Magnetophotoselection Applied to the Triplet State Observed by EPR in Photosynthetic Bacteria*

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SUMMARY

Magnetophotoselection (MPS) techniques have been used to study the triplet state observed by electron paramagnetic resonance (epr) in photosynthetic bacteria. Intact R. rubrum chromatophores and systems in which the effects of energy transfer via antenna chlorophyll molecules have been minimized were examined. These preliminary results indicate that there is order in the bacteriochlorophyll antenna system, that the optical transition at 890 nm appears to be along the triplet y axis of the bacteriochlorophyll special pair ($Bchl_{SP}$), and that the resultant transition moment associated with 800 nm is approximately parallel to the long wavelength transition moment of the $Bchl_{SP}$.

Since the original observation by electron paramagnetic resonance (epr) spectroscopy of triplet states in photosynthetic systems by Dutton et al. (1) magnetic resonance investigations of the triplet state have been employed to probe the dynamic and structural features of the primary process in bacterial photosynthesis (2-5). The information content of triplet epr spectra can be increased by selective excitation of the molecules to the triplet state with plane polarized light oriented in specific directions relative to the magnetic field (H) used in the epr experiment. This process of magnetophotoselection (MPS)** has now been applied to triplet states observed in intact chromatophores of photosynthetic bacteria, and to chromatophores treated chemically to disrupt energy transfer from the antenna to the photo-reaction center.

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**A description of triplet state epr spectroscopy and MPS is given in (6) and references therein.

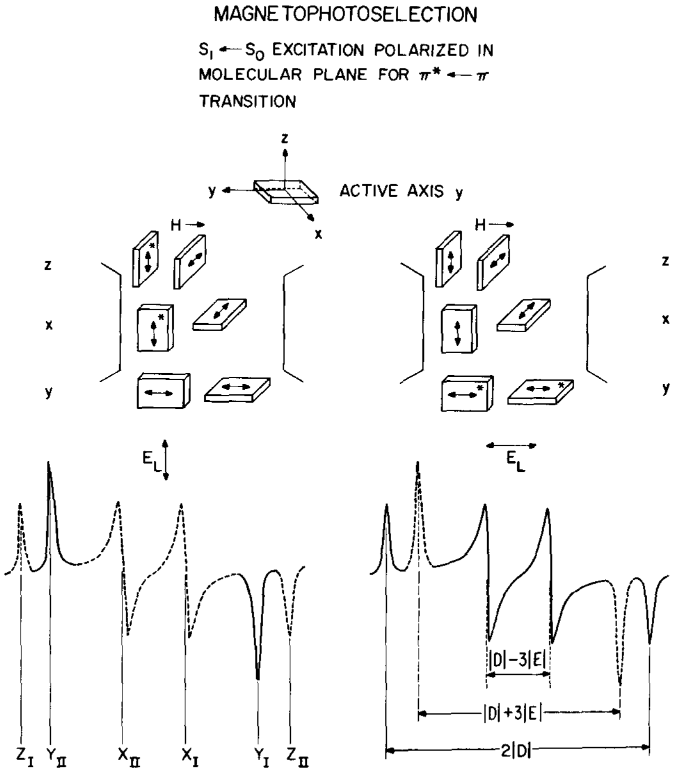

Fig. 1. Magnetophotoselection is illustrated for excitation polarized along the in-plane y axis (signified by the double-headed arrow in the boxes). The orientations with molecular axes along the magnetic field (H) give rise to the epr transitions in randomly oriented triplet systems. Each orientation gives rise to two transitions. The six transitions are labeled in the lower left hand spectrum. Here the z orientation is defined as that for which the fine-structure splitting is maximized. Furthermore, the splittings are described by the zero field splitting parameters D and E as shown in the lower right hand spectrum. In the extreme, there are two ways for a molecular axis to be along H as illustrated by the six orientations of the boxes shown in both the left and right side of figure. When the molecules are excited with plane polarized light with the electric vector of the light (E_L) either perpendicular (left hand side of figure) or parallel (right hand side of figure) to H only molecules with certain orientations will be excited (indicated by an asterisk (*)). The spectra below show which transitions will appear enhanced (dashed lines) and attenuated (full lines) for the two cases.

The utility of MPS is based on the fact that in a first derivative epr absorption spectrum recorded on a randomly oriented system of triplet state species the peaks in the spectrum arise only from those relatively few molecules that have one of their magnetic principal axes* oriented along the applied magnetic field. This axis system can, in general, be related to the molecular framework. For example, in monomeric chlorophylls the x and y triplet axes are generally taken to be in the plane of the macrocycle. Although the detailed assignment of the triplet axes in chlorophyll _in vivo_ aggregates is not completely confirmed (5), exact knowledge of the coordinate system in molecular terms fortunately is not essential in order to interpret MPS results. Ultimately any given axis system, in this case the triplet axis system (see Fig. 1), is sufficient to determine the directions of optical transitions.

A second important aspect of MPS is that the excitation which populates the singlet state (ultimately leading to the triplet state by intersystem crossing) in many aromatic compounds is polarized dominantly in the molecular x,y plane (*$\pi \leftarrow \pi$ transition). This is expected to be the case for the chlorophylls (7). Thus, excitation of chlorophyll with plane polarized light in which the electric vector of the light (E_L) is either parallel or perpendicular to the magnetic field (H) used in a triplet epr experiment leads to selective excitation of molecules with different orientations relative to the magnetic field. The principles of MPS are illustrated for monomeric chromophores in Fig. 1. In this schematic the transition moment is polarized along one axis in the molecular plane.

METHODS AND MATERIALS

The methods used for observing the triplet spectra at near 5°K have been previously described (2,8). The samples were irradiated with a Varian Eimac 300 W xenon lamp. The "broad" band light excitation was achieved with a Corning 2-64 filter or an Ealing 26-4465 filter. "Narrow" band excitation at 800 nm (half-bandwidth (HBW) 11.3 nm) and 890 nm (HBW 12.5 nm) was achieved with Ditric Optics 3 cavity near-infrared bandpass filters. A Polaroid Corp. HR 2.5 polarizing film was used to obtain polarized light.

Chromatophores of _R. rubrum_ were treated with K_2IrCl_6 to oxidize and inactivate the antenna bacteriochlorophyll (9,10).

All epr samples were prepared in 50% glycerol:50% buffer solution.

RESULTS AND DISCUSSION

The results for intact and antenna-inactivated chromatophores of _R. rubrum_ are summarized in Table I. Figure 2 shows some typical epr spectra on these systems obtained by using the magnetophotoselection technique.

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*The axis system in this case is defined by the electron spin-spin coupling tensor and does not necessarily coincide with the axis system defined by the optical transitions.

Table I. Effect of Plane Polarized Light on the EPR Triplet Spectral Intensities for *R. rubrum*.

	Excitation		
	Broad $\sim$700 nm-2500 nm	800 nm Δ^a 11.3 nm	890 nm[b] Δ 12.6 nm
Untreated[c] chromatophores			
MPS(x)[d]	-	-	-
MPS(y)	+	+	+
MPS(z)	e	-	+
Treated chromatophores			
MPS(x)	-	-	-
MPS(y)	+	+	+
MPS(z)	-	-	-

[a] Δ is the spectral width at which the transmission is 50% of the peak value. [b] Low temperature absorption of P865 (11-13). [c] Untreated and treated refers to treatment with K_2IrCl_6. [d] MPS(i), i = x,y,z refers to sign of polarization ratio observed for the triplet epr x, y, or z transitions, respectively. The polarization ratio is given by (14): $P_i = (I_{i\parallel} - I_{i\perp})/(I_{i\parallel} + I_{i\perp})$, i = x,y,z where $I_{i\parallel}$ and $I_{i\perp}$ refer to the intensity of a given epr transition excited by light polarized either parallel or perpendicular to the magnetic field. [e] $P_z = 0$, corresponding to no MPS effect.

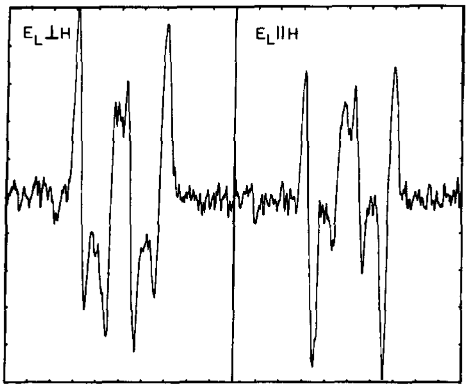

Fig. 2. Triplet epr spectra of chromatophores of *R. rubrum* treated with K_2IrCl_6 excited with polarized light at 800 nm with a half-bandwidth of 11.3 nm. Light vector (E_L) orientated relative to the magnetic field (H) as indicated.

Table I shows that there are obvious differences in the signs, MPS(i), i = x,y,z, of the polarization ratios, P_i, (see Table I) between the treated and untreated systems for both broad and narrow band excitation. In general, untreated systems with broad band excitation exhibit smaller MPS effects than treated cases. We interpret these differences, together with the differences in magnitude of polarization in the two cases, as the result of excitation transfer via antenna chlorophyll to the Bchl_{sp} whose triplet state is being observed. The MPS effects which are observed in the untreated case in the epr x and y transitions for broad band excitation, and for all epr transitions when 890 nm excitation is used indicate that there is order in the orientation of antenna chromophores or at least non-random directionality in the energy transfer process. Similar observations by other techniques have been made in bacteria (15) and green plant systems ((16) and references therein).

The MPS effects observed with narrow band excitation at 890 nm in chromatophores treated with K_2IrCl_6 can be rationalized as the result of direct excitation of the Bchl_{sp} in the reaction center. The singlet excitation appears polarized primarily along the direction of the triplet y axis of the Bchl_{sp}. A more quantitative analysis of the data indicates possibly a small contribution to the singlet transition which is along the triplet z axis.* This would result, for instance, if the two molecules of the Bchl_{sp} are not parallel or if there are charge-transfer effects (17).

Our results with narrow band excitation at 800 nm show that the resultant transition moment associated with this absorption is approximately parallel to the 890 nm long wavelenth transition moment of the Bchl_{sp}.

We have also observed substantial MPS effects in various _R. sphaeroides_ R26 systems (whole cells, treated chromatophores, and reaction centers) and in _R. viridis_ whole cells. These preliminary results show that this method provides a useful, additional tool to study the structural similarities and differences in various bacterial systems. Furthermore, it should ultimately be possible

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*This is assuming that all effects of antenna have been eliminated in the K_2IrCl_6 treated systems.

to establish the relative geometrical relationship between the optical transition moments of the various reaction center pigments and the Bchl_{sp}.

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