observed in this bacterium. Several investigations [4,7,8] were done under these circumstances which have to be interpreted not only in the light of the radical pair mechanism but also considering the fission process. First, the absence of magnetic-field-dependent emission in oxidized samples upon BChl excitation reported earlier in some preparations [7,8] does not imply that magnetic-field-dependent emission is only present in reduced samples. We have shown that magnetic-field-dependent emission can be induced upon carotenoid excitation in oxidized samples (Refs. 32 and 33; see also this work). The initial decrease of magnetic-field-dependent emission in low field observed in several bacteria under reducing conditions [7,8] (Figs. 4B and 5B) is not associated withthe reaction center as has been suggested by Elfimov et al. [71] or due to an interaction between the energy-transfer process in the reaction center and the antenna (Ref. 72; see also Frank, H.A., personal communication), but arises exclusively from the fission process in the antenna. The temperature dependences of magnetic field-dependent emission and $H_{1/2}$ reported earlier [3,4] reflect both the reaction center and the antenna associated processes due to the direct carotenoid excitation. For instance, the increase of $H_{1/2}$ between 250 and 77 K reported by Rademaker et al. (Fig. 4B of Ref. 3) was found to be due to the elimination of the reaction center contribution with a relatively low $H_{1/2}$ value upon cooling. The initial high value of 500 gauss at 300 K (obtained from Fig. 3B of Ref. 3) and the estimated decrease of $H_{1/2}$ between 320 and 250 K are due to an incorrect calculation of $H_{1/2}$ from the complex saturation curves using carotenoid excitation. The temperature dependence of magnetic-field-dependent emission reported by Rademaker et al. (Fig. 4A of Ref. 3) is different from our results and probably reflects both categories of magnetic-field-dependent emission. The remaining magnetic-field-dependent emission at temperatures below 150 K has to be ascribed to the magnetic-field-dependent emission associated with the antenna and/or orientation effects. In *Chlorella vulgaris* and spinach chloroplasts (Fig. 5A and B of Ref. 3) extremely high $H_{1/2}$ values of about 1000 gauss were observed. Again, this may be ascribed to orientational effects in the large stationary magnetic field applied and/or an antenna-associated magnetic-field-dependent emission. The magnetic-field-dependent emission increase in PS I spinach particles measured by a 50 Hz modulated field indeed showed lower $H_{1/2}$ values of about 300–650 gauss [73]. Recently, Elfimov et al. [32] suggested that the fission process in non-sulfur bacteria (e.g., *Rps. capsulata*) depends on the temperature, but these results [71] were obtained under conditions where both the antenna and the reaction center associated processes were excited. In addition, as was discussed in Section The Magnetic-Field-Dependent Emission Associated With The Antenna Complex, changes of the total emission spectrum could also account for the observed temperature dependence. The curves shown in Fig. 10A and B clearly indicate the difference of the temperature dependence between BChl and carotenoid excitation. In the latter case both categories of magnetic-field-dependent emission are observed.

Some concluding remarks

From our results we conclude that the fission process is operative in both the B850 and the B880 complex. It is remarkable that the shape of the saturation curves observed in *Rps. sphaeroides* in whole cells/chromatophores and the various antenna complexes in *Rps. capsulata* [9,32] and in organic crystals like anthracene or pentacene-doped tetracene [15] are very similar. The H_0 values are about 400 gauss. Apparently, these curves do not strongly depend on the type of molecules involved or the type of fission process (homo- or heterofission). In contrast, the saturation curves observed in the B880 complex and oxidized cells or chromatophores of *R. rubrum* are clearly different. In addition, the yield of the carotenoid triplet formed by fission is high in *R. rubrum* (approx. 0.3), very low in *Rps. sphaeroides* 2.4.1 and G1C (less than 0.01) [12], but again high in organic crystals [15]. Besides the structural differences of the antenna between the two families, the energy-transfer efficiencies are very different (30% in *R. rubrum* and about 90% in *Rps. sphaeroides*) [14]. It may be proposed that this is somehow related to differences in the carotenoid-carotenoid or carotenoid-bacteriochlorophyll organization.

This work indicates that the formation of the triplet pair competes with energy transfer (although no proof was given) and that the triplet pair is not an intermediate in the energy-transfer process. In this light the biological meaning of the fission process remains obscure.

Acknowledgements

We wish to thank Mr. F.W. van der Kooij, Mr. E.J. Lous and Dr. T. Swarthoff for the assistance with and the performance of some of the experiments. The authors are indebted to Mrs. L.M. Blom, Mr. A.H.M. de Wit and Mr. F.T.M. Zonneveld for culturing the bacteria and skillful preparative work. Thanks are also due to Prof. Dr. J. Amesz for valuable suggestions and many stimulating discussions. The investigation was supported by the Netherlands Foundation for Biophysics, financed by the Netherlands Organization for the Advancement of Pure Research (ZWO).

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