tribution in the lattice diminishes from nearly complete order in albite through orthoclase and adular to the disordered sanidine.[22] In the last three cases the ^{29}Si lines are no longer separated, but the presence of several separate resonances is still apparent; see Figure 4.

In accordance with the Loewenstein rule,[24] only Q^4 (4Al) units should be present in pure nepheline with a 1:1 Al:Si ratio. The strongest line at −84.8 ppm fits this assumption well, and the small shoulder at −88.4 ppm is likely to be caused by Q^4 (3Al) groups, present in the natural mineral owing to deviations from the ideal 1:1 Al:Si ratio.[25] The Q^4 (4Al) groups are the main structure elements of the anorthite lattice as well. The ^{29}Si chemical shift

(−83.1 ppm) is close to that found in nepheline. No additional lines were found, but the resonance was asymmetrically broadened with some weak, unresolved contribution from diamagnetically shifted Q^4 (3Al) units.

In all cases, a regular paramagnetic shift accompanies increasing substitution of silicon by aluminum in the Q^4 units. These shifts, and the corresponding shift increments, are peculiar to the solid state and have no counterparts in the ^{29}Si NMR spectra of solutions. Preliminary studies of zeolites have shown that the above-mentioned relationships are quite general and that ^{29}Si chemical shifts can well be used in structural studies of aluminosilicates and related inorganic compounds.

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Electron Paramagnetic Resonance Detection of Carotenoid Triplet States

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Abstract: Triplet states of carotenoids have been detected by X-band electron paramagnetic resonance (EPR) and are reported here for the first time. The systems in which carotenoid triplets are observed include cells of photosynthetic bacteria, isolated bacteriochlorophyll–protein complexes, and detergent micelles which contain β-carotene. It is well known that if electron transfer is blocked following the initial acceptor in the bacterial photochemical reaction center, back reaction of the primary radical pair produces a bacteriochlorophyll dimer triplet. Previous optical studies have shown that in reaction centers containing carotenoids the bacteriochlorophyll dimer triplet sensitizes the carotenoid triplet. We have observed this carotenoid triplet state by EPR in reaction centers of *Rhodopseudomonas sphaeroides*, strain 2.4.1 (wild type), which contain the carotenoid spheroidene. The zero-field splitting parameters of the triplet spectrum are $|D| = 0.0290 \pm 0.0005$ cm^{-1} and $|E| = 0.0044 \pm 0.0006$ cm^{-1}, in contrast with the parameters of the bacteriochlorophyll dimer triplet, which are $|D| = 0.0189 \pm 0.0004$ cm^{-1} and $|E| = 0.0032 \pm 0.0004$ cm^{-1}. Bacteriochlorophyll in a light harvesting protein complex from *Rps. sphaeroides*, wild type, also sensitizes carotenoid triplet formation. In whole cells the EPR spectra vary with temperature between 100 and 10 K. Carotenoid triplets also have been observed by EPR in whole cells of *Rps. sphaeroides* and cells of *Rhodospirillum rubrum* which contain the carotenoid spirilloxanthin. Attempts to observe the triplet state EPR spectrum of β-carotene in numerous organic solvents failed. However, in nonionic detergent micelles and in phospholipid bilayer vesicles β-carotene gives a triplet state spectrum with $|D| = 0.0333 \pm 0.0010$ cm^{-1} and $|E| = 0.0037 \pm 0.0010$ cm^{-1}.

Introduction

The ubiquity of carotenoids in biological systems is matched in degree by the magnitude of their functional importance. The primary photochemistry in vision is initiated by the absorption of light by the carotenoid retinal[1] The photosynthetic apparatus supplements its light-capturing ability with carotenoid molecules functioning as antenna or light-harvesting pigments which transfer their energy to the reaction center where the primary events of the photosynthetic process occur.[2] The role of carotenoids as protective devices against irreversible photodestruction from singlet oxygen is well known in photosynthetic bacteria, green plants, and algae.[3] However, surprisingly little is known about the excited-state structure of this class of molecules. Recent two-photon and high-resolution vibrational spectroscopic experiments have revealed low-lying excited singlet states of linear polyenes from which fluorescence occurs but into which absorption is forbidden.[4,5] These observations have challenged theoreticians to explain the

exact origin of these states, and numerous interpretations have been offered.[6,7]

The triplet state manifold in carotenoid molecules is even less understood. This is due in part to the fact that direct population of the triplet states of isolated carotenoids via singlet–triplet intersystem crossing is not very efficient.[8,9] Only optical flash photolysis techniques applied to photosensitized carotenoid systems have succeeded in populating the triplet states of these mole-

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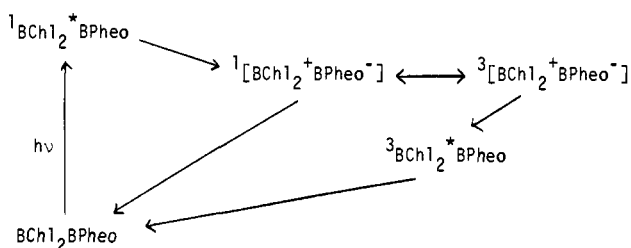(8) M. Chessin, R. Livingston, and T. G. Truscott, *Trans. Faraday Soc.*, **62**, 1519 (1966).
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Scheme I

Scheme II

Figure 1. (a) *Rps. sphaeroides* wild type reaction center, triplet-state spectrum taken with the following conditions: temperature, 160 K; receiver gain, 50; microwave power, 5 mW; light modulation frequency, 33 Hz; sweep time, 1 h; recorder time constant, 30 s. (b) *Rps. sphaeroides* R-26 reaction center, triplet-state spectrum. The conditions are the same as in (a) except: receiver gain, 32; sweep time, 8 min; recorder time constant, 3 s.
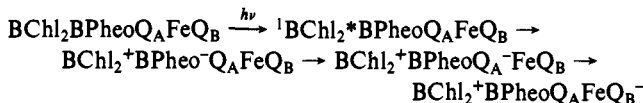
cules,[8-10] and no electron paramagnetic resonance (EPR) studies have been reported in the literature.

In the present work, we offer the first EPR observation of the triplet states of carotenoids. The systems that we have studied include β-carotene and numerous photosynthetic carotenoid pigments in vivo. In isolated pigment protein complexes from photosynthetic bacteria, photosensitization of the carotenoid triplet states by bacteriochlorophyll is accomplished. Our motivation for this study stems from the possibility that we observed a carotenoid triplet state in green-plant preparations previously,[11] and also from the abundance of literature on the optical detection of the triplet states of carotenoids in photosynthetic preparations.[12-16] Our choice of sample conditions closely parallels that of these optical experiments and is based on present knowledge about the structure and properties of the bacterial photosynthetic apparatus.

The reaction centers of photosynthetic bacteria are known to contain a primary donor consisting of a bacteriochlorophyll dimer (sometimes called the "special pair"), an initial electron acceptor thought to be a bacteriopheophytin molecule, and a following electron acceptor comprised of a quinone interacting with an iron atom.[17] Another quinone acts as a secondary acceptor. After absorption of light, the primary donor is promoted to an excited singlet state. The donor becomes oxidized and the acceptors reduced in rapid sequence, i.e.,

$$BChl_2BPheoQ_AFeQ_B \xrightarrow{h\nu} {}^1BChl_2^*BPheoQ_AFeQ_B \rightarrow$$
$$BChl_2^+BPheo^-Q_AFeQ_B \rightarrow BChl_2^+BPheoQ_A^-FeQ_B \rightarrow$$
$$BChl_2^+BPheoQ_AFeQ_B^-$$

At room temperature under ambient redox conditions, the charge on the second quinone may then proceed to various secondary acceptors to initiate the chemistry of bacterial photosynthesis. At low temperatures, this chemistry is inhibited, and the reaction center remains in the charge-separated state for seconds before charge recombination occurs. Under reducing conditions (~-300 mV) or in reaction center preparations devoid of quinones, the primary photochemistry is blocked, and the photoinduced charge-separated state of $BChl_2^+BPheo^-$ undergoes a rapid back reaction (~10 ns). Not all of the reaction centers which back react in this manner return directly to the ground state; many proceed via a triplet state which develops on the $BChl_2$ pair. The overall scheme is given in Scheme I. At low temperatures the bacteriochlorophyll triplet yield is near unity. At higher temperatures it is less.[17]

Scheme I applies to the photosynthetic bacteria which are lacking carotenoid pigments. In the carotenoid-containing systems
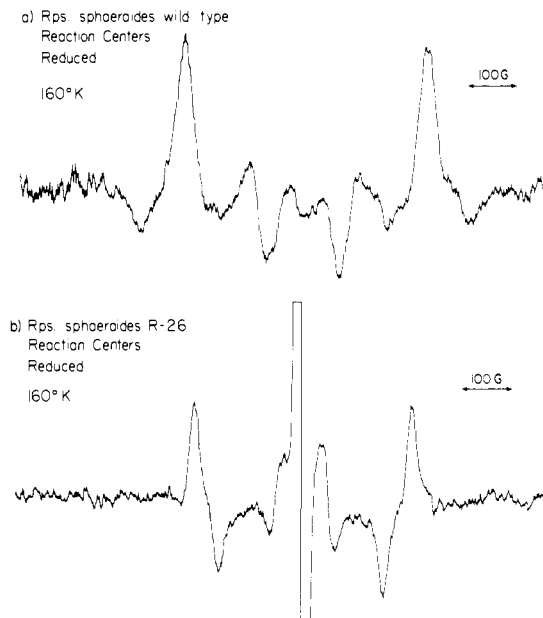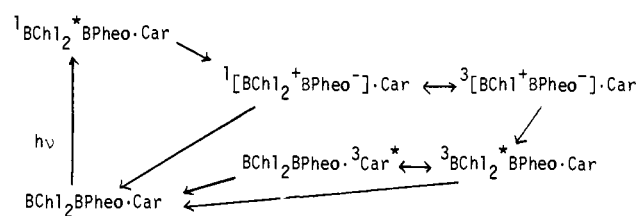
the carotenoid triplet state is also involved in the sequence of back reactions which can occur when photochemistry is blocked. Parson and Monger[18] have proposed that an equilibrium triplet energy exchange occurs between the $BChl_2$ and the carotenoid which can be illustrated as in Scheme II. The equilibrium has been suggested to shift toward the $^3BChl_2^*$ at the low temperatures. We have tested these ideas by examining the effect of temperature and the state of reduction on the triplet state EPR spectra of carotenoid-containing and carotenoidless photosynthetic bacteria.

Experimental Section

Photosynthetic bacteria were grown as described previously and stored as frozen pastes at -20 °C.[19,20] Reaction center proteins of carotenoidless mutant R-26 of *Rhodopseudomonas sphaeroides* were prepared according to Clayton and Wang.[21] Reaction centers of wild type *Rps. sphaeroides*, strain 2.4.1, were prepared in a similar manner.[22] Initial samples of the light-harvesting protein from wild-type *Rps. sphaeroides* were a gift of R. J. Cogdell. The protein was also prepared as described by Clayton and Clayton.[23] All biological samples contained 50% ethylene glycol. Untreated samples were prepared at ambient redox potential. Reduced samples were treated with 0.02 M sodium dithionite and 1.0 × 10⁻⁵ M methylviologen in 0.025 M Tris·HCl buffer, pH 8.0.

Triplet state EPR spectra were detected by light modulation at 33.5 or 11 Hz as described previously.[19] Excitation from a 1000-W mercu-

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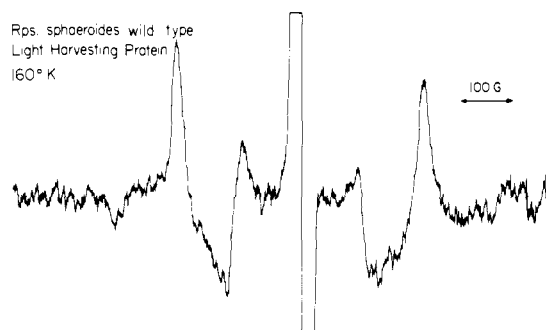
Figure 2. *Rps. sphaeroides* wild type light harvesting protein, triplet-state spectrum. Experimental conditions: temperature, 160 K; receiver gain, 63; microwave power, 5 mW; light modulation frequency, 11 Hz; sweep time, 1 h; recorder time constant, 30 s.

ry–xenon dc arc lamp was filtered through 5 cm of water in a Pyrex container and focused through the 75% transmitting grid of a Varian TM_{110} (E-238) microwave cavity. Magnetic field modulation amplitude and frequency were 16 G and 100 kHz, respectively. Measurements at 10 and 100 K utilized an Air Products Helitron cryostat. Measurements at 160 K were made with the use of a Varian Associates nitrogen gas flow Dewar.

Carotenoids were suspended in micelles by dropwise addition of 50 μL of carotenoid solution in THF to 2 mL of phosphate buffer containing 0.10 M detergent. The nonionic detergent IGEPAL-CO-630 (GAF) was used. The detergent solution was vigorously stirred and gently heated to remove THF. Carotenoids were incorporated in phospholipid vesicles by injection of a known volume of THF or ethanol solution containing both lipid and carotenoid into rapidly vortexing buffer solution. Egg phosphatidylcholine was purified by published procedures.[24] β-Carotene was obtained from Sigma. The samples were purged of oxygen by bubbling nitrogen through the solutions for a few minutes before freezing.

Results

Reaction centers from *Rps. sphaeroides* wild type display a triplet-state spectrum at 160 K which is distinct from that observed in reaction center preparations of the carotenoidless mutant, *Rps. sphaeroides* R-26 (Figure 1). The *Rps. sphaeroides* wild type triplet spectrum is characterized by the zero-field splitting parameters $|D| = 0.0290 \pm 0.0005$, $|E| = 0.0044 \pm 0.0006$ and the polarization pattern *eaa eea*, where *e* denotes a signal in emission and *a* denotes a signal in absorption. The *Rps. sphaeroides* R-26 triplet state spectrum displays zero-field splitting parameters $|D| = 0.0189 \pm 0.0004$, $|E| = 0.0032 \pm 0.0004$ and the polarization pattern *aee aae*. The zero-field splitting parameters and the polarization patterns of both spectra are invarient with temperature down to 10 K.

The triplet-state spectrum of the light-harvesting protein isolated from *Rps. sphaeroides* wild type is shown in Figure 2. Its polarization pattern is *eae aea*, and its zero-field splitting parameters are $|D| = 0.0326 \pm 0.0007$, $|E| = 0.0036 \pm 0.0007$. The features of this spectrum also do not vary with temperature down to 10 K. The acetone–methanol extract of this protein complex gave rise to a bacteriochlorophyll triplet state upon illumination at the low temperatures. The former spectrum did not appear after extraction.

Untreated whole cells of *Rps. sphaeroides* wild type display a marked change in their triplet-state spectra upon raising the temperature from 10 to 100 K (Figure 3). At 10 K the spectrum has a $|D|$ value of 0.0189 ± 0.0005, an $|E|$ value of 0.0030 ± 0.0005, and a polarization pattern of *aee aae* and is identical with the triplet-state spectrum of *Rps. sphaeroides* R-26 reaction centers or whole cells. At higher temperatures the line shape is transformed into a triplet spectrum described by $|D| = 0.0323 \pm 0.0010$, $|E| = 0.0033 \pm 0.0010$, and a polarization pattern *(e)ae aea* and bears a resemblance to the *Rps. sphaeroides* light harvesting protein spectrum shown in Figure 2.

Reduced whole cells of *Rps. sphaeroides* wild type also show a change in their triplet-state spectra upon raising the temperature
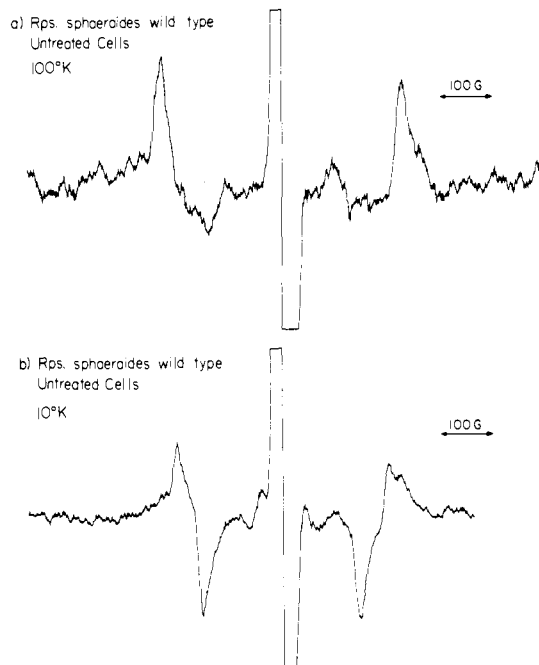
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Figure 3. *Rps. sphaeroides* wild type untreated whole cells, triplet-state spectra taken with the following conditions: receiver gain, 25; microwave power, 1 mW; light modulation frequency, 33 Hz; sweep time, 30 min; recorder time constant, 30 s; temperature, (a) 100 K, (b) 10 K.

from 10 to 100 K (Figure 4). At 10 K, however, the spectrum appears to be a convolution of two triplet signals, which makes an accurate determination of the zero-field splitting parameters difficult. At 100 K one triplet species is observed with zero-field splitting parameters $|D| = 0.0289 \pm 0.0010$ and $|E| = 0.0044 \pm 0.0010$ and the polarization pattern *eaa eea*.

Also shown in Figure 4 is the triplet-state spectrum of reduced cells of *Rps. sphaeroides* R-26 taken at 10 K. Its polarization pattern and zero-field splitting parameters are independent of temperature (between 160 and 10 K). They are *aee aae* and $|D| = 0.0189 \pm 0.0003$, $|E| = 0.0030 \pm 0.0003$, essentially identical with the corresponding values for reduced reaction centers (Figure 1b).

Figure 5 shows the effect of temperature and reduction on the triplet-state spectrum of *Rhodospirillum rubrum* wild type. The untreated cells display one triplet species at 100 K having the polarization pattern *eae aea* and $|D| = 0.0233 \pm 0.0007$ and $|E| = 0.0026 \pm 0.0007$. This spectrum does not change with temperature down to 10 K. The reduced cells exhibit one triplet spectrum at 100 K which is characterized by zero-field splitting parameters $|D| = 0.0180 \pm 0.0004$ and $|E| = 0.0040 \pm 0.0004$ and the polarization pattern *eaa eea*. At 10 K the line shape becomes complex owing to the presence of more than one triplet signal.

The triplet-state spectrum of β-carotene in detergent micelles at 160 K is given in Figure 6. Its polarization pattern is *eae aea* and its zero-field splitting parameters are $|D| = 0.0333 \pm 0.0010$ and $|E| = 0.0037 \pm 0.0010$. The spectrum showed little change when the β-carotene was suspended in phospholipid vesicles. We failed to observe the triplet-state spectrum of β-carotene dissolved in numerous organic solvents (e.g., 2-methyltetrahydrofuran, hexane, EPA, and cyclohexane).

We also recorded the triplet spectra and analyzed the effect of reduction and temperature for the carotenoidless mutant *Rsp. rubrum* G-9, for *Rps. viridis*, and for *Rps. palustris*. Only the *Rps. palustris* showed an effect upon variation of these factors. These results and all of our experimental findings are summarized in Table I.

Discussion

The triplet-state species that arises after illumination of cells or reaction-center preparations from the carotenoidless mutants

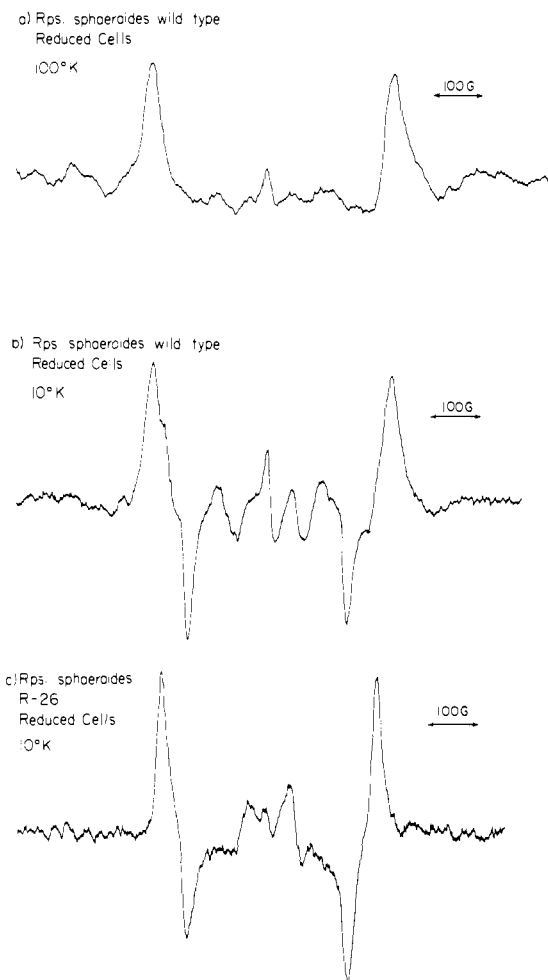4896   *J. Am. Chem. Soc., Vol. 102, No. 15, 1980*

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Figure 4. (a) *Rps. sphaeroides* wild type reduced whole cells, triplet-state spectrum taken with the following conditions: receiver gain, 80; microwave power, 1 mW; light modulation frequency, 33 Hz; sweep time, 8 min; recorder time constant, 10 s; temperature, 100 K. (b) *Rps. sphaeroides* wild type reduced whole cells, triplet-state spectrum taken with the experimental conditions of (a) except: receiver gain, 63; sweep time, 16 min; temperature, 10 K. (c) *Rps. sphaeroides* R-26 reduced whole cells, triplet-state spectrum taken with the experimental conditions of (b).

Rps. sphaeroides R-26 or *Rsp. rubrum* G-9 is known to be a bacteriochlorophyll dimer triplet state localized on the primary donor.[17] Its *aee aae* polarization pattern indicates that a charge separation/recombination process is involved in the mechanism of its formation.[25] Such a process for triplet-state formation is best understood in terms of the radical-pair mechanism.[25] According to this mechanism, the system is initially prepared in an excited singlet state. After one electron is transferred from a donor to an acceptor, a change in spin correlation between the spins may result in significant quantum-mechanical mixing between the singlet and middle-energy high-field triplet spin sublevel, T_0. The effect of this process is to distribute the spin population of the triplet state heavily in favor of the T_0 level. If the triplet state is then observed by EPR prior to spin–lattice relaxation, all T_0 to T_{+1} transitions are in absorption and all T_0 to T_{-1} transitions are in emission. Hence, for systems where the zero-field splitting parameter, D, is positive, the polarization pattern *aee aae* is observed. If D is negative, one observes the inverted polarization pattern *eaa eea*.

The triplet-state spectrum which we observed in the reaction centers of the carotenoid-containing *Rps. sphaeroides* wild type (Figure 1a) is most likely due to the triplet state of the carotenoid spheroidene, which is known to be associated with the reaction
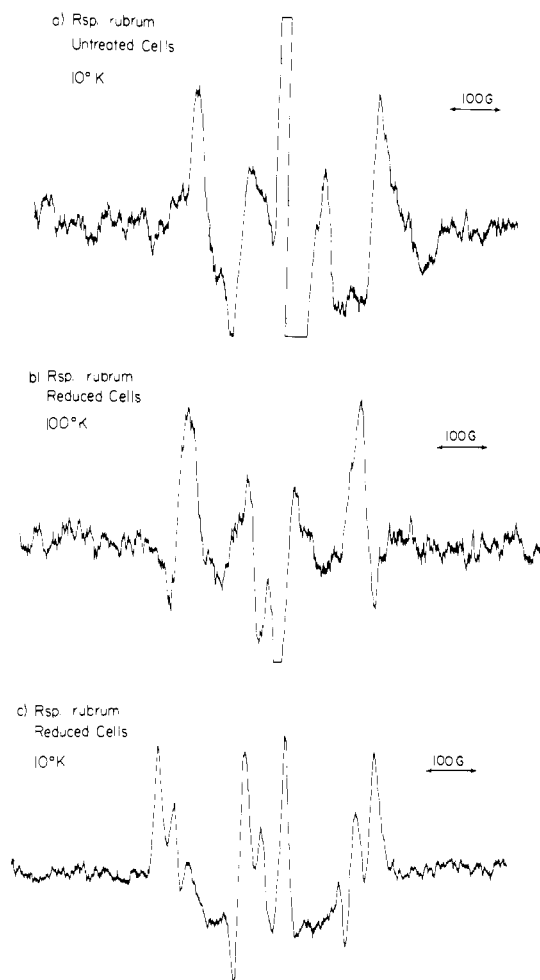
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Figure 5. *Rps. rubrum* wild type, triplet-state spectra of (a) untreated cells. The spectrum was taken with the following conditions: temperature, 10 K; receiver gain, 125; microwave power, 1 mW; light modulation frequency, 33 Hz; sweep time, 16 min; recorder time constant, 10 s. (b) Reduced cells. The spectrum was taken with the experimental conditions of (a) except: temperature, 100 K; receiver gain, 20; sweep time, 8 min; recorder time constant, 3 s. (c) Reduced cells. The spectrum was taken with the experimental conditions of (a) except: receiver gain, 25.
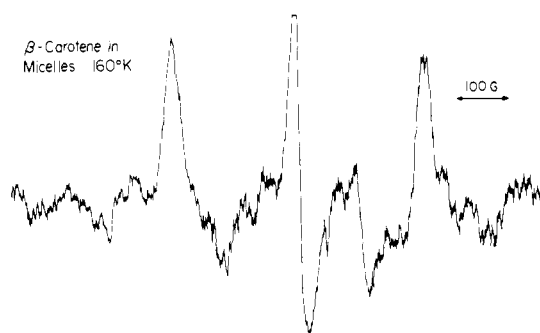

Figure 6. β-Carotene in micelles, triplet-state spectrum taken with the following conditions: temperature, 160 K; receiver gain, 200; microwave power, 5 mW; light modulation frequency, 11 Hz; sweep time, 30 min; recorder time constant, 30 s.

center protein complex.[16] This assignment is supported by the following arguments.

I. The spectrum shown in Figure 1a is not observed in the carotenoidless *Rps. sphaeroides* R-26 species under a wide variety of preparative, redox, and temperature conditions (Table I). This implies that it is not characterstic of one of the other reaction center pigments. Four bacteriochlorophyll molecules and two bacteriopheophytin molecules comprise the reaction center, but the zero-field splitting parameters of the spectrum shown in Figure

Table I. Zero-Field Splitting Parameters, Polarization Patterns, and Assignments of the Observed Triplet States[a]

sample	temp, K	$\|D\|$	$\|E\|$	polarization pattern	triplet assignment
Rhodopseudomonas sphaeroides wild type					
reduced reaction centers	160, 100, 10	0.0290 ± 0.0005	0.0044 ± 0.0006	*eaa eea*	reaction center carotenoid
light-harvesting protein (LHP)	160, 100, 10	0.0326 ± 0.0007	0.0036 ± 0.0007	*eae aea*	light-harvesting carotenoid
LHP pigment extract	160	0.0220 ± 0.0005	0.0053 ± 0.0005	*eea eaa*	bacteriochlorophyll monomer
reduced cells	100	0.0289 ± 0.0010	0.0044 ± 0.0010	*eaa eea*	reaction center carotenoid
	10	convoluted			RC carotenoid and BChl$_2$
untreated cells	100	0.0323 ± 0.0010	0.0033 ± 0.0010	*eae aea*	light-harvesting carotenoid
	10	0.0189 ± 0.0005	0.0030 ± 0.0005	*aee aae*	BChl$_2$
Rhodopseudomonas sphaeroides R-26					
reduced reaction centers	160, 100, 10	0.0189 ± 0.0004	0.0032 ± 0.0004	*aee aae*	BChl$_2$
reduced cells	100, 10	0.0189 ± 0.0003	0.0030 ± 0.0003	*aee aae*	BChl$_2$
untreated cells	100, 10	0.0189 ± 0.0003	0.0030 ± 0.0003	*aee aae*	BChl$_2$
Rhodospirillum rubrum wild type					
reduced cells	100	0.0180 ± 0.0004	0.0040 ± 0.0004	*eaa eea*	reaction center carotenoid
	10	convoluted			RC carotenoid and BChl$_2$
untreated cells	100, 10	0.0233 ± 0.0007	0.0026 ± 0.0007	*eae aea*	light-harvesting carotenoid
Rhodospirillum rubrum G-9					
reduced cells	100, 10	0.0192 ± 0.0004	0.0033 ± 0.0004	*aee aae*	BChl$_2$
untreated cells	100, 10	0.0192 ± 0.0004	0.0033 ± 0.0004	*aee aae*	BChl$_2$
Rhodopseudomonas viridis					
reduced cells	100, 10	0.0154 ± 0.0003	0.0036 ± 0.0003	*aee aae*	BChl$_2$
untreated cells	100, 10	0.0154 ± 0.0004	0.0036 ± 0.0004	*aee aae*	BChl$_2$
Rhodopseudomonas palustris					
reduced cells	100, 10	0.0190 ± 0.0005	0.0034 ± 0.0005	*aee aae*	BChl$_2$
untreated cells	100	convoluted			LH carotenoid and BChl$_2$
	10	0.0190 ± 0.0005	0.0034 ± 0.0005	*aee aae*	BChl$_2$
β-carotene					
in micelles	160	0.0333 ± 0.0010	0.0037 ± 0.0010	*eae aea*	β-carotene
in vesicles	160	0.0331 ± 0.0010	0.0037 ± 0.0010	*eae aea*	β-carotene

[a] $\|D\|$ and $\|E\|$ are given in cm^{-1}.

1a are well outside the range of those expected for bacteriochlorophyll or bacteriopheophytin monomers or aggregates.[25]

II. The polarization pattern of the triplet in Figure 1a is *eaa eea*. This means that the triplet is either directly involved in or closely coupled to an electron-transfer process. The latter possibility has been discussed for this bacterium by Monger, Cogdell, and Parson.[14] They concluded from extensive optical experimentation on this species that the carotenoid triplet was formed by energy transfer from the BChl$_2$ triplet state. If the energy transfer from the donor triplet to the acceptor triplet occurs on a time scale which is fast compared to spin–lattice relaxation, the acceptor triplet spectrum may also display a radical pair polarization pattern. Indeed, the BChl$_2$ polarization pattern is *aee aae* and the observed triplet is *eaa eea*. The difference between these patterns may then be accounted for if the D value of the energy acceptor (carotenoid) is opposite that of the BChl$_2$ and energy transfer occurs from the T$_0$ level of BChl$_2$ to the T$_0$ level of the acceptor. Alternatively, if the D values of both BChl$_2$ and acceptor have the same sign and the energy transfer proceeds from the T$_0$ level of the BChl$_2$ to the T$_{\pm 1}$ levels of the acceptor, the observed polarization pattern would also be found.

III. Our observations of a temperature dependence of the triplet signals from reduced cells of *Rps. sphaeroides* wild type show that the BChl$_2$ triplet state signal amplitudes increase relative to the triplet in Figure 1a as the temperature is lowered (Figure 4). Precisely the same effect was reported by Cogdell, Monger, and Parson[13,14] using optical techniques. Their findings were that the carotenoid triplet yield was near 100% at room temperature, somewhat lower at 77 K, and effectively replaced by the formation of the BChl$_2$ triplet below 77 K. Figure 4 shows that at 100 K one triplet species (presumably a carotenoid) dominates the spectrum. As the temperature is lowered to 10 K the BChl$_2$ triplet signals are evident (compare Figures 4b and 4c). The fact that all preceding triplet state EPR investigations of *Rps. sphaeroides* wild type were carried out at temperatures below 10 K explains why the carotenoid triplet state was not reported until this time.

Figure 3 shows that untreated cells of *Rps. sphaeroides* wild type display a dramatic change in their triplet-state spectrum with temperature. At 10 K the predominant triplet signal belongs to

the BChl$_2$ species. Because the forward photochemistry is not blocked at the iron quinone acceptor, the triplet signals are substantially smaller than when the photochemistry is inhibited by chemical reduction (Figure 4). Also, large free-radical signals appear in the central region of the spectrum indicating that radical formation and decay are occurring at a frequency comparable to our chopping rate. The 100 K triplet state spectrum (Figure 3a) is also quite weak, but noticeably different from both the 10 K spectrum of the untreated cells (Figure 3b) and the 100 K spectrum of the reduced *Rps. sphaeroides* wild type cells (Figure 4a). We believe that the triplet-state spectrum of Figure 3a belongs to a carotenoid species which acts as a trap outside the reaction center. To test the plausibility of this assignment we examined the light-induced triplet state spectrum of a light-harvesting protein complex prepared from *Rps. sphaeroides* wild type (Figure 2). The light-harvesting complex is known to contain three bacteriochlorophyll molecules and one carotenoid molecule.[20,27] It is also known that the carotenoid molecule sensitizes the bacteriochlorophyll fluorescence.[26] The spectrum of the intact protein complex matches nicely that of the untreated *Rps. sphaeroides* wild type at 100 K. Also, the polarization pattern observed for these triplet species is *eae aea*, which indicates that a charge separation/recombination process is not involved in the mechanism for triplet formation. Because the extracted pigment solution exhibits only bacteriochlorophyll monomer triplet state signals, it apears that the carotenoid molecular triplet state is sensitized by the bacteriochlorophyll triplet. Generation of the carotenoid triplet state in the intact complex by selective excitation of the bacteriochlorophyll (λ >545 nm) showed this to be the case. In the untreated cells the triplet excitation is trapped in the antenna, presumably because on the time scale of our experiment (determined by our chopper speed to be 30–100 ms) the reaction center remains closed; i.e., the primary donor is oxidized. Monger, Codgell, and Parson, using optical techniques, found carotenoid triplet states in the antenna of *Rps. sphaeroids* wild type under

(26) R. J. Cogdell, M. F. Hipkins, and W. MacDonald, private communication.

(27) R. J. Cogdell and J. P. Thornber, *Ciba Found. Symp.*, **61**, 61 (1979).

the same experimental conditions (i.e., when the photosynthetic apparatus was oversaturated with light).[14]

Figure 5 shows the results of studies of *Rps. rubrum* wild type, where the same interpretation as given above seems likely, namely, that in the untreated cells of *Rps. rubrum* wild type a carotenoid triplet lying outside the reaction center is excited owing to the closure of the reaction center trap during the light modulated experimental sampling time. This assignment is supported by the fact that the polarization pattern for this triplet is *eae aea* (i.e., not radical pair polarized). Reduction of the cells, however, leads to the observation of a completely different triplet state at the higher temperature which is polarized *eaa eea* (radical pair polarized), reflecting its ability to trap excitation from the BChl$_2$ special pair. The spectrum of the reduced cells at lower temperatures shows a convolution of two triplet states, one of which clearly belongs to the BChl$_2$, the other being residual signals from the carotenoid system.

It is interesting to note the trends in the magnitudes of the zero-field splitting parameters of the triplets assigned to the carotenoids. The carotenoid in the reaction center of *Rps. sphaeroides* wild type has a significantly larger $|D|$ value than the reaction center carotenoid from *Rps. rubrum* wild type. Thin layer chromatography[16] of pigments from *Rps. rubrum* cells grown in our laboratory revealed only one carotenoid, identified as spirilloxanthin.[27] Reaction centers of *Rps. sphaeroides* wild type contain only spheroidene.[16] The observed differences in the $|D|$ values may be understood in terms of the extent of electron delocalization within the carotenoid molecules. Spirilloxanthin contains a chain of 13 conjugated carbon–carbon double bonds, whereas spheroidene contains only 10. The lesser extent of delocalization in the spheroidene molecule could lead to increased dipolar interaction between the unpaired electrons in the triplet state of this system, and hence a larger $|D|$ value. Because cells of *Rps. rubrum* wild type contain only one carotenoid species, we might expect the $|D|$ parameter to remain the same in both the reaction center and antenna systems. This is not the case (Table I). The reaction center carotenoid $|D|$ value is significantly smaller than that of the antenna carotenoid, suggesting that environmental or conformational effects may be important in this analysis. *Rps. sphaeroides* triplets show the same trend. More studies on the triplet states of carotenoids explaining the effects of conjugation and environment on the zero-field splitting parameters must be done before further discussion can be made.

The final system to be discussed is the triplet state of β-carotene in micelles. Despite numerous attempts to view the triplet state in organic solvents by EPR, we were unable to detect the triplet state of β-carotene in these media. It is well known that the excitation of the triplet state of β-carotene via singlet–triplet intersystem crossing is not a highly favored process.[8,9] As previously mentioned most sucessful attempts to see the triplet state of β-carotene have been through the use of triplet sensitizers in solution along with the β-carotene and using flash photolysis techniques. The structure of the β-carotene molecule may be such that photochemical or vibrational (radiationless) relaxation competes even at 77 K with the singlet–triplet intersystem crossing process. Only when these modes of relaxation are made less probable can the intersystem crossing process respond favorably. Incorporation of the β-carotene in micelles or vesicles allows this to occur. Similar effects have been observed for the triplet states of various aromatic hydrocarbons.[28]

It is known that β-carotene is a component of green plant reaction center preparations.[29] Because of the similarity between the β-carotene triplet state spectrum presented here and that observed in green-plant preparations and published previously,[11] we conclude that the triplet state viewed in these preparations is likely to be that of β-carotene. This same triplet state was also observed in green-plant preparations using optical detection of magnetic resonance techniques.[30]

This new method of detecting carotenoid triplet states can now be used to probe the structure and function of carotenoids not only in photosynthetic systems but in other carotenoid-containing biological samples as well.

(28) K. Kalyanasundaram, F. Grieser, and J. K. Thomas, *Chem. Phys. Lett.*, **51**, 501 (1977).
(29) J. P. Thornber, R. S. Alberte, F. A. Hunter, J. A. Shiozawa, and K-S. Kan, *Brookhaven Symp. Biol.*, **28**, 132 (1977).
(30) A. J. Hoff, Govindjee, and J. C. Romijn, *FEBS Lett.*, **73**, 191 (1977).

A Thermodynamic Derivation of the Cross-Relations for Rates of Electron-Transfer Reactions

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Abstract: A derivation of the cross-relations first given by Marcus, which predict the rate of electron-transfer reactions from the rates of electron-exchange reactions and the standard free energy change, is given. The derivation is based solely on thermodynamic cycles and the principle of detailed balance; the usual microscopic or mechanistic assumptions are not necessary, but we do assume independent activation of the reaction partners. Thus cross-relations should hold even in many cases for which other predictions of any given electron-transfer theory may fail. Comments are made on the possible causes for breakdown of the cross-relations; in particular, a correction factor akin to the one introduced by Marcus becomes apposite when the overall exoergicity is large, and under these conditions independent activation is no longer a valid assumption.

Introduction

Measurements of electron-transfer (ET) rates are usually discussed in terms of the theoretical framework developed by

Marcus.[1–4] In particular, the Marcus cross-relations between the rates of electron exchange (EE) and ET reactions are often em-

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(1) For a convenient general review, cf. W. L. Reynolds and R. W. Lumry, "Mechanisms of Electron Transfer", Ronald Press, New York, 1966.