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Magnetic-field effects in photosynthetic bacteria. II. Formation of triplet states in the reaction center and the antenna of *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides*. Magnetic-field effects

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In cells, chromatophores, reaction centers and antenna complexes of various strains of *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides*, we determined by absorbance difference spectroscopy the triplet yield, ϕ_T, induced by a laser flash, as a function of the strength of a magnetic field, the redox state of the reaction center, the temperature and the excitation and detection wavelength. Three categories of triplet formation can be distinguished. Category I is caused by the charge recombination of the triplet state of the radical pair in the reaction center. The triplet yield decreases in a magnetic field and increases upon cooling. The magnetic field dependence of ϕ_T is complementary to that of the magnetic-field-induced bacteriochlorophyll emission change associated with the reaction center (see preceding paper: Kingma, H., Van Grondelle, R. and Duysens, L.N.M. (1985) Biochim. Biophys. Acta 808, 363–382) and is discussed in terms of the radical pair mechanism. Category II is formed in the antenna complex of the carotenoid-containing purple bacteria. The triplet yield depends on the strength of a magnetic field, but does not vary with the temperature between 100 and 300 K. The triplet is formed only upon direct carotenoid excitation and is not observed in reaction centers. The triplet formation is closely related to the antenna-associated magnetic-

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Abbreviations: A, absorbance, ABChl, antenna bacteriochlorophyll; ACar, antenna carotenoid; BChl, bacteriochlorophyll; BPh, bacteriopheophytin; B-800, B-850 and B-880, 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, P-880, 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.

field-dependent emission (see the above-mentioned preceding paper) and is discussed in terms of the process of singlet fission of the excited carotenoid singlet state into a triplet pair. Homofission, involving two carotenoid molecules, explains all the pertinent data in *Rps. sphaeroides*, while heterofission might account for the observed magnetic-field effects in the antenna complex of *R. rubrum*. The triplet of the third category is formed upon both carotenoid and bacteriochlorophyll excitation in the antenna of all bacteria studied. The yield in antenna complexes does not depend on the temperature. No direct effect of a magnetic field upon the triplet yield is observed. Triplet formation in this category is probably due to intersystem crossing of bacteriochlorophyll, followed by triplet energy transfer to an antenna carotenoid molecule, if present. Upon carotenoid excitation of reduced cells or chromatophores, a mixture of all three categories is observed.

Introduction

Several years ago Blankenship et al. [2] showed that the yield of the triplet state (ϕ_T) that is formed in reduced reaction centers of *Rhodopseudomonas sphaeroides* R26 depends on the presence of a magnetic field. Qualitatively similar results were obtained in reduced reaction centers, chromatophores and cells of *Rps. sphaeroides* 2.4.1 and *R. rubrum* [3,4]. The process of triplet formation and the magnetic-field-dependent triplet yield were discussed in terms of the so-called radical pair mechanism [5–7].

In addition to a reaction center carotenoid triplet state ($RCar^T$), in several purple bacteria an antenna carotenoid triplet state ($ACar^T$) can be formed [4,11,12]. One of the possible mechanisms of $ACar^T$-formation is by intersystem crossing of an excited antenna bacteriochlorophyll (ABChl*) to an antenna BChl triplet state ($ABChl^T$), followed by triplet energy transfer from $ABChl^T$ to $ACar^T$.

A few years ago, Rademaker et al. [4] discovered a magnetic-field-induced decrease of about 40% of the $ACar^T$ yield upon excitation in whole cells of *R. rubrum* S1. In the preceding paper [1] we showed that in oxidized cells or chromatophores and in the B880 antenna complex of *R.*

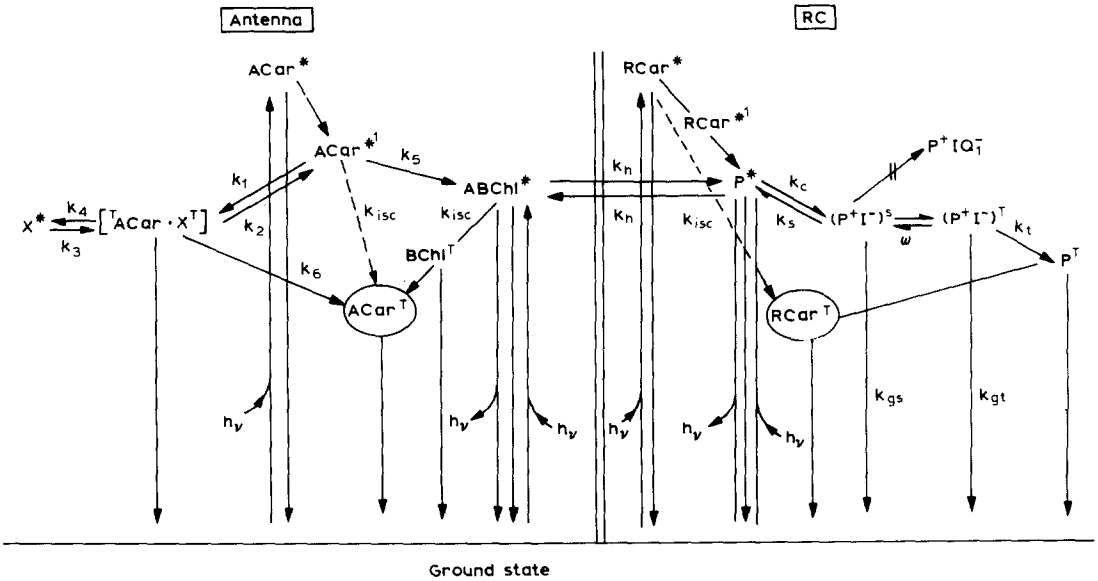


Fig. 1. Scheme of energy transfer in a carotenoid-containing purple bacterium with Q_1 reduced. ACar*′ represents the lowest excited singlet state of a carotenoid molecule. Transitions to this state from the ground state ACar are not allowed. The energy of ACar*′ is for β-carotene approx. 25% lower than for the allowed transition [37]. See text and preceding paper [1] for further explanation.

rubrum S1, a magnetic-field-induced emission charge occurs upon antenna carotenoid excitation. In several photosynthetic strains of *Rps. sphaeroides* [12–15] similar phenomena were observed. As a possible explanation for the antenna-associated magnetic-field-dependent triplet yield and emission, the process of singlet fission has been proposed. Rademaker et al. [4] suggested as an explanation of such phenomena that fission of ACar* forms a triplet pair (^{T}ACar.X^{T}) (for a discussion of this process, see Refs. 1, 15 and 16), where X is an unspecified adjacent molecule (see Fig. 1).

In the preceding paper [1], we have shown that the observed magnetic-field-dependent emission has to be discussed in terms of both an antenna-associated and a reaction-center-associated process, which can be distinguished on the basis of the excitation wavelength, the redox state of the reaction center and the observed $H_{1/2}$ value. In this paper, we show that the magnetic-field-dependent triplet yield associated with the light-harvesting antenna exists in all purple bacteria examined and is well correlated with magnetic-field-dependent emission observed under the same conditions. Reaction center and antenna-associated magnetic-field-dependent triplet yield can be separated on a similar basis as the magnetic-field-dependent emission. There is a third path of triplet formation, i.e., intersystem crossing of BChl*, of which the intrinsic yield probably does not depend on the strength of a magnetic field.

Materials and Methods

The growth of *R. rubrum* S1 (wild type), *R. rubrum* FR1 VI, *Rps. sphaeroides* G1C and *Rps. sphaeroides* R26 and the isolation of the various preparations is described in the preceding paper [1]. Reduced samples were prepared by adding 1 mg/ml $Na_2S_2O_3$ (5 mM) and oxidized samples were prepared by adding $K_3Fe(CN)_6$ up to a final concentration of 1–2 mM. For measurements at low temperature glycerol (60% v/v) and 0.5 M sucrose were present to prevent crystallization upon cooling. The absorbance changes were measured in a single beam spectrophotometer described elsewhere [17] designed to measure laser flash-induced absorbance changes with a time resolution of $3 \cdot 10^{-8}$ s as a function of the strength of a magnetic field, which was sinusoidally modulated with a frequency of 50 Hz. Samples placed between the poles of the magnet were excited by a frequency-doubled Nd-YAG laser ($\lambda = 532$ nm; pulse width, 30 ns; maximum energy, 0.2 J), by a home-built tunable dye laser using rhodamine 6G or rhodamine B ($\lambda = 560$–620 nm; pulse width, 15 ns; maximum energy, 0.1 J) pumped by the Nd-YAG laser, or by a xenon flash-pumped Phase-R dye laser system using coumarin 503 ($\lambda = 490$–510 nm; pulse width, 0.2 μs; maximum energy, 10 mJ). The laser beam passed through a transparent glass plate and the laser energy was determined by four silicon photodiodes placed at the four edges of the glass plate by measuring the scattered laser light. The photodiode currents were electronically summed and integrated, and via an 8-bit a.d.-converter stored in an LSI-11 local computer system.

The measuring light pulse (3 ms), the excitation pulse and the magnetic field were phase-locked. The chopped measuring light, provided by a 250 W tungsten-iodine lamp, reached the sample after passing through narrow-band interference filters (Schott AL or Baltzers B40). The transmitted light was detected by an S20-type photomultiplier through a Bausch and Lomb monochromator (halfwidth, 4.8 nm) and addtional narrow-band interference filters or absorbance filters, to prevent scattered laser light and fluorescence from reaching the photomultiplier. The photomultiplier was connected with a current-voltage converter and a differential amplifier to a Biomation 8100 transient recorder (sample rate 100 MHz), used in the pretrigger mode. If the laser energy detected was found to be within the selected energy window (deviation from linearity less than 2%), the memory of the transient recorder was stored in the LSI buffer and averaged, if required. The LSI buffer content was stored on magnetic tape or read out to the central PDP 11-44 computer system for further data processing. The temperature was controlled using a cuvette with an optical path length of 1 mm [17], in which a heating electrode was mounted. Cold nitrogen flowed through the cuvette walls. The temperature could be controlled within 1 K by a feedback system. If not indicated otherwise the temperature was kept constant at 290 K.

Results and Interpretation

In cells/chromatophores of several strains of the photosynthetic bacteria *R. rubrum* and *Rps. sphaeroides*, triplet states are formed within the time resolution of the measurements (30 ns) upon excitation with a laser flash, under various redox conditions. In the carotenoidless mutants *R. rubrum* FR1 VI and *Rps. sphaeroides* R26 under reducing conditions, a state has been observed which at room temperature decays within 9 and 6 μs, respectively, and which can be ascribed to a reaction center bacteriochlorophyll triplet state ($RBChl^T$) [11,18,19]. In the carotenoid-containing strains *R. rubrum* S1, *Rps. sphaeroides* 2.4.1 and G1C a state is observed which decays within 1–10 μs, that has been attributed to a reaction center carotenoid triplet state ($RCar^T$) [4,8,11,18]. Triplet states are also observed in reduced reaction centers and these most likely arise from charge recombination of the radical pair state $(P\text{-}880^+I^-)^T$ followed by triplet energy transfer in carotenoid-containing strains [8,18]. With saturating flashes, the amount of triplets formed is equal to the amount of reaction centers in the state P-880 Q_1^-; the yield decreases in a magnetic field and increases upon cooling. These triplets will be described in the next section, which deals with experiments performed on isolated reaction centers.

A second mechanism of triplet formation occurs in antenna complexes and, as will be discussed below, involves intersystem crossing, triplet energy transfer and fission/fusion processes in the antenna. The yield of these triplets does not depend significantly on the temperature between 293 and 77 K, and the magnetic-field-dependent triplet yield is only found upon direct carotenoid excitation. These triplets will be described in the subsequent section which deals with the experiments performed on isolated antenna complexes and oxidized cells/chromatophores.

The Section Triplet Formation in Reduced Cells/Chromatophores discusses the triplet formation in reduced cells and chromatophores, in which both reaction center (Section Triplet Formation in Isolated Reaction Centers) and antenna (the subsequent Section) triplets are formed.

Triplet formation in isolated reaction centers

R. rubrum S1

Fig. 2A shows the absorbance difference spectra of the 3.2 μs decaying component in reduced reaction centers of *R. rubrum* S1 upon 600 nm (●) and 532 nm (○) excitation, and these spectra are identical to those reported earlier [8]. The absorbance increases at 585 and 430 nm can be attributed to the formation of spirilloxanthin triplet bands. The absorbance decreases at 485 and 505 nm reflect the bleaching of the singlet absorption bands of spirilloxanthin [4,8,20]. The 430 nm absorbance increase is possibly only present if the spirilloxanthin is in a 15-*cis* configuration [21,22].

Fig. 2B shows the $RCar^T$ yield measured at 430 nm as a function of the laser pulse energy, which saturated when about 50 photons were absorbed per reaction center. Using the fact that each reaction center contains one carotenoid molecule [23,24], we calculate $\Delta\varepsilon_{430} = 63\ mM^{-1} \cdot cm^{-1}$ and in a similar way $\Delta\varepsilon_{585} = 41\ mM^{-1} \cdot cm^{-1}$ from the plateau values assuming that each reaction center carotenoid can be converted into a triplet state. The maximum molar extinction coefficient of spirilloxanthin triplet-minus-singlet in cyclohexane (at 560 nm) is $43\ mM^{-1} \cdot cm^{-1}$ [20], and this agrees well with the above value.

The quantum yield of $RCar^T$ measured with a weak laser flash (less than 0.1 $RCar^T$ per reaction center) was calculated to be 0.13. In 'old' reaction centers (stored for more than 6 days at −40°C), quantum yields varying between 0.31 and 0.43 were obtained, in line with similar observations by Wasielewski (Wasielewski, M.R., personal communication) and the wide range of quantum yields reported [4,8,18]. The quantum yield of $RCar^T$ formed by direct carotenoid excitation was about the same as by direct BChl excitation, which indicates that, if the $RCar^T$ is formed via $(P\text{-}880^+I^-)^T$ recombination, the efficiency of transfer of singlet energy from RCar* to P-880 is close to 1, in contrast with the energy-transfer efficiency of ACar* to antenna BChl of 0.3 (Refs. 4 and 25; see also below).

Fig. 2C shows the relative magnetic-field-dependent triplet yield decrease, $\{\phi_T(H) - \phi_T(H = 0)\}/\phi_T(H = 0)$, as a function of the magnetic-field strength in reduced reaction centers of *R. rubrum*

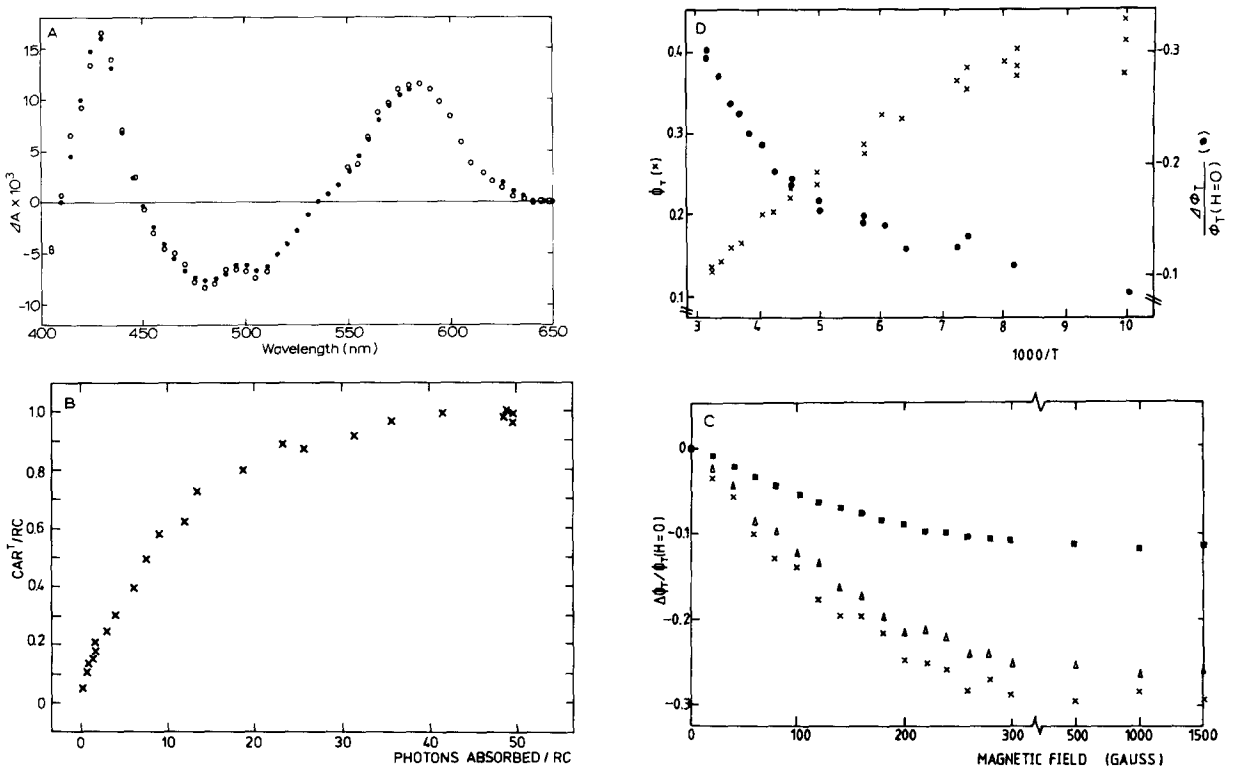


Fig. 2. (A) Spectrum of the absorbance change ($\tau = 3.2$ μs) in reaction centers (RC) of *R. rubrum* S1, induced by a non-saturating 600 nm laser flash. Optical pathlength, 1 mm; $A_{865-960} = 0.29$; 5 mM dithionite added; (●), 600 nm excitation; (○), 532 nm excitation. (B) Relative Car^T yield in reaction centers of *R. rubrum* S1, detected at 430 nm as a function of the laser energy ($\lambda = 600$ nm). Conditions as in Fig. 2A. (C) Relative magnetic-field-dependent triplet yield in reaction centers of *R. rubrum* S1 detected at 430 nm as a function of the magnetic-field strength, induced by a 600 nm laser flash resulting in 0.2 (×), 0.3 (△) and 1.0 (■) $RCar^T$ per reaction center in zero field. Conditions as in Fig. 2A. (D) $RCar^T$ yield ϕ_T (×) and relative magnetic-field-dependent triplet yield (○) in reaction centers of *R. rubrum* S1, induced by a weak 600 nm laser flash, detected at 430 nm as a function of the temperature. Conditions as in Fig. 2A.

S1, detected at 430 nm upon 600 nm excitation. Three saturation curves are shown, using excitation energies that resulted in 0.1 (×), 0.3 (△) and 1.0 (■) $RCar^T$ per reaction center in zero field ($\Delta\varepsilon_{430} = 63$ $mM^{-1} \cdot cm^{-1}$). Similar results were obtained upon 532 nm excitation. The $H_{1/2}$ value, i.e., the magnetic-field strength at which the magnetic-field-induced change in the triplet yield is half maximal, is about 90 gauss in all cases, in good agreement with the $H_{1/2}$ value of 100 gauss of the magnetic-field-dependent emission observed in reduced reaction centers of *R. rubrum* S1 [1]. The amplitude of the relative magnetic-field-dependent triplet yield decreased with increasing excitation light intensity, in agreement with similar observations by Schenck et al. [18] in reaction centers of *R. rubrum* S1, but in contrast with the relative magnetic-field-dependent triplet yield in reduced chromatophores at various pulse intensities (see below). The magnetic field effect on the $RCar^T$ yield is partly undone by using high laser pulse intensities, probably due to saturation effects.

Fig. 2D shows the $RCar^T$ yield as a function of the temperature in reduced reaction centers of *R. rubrum* S1 (×). In old reaction centers the quantum yield increases from 0.4 to 0.8 upon cooling from 293 K to 100 K (not shown). The decay rate of the $RCar^T$ did not depend on the temperature over the temperature range studied. Part of the increase of the $RCar^T$ yield upon cooling may be due to band sharpening. The closed circles (■)

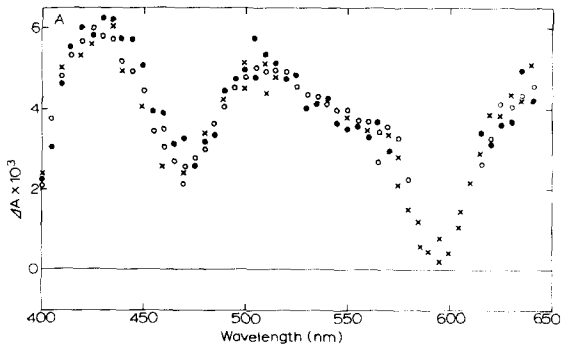
show the relative magnetic-field-dependent triplet yield, $\Delta\phi_T/\phi_T(H=0)$ in reaction centers as a function of the temperature. The absolute magnetic-field-dependent triplet yield, $\Delta\phi_T$, did not vary significantly with temperature, in agreement with Schenck et al. [26]. Therefore, the temperature dependence of the relative magnetic-field-dependent triplet yield is the inverse of the temperature dependence of ϕ_T. The $H_{1/2}$ value of the saturation curve did not depend on the temperature, again in agreement with the observation that the $H_{1/2}$ value of the magnetic-field-dependent emission was independent of the temperature [1].

Rps. sphaeroides G1C and R26

In contrast with *R. rubrum,* the absorbance changes observed in reduced reaction centers of *Rps. sphaeroides* G1C, induced by a 600 nm dye laser flash, showed a two-exponential decay.

Fig. 3A shows the absorbance difference spectrum of the 80 μs component (○,×), which is very similar to the BChl triplet-minus-singlet spectrum found in reduced chromatophores [11] and in reduced reaction centers of the carotenoidless mutant *Rps. sphaeroides* R26 (●).

The quantum yield of the 80 μs component in reaction centers of *Rps. sphaeroides* G1C was calculated from the absorbance change at 430 nm induced by a weak dye laser flash (less than 0.1 triplet/reaction center) using $\Delta\varepsilon_{430} = 65\ mM^{-1} \cdot cm^{-1}$ [18,26,27]. Under reducing conditions the quantum yield was calculated to be 0.03 in reaction centers of *Rsp. sphaeroides* G1C and 0.14 in reaction centers of *Rps. sphaeroides* R26. For several reasons we believe that the 80 μs decay component in reaction centers of G1C reflects a



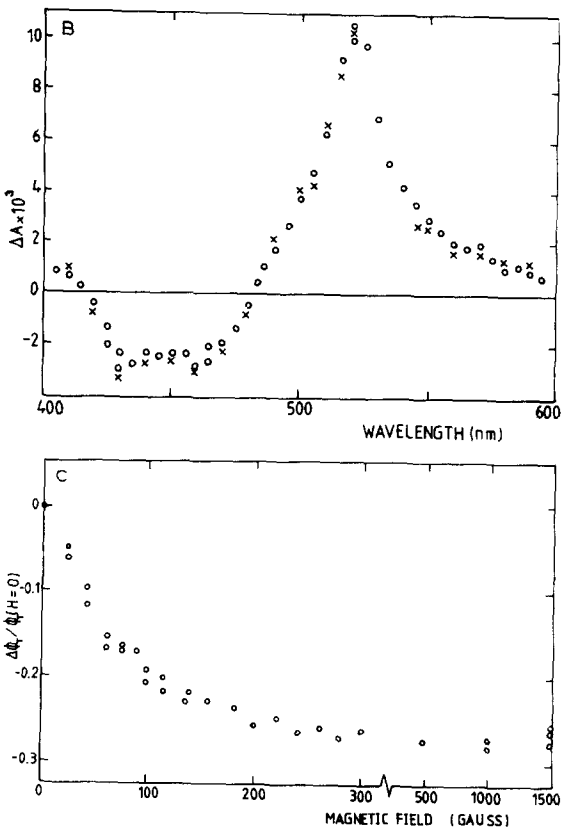


Fig. 3. (A) Absorbance difference spectra induced by a non-saturating laser flash in reduced reaction centers. (a) Spectrum of the 80 μs component in *Rps. sphaeroides* G1C upon 600 nm (○) and 532 nm excitation (×). (b) Spectrum of the 6 μs component in *Rps. sphaeroides* R26 upon 600 nm excitation (●). The spectra are normalized at 515 nm, 5 mM dithionite present. (B) Spectrum of the 9 μs component of the absorbance change in reaction centers of *Rps. sphaeroides* G1C induced by a weak laser flash. Optical pathlength, 1 mm; $A_{865-960} = 0.56$; 5 mM dithionite added; (○), 600 nm excitation; (×), 532 nm excitation; spectrum scaled to the 600 nm laser flash-induced spectrum. (C) Relative magnetic-field-dependent triplet yield in reaction centers of *Rps. sphaeroides* G1C detected at 520 nm as a function of the magnetic-field strength, induced by a 600 nm laser flash resulting in 0.09 $RCar^T$ per reaction center in zero field. Conditions as in Fig. 3B.

$RBChl^T$ state and not a free $BChl^T$ state: (i) the spectrum is identical to that of the $BChl^T$ reaction center state in reaction centers of R26; (ii) under intermediate redox conditions no triplet state was observed in reaction centers of *Rps. sphaeroides* R26 and G1C; (iii) the $BChl^T$ yield decreased by about 22% ($H_{1/2} = 50$ G) in reaction centers of

Rps. sphaeroides R26 and by about 25% ($H_{1/2}$ = 65 G) in reaction centers of *Rps. sphaeroides* G1C in a magnetic field of 1500 G; (iv) the $BChl^T$ yield showed a similar temperature dependence as the $RCar^T$ yield (see below). An explanation may be that the $RBChl^T$ in G1C reaction centers reflects a population of reaction centers that either have lost their reaction center carotenoid or in which transfer of the triplet energy from $RBChl^T$ to carotenoid is not possible.

Fig. 3B shows the absorbance difference spectra of the 9 μs component in reduced reaction centers of *Rps. sphaeroides* G1C upon 600 and 532 nm excitation. The maximum near 520 nm can be ascribed to Car^T absorption [20] and the minima near 430 and 460 nm to the peaks in the absorbance spectrum of neurosporene, the major carotenoid in G1C. The spectrum is very similar to the carotenoid triplet-minus-singlet difference spectrum observed in reduced reaction centers of *Rps. sphaeroides* GA [8] although the main carotenoid associated with the reaction center in this bacterium is chloroxanthin and not neurosporene. These two carotenoids, however, are only slightly different, because neurosporene, a precursor of chloroxanthin in the carotenoid synthesis, is converted into chloroxanthin by a single hydration step only [28].

The maximum yield of the 80 μs component, $P\text{-}880^T$, was about 0.4 triplet per reaction center using a saturating 600 nm dye laser flash. If we assume that these triplets are formed in reaction centers lacking a reaction center carotenoid molecule and that the remaining 60% of the reaction centers is able to generate a Car^T, the molar extinction coefficient $\Delta\varepsilon_{520}$ is calculated to be 26 $mM^{-1} \cdot cm^{-1}$ from the maximum 520 nm absorbance change. The quantum yield of $RCar^T$ formation measured in a weak 600 nm flash is then calculated to be 0.15. Upon cooling the $RCar^T$ yield increased to 0.46 at 100 K and the $RBChl^T$ yield from 0.03 to 0.15 at 100 K. It must be noted, however, that the ratio of the yields of $RCar^T$ and $RBChl^T \approx 0.15/0.03 = 5$ does not agree with the ratio of the fraction of reaction centers with a reaction center carotenoid and those, which lack triplet energy transfer to reaction center carotenoid ($0.6/0.4 = 1.5$). The $RBChl^T$ yield in *Rps. sphaeroides* R26 increased upon cooling from 0.14 at 293 to 0.4 at 100 K.

Fig. 3C shows the relative magnetic-field-induced change in the triplet yield as a function of the magnetic-field strength in reduced G1C reaction centers detected at 520 nm, using a weak 600 nm excitation flash. As with the magnetic-field-dependent emission in reduced rection centers [1] a $H_{1/2}$ value of about 60 G is observed for both the $RBChl^T$ and the $RCar^T$. The relative magnetic-field-dependent triplet yield decreased upon cooling to about 5% at 100 K and $H_{1/2}$ remained about the same (55 G, data not shown).

Triplet formation in oxidized cells / chromatophores and isolated antenna complexes

In oxidized cells and chromatophores and antenna complexes of all purple bacteria studied, the yield for the formation of the triplet states did not depend on the temperature between 100 and 293 K, regardless the excitation wavelength. In addition, the $ABChl^T$ and the $ACar^T$ yield, upon direct BChl excitation, did not depend on the magnetic-field strength within the accuracy of the measurements (approx. 2% of the triplet yield).

R. rubrum S1

Fig. 4A shows the absorbance difference spectrum of the 1.8 μs decay component in the B-880 complex of *R. rubrum* S1 in the presence of 1 mM $K_3Fe(CN)_6$ upon 600 nm (×) and 532 nm (○) excitation. Similar results were obtained in oxidized cells and chromatophores (see also Ref. 4). The spectrum of the $ACar^T$ in the B-880 complex did not depend on the redox compounds added (ferricyanide or dithionite), but the decay time increased to 3.6 μs upon addition of 5 mM dithionite or after flushing with nitrogen. The spectrum of Fig. 4A is very similar to the spirilloxanthin triplet-minus-singlet spectrum in cyclohexane [28] and resembles the spectrum of Fig. 2A measured in isolated reaction centers, apart from the absence of the absorbance increase at 430 nm. It appears that the 430 nm absorbance increase is associated only with the Car^T formation in the reaction center (see the next section).

Fig. 4B shows the magnetic field dependence of the relative magnetic-field-dependent triplet yield in the oxidized B-880 complex (○) of *R. rubrum*

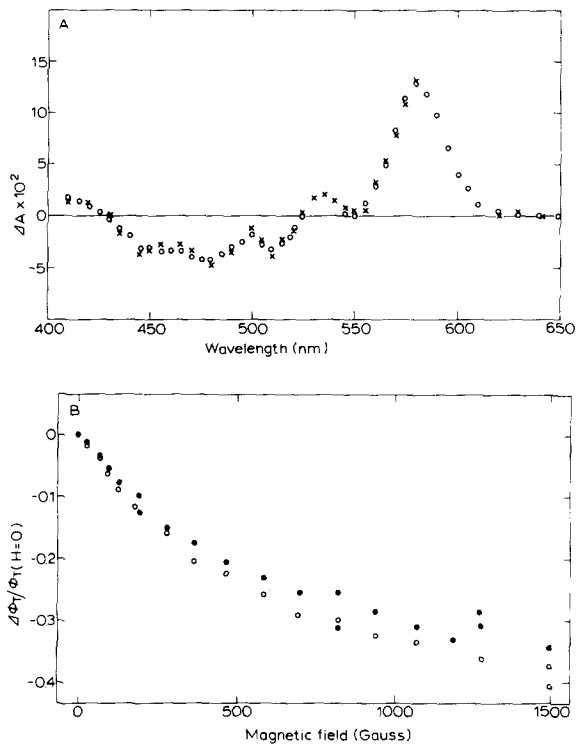


Fig. 4. (A) Spectrum of the absorbance change ($\tau = 1.8$ μs) in the B-880 complex of *R. rubrum* S1, induced by a laser flash. Optical pathlength, 1 mm; $A_{880-960} = 0.52$, 1 mM $K_3Fe(CN)_6$ added. (×), 600 nm excitation; (○), 532 nm excitation; spectrum scaled to the 600 nm laser flash-induced spectrum. (B) Relative magnetic-field-dependent triplet yield in the B-880 complex (○) and in oxidized chromatophores (●) of *R. rubrum* S1, detected at 580 nm as a function of the magnetic field strength, induced by a 532 nm non-saturating laser flash. $A_{880-960} = 0.57$ (●) and0.52 (○). Other conditions as in Fig. 4A.

S1 upon carotenoid excitation at 532 nm and detected at 580 nm. Similar results were obtained in oxidized chromatophores (●).

The $H_{1/2}$ values of more than 400 G and the shape of the saturation curves of the magnetic field-dependent triplet yield are very similar to those observed for the magnetic-field-dependent emission in the same samples [1]. In contrast with Rademaker et al. [4] we did not observe an initial threshold of 200 G, either with the magnetic-field-dependent emission [1] or with the magnetic-field-dependent triplet yield. The use of large stationary magnetic fields by Rademaker et al. [4] may have introduced a shift of the abscissa due to residual magnetism, which is absent in the experiments described here, because a 50 Hz modulated magnetic field was used to determine magnetic-field-dependent triplet yield and emission. The amplitude of the relative magnetic-field-dependent triplet yield did not depend on the excitation energy between 0.1 and 13 photons absorbed per carotenoid molecule.

By exciting at different wavelengths, we rouhgly determined the excitation spectrum of the relative magnetic-field-dependent triplet yield between 560 and 620 nm. Within the resolution of the experiment, the spectrum followed the spirilloxanthin absorbance spectrum, but not the BChl absorption band at 590 nm (data not shown). Excitation light above 580 nm did not result in a magnetic-field-dependent triplet yield. The ratio $\Delta\phi_T/\phi_T(H = 0)$ was maximum upon direct antenna carotenoid excitation. The quantum yield of the triplet formation depended on the excitation wavelength. With 532 nm excitation a quantum yield of 0.3 was calculated using $\Delta\varepsilon_{580} = 41\ mM^{-1} \cdot cm^{-1}$ and with 600 nm excitation the quantum yield was about 0.2, in good agreement with Rademaker et al. [4].

In chromatophores of the carotenoidless mutant *R. rubrum* FR1 VI $ABChl^T$ is formed under oxidizing conditions with a quantum yield of 0.17 upon 600 nm excitation. The absorbance difference spectrum was similar to the one shown in Fig. 3A and the absorbance change decayed with a single exponent with $\tau = 60$ μs (data not shown). Regardless the wavelength of excitation no magnetic-field-dependent triplet yield was observed. Similar results were obtained in oxidized chromatophores of *Rps. sphaeroides* R26 (see Discussion and Table I).

The presence of a magnetic-field-dependent triplet yield upon antenna carotenoid excitation in *R. rubrum* S1 and the absence of a magnetic-field-dependent triplet yield upon bacteriochlorophyll excitation in *R. rubrum* FR1 VI are in agreement with the results presented in the preceding paper [1] concerning the antenna-associated magnetic-field-dependent emission. Upon 600 nm excitation the $ACar^T$ is formed by intersystem crossing via $ABChl^T$, but upon 532 nm excitation the $ACar^T$ is partly formed by a magnetic-field-dependent fission from ACar*. If we assume that about 30% of

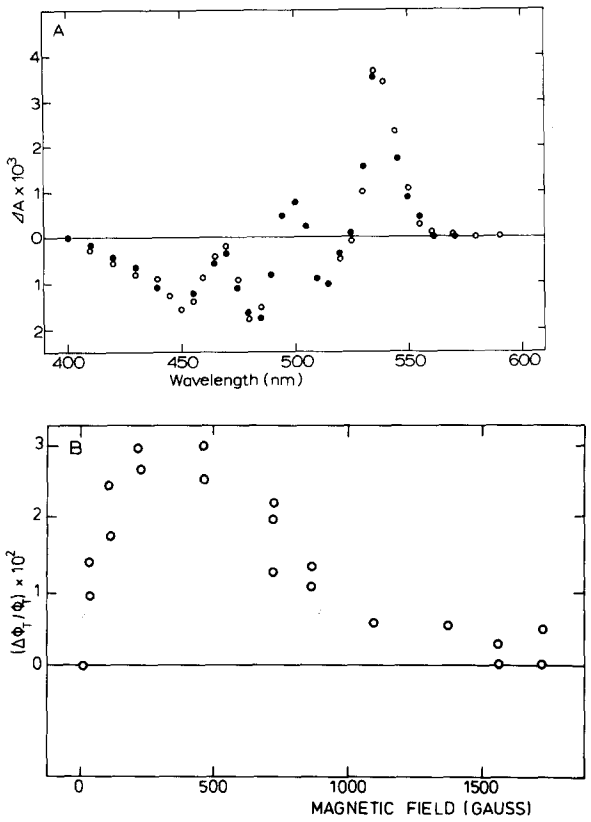


Fig. 5. (A) Spectrum of the absorbance change ($\tau = 6$ μs) in the B-800–850 complex of *Rps. sphaeroides* 2.4.1, induced by a laser flash. Optical pathlength, 1 mm. $A_{850-960} = 0.5$, 1 mM $K_3Fe(CN)_6$ added. (●), 600 nm excitation; (○), 495 nm excitation. (B) The relative magnetic-field-dependent triplet yield in the B-800–850 complex of *Rps. sphaeroides* 2.4.1, detected at 535 nm, as a function of the magnetic-field strength. Conditions as in Fig. 5A. Excitation at 495 nm.

the excitations absorbed at 532 nm are transferred to antenna bacteriochlorophyll [25], the remaining excitations are calculated to give $ACar^T$ formation via fission with a yield of 0.34.

Rsp. sphaeroides

Fig. 5A shows the absorbance difference spectrum of the 6 μs component observed upon 495 nm (○) and 600 nm (●) excitation in the B-800–850 complex of *Rps. sphaeroides* 2.4.1. The spectrum shows a maximum absorbance increase at 540 nm, which can be ascribed to triplet-triplet absorption by sphaeroidene, and two minima at 450 and 480 nm, which are due to the bleaching of the singlet absorption bands of sphaeroidene [20,28]. The quantum yield of triplet formation is calculated to be 1.8% upon 495 nm excitation and 1.9% upon 595 nm excitation ($\Delta\varepsilon_{540} = 29\ mM^{-1} \cdot cm^{-1}$ [20]), which is surprisingly low compared to the values observed in *R. rubrum.*

Fig. 5B shows the relative magnetic-field-dependent triplet yield change as a function of the magnetic field strength upon 495 nm excitation and detected at 540 nm in the B-800–850 complex of *Rps. sphaeroides* 2.4.1. The maximum relative magnetic-field-dependent triplet yield change observed at $H \approx 250$ G is about 3%, which is very low as compared to the relative magnetic-field-dependent triplet yield change in the B-880 complex of *R. rubrum* S1 (40%). At low-magnetic-field

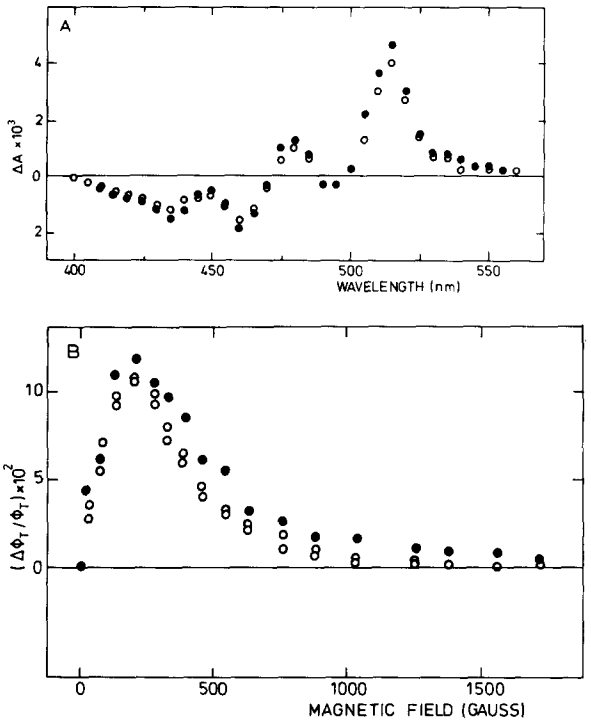


Fig. 6. (A) Spectrum of the absorbance change ($\tau = 6.4$ μs) in the B-800–850 complex of *Rps. sphaeroides* G1C, induced by a laser flash. Optical pathlength, 1 mm; $A_{850-960} = 0.72$; 1 mM $K_3Fe(CN)_6$ added. (●), 595 nm excitation; (○), 495 nm excitation. (B) Relative magnetic-field-dependent triplet yield in the B-800–850 complex (○) and in oxidized cells (●) of *Rps. sphaeroides* G1C, detected at 515 nm, as a function of the magnetic-field strength. Excitation at 495 nm. Conditions as in Fig. 6A.

strengths an increased triplet yield in a magnetic field is observed, in contrast with the large decrease observed in *R. rubrum*. With 600 nm excitation no magnetic-field-dependent triplet yield could be found.

Fig. 6A shows the absorbance difference spectrum of the 6.4 μs decay component observed upon 495 nm excitation (○) and 595 nm (●) excitation in the B-800–850 complex of *Rps. sphaeroides* G1C. The spectrum shows an absorbance increase at 515 nm, which is most likely due to the triplet–triplet absorption of neurosporene. Bleaching of the peaks in the neurosporene absorption spectrum are observed at 430, 460 and 495 nm. The spectrum is, however, clearly different from the neurosporene triplet–singlet spectrum observed in reaction centers (Fig. 3B), where the bleaching at 495 nm is absent. The quantum yield of $ACar^T$ formation in this complex was calculated to be 1.4% with 495 nm excitation and 1.5% with 600 nm excitation, taking $\Delta\varepsilon_{515} = 26\ mM^{-1} \cdot cm^{-1}$.

Fig. 6B shows the relative magnetic-field-dependent triplet yield as a function of the magnetic-field strength in the B-800–850 complex of *Rps. sphaeroides* G1C detected at 515 nm upon 495 nm excitation (○). As with the wild type (Fig. 5B), the magnetic-field-dependent triplet yield is only observed upon carotenoid excitation. The triplet yield in a magnetic field is higher than or equal to the triplet yield in zero field. Similar results were obtained in oxidized chromatophores/cells (Fig. 6B, (●)). The relative amplitude of the magnetic-field-dependent triplet yield did not depend significantly on the excitation energy between 0.06 and 3 photons absorbed per carotenoid molecule.

Triplet formation in reduced cells/chromatophores

R. Rubrum

Fig. 7A shows the flash-induced absorbance difference spectrum of the 3.2 μs component upon 600 nm excitation in reduced chromatophores of *R. rubrum* S1. An absorbance increase at 430 nm is observed and the spectrum is very similar to a spirilloxanthin triplet-minus-singlet spectrum. Again the 430 nm absorbance increase probably reflects the $RCar^T$ formation generated by charge recombination in the reaction center.

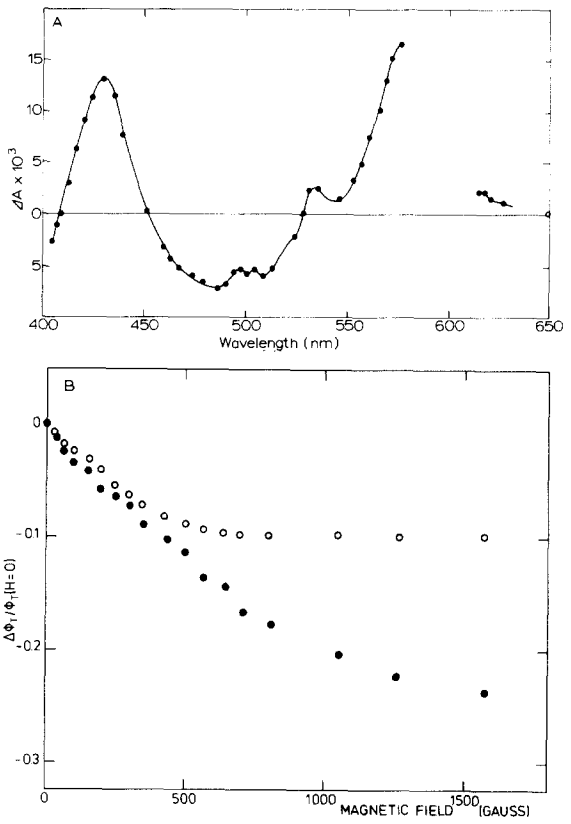


Fig. 7. (A) Spectrum of the absorbance change ($\tau = 3.2$ μs) in reduced chromatophores of *R. rubrum* S1, induced by a 600 nm laser flash. Optical pathlength, 1 mm. $A_{880-960} = 0.52$; 5 mM dithionite added. (B) Relative magnetic-field-dependent triplet yield in chromatophores of *R. rubrum* S1, as a function of the magnetic-field strength. (○), Excitation wavelength, 600 nm (0.1 Car^T per reaction center); detection wavelength, 430 nm. (●) Excitation wavelength, 432 nm (0.08 Car^T per reaction center); detection wavelength, 580 nm.

The quantum yield of triplet formation in reduced chromatophores was calculated to be 0.12 from the 430 nm absorbance change ($\Delta\varepsilon_{430} = 63\ mM^{-1} \cdot cm^{-1}$), very similar to the quantum yield observed in isolated reaction centers. The 580 nm absorbance change reflects both the $RCar^T$ and the $ACar^T$ formation (see Figs. 2A and 5A). If we assume that the molar extinction coefficient at 580 nm is the same for both the $RCar^T$ and the $ACar^T$ (both spirilloxanthin [28]), the total Car^T yield calculated from the absorbance change at 580 nm is found to be 0.24 ($\Delta\varepsilon_{580} = 41\ mM^{-1} \cdot cm^{-1}$, see Section Triplet Formation in Isolated Reaction

Centers), which results in a quantum yield of $ACar^T$ formation of 0.24–0.12 = 0.12. In chromatophores of *R. rubrum* S1 at moderate redox potentials (reaction center redox state: P-880 I Q_1) a triplet yield of 0.06 is found upon 600 nm excitation. The quantum yields of $ACar^T$ obtained for the various redox states of the reaction center, 0.2 in the state P^+ I Q_1 (see the previous Section), 0.12 in the state P I Q_1^- and 0.06 in the state P I Q_1 are in good agreement with the assumption that the $ACar^T$ yield is proportional to the relative BChl emission yield observed in these redox states, i.e., 3.4, 2.05 and 1.0, respectively (see preceding paper [1]).

In chromatophores of the carotenoidless mutant FR1 VI of *R. rubrum*, we calculated that triplet formation under reducing conditions has a quantum yield of 0.25 upon BChl excitation, by comparison with the bleaching of the 880 nm absorbance band under intermediate redox conditions (reflecting photooxidation of P-880). This is very similar to the triplet yield observed in the wild type. If we assume that the $RBChl^T$ yield in reduced chromatophores is similar to that in isolated reaction centers, i.e. 0.14, the $ABChl^T$ yield in reduced chromatophores is 0.25–0.14 = 0.11, very similar to the $ACar^T$ in the wild type (0.12). This indicates that the molar extinction coefficient used in *R. rubrum* S1, i.e. $\Delta\varepsilon_{580} = 41\ mM^{-1} \cdot cm^{-1}$, is indeed the same for $ACar^T$ and $RCar^T$. From the $BChl^T$ yield of 0.25 in *R. rubrum* FR1 VI, we calculate a molar extinction coefficient of about 16 $mM^{-1} \cdot cm^{-1}$ at 510 nm, in good agreement with earlier proposals [21].

Fig. 7B shows the relative magnetic-field-dependent triplet yield as a function of the magnetic-field strength in reduced chromatophores of *R. rubrum* S1 detected at 430 nm upon 600 nm excitation (○), and detected at 580 nm upon 532 nm excitation (●). The saturation curves are markedly different and depend both on the wavelength of excitation and detection. With 600 nm excitation an $H_{1/2}$ value of about 250 G is found regardless the wavelength of detection, but the amplitude of the relative magnetic-field-dependent triplet yield is maximum at 430 nm. With 532 nm excitation (in the carotenoid region of the absorbance spectrum) $H_{1/2}$ shifts to larger values depending on the detection wavelength: around 430 nm $H_{1/2}$ = 250 G, and at 580 nm $H_{1/2}$ exceeds 400 gauss. The $H_{1/2}$ values of 250 and 400 G correspond reasonably well with the $H_{1/2}$ values observed for the reaction-center-associated magnetic-field-dependent emission (240 gauss) and the antenna-associated magnetic-field-dependent emission (more than 400 gauss) (see the preceding paper [1]).

These experiments can be explained as follows. Upon 600 nm excitation $RCar^T$ and $ACar^T$ are formed, the latter only via intersystem crossing from ABChl*. The $RCar^T$ yield is magnetic-field-dependent ($H_{1/2} = 250$ G, $\Delta\phi_T/\phi_T \approx 0.10$) and the $RCar^T$ is the only contributor to the signal at 430 nm. Therefore, the same $H_{1/2}$ value is observed through the whole spectrum, but the relative magnetic-field-dependent triplet yield varies, being large around 430 nm and small around 580 nm. Upon 532 nm excitation additional $ACar^T$ is formed via fission of ACar*. The yield of $ACar^T$ is now also magnetic-field-dependent ($H_{1/2} \geqslant 400$ G) and therefore both $H_{1/2}$ and $\Delta\phi_T/\phi_T$ vary over the spectrum. A quantitative analysis of the magnetic-field-dependent triplet yield detected at various wavelengths was in good agreement with the extinction coefficient calculated from the absorbance difference spectra (see the two preceding sections).

Rps. sphaeroides

Fig. 8A shows the absorbance difference spectrum of the 9 μs decaying component induced by a 600 nm dye laser flash in reduced chromatophores of *Rps. sphaeroides* G1C. Apart from the absorbance increase at 550 nm, the spectrum is very similar to the absorbance difference spectrum observed in the B-800–850 complex (see Fig. 6A). Note that the increase at 550 nm is also absent in the spectrum obtained in G1C reaction centers (Fig. 3B). It may be speculated that the 550 nm absorbance increase arises from an $ACar^T$ state generated in the B-880 complex. Both the 515 nm and the 550 nm absorbance change depend on a magnetic field in the reduced chromatophores. ϕ_T detected at 515 nm showed a magnetic-field dependence similar to that observed in reduced chromatophores of *R. rubrum* S1 (Fig. 7B, (○)) with an $H_{1/2}$ value of 230 G and $\Delta\phi_{max}/\phi_T = 0.12$. In contrast to *R. rubrum*, the saturation curves do not depend on the excitation wavelength. The reason

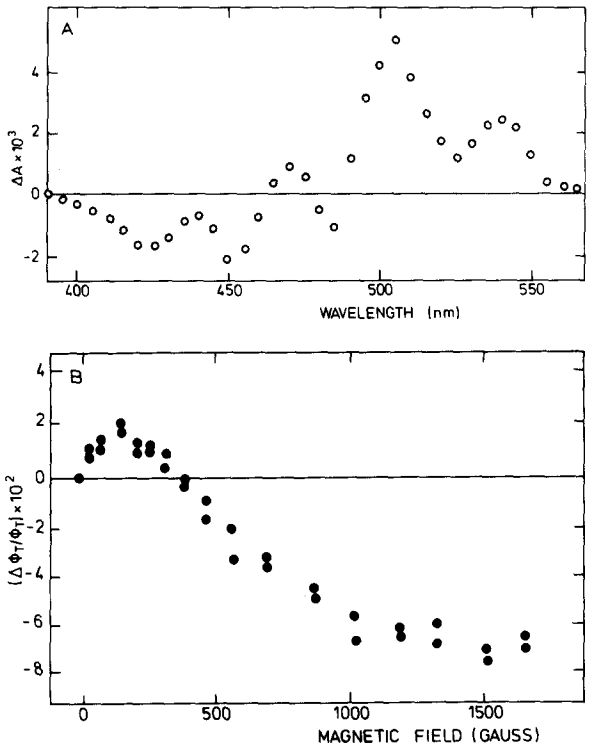


Fig. 8. (A) The spectrum of the absorbance change ($\tau = 9$ μs) in reduced chromatophores of *Rps. sphaeroides* G1C, induced by a 600 nm laser flash. Optical pathlength, 1 mm; $A_{870-960} = 0.92$, 5 mM dithionite added. (B) Relative magnetic-field-dependent triplet yield in reduced chromatophores of *Rps. sphaeroides* G1C as a function of the magnetic-field strength. Excitation wavelength, 430 nm; detection wavelength, 540 nm. Conditions as in Fig. 8A.

for this is mainly that the quantum yield of $ACar^T$ formation via singlet fission is essentially zero in zero field and maximally $1.4 \cdot 10^{-3}$ at $H = 200$ G. In addition, the quantum yield of $ACar^T$ formation via intersystem crossing is only about 1.8%. Therefore, at all wavelengths of excitation, the $RCar^T$ is the dominating species being formed and $H_{1/2}$ and $\Delta\phi_T/\phi_T$ are more or less constant.

In contrast, the shapes of the relative magnetic-field-dependent triplet yield saturation curves detected at 550 nm did depend on the excitation wavelengths (Fig. 8). With BChl excitation a curve typical for the reaction-center-associated magnetic-field-dependent triplet yield is observed, but with carotenoid excitation ($\lambda = 495$ nm) the shape of the saturation curve (Fig. 8B) reflects contributions of both the reaction-center-associated and the antenna-associated magnetic-field-dependent triplet yield. Either the 550 nm absorbance change arises predominantly from the antenna triplet formation or the magnetic-field-dependent triplet yield with this antenna carotenoid is relatively large (see also the preceding paper [1]). The difference of the maximum absorbance changes, at 515 and 550 nm, is remarkable and hard to explain. Although only one type of antenna carotenoid, neurosporene, is supposed to be present in this bacterium [29], the presence of a small amount of sphaeroidene or oxidized neurosporene could explain the data.

Discussion

Triplet formation in the reaction center

In isolated reaction centers with Q_1 in the oxidized state no triplet state is formed with a significant yield, either by intersystem crossing or by singlet fission, independent of the strength of a magnetic field up to 1500 gauss. However, in all reduced, reaction-center-containing preparations a reaction center triplet state is formed. In isolated reaction centers the triplet yield is 0.15–0.18 and decreases by 22–30% in high magnetic field. In cells and chromatophores the reaction center triplet yield is 0.12–0.15 and decreases by about 10% in a magnetic field of 1500 gauss (see Table I). The magnetic-field-dependent triplet yield shows a magnetic-field dependence complementary to that of the reaction-center-associated magnetic-field-dependent emission (see Figs. 2B and 3C of the preceding paper [1]). Therefore, in agreement with earlier reports [2,3,4,9,11,18,24], we conclude that the reaction-center triplet state is formed by charge recombination of a radical-pair triplet state. In the carotenoid-containing bacteria the $BChl^T$, probably shared between P-800 and P-880 [18,30,31], is rapidly transferred into the $RCar^T$. The decay time of the reaction center triplet state is 3–9 μs, except for the $BChl^T$ in reaction centers of *Rps. sphaeroides* G1C, which shows a remarkably long decay time of 80 μs. Such a long lifetime has been observed also in the carotenoidless mutants *R. rubrum* G9 (approx. 50 μs) and in *P. aestuarii* (approx. 80 μs [32]). That we are dealing with a reaction-center triplet state is shown by the follow-

ing observations: (i) the magnetic field dependence of the $BChl^T$ is very similar tothat of the $RCar^T$ in G1C reaction centers; (ii) the spectrum of the 80 μs component is identical to that of the 6 μs component of R26 reaction centers, which has been attributed to the formation of a $BChl^T$ state; (iii) in oxidized reaction centers no such state is observed.

The large range of quantum yields of the reaction center triplets [2–4,9–11,18,24] ranging from 0.1 to 0.6 may be explained at least partly by our observation that in reaction centers the triplet yield increases by a factor of 2–4 upon aging. In reduced cells and chromatophores of *R. rubrum* the quantum yield of reaction center triplet formation may have been overestimated because also antenna triplet states are formed. In cells and chromatophores of *Rps. sphaeroides* the latter effect can be neglected, because the antenna triplet yield is an order of magnitude smaller than the reaction center triplet yield (Table I). However, in *R. rubrum* S1 the reaction center triplet state (Figs. 2A, 4A and 7A) is estimated from the 430 nm absorbance increase to have a quantum yield of

TABLE IA

REACTION-CENTER-ASSOCIATED TRIPLET STATES

BChl excitation, reducing conditions.

	Isolated reaction centers				Cells/chromatophores			
	T (μs)	ϕ_T	$\Delta\phi_T/\phi_T$	$H_{1/2}$ (G)	T (μs)	ϕ_T	$\Delta\phi_T/\phi_T$	$H_{1/2}$ (G)
R. rubrum S1	3.2	0.13	0.3	90	3.2	0.12	0.1	250
Rps. sphaeroides G1C	9 80	0.15 0.03	0.27 0.25	60 65	9	0.15	0.1	230
R. rubrum FR1 VI					9	0.13 [a]	0.1	240
Rps. sphaeroides R26	6.0	0.14	0.22	50				

TABLE IB

ANTENNA-ASSOCIATED TRIPLET STATES

n.o., not observed.

	Magnetic-field-dependent carotenoid excitation				Magnetic-field-dependent BChl excitation	
	T (μs)	ϕ_T(max)	$\Delta\phi_T/\phi_T$	Hmax (G)	T (μs)	ϕ_T
R. rubrum S1	1.8 [b]	0.3 [b]	0.4 [b]	> 400 [b]	1.5–3.6	0.06 [c] 0.12 [d] 0.20 [b]
Rps. sphaeroides 2.4.1	6.0 [e]	$6\cdot10^{-4}$ [e]	+1.0 [e]	300 [e]	6.0 [e]	0.018–0.019 [e]
Rps. sphaeroides G1C	6.4 [f] 9 [g]	$1.4\cdot10^{-3}$ [f] ?	+1.0 [f] ?	250 [f]	6.4 [f] 9 [g]	0.014–0.015 [f] 0.07 [g]
R. rubrum FR1 VI	n.o.	n.o.	n.o.	n.o.	90 [b]	0.17 [b]
Rps. sphaeroides R26	n.o.	n.o.	n.o.	n.o.	70 [b]	0.19 [b]

[a] Assuming that the total triplet yield measured (0.25) reflects both the antenna (0.12) and reaction center triplet state (0.13).
[b] Reaction center in state P^+Q_1.
[c] Reaction center in state PQ_1.
[d] Reaction center in state PQ_1^-.
[e] B-800–850 complex.
[f] B-800–850 complex detected at 515 nm.
[g] Reduced chromatophores detected at 540 nm.

0.12, while the 580 nm absorbance change, reflecting both the antenna and the reaction center triplet, results in a quantum yield of 0.25 to 0.38 depending on the excitation wavelength.

The amplitude of the relative magnetic-field-dependent triplet yield associated with reaction center triplets is about the same for all species, about 10% in cells and chromatophores and about 25% in isolated reaction centers. This agrees with the observation that the relative reaction center-associated magnetic-field-dependent emission is about 1–2% in cells and chromatophores and about 1–3% in reaction centers [1]. In addition, the $H_{1/2}$ values of the reaction-center-associated magnetic-field-dependent triplet yield and emission are about the same. The $H_{1/2}$ values are 50–100 gauss in isolated reaction centers, but 230–250 gauss in cells and chromatophores. The absence of fast energy transfer from the reaction center back to a surrounding antenna in isolated reaction centers might increase the effective lifetime of the radical pair state and to some extent account for the low $H_{1/2}$ value observed in reaction centers [1,33]. However, the observed difference between reaction centers and cells or chromatophores seems to be much too large, and so far we have only an ad hoc explanation (see Ref. 1 for a detailed discussion).

The decrease of the relative magnetic-field-dependent triplet yield with increasing excitation energy observed in reaction centers probably reflects the occurrence of multiple hits at high pulse intensities. The decreased probability of triplet formation in a magnetic field will be partly compensated by saturation of the triplet yield, leading to a smaller magnetic-field-dependent triplet yield. In addition, at high pulse intensities (50 photons per reaction center) multiple excitation processes will occur, which could give rise to additional magnetic field effects [16]. In reduced cells and chromatophores the variation of the magnetic-field-dependent triplet yield with the strength of the magnetic field appeared to be more or less independent of the excitation energy. However, the observed magnetic-field-dependent triplet yield is relative small (up to 10%) compared to that in reaction centers (approx. 30%), and therefore a possible decrease of the relative magnetic-field-dependent triplet yield with increasing excitation energy might be less apparent.

The increase of the reaction center triplet yield in reduced reaction centers of *R. rubrum* S1 upon cooling is about the same as the corresponding increase observed in reduced cells and chromatophores (Refs. 9 and 17; Kingma, H., unpublished results) and can partly be ascribed to a decrease of the recombination rate constant k_s (Fig. 1, in Ref. 1). Below 150 K no recombination luminescence [9] or reaction-center-associated magnetic-field-dependent emission [1] is observed, which has been explained by assuming that at these temperatures no significant recombination of $(P^+I^-)^S$ to P-880* occurs. Therefore, the decrease of the magnetic-field-dependent triplet yield and the increase of ϕ_T may be related to an increase in k_c, k_t, the singlet-triplet mixing, or by a decrease in k_{gs} and/or k_{gt}. A decrease in k_{gt} as a function of the temperature was recently suggested by Schenck et al. [18] to explain the temperature dependence of ϕ_T in reaction centers of *Rps. sphaeroides* and *R. rubrum*. However, the absolute magnetic-field-dependent triplet yield is independent of the temperature, which argues against the occurrence of large changes in k_c, k_t or k_{gt} [6]. Moreover, temperature-dependent S–T mixing would probably also affect the $H_{1/2}$ value [6,7], which is contrary to our observations. According to the work of Werner et al. [6], a decrease in k_{gs} by a factor of 4–5 could result in the decrease of $\Delta\phi_T/\phi_T$ in increase of ϕ_T, while the $H_{1/2}$ value will remain more or less constant in agreement with our observations (see Ref. 7). At 100 K the reaction center triplet yield is about 50%, indicating approx. equal probabilities for P^+I^- to recombine via k_{gs} directly to the ground state P I or via k_t to $BChl^T$. At room temperature we have estimated the former probability to be about 7-times larger than the latter [33] and these numbers support the proposed substantial decrease in k_{gs} upon lowering the temperature. However, this is in disagreement with the earlier proposals [9] that a major fraction of the $P\text{-}880^+I^-$ occurs via the excited states P-880* and B-880*. This fitted for instance the observed emission yield ϕ_E as a function of the temperature well. In addition the energy scheme proposed in Ref. 9 could not be used to explain the observed variation of ϕ_T and $\Delta\phi_T/\phi_T$ with temperature. We will return to this question elsewhere (Ref. 17 and Kingma, H. and Van Grondelle, R., unpublished results).

Antenna triplet formation

The magnetic-field-dependent triplet formation in the antenna is only found upon antenna carotenoid excitation in antenna complexes and oxidized cells or chromatophores and is not observed in the carotenoidless mutants *R. rubrum* FR1 VI and *Rps. sphaeroides* R26. Thus, as for the antenna-associated magnetic field-dependent emission [1], the presence of an antenna carotenoid appears to be required. It has been argued that the absence of a spin-polarized triplet state in the antenna of *Rps. capsulata* [34] might exclude the formation of a radical-pair state, which could possibly account for the magnetic-field-dependent processes observed in the antenna [10]. However, it seems hazardous to exclude a radical-pair mechanism based upon negative evidence. In the species studied here the antenna-associated magnetic-field-dependent emission and triplet yield can be well explained by singlet fission of an excited antenna carotenoid into a triplet pair. The absence of a temperature dependence of the triplets formed by fission indicates that fusion (rate constants, k_2 and k_4) is not important.

R. rubrum S1

The magnetic field-dependent triplet yield in *R. rubrum* S1 shows a magnetic field dependence complementary to that of the antenna-associated magnetic-field-dependent emission [1]. The shapes of the curves do not permit one to distinguish between homofission, involving the triplet pair (^{T}ACar.ACar^{T}) and heterofission, e.g. (^{T}ACar.ABChl^{T}) (Ref. 16 and Geacintov, N.E. and Moore, T.A., personal communication). However, our work favors homofission for the following reasons: (i) magnetic-field-dependent triplet yield and emission are only observed upon carotenoid excitation in all species, (ii) the spirilloxanthin excited state (approx. 2.2 eV [20]) has sufficient energy to populate the (^{T}ACar.ACar^{T}) pair (approx. 1.3 eV), even if fission would take place from the lower lying, optically forbidden, carotenoid excited state at about 1.65 eV [20,37]. In contrast, the (^{T}ACar.ABChl^{T}) pair (approx. 1.65 eV (Ref. 16 and Geacintov, N.E. and Moore, T.A., personal communication)) would then be just accessible, (iii) magnetic-field-dependent emission is temperature-independent, suggesting that formation of the (^{T}ACar.ACar^{1}) triplet pair at a relatively low energy is not reversible and competes with energy transfer to antenna BChl. In the case of heterofission, once the triplet pair is formed from ACar*, fusion to ABChl* would be expected to occur, which might lead to a temperature-dependent magnetic-field-dependent emission [16]. The large difference in amplitude between magnetic-field-dependent triplet yield (40%) and emission (0.1%) is hard to explain in both schemes. Assuming homofission to occur we have to propose that in Fig. 1 not only the rate constant k_1 depends on the magnetic field, but also that the ratio k_4/k_6 varies with the magnetic field in a very similar way.

Finally, there is one argument which suggests that strong interactions between carotenoid molecules in a B-880 complex do not occur. Although the smallest B-880 complex contains 6–8 BChl *a* molecules [35] and therefore 6–8 carotenoid molecules, no carotenoid circular dichroism can be observed; this in contrast to the circular dichroism of the B-8880 complex of *Rps. sphaeroides* 2.4.1, which shows a strong conservative signal in the carotenoid region [35,36].

Rps. sphaeroides

In contrast to *R. rubrum*, both antenna complexes of *Rps. sphaeroides* G1C and 2.4.1 exhibit a magnetic-field-dependent triplet yield increase. The relative magnetic-field-dependent triplet yield is small, 3–10%, and is only observed upon antenna carotenoid excitation. No magnetic-field-dependent triplet yield is found in the carotenoidless mutant R26. If we assume that the efficiency of energy transfer from ACar* to antenna BChl exceeds 90%, the similarity of the difference spectra (Fig. 6A), the equality of the quantum yields and the absence of the magnetic field dependence upon BChl excitation can be explained by the hypothesis that the antenna carotenoid triplet in zero field is formed predominantly via intersystem crossing of BChl* (Car* → BChl* → $^{k_{isc}}$ BChl^{T} → Car^{T}). Apparently, little or no triplet state is formed by singlet fission in zero field. Together with the initial increase of the triplet yield with increasing magnetic field and the need for specific carotenoid excitation, this can be understood by assuming that the triplet pair consists of two identical caro-

tenoid molecules (sphaeroidene in *Rps. sphaeroides* 2.4.1 and neurosporene in *Rps. sphaeroides* G1C) with a special mutual oritentation [16]. Recent observations by Kramer et al. [35] showed a strong conservative antenna carotenoid circular dichroism spectrum in the B-800–850 complex of *Rps. sphaeroides* 2.4.1, which suggests that the antenna carotenoids in this bacterium occur in pairs. The shape of the curves shown in Figs. 5B and 6B can then be explained as follows: in zero field a triplet cannot be formed by homofission, because fission is a spin-conserving process and the nine possible substates of the triplet pair are now pure quintet, triplet and mixed singlet-quinted states. As the magnetic field increases, the substates will be mixed and there will be substates with a mixed singlet-triplet character, resulting in triplet formation. At high fields the rate constants of fission decrease due to a lower number of substates with some singlet character. The triplet yield in zero field will therefore be equal to or lower than that in the presence of a magnetic field.

Heterofission cannot account for the observed magnetic field dependence of the magnetic-field-dependent triplet yield, because then the triplet pair state can have triplet character even in zero field, and a single triplet state would be formed [16].

Acknowledgements

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