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MAGNETIC FIELD EFFECTS ON THE FLUORESCENCE OF MUTANT STRAINS OF *RHODOPSEUDOMONAS CAPSULATA*

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The magnetic field effects on bacteriochlorophyll fluorescence in six strains of *Rhodopseudomonas capsulata* were investigated. All strains exhibit an increase in fluorescence upon application of a magnetic field. Large magnetic field effects are shown to arise in mutants which contain the B800-850 complex as the only bacteriochlorophyll-containing protein. These fluorescence increases are observed only with carotenoid excitation and are best described by a carotenoid singlet heterofission mechanism. Variations in the magnitudes of the magnetic field effects for the *Rps. capsulata* strain arise from energy differences in the excited states of the molecules involved in the process. In order to determine the contribution from reaction centers to the magnetic field effects observed in the mutants which contain all three pigment-protein complexes, reaction centers were isolated from these strains. The reaction center contribution to the magnetic field effect on fluorescence in whole cells was determined to be smaller than the antenna contribution when carotenoid excitation was employed.

Introduction

Photosynthetic bacteria contain membrane-bound antenna and reaction center pigment-protein complexes [1]. Light energy captured by the antenna complexes, B800-850 and B870, is channelled to the reaction center by excitation transfer [1]. This energy is then used to promote electron transfer in the reaction center [1]. The formation of a radical ion pair between the primary electron donor (a bacteriochlorophyll dimer) and the primary electron acceptor (a bacteriopheophytin molecule) occurs in less than 10 ps [2].

Charge recombination between the bacteriochlorophyll dimer and bacteriopheophytin results in the formation of the bacteriochlorophyll dimer excited singlet state, $^1BChl_2^*$, triplet state, $^3BChl_2^*$, or ground state, $BChl_2$ [2]. The bacteriochlorophyll dimer triplet state is populated via the so-called radical pair mechanism, [3]. According to this mechanism, quantum-mechanical mixing of the singlet and triplet radical pair states owing to dephasing of the radical spins gives rise to a non-Boltzmann distribution of spins in the bacteriochlorophyll dimer triplet state. The application of an external magnetic field splits the triplet spin sublevels of the radical pair and reduces the singlet-triplet mixing. Because the initial charge separation involves $^1BChl_2^*$ as a precursor, the application of a magnetic field leads to a decrease in the yield of $^3BChl_2^*$ or an increase in the fluorescence yield from $^1BChl_2^*$.

Hoff et al. [4] have investigated the magnetic field-induced changes on the triplet yield of *Rhodopseudomonas sphaeroides* wild type. They reported substantial differences between the mag-

Abbreviations: BChl, bacteriochlorophyll; B800-850 and B870, light-harvesting pigment-protein complexes denoted by the major bacteriochlorophyll absorption bands.

netic field effects observed for chromatophore and reaction center preparations. They suggested that the exchange interaction between members of the radical ion pair may vary with sample preparation and give rise to the observed differences. More recently, Rademaker et al. [5] have observed a magnetic field effect on the antenna carotenoid triplet yield in chromatophores of *Rhodospirillum rubrum*. They suggested that the effect arises from the generation of a spin pair state in the antenna and that this state may be either radical pair or triplet pair in nature. Voznyak et al. [6] have implicated the triplet pair as giving rise to a magnetic field effect on the fluorescence in whole cell preparations of *Rps. capsulata*. These authors monitored the magnetic field effects on the fluorescence of whole cells as a function of the excitation wavelength and redox state of the reaction center. Their interpretation of these results was that excitation into bacteriochlorophyll absorption bands at 600 nm yielded a carotenoid triplet only when the reaction center was chemically reduced prior to the fluorescence experiment. Furthermore, excitation into the carotenoid absorption bands at 510 nm gave rise to a carotenoid triplet state independent of the redox state of the reaction center. Recently, we have demonstrated that magnetic field effects on bacteriochlorophyll fluorescence can be observed in strains of *Rps. capsulata* which lack reaction centers [7]. These findings pointed to the need for further investigations to answer the question of how much of these magnetic field effects originates in the reaction center and how much arises in the antenna.

In this paper we present an investigation of the magnetic field effects on the fluorescence from six mutant strains of *Rps. capsulata* denoted SB1003, MT1131, MW442, MW4422, Y142 and BY1424. These strains are ideal to test the sources of the observed fluorescence magnetic field effects, in that each one contains one or more of the three pigment-protein complexes and either neurosporene or spheroidene as the major carotenoid pigment. The SB1003 and MT1131 strains contain the B800-850 and B870 complexes, and reaction centers. The MW442 and MW4422 strains contain the B870 and reaction center protein complexes while Y142 and BY1424 contain only the B800-850 protein complexes. The strains SB1003, MW442 and Y142 contain spheroidene whereas MT1131, MW4422 and BY1424 contain neurosporene. (Table I summarizes this.) By analyzing this series of mutants, where a systematic variation of the pigment-protein complex and carotenoid composition is accomplished, insight into the sources of the magnetic field effects on fluorescence for various preparations of photosynthetic bacteria can be afforded.

In this work we will show that the fluorescence magnetic field effects observed in *Rps. capsulata* are best described by a singlet exciton fission model [8]. This model is part of a more general theory of cooperative exciton processes first used to describe magnetic field effects on the fluorescence and on triplet annihilation in molecular crystals [9–13].

Materials and Methods

The *Rps. capsulata* mutant strains were obtained as a gift from the laboratory of Professor Barry Marrs at the St. Louis University School of Medicine. Reaction center-containing strains were

TABLE I

CAROTENOID AND PIGMENT-PROTEIN COMPLEX COMPOSITION OF MUTANT STRAINS OF *RPS. CAPSULATA*

Rps. capsulata strain	Carotenoid composition	Pigment-protein composition
SB1003	spheroidene spheroidenone	B800-850, B870, reaction centers
MW442	spheroidene spheroidenone	B870, reaction centers
Y142	spheroidene spheroidenone	B800-850
MT1131	neurosporene hydroxyneurosporene methoxyneurosporene	B800-850, B870, reaction centers
MW4422	neurosporene hydroxyneurosporene methoxyneurosporene	B870, reaction centers
BY1424	neurosporene hydroxyneurosporene methoxyneurosporene	B800-850

grown anaerobically in the light in a 3% yeast extract/3% bactopeptone medium. The reaction centerless strains were grown aerobically in the dark in the same medium. After harvesting the cells were washed with 25 mM Tris (pH 7.5), and pelleted by centrifugation at $25\,000 \times g$.

Isolated reaction centers from SB1003 and MT1131 were prepared essentially according to the method of Jolchine and Reiss-Husson [14], the only modification being that the pellet obtained from the $150\,000 \times g$ centrifigation was resuspended in 0.1 M phosphate buffer (pH 7.5), adjusted to an absorbance of 50 at 850 nm, treated a second time with lauryldimethylamine *N*-oxide (0.05%) and recentrifuged at $150\,000 \times g$ for 2 h.

The whole cell samples used in the fluorescence experiments were prepared by dilution of the pelleted cells with 25 mM Tris containing sodium dithionite ($5.0 \cdot 10^{-2}$ M) and approx. 10^{-5} methyl viologen. The pH of the sample was not altered by more than 0.5 pH units upon the addition of dithionite. Ethylene glycol (50%, v/v) was added to prevent sample cracking at the lower temperatures. The final sample concentration was adjusted to an absorbance of 0.5 at 590 nm. The isolated reaction center samples were diluted with 0.1 M phosphate buffer containing $5 \cdot 10^{-2}$ M sodium dithionite and 50% (v/v) ethylene glycol. All of the solutions were degassed with argon and freeze-pump-thawed (four cycles) to eliminate any irreversible photobleaching due to oxygen.

Fluorescence spectroscopy was carried out in an external magnetic field using a Spectra Physics argon laser (Model 165) for carotenoid excitation and an Ealing 100 W mercury arc lamp with a 550 nm cutoff filter for bacteriochlorophyll excitation. The fluorescence was detected by an RCA S1 type photomultiplier tube in conjunction with a half-meter Jarrell Ash monochromator (Model 82-020). The photomultiplier output was amplified by a Keithley 414S picoammeter and fed to a Varian C-1024 signal averager for processing. The detection wavelengths were 911 nm for the isolated reaction centers and the reaction center-containing whole cells, and 885 nm for whole cells of the reaction centerless mutants. These wavelengths correspond to the maximum fluorescence intensity in each case. No magnetic field effect on the photomultiplier tube was observed as checked by samples of bacteriochlorophyll in methanol and rhodamine-6G in ethanol. The magnitudes of the observed magnetic field effects were unaffected by sample concentration (to 0.08*A* at 590 nm), laser intensity (to 2 $mW \cdot cm^{-2}$) and detection wavelength (± 30 nm). All samples were sealed in 3-mm tubes for the fluorescence experiments.

Results

The data for the magnetic field-induced fluorescence changes for whole cell preparations of the *Rps. capsulata* strains excited both into the carotenoid bands and bacteriochlorophyll bands are given in Table II. The magnitude of the magnetic field effect ($\Delta F/F$) is given by:

$$\Delta F/F = [F(H=\infty) - F(H=0)]/F(H=0) \quad (1)$$

where $F(H=\infty)$ represents the fluorescence intensity at infinite magnetic field and $F(H=0)$ represents that at zero magnetic field. The half-saturation value ($B_{1/2}$) qualitatively describes the shape of the fluorescence magnetic field effect curve. The $\Delta F/F$ and $B_{1/2}$ values were extracted from the data by curve fitting single- or double-exponential functions to the observed fluorescence changes. Although there is no theoretical basis for the assignment of the curves to exponential functions, the fitting procedure facilitated the calculation of the $\Delta F/F$ and $B_{1/2}$ values, particularly in cases where the fluorescence change had not yet levelled off at the maximum applied field value. In all cases the fits were excellent.

With carotenoid band excitation the $\Delta F/F$ value for all bacteria decreases as the temperature is lowered from 240 to 100 K. The $B_{1/2}$ value increases as the temperature is lowered over this same range. A comparison of the magnetic field effects on fluorescence in whole cells of the spheroidene-containing strains Y142, MW442 and SB1003 which contain various combinations of pigment-protein complexes addresses the question of whether or not different pigment-protein complexes contribute differently to the overall fluorescence effects. The magnetic field effects on the fluorescence of the Y142 and BY1424 strains which contain only the B800-850 pigment-protein complex unambiguously demonstrate that such effects

can be observed in the antenna system of photosynthetic bacteria. The SB1003 strain which contains the B800-850, B870 and reaction centers shows an effect with a larger $\Delta F/F$ and $B_{1/2}$ value than Y142. The MW442 strain which contains the B870 and reaction centers shows a $\Delta F/F$ intermediate between Y142 and SB1003 and $B_{1/2}$ value which is larger than those observed in Y142 and SB1003. A similar trend is observed by comparison of the neurosporene-containing strains BY1424, MW4422 and MT1131; namely, the MT1131 strain displays a larger $\Delta F/F$ and $B_{1/2}$ value than BY1424, and MW4422 shows an effect similar to that of BY1424. At temperatures around 150 K and below, however, MT1131 and MW4422 display markedly biphasic magnetic field effects (Fig. 1a and b). This biphasic behavior is observed only in reduced whole cells of these bacteria (see Table II).

A comparison of two strains with identical pigment-protein composition and different carotenoids addresses the question of the role of caro-

TABLE II

THE MAGNITUDE ($\Delta F/F$) AND HALF-SATURATION $B_{1/2}$ VALUES OF THE MAGNETIC FIELD EFFECTS ON THE FLUORESCENCE OF *RPS. CAPSULATA*

Excitation wavelengths were obtained by use of the multiline mode of an argon ion laser unless noted otherwise.

Rps. capsulata strain	Temperature (K)	$\Delta F/F$	$B_{1/2}$ (G)	Comments
SB1003				
reduced whole cells	240	0.014 ± 0.004	225 ± 68	> 550 nm excitation
	240	0.018 ± 0.003	350 ± 46	–
	150	0.015 ± 0.002	423 ± 28	–
	100	0.008 ± 0.003	592 ± 40	–
unreduced whole cells	240	0.018 ± 0.002	323 ± 35	–
reduced reaction centers	240	0.008 ± 0.003	50 ± 32	–
MT1131				
reduced whole cells	240	0.019 ± 0.004	240 ± 81	> 550 nm excitation
	240	0.036 ± 0.002	876 ± 33	–
	150	0.021 ± 0.004	–	biphasic
	100	0.012 ± 0.002	–	biphasic
oxidized whole cells	150	0.022 ± 0.003	975 ± 30	–
B800-850 complexes	240	0.020 ± 0.002	800 ± 30	–
reduced reaction centers	240	0.008 ± 0.003	50 ± 27	–
MW442				
reduced whole cells	240	0.016 ± 0.002	539 ± 35	–
	150	0.012 ± 0.002	675 ± 38	–
	100	0.010 ± 0.002	902 ± 41	–
MW4422				
reduced whole cells	240	0.021 ± 0.002	633 ± 35	–
	150	0.019 ± 0.002	–	biphasic
	100	0.008 ± 0.002	–	biphasic
Y142				
unreduced whole cells	240	0.006 ± 0.002	246 ± 36	–
	150	0.001 ± 0.0005	–	
	240	no effect	–	> 550 nm excitation
BY1424				
unreduced whole cells	240	0.021 ± 0.001	769 ± 20	–
	150	0.019 ± 0.001	937 ± 25	–
	100	0.015 ± 0.002	1064 ± 33	–
	240	no effect	–	> 550 nm excitation

tenoids in affecting the magnetic field-induced fluorescence changes. The neurosporene-containing strains (Fig. 1a–c) give rise to magnetic field effects with significantly larger $\Delta F/F$ and $B_{1/2}$ values than the effects shown for the spheroidene strains (Fig. 1d–f).

With excitation into the bacteriochlorophyll absorption bands beyond 550 nm, one observed no

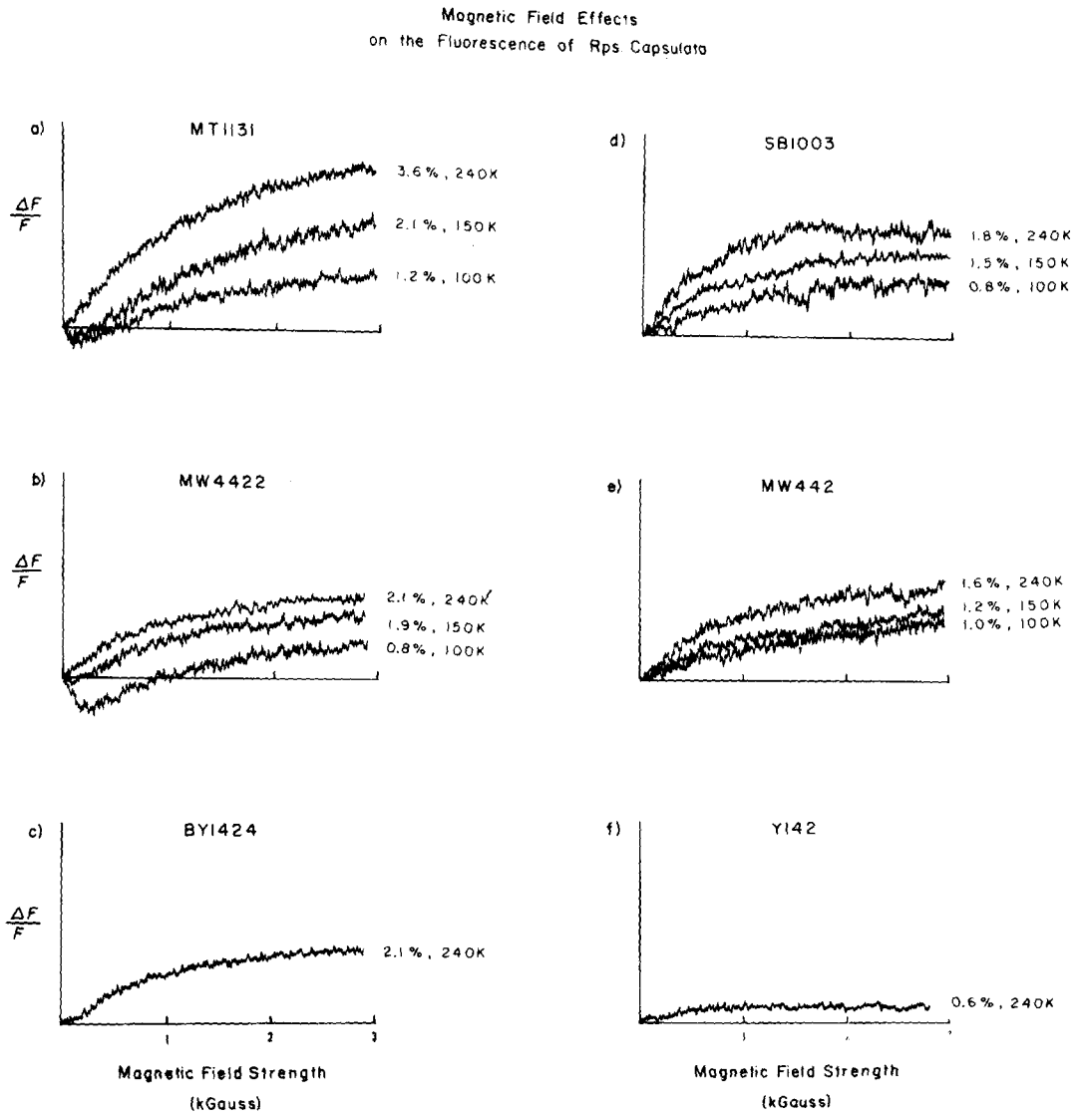

Fig. 1. Magnetic field effects on the fluorescence of mutant strains of *Rps. capsulata*. The laser power was 30 mW using the multiline mode of an argon ion laser. The magnetic field sweep rate was 33 $G \cdot s^{-1}$. (a) Reduced cells of MT1131 using a fluorescence detection wavelength of 911 nm; The upper two traces are averages of 30 sweeps, and the lower trace is an average of 20 sweeps. (b) Reduced cells of MW4422; fluorescence detection wavelength, 911 nm. The upper trace is an average of 11 sweeps, the middle trace is an average of 25 sweeps, and the bottom trace is an average of 30 sweeps. (c) Cells of BY1424. The trace is an average of 15 sweeps using a detection wavelength of 885 nm. (d) Reduced cells of SB1003. Fluorescence detection wavelength, 911 nm; the upper two traces are averages of 50 sweeps and the lower trace is an average of 25 sweeps. (e) Reduced cells of MW442. Fluorescence detection wavelength, 911 nm; the upper trace is an average of 30 sweeps, the middle trace is an average of 40 sweeps and the bottom trace is an average of 20 sweeps. (f) Cells of Y142. Fluorescence detection wavelength, 885 nm; the trace is an average of 15 sweeps.

magnetic field effects on the fluorescence in the B800-850-containing strains of Y142 and BY1424. Also, with bacteriochlorophyll excitation the fluorescence magnetic field effects in the reaction center-containing strains of SB1003 and MT1131 are observed only when the reaction centers are chemically reduced. This is consistent with the observations by Voznyak et al. [6] on whole cells of *Rps. capsulata*. In contrast, the net magnetic field-induced increase in the fluorescence from all of these strains of *Rps capsulata* arises independently of the redox state of the reaction center when carotenoid band excitation is used.

Isolating reaction centers from the two strains SB1003 and MT1131 allows one to distinguish between reaction center effect and antenna effects. The magnetic field effects on the fluorescence of isolated reaction centers are observed only when the samples are chemically reduced and show little or no difference between species (see Table II). However, the isolated, reaction center effects are dramatically different from the SB1003 or MT1131 whole cell effects. The isolated reaction centers show fluorescence magnetic field effects with $\Delta F/F$ values of less than 1% and $B_{1/2}$ values of approx. 50 G and resemble the magentic field effects observed from *Rps. sphaeroides* R26 [15]. The whole cell preparations of SB1003 and MT1131 show effects with $\Delta F/F$ values of the order of 2–3% and $B_{1/2}$ values substantially larger than 50 G (Table II).

B800-850 pigment-protein complexes were isolated from MT1131 to address the question of whether or not the antenna magnetic field effects arise from a cooperative effect between pigment-protein complexes linked by the presence of a membrane. The $\Delta F/F$ value for the isolated B800-850 pigment-protein complex of MT1131 at 240 K was found to be 0.020, virtually identical to the $\Delta F/F$ value for whole cells of BY1424 at 240 K (see Table II). Also, the $B_{1/2}$ value for the B800-850 complex from MT1131 (800 G) is very similar to that of BY1424 (769 G). This indicates that for these bacteria the effects arise within a single pigment-protein complex and are not dependent on cooperativity between complexes held together by the photosynthetic membrane.

Discussion

Theoretical model

Singlet exciton fission, a well known process in the photophysics of molecular crystals [8], is represented by:

$$S_1^* + S_0 \rightarrow [T \cdot T'] \tag{2}$$

where S_1^* and S_0 are an excited singlet state and the ground state, respectively. $[T \cdot T']$ is a triplet pair consisting of nine spin substates. When the colliding singlet excitons occupy identical molecules, the process is termed homofission, otherwise it is considered to be heterofission [8].

The spin hamiltonian describing the triplet exciton pair in an external magnetic field is given by:

$$\mathscr{H} = g\beta \boldsymbol{H} \cdot (\hat{\boldsymbol{S}}_1 + \hat{\boldsymbol{S}}_2) + \hat{\boldsymbol{S}} \cdot \underline{\boldsymbol{D}} \cdot \hat{\boldsymbol{S}} \tag{3}$$

where the first term represents the Zeeman interaction and the second term represents the dipolar spin-spin interaction. A generalized wave function for any one of the nine possible triplet exciton pair states is written as:

$$\psi = \phi_S C_S \sigma_S + \phi_T C_T \sigma_T + \phi_Q C_Q \sigma_Q \tag{4}$$

where ϕ_S, ϕ_T and ϕ_Q represent the orbital parts of the wave function, and $C_S\sigma_S$, $C_T\sigma_T$ and $C_Q\sigma_Q$ represent the products of the coefficients and the spin wave functions for the singlet, triplet and quintet states, respectively.

The rate of singlet exciton fission was found by Merrifield [9] to depend on the magnitude of an externally applied magnetic field. He concluded that the fission rate is determined by the quantum-mechanical mixing between the singlet exciton states and the nine triplet spin pair states (e.g., the rate is enhanced by increased singlet character in the triplet pair states). In order to determine the extent of this mixing, the coefficients of the spin wave functions must be obtained by diagonalization of the above hamiltonian (Eqn. 3).

For homofission one can write $T = T'$. In this case three of the nine spin sublevels of the triplet exciton pair are found to contain singlet character of zero field [8]. At high magnetic fields, however, where the Zeeman splitting is much larger than the

dipolar splitting ($g\beta H \gg |D|$), the spins are quantized along the magnetic field, and it is found that only two substates of the triplet exciton pair have singlet character [8]. Because the rate of singlet exciton fission depends on the mixing between the singlet exciton states and the triplet exciton pair states, the application of a magnetic field results in a slower rate of homofission.

For heterofission ($T \neq T'$) it is found that all nine triplet exciton pair states may have singlet character at zero field [8]. At high magnetic fields the number of spin sublevels containing singlet character is reduced to two. Thus, it is expected that the rate of singlet homofission be decreased from the rate at zero field.

For molecular systems where S_1^* is a fluorescing level undergoing singlet exciton fission, an increase in the prompt fluorescence of the system with increasing magnetic field is observed [8]. This effect is observed in both the homofission and heterofission cases.

The inverse of singlet fission is triplet exciton fusion [8]. It is represented by the collision of two triplet excitons followed by fusion into two singlets.

$$[T \cdot T'] \rightarrow S_1^* + S_0 \tag{5}$$

The $[T \cdot T']$ state may result from a collision between two migrating triplets, previously uncorrelated, or singlet exciton fission (Eqn. 2). Analogous to singlet exciton fission, the rate of triplet exciton fusion is magnetic field dependent. The rate depends on the mixing between singlet exciton and triplet spin pair states. Again, when $T = T'$ (homofusion) at zero field three substates of the triplet exciton pair will have singlet character whereas only two substates will have singlet character at high magnetic fields. For triplet exciton heterofusion ($T \neq T'$) at zero field, all nine substates of the triplet exciton pair may contain singlet character. The number of such states is reduced to two as a magnetic field is applied. Thus, the triplet exciton fusion rate will decrease with application of a magnetic field. If S_1^* is a fluorescing level then the molecular system will exhibit a decrease in the delayed fluorescence with increasing magnetic field [8].

For the process:

$$S_1^* + S_0 \underset{k_{\text{fusion}}}{\overset{k_{\text{fission}}}{\rightleftharpoons}} T + T' \tag{6}$$

where T and T′ are uncorrelated triplet states, Merrified [9] has proposed that the ratio of the fission to the uncorrelated fusion rate constant obeys the following relation:

$$k_{\text{fission}}/k_{\text{fusion}} = 9\exp\{-[(E_T + E_{T'}) - E_S]/kT\} \tag{7}$$

where E_T and $E_{T'}$ are the energies of triplet excitons and E_S represents the singlet exciton energy, k is Boltzmann's constant and T is the absolute temperature. From this equation one can see that the fission/fusion processes are temperature dependent and also depend on the energetics of the states involved in the process. For fission to be energetically favored over fusion the energy of the triplet exciton pair ($E_T + E_{T'}$) must be approximately equal to or less than the singlet exciton energy (E_S). For fusion to be energetically favored over fission E_S must be less than $E_T + E_{T'}$.

Application of the theoretical model to the observed magnetic field effects

The theory of cooperative exciton processes provides a useful working model for understanding the magnetic field effects on fluorescence observed in the antenna strains of *Rps. capsulata* BY1424 and Y142 (Fig. 1c and f). It is observed that the bulk fluorescence intensity increases with magnetic field strength in both of these strains of bacteria. A net increase in fluorescence intensity is consistent with singlet fission being active in these bacteria. Fusion as the dominant process (Eqn. 5) would result in a net decrease in the bulk fluorescence intensity. An increase in the fluorescence can also be explained by invoking the radical pair mechanism as being active in the antenna of these bacteria. However, no radical pair polarized triplet state signals were observed in the ESR spectra of BY1424 and Y142 [7]. These types of signals would be expected if an electron-transfer event generated a radical pair which subsequently underwent charge recombination. Also, no $g = 2.00$ light-induced signal was observed in the ESR experiments, suggesting that charge separation is not occurring in these bacteria.

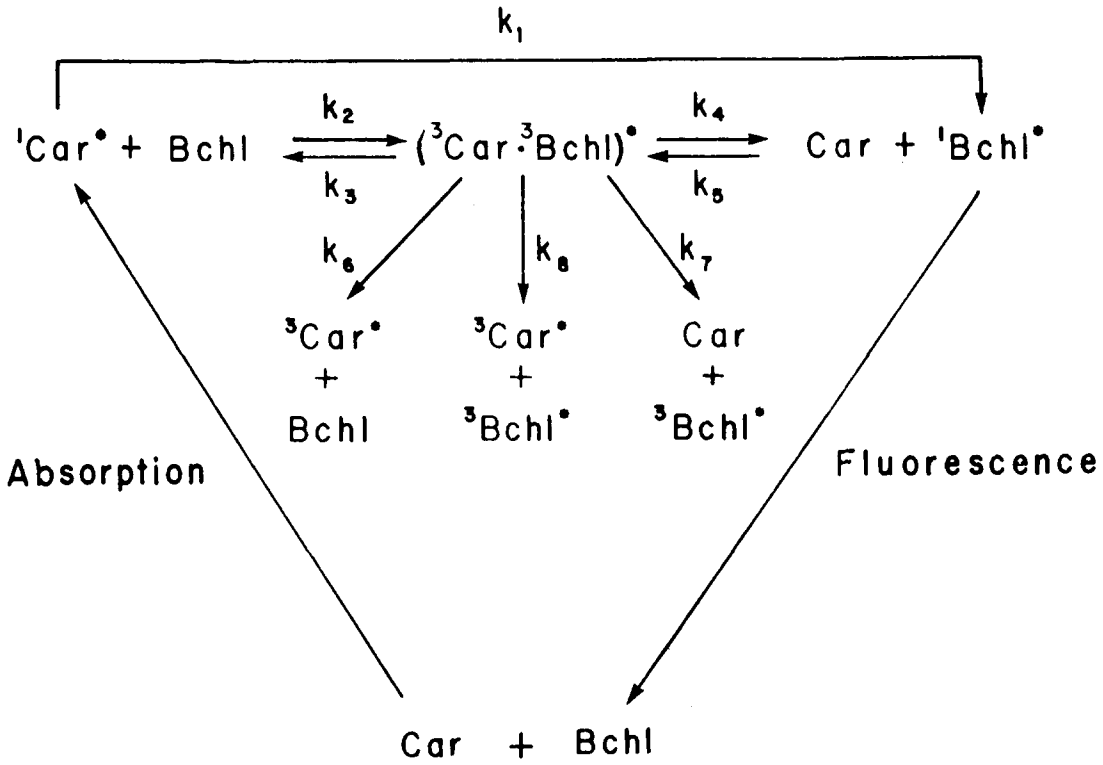

Fig. 2. Schematic representation of some of the various rate processes which can occur following absorption of light by the carotenoids in the antenna pigment-protein complexes of *Rps. capsulata*.

In order to determine whether the effect constitutes a homofission or a heterofission and which excited singlet states are involved in the process, experiments were performed using either carotenoid or bacteriochlorophyll excitation. As mentioned in Results, fluorescence magnetic field effects were observed upon excitation into the carotenoid absorption bands of BY1424 and Y142. No such effects were observed in these same strains upon excitation into the bacteriochlorophyll absorption bands (greater than 550 nm). This indicates that carotenoid excited singlet states are involved in the singlet fission process and that bacteriochlorophyll excited singlet states can be involved only in the sense that they contain the fluorescing levels. They do not appear to be undergoing singlet fission themselves. Fig. 2 illustrates schematically what the competing processes are likely to be. In this scheme the rate constant k_5 is probably small compared to k_2.

As previously mentioned, fluorescence magnetic field effects were observed in B800-850 pigment-protein complexes isolated from the strain MT1131 (see Table II). In this experiment the complexes were studied in a preparation where they were released from the chromatophore membrane. Because the carotenoid is already implicated in the magnetic field effect from the selective excitation experiments, and because the isolated complexes are most likely not aggregates to any significant degree and contain only one carotenoid per three bacteriochlorophyll molecules [16], it is concluded that the observed magnetic field effect on bacteriochlorophyll fluorescence arise from a heterofission process involving one carotenoid excited singlet state and one bacteriochlorophyll ground state, i.e.:

$$^1\text{Car}^* + \text{BChl} \rightleftarrows [^3\text{Car}^* \cdot ^3\text{BChl}^*] \qquad (8)$$

This conclusion is supported by the energetics of the states involved in the process. Fission requires that the energy of the triplet exciton pair be approximately equal to or less than the singlet exci-

ton energy. In the B800-850 of Y142 and BY1424, the energies of the lowest excited carotenoid singlet states of spheroidene and neurosporene calculated from their singlet absorption spectra are 2.4 and 2.7 eV, respectively. The bacteriochlorophyll triplet state energy has been estimated from phosphorescence studies to be approx. 1.0 eV [17,18]. The triplet state energies of spheroidene and neurosporene have been determined from flash photolysis, triplet state sensitization experiments to be 0.8 and 0.9 eV, respectively [19]. Thus, the triplet exciton pair energies in Y142 (1.8 eV) and BY1424 (1.9 eV) are low lying with respect to their carotenoid excited singlet state energies favoring fission over fusion in both strains.

Once the [^{3}Car* $\cdot$ ^{3}BChl*] triplet exciton pair is generated by fission it may decay via many pathways (Fig. 2). Correlated fusion can occur regenerating the excited singlet state of the carotenoid (rate constant k_3 in Fig. 2). This is not likely in that it is not favored energetically. The energy of the triplet exciton pair (approx. 1.8 and 1.9 eV for spheroidene and neurosporene, respectively) is not large enough to regenerate the excited singlet states of either carotenoid. Fusion may also generate the excited singlet state of bacteriochlorophyll (rate constant k_4 in Fig. 2). The energy of this state is approx. 1.5 eV as calculated from the 850 nm absorption band. Here correlated fusion is energetically favored and will contribute a delayed fluorescence component to the bulk fluorescence. The triplet exciton pair can also decay to form a carotenoid triplet state and bacteriochlorophyll ground state (rate constant k_6 in Fig. 2), a carotenoid ground state and a bacteriochlorophyll triplet state (rate constant k_7) or both a carotenoid and a bacteriochlorophyll triplet state (rate constant k_8). Bacteriochlorophyll triplet states which are formed will most likely be quenched by the carotenoid triplet states through triplet-triplet energy transfer [20].

The question still remains as to the origin of the differences in the magnetic field efffects arising in the strains of bacteria differing only in their carotenoid composition. It is known that the intensity of bacteriochlorophyll fluorescence is dependent on the concentration of carotenoid singlet states through singlet energy transfer [21]. The concentration of carotenoid excited singlet states is enhanced as the magnetic field is increased owing to a reduction in the mixing of the singlet exciton states with the triplet pair states. Thus, one observes an increase in the bulk bacteriochlorophyll fluorescence with increasing magnetic fields. Because it has been determined that spheroidene and neurosporene have similar efficiencies for singlet energy transfer [21], the differences in the fluorescence magnetic field effects observed for BY1424 and Y142, for example, cannot be explained on this basis. The differences are best explained with the help of Fig. 2 and an expression which can be derived from the scheme assuming: (i) photostationary behavior of the system (the experiments were done with continuous illumination); (ii) k_3 and k_5 are small. k_3 is likely to be small based on the energetics arguments presented above (i.e., it corresponds to an uphill process). k_5 is certainly small in that no magnetic field effects on the fluorescence are observed upon bacteriochlorophyll excitation of the reaction centerless mutants. Thus, we may obtain the following expression:

$$\frac{[^1\text{BChl*}]}{[^1\text{Car*}][\text{BChl}]} = \frac{k_1k_6 + k_1k_7 + k_1k_8 + k_1k_4 + k_2k_4}{k_f(k_4 + k_6 + k_7 + k_8)} \qquad (9)$$

which represents the ratio of the concentration of ^{1}BChl* to ^{1}Car* and BChl. k_f is the rate constant for fluorescence. Note that if k_6, k_7 and k_8 are small the ratio will not depend on k_4; i.e., fusion will not contribute to the observed changes in fluorescence. Because singlet fission is an activated process [8] one may write:

$$k_i = k_i' e^{-\Delta E_i/kT} \quad i = 2,4 \qquad (10)$$

where k_i' is the frequency factor and ΔE_i the activation energy for the process. Because ΔE_2 depends both on the energy of the excited singlet state of the carotenoid and on the transition state energy, and ΔE_4 depends on the energies of the triplet pair states and the transition state energy, bacteria containing different carotenoids are likely to show different magnetic field effects on fluorescence. Indeed, this is what is experimentally observed (Table II).

The temperature dependence of the $\Delta F/F$ values (the magnitude of $\Delta F/F$ decrease as the temperature is lowered) may be explained from a

consideration of the populations of the triplet pair levels. The fission/fusion pathway via k_2 and k_4 (Fig. 2) which generates ^{1}BChl* is driven by singlet mixing with the triplet pair states. When a magnetic field is applied to the system the levels with net spin angular momentum (the triplets and quintets) will split to higher and lower energy than at zero field. If the triplet pair has singlet energies which are greater than or equal to the triplet and quintet energies, a decrease in the temperature will reduce the Boltzmann population of the singlet levels. The result would be a decrease in the rate of mixing between the triplet pair and ^{1}BChl*. Thus, one would observed a decrease in the $\Delta F/F$ values as the temperature is lowered.

As previously stated, reaction centers from MT1131 and SB1003 were isolated to determine whether the fluorescence magnetic field effects observed in these strains arise from the antenna, the reaction centers or a combination of both. The fluorescence magnetic field effects from isolated MT1131 and SB1003 reaction centers are not dependent on the carotenoid composition in the reaction center (see Table II). Furthermore, the reaction center fluorescence magnetic field effects are small compared to the antenna magnetic field effects and are observed upon carotenoid excitation and only when the preparation is chemically reduced. These effects are very similar to those reported previously for *Rps. sphaeroides* R26 [15]. The process giving rise to these effects has been explained by many groups using the radical pair mechanism [22]. The whole cell preparations of MT1131 and SB1003, however, show magnetic field effects on fluorescence independent of the reaction center redox potential when carotenoid excitation is used (Table III). Therefore, it is concluded that the magnetic field effects on fluorescence observed in the whole cell preparations of MT1131 and SB1003 arise predominantly in the antenna. The mechanism giving rise to the effects as previously discussed is most likely a singlet heterofission mechanism, analogous to the explanation offered for the behavior of the BY1424 and Y142 strains.

A comparison of the $\Delta F/F$ values for MT1131 and BY1424 as well as for SB1003 and Y142 at 240 K (Table II) shows the dependence of the magnetic field effects on the pigment-protein composition of the bacteria. The magnitudes of the magnetic field effects on fluorescence in MT1131 (3.6%) and SB1003 (1.8%) are larger than those observed in BY1424 (2.1%) and Y142 (0.6%), respectively. The reaction centers appear to make only a small contribution to the observed magnetic field effects in MT1131 and SB1003 based on results from the isolated reaction center studies. The rather large differences in the $\Delta F/F$ values noted above (for BY1424 vs. MT1131 and Y142 vs. SB1003) most likely originate in the B870 pigment-protein complexes of MT1131 and SB1003. This is supported by the observation of magnetic field effects on the fluorescence in whole cells of MW442 and MW4422 which remain invariant to the redox poise of the sample when carotenoid excitation is exployed. These bacteria contain only the B870 pigment-protein complex and reaction centers.

Analogous to the $\Delta F/F$ values, the $B_{1/2}$ values for the fluorescence magnetic field effects depend on the type of carotenoid present, and the pigment-protein complex composition. To understand the molecular features affecting the magnitude of the $B_{1/2}$ value a theoretical description involving the dynamics and energetics of the excited states is required. Such a description is beyond the scope of the present work. However, qualitative deductions concerning what to include in the description can be drawn from the data presented in Table II. The $B_{1/2}$ values increases as the temperature decreases and is larger at any given temperature for the neurosporene-containing strains compared to the spheroidene-containing strains. Furthermore, the $B_{1/2}$ value is seen to increase with the presence of the B870 complex and reaction centers as shown in the data for the fluorescence magnetic field effects in BY1424 and Y142 relative to their parent strains MT1131 and SB1003 (see Table II). These trends in the $B_{1/2}$ values suggest that the theoretical model should incorporate such factors as the carotenoid energy, pigment-protein complex trapping dynamics and temperature.

An interesting feature of the data presented in Table II for MT1131 and MW4422 is the biphasic behavior of the magnetic field effects on the fluorescence observed at temperatures below 150 K. As the magnetic field is applied the fluorescence is observed first to decrease and then increase as the

magnetic field strength is increased (Fig. 1a and b). This biphasic behavior has been observed previously by other groups [23,24] in neurosporene-containing bacteria, but no satisfactory explanation of its occurrence has been offered. Because the biphasic behavior disappears with oxidation of the primary donor (see Table II) and because no such behavior is observed in the reaction centerless mutant BY1424, it is likely that the effect has its origin in the reaction centers of MT1131 and MW4422. Reaction center charge recombination could give rise to a decrease in magnetic field-induced fluorescence if the singlet-triplet mixing of the reaction center radical pair is enhanced at $g\beta H \approx |D|$. At 240 K these reaction center recombination fluorescence effects may be masked by the fission/fusion (k_2 and k_4) fluorescence effects. As the temperature is lowered from 240 to 100 K the rate of singlet fission and correlated fusion is reduced. Thus, the biphasic reaction center magnetic field effect may become more prominant. The presence of neurosporene also appears to be important in that the effect is absent from the spheroidene-containing strains. This carotenoid dependence is probably due to the variation in the rates of singlet fission for the different carotenoids; such rates being competitive with the reaction center processes.

By virtue of the pigment-protein complex and carotenoid composition of the *Rps. capsulata* mutants studied, we have been able to assign unambiguously fluorescence magnetic field effects as occurring in the B800-850 pigment-protein complexes. Selective excitation into the carotenoid and bacteriochlorophyll absorption bands along with the obsevation that all magnetic field-induced fluorescence changes result in an enhancement of fluorescence intensity has led us to conclude that the fluorescence magnetic field effects in the B800-850 pigment-protein complexes arise from carotenoid singlet heterofission. Furthermore, the studies involving isolated reaction centers from MT1131 and SB1003 indicate that the B800-850 contribution to the magnetic field effect on fluorescence in whole cells of these parent strains is large in comparison to the reaction center contribution.

In summary, there are three types of magnetic field effects on bacteriochlorophyll fluorescence active in these systems: (i) Those reported by Hoff et al. [4,25] and Voznyak et al. [15] in reduced whole cells and chromatophores of *Rps. sphaeroides* wild type. These magnetic field effects on the fluorescence have been described by the radical pair mechanism; (ii) the magnetic field effects on the fluorescence of isolated reaction centers (Refs. 4 and 26 and the present work). These are effects typified by *Rps. sphaeroides* R-26 [4,26] and are also described by the radical pair mechanism; (iii) those magnetic field effects observed using carotenoid excitation, which exhibit no redox dependence and are best described by a carotenoid singlet heterofission model (Refs. 5 and 6 and the present work).

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