GEOMETRY FOR THE PRIMARY ELECTRON DONOR AND THE BACTERIOPHEOPHYTIN ACCEPTOR IN *RHODOPSEUDOMONAS VIRIDIS* PHOTOSYNTHETIC REACTION CENTERS

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ABSTRACT The tetrapyrrole electron donors and acceptors (bacteriochlorophyll, BCh; bacteriopheophytin, BPh) within the bacterial photosynthetic reaction center (RC) are arranged with a specific geometry that permits rapid (picosecond time scale) electron tunneling to occur between them. Here we have measured the angle between the molecular planes of the bacteriochlorophyll dimer (primary donor), B_2, and the acceptor bacteriopheophytin, H, by analyzing the dichroism of the absorption change associated with H reduction, formed by photoselection with RCs of *Rhodopseudomonas viridis.* This angle between molecular planes is found to be 60° ± 2. This means that the ultrafast electron tunneling must occur between donors and acceptors that are fixed by the protein to have a noncoplanar alignment. Nearly perpendicular alignments have been determined for other electron tunneling complexes involving RCs. These geometries can be contrasted with models proposed for heme-heme electron transfer complexes, which have emphasized that mutually parallel orientations should permit the most kinetically facile transfers.

INTRODUCTION

The electron transfers between donors and acceptors within the bacterial photosynthetic reaction center (RC) are extremely fast. A light-generated, excited state of bacteriochlorophyll dimer, B_2^*, likely reduces a bacteriochlorophyll, B, in ~1 ps (6–8), followed by a transfer from B^- to a bacteriopheophytin, H, in ≤10 ps (7, 8). The RC provides an experimental opportunity to examine complexes of cyclic tetrapyrroles that are fixed within a protein to permit efficient electron transfer. The most detailed models for the geometry of electron transfer complexes involving porphyrins have been discussed for heme-heme electron transfers, in which coplanar complexes are proposed (1–5). Here we have used photodichroism techniques to measure the angle between the B_2 and H donor and acceptor molecular planes in RC of *Rhodopseudomonas (Rps.) viridis.*

The bacterial RC electron donor B_2 and acceptor H have been found to be similarly oriented for all of the species studied (for reviews see references 9–11). A nearly perpendicular orientation of the BChl planes for the B_2 with respect to the membrane surface is shown by the fact that a plane formed by both the optical Q_X, Q_Y (12–15) and spin-polarized triplet magnetic X, Y axes is found to be tilted at 10–20° from the membrane normal (16, 17). A well-supported model suggests that the B_2 is composed of a staggered pair of parallel BChl molecules, overlapping along the Y (pyrrole ring I-III) direction (18–20). The linear dichroism of oriented membranes has shown that the B_2 Q_Y transition lies nearly parallel to the membrane plane (12–15).

The acceptor H molecular plane is also found to lie nearly perpendicular to the membrane surface, with the Q_Y transition lying along the normal to the membrane and the Q_X transition lying in plane (12–14). This arrangement has also been confirmed by photoselection experiments, where an angle of 90° has been measured between the B_2 Q_Y and H Q_Y directions (21–24).

These orientations for the B_2 and H are depicted in Fig. 1, where the X, Y plane represents a plane parallel to the membrane surface. As a result of this geometry, it can be seen that the angle between the B_2 and H planes can be determined by a measurement of the angle between the B_2 Q_Y and H Q_X transition moments. This has been estimated previously to be ~60° from photoselection experiments in which RCs of *Rps. sphaeroides* were excited within the

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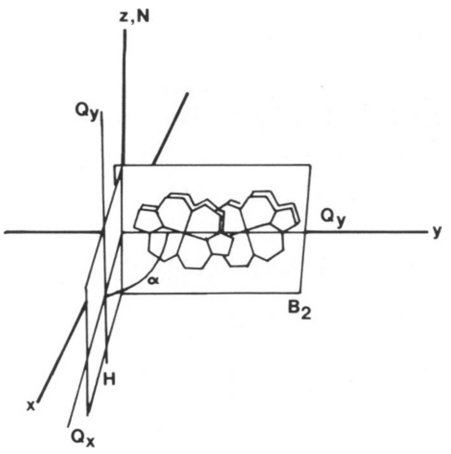

FIGURE 1 Orientations of the reaction center bacteriochlorophyll dimer, B_2, and acceptor bacteriopheophytin, *H*, with respect to the membrane plane. The plane *X, Y* represents a plane parallel to that of the membrane surface. A plane formed by both the *B* optical $Q_{X,Y}$ (12–15) and triplet magnetic *X, Y* directions lies nearly perpendicular to the membrane surface. The B_2 Q_Y transition lies essentially parallel to the membrane surface (12–15). The *H* molecular plane is also essentially perpendicular to the membrane, with the Q_Y and Q_X transitions lying, respectively, parallel and perpendicular to the membrane normal. The B_2 Q_Y and *H* Q_X transitions make an angle α.

BPh Q_X bands and the dichroism of the B_2 Q_Y absorption changes were measured (21). However, certainty in this measurement will depend upon the extent to which the H Q_X band is overlapped by the absorptions of other RC chromophores. Here we have determined this angle directly by photoselection within the B_2 Q_Y transition in RCs of *Rps. viridis* and measurement of the dichroism (with respect to this axis) of the absorption changes associated with H reduction. The geometry derived here for the B_2/H, donor/acceptor pair should compliment a forthcoming x-ray crystal structure of *Rps. viridis* RCs (25), by providing a criterion for identifying which of the two RC BPhs functions as the electron acceptor.

METHODS

In *Rps. viridis*, the H can be trapped in a reduced state by illumination of samples in which the electron transfer to subsequent acceptors has been blocked by previous chemical reduction (26, 27). Following prolonged illumination H^- accumulates as a result of irreversible (at $T < 200$°K) electron transfer from an RC-associated cytochrome *c* (cyt *c*) to the light-generated $B_2^+H^-$ state, according to the following scheme (26, 27):

$$\text{cyt}\ c^{2+}\ B_2H \xrightleftharpoons[10\ \text{ns}]{h\nu < 10\ \text{ps}} \text{cyt}\ c^{2+}\ B_2^+H^- \xrightarrow{>0.1\ \mu\text{s}} \text{cyt}\ c^{3+}\ B_2H^-.$$

The inefficiency of the cyt *c* donation by comparison to the $B_2^+H^-$ charge recombination means that many cycles of charge separation must occur before an appreciable amount of the stably trapped B_2H^- state accumulates (26, 27).

By exciting immobilized RCs at 100°K within the B_2 Q_Y transition (peak 990 nm) with linearly polarized light in a vertical direction, the angle, α, between the B_2 Q_Y axis and other transitions can be determined from their dichroism according to (9, 21–24):

$$p = \frac{\Delta A_V - \Delta A_H}{\Delta A_V + \Delta A_H} = \frac{3\cos^2\alpha - 1}{3 + \cos^2\alpha}.$$

ΔA_V and ΔA_H are the absorption changes measured with light vertically and horizontally polarized. The polarization, *p*, will be positive for $\alpha < 55°$ (limit = 0.5 for $\alpha = 0°$) and negative for $\alpha > 55°$ (limit = −0.33 for $\alpha = 90°$).

RESULTS

The absorption spectrum for dithionite-reduced RCs of *Rps. viridis* at 100°K is shown in Fig. 2 *A*. The B_2 Q_Y transition moment at 990 nm is well separated from other transitions. The H Q_X (545 nm) and Q_Y (805 nm) transitions are largely obscured by the overlying absorptions of other RC chromophores (14, 22, 23, 28, 29). Notable also in the spectrum are the cyt *c* α-band absorptions at 556 nm and a split peak at 548 and 550 nm.

Illumination for 15 min at 100°K with vertically polarized light ($\lambda > 900$ nm) produced the absorbance changes ($\Delta A_V + \Delta A_H$) shown in Fig. 2 *B*. The reduction of H is clearly indicated by the bleaching at 805 and 545 nm (14, 22–24, 26–29) and the increase at 688 nm associated with BPh anion formation (30). Also evident are the absorbance shifts of the B_2 Q_Y, and the complex absorbance changes near 832 and 610 nm. The bleaching near 545 nm also contains contributions due to the oxidation of cyt *c*, which absorbs at 548 and 550 nm in these preparations.

Fig. 2 *C* shows the dichroism ($\Delta A_V - \Delta A_H$) accompanying these absorbance changes. The polarizations for both the H Q_X and Q_Y transitions are clearly seen to be negative (i.e., dichroism positive, but absorption changes negative), showing that $\alpha > 55°$ for both transitions. Complex dichroism signals in the 545 nm region arise from the cyt *c* contribution. These can be eliminated by including an excess of a redox mediator (200 μM, methyl viologen) in the RC solution that is capable of re-reducing the cyt *c* upon warming the sample to 200°K after H^- formation. This is shown in the spectra in Fig. 3, taken after the sample was cooled back to 100°K. These spectra of the H Q_X absorption changes in the absence of contributions by cyt *c* clearly show the negative *p* value for the H Q_X transition with respect to the B_2 Q_Y axis.

The values of *p* calculated from these absorption changes are very low, as shown in Table I, even for short periods of illumination (t = 1 min, <5% H reduced). This arises from the fact that in order to accumulate a detectable amount of H^-, the excitation light partially saturates the cycle $B_2H \rightleftharpoons B_2^+H^-$. This diminishes the dichroism due to photoselection (see reference 21) because, as saturation is approached, the distribution of RCs undergoing photochemistry progressively has the B_2 Q_Y direction less uniquely aligned along the vertical direction. An analogous decrease in the orientational ordering of the RCs could be produced by decreasing the order parameter (9, 31) for excitation, S_E, which is a measure of the extent to which an absorption dipole is uniquely projected along a single

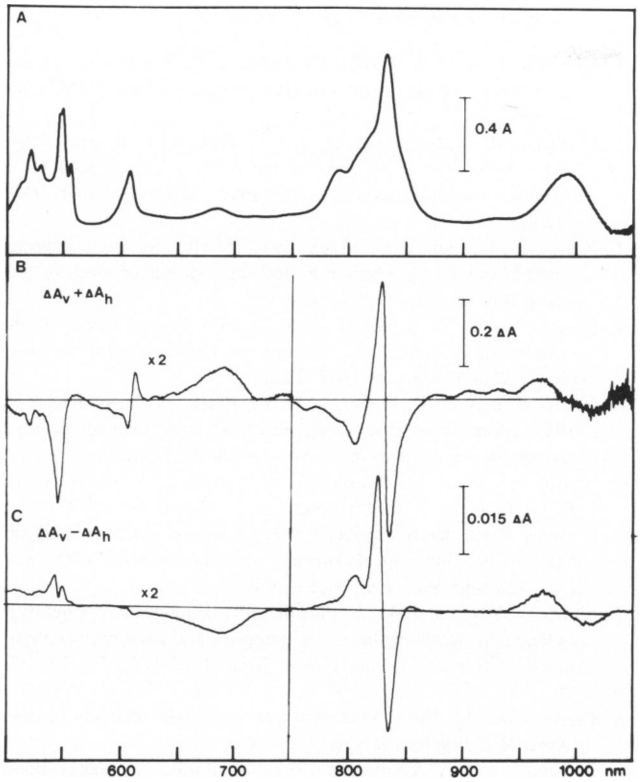

FIGURE 2 Optical spectra for the *Rps. viridis* reaction center at 100°K. (*A*) The absorbance spectrum of the reaction center in 60% glycerol (vol/vol), 10 mM Tris-HCl, 0.5% cholate, pH 8, and reduced with a few crystals of dithionite. The optical pathlength was 1 mm. (*B*) An optical difference ($\Delta A_V + \Delta A_H$) between the absorption spectrum measured at 100°K after 15 min of illumination with vertically polarized light, $\lambda > 900$ nm, minus the spectrum before illumination (i.e., Fig. 2 *A*). The actinic light was obtained from a xenon arc, filtered through a series of filters (R 715, Schott Optical Glass Inc., Duryea, PA; and 88C, Wratten, Rochester, NY) to remove light of $\lambda < 830$ nm, and also passed through a stirred 2.5-cm water filter containing chromatophores of *Rps. sphaeroides* GA ($A_{850} > 10$/cm) to remove light of $\lambda < 900$ nm. Polarization of the light was produced by a pair of infrared polarizing filters (HN7; Polaroid Corp., Cambridge, MA), mounted just before the sample. (*C*) The linear dichroism spectrum ($\Delta A_V - \Delta A_H$) produced by the 15-min illumination with vertically polarized light as described in *B*. For both *B* and *C*, the absorbance scale is expanded by a factor of 2 between 500 and 750 nm. The reaction centers were prepared by the method of Clayton and Clayton (27). Both absorbance and dichroism spectra were measured with the same apparatus, which has been described previously (33). The apparatus consists of a monochromator and prism polarizer with which the exiting light is linearly polarized, and then rotated between the horizontal and vertical positions at a frequency of 100kHz by a photoelastic modulator. The dichroism signal is measured with a synchronized, modulated detection to produce the $A_V - A_H$ difference, while the absorption is measured with an unmodulated detection, and by taking the logarithm of the transmission ratios with appropriate reference samples, to yield the average: $(A_V + A_H)/2$.

molecular (RC) direction. Equations showing the dependence of fluorescence polarization on S_E have been derived (31). Since the analysis of photoselection by polarized fluorescence or photodichroism are mathematically equivalent (9), these equations (31) can be used to compensate

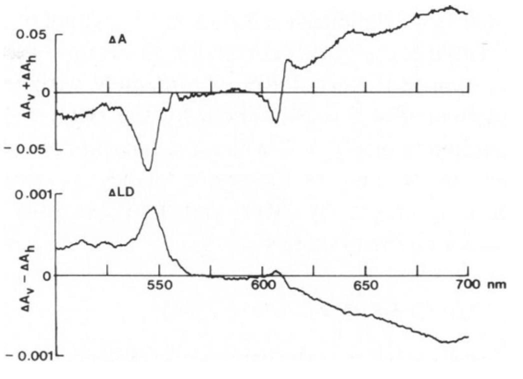

FIGURE 3 Absorption difference ($\Delta A_V + \Delta A_H$) and dichroism ($\Delta A_V - \Delta A_H$) spectra measured at 100°K and produced by the illumination as described in Fig. 2, but also following a warming of the sample to 200°K for 1 min before cooling the sample back to 100°K. The sample also contained 200 μM methyl viologen, which was capable of re-reducing the oxidized cyt *c* at temperatures near 200°K. Other conditions were as described in Fig. 2.

for the depolarization due to saturation, although here we use S_E to represent a saturation-dependent parameter describing the extent of order for the photochemically activated RCs:

$$p = \frac{3\,S_E\,(3\cos^2\alpha - 1)}{10 + S_E\,(3\cos^2\alpha - 1)}.$$

S_E can be determined from the fact that the B_2 Q_Y absorption at 990 nm is an uniformly polarized band (22, 23), and by definition is polarized along the excitation direction.

As a result, the angle α for the absorption changes at 970 nm should be zero, which can be substituted into the above equation along with the measured value of p to yield S_E. The values of the unknown α's at other wavelengths can be

TABLE I
ANGLES BETWEEN TRANSITION MOVEMENTS AND THE B_2, Q_Y DIRECTION IN REACTION CENTERS OF *RPS. VIRIDIS*

λ	Transition	p (±0.003)	α	α_C (±2°, S_E = 0.185)
nm				
970	B_2, Q_Y	0.107	46°	0°
837	—	0.065	51°	31°
826	—	0.08	49°	25°
805	H, Q_Y	−0.057	59°	90°
688	H^-	−0.058	59°	90°
545	H, Q_X	−0.014	56°	60°

List of the dichroism values, p, for the absorption changes associated with H reduction. From these, the angles, α, between the B_2Q_Y axis (excitation axis) and the transition moments associated with the absorption changes are calculated. The angles α_C, corrected for depolarization in the photoselection are also given. The order parameters for the excitation, S_E = 0.185, was calibrated by assuming that the B_2 970-nm adsorption changes should be polarized along the excitation direction (see text).

calculated from the measured p using this value of S_E. As seen in Table I, use of this correction yields an α for the H Q_Y transition (805 nm) of 90°, in agreement with previous measurements (14, 21–24). The α for the H Q_X (545 nm) band is found to be 60° ± 2, which is a measure of the angle between the B_2 and H molecular planes. A correction procedure proposed by Mar and Gingras (34) yields numerically identical angles.

DISCUSSION

These results show that the orbital overlap necessary for an efficient electron transfer can be readily established with a noncoplanar arrangement of redox centers. The possibility remains that the oblique angle may prevent a rapid, direct, back reaction between B_2^+ and H^- without the participation of B, a monomeric bacteriochlorophyll that possibly functions as a redox center between B_2 and H (6, 7). Because the absorption spectrum of B is overlapped by that of the other RC monomeric bacteriochlorophyll, the orientation of B has not yet been identified spectroscopically. However, linear dichroism measurements show that the average for the two Q_Y (12–14) and two Q_X (Tiede, D. M., and J. Breton, unpublished results) transitions must make an angle greater than 55° with respect to the membrane normal (with 35° of membrane surface). The normals to both the B_2 and H optical $Q_{X,Y}$ planes are nearly perpendicular to the membrane normal, at angles of 70–80° (12–17) and ~90° (12–14), respectively (for reviews references 9–11).

Electron tunneling has also been demonstrated to occur between the RC-associated, low potential cyt *c* hemes and B_2^+ in *Rps. viridis* and *C. vinosum*. Previous EPR measurements in *C. vinosum* (32) have shown that these heme planes are nearly perpendicular to that of the B_2 cation. Qualitatively, the negative p values found here for the *Rps. viridis* cyt *c* (Fig. 2 *C*) also suggest an angle $\alpha > 55°$. In addition, in the RC of green plants the plane of the P680 chlorophyll has recently been shown to be perpendicular to those of its acceptor (pheophytin) and a donor (cyt *b* 559) (A. W. R. Rutherford, personal communication).

Hypothetical models for interprotein heme-heme electron transfer (1, 3–5) and a slow intraprotein transfer between perpendicularly oriented hemes (2) have led to the hypothesis that a parallel alignment of hemes will permit the most kinetically facile transfers by maximizing the extent of orbital overlap (1–5). However, we have shown here that the extremely fast RC intraprotein electron transfers occur through noncoplanar geometries. We suggest that either a sufficient p-orbital overlap can be obtained by a nonparallel alignment of macrocycles, or that electron transfer pathways involve more than just the carbon, π-bonding porphyrin cores.

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