Structure-based analysis of the initial electron transfer step in bacterial photosynthesis: Electric field induced fluorescence anisotropy

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The fluorescence intensity from randomly oriented, immobilized *Rb. sphaeroides* reaction centers at 77 K increases in the presence of an externally applied electric field. We have proposed that this increase is due to a net decrease in the rate of the forward electron transfer reaction which competes with the prompt fluorescence. This decrease results from the change in the free energy difference between the reactant and very dipolar product state in the presence of the electric field. Because the free energy change and thus the electron transfer rate for a given reaction center depends on its orientation relative to the field, the intensity of the competing fluorescence likewise becomes orientation dependent. An expression is derived relating the degree of this electric field induced fluorescence anisotropy to the angle ζ_{et} between the fluorescence transition moment and the effective dipole moment whose interaction with the field results in the change in the rate of the electron transfer reaction which competes with fluorescence. ζ_{et} is determined to be about 69°. This angle can be estimated from the x-ray crystal structure coordinates for possible identities of the initial electron acceptor. The results are inconsistent with a two-step hopping mechanism in which the bacteriochlorophyll on the *L* side is the initial electron acceptor whose formation competes with fluorescence. Effects of an electric field on the electronic coupling for a superexchange mechanism are discussed. The theoretical and experimental approach should be generally applicable for studying long-range electron transfer reactions.

I. INTRODUCTION

Bacterial photosynthesis is initiated by electronic excitation of the primary electron donor, which is a dimer of bacteriochlorophylls (called the special pair or P). Within a few ps following excitation of the special pair an electron is transferred over a long distance to the bacteriopheophytin monomer on the *L* side (denoted H).[1–3] No evidence has been found from transient absorption experiments to indicate that the monomeric bacteriochlorophyll on the *L* side (denoted B) first receives the electron from the special pair prior to H. This observation is somewhat surprising since B is considerably closer than H to the special pair as determined by x-ray crystallography[4–6] (Fig. 1). Possible shortcomings of such measurements are uncertainties regarding transient spectral assignments and the limits of sensitivity to small changes in absorbance. In this paper we present results from a new experimental approach to this problem which takes advantage of the fact that the positions of B and H relative to the special pair[33] are different and that the initial electron transfer reaction which competes with the prompt fluorescence from the special pair can be affected by an externally applied electric field.[7] We conclude that the formation of $P^{\cdot +} H^{\cdot -}$ and not $P^{\cdot +} B^{\cdot -}$ competes with fluorescence.

In an applied electric field the energy gap between the reactant and product states of the initial electron transfer step which competes with fluorescence will change. Because the rate of the electron transfer reaction is expected to depend on the energy gap,[8,9] it will also change. The extent to which the energy gap is affected depends on the magnitude of the difference dipole moment $\Delta\mu_{et}$ between the initial and final states and the orientation of this difference dipole relative to the applied electric field. Reaction centers which are

oriented such that the electron transfer rate is decreased the most by the field will have the greatest increase in fluorescence yield. As a result, the prompt fluorescence from the isotropic, immobilized sample, even when excited with unpolarized light, will become anisotropic in an electric field.[34]
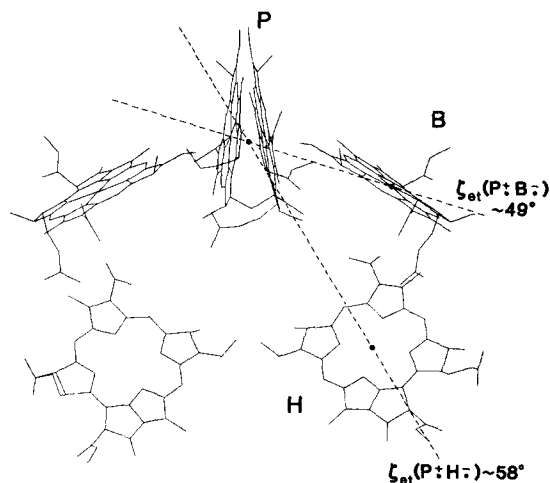

FIG. 1. Arrangement of the chromophores participating in the initial electron transfer reaction taken from the x-ray coordinates for *R. viridis* reaction centers (Ref. 4). A nearly identical figure is obtained for *Rb. sphaeroides* (see Refs. 5 and 6). The absorption (fluorescence) transition moment direction is approximately perpendicular to the page passing through the point at the geometric center of the special pair (P). The angle ζ_{et} is between the direction of the transition moment and a line drawn from the geometric center (Ref. 36) of P to that of either B or H using the coordinates from the crystal structure of *R. viridis* (see Table I). This figure is for the purpose of illustration only. No two-dimensional representation can adequately represent this three-dimensional problem.

The physical picture is illustrated schematically in Fig. 2.

Based on the theoretical treatment developed in the next section, a quantitative analysis of the electric field induced fluorescence anisotropy can be performed to determine the angle ζ_{et} between $\Delta\mu_{et}$ and the transition dipole moment of the competing fluorescence. Because the dipole moment of the electron transfer product state is expected to be very large relative to that of the initial state, it is reasonable to assume that $\Delta\mu_{et}$ is primarily determined by the product state dipole (the dipole moments of the states $P^{+}B^{-}$ and $P^{+}H^{-}$ for a full electron charge separated by the center-to-center distance are about 50 and 80 D, respectively). Thus, $\Delta\mu_{et} = \mu(P^{+}I^{-}) - \mu(^{1}P) \simeq \mu(P^{+}I^{-})$ if $\mu(^{1}P) \ll \mu(P^{+}I^{-})$, where I^{-} is either B^{-} or H^{-}. The direction of $\Delta\mu_{et}$ for these two possibilities can be determined from the x-ray crystal structure and turns out to be different. The orientation of the fluorescence transition dipole moment relative to the molecular axes can be determined by combining the results of single crystal polarized absorption measurements or theoretical calculations with fluorescence polarization

studies. Thus, by using the RC structure as determined by x-ray crystallography, the results of the electric field induced fluorescence anisotropy experiments allow one to determine the likely identity of the initial electron acceptor.

II. THEORY

When the dominant effect of an applied electric field on an absorption or emission spectrum of an immobilized, randomly oriented sample is due to a dipole moment difference between the ground and excited states, the change in absorption or emission has a second derivative shape. The effect of a field on the Q_{y} region of the special pair absorption band in Fig. 3(D) provides a good example.[11–13] In addition, the magnitude of the relative change in absorption or fluorescence in a field has a very specific dependence on the experimental angle χ between the applied electric field and the electric vector of the probing light and on the internal angle ζ_{A} between $\Delta\mu_{A}$, the difference dipole between the ground and excited state, and the transition dipole for the transition of interest[14]:

$$\Delta A(\chi) \propto 5|\Delta\mu_{A}|^{2}$$
$$+ (3\cos^{2}\chi - 1)[3(|\Delta\mu_{A}|\cos\zeta_{A})^{2} - |\Delta\mu_{A}|^{2}]. \quad (1)$$

An identical expression can be written for $\Delta F(\chi)$, the change in fluorescence due to a permanent dipole moment difference $\Delta\mu_{F}$ between the fluorescing and ground state.[15]

The observed effect of an applied electric field on the prompt *fluorescence* from RCs is distinctly different as is clear from the fact that the change due to the field has very little of a second derivative component[7] and is very similar in shape to the fluorescence spectrum itself [Figs. 3(A) and 3(B)]. We have proposed[7] that the new effect in the RC is primarily due to a change in the rate of the initial electron transfer reaction which competes with fluorescence. In the following, we derive an expression analogous to Eq. (1) which applies to this different mechanism, so that ζ_{et}, the angle between $\Delta\mu_{et}$ and the fluorescence transition moment, can be determined. This model applies to direct electron transfer from ^{1}P to either B or H. Possible effects of an elec-
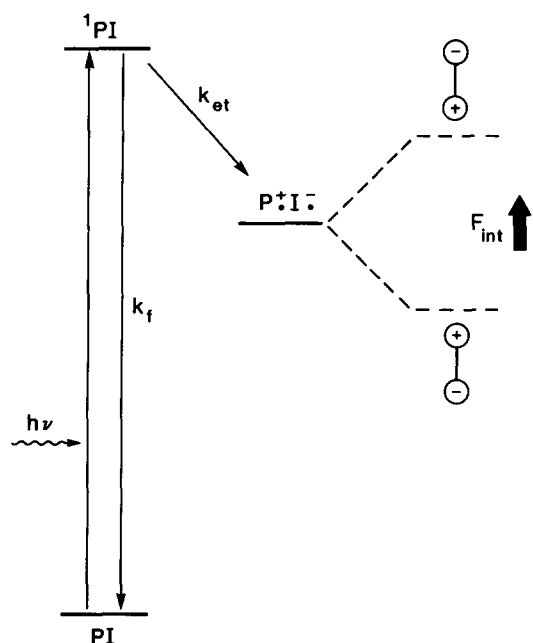

FIG. 2. Reaction scheme describing the initial charge separation step in *Rb. sphaeroides* reaction centers (not to scale). P is the special pair electron donor; I is the intermediate electron acceptor, either the monomeric BChl (B) or monomeric BPheo (H) on the L side. k_{et} is the rate constant for electron transfer ($\sim 6 \times 10^{11}$ s^{-1} at 77 K, negligible recombination to ^{1}P at this temperature), and k_{f} is the rate of fluorescence ($\sim 2 \times 10^{8}$ s^{-1}). The solid lines are schematic energy levels in zero electric field. For an isotropic immobilized sample in an applied electric field, the potential energy of the $P^{+}I^{-}$ state increases or decreases depending on the orientation of the $P^{+}I^{-}$ dipole; the largest changes occur for dipoles oriented parallel or antiparallel to the field and are illustrated with the dotted levels. The ground and excited states of P may also have nonzero dipole moments, however, these are assumed to be much smaller than the $P^{+}I^{-}$ dipole moment and are not considered. The change in the energy of the $P^{+}I^{-}$ state relative to that of ^{1}P leads to a change in k_{et} which depends on the orientation of the dipoles in the field. This leads to an orientation dependent change in the fluorescence which competes with k_{et}. Thus, the fluorescence which was isotropic in zero electric field is expected to become anisotropic in an applied field.
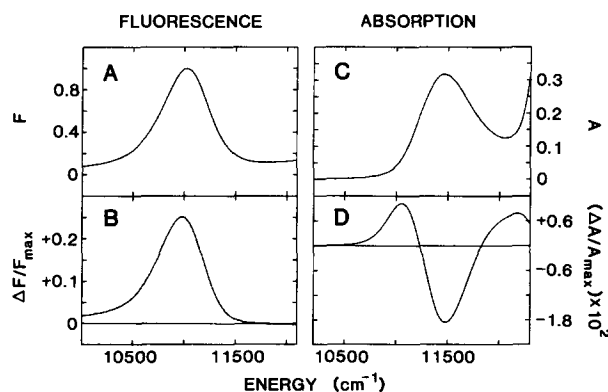

FIG. 3. (A) The fluorescence spectrum of quinone-depleted *Rb. sphaeroides* RCs in PVA at 77 K in the absence of a field and (B) the change in the fluorescence intensity for the same sample in a field of 8.9×10^{5} V/cm. (C) The absorption spectrum of the Q_{y} transition of the special pair in quinone-depleted reaction centers in PVA at 77 K and (D) the change in the absorbance for the same sample in a field of 8.9×10^{5} V/cm.

tric field on the electronic coupling in a superexchange mechanism are discussed in Sec. V.

The probability that a molecule with a fixed orientation spontaneously emits a photon at wave number ν observed at a polarization direction defined by unit vector $\hat{e}$ is given by

$$\Pi(\nu) = K\nu^3(\hat{e}\cdot\hat{p})^2 S(\nu), \tag{2}$$

where K is a constant, $\hat{p}$ is a unit vector in the direction of the transition dipole of the molecule, and $S(\nu)$ is a normalized shape function. For an isotropic immobilized sample in an electric field F_{int}:

$$\Pi(F_{int},\nu) = K\nu^3\langle\Phi_f(F_{int},\beta)(\hat{e}\cdot\hat{p})^2\rangle S(\nu), \tag{3}$$

where $\Phi_f(F_{int},\beta)$ is the normalized fluorescence quantum yield which is a function of the electric field and the angle β between $\Delta\mu_{et}$ and F_{int}, $S(\nu)$ is assumed to be independent of orientation and field (only the intensity of the band is observed to change, not the shape), and the brackets denote an average over all molecular orientations. F_{int} is the actual field experienced by the molecules under investigation, which is larger than the applied electric field F_{ext} by approximately a factor of 1.2 to 1.5 due to the dielectric properties of the environment.[11] This factor does *not* enter into the subsequent analysis. In this model, the field only affects $\Phi_f(F_{int},\beta)$, i.e., only the observed zeroth derivative effect is modeled. Using the reaction scheme in Fig. 2:

$$\Phi_f(F_{int},\beta) = k_f/(k_f + k_{et}), \tag{4}$$

where k_f is the rate of the radiative transition, k_{et} is the rate of the competing initial electron transfer reaction, and we assume that there are no other decay pathways from ^1P. Consistent with this assumption, at room temperature and zero field, Φ_f is about 4×10^{-4}[16] and k_{et} is about 3×10^{11} s^{-1}[1,2] giving a reasonable value for k_f of 2×10^8 s^{-1}. k_f is proportional to the oscillator strength for this transition. If the oscillator strength (and thus k_f) were sufficiently electric field dependent to produce the observed magnitude of the change in fluorescence intensity, the effect of an electric field on the Q_y absorption band of the special pair (same transition as the fluorescence) would be expected to have a primarily zeroth derivative shape. However, as shown in Fig. 3(D), the shape of the absorption electric field effect spectrum is approximately that of the second derivative of the absorption spectrum [Fig. 3(C)]. In addition, we have shown that the electric field effect on both the Q_y absorption and fluorescence from bacteriochlorophyll a and bacteriopheophytin a[7] in PMMA show little or no zeroth derivative contribution. Therefore, we reasonably assume that k_f is independent of electric field. At low enough fields such that $k_{et} \gg k_f$ for all orientations:

$$\Phi_f(F_{int},\beta) \propto 1/k_{et}. \tag{5}$$

The rate of electron transfer k_{et} depends on the free energy difference between the initial and final states of the reaction[8,9] which depends on both F_{int} and β. We stress that, as shown below, the details of this dependence are not crucial to the form of the predicted *angle* dependence; the details will affect the absolute magnitude of the effect but not the rela-

tive magnitude as a function of experimental and molecular angles which is what is required to determine ζ_{et}. Important examples of electric field effects on other reactions in oriented systems have been presented by several investigators.[17-19] The change in the free energy difference in the presence of an electric field is given by $-\Delta\mu_{et}\cdot F_{int}$. As shown schematically in Fig. 2, when $\Delta\mu_{et}$ is dominated by the product state dipole moment and this dipole is aligned with the field, the final state is lowered in energy relative to the initial state and the free energy difference is more negative since the reaction is exergonic in zero field.

To illustrate the approach for obtaining an expression analogous to Eq. (1), we consider as a simple example the case where k_{et} can be modeled using classical Marcus electron transfer theory.[8] Then

$$1/k_{et} = C_1\exp\{(\lambda + \Delta G_{et}^0 - \Delta\mu_{et}\cdot F_{int})^2/4\lambda kT\}, \tag{6}$$

where λ is the reorganization energy, k is the Boltzmann constant, ΔG_{et}^0 is the standard free energy difference at *zero field*, $\Delta\mu_{et}$ is the permanent dipole moment difference between the initial and final states of the electron transfer reaction, T is the temperature, F_{int} is the electric field experienced by the reacting system due to an externally applied electric field, k_{et} is the rate normalized to 1 at $F_{int} = 0$ and C_1, which contains the electronic coupling matrix element and the reorganization energy, is assumed to be independent of electric field.[35] At fields for which the exponent is less than about 0.5, it can be expanded in a series keeping terms to second order in field with less than a few percent error

$$1/k_{et} \simeq 1 + C_2(\Delta\mu_{et}\cdot F_{int}) + C_3(\Delta\mu_{et}\cdot F_{int})^2, \tag{7}$$

where C_2 and C_3, which contain λ and ΔG_{et}^0, are assumed to be constants.[35] Then

$$\Pi(F_{int},\nu) = K\nu^3\langle(\hat{e}\cdot\hat{p})^2\{1 + C_2(\Delta\mu_{et}\cdot F_{int})$$
$$+ C_3(\Delta\mu_{et}\cdot F_{int})^2\}\rangle S(\nu) \tag{8}$$

and

$$\Pi(F_{int},\nu)$$
$$= \Pi(F_{int} = 0,\nu) + K\nu^3 C_3\langle(\hat{e}\cdot\hat{p})^2(\Delta\mu_{et}\cdot F_{int})^2\rangle S(\nu), \tag{9}$$

where terms linear (and all higher odd power terms) in F_{int} can be dropped because they average to zero for an isotropic distribution. Therefore, the observed change in the fluorescence intensity is given by

$$\Delta F_{et} \propto \langle(\hat{e}\cdot\hat{p})^2(\Delta\mu_{et}\cdot F_{int})^2\rangle. \tag{10}$$

The term in Eq. (10) which gives rise to the dependence of ΔF_{et} on experimental and internal angles is *identical* to that which appears in the derivation of Eq. (1) (an outline of the orientational averaging procedure can be found in Ref. 20). This analysis predicts that the fluorescence change ΔF_{et} should be quadratic with field up to reasonably large fields and that the dependence of ΔF_{et} on χ and ζ_{et} will be exactly analogous to Eq. (1)

$$\Delta F_{et}(\chi) \propto 5|\Delta\mu_{et}|^2$$
$$+ (3\cos^2\chi - 1)\{3(|\Delta\mu_{et}|\cos\zeta_{et})^2 - |\Delta\mu_{et}|^2\}. \tag{11}$$

The above analysis was for a particular dependence of k_{et} (or Φ_f) on the driving force and applied field. We stress, however, that any dependence of k_{et} on ΔG^0_{et} and F_{int} which can be reasonably well fit to a general third order polynomial in powers of $\Delta\mu_{et}\cdot F_{int}$ around the zero field value over the limited range of the curve which is sampled when the electric field is applied (this range depends on the specific values of $|\Delta\mu_{et}|$ and $|F_{int}|$), will result in the angle dependence found in Eq. (11). A third order polynomial is a very versatile fitting function for curves which are relatively smooth over the range being fit, as is expected for the dependence of k_{et} on ΔG^0_{et} and F_{int} for the RC problem or any other long range electron transfer reaction in a condensed phase.[8] Because the expressions derived are independent of the particular model used, Eq. (11) should be generally useful for studying electron transfer reactions which compete with radiative transitions.

The validity of this treatment can be supported by experimentally verifying that ΔF_{et} is quadratic with field strength over an appropriate range and that the observed dependence of the fluorescence intensity on the experimental angle χ can be fit to the expression in Eq. (11) for some value of ζ_{et}. Note that the constant of proportionality in Eq. (10) which determines the absolute magnitude of the effect does not affect the predicted angle dependence in Eq. (11), i.e., the relative magnitude as a function of experimental and internal angles can be treated independently of the absolute magnitude. The same applies to the angular dependence of the conventional Stark effect on absorption or fluorescence which we,[11,12] and others[13-15] have used.

III. METHODS

Quinone-depleted *Rhodobacter sphaeroides* reaction centers, R26 mutant, were prepared by standard methods.[21,22] The RCs in 0.1% Triton X-100/10 mM Tris·HCl, pH 8.0, were embedded in poly(vinyl alcohol) films (PVA) which were coated with semitransparent Ni electrodes as described previously.[11]

Quinone-containing RC samples were prepared by adding a five to tenfold excess of menadione dissolved in ethanol (20 mM). The films prepared with menadione were about 90% quinone containing as determined by room temperature transient absorption experiments. Menadione is used because it is more strongly bound than the native ubiquinone which has a tendency to be lost during film preparation. Menadione-containing *Rb. sphaeroides* RCs have properties very similar to those which contain the native ubiquinone. The optical density for the special pair Q_y band for the various samples was between 0.06 and 0.26 at room temperature. BChla was dissolved in pyridine and added to toluene. The BChla solution was then added to a solution of poly(methyl methacrylate) (PMMA) in toluene and cast into a film. The optical density of the films used was less than 0.12 at room temperature. All films were between 30 and 80 μm thick.

The samples were immersed in liquid nitrogen and excited through the nickel electrode surface. The fluorescence was detected either from the front face at an angle of 50° or through the opposing electrode. The excitation source used was a 450 W xenon arc lamp which was filtered to pass either 350–600 or 532 $\pm$ 5 nm light. Excitation at these wavelengths is used because it provides nearly isotropic excitation of the special pair[34] and minimizes the contribution to the fluorescence change from the effect of the electric field on the absorption of the excitation light. The latter is true because the electric field effect on the absorption at any wavelength in this region is relatively small[12] and the net effect (integrated over the second derivative-shaped contributions) is negligible. The results obtained using either excitation conditions are identical within the experimental uncertainty. The fluorescence was dispersed in a single monochromator and detected with a Si-avalanche photodiode. For quinone-depleted RCs, no change in the fluorescence intensity was detected over a period of many hours of illumination at 77 K. For quinone-containing samples, lower excitation intensity (< 10 mW/cm^2) was required to avoid saturation and slow changes in the fluorescence and ΔF_{et} signals. The fluorescence was modulated by an electric field generated by a high voltage power supply operating at around 300 Hz and the signal at the second harmonic was measured using a lock-in amplifier.

For the angle dependence measurements the fluorescence was collected through a Glan–Thompson polarizer positioned in front of the entrance slit of the monochromator. Two methods were used to change the experimental angle χ between the polarization direction and the applied electric field: either the sample was held fixed at some angle relative to the polarizer which was rotated, or the polarizer was set to transmit horizontally polarized light and the sample was rotated about a vertical axis. At every angle χ, the change in fluorescence due to the field was divided by the total fluorescence intensity in the absence of the field to normalize out the effects of excitation intensity and collection efficiency. The angle dependence data for quinone-depleted RCs shown in Fig. 5(A) were obtained using a relatively low field ($F_{ext} \simeq 2.7 \times 10^5$ V/cm) to insure that the assumptions made in the derivation of Eq. (11) are valid (no systematic difference at higher fields up to $F_{ext} \simeq 9 \times 10^5$ V/cm has been observed; subsequent data were obtained using fields up to 9×10^5 V/cm). As a result, a large emission bandwidth (about 38 nm) centered at 910 nm was required to increase the signal-to-noise ratio of the modulated fluorescence signal. The large bandwidth was acceptable because the dependence of ΔF_{et} on χ was observed to be homogeneous over this wavelength range (data not shown). Because the fluorescence passes through several materials of different refractive indices prior to collection, the angle relative to the sample normal at which the fluorescence was collected is not the angle at which it was emitted. This has been accounted for in the determination of the relevant angles.

IV. RESULTS

The fluorescence spectrum and the change in fluorescence in the presence of an applied electric field at 77 K for quinone-depleted *Rb. sphaeroides* RCs embedded in a PVA film are shown in Figs. 3(A) and 3(B). The effect of field on the absorption spectrum of quinone-depleted RCs is shown in Fig. 3(D) for comparison. The dependence of ΔF_{et} and ΔA on field is shown in Fig. 4. The dependence of
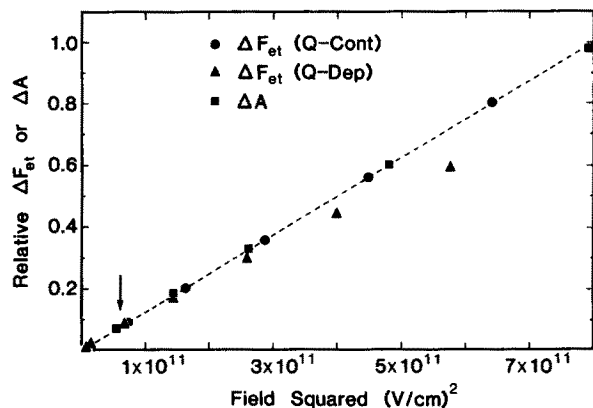
FIG. 4. The dependence of the change in absorbance (squares) and the fluorescence intensity for quinone-depleted (triangles) and quinone-containing (circles) RCs on the square of the applied field strength for the Q_y transition of the special pair in *Rb. sphaeroides* reaction centers in PVA at 77 K. The same dependence of ΔF_{et} for quinone-depleted RCs is observed using a dc applied electric field. The dotted line is a straight line drawn through the four lowest field ΔA points. The arrow indicates the field at which the dependence of $\Delta F_{et}(\chi)/\Delta F_{et}(\chi = 90)$ on χ shown in Fig. 5 was measured.

$\Delta F_{et}(\chi)/\Delta F_{et}(\chi = 90)$ on the experimental angle χ for quinone-depleted *Rb. sphaeroides* RCs and the dependence of $\Delta F(\chi)/\Delta F(\chi = 90)$ on χ for six-coordinate bacteriochlorophyll a in PMMA are shown in Fig. 5(A) along with the best fit of the data for ζ_{et} or ζ_F using Eq. (11) or (1), respectively. In addition, plots of $\Delta F_{et}(\chi)/\Delta F_{et}(\chi = 90)$ calculated from Eq. (11) for ζ_{et} equal to 0°, 54.7°, and 90° are shown (dashed lines) to illustrate the sensitivity of the measurement to this angle. The dependence of $\Delta F_{et}(\chi)/\Delta F_{et}(\chi = 90)$ on χ is the same within the experimental uncertainty for menadione-containing RCs (data not shown) as for quinone-depleted RCs. Note that the RC absorption [Fig. 3(D)], and the bacteriochlorophyll a absorption[12] *and* fluorescence (data not shown) electric field effects have the expected second derivative line shape, whereas the change in the RC fluorescence [Fig. 3(B)] has a primarily zeroth derivative line shape.

Figure 4 shows that ΔF_{et} is quadratic with field strength for quinone-containing RCs and slightly subquadratic at high field for quinone-depleted RCs. It is possible that this difference is caused by a contribution from delayed fluorescence (fluorescence following charge recombination of $P^{\cdot+}I^{\cdot-}$ to regenerate ^1P) from quinone-depleted RCs, which is impossible for quinone-containing RCs because of a much shorter radical pair $(P^{\cdot+}I^{\cdot-})$ lifetime. Even though there is very little delayed fluorescence from quinone-depleted RCs in zero field at 77 K, it is possible that the barrier for charge recombination could be reduced enough at the highest fields for RCs oriented such that the $P^{\cdot+}I^{\cdot-}$ dipole is opposed to the electric field to lead to some delayed fluorescence. As a result, there would be a contribution to ΔF_{et} for quinone-depleted RCs which may not have the same field dependence as the contribution due to the change in the rate of the forward electron transfer process. This suggests that the slightly subquadratic field dependence of ΔF_{et} for quinone-deplet-

ed RCs is due to a contribution from delayed fluorescence at high electric fields.

Figure 5(A) shows that the angle dependence data can be very well fit to the expression found in Eq. (11) with ζ_{et} = 71°. The uncertainty in the fit is very small and repeated measurements on the same sample gave values which were within 2°. We observed somewhat larger variations from sample to sample; in each case the angle dependence gave an excellent fit to Eq. (11). The data obtained using four samples (including a menadione-containing RC sample) could all be fit to Eq. (11) using a value of ζ_{et} that is within 3° of 69°. The uncertainty in the value ζ_{et} = 69° ± 4° is very conservatively estimated by including this sample-to-sample variation. For comparison, the angle dependence of $\Delta A(\chi)/\Delta A(\chi = 90)$ for RCs [shown in Fig. 5(B)] was fit to Eq. (1) using ζ_A = 38° ± 2°.[12,13] This is the angle between the absorption transition moment and the difference dipole between the ground state and the first excited singlet state of P. ζ_{et} is the angle between the fluorescence transition dipole moment and the difference dipole between the product state of the electron transfer reaction ($P^{\cdot+}B^{\cdot-}$ or $P^{\cdot+}H^{\cdot-}$) and the fluorescing state (the initial state of the electron transfer reaction).
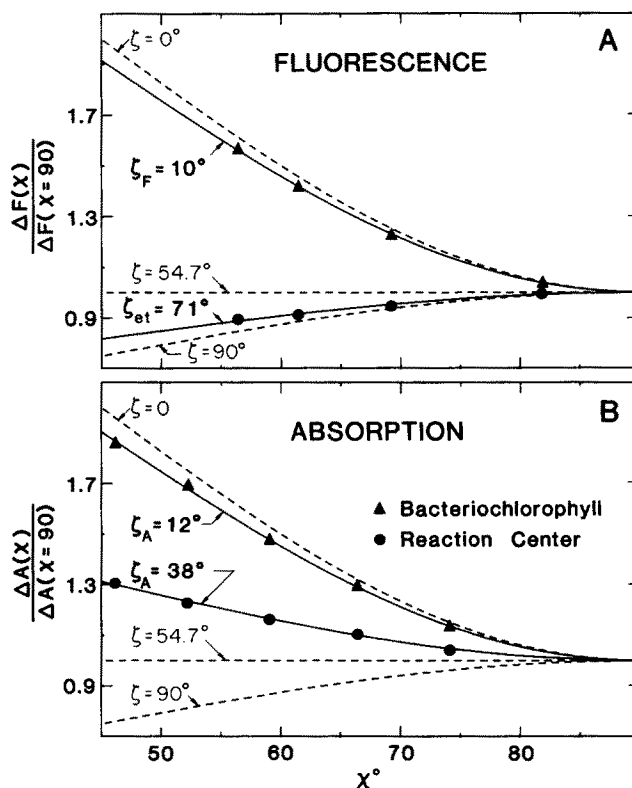

FIG. 5. (A) The dependence of $\Delta F_{et}(\chi)/\Delta F_{et}(\chi = 90)$ on the experimental angle χ for quinone-depleted *Rb. sphaeroides* reaction centers (circles) and the dependence of $\Delta F(\chi)/\Delta F(\chi = 90)$ on χ for six-coordinate bacteriochlorophyll a in PMMA (triangles) along with the best fit of the data for ζ_{et} or ζ_F using Eq. (11) or (1), respectively. (B) The dependence of $\Delta A(\chi)/\Delta A(\chi = 90)$ on the experimental angle χ for the special pair Q_y absorption band of *Rb. sphaeroides* reaction centers (circles) and six-coordinate bacteriochlorophyll a in PMMA (triangles) along with the best fit of the data for each to Eq. (1) (taken from Ref. 12). In (A) and (B), plots of $\Delta F(\chi)/\Delta F(\chi = 90)$ and $\Delta A(\chi)/\Delta A(\chi = 90)$ vs χ calculated from Eq. (1) for ζ equal to 0°, 54.7°, and 90° are shown (dashed lines) to illustrate the sensitivity of the measurement to this angle.

V. DISCUSSION

A. Analysis of the observed ζ_{et} using the x-ray structure

From the excellent fit of the angle dependent data to Eq. (11) in Fig. 5(A) it appears that the theoretical development leading to this expression is appropriate to this problem. This method of analysis should be generally useful for probing electron transfer reactions. The experimentally obtained value of ζ_{et} can be interpreted using information available from the crystallographic coordinates and independent spectroscopic measurements. We obtain the directions of the dipole moments of the $P^{+}B^{-}$ and $P^{+}H^{-}$ states by drawing a vector between the geometric center of P and the center of either the B or H macrocycle using the x-ray structure coordinates for *Rhodopsuedomonas viridis*.[4,36] The direction of the special pair Q_y transition moment relative to the crystallographic axes can be determined from polarized absorption measurements performed on RC single crystals. This has been done semiquantitatively for *R. viridis* RCs,[24] and the special pair Q_y transition dipole moment was found to lie approximately along the direction of the vector sum of the Q_y directions of the monomers comprising the dimer (see Table I). The angle between the Q_y absorption and fluorescence transition dipole moments in *Rb. sphaeroides* has been shown by fluorescence polarization measurements to be very small.[25] For subsequent analysis, the absorption and emission transition dipoles will be assumed to be parallel. Using this independent structural and experimental information we obtain estimates for ζ_{et} depending on whether the initial electron acceptor is B or H. Since there is only limited single crystal polarized absorption data to date, we also consider the results of recent theoretical calculations of the transition moment orientation. The simplest involves taking the vector sum of the monomer Q_y directions. In addition we

have used the results of sophisticated calculations which have been provided to us by Dr. M. Plato and Dr. W. Parson (personal communications). The results are shown schematically in Fig. 1 and in Table I.

As shown in Table I all of these approaches give approximately the same result. Irrespective of the method used to estimate the angle, the hypothetical values of $\zeta_{et}(P^{+}B^{-})$ and $\zeta_{et}(P^{+}H^{-})$ fall below and above the magic angle, 54.7°, respectively. As can be seen from Fig. 5, differentiating between values of ζ_{et} which are on opposite sides of the magic angle is relatively easy because the sign of the change in $\Delta F_{et}(\chi)/\Delta F_{et}(\chi=90)$ with χ is opposite in the two cases. Therefore, the electric field induced fluorescence anisotropy data in Fig. 5(A) shows that ζ_{et} is considerably greater than the magic angle, which is inconsistent with a two-step hopping mechanism in which the initial electron acceptor is the monomeric BChl (B). Possible explanations for the observation that the experimentally determined value of ζ_{et} is somewhat larger than the estimates of $\zeta_{et}(P^{+}H^{-})$ in Table I are discussed in the following section.

B. Mechanism of charge separation

Since the availability of the x-ray coordinates for photosynthetic RCs,[4–6] there has been much discussion of the role of the monomeric BChl which is located between the special pair electron donor and BPheo electron acceptor. Based on theoretical and experimental analyses[8] of the distance dependence of the rate of electron transfer, it is unlikely that an electron from ^1P is transferred directly to H (edge-to-edge separation ~ 10 Å) within a few ps. An alternative mechanism involves initial transfer of the electron to B to form $P^{+}B^{-}$ which decays very rapidly by a second electron transfer to form $P^{+}H^{-}$. The second much more rapid step is required to explain the apparent absence of buildup of measurable quantities of $P^{+}B^{-}$.[26] As discussed at the outset, it is not entirely clear how to define the limits of sensitivity for detection of an intermediate whose spectrum is not known.[27] The results reported in this paper provide an entirely independent approach to this question and a positive demonstration that the angle between the special pair Q_y transition dipole moment and the dipole moment of the state whose potential energy change in an electric field results in a change in the yield of fluorescence is about 69°, substantially larger than expected if the electron is first transferred to B. This implies that the formation of the $P^{+}B^{-}$ state does not compete with fluorescence from ^1P.

Having ruled out a two-step electron hopping scheme involving B, we consider another widely discussed hypothesis to explain the rapid long-range electron transfer. The hypothesis is that the intervening BChl monomer mediates electron transfer between ^1P and H by superexchange.[28,29] In one view of this model the initial excited state is considered to be a quantum mechanical mixture of ^1P and $P^{+}B^{-}$.[28,29] The electronic coupling between the initial and final states can no longer be considered to be independent of electric field because it is expected to be sensitive to the energy gap between the ^1P and the $P^{+}B^{-}$ states[28] which will be field

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TABLE I. Calculated angles between the Q_y transition dipole moment of the special pair and hypothetical $P^{+}B^{-}$ or $P^{+}H^{-}$ dipole moments.[a]

Method used to obtain Q_y transition	Reference	$\zeta_{et}(P^{+}B^{-})$	$\zeta_{et}(P^{+}H^{-})$
Single crystal spectroscopy	b	45.9°	57.7°
Vector sum of monomer Q_y transitions	c	48.0°	55.6°
Vector sum of monomer Q_y transitions	d	53.1°	58.3°
QCF-PP calculations	e	46.6°	57.2°
INDO/S with CI	f	53.2°	61.0°

[a] The direction of $\Delta\mu_{et}$, the $P^{+}B^{-}$, or $P^{+}H^{-}$ state dipole moment, is obtained from the *R. viridis* x-ray coordinates (see Ref. 36) and the direction of the transition dipole moment p is obtained by the variety of methods indicated.

[b] The measurements of Zinth *et al.* (Ref. 24) were done on *R. viridis* single crystals.

[c] The monomer Q_y transition moment direction defined by a line between the ring I and ring III nitrogens.

[d] Monomer Q_y transition moment direction defined by a line between the ring I nitrogen and the ring V keto-carbonyl oxygen.

[e] W. W. Parson (personal communication) based on calculations using the *Rb sphaeroides* x-ray coordinates (see Ref. 29).

[f] M. Plato (personal communication) calculated for *R. viridis*.

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dependent. As before, $\Delta G_{et}(\mathbf{F}_{int}) = \Delta G_{et}^0 - \Delta \mu_{et} \cdot \mathbf{F}_{int}$ will depend on the difference dipole between the initial state (a mixture of ^{1}P and P$^{.+}$B$^{.-}$) and the final state, P$^{.+}$H$^{.-}$. The relative contributions to the change in fluorescence from these two effects will determine the observed electric field induced fluorescence anisotropy. If the electron transfer rate is much more sensitive to a small change in $\Delta G_{et}(\mathbf{F}_{int})$ than to a small change in the energy of the P$^{.+}$B$^{.-}$ state, Eq. (11) will apply and ζ_{et} is expected to be slightly larger than 58° because of the contribution from P$^{.+}$B$^{.-}$ to the initial state and the fact that the angle between $\Delta \mu = \mu(P^{.+}H^{.-}) - \mu(P^{.+}B^{.-})$ and the special pair Q_y transition dipole moment direction is about 80°. If the two effects make comparable contributions, a more detailed analysis is required. Such a detailed analysis of the effect of an electric field on V for a superexchange mechanism shows that its contribution depends critically on the zero field values of the energy of the P$^{.+}$B$^{.-}$ state, λ, ΔG_{et}^0, and temperature.[30] If the P$^{.+}$B$^{.-}$ state is far separated in energy from ^{1}P, then the effect of an electric field on V is expected to be small. Interestingly, the value of ζ_{et} we have obtained is greater than that predicted from the reaction center coordinates assuming no contribution from P$^{.+}$B$^{.-}$ to ^{1}P. Although we are confident of the precision of the measurements, it is not clear whether our simple approach to obtaining the $\Delta \mu_{et}$ dipole moments[36] or the transition moment of P (see Table I) is accurate enough to estimate the superexchange contribution. Furthermore, this crude physical analysis of the effect of superexchange on the angle ζ_{et} needs to be supplemented by a more formal treatment of the effect, which is in progress.[30] In principle such an analysis may offer an experimental approach to estimating the superexchange contribution.

Fischer and Scherer have advanced the interesting hypothesis that the initial step following excitation of the special pair can be described as a combined excitation–electron transfer process in which deactivation of ^{1}P is coupled to the charge transfer transition BH$\rightarrow$B$^{.+}$H$^{.-}$.[31,32] The rate of this process would also be expected to be sensitive to an electric field, leading to a change in the yield of fluorescence. It is not immediately obvious whether such a mechanism would give rise to the observed net *increase* in the fluorescence intensity; the sign and absolute magnitude of the change in fluorescence intensity depends on the shape of the curve (around the zero field value) relating the fluorescence yield to the energy of the relevant states. However, the *relative* change in fluorescence intensity as a function of experimental and molecular angles will again be described by Eq. (11) if the dependence of Φ_f on electric field can be fit to a general third order polynomial in powers of $\Delta \mu \cdot \mathbf{F}_{int}$. In this model, $\Delta \mu$ is expected to be largely determined by $\mu(B^{.+}H^{.-})$, and the angle between $\mu(B^{.+}H^{.-})$ and the special pair Q_y transition moment direction is about 80°. The results of the experiments described in this paper provide a good test for this and other hypotheses regarding the initial step in bacterial photosynthesis.

VI. SUMMARY

In summary, the underlying physics leads to the expectation that the rate of long range electron transfer can be affected by an electric field. This is because the field changes the energy gap between the reactant and dipolar product state, and the rate of electron transfer depends on this energy difference. When fluorescence competes with electron transfer, the fluorescence quantum yield also becomes dependent on electric field, the magnitude of the effect depending on the form of the relationship between k_{et}, ΔG_{et}^0, and $\mathbf{F}_{int}$ for the problem being considered. At the same time, the orientation dependence of k_{et} in an electric field gives rise to an orientation dependence of the fluorescence yield, a new effect which we call electric field induced fluorescence anisotropy. Using the relationship between k_{et} and ΔG_{et}^0 originally proposed by Marcus,[8] we showed analytically that the dependence of ΔF_{et} in an applied electric field due to this mechanism on the experimental angle χ and on the molecule-fixed angle ζ_{et} is identical in form to that derived earlier for conventional absorption and fluorescence Stark effects.[14,15] We further showed that the same angle dependence is predicted for rather general forms of the dependence of k_{et} on ΔG_{et}^0 and F_{int}. We demonstrate that the fluorescence does indeed become anisotropic in an electric field and that ζ_{et} is about 69°. Using the x-ray coordinates of the reaction center to estimate the direction of the line between the donor cation and the acceptor anion for the two likely candidates for the acceptor, as well as the direction of the transition dipole moment obtained from single crystal polarized absorption spectroscopy[24] or calculations, we find that ζ_{et} is predicted to be less than the magic angle (54.7°) if B is the initial electron acceptor and larger than the magic angle if H is the initial acceptor. Thus, this experiment implies that formation of the P$^{.+}$B$^{.-}$ state does not compete with fluorescence from ^{1}P and that the monomeric BPheo is the initial electron acceptor. Further refinement of the analysis awaits improved information on the direction of the transition moment and a theoretical treatment which predicts the effects of an electric field within a superexchange[30] or a coupled excitation–electron transfer model.

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[33]Since there is no evidence at the present time that the monomeric BChl and BPheo on the M side participate in electron transfer under ordinary conditions, we neglect these chromophores and simplify the notation to P, B, and H; when discussing a generic initial electron acceptor, we use the traditional notation I. In any event, the experiments described in this paper cannot distinguish the L- and M-side electron transport chains because the calculated value of the angle ζ_{et} would be approximately the same for the two branches.

[34]Perfectly isotropic excitation of the special pair is not necessary. The required observable is the *fractional change* in the fluorescence intensity due to the applied electric field for light emitted from the sample at a given angle and with a given polarization relative to the applied field. Furthermore, the low excitation light intensity required for conventional fluorescence polarization studies is not necessary for the experiment described in this paper because the degree of excitation polarization is irrelevant.

[35]C_1 contains the electronic coupling matrix element V and the reorganization energy λ, both of which are assumed to be field independent. This assumption has been implicit in previous studies of electric field effects (see Refs. 17–19). Although V and λ may in fact be dependent on electric field, their dependence in a direct electron transfer model is expected to be very small relative to that of the free energy difference between ^{1}P and

P^+I^- at the low fields used in these experiments. V, the electronic coupling matrix element, is determined by the orbital overlap between the donor excited state and the acceptor ground state. For V to be affected by an electric field, the electronic distributions of the states relative to one another would have to change. The extent to which the electronic configurations can be affected by an electric field along any axis is determined by the respective polarizability tensors. The change in the orbital overlap is thus related to the *difference* polarizability tensor between the donor excited state and the acceptor ground state. Any angle dependence to the change in fluorescence in the presence of an electric field would arise only from *anisotropy* in the difference polarizability tensor, for which no information is currently available. If the difference polarizability tensor is isotropic, any effect on V due to the applied field would come in as a scalar quantity which would not affect the predicted form of the angle dependence found in Eq. (11), but only the absolute magnitude of ΔF_{et} which is irrelevant to the presented analysis. Since the fields used in these experiments ($\sim 5 \times 10^5$ V/cm) are fairly small (the field due to a point charge at a distance of 5 Å is about 6×10^7 V/cm for a dielectric constant of 1) this anisotropic difference polarizability effect can be reasonably neglected relative to the large and anisotropic effect of an electric field on ΔG_{et} (F_{int}) and the rate of electron transfer reactions between very dipolar states. The effects of an electric field on V for a superexchange mechanism are outlined in the text in Sec. V. λ is assumed to be independent of ΔG_{et}^0 in almost all studies of the effect of ΔG_{et}^0 on k_{et}, where ΔG_{et}^0 is changed by changing the identity (and thus redox potential) of the donor or the acceptor. In the experiments described in this paper the identity of all molecules remains the same, so the assumption of constant λ over the limited range of ΔG_{et} (F_{int}) values sampled by the RCs in the presence of the electric field is at least as valid.

[36]The *Rb. sphaeroides* (Refs. 5 and 6) and *R. viridis* (Ref. 4) structures are taken to be identical, which is true at the level of accuracy required for this analysis. The fully refined *R. viridis* coordinates used in this paper were kindly provided by Dr. J. Deisenhofer (dated Feb. 1, 1988). The geometric center of P is defined as the point midway between the centers of rings I of the monomeric bacteriochlorophylls which comprise the special pair. The geometric center of B is defined as the point which is equidistant from both the ring I and ring III nitrogens and the ring II and ring IV nitrogens (similarly for the geometric center of H). A more refined analysis could include the charge distribution within each ion, but this should make only a very small difference since the charge is spread out quite evenly around the macrocycles and the distance between the molecular centers is fairly large. Although there is a substantial body of data on the spin density distributions in P^+ and the acceptor anion (Ref. 23) (and on pure BChl$^-$ and BPheo$^-$) (Ref. 23), such measurements are performed on the radicals at equilibrium, not immediately (within a few ps) after their formation from ^{1}P, and not in the presence of the other ion. The interpretation of the experimentally determined value of ζ_{et} can be readily refined if such information becomes available, although it is not obvious how such information could be obtained on the appropriate time scale. Time dependent electrochromic shifts of the monomer absorption bands due to the presence of P^+ measured on the picosecond time scale could bear on this point. In addition, in our analysis we assume that the magnitude of $\mu(^1$P) is small relative to that of $\mu(P^+I^-)$. In a previous paper we determined that $\Delta\mu_F = \mu(^1$P) $- \mu(^gP)$, where ^gP is the ground electronic state of P, is less than about 7 D (and perhaps much less) (Ref. 7). As long as $\mu(^gP)$ is not large *and* in the same direction as $\mu(^1$P), this result indicates that $\mu(^1$P) is small compared to $\mu(P^+I^-)$.