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# MAGNETOPHOTOSELECTION STUDIES ON *RHODOPSEUDOMONAS VIRIDIS* REACTION CENTERS

T.L. TROSPER [a*], HARRY A. FRANK [b], J.R. NORRIS [a] and M.C. THURNAUER [a]

[a] *Chemistry Division, Argonne National Laboratory, Argonne, IL 60439 and* [b] *Department of Chemistry, University of Connecticut, U-60, Storrs, CT 06268 (U.S.A.)*

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**Magnetophotoselection experiments were performed on the triplet state of *Rhodopseudomonas viridis* reaction center complexes isolated with the detergent sodium dodecyl sulfate. The angles between the optical transition moments of the reaction center chromophores and the triplet magnetic axes were determined. None of the transition moments of the spectroscopic forms of bacteriochlorophyll *b* in the reaction center lies along any of the triplet magnetic axes, in contrast to the situation in *Rhodopseudomonas sphaeroides* and *Rhodospirillum rubrum*. The magnetophotoselection results for *Rps. viridis* are combined with data from other orientation studies (Paillotin, G., Vermeglio, A. and Breton, J. (1979) Biochim. Biophys. Acta 545, 249–264; Frank, H.A., Friesner, R., Nairn, J.A., Dismukes, G.C. and Sauer, K. (1979) Biochim. Biophys. Acta 547, 484–501) to construct a diagram showing the relative orientations of the reaction center optical transition moments and the triplet magnetic axes of the primary donor with respect to the membrane plane.**

## Introduction

The composition of bacterial photosynthetic reaction centers, where the initial steps in the conversion of electromagnetic to chemical energy occur, has been thoroughly investigated (see, e.g. Ref. 1). A detailed study of *Rhodopseudomonas viridis* reaction centers has recently confirmed that the pigment composition of these complexes is similar to those of other organisms, although *Rps. viridis* contains BChl *b* instead of BChl *a* [2]. Lately, attention has been focused on arrangements and orientations of the chromophores within a reaction center complex, because a specific organization of the pigment molecules appears to be required for efficient performance of the primary steps in photosynthetic energy conversion.

In all organisms investigated so far, the porphyrin chromophores present are a special pair of bacteriochlorophylls which serves as the primary electron donor, two other bacteriochlorophylls with undetermined functions, and two bacteriopheophytins, at least one of which is an intermediate electron acceptor [3].

Three types of measurement have been used to study orientations of these pigments in reaction centers or chromatophore membranes. First, optical studies using polarized light – linear dichroism and photoselection measurements – have been made on oriented membrane samples from several organisms, and on *Rps. sphaeroides* reaction centers partially oriented in stretched polymer films. These investi-

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Abbreviation: BChl, bacteriochlorophyll; SDS, sodium dodecyl sulfate; ENDOR, electron-nuclear double resonance.

* Permanent address: Department of Physics, University of Missouri, Kansas City, MO 64110, U.S.A.

gations have given the relative orientations of chromophore transition moments with respect to the membrane plane, or the angles between projections of the transition moments onto the membrane plane [4–8]. Second, EPR spectroscopy of the triplet state of the reaction center special pair in oriented cells or membranes has indicated the orientations of the triplet magnetic axes with respect to the membrane plane in *Rhodospirillum rubrum* chromatophores [9] and in whole cells of *Rps. palustris* and *Rps. viridis* [10]. The third type of experiment is magnetophotoselection, in which light polarized parallel or perpendicular to the Zeeman magnetic field is used to generate the triplet state spectrum detected in an EPR experiment [11]. This technique was first applied to the *R. rubrum* chromatophores by Thurnauer and Norris [11,13] and has since been used to study *Rps. sphaeroides* reaction centers [14,15]. Data from these studies were used to calculate the angles between optical transition moments and the triplet magnetic axes of the excited special pair. Thus, magnetophotoselection can serve as a link between the optical and the other EPR investigations, providing orientation information that should be consistent with both. Together, these data allow a determination of the three-dimensional arrangement of the reaction center bacteriochlorophyll transition moments with respect to the membranes in which they are located.

*Rps. viridis* is the only organism for which both polarized optical and triplet EPR investigations have been made with oriented membrane samples [7,10]. We report here results of magnetophotoselection experiments on reaction centers isolated from this organism. The data are consistent with the results of the other studies. A possible arrangement of the optical transition moments of the special pair and the other BChl *b* molecules, and of the triplet magnetic axes, with respect to the membrane plane is presented. It successfully incorporates results from the different types of investigation on *Rps. viridis*.

## Materials and Methods

*Rps. viridis* cells grown photosynthetically in the medium of Eimhjellen et al. [16] were harvested after 5–7 days and stored frozen. Reaction centers were isolated from thawed cells according to the method of Trosper et al. [17] except that 40 mg SDS/$\mu$mol BChl *b*, at a final detergent concentration of 1.8% was used to solubilize membranes from broken cells. Reaction center complexes precipitated with $(NH_4)_2SO_4$ were resuspended in 50 mM Tris–HCl, pH 8.0, and dialyzed extensively against the same buffer. Samples for EPR spectroscopy were prepared under $N_2$ in dim light. Combined aliquots of reaction center suspension and 40 mg/ml sodium dithionite solution were bubbled with $N_2$ gas, diluted with an equal volume of ethylene glycol, and transferred to 3 mm diameter quartz sample tubes. Aliquot volumes were chosen so that the final concentrations of reaction center and dithionite were $(2–5) \cdot 10^{-5}$ M and approx. 10 mg/ml, respectively. The samples were then frozen by slow immersion in liquid $N_2$. Badly cracked, bubbled, or cloudy glasses were not used for EPR spectroscopy.

Triplet EPR spectra were obtained with a Varian E9 X-band spectrometer equipped with an Air Products Helitran cryostat which maintained sample temperature between 5 and 6 K. Microwave power of 50 $\mu$W, below the saturation level, was used. The sample was excited by a 300 W xenon arc illuminator (Varian-Eimac) which was electronically modulated at 140 Hz. The output signal from the spectrometer (100 kHz field modulation frequency and 32 G field modulation amplitude) was referenced to the light signal using an Ithaco Model 393 lock-in amplifier for phase-sensitive detection. Signal averaging was accomplished with a Fabritek 1072 multichannel analyzer interfaced to the Sigma 5 computer of the Chemistry Division, ANL.

Values of the maxima and minima in the triplet spectra used to compute the projections of the optical transition moments onto the principal magnetic axes were obtained as described previously [10,14], by manually setting the magnetic field at these peak positions and averaging signal amplitudes over a time equal to several instrumental time constants. Zero-field splitting parameters were calculated by computer simulation of an experimental triplet spectrum obtained by exciting a sample with broad band far red, unpolarized light (Fig. 1B). Peak positions shifted less then 2 G when the interference filters and polarizers were in place during photoselection measurements.

For the photoselection experiments, light passed through a collimating lens, a 2 cm water filter

(omitted for 990 nm excitation), a Corning 2-64 filter, a focusing lens, the appropriate Ditric narrow band (±3 nm) interference filter, and an appropriate Polaroid polarizer immediately in front of the cavity. The Polarizer could be manually turned through 90°. For excitation at 830, 850 and 990 nm, Polaroid HR 2.0 was used; at 790 nm, a nonstandard polaroid sheet, a gift of Polaroid Corp., which was less optically dense then the HR type but provided similar polarization, was used.

Computer simulations of the observed spectral lineshapes were performed as described in Ref. 14. The procedures involved numerical diagonalization of the triplet state Hamiltonian and orientational averaging of the calculated oscillator strengths over the ensemble distribution [18].

Technical grade SDS was obtained from Matheson, Coleman, Bell. Other chemicals used were reagent grade.

Absorption spectra center preparations and of the polarizers were recorded with a Cary 14R spectrophotometer.

## Results

The triplet EPR spectrum excited with unpolarized far red light is shown in Fig. 1A, and the simulated spectrum in Fig. 1B. Zero-field splitting parameters for this triplet were calculated to be $|D| = 0.0155 \pm 0.0001$ $cm^{-1}$ and $|E| = 0.0036 \pm 0.0001$ $cm^{-1}$, which are within the experimental error of those previously reported [19,20]. The large modulation amplitude, which was used to increae sensitivity, apparently did not alter peak positions. As is the case for reaction center preparations from other organisms [20,21], the average rate constant for triplet decay at 5 K is consistent with a lifetime significantly longer than that observed optically at room temperature [22].

An example of triplet state spectra excited with 990 nm light parallel or perpendicular to the EPR magnetic field, and obtained by averaging 16 30-s scans, is shown in Fig. 2. These spectra have a signal-to-noise ratio too low to obtain accurate values for peak amplitudes. A similar phenomenon was observed in triplet spectra excited with light of other wavelengths. In order to reduce the signal-to-noise ratio without introducing possible peak height distortions

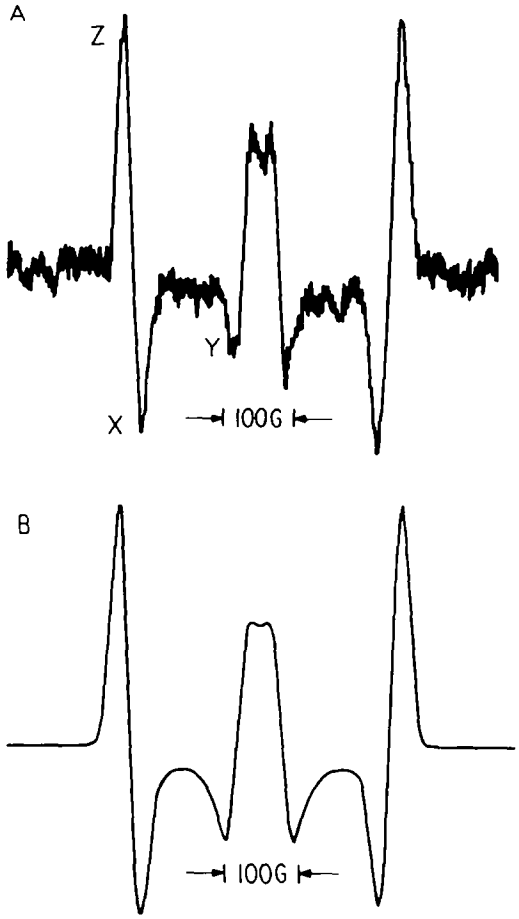


Fig. 1. (A) Triplet state EPR spectrum of *Rps. viridis* reaction center observed at 5 K, generated by unpolarized broad band far red light, and recorded under conditions described in text. The peaks have been assigned in the usual way to transitions in special pairs with their *Z*, *X* or *Y* principal magnetic axes, respectively, aligned parallel to the external magnetic field [20]. (B) Computer-simulated triplet spectrum.

caused by the modulation amplitude and without using inconveniently long instrumental time constants, the data required for calculation of the projections of the optical transition moments onto the triplet magnetic axes were obtained with the field held constant at the peak positions, as previously described [10,14]. Samples were illuminated with 790, 830, 850 or 990 nm polarized light. Signals observed for the 790 nm excitation were rather small, and the differences for polarizations parallel and perpendicular to the magnetic field were not significant compared to the noise level, even at constant field.

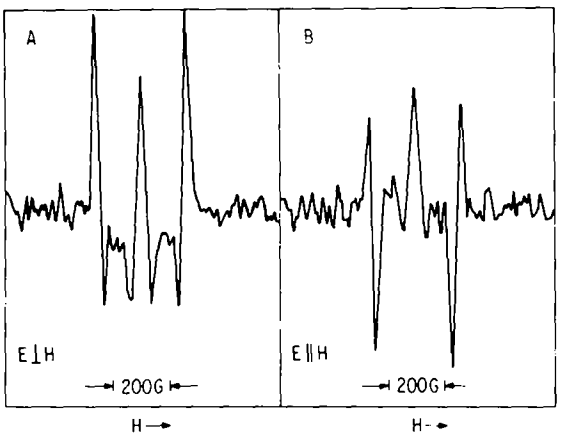


Fig. 2. Triplet state spectra excited with polarized 990 nm light and recorded under conditions described in text. (A) Electric vector, $\vec{E}$, of exciting light polarized perpendicular to magnetic field. (B) $\vec{E}$ parallel to magnetic field.

Results for the other three wavelengths are presented in Table I. The projections are the direction cosines for the optical transition moments of the reaction center chromophores absorbing at those wavelengths. The regions of solution result in uncertainties in the orientation angles of ±7° for the 990 nm transition moment, ±4° for the 850 nm transition moment and ±3° for the 830 nm transition moment. Errors owing to incomplete polarization by the polaroids would fall within these limits. Some uncertainty is also contributed by overlap of the 830 and 850 nm absorption bands, even at liquid helium temperature [28], and by residual light scattering in or localized heating of the sample glasses. These uncertainties cannot be quantitated easily; the usual steps were taken to minimize the latter effects.

As seen from the data in Table I, the long wavelength transition of the special pair does not lie primarily along any one of the magnetic axes. This result is in contrast to the orientation of the corresponding transition moments in *Rps. sphaeroides* and *R. rubrum* [14,20]; however, the present results are consistent with *Rps. viridis* data from other types of measurement (see below). This is of particular interest because the reaction center complexes used in the present study were solubilized with SDS. The procedure causes alterations in the EPR spectra of the quinone-Fe acceptors associated with the reaction center [19], but does not remove carotenoid [17]. In the other investigations on *Rps. viridis*, whole cells, chromatophores or reaction centers isolated by dodecyldimethylamine oxide treatment were used [7,10]. We can thus conclude that SDS treatment does not cause significant reorientation of the BChl *b* molecules in the complex.

## Discussion

The above results can be used to construct a three-dimensional picture of the magnetic axes and optical transition moments in *Rps. viridis* reaction centers. Combination of these data with the results of optical photoselection studies [7] and of EPR measurements on aligned whole cells [10] permits visualization of the vector orientations with respect to the photosynthetic membrane, in an arrangement consistent with all three investigations and with other dichroic studies as well [8].

The principal magnetic axes of the special pair triplet state, which are the principal axes of the diagonalized spin-spin coupling tensor [23], have been

TABLE I

ORIENTATION DATA

Projections (direction cosines) of the optical transition moments of reaction center chromophores onto the magnetic axes of the triplet. The absolute values listed provided the best fit of calculated spectra to experimental data.

| Excitation wavelength (nm) | Projection on magnetic axis | | | Region of solution for axis | |
|---|---|---|---|---|---|
| | *X* | *Y* | *Z* | *X* | *Y* |
| 990 | 0.50 (60°) | 0.75 (41°) | 0.43 (65°) | 0.40–0.60 | 0.70–0.80 |
| 850 | 0.70 (46°) | 0.48 (61°) | 0.52 (59°) | 0.65–0.75 | 0.41–0.55 |
| 830 | 0.50 (60°) | 0.70 (46°) | 0.52 (59°) | 0.45–0.55 | 0.67–0.73 |

chosen as the coordinate axis system (Fig. 3A). Then the 990 nm optical transition moment is placed in the diagram assuming all projections are positive; i.e., that the vector lies in the first quadrant. Paillotin et al. [7] concluded from photoselection measurements on magnetically oriented cells that this optical transition lies within 10° in the membrane plane, and the EPR measurements of Frank et al. [10] on similarly oriented cells suggested that the $Z$ magnetic axis of the triplet also lies approximately in the membrane. Thus, the 990 nm vector and the $z$ axis of the coordinate system in Fig. 3A may be assumed to define the membrane plane, within about 10°. The position of the plane is indicated in the figure. The normal to the membrane plane lies in the $x$–$y$ plane and must be perpendicular to the projection of the 990 nm transition moment in the same plane, i.e., at an angle of $(90°-\phi_{990})$ or 34 ± 10° with respect to the $X$ magnetic axis. The membrane normal is also shown in Fig. 3. Within the combined experimental errors of the measurements, this position falls in the range of solution found by Frank et al. [10] for oriented whole cells.

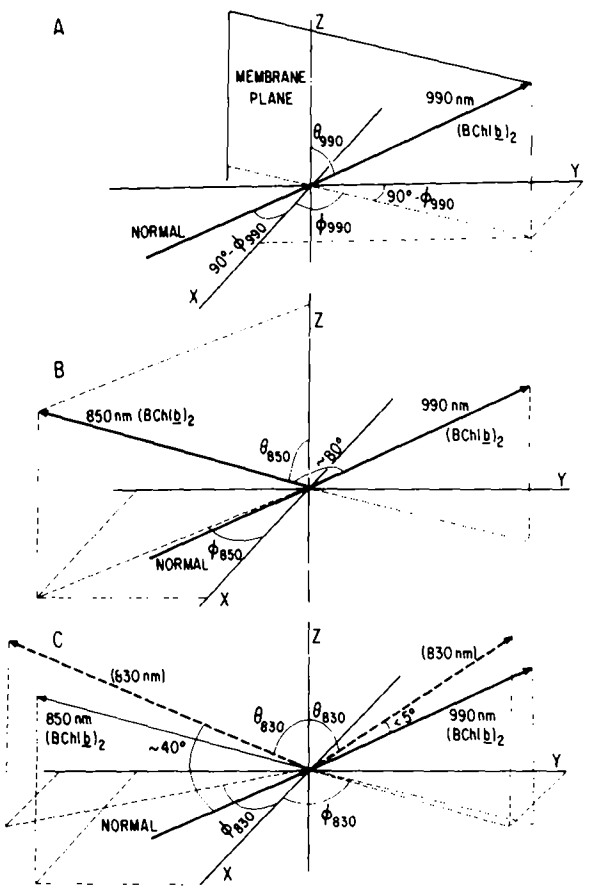


Fig. 3. Diagrams of the triplet magnetic axes and optical transition moments of some chromophores in the *Rps viridis* reaction center. The coordinate axes are the triplet $X$, $Y$ and $Z$ magnetic axis. (A) The orientations of the special pair 990 nm transition moment, of the membrane plane, and of its normal. (B) The orientation of the 850 nm transition moment with respect to the 990 nm transition and the membrane normal; (C) Two possible orientations of the BChl *b* 830 nm transition moments. The geometrical quantities used in these constructions are given in Table II. See text for discussion.

The 850 and 830 nm transition moments in the membrane-bound reaction center could also be placed on the diagram in the first quadrant. However, the projections listed in Table II are all absolute values, and locations in other quadrants are possible because the method does not distinguish positive and negative projections. It is thus useful to consider how the 850 nm transition moment would be oriented if any of the projections were negative. If either the $x$ or $y$ projection of the 850 nm vector is assumed to be negative, the 850 nm vector will be placed at an angle of approx. 80 ± 10° to the 990 nm transition moment. This relative orientation is in close agreement with that found by Shuvalov and Asadov [8] in their dichroism investigations of *Rps. viridis* reaction centers, as well as being within experimental error of the orientation suggested by optical photoselection studies [7]. In the diagram in Fig. 3B, the 850 nm transition moment has been added and the membrane plane omitted for clarity. The transition moment lies at an angle of about 30° with respect to the membrane normal, in agreement with the result of the optical photoselection studies which suggested that tha 850 nm transition is tilted out of the membrane plane by more than 50° [7].

If the 830 nm transition moment is placed in the first quadrant (all positive projection values), it lies, within experimental error, in the membrane plane and at a small angle to the 990 nm transition moment. If either the $x$ or $y$ projection of this transition moment is assumed to be negative, the vector lies at about a 40° angle with respect to the membrane normal (Fig. 3C). Neither of these orientations is in agreement with the average angle of approx. 25° out

TABLE II

ORIENTATION FACTORS

Parameters for optical transition moments in magnetic axis coordinate system. See text and Fig. 3 for definitions of angles.

| Excitation wavelength (nm) | $\cos\theta$ [a] | $\theta$ | Projection on $x$-$y$ plane | $\cos\phi$ [b] | $\phi$ | Angle to membrane normal [c] |
|---|---|---|---|---|---|---|
| 990 | 0.43 | $65 \pm 7°$ | 0.90 | 0.55 | $56 \pm 10°$ | assume 90° |
| 850 | 0.52 | $59 \pm 5°$ | 0.85 | 0.82 | $-35 \pm 8°$ | ≈35° |
| 830 | 0.52 | $59 \pm 3°$ | 0.85 | 0.58 | $\pm 54 \pm 6°$ | ≈90 or $37 \pm 6°$ |

[a] $\cos\theta = z$ projection.

[b] $\cos\phi = (x\text{-projection})/\sin\theta$.

[c] From $\vec{A} \cdot \vec{B} = \cos\alpha = A_xB_x + A_yB_y + A_zB_z$; where $\vec{A}$ = transition moment, $\vec{B}$ = normal, and $\alpha$ = angle between $\vec{A}$ and $\vec{B}$. The normal has projections of 0.83 on the $x$-axis and −0.56 on the $y$-axis.

of the membrane plane reported by other investigators on the basis of their optical measurements [7,8]. However, it must be remembered that the 830 nm absorption band is a convolution of bands resulting from absorptions by two BChl *b* molecules which do not necessarily have the same orientation with respect to the membrane plane. Analysis of their linear dichroism data has led Paillotin et al. [7] to suggest that the transition moment for one of these BChl *b* molecules may in fact lie approximately in the plane, and the other protrude at an angle of about 50° to it. The magnetophotoselection results reported here would be consistent with their interpretation for either of the 830 nm transition moments. Partial overlap of several absorption bands in this wavelength region precludes more detailed consideration of these results.

Fig. 3 represents a synthesis of information from three different types of measurement made in three different laboratories on samples prepared in different ways. The combination of these results (Refs. 7 and 10, and the present work) into a single picture without significant contradictions or inconsistencies is noteworthy. The diagram is not elaborated, however, to show an actual arrangement of reaction center chromophores. The spacing between the pigment molecules is not yet known, and data available [2,7,8, 26] do not unequivocally determine a particular arrangement of the two special pair BChls *b* which results in the observed exciton bands.

Despite several similarities between *Rps. viridis* and other extensively investigated photosynthetic bacteria, particular differences continue to become apparent. Some of these have been revealed by EPR studies. The evidence that the linewidth of the oxidized special pair is not narrower by a factor of $\sqrt{2}$ than that of the oxidized monomer, BChl $b^+$, as it is in BChl *a*-containing organisms [24,25], suggested that the special pair might not be a dimer [25]. However, recent extensive EPR and ENDOR experiments [26] led to the conclusion that the special pair in *Rps. viridis* is most likely a dimer of BChl *b* with distorted porphyrin rings and possibly a different intermolecular spacing. Also, the absolute values of the zero-field splitting parameters of the triplet are rather different in *Rps. viridis* from those of BChl *a* bacteria [19,20], again suggesting different orientations for the special pair molecules or possibly a different degree of charge transfer character in the triplet.

To this list of distinctive properties of *Rps. viridis* the present work adds another – the fact that the optical transition moments of the special pair do not lie primarily along any of the principal triplet magnetic axes. A colinear arrangement had been deduced from earlier investigations of *Rps. sphaeroides* [14] and *R. rubrum* [20] reaction centers. The difference between *Rps. viridis* and these other bacteria could again be due to slightly different dimer geometries. In this regard magnetophotoselection experiments on *Rps. palustris* are expected to be informative, because the triplet magnetic axes in reaction centers of this organism are similarly oriented with respect to the membrane as in *Rps. viridis* [10], but the zero-field splitting parameters are the same in *Rps. palustris*

as in *Rps. sphaeroides* and *R. rubrum* [27]. Also, determination of the sign of the zero-field splitting parameter *D* in *Rps. viridis* may provide information on the extent of charge transfer character of the special pair triplet state [20]. Use of all the EPR and optical data, and values of the oscillator strengths of the individual absorption bands [8] in molecular exciton calculations [29], will eventually permit construction of a three-dimensional model of the BChl *b* molecules in the *Rps. viridis* reaction center complex.

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