TRIPLET STATES IN PHOTOSYNTHESIS*

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SUMMARY

A comparison of zero field splitting (ZFS) and spin polarization of triplet spectra of bacteriochlorophyll a in vitro and in vivo provides support for the special pair model of photoreactive chlorophyll in photosynthetic bacteria. Spin polarization of the triplet spectra is a new and unique probe of primary events in the light conversion act in photosynthesis.

INTRODUCTION

The recent important observation by Dutton et al. (1) of electron spin resonance (esr) triplet signals in photosynthetic bacteria has stimulated new interest in suggestions advanced long ago that chlorophyll triplet states may be involved in photosynthesis (2,3). Leigh, Dutton et al. (4-7) were successful in recording triplet spectra at low temperature in photosynthetic bacteria, provided electron transfer from the photoreaction center was prevented by reduction (sodium dithionite) and saturation of the usual electron acceptors. The in vivo triplet data were analyzed by comparison of the zero field splitting (ZFS) parameters D and E with those determined for chlorophyll triplets in vitro. In large organic compounds with π-systems of delocalized electrons, D is a rough measure of the size of the π-system (or the area over which the triplet electron

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spins are delocalized) and E is related to the axial symmetry of the system (8). Because no triplet spectra had ever been recorded on bacteriochlorophyll *in vitro*, Leigh and Dutton (7) were compelled to use ZFS of chlorophylls *a* and *b*, or the ever more remotely related porphyrins, for the comparison. Leigh and Dutton concluded that their *in vivo* ZFS triplet parameters could best be explained on the basis of the special pair model of photo-reactive chlorophyll proposed by Norris *et al*. (9,10). In this model, a chlorophyll-water-chlorophyll "sandwich" is invoked to account for the optical, esr, and endor properties characteristic of *in vivo* photo-reaction centers (11).

We have been successful in recording *in vitro* complete (full-field) esr photo-induced triplet spectra for all of the major, well-characterized chlorophylls, including bacteriochlorophyll *a* (12) (Table I), and are now able to make a direct comparison between *in vitro* and *in vivo* bacteriochlorophyll triplet state parameters (Table I). The *in vivo* D value of 0.0187 cm^{-1} is significantly smaller than the *in vitro* D value of 0.0224 cm^{-1}. The 20% decrease D is entirely compatible with the special pair model, which requires sharing of the triplet by two chlorophyll molecules, with a consequent decrease in D because the size of π-system in which the triplet exists has been increased.

The *in vivo* triplet spectrum of bacteriochlorophyll (Fig. 1) is polarized, i.e., has anomalous intensities indicative of both absorption and emission, which are a consequence of non-Boltzmann populations of the three energy sub-levels of the triplet. The *in vivo* spectra indicate that the triplet energy levels are populated by processes that are nearly independent of the orientation of the excited chlorophylls relative to the magnetic field. The *in vitro* and *in vivo* spectra exhibit basically

TABLE I

ZFS Parameters of *In Vitro* and *In Vivo* Chlorophylls[a]

Chlorophyll Species	Aggregation State	ZFS |D| (cm^{-1})	ZFS |E| (cm^{-1})
In Vitro[b]:			
Bacteriochlorophyll *a*	monomer	0.0224	0.0055
Chlorophyll *a*	monomer	0.0275	0.0036
Chlorophyll *a*	dimer[c]	0.0282	0.0032
Chlorophyll *b*	monomer	0.0287	0.0037
Chlorophyll *b*	oligomer[c]	0.0277	0.0032
In Vivo:			
R. rubrum cells		0.0187	0.0034
Spinach chloroplasts		0.0284	0.0039

[a]Limits of error for |D| and |E|: $\pm$ 0.0005 cm^{-1}.
[b]Average of both ^{1}H and ^{2}H compounds.
[c]One determination.

different polarization. The *in vivo* polarization is unusual because only the middle energy level of the triplet state appears to be populated. (In attempting to account for the *in vivo* experimental results, Leigh and Dutton (7) appear to have incorrectly evaluated the contribution of the magnetic field angular dependence to the singlet-triplet cross-over, and thus did not recognize the unusual aspects of the polarization in the *in vivo* triplet spectra.) On the basis of present knowledge about polarization phenomena in singlet-triplet intersystem crossing, there appears to be no way in which the *in vivo*

polarization can be explained in terms of single bacteriochlorophyll molecules. All the *in vitro* chlorophyll triplet spectra we have obtained show polarization that can be explained in terms of spin-orbit facilitation of the singlet-triplet transition. However, the standard spin-orbit mechanism for the birth and decay of the triplet state cannot account for the observed polarization in photosynthetic bacteria.

Because spin-orbit coupling in a single molecule appears to be basically incapable of accounting for the observed spin polarization, it is natural to consider the polarization in the chlorophyll special pair context. Our earlier proposal suggests that the primary light conversion event in the chlorophyll special pair forms the species $Chl^{+\cdot}$ H_2O $Chl^{-\cdot}$, or the equivalent neutral species $[(Chl^{+\cdot}\ OH^{-})(Chl^{-}\ H^{+\cdot})]$, in which one chlorophyll molecule is oxidized and the other reduced (9,10). The formation of these species in the primary light conversion act leads to triplet states in which only the middle energy level is populated, and explains the polarization observed in the *in vivo* *R. rubrum* triplet spectra (Fig. 1B). The special pair proposal for *in vivo* photo-reactive bacteriochlorophyll is consistent with the triplet ZFS and polarization data, as well as the optical, esr, and endor properties of *in vivo* photo-reactive chlorophyll.

The situation in green plants is considerably more complex. Leigh and Dutton (7) report photo-induced triplet signals in a spinach chloroplast photosystem I preparation. We have also been able to observe triplet spectra in intact algae and in intact spinach chloroplasts (Fig. 1D), but the triplet spectra are different from those described for green plant active center preparations by Leigh and Dutton (7). Our spectra resemble in ZFS (but not polarization) triplet spectra obtained from Chl *a*

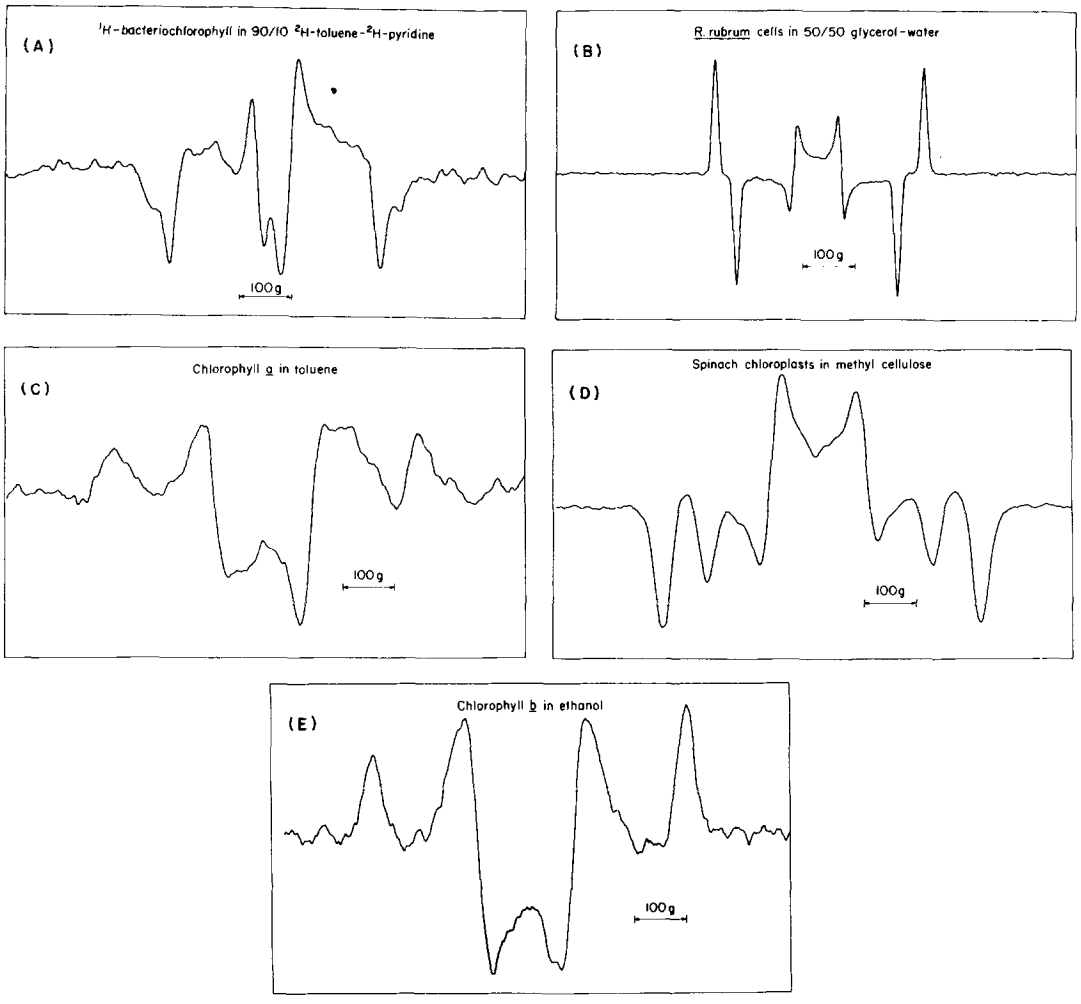

Figure 1. Triplet esr spectra of chlorophylls, recorded at 5°-20°K. The spectra were generated by light modulation: (A) Bacteriochlorophyll in 90% toluene-10% pyridine; (B) Whole cells of R. rubrum in 50:50 glycerol/water; (C) Chlorophyll a in toluene; (D) Chloroplasts in aqueous methyl cellulose; (E) Chlorophyll b in ethanol.

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or Chl b monomers or oligomers, $(\text{Chl } a_2)_n$ (13). We are unable to state with certainty whether the green plant triplet spectra we have obtained arise in chlorophyll a or b, because the ZFS parameters of a and b triplets in vitro are sufficiently similar as to preclude positive differentiation. It is possible that the in vivo triplet we see in green plants may arise from aggregated

chlorophyll species in antenna chlorophyll. Further, because disaggregated (monomeric) chlorophylls can be produced by treatment of chloroplasts with detergents, we see no way at present in which the origin of green plant triplet spectra can be unambiguously assigned.

Triplets in chlorophyll a and chlorophyll b oligomers have ZFS slightly different from those of the corresponding monomers. However, the triplet polarization in the aggregated chlorophyll spectra are quite different from those produced in monomer chlorophyll, and provide sufficient additional information to make it very likely that a more definitive interpretation of the in vivo spectra will be possible. Polarization is a kinetic process and kinetic data on the rate processes involved in population and depopulation of the triplet energy sub-levels will be required for full interpretation and utilization of the polarization information available in both the in vivo and in vitro triplet spectra. Quantitative kinetic data on spin polarization, in our opinion, will make it possible to use photo-induced and esr detected triplet state data as a new and powerful probe of green plant photosynthesis.

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