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Chemistry

The triplet state in bacterial photosynthesis: Possible mechanisms of the primary photo-act

(electron paramagnetic resonance/electron spin polarization/radical pair/special pair)

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ABSTRACT ***In vitro* and *in vivo* triplet state electron paramagnetic resonance (epr) spectra of bacteriochlorophylls (Bchls) show important differences in (*a*) electron spin polarization (esp), and (*b*) zero field splitting (ZFS) parameters. The unusual esp and ZFS properties of the observed *in vivo* triplet state are best interpreted as arising from a short-lived radical pair precursor (not directly observable by epr) formed in or with the special pair of bacteriochlorophyll molecules involved in the primary photo-act.**

The successful observations of electron paramagnetic resonance (epr) triplet signals in photosynthetic bacteria by Dutton, Leigh, and coworkers (1–4) have revived interest in speculations advanced many years ago [e.g., Franck *et al.* (5)] that chlorophyll (Chl) triplet states may be involved in the primary events of photosynthesis. The magnetic properties of the lowest excited triplet state of all of the important chlorophylls have now been characterized *in vitro* (6–12), which makes it possible to compare *in vivo* and *in vitro* triplet spectra. In this communication we show that the bacteriochlorophyll (Bchl) special pair (BB)† previously postulated to participate in the primary light conversion step (13, 14) provides an eminently suitable framework for the interpretation of the *in vivo* triplet state electron spin polarization (esp) and zero field splitting (ZFS). We propose that if normal chemistry of photosynthesis is blocked, the radical pair state formed in the special pair in the primary photo-act decays to a triplet state (observable by epr) whose esp reflects the unusual spin population of the radical pair intermediate.

METHODS

The methods we used for observing the triplets (11, 12) are basically those of Leigh and Dutton (4).

RESULTS AND DISCUSSION

Electron Spin Polarization. In zero magnetic field, T_1 (the lowest triplet excited state of Bchl) is split into three spin states with eigenfunctions $|T_x^1\rangle$, $|T_y^1\rangle$, and $|T_z^1\rangle$, with an energy splitting described by the parameters D and E. In high magnetic field (about 3500 gauss) the eigenfunctions of the triplet spin states are given by $|T_{+1}^1\rangle$, $|T_0\rangle$, and $|T_{-1}^1\rangle$ and can be related to those at zero field by mixing coefficients that depend on the strength and direction of the magnetic field. The selective population and depopulation of the triplet spin sublevels results in a non-Boltzmann distribution of spin populations in the triplet manifold. This manifests itself in triplet spectra that differ from the normal intensity pattern in that some of the transitions show enhanced absorption (*a*) while the others are in emission (*e*), i.e., show esp.

The relative intensity patterns of the epr spectra can be predicted from the initial population or depopulation rates (Table 1). In all of the *in vitro* chlorophylls (Table 2), as expected, a change in the sign of the polarization of the signals occurs when the external magnetic field is along the axis or axes of the largest population:depopulation rates.

Table 2 gives the esp patterns for the systems that we have studied and examples of typical triplet spectra we have observed are given in Fig. 1 and in ref. 11. The dominant relative population:depopulation rates are assigned in Table 2. Our results for *in vitro* Chl *a* and Chl *b* show qualitative agreement with the results obtained by other workers (9, 10, 18, 19), in that the population and decay occurs mainly through the in-plane spin eigenfunctions of the macrocycle.

As has been pointed out before, the esp appears to be very sensitive to the aggregation state of the chlorophylls (12). The *in vivo* esp is unusual in that there is no change in the sign of the polarization along a particular axis. In the ~3500 gauss magnetic field in which the epr triplet spectra are recorded, it is principally the T_0 sublevel that is being populated relative to T_{+1} and T_{-1}, for all three orientations. Note that this polarization scheme does not appear in Table 1.

Radical Pair Intermediate Mechanism. We propose to explain the esp of the *in vivo* triplet spectrum by the formation of a radical pair intermediate in the initial photo-act (Mechanism 1).

$$\begin{array}{lcl} & & \text{Chemistry} \\ & & \uparrow b' \\ (BB)X^r \xrightarrow{h\nu} {}^1*(BB)X^r \xrightarrow{a} & & \alpha_S{}^1(B^+B^-)X^r \\ & & + \\ & & \beta_T{}^3(B^+B^-)X^r \\ & & \downarrow b \\ & & (1-\gamma_{CT})^3*(BB)X^r + \gamma_{CT}{}^3(B^+B^-)X^r \end{array}$$

Abbreviations: epr, electron paramagnetic resonance; ZFS, zero field splitting; D, E, zero field splitting parameters; esp, electron spin polarization; *a*, absorption; *e*, emission; S, singlet states; T, triplet states; ISC, intersystem crossing; Bchl, bacteriochlorophyll; Chl *a*, *b*, chlorophylls *a* and *b*; Bp, bacteriopheophytin; BB, bacteriochlorophyll special pair.

† We have earlier suggested that the *in vivo* photo-active special pair is a chlorophyll-water sandwich (15). No direct evidence that water is present in the *in vivo* special pair exists, although this is a highly plausible assumption (16). In this communication we will, therefore, use the neutral symbol BB for the special pair. The role of water in the special pair has been discussed elsewhere (15, 17).

Table 1. Polarization patterns predicted from relative zero field population:depopulation rates[a]

Relative population:depopulation rate[b]			Polarization at canonical orientations[c]					
k_x	k_y	k_z	Z_I	X_{II}	Y_{II}	Y_I	X_I	Z_{II}
1	0	0	*e*	*e*	*a*	*e*	*a*	*a*
0	1	0	*e*	*a*	*e*	*a*	*e*	*a*
0	0	1	*a*	*a*	*a*	*e*	*e*	*e*
1	1	0	*e*	*e*	*e*	*a*	*a*	*a*
1	0	1	*a*	*e*	*a*	*e*	*a*	*e*
0	1	1	*a*	*a*	*e*	*a*	*e*	*e*
2	1	0	*e*	*e*	—	—	*a*	*a*
2	0	1	—	*e*	*a*	*e*	*a*	—
1	2	0	*e*	—	*e*	*a*	—	*a*
0	2	1	—	*a*	*e*	*a*	*e*	—
1	0	2	*a*	—	*a*	*e*	—	*e*
0	1	2	*a*	*a*	—	—	*e*	*e*

[a] This table gives representative population:depopulation rates for spin-orbit intersystem crossing (ISC) for isolated molecules and is not all inclusive.

[b] Valid if certain restrictions can be made in the value of T_{1e} (electron spin lattice relaxation time) relative to the population:depopulation rate constants (10).

[c] The assignments are made assuming $D > 0$ (the usual case for $\pi\pi^*$ triplet states) and $D > -3E > 0$.

Here BB stands for the Bchl special pair, X^r is the fully reduced primary electron acceptor, α_S and β_T are the respective fractions of singlet and triplet in the radical pair state, and γ_{CT} is the fraction charge transfer character in the epr-observed triplet state. The superscripts indicate a singlet (1), triplet (3), excited (*) or radical pair [(+) and (−)] state. It is expected that the triplet excitation is delocalized over both molecules in the ^{3}*(BB) state: ^{3}(*BB) ↔ ^{3}(B*B).

In Mechanism 1, electron transfer occurs (via path a) from the photo-excited singlet state within the Bchls of the special pair from one to the other Bchl molecule forming an initial charge-transfer radical pair. For such a pair in high magnetic fields the singlet and T_0 states of the radical pair manifold are closest in energy (20, 21) so that ISC occurs mainly to the T_0 sublevel. This mixed radical pair state has too short a lifetime to be observed by ordinary epr. Because the normal photosynthetic path (b′) is blocked in our *in vivo* experimental systems, in step (b) the excitation goes to the epr-observed triplet, which has partial charge transfer character as indicated by γ_{CT}. We emphasize that it is this state which is observed in the epr experiment, not the initial radical pair. The spin selection that occurs in the initial radical pair is preserved, and the unusual polarization in the triplet spectra thus gives indirect information on the nature of the initial radical pair. A radical pair mechanism for the photoactivity of chlorophyll–water adducts (15) and for the primary event in chlorophyll special pairs has been proposed previously (16). Mechanism 1 as written implies a basic asymmetry in the special pair such that one Bchl in the special pair acts as electron acceptor for the other acting as donor in the primary event.

Very recent optical studies of Parson *et al.* (22–24) provide experimental evidence for a radical pair intermediate in photosynthesis (via b′). Parson and coworkers have measured optical absorbance changes after flash excitation of reaction center Bchl when the usual electron acceptor is fully reduced, and observed two different optical transient states.

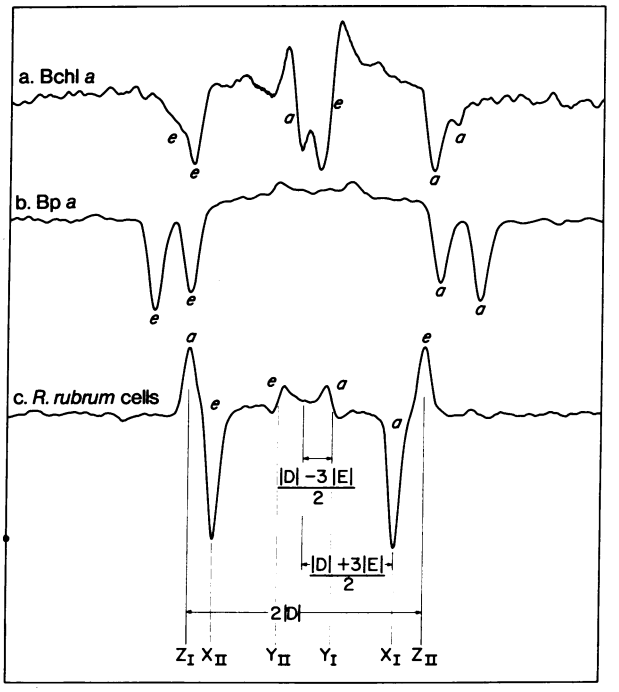

FIG. 1. (a) Triplet epr spectrum of bacteriochlorophyll *a* in 10% pyridine:90% toluene; (b) triplet epr spectrum of bacteriopheophytin *a* in 10% pyridine:90% toluene; (c) triplet epr spectrum of whole cells of *R. rubrum* in 50% glycerol:50% buffer. The intensity patterns are indicated on all spectra. The total scan range is 1000 gauss, temperature = about 5°K. The peaks are labeled in (c) to correspond to the definitions in Tables 1 and 2.

One state with a half-life of approximately 6 nsec has spectral properties of both the Bchl anion and cation (which we believe corresponds to the initial radical pair of Mechanism 1). The second transient with a half-life of 120 μsec has an optical spectrum similar to that of Bchl triplets (which we identify with the triplet observed in the epr experiments). Quantum yield studies as a function of temperature have been interpreted by Parson *et al.* (23, 24) in terms of electron transfer in the special pair. By the use of picosecond kinetic techniques on bacterial reaction center preparations under conditions in which electron transfer can occur, Rockley *et al.* (24) have provided optical evidence that a Bchl radical pair state is an intermediate in the normal initial electron transfer reaction. Further, Zubkov (25) has observed a system exhibiting unusual esp similar to *in vivo* Bchl which is explained to result from a biradical state. It is encouraging that both the optical and triplet data can be interpreted by Mechanism 1, in which the short-lived transient is identified with the initial radical pair, and the long-lived transient with the triplet observed by epr.

The lifetime of 120 μsec for the long-lived transient of Parson *et al.* (23) must, however, be compared to the 6 μsec lifetime observed for reaction center triplets (4). The optical experiments measure the true lifetime of the triplet state, whereas the epr experiment reflects not only the true triplet lifetime, but also spin lattice relaxation in competition with microwave saturation of the triplet levels. To observe the true triplet lifetime by epr, extremely low microwave powers must be used, a condition which is very difficult to achieve in these *in vivo* systems with ordinary epr equipment. The lifetime observed in the epr experiment is, therefore, related to transitions within the triplet manifold,

Table 2. *In vitro* and *in vivo* triplet spectra

Species	ZFS[a]		Polarization[b]						Relative population: depopulation[c]			
	|D| (cm^{-1})	|E| (cm^{-1})	Z_I	X_{II}	Y_{II}	Y_I	X_I	Z_{II}	k_z	k_y	k_x	D/E
In vitro[d]												
Chlorophyll *a*	0.0275	0.0036	*e*	*e*	*e*	*a*	*a*	*a*	0	1	1	7.6
Pheophytin *a*	0.0339	0.0033	*e*	—	*e*	*a*	—	*a*	0	2	1	10.3
Chlorophyll *b*	0.0287	0.0037	*e*	*e*	*e*	*a*	*a*	*a*	0	1	1	7.8
Pheophytin *b*	0.0332	0.0028	*e*	*e*	*e*	*a*	*a*	*a*	0	1	1	11.9
Chlorophyll c_1	0.0269	0.0055	*e*	*e*	*e*	*a*	*a*	*a*	0	1	1	4.9
Chlorophyll c_2	0.0276	0.0058	*e*	*e*	*e*	*a*	*a*	*a*	0	1	1	4.8
Bacteriochlorophyll *a*	0.0224	0.0055	*e*	*e*	*e*	*a*	*a*	*a*	0	1	1	4.1
Bacteriopheophytin *a*	0.0256	0.0045	*e*	*e*	—	—	*a*	*a*	0	1	2	5.7
Bacteriochlorophyll *b*	0.0252	0.0059	*e*	*e*	*e*	*a*	*a*	*a*	0	1	1	4.3
Bacteriopheophytin *b*	0.0247	0.0050	*e*	*e*	*e*	*a*	*a*	*a*	0	1	1	5.0
In vivo (whole cells)[e]												
Rhodospirillum rubrum (H)	0.0185	0.0033	*a*	*e*	*e*	*a*	*a*	*e*	—	—	—	5.6
R. rubrum (D)	0.0185	0.0034	*a*	*e*	*e*	*a*	*a*	*e*	—	—	—	5.4
Rhodopseudomonas sphaeroides (H)	0.0182	0.0035	*a*	*e*	*e*	*a*	*a*	*e*	—	—	—	5.2
R. sphaeroides (D)	0.0183	0.0032	*a*	*e*	*e*	*a*	*a*	*e*	—	—	—	5.7
Rhodopseudomonas palustris (H)	0.0182	0.0035	*a*	*e*	*e*	*a*	*a*	*e*	—	—	—	5.2
R. palustris (D)	0.0184	0.0031	*a*	*e*	*e*	*a*	*a*	*e*	—	—	—	5.9
Rhodopseudomonas gelatinosa	0.0184	0.0028	*a*	*e*	*e*	*a*	*a*	*e*	—	—	—	6.6
Rhodopseudomonas viridis (H)[f]	0.0184	0.0033	*a*	*e*	*e*	*a*	*a*	*e*	—	—	—	5.6
R. viridis (D)	0.0184	0.0033	*a*	*e*	*e*	*a*	*a*	*e*	—	—	—	5.6

[a] Obtained at ~ 5°K.
[b] The "y" orientation cannot be assigned to a specific in-plane direction, and the transition is assigned by defining $E < 0$.
[c] Assignments made by comparing with Table 1.
[d] We have tried to make sure that the *in vitro* chlorophyll systems are monomeric by using a variety of strongly basic solvents, all of which yield identical results. However, some questions still remain about the state or states of the chlorophylls at cryogenic temperatures. Spectra were obtained using 1 kHz light modulation and 100 kHz field modulation with appropriate phase-sensitive detection. The signal phase is given relative to *R. rubrum*.
[e] Spectra were recorded using 100 Hz light modulation and 100 kHz field modulation with appropriate phase-sensitive detection. (H) and (D) designate proton- and deuterium-containing organisms.
[f] Cultivated in a modification of the medium described by Eimhjellen *et al.* (36).

whereas the optical experiment measures the true lifetime of the triplet state.

Zero Field Splitting Parameters. Since the magnitude of D is a qualitative measure of the average distance between the unpaired electrons, it is not surprising that the chlorophylls with extensive electron delocalization have relatively small values of D (Table 2). The magnitude of E gives a measure of the deviation from axial symmetry in a molecule.

A comparison of *in vivo* and *in vitro* triplet data in Table 2 reveals that the ZFS parameters in the bacterial photo-reaction centers are reduced relative to monomeric Bchl. This suggests a participation of more than one Bchl molecule in the genesis of the triplet signal (4, 12), and it is reasonable to consider the special pair to account for the decrease in D. However, the observed 20% reduction in the value of D is probably too small to be explained only in terms of a simple radical pair formed by electron transfer between the two Bchl molecules in the special pair. Reductions in ZFS parameters have been explained in several other systems by a rapid averaging of the triplet excitation over two electronic states (26) or over two molecules appropriately oriented (27). Reduced ZFS parameters have also been observed in the lowest excited triplet state of either donor or acceptor in systems that form charge transfer complexes (28–30). In this latter situation, both D and E are simultaneously reduced by a fraction of charge transfer character, thus maintaining a constant D/E ratio.

Thus, a process that combines the rapid transfer (on the epr time scale) of excitation between two suitably oriented Bchl molecules, modified by inclusion of charge transfer character in the triplet state, can explain the ZFS results in Table 2, as well as those of Leigh and Dutton (4) on reaction center preparations. One can account for the essentially constant D/E ratio of *in vivo* preparations (Table 2, ref. 4) by considering the postulated model for the special pair (16, 31) in which one Bchl molecule is rotated relative to the other one, maintaining the z axes of the Bchls parallel. Thus, a rotation of approximately 40° yields the average D/E ratio observed in the *in vivo* experiments. Adding appropriate charge transfer contributions to the triplet state then gives the observed ZFS. Thus, for *R. rubrum* whole cell prepara-

tions a charge transfer contribution of about 18%[‡] gives the correct ZFS, and a combination of charge transfer character (up to 30%) and small adjustments in rotation can give the ZFS reported in other intact organisms (Table 2) and in reaction center preparations (4).

Bacteriopheophytin Participation. Mechanism 1 satisfactorily explains the ZFS and esp experimental data, but an alternative possibility must also be considered. Bacterial reaction center preparations contain both Bchl and bacteriopheophytin (Bp) (32). The close similarity in the ratio D/E for the *in vivo* Bchl preparations with that for Bp (Table 2 and Fig. 1) suggests a possible role for Bp as the primary electron acceptor. We can suppose that the initial electron transfer goes from the special pair (BB) to Bp, and the radical pair precursor of the triplet state in this case is formed between the oxidized (BB) special pair and the reduced Bp. Intersystem crossing would take place as in Mechanism 1, only now the average distance between the two unpaired electrons is possibly greater, thereby reducing the energy difference between S and T_0 and facilitating ISC. The observed *in vivo* triplet in this formulation is then actually the triplet of bacteriopheophytin with partial charge transfer character.

A difficulty with this interpretation is the energy difference between Bchl and Bp excited states. Since it has been shown that correlations between ZFS and triplet state energies can be made (33), the data of Table 1 suggest that the excited Bchl triplet lies below that of Bp, and consequently the triplet energy would be expected to be trapped in BB. Nevertheless, until the relative triplet state energies are determined with more precision, a mechanism involving Bp in the primary act cannot be excluded, and indeed, some combination of mechanisms with and without Bp participation may be operative in some situations.

Singlet Fission. Yarmus *et al.* (34) have reported that triplet excitons can be formed by an extremely efficient singlet fission process: $S_0 + S_1 \rightarrow (T_1T_1) \rightarrow T_1 + T_1$ where S and T represent the singlet and triplet states, respectively, and (T_1T_1) denotes a correlative triplet exciton pair formed between two molecules in the T_1 state.

Swenberg *et al.* (35) showed that this process implies selective population of the T_0 (high field) triplet substate for all orientations of the molecular axes relative to the field, thus providing still another possible route to the esp observed in the *in vivo* experiments. The special pair in Bchl reaction centers may provide a favorable situation for a singlet fission to the triplet state to occur, but here again the experimental data required to support or exclude singlet fission are not yet available.

SUMMARY

The unusual ZFS and esp of triplet states in photosynthetic bacteria are interpreted here by an initial radical pair formed in the primary photo-act (Mechanism 1). This mechanism seems to be highly compatible with the recent optical studies of Parson (22–24). Other possible mechanisms, involving bacteriopheophytin and singlet fission, are also discussed. These appear to be less plausible, but additional data will be required for a more definitive decision. The technique of optically detected magnetic resonance introduced into this field by Clarke *et al.* (18, 19) appears to offer the best possibilities for providing the information necessary for selection between the mechanisms discussed here.

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‡ Percent charge transfer character was determined by the method described by Möhwald (30), which gives the percent charge transfer character within 20%.

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