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Triplet–triplet energy transfer in B800–850 light-harvesting complexes of photosynthetic bacteria and synthetic carotenoporphyrin molecules investigated by electron spin resonance

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Triplet state electron spin resonance (ESR) spectroscopy has been used to study triplet–triplet energy transfer in B800–850 light-harvesting complexes and carotenoporphyrin molecules. The B800–850 complexes were isolated from the photosynthetic bacteria *Rhodobacter sphaeroides* GA, aerobically and anaerobically grown *Rb. sphaeroides* wild type and *Rhodopseudomonas acidophila* 7750. Free-base and zinc-substituted carotenoporphyrins featuring various linkage structures and different orientations of the pigments were studied. The carotenoporphyrins which contain a short bridging link display ESR spectra which resemble those of the B800–850 complexes. The results indicate a close spatial proximity between the bacteriochlorophyll and carotenoid pigments in the B800–850 complexes which leads to efficient triplet-triplet energy transfer. Computer simulations of the observed ESR spectral line-shapes yielded values for the zero-field splittings which can be understood in terms of the varying extents of π-electron conjugation in the carotenoids. The rate constants for population and decay of the observed triplet states were also obtained from the computer simulations. All of the carotenoid triplet states exhibit similar ESR spectral lineshapes and are characterized by the spin polarization pattern *eae aea*. The molecular basis for the spectral uniformity may be explained by triplet energy transfer according to the exchange mechanism and conformational changes of the carotenoid which lead to triplet spin relaxation.

Introduction

The antenna systems of purple photosynthetic bacteria are comprised of discrete pigment-protein complexes in which non-covalently bound bacteriochlorophyll (BChl) and carotenoid molecules are capable of transferring excitation energy to the reaction center [1]. One such complex, denoted B800–850 for its approximate wavelengths of maximum absorption in the near infrared spectral region, makes up a large part of the antenna system in several photosynthetic bacteria [2]. Excitation transfer between the pigments in the B800–850 complex has been studied extensively by optical spectroscopic methods [3]. Several of these studies have focussed on the participation of carotenoids which are known to act as light harvesting molecules by transferring singlet energy

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to BChl [4–6]. Also, owing to their low-lying triplet states, carotenoids can protect the photosynthetic apparatus by quenching BChl triplet states before they can sensitize the formation of singlet-state oxygen – a powerful and destructive oxidizing agent [7].

One approach to examining the molecular features which control both singlet and triplet excitation transfer between carotenoids and chlorphylls is to study covalently linked carotenoporphyrin molecules [8–13]. These molecules consist of carotenoid polyenes linked to porphyrin derivatives and are capable of mimicking both the light-harvesting and photoprotective functions of carotenoids [10]. Flash photolysis and pulse radiolysis techniques have been applied to these molecules in order to understand the relationship between the structure of the complex and the rate and efficiency of energy transfer [10–13]. These studies have shown that upon photoexcitation a porphyrin triplet state is formed by intersystem crossing from an excited singlet state. Subsequently, the porphyrin triplet energy is transferred rapidly to yield a carotenoid triplet state.

Recently, electron spin resonance (ESR) techniques have been used to probe the structures, geometries and dynamics of carotenoids in whole cells, membranes and isolated reaction center complexes of several strains of photosynthetic bacteria [14–19]. In this paper we present a parallel investigation of the triplet state ESR properties of a series of B800–850 complexes and synthetic carotenoporphyrin molecules. The data offer the means for assigning the identities of the triplet state species, elucidating the structural requirements for triplet energy transfer in the B800–850 complexes, and examining the magnetic properties of carotenoporphyrins.

Materials and Methods

Cells of the photosynthetic bacteria were grown as previously described [4]. In each case the cells were harvested by centrifugation at $15\,000 \times g$. Chromatophores of the *Rb. sphaeroides* strains were prepared as described by Clayton and Clayton [20]. Broken cells of *Rps. acidophila* 7750 were prepared according to Cogdell et al. [21].

The B800–850 complexes were isolated from *Rb. sphaeroides* wild-type strains 2.4.1 and GA as follows. Chromatophores were adjusted to an optical absorbance of 50 cm^{-1} at the absorbance maximum near 850 nm using 20 mM Tris-HCl (pH 8.0). The chromatophore solution was made 150 mM in NaCl, lauryl dimethylamine oxide (LDAO) was added to 0.3%, and the mixture was incubated at 28 °C for 30 min. The solubilized chromatophores were centrifuged at $15\,000 \times g$ for 15 min. The pellet, which is enriched in the B800–850 complex, was retained, while the supernatant, which contains reaction center and antenna complexes, was centrifuged at $250\,000 \times g$ for 60 min. The pellets from the high- and low-speed centrifugations were combined and resuspended in 20 mM Tris-HCl to an optical absorbance of 50 cm^{-1} at the 850 nm absorption maximum. The supernatant from the high speed centrifugation is rich in reaction centers and can be frozen for purification at a later date. The resuspended pellets were treated with an additional 1% LDAO, incubated at room temperature for 5 min, and centrifuged at $10\,000 \times g$ for 10 min. The pellet was discarded and the supernatant was diluted to approx. 4-times the original volume with 20 mM Tris-HCl (pH 8.0). The diluted supernatant was applied to a DEAE-Sephacel column (150 ml bed volume) and eluted with 20 mM Tris-HCl (pH 8.0), 0.1% LDAO and 0 to 320 mM NaCl (in 40 mM steps). Pure B800–850 complex, as evidenced by an A_{850}/A_{280} absorption ratio of greater than 2.5, was eluted from the column at approx. 200 mM NaCl. B800–850 complex from *Rps. acidophila* 7750 was isolated from the membranes as previously described [21]. The pigment-protein complexes were concentrated by dialysis to an absorbance of approx. 320 cm^{-1} at the 850 nm absorption maximum against a slurry of Aquacide I (Calbiochem) in 100 mM Tris-HCl/0.1% LDAO (pH 8.0). The B800–850 complex samples were prepared for triplet state ESR experiments by mixing approx. 20% ethylene glycol with the pigment-protein complex (final A_{850} greater than 250 cm^{-1}), placing in a quartz ESR sample tube (3 mm i.d. × 4 mm o.d.) and freezing in liquid nitrogen.

The synthesis of the model porphyrins and covalently-linked carotenoporphyrins will be described elsewhere. Samples were prepared by dis-

solving the molecules in 2-methyltetrahydrofuran (Aldrich) which had been purified by distillation from sodium metal. All of the samples had a concentration of approx. $5 \cdot 10^{-3}$ M and were subjected to three freeze-pump-thaw cycles before freezing in liquid nitrogen for use in the ESR experiments.

Triplet state ESR spectroscopy was crried out with a Varian X-band spectrometer. The magnetic field modulation was kept at a frequency of 100 kHz. Excitation was provided by light from a 1000 W xenon arc lamp (Kratos LH151N/1S) filtered through 3.5 cm of water in a Pyrex bottle. The light was focussed into a Varian TE microwave cavity fitted with an open-ended flange. The light-induced signals were detected using a lock-in amplifier (PAR 128A) referenced to the light excitation which was modulated by a chopper. The light modulation frequency was kept low enough to avoid phase shifts in the lock-in detected signals. The lock-in amplifier phase angle was tuned to maximize the overall spectral intensity. The ESR spectra were analyzed via computer simulations of the experimental lineshapes [22].

Results

The triplet state ESR spectra of the B800–850 light harvesting complexes isolated from *Rb. sphaeroides* GA, anaerobically and aerobically grown *Rb. sphaeroides* wild type and *Rps. acidophila* 7750 are shown in Fig. 1. All of the spectra display an *eae aea* polarization pattern, and exhibit no temperature dependence between 160 K and 8 K. The triplet spectrum from the *Rb. sphaeroides* GA B800–850 complex is characterized by the zero-field splitting parameters, $|D| = 0.0365 \pm 0.0002$ cm^{-1} and $|E| = 0.0035 \pm 0.0002$ cm^{-1}. The triplet spectrum from the anaerobically grown *Rb. sphaeroides* wild-type B800–850 complex has zero-field splitting parameters, $|D| = 0.0324 \pm 0.0002$ cm^{-1} and $|E| = 0.0036 \pm 0.0002$ cm^{-1}, whereas the spectrum of the complex isolated from aerobically grown cells gives rise to the parameters, $|D| = 0.0318 \pm 0.0002$ cm^{-1} and $|E| = 0.0032 \pm 0.0002$ cm^{-1}. The triplet spectrum from the *Rps. acidophila* 7750 B800–850 complex is characterized by the zero-field splitting parameters, $|D| = 0.0279 \pm 0.0002$ cm^{-1} and $|E| = 0.0029 \pm 0.0002$ cm^{-1}.

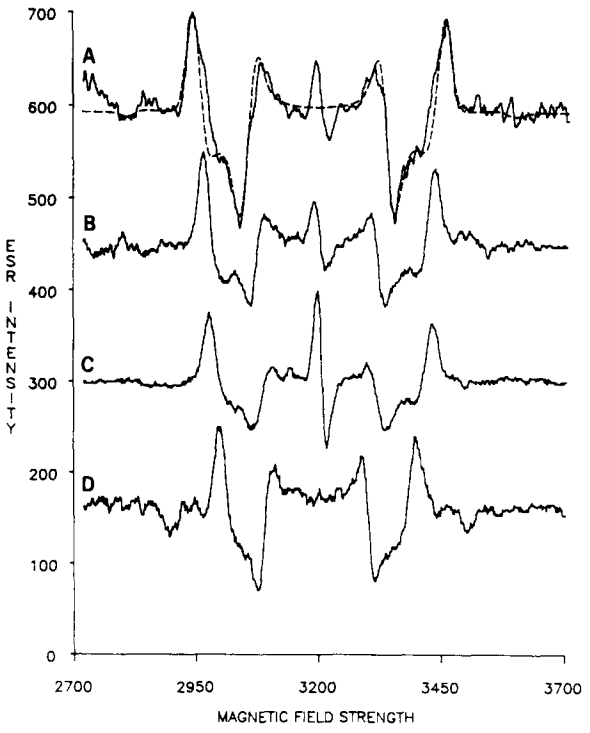

Fig. 1. Triplet state ESR spectra of B800–850 complexes. (a) ESR spectrum of *Rb. sphaeroides* GA taken under the following conditions: temperature, 100 K; modulation amplitude, 29G; microwave frequency, 9.050 GHz; microwave power, 16 mW; light modulation frequency, 100 Hz; lock-in amplifier sensitivity, 2.5 mV; sweep time, 90 min; recorder time constant, 30 s. The dashed line represents an example of a computer generated spectrum using the parameters given in Tables I and II with an intrinsic linewidth paraemter of 20 ± 2 G. The following experimental spectra were calculated in the same manner. (b) ESR spectrum of *Rb. sphaeroides* wild type (anaerobically grown) taken under the same conditions as (a) except: sweep time, 60 min. (c) ESR spectrum of *Rb. sphaeroides* wild type (aerobically grown) taken under the same conditions as (a) except: sweep time, 70 min. (d) ESR spectrum of *Rps. acidophila* 7750 taken under the same conditions as (b). The $g = 2.0$ signals are due to the presence of a small (under 1%) amount of reaction center protein in some of the preparations.

Fig. 2 shows the structures of the model compounds studied here. Compounds 1–3 consist of a tetraarylporphyrin linked via an amide functional group to a synthetic carotene derivative. Carotenoporphyrins 1, 2 and 3 have the carotenoid linkage *ortho, meta* and *para* to the point of

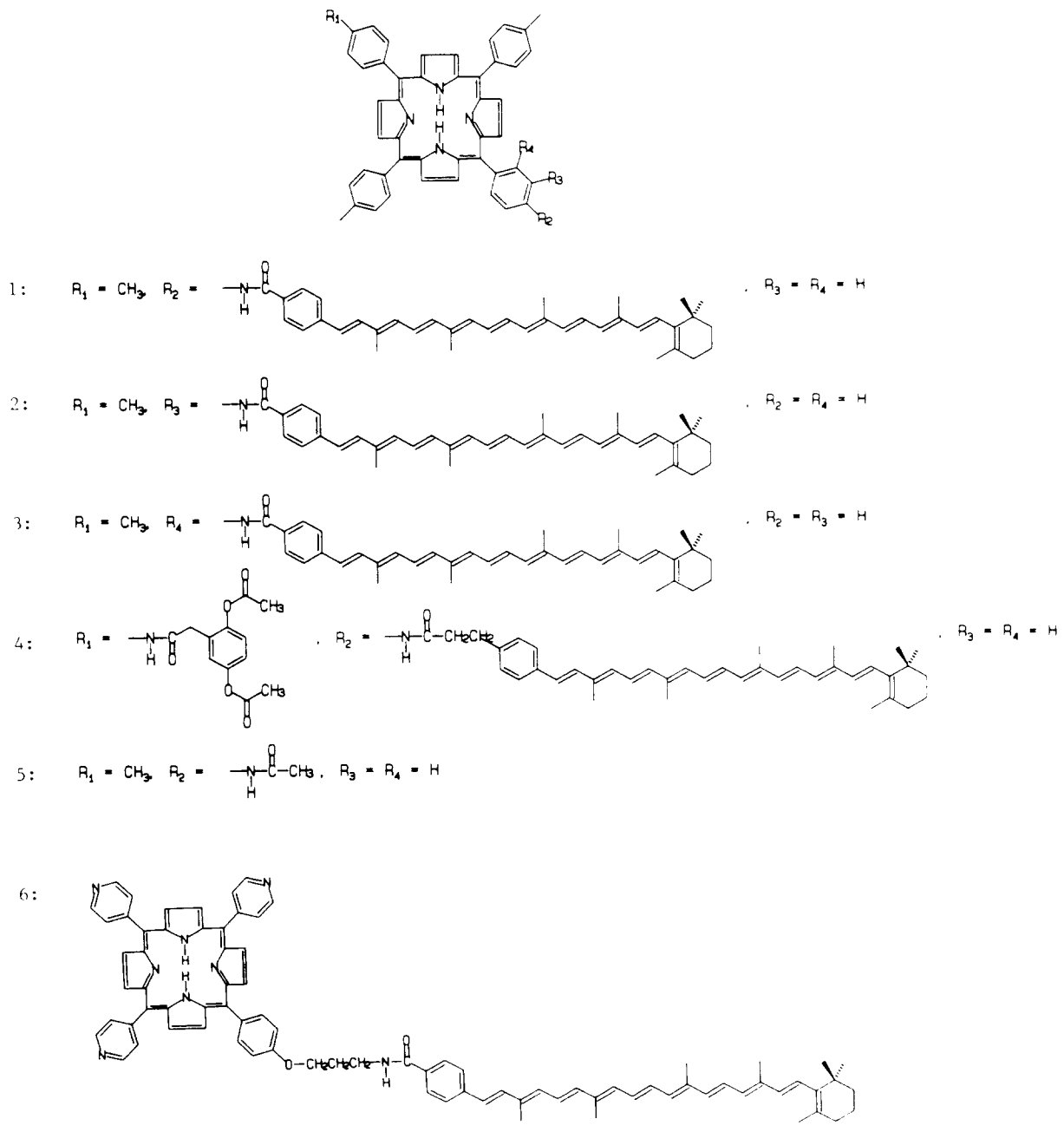

Fig. 2. Structures of the carotenoporphyrins used in the present study.

attachment of the *meso*-aryl ring to the porphyrin, respectively. Compound 4 is a molecular triad consisting of a tetraarylporphyrin linked to both an hydroquinone diacetate and a carotenoid. Two methylene groups in the carotenoid link position the polyene farther away from the porphyrin than in compounds 1–3. Compound 5 is tetraarylporphyrin with an acetamido functional group. Carotenoporphyrin 6 consists of a *meso*-tripyridylporphyrin linked to a carotene derivative via

an ether oxygen, three methylenes and an amide group.

The triplet state ESR spectra from the model compounds are shown in Fig. 3. All of these ESR spectra display the polarization pattern *eae aea*, where *e* denotes a signal in emission and *a* denotes a signal in absorption. The spectra of compounds 1–3 are all identical and are characterized by zero-field splitting parameters $|D| = 0.0356 \pm 0.0002$ cm^{-1} and $|E| = 0.0036 \pm 0.0002$ cm^{-1}. Compound 4 displays an ESR lineshape that is a convolution of two triplet species. One triplet has zero-field splitting parameters identical to those of carotenoporphyrins 1–3, whereas the other triplet has zero-field splitting parameters $|D| = 0.0378 \pm 0.0002$ cm^{-1} and $|E| = 0.0080 \pm 0.0002$ cm^{-1} and is similar in shape to the spectra observed for porphyrin 5 and carotenoporphyrin 6 (see Fig. 3). The spectrum of carotenoporphyrin 6 is characterized by zero-field splitting parameters $|D| = 0.0398 \pm 0.0002$ cm^{-1} and $|E| = 0.0078 \pm 0.0002$ cm^{-1}, whereas the spectrum of the tetraarylporphyrin 5 has zero-field splitting paramters $|D| = 0.0378 \pm 0.0002$ cm^{-1} and $|E| = 0.0080 \pm 0.0002$ cm^{-1}. The lineshape of this spectrum and the zero-field splittings are similar to those reported previously for tetraphenylporphyrin [23–24].

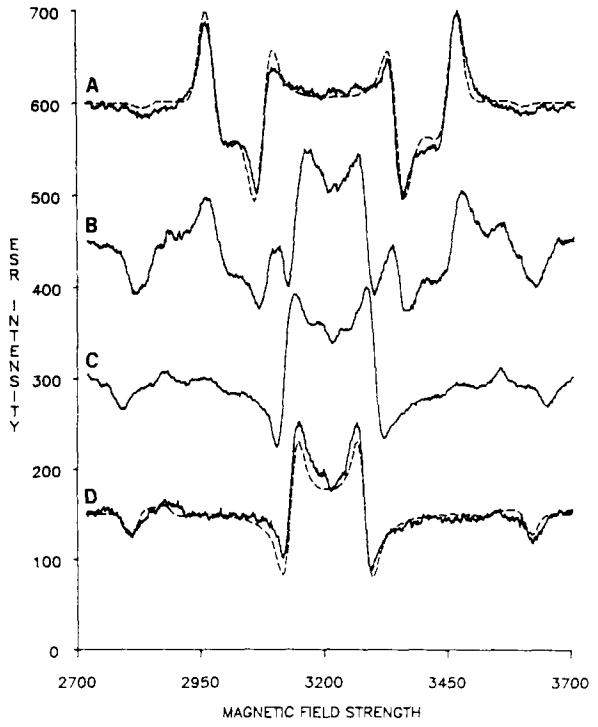

Fig. 3. Triplet state ESR spectra of the carotenoporphyrin molecules shown in Fig. 1. (a) ESR spectrum of carotenoporphyrin 1 taken under the following conditions: temperature, 100 K; modulation amplitude, 25 G; microwave frequency, 9.050 GHz; microwave power, 16 mW; light modulation frequency, 100 Hz; lock-in amplifier sensitivity, 10 mV; sweep time, 90 min; recorder time constant, 10 s. The dashed line represents the computer generated spectrum using the parameters given in Tables I and II with an intrinsic linewidth parameter of 20 ± 2 G. (b) ESR spectrum of carotenoporphyrin 4 taken under the same conditions as (a) except: modulation amplitude, 22 G; sweep time, 60 min. (c) ESR spectrum of carotenoporphyrin 6 taken under the same conditions as (a) except: temperature, 115 K; modulation amplitude, 22 G; sweep time, 120 min; recorder time constant, 100 s. (d) ESR spectrum of tetraarylporphyrin 5 taken under the same conditions as (a) except: temperature, 110 K; modulation amplitude, 22 G; light modulation frequency, 33 Hz; sweep time, 15 min; recorder time constant, 3 s. The dashed line represents the computer generated spectrum using the parameters given in Tables I and II with an intrinsic linewidth parameter of 20 ± 2 G.

In addition to the free-base carotenoporphyrin systems, the zinc-substituted porphyrin analogs of the compounds 1 and 4 were analyzed by triplet state ESR. Zinc-substituted 1 displayed precisely the same spectrum as free-base 1 shown in Fig. 3A. The zinc-substituted 4, however, displayed an ESR spectrum that was markedly different from the corresponding free-base compound (compare Figs. 3B and 4.) The lineshape arising from the zinc-substituted 4 is consistent with two triplet species giving rise to the lineshape. The first of these triplets has precisely the same zero-field splitting parameters as compound 1, whereas the second triplet species is characterized by large peaks on the extreme low-field and high-field ends of the spectrum and a pronounced dip in the center. The lineshape of this second triplet resembles that of zinc-tetraphenylporphyrin reported previously [25]. The zero-field splitting parameters for this triplet were found here to be $|D| = 0.0308 \pm 0.0005$ cm^{-1} and $|E| = 0.0103 \pm 0.0005$ cm^{-1}. All of the triplet state zero-field splittings are summarized in Table I

The triplet state ESR spectra are sensitive functions of the dynamics of population and depopulation of the individual triplet state spin sublevels [19]. Thus, computer simulations of the ESR line-

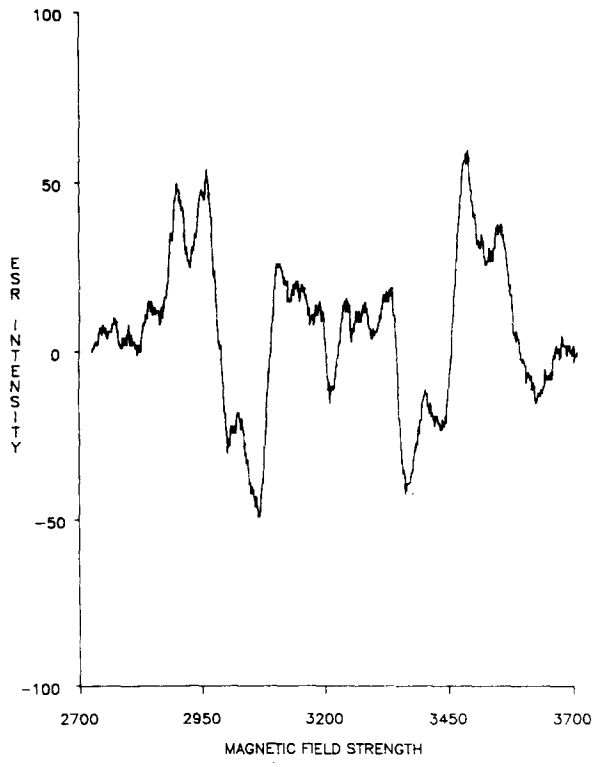

Fig. 4. Triplet state ESR spectrum of zinc-carotenoporphyrin 4. The ESR spectrum was taken under the following conditions: temperature, 12 K; modulation amplitude, 22 G; microwave frequency, 9.070 GHz; microwave power, 16 mW; light modulation frequency, 90 Hz; lock-in amplifier sensitivity, 2.5 mV; sweep time 45 min; recorder time constant, 30 s.

shapes will yield these rate constants if they are treated as variable parameters in the model. It has been established for carotenoids that the rate constants extracted from computer simulations of the high-field ESR lineshapes agree with those measured by transient ESR techniques [19]. The procedure for simulating the triplet state ESR spectra of the model compounds was as follows: First, the ESR spectrum of tetraarylporphyrin 5 was simulated using the zero-field rate constant for population and decay measured for tetraphenylporphyrin and reported in the literature [24,26]. The values are $k_1 = 50 \pm 10\ s^{-1}$, $k_2 = 168 \pm 40\ s^{-1}$, and $k_3 = 692 \pm 50\ s^{-1}$ for the depopulating rate constants and $P_1 = 0.07$, $P_2 = 0.24$ and $P_3 = 1.00$ for the relative populating rate constants. The subscripts correspond to the first, second and third canonical peaks in the triplet ESR spectrum. The spin lattice relaxation rate constants were varied over the range zero to $10000\ s^{-1}$. Agreement between the experimental and calculated lineshapes (Fig. 3) was obtained with the values $W_1 = 450 \pm 100\ s^{-1}$ and $W_2 = 1400 \pm 100\ s^{-1}$ where W_1 and W_2 repre-

TABLE I

ZERO-FIELD SPLITTING PARAMETERS OF THE OBSERVED TRIPLET STATES

$|D|$ and $|E|$ are the triplet state zero-field splitting parameters (in cm^{-1}) which characterize the ESR spectra. The parameters were obtained from computer simulations of the experimental triplet state spectra. The errors in the numbers define the range of parameters for which the simulations fell within the reproducibility of the experimental spectra.

Sample	$\|D\|$	$\|E\|$	Triplet assignment
B800–850 complexes			
Rb. sphaeroides GA	0.0365 ± 0.0002	0.0035 ± 0.0002	neurosporene
Rb. sphaeroides wild type (anaerobic)	0.0324 ± 0.0002	0.0036 ± 0.0002	spheroidene
Rb. sphaeroides wild type (aerobic)	0.0318 ± 0.0002	0.0032 ± 0.0002	spheroidenone
Rps. acidophila 7750	0.0279 ± 0.0003	0.0029 ± 0.0003	rhodopin
Model compounds			
carotenoporphyrins 1, 2, 3	0.0356 ± 0.0002	0.0036 ± 0.0002	carotenoid
Zn-carotenoporphyrin 1	0.0356 ± 0.0002	0.0036 ± 0.0002	carotenoid
carotenoporphyrin 4			
Triplet 1	0.0356 ± 0.0002	0.0036 ± 0.0002	carotenoid
Triplet 2	0.0378 ± 0.0002	0.0080 ± 0.0002	tetraarylporphyrin
Zn-carotenoporphyrin 4			
Triplet 1	0.0356 ± 0.0002	0.0036 ± 0.0002	carotenoid
Triplet 2	0.0308 ± 0.0005	0.0103 ± 0.0005	Zn-tetraarylporphyrin
carotenoporphyrin 6	0.0398 ± 0.0002	0.0078 ± 0.0002	tripyridylporphyrin
tetraarylporphyrin 5	0.0378 ± 0.0002	0.0080 ± 0.0002	tetraarylporphyrin

sent the rate constants for spin-lattice relaxation between adjacent and nonadjacent spin sublevels, respectively. The rate constants for spin-lattice relaxation are in reasonable agreement with the average value of 3300 s^{-1} which has been determined for tetraphenylporphyrin from transient ESR experiments [27].

The carotenoporphyrin triplet state spectra were calculated phenomenologically by treating the zero-field populating and depopulating rate constants as simulation parameters. The depopulating rate constants were varied over the range zero to 300000 s^{-1} with the restriction that $[(k_1 + k_2 + k_3)/3]^{-1} = 10$ μs (the approximate triplet-state lifetime of compound 1). The relative populating rate constants were varied over the range zero to 1.0. Because the carotenoporphyrins exhibit short triplet lifetimes and display extensive spin polarization in their triplet spectra, the spin-lattice relaxation rate constants were set equal to zero. The zero-field populating and depopulating rate constants were extrapolated to high-field using the random phase approximation [28]. A simulation of the spectrum of compound 1 is shown in Fig. 3A and was obtained with depopulating rate constants equal to $k_1 = 35000 \pm 15000$ s^{-1}, $k_2 = 165000 \pm 15000$ s^{-1} and $k_3 = 90000 \pm 10000$ s^{-1}, and relative populating rate constants equal to $P_1 = 0.05 \pm 0.02$, $P_2 = 0.14 \pm 0.10$ and $P_3 = 0.96 \pm 0.04$. The errors in the rate constants give the range of parameters for which the simulations fell within the reproducibility of the experimental spectra. Table II summarizes the rate constants deduced for the porphyrin and acrotenoid triplets from the computer simulations of their ESR spectra.

Discussion

The triplet state ESR spectra which are observed in the B800–850 light-harvesting complexes of photosynthetic bacteria (Fig. 2) are due to carotenoids. This assignment is supported by the following arguments:

(i) The zero-field splitting parameters which characterize the triplet state spectra correlate with the structures of the various carotenoids present in the different B800–850 complexes. *Rb. sphaeroides* GA contains neurosporene, methoxyneurosporene and hydroxyneurosporene [29]. Anaerobically and aerobically grown wild-type *Rb. sphaeroides* contain spheroidene and spheroidenone, respectively, as their major carotenoid pigments [29,30]. *Rps. acidophila* 7750 contains primarily rhodopin [31]. The differences in the zero-field splitting parameters presented in Table I may be understood in terms of variations in the extent of dipole-dipole interaction arising from different amounts of π-electron conjugation in the various carotenoids. The neurosporene chromophore in *Rb. sphaeroides* GA has the least extent of π-electron conjugation (nine carbon–carbon double bonds) and was found

TABLE II

KINETIC PARAMETERS USED IN THE ESR SPECTRAL SIMULATIONS

k_1, k_2 and k_3 are the zero-field depopulating rate constant (in s^{-1}). The subscripts refer to the first, second and third canonical peaks in the ESR spectra. W_1 and W_2 are the rate constants for spin-lattice relaxation (in s^{-1}) between adjacent and nonadjacent triplet spin sublevels, respectively. P_1, P_2 and P_3 are zero-field populating rate constants. Because of the similarity in the lineshapes of the ESR spectra for the B800–850 complexes and carotenoporphyrin 1, all of those spectra were able to be simulated using the same range of kinetic parameters. The errors in the numbers give the range of parameters for which the simulations fell within the reproducibility of the experimental spectra.

System	k_1	k_2	k_3	W_1	W_2
Tetraarylporphyrin 5 [a]	50 ± 10	168 ± 40	692 ± 50	450 ± 100	1400 ± 100
	$P_1 : P_2 : P_3 = 0.07 : 0.24 : 1.00$				
B800–850 complexes and carotenoporphyrin 1	35000 ± 15000	165000 ± 15000	90000 ± 10000	0.0	0.0
	$P_1 : P_2 : P_3 = 0.05 \pm 0.02 : 0.14 \pm 0.10 : 0.96 \pm 0.04$				

[a] Populating and depopulating rate constants taken from Ref. 26.

to have the largest $|D|$ value (largest dipolar interaction). The $|D|$ values follow the order neurosporene (nine carbon–carbon double bonds) > spheroidene (ten carbon–carbon double bonds) > spheroidenone (ten carbon–carbon double bonds plus one carbon–oxygen double bond) > rhodopin (eleven carbon–carbon double bonds). These results indicate that within the B800–850 complex class, the protein exerts little influence on the trends in the carotenoid zero-field splitting parameters.

(ii) There is a correlation between the trends in the wavelength maxima observed in the optical spectroscopic experiments carried out previously on B800–850 complexes and the trends in the zero-field splittings presented here. Intense carotenoid triplet-triplet absorption signals have been observed using transient optical spectroscopic methods applied to B800–850 complexes [32]. The triplet-triplet absorptions have been shown to blue shift as the length of the π-electron conjugation is shortened. This is consistent with the ESR data which show that the zero-field splitting parameters increase as the extent of π-electron conjugation decreses.

(iii) No signals resembling those in Fig. 1 have been seen in antenna complexes isolated from the carotenoidless mutant *Rb. sphaeroides* R26.1 (Frank and Chadwick, unpublished results).

(iv) The ESR spectra of the B800–850 complexes shown in Fig. 1 bear a marked similarity in lineshape to the ESR spectra observed from the carotenoids in synthetic carotenoporphyrin molecules (Fig. 3). Optical spectroscopic studies reveal that carotenoporphyrins 1–3 (Fig. 2) form a carotenoid triplet within 20 ns after photoexcitation. Compound 6 displays only a porphyrin triplet state (no carotenoid triplet) in its optical triplet-triplet absorption spectrum in a glass at 77 K. The same effect was observed for these molecules in the ESR experiments. When the carotenoid is absent (e.g., in tetraarylporphyrin 5) or when it is linked by a long chain (i.e., carotenoporphyrin 6), one observes only the porphyrin triplet state spectrum either optically or by ESR. The zero-field splitting parameters of compound 6 are larger than those of tetraarylporphyrin 5 most likely because of the electron-withdrawing effects of the pyridyl functional groups present in 6 (see Fig. 2.) As the link between the porphyrin and the carotenoid is shortend, the rate and efficiency of energy transfer from the porphyrin to the carotenoid increases. If the rate of energy transfer is comparable to the decay of the carotenoid triplet to the ground state (about 10 μs), both the porphyrin and carotenoid triplets can be observed simultaneously (see Fig. 3.) If the carotenoid is linked in very close proximity to the porphyrin, such as provided by the amide link in compounds 1–3, the triplet transfer rate is extremely fast (no more than 20 ns), the transfer efficiency is very high (about 100%) and one observes only the carotenoid triplet state.

The requirement of close proximity for fast triplet-triplet transfer between spatially fixed donor-acceptor pairs of molecules in rigid media has been established from the optical spectroscopic studies on carotenoporphyrins [13]. These results show that a close proximity is an essential structural feature for efficient porphyrin-to-carotenoid triplet-triplet energy transfer because the triplet-triplet transfer rate decreases as the length of the link is increased [13]. As discussed above, the ESR spectra of the synthetic carotenoporphyrins are also consistent with this behavior. There are striking similarities in the carotenoid triplet state ESR spectra of the B800–850 complexes (Fig. 1) and the carotenoporphyrin molecules with the shortest porphyrin to carotenoid linkages, the amide-linked carotenoporphyrins 1–3 (Fig. 3A). In both cases the carotenoid triplet state is formed with very high efficiency (approx. 100%) which strongly implies that a very close geometric proximity is also achieved between the BChl and the carotenoid in the B800–850 complexes.

The most conspicuous feature of the data presented here is the fact that all of the triplet state ESR spectra attributable to carotenoids exhibit the spin polarization pattern *eae aea*. These data show that the carotenoid triplet ESR lineshapes are not affected by changes in the structure of the donor (porphyrin, zinc-porphyrin or BChl), are insensitive (apart from the spectral line splittings) to the extent of conjugation of the carotenoid acceptor, and are not dependent on the relative orientation of the pair (i.e., the *para, meta* and *ortho* isomers 1–3 gave rise to the same ESR spectra). The molecular basis for this spectral uni-

formity may be considered in the following way:

The magnitude of the interchromophore exchange interaction (U_{ex}) relative to the zero-field splittings gives rise to two limiting cases [33]. If the exchange interaction is small compared to the zero-field splittings, the spin states of the donor and acceptor pair are those of the individual molecules. In this case during triplet-triplet energy transfer the spatial orientation of the triplet state angular momentum is conserved. At zero magnetic field the probabilities for populating the individual triplet spin sublevels of the energy acceptor are proportional to the squares of the direction cosines relating the orientations of the donor and acceptor triplet magnetic axes [34–36]. In the high-field limit the spin states of both donor and acceptor are essentially quantized along the external field conserving the high-field quantum number during the excitation transfer [37,38]. On the other hand, if the exchange interaction is large compared to the zero-field splittings, the donor-acceptor pair is best thought of as an excited state dimer where the strength of the exchange interaction forces quantization of the dimer spin system about a new set of magnetic axes whose orientations are the average of the relative positions of the two monomer principal axis systems.

If the rate of triplet energy transfer from donor to acceptor is fast relative to the rates of spin lattice relaxation or decay of the triplet donor to the ground state (as suggested here by the computer simulations and using the energy transfer kinetic data from reference 13), the probability of populating the triplet spin sublevels of the acceptor would depend on the relative orientation of the donor and acceptor principal magnetic axes. As stated above the *para, meta* and *ortho* isomers 1, 2 and 3 display the same ESR spectrum; i.e., these spectra are insensitive to changes in the relative orientation of the pair.

If the spins were quantized by the Zeeman field (about 3000 G) one might not expect the spectra to be sensitive to the relative orientation of the donor and acceptor. One would expect, however, that changing the the individual triplet spin sublevel populating rates of the donor, accomplished here by substituting zinc into the porphyrin ring systems of carotenoporphyrins 1 and 4 (evident by comparing the lineshapes displayed in Figs. 3B and 4), should affect the ESR spectrum of the carotenoid acceptor. It is known from previous optical detection of magnetic resonance studies on porphin [39] that substituting zinc for the two central hydrogens in this molecule changes its populating and depopulating rate constants. With regard to the free base and Zn-substituted carotenoporphyrins analyzed in the present study, according to the exchange mechanism of triplet energy transfer [40] which includes no spin-dependent terms, a change in the manner in which the porphyrin is populated should manifest itself as a change in the carotenoid spin sublevel population distribution and be observed in its ESR spectrum.

The weak coupling case is exemplified by Imamura, et al. [41] who have observed changes in the spin polarization patterns of the triplet acceptors biacetyl and naphthalene brought about by energy transfer from the weakly coupled triplet donors benzophenone and acetophenone. These effects are observed because the high-field ESR spin polarization patterns depend on the relative zero-field spin sublevel populations which are sensitive to changes in the donor populating rate constants [42]. Similar effects on the high field polarization patterns of the carotenoid triplet spectra were not observed here. The carotenoid spectra observed in the free-base and zinc-substituted carotenoporphyrins 1 and 4 were indistinguishable. Other factors must be considered.

In the other limit, a rather small exchange interaction (by optical spectroscopic standards) would fulfill the condition of $U_{ex} > |D|, |E|$, because the $|D|$ and $|E|$ values for the triplet states reported here are on the order of 0.01 cm^{-1}. An exchange interaction as large as 10 to 100 cm^{-1}, which would also be larger than the Zeeman interaction (about 0.3 cm^{-1}), and comparable to typical exciton splittings, may not be resolved in the broad absorption spectra which are characteristic of these molecules [13]. A large exchange interaction would force quantization of the spin system about an essentially dimeric triplet axis system and be responsible for the manner in which the triplet state is populated. The optical spectra would appear essentially monomeric in shape while the ESR lineshapes would appear dimeric. As in the weak coupling case, the rate constants for triplet population and decay would depend on the rela-

tive orientation of the pair and on the symmetry of the spin system [43,44]. It is difficult, however, to reconcile a strongly coupled spin system with the following experimental observations. (1) The ESR lineshapes are independent of the relative orientation of the triplet pair (i.e. the *para-*, *meta-* and *ortho*-linked carotenoporphyrins 1, 2 and 3 displayed the same spectra.). (2) There is no effect of zinc-substitution on the ESR spectra. A triplet state formed from a strongly coupled pair should have zero-field splitting parameters that are affected by a change in the structure of either the donor or the acceptor. The present results indicate no change in the zero-field splittings of the resulting triplet upon zinc substitution. Moreover, the zero-field splittings correlate with the structures of the carotenoids only. (3) The free-base and zinc-substituted carotenoporphyrin 4 molecules display both porphyrin and carotenoid lineshapes simultaneously. (4) Even though the energy transfer rate for compound 4 is about 100-times less than that for compound 1 and therefore the exchange coupling is markedly changed in going from 4 to 1, their carotenoid triplet state ESR spectra are identical. For these reasons the possibility of a strongly coupled spin system is ruled out.

The ever-present carotenoid polarization pattern (*eae aea*) seen herein may be explained if one considers that upon trapping the porphyrin triplet energy, the carotenoid undergoes a characteristic structural alteration which changes its nuclear conformation and results in a redistribution of its spin sublevel populations. Nuclear displacements which modulate the triplet magnetic axes and zero-field splittings have been invoked to explain spin lattice relaxation [45]. Fast conformational changes, such as a twists about carbon-carbon bonds and changes in the single and double bond lengths in the carotenoid π-electron chain in response to the molecule entering its triplet state, could alter the spin sublevel populations in an analogous fashion to spin lattice relaxation. In this connection, resonance Raman studies have suggested that β-carotene does undergo changes in its nuclear conformation upon entering its triplet state [46]. The structural changes are thought to be completely reversible upon deactivation of the carotenoid to its ground state, leading to no accumulation of isomeric photoproducts. This effect would not drive the carotenoid triplet spin system to thermal equilibrium, because the triplet state lifetimes of carotenoids are too short. It would, however, render the distribution of population in the acceptor triplet spin sublevels different from what one would expect to observe based on triplet donor populations. The fact that all carotenoid ESR spectra are very similar, regardless of the structure, orientation or any other feature of the triplet-state energy donor, suggests that this activity is a general property of these molecules.

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References

1 Cogdell, R.J. and Thornber, J.P. (1980) FEBS Lett. 122, 1–8
2 Thornber, J.P., Cogdell, R.J., Pierson, B.K. and Seftor, R.E.B. (1983) J. Cell. Biochem. 23, 159–169
3 Van Grondelle, R. (1985) Biochim. Biophys. Acta 811, 147–195
4 Cogdell, R.J., Hipkins, M.F., MacDonald, W. and Truscott, G. (1981) Biochim. Biophys. Acta 634, 191–202
5 Kramer, H.J.M., Pennoyer, J.D., Van Grondelle, R., Westerhuis, W.H.J., Niederman, R.A. and Amesz, J. (1984) Biochim. Biophys. Acta 767, 335–344
6 Kramer, H.J.M., Van Grondelle, R., Hunter, C.N., Westerhuis, W.H.J. and Amesz, J. (1984) Biochim. Biophys. Acta 765, 156–165
7 Krinsky, N.I. (1971) in Carotenoids (Isler, O., Guttman, G. and Solms, U., eds.), pp. 669–716, Birkhauser, Basel
8 Dirks, G., Moore, A.L., Moore, T.A. and Gust, D. (1980) Photochem. Photobiol. 32, 277–280
9 Moore, A.L., Dirks, G., Gust, D. and Moore, T.A. (1980) Photochem. Photobiol. 32, 691–695
10 Bensasson, R.V., Land, E.J., Moore, A.L., Crouch, R.L., Dirks, G., Moore, T.A. and Gust, D. (1981) Nature 290, 329–332
11 Gust, D., Moore, A.L., Joy, A., Tom, R., Moore, T.A., Bensasson, R.V. and Land, E.J. (1982) Science 216, 982–984
12 Liddell, P.A., Nemeth, G.A., Lehman, W.R., Joy, A.M., Moore, A.L., Bensasson, R.V., Moore, T.A. and Gust, D. (1982) Photochem. Photobiol. 36, 641–645

13 Gust, D., Moore, T.A., Bensasson, R.V., Mathis, P., Land, E.J., Chachaty, C., Moore, A.L., Liddell, P.A. and Nemeth, G.A. (1985) J. Am. Chem. Soc. 107, 3631–3640
14 Frank, H.A., Bolt, J.D., de B. Costa, S.M. and Sauer, K. (1980) J. Am. Chem. Soc. 102, 4893–4898
15 Frank, H.A., Machnicki, J. and Felber, M. (1982) Photochem. Photobiol. 35, 713–718
16 Frank, H.A., Machnicki, J. and Friesner, R. (1983) Photochem. Photobiol. 38, 451–455
17 Frank, H.A., Machnicki, J. and Toppo, P. (1984) Photochem. Photobiol. 39, 429–432
18 McGann, W.J. and Frank, H.A. (1985) Biochim. Biophys. Acta 807, 101–109
19 McGann, W.J. and Frank, H.A. (1985) Chem. Phys. Lett. 121, 253–261
20 Clayton, R.K. and Clayton, B.J. (1972) Biochim. Biophys. Acta 283, 492–504
21 Cogdell, R.J., Durant, I., Valentine, J., Lindsay, J.G. and Schmidt, K. (1983) Biochim. Biophys. Acta 772, 427–435
22 Frank, H.A., Friesner, R., Nairn, J.A., Dismukes, G.C. and Sauer, K. (1979) Biochim. Biophys. Acta 547, 484–501
23 Levanon, H. and Wolberg, A. (1974) Chem. Phys. Lett. 24, 96–98
24 Clarke, R.H. and Connors, R.E. (1975) J. Chem. Phys. 62, 1600–1601
25 Kleibeuker, J.F., Platenkamp, R.J. and Schaafsma, T.J. (1976) Chem. Phys. Letters 41, 557–561
26 Van der Bent, S.J. and Schaafsma, T.J. (1975) Chem. Phys. Lett. 35, 45–50
27 Levanon, H. and Vega, S. (1974) J. Chem. Phys. 61, 2265–2274
28 Felix, C.C. and Weissman, S.I. (1975) Proc. Natl. Acad. Sci. USA 72, 4203–4204
29 Cogdell, R.J. and Crofts, A.R. (1978) Biochim. Biophys. Acta 502, 409–416
30 Schneour, E.A. (1962) Biochim. Biophys. Acta 62, 534–540
31 Schmidt, K. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, R.W., eds.), pp. 729–750, Plenum Press, New York
32 Kingma, H., Van Grondelle, R. and Duysens, L.N.M. (1985) Biochim. Biophys. Acta 808, 383–399
33 Brenner, H.C., Brock, J.C. and Harris, C.B. (1978) Chem. Phys. 31, 137–164
34 El-Sayed, M.A., Tinti, D.S. and Yee, E.M. (1969) J. Chem. Phys. 51, 5721–5723
35 Clarke, R.H. (1970) Chem. Phys. Lett. 6, 413–416
36 Brenner, H.C. (1973) J. Chem. Phys. 59, 6362–6379
37 Soos, Z.G. (1969) J. Chem. Phys. 51, 2107–2112
38 Suna, A. (1970) Phys. Rev. B1, 1716–1739
39 Chan, I.Y., Van Dorp, W.G., Schaafsma, T.J. and Van der Waals, J.H. (1971) Mol. Phys. 22, 741–752
40 Dexter, D.L. (1953) J. Chem. Phys. 21, 836–850
41 Imamura, T., Onitsuka, O., Murai, H. and Obi, K. (1984) J. Phys. Chem. 88, 4028–4031
42 Kleibeuker, J.F. (1977) Doctoral Thesis, Agricultural University, Wageningen, The Netherlands
43 Van der Waals, J.H. and De Groot, M.S. (1967) in The Triplet State (Zahlan, A., ed.), p. 101, Cambridge University Press, London
44 Clarke, R.H. and Frank, H.A. (1976) J. Chem. Phys. 65, 39–47
45 Wolfe, J.P. (1971) Chem. Phys. Lett. 10, 212–218
46 Jensen, N-H., Wilbrandt, R., Pagsberg, P.B., Sillesen, A.H. and Hansen, K.B. (1980) J. Am. Chem. Soc. 102, 7441–7444