

## Electric Field Effects on the Initial Electron-Transfer Kinetics in Bacterial Photosynthetic Reaction Centers

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The effect of an applied electric field on the kinetics of the initial picosecond electron-transfer reaction in *Rb. sphaeroides* reaction centers has been measured in isotropic samples at 77 K. The net rate of formation of  $H_L^-$  is reduced upon application of an electric field of  $10^6$  V/cm, consistent with the previously observed increase in the quantum yield of the competing prompt fluorescence. The observed magnitude of the effect on the initial reaction is compared with the predictions of various models, and the consequences of including indirect electronic coupling between the initial and final states through a third state (superexchange) are investigated. It is found that the treatments of the initial electron-transfer reaction commonly in use greatly overestimate the magnitude of the field effect because they are based on a dependence of the rate of electron transfer on the free energy change which is steeper than appears to be appropriate for this process. No evidence was found for electron transfer down the M side of the reaction center at the highest applied field, indicating that unidirectionality of the initial electron transfer is not due to small energetic differences between charge-separated states involving the chromophores on the L and M sides.

The mechanism of the initial charge separation step in bacterial photosynthetic reaction centers (RCs) is a subject of continuing debate and interest. In this paper we address this issue by measuring the effect of an externally applied electric field on the rate of the initial electron-transfer reaction. The arrangement of the reactive chromophores as revealed in the X-ray crystal structure of the *Rps. viridis* RC is shown in Figure 1.<sup>1-3</sup> A similar structure is obtained for *Rb. sphaeroides* RCs.<sup>4-6</sup> Following electronic excitation of the special pair, denoted P, to its lowest energy singlet excited state,  $^1P$ , an electron is donated within a few picoseconds (even at cryogenic temperatures) to the bacteriochlorophyll monomer labeled  $H_L$  to form the state  $P^{+}H_L^{-}$ .<sup>7-13</sup> P and  $H_L$  are separated by about 10 Å edge to edge and about 17 Å center to center. While a second bacteriochlorophyll molecule (labeled  $H_M$ ) is approximately the same distance from P (Figure 1), there is no evidence that electron transfer occurs in appreciable yield to form the state  $P^{+}H_M^{-}$ .<sup>14,15</sup>

There has been considerable conjecture on the physical origin of this remarkably rapid, long-distance, unidirectional electron transfer from  $^1P$  to  $H_L$ . Most conjecture has focused on the role of the monomeric bacteriochlorophyll labeled  $B_L$  in Figure 1. The other monomeric bacteriochlorophyll labeled  $B_M$  appears not to play a significant role since it can be removed or greatly modified<sup>16</sup> without any effect on the rate of charge separation.<sup>17</sup> Two classes of mechanisms have emerged, with several variations. The first is a sequential, two-step mechanism in which the electron hops from  $^1P$  to  $B_L$  (with rate constant  $k_{P \rightarrow B}$ ) forming  $P^{+}B_L^{-}$  as a discrete intermediate, followed by a second hop from  $B_L^{-}$  to  $H_L$  (with rate constant  $k_{B \rightarrow H}$ ) to form  $P^{+}H_L^{-}$ . There is general agreement from the results of transient absorption spectroscopy that if this mechanism is applicable,  $k_{B \rightarrow H}$  is greater than  $k_{P \rightarrow B}$ . The question of how much greater is the critical issue, and one for which there are significant differences among the results from recent measurements. All would place  $k_{P \rightarrow B}$  at roughly  $(3 \text{ ps})^{-1}$  at room temperature, but the limits on  $k_{B \rightarrow H}$  estimated from the data are about  $(50 \text{ fs})^{-1}$ ,<sup>10</sup>  $(150 \text{ fs})^{-1}$ ,<sup>11</sup> and  $(1 \text{ ps})^{-1}$ .<sup>13</sup> The first two laboratories find no spectral or kinetic evidence for the formation of state  $P^{+}B_L^{-}$ , whereas the latter study<sup>13</sup> claims evidence for a  $\sim 15\%$  maximal transient population of this state.

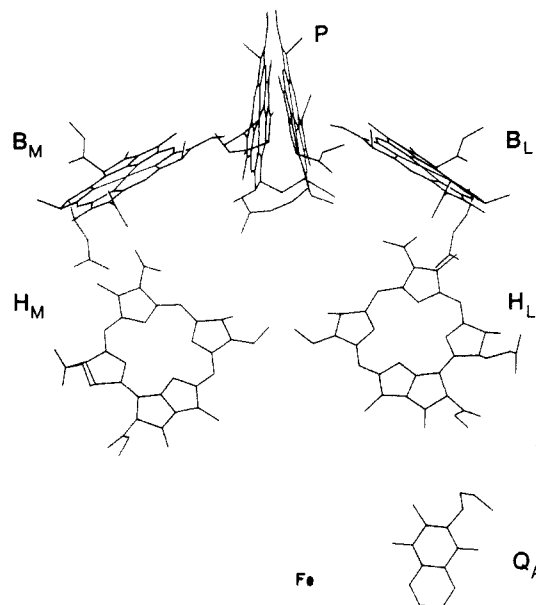
An analysis from a completely different kind of measurement, the anisotropy induced in the fluorescence from  $^1P$  upon application of an external electric field, suggests that formation of an intermediate state whose permanent electric dipole moment is oriented as expected for  $P^{+}H_L^{-}$ , and not as for  $P^{+}B_L^{-}$ , competes

with fluorescence from  $^1P$ .<sup>18</sup> Hence, this measurement and the transient absorption measurements which have found a lack of evidence for formation of  $P^{+}B_L^{-}$ <sup>7-12</sup> favor a second type of electron-transfer mechanism in which an electron is transferred directly from  $^1P$  to  $H_L$  with the electronic coupling between  $^1P$  and  $P^{+}H_L^{-}$  possibly enhanced by coupling between each of these states and states involving  $B_L$ .<sup>15,19-27</sup> Most widely discussed is

- (1) Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *J. Mol. Biol.* **1984**, *180*, 385-398.
- (2) Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *Nature* **1985**, *318*, 618-624.
- (3) Michel, H.; Epp, O.; Deisenhofer, J. *EMBO J.* **1986**, *5*, 2445-2451.
- (4) Allen, J. P.; Feher, G.; Yeates, T. O.; Komiya, H.; Rees, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5730-5734.
- (5) Allen, J. P.; Feher, G.; Yeates, T. O.; Komiya, H.; Rees, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 6162-6166.
- (6) Chang, C. H.; Tiede, D.; Tang, J.; Smith, U.; Norris, J. *FEBS Lett.* **1986**, *205*, 82-86.
- (7) Woodbury, N. W.; Becker, M.; Middendorf, D.; Parson, W. W. *Biochemistry* **1985**, *24*, 7516-7521.
- (8) Martin, J. L.; Breton, J.; Hoff, A. J.; Migus, A.; Antonetti, A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 957-961.
- (9) Breton, J.; Martin, J. L.; Migus, A.; Antonetti, A.; Orszag, A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 5121-5125.
- (10) Fleming, G. R.; Martin, J.-L.; Breton, J. *Nature* **1988**, *333*, 190.
- (11) Kirmaier, C.; Holten, D. *FEBS* **1988**, *239*, 211-218.
- (12) Kirmaier, C.; Holten, D. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3552-56.
- (13) Holzapfel, W.; Finkle, U.; Kaiser, W.; Oesterheld, D.; Scheer, H.; Stiltz, H. U.; Zinth, W. *Chem. Phys. Lett.* **1989**, *160*, 1-7; *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5168-72.
- (14) Kirmaier, C.; Holten, D. *Photosynth. Res.* **1987**, *13*, 225-260.
- (15) Michel-Beyerle, M. E.; Plato, M.; Deisenhofer, J.; Michel, H.; Bixon, M.; Jortner, J. *Biochem. Biophys. Acta* **1988**, *932*, 52-70.
- (16) Ditson, S. L.; Davis, R. C.; Pearlstein, R. M. *Biochim. Biophys. Acta* **1984**, *766*, 623-629.
- (17) Maroti, P.; Kirmaier, C.; Wraight, C.; Holten, D.; Pearlstein, R. M. *Biochim. Biophys. Acta* **1985**, *810*, 132-139.
- (18) Lockhart, D. J.; Goldstein, R. F.; Boxer, S. G. *J. Chem. Phys.* **1988**, *88*, 1408-1415.
- (19) Plato, M.; Möbius, K.; Michel-Beyerle, M. E.; Bixon, M.; Jortner, J. *J. Am. Chem. Soc.* **1988**, *110*, 7279-7285.
- (20) Bixon, M.; Jortner, J.; Michele-Beyerle, M.; Ogrodnik, A.; Lersch, W. *Chem. Phys. Lett.* **1987**, *140*, 622-630.
- (21) Bixon, M.; Jortner, J. *J. Phys. Chem.* **1988**, *92*, 7148-7156.
- (22) Won, Y.; Friesner, R. A. *Biochim. Biophys. Acta* **1988**, *935*, 9-18.
- (23) Bixon, M.; Michel-Beyerle, M. E.; Jortner, J. *Isr. J. Chem.* **1988**, *28*, 155-168.
- (24) Marcus, R. A. *Chem. Phys. Lett.* **1987**, *133*, 471-477.

<sup>†</sup>Stanford University.

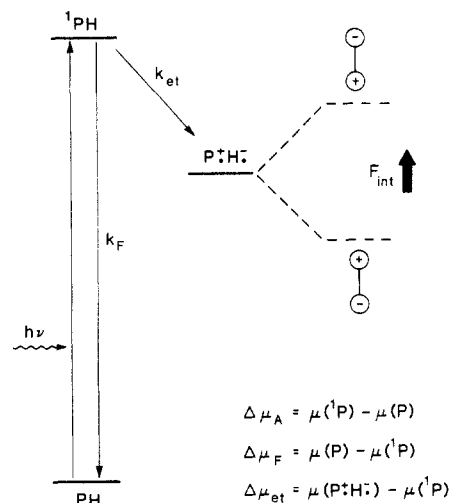
<sup>‡</sup>Washington University.



**Figure 1.** Arrangement of the chromophores in the RC taken from the X-ray coordinates for *Rps. viridis* RCs.<sup>1-3</sup> A nearly identical figure is obtained for *Rb. sphaeroides*.<sup>4-6</sup> P is a dimer of bacteriochlorophyll molecules. B<sub>M</sub> and B<sub>L</sub> are bacteriochlorophyll monomers, and H<sub>M</sub> and H<sub>L</sub> are bacteriopheophytins. An approximate C<sub>2</sub> symmetry axis is directed along a line which runs from the geometric center of P to the non-heme iron atom.

a mechanism in which the state P<sup>++</sup>B<sub>L</sub><sup>-</sup> mediates the coupling between the initial state <sup>1</sup>P and the final state P<sup>++</sup>H<sub>L</sub><sup>-</sup>. In this mechanism, P<sup>++</sup>B<sub>L</sub><sup>-</sup> is postulated to be higher in energy than <sup>1</sup>P and likely not detectable by transient absorption spectroscopy because it is not actually populated as a discrete intermediate. The energy of this state and its coupling to the reactant and product states are critical in any mechanism; unfortunately, there is little direct information on these parameters at the present time.

One approach to gaining further insight into the mechanism of the initial step is to perturb the system with an electric field. An electric field will change the energy of dipolar states such as P<sup>++</sup>B<sub>L</sub><sup>-</sup> and P<sup>++</sup>H<sub>L</sub><sup>-</sup>, or the equivalent charge-separated states on the M side, P<sup>++</sup>B<sub>M</sub><sup>-</sup> and P<sup>++</sup>H<sub>M</sub><sup>-</sup>, by an amount given by the dot product of the field and the permanent electric dipole moment of the state. There will also be an effect on the energy of <sup>1</sup>P because this state appears to be somewhat dipolar,<sup>28-34</sup> but this will generally be neglected in the following because the dipole of <sup>1</sup>P is likely to be small relative to that of the charge-separated states. In an isotropic sample such as we use here all orientations of the relevant dipoles relative to the applied field direction are present, and the field produces a spread in the energies of states. The state energies for any particular RC depend on the relative directions of the field and the state dipoles. It is important to realize that with an applied field of 10<sup>6</sup> V/cm (10 meV/Å) the energy of a state with a 50-D dipole such as P<sup>++</sup>B<sub>L</sub><sup>-</sup> can be increased or decreased by as much as about 800 cm<sup>-1</sup> (100 meV) depending on orientation. This is a sizable fraction of the energy



**Figure 2.** Reaction scheme describing the initial events in bacterial photosynthesis. The largest energetic changes caused by an electric field occur for dipoles oriented parallel or antiparallel to the field and are illustrated with dashed lines (approximately to scale for P<sup>++</sup>H<sub>L</sub><sup>-</sup> in a field of 10<sup>6</sup> V/cm).

change associated with the initial charge separation step which is estimated to be between 170<sup>35</sup> and 260 meV<sup>36</sup> at room temperature, and perhaps even less at 77 K.<sup>35</sup>

For an applied field of 1 × 10<sup>6</sup> V/cm the fluorescence from an isotropic, immobilized sample of Q<sub>A</sub>-containing *Rb. sphaeroides* R-26 RCs in a poly(vinyl alcohol) matrix has been found to increase by 40% at 77 K.<sup>18,37</sup> In addition, the magnitude of the electric field effect on the fluorescence from Q<sub>A</sub>-containing RCs was found to be quadratic with applied field strength (ac or dc applied fields) for applied fields between 1 × 10<sup>5</sup> and 1 × 10<sup>6</sup> V/cm at 77 K.<sup>37</sup> A reasonable interpretation of this result is that, on average in the isotropic sample, the rate of the forward electron-transfer reaction which competes with fluorescence, *k*<sub>et</sub>, is reduced so the fluorescence yield increases (see Figure 2). Bixon and Jortner<sup>38</sup> have analyzed these data using various models for the initial charge separation step and predict both a substantially larger value of the relative change in the fluorescence intensity, Δ*F*/*F*, than observed and a superquadratic dependence of Δ*F*/*F* on field. (Δ*F*/*F* is the change in the fluorescence intensity in the presence of the field divided by the fluorescence intensity in zero applied field.) This treatment and elaborations are discussed in detail below.

An important consideration in any electric field effect experiment involves the determination of the strength of the additional electric field experienced by the reactive system due to the applied electric field perturbation. Although the externally applied electric field, *F*<sub>ext</sub> (given by the applied voltage divided by the separation between the electrodes), is known quite accurately, the additional field actually experienced by the reacting system due to the applied field, *F*<sub>int</sub>, is less well understood. *F*<sub>int</sub> is not the total internal electric field; it is rather the change in the electric field experienced by the system due to the externally applied voltage.<sup>59</sup> The local field correction *f* accounts for the difference between *F*<sub>ext</sub> and *F*<sub>int</sub>: *F*<sub>int</sub> = *fF*<sub>ext</sub>. In most treatments, this local field correction factor is *greater* than unity,<sup>29,32,33,39</sup> with typical values of *f* ranging from about 1.2 to 1.8. If this is the case in the RC, then the change in the energies of dipolar state in the presence of the applied voltage is *larger* than that given by the externally applied field.

In order to further our understanding of the mechanism of the initial electron-transfer reaction, we have directly measured via

- (25) Marcus, R. A. *Chem. Phys. Lett.* **1988**, *146*, 13-21.  
 (26) Scherer, P. O. J.; Fischer, S. F. *J. Phys. Chem.* **1989**, *93*, 1633-1637.  
 (27) Warshel, A.; Creighton, S.; Parson, W. W. *J. Phys. Chem.* **1988**, *92*, 2696-2701.  
 (28) DeLeeuw, D.; Malley, M.; Butterman, G.; Okamura, M. Y.; Feher, G. *Biophys. Soc., Abstr.* **1982**, *37*, 111a.  
 (29) Lockhart, D. J.; Boxer, S. G. *Biochemistry* **1987**, *26*, 664-668, 2958.  
 (30) Boxer, S. G.; Lockhart, D. J.; Middendorf, T. R. *Springer Proc. Phys.* **1987**, *20*, 80-90.  
 (31) Lockhart, D. J.; Boxer, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 107-111.  
 (32) Lösche, M.; Feher, G.; Okamura, M. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7537.  
 (33) Lösche, M.; Feher, G.; Okamura, M. Y. In *The Photosynthetic Bacterial Reaction Center—Structure and Dynamics*; Breton, J., Vermeglio, A., Eds.; Plenum: New York, 1988; pp 151-164.  
 (34) Braun, H. P.; Michel-Beyerle, M. E.; Breton, J.; Buchanan, S.; Michel, H. *FEBS Lett.* **1987**, *221*, 221-225.

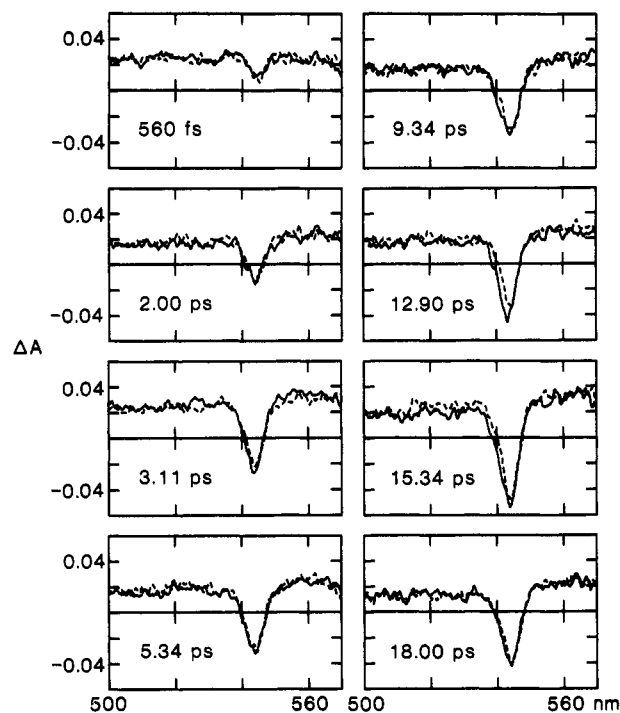
- (35) Woodbury, N. W.; Parson, W. W. *Biochim. Biophys. Acta* **1984**, *767*, 345-361.  
 (36) Goldstein, R. A.; Takiff, L.; Boxer, S. G. *Biochim. Biophys. Acta* **1988**, *934*, 253-63.  
 (37) Lockhart, D. J.; Boxer, S. G. *Chem. Phys. Lett.* **1988**, *144*, 243.  
 (38) Bixon, M.; Jortner, J. *J. Phys. Chem.* **1988**, *92*, 7148-7156.  
 (39) Chen, F. P.; Hanson, D. M.; Fox, D. *J. Chem. Phys.* **1975**, *63*, 3878-3885.

transient absorption spectroscopy the effects of an electric field on the kinetics of the initial charge separation step. The basic interpretation of the observed increase in fluorescence upon application of an electric field predicts a direct relationship between the steady-state fluorescence results and a time-resolved absorption measurement. If the change in the fluorescence intensity is due solely to a change in  $k_{et}$ , then the relative change in the integral of the  $^1P$  decay or  $P^{*+}H_L^{-}$  formation curve upon application of a field is predicted to be equal to  $\Delta F/F$ . Because an applied electric field produces a range of state energies in an isotropic sample, there will also be a distribution of electron-transfer rate constants. Hence, the electron-transfer kinetics, which at zero field are approximately exponential to within the signal-to-noise,<sup>7-12</sup> are expected to become nonexponential as the field is applied. For example, this has been observed and analyzed for the millisecond  $P^{*+}Q_A^{-}$  charge recombination reaction.<sup>40-42</sup> If the previously measured value of  $\Delta F/F$  is indicative of the change in  $k_{et}$  alone, then in an applied field of  $10^6$  V/cm the largest difference between the field-on and field-off kinetic curves at any given time is expected to be only about 10% of the initial amplitude. (This is because the integrated difference between the field-on and field-off curves is expected to be spread out over many zero-field  $1/e$  times; see Discussion section.) On the other hand, if commonly used treatments of the initial electron-transfer reaction are applicable, much larger changes are expected. As shown here, we find a small but nonzero effect of a  $10^6$  V/cm applied electric field on the rate of  $P^{*+}H_L^{-}$  formation, with the observed magnitude of the effect on the kinetics in satisfactory agreement (within about a factor of about 2) with the fluorescence electric field effect results.

The transient absorption measurements also allow us to address the question of whether the electron can be forced to go down the M side to form  $P^{*+}H_M^{-}$  in a very large applied electric field. Examination of the X-ray structure coordinates<sup>1-6</sup> shows that the direction of the  $P^{*+}B_L^{-}$  dipole moment is nearly antiparallel to the  $P^{*+}B_M^{-}$  dipole moment. (The angle between them is estimated to be  $\sim 155^\circ$ ; see Figure 1.) In an isotropic sample, this fortuitous situation can be exploited because for those RCs oriented in the field such that the  $P^{*+}B_L^{-}$  state energy is most increased by the field, the  $P^{*+}B_M^{-}$  state energy is most decreased (approximately), and vice versa. Assuming a 50-D dipole for both  $P^{*+}B_L^{-}$  and  $P^{*+}B_M^{-}$  based on the X-ray structure,<sup>60</sup> for a field of  $10^6$  V/cm, the energy difference between these two states in a given RC can be changed by more than  $1600\text{ cm}^{-1}$  (200 meV), a significant amount on the scale of energy differences believed to be relevant to this problem.<sup>35,36</sup> Note that working with isotropic samples is the key to this experiment: the L and M sides are not differentially affected in samples oriented such that the RC local  $C_2$  axis is parallel to the applied electric field direction. A preliminary account of this work was presented elsewhere.<sup>43</sup>

### Experimental Methods

The measurement of the electric field effect on the initial kinetics was performed at 77 K on  $Q_A$ -containing *Rb. sphaeroides* RC samples in poly(vinyl alcohol) (PVA) matrices. In order to perform electric field effect measurements, it is necessary to dry the sample, especially if the PVA film is coated with Ni electrodes by vapor deposition.<sup>29</sup> It was observed that a fraction of the initially excited RCs returned to the ground state on the time scale of 2–10 ns in such very dry films, in contrast to  $Q_A$ -containing RC samples in frozen glasses or PVA films that have not been subjected to extensive drying.<sup>14</sup> The mechanism leading to this change in very dry films is not understood, though effects of the



**Figure 3.** Transient absorption spectra of an isotropic sample of *Rb. sphaeroides* RCs at 77 K in the region of the  $Q_x$  bands of  $H_L$  and  $H_M$  taken between 0 and 18 ps ( $\sim 0$ –10 zero-field  $1/e$  times), in zero field (solid lines), and in an applied field of  $10^6$  V/cm (dashed lines).

degree of hydration on RCs have been noted before.<sup>44</sup> (There is little change in the absorption or fluorescence properties of our dry films.) The observed value of  $\Delta F/F$  was only slightly sensitive to the degree of drying and did not correlate with the extent of the observed ground-state recovery, an observation which is in keeping with the fact that this ground-state recovery occurs on a much longer time scale than the initial electron-transfer reaction. In order to minimize this problem, some samples were dried for less than 1 day. For these samples, less than 20% of the initially excited RCs were observed to have returned to the ground state in less than 10 ns, and our zero-field decay kinetics at 77 K are comparable with previous studies of RCs in PVA films and in glycerol/water glasses at this temperature.<sup>7,10</sup> Instead of using vapor-deposited Ni electrodes with these samples, glass slides coated with a conductive layer of indium–tin oxide (ITO) were attached to both sides of the film by means of a cyanoacrylate bonding material (Loctite Super Bonder 495).<sup>33</sup> For both of the films used in these experiments, the distance between the electrodes was measured to be  $85 \pm 6\ \mu\text{m}$ , and the applied dc voltage was 8500 V.  $\Delta F/F$  was measured for these samples (mounted in the Dewar and cooled) just prior to the transient absorption measurements.

The transient absorption changes between 450 and 560 nm were measured as a function of time after exciting the samples with 350-fs flashes at 582 nm. Acquisition of data proceeded pairwise: for each delay time setting, the transient absorption spectrum was first measured with the field off, followed by application of the voltage which was left on while a new spectrum was acquired. The absorption Stark effect in the 450–560-nm region in *Rb. sphaeroides* RCs is very small,<sup>31</sup> and consequently the field effect on the absorption of either the exciting or probing light makes no appreciable contribution to the differences between the field-off and field-on kinetic curves. The data were obtained by using two different samples of similar optical density and were combined for analysis.

The temperature dependence of  $\Delta F/F$  was measured between 24 and 220 K in the presence of a field of  $7 \times 10^5$  V/cm. These measurements were performed using different samples than those used for the transient absorption experiments. The samples were

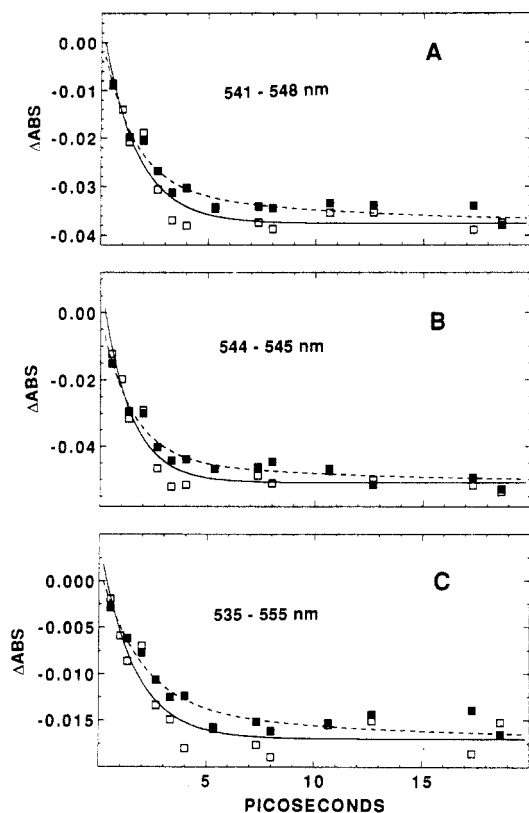
(40) Boxer, S. G.; Goldstein, R. A.; Franzen, S. In *Photoinduced Electron Transfer*; Fox, M. A., Chanon, M., Eds.; Elsevier: Amsterdam, 1988; Vol. B, pp 163–215.

(41) Boxer, S. G.; Lockhart, D. J.; Franzen, S. In *Photochemical Energy Conversion*; J. R. Norris, Jr., Meisel, D., Eds.; Elsevier: Amsterdam, 1989; pp 196–210.

(42) Franzen, S.; Goldstein, R. F.; Boxer, S. G. *J. Phys. Chem.* **1990**, *94*, 5135–49.

(43) Boxer, S. G.; Lockhart, D. J.; Kirmaier, C.; Holten, D. In *Perspectives in Photosynthesis*; Jortner, J., Pullman, B., Eds.; Kluwer: Dordrecht, 1990; pp 39–51.

(44) Clayton, R. K. *Biochim. Biophys. Acta* **1978**, *504*, 255.



**Figure 4.** Time dependence of the transient absorption changes due to the formation of  $H_L^{\bullet-}$  in the absence (open symbols) and presence (closed symbols) of an applied electric field of  $10^6$  V/cm at 77 K. The  $y$  values are the differences between the average absorbance in the wavelength regions (A) 541–548 nm, (B) 544–545 nm, and (C) 535–555 nm, referenced to the transient absorption between 500 and 530 nm (see Figure 3). The zero-field data are fit to a single-exponential function (solid black lines) with  $1/e$  times of (A) 1.6 ps, (B) 1.4 ps, and (C) 1.6 ps. As a guide to the eye and to estimate the extent of the field effect on the kinetics, the field-on data are fit to a sum of two exponentials (dashed lines) with the long time (asymptotic) values fixed at the values determined by the single-exponential fits of the zero-field data and the time constant for the second exponential fixed at 10 ps.

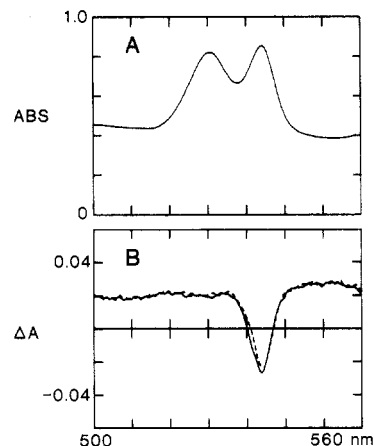
cooled through contact with a cold finger in a closed cycle helium refrigerator.

### Results and Methods of Analysis

**Results.** Transient absorption spectra in the bacteriochlorophyll  $Q_x$  region at a series of times after a 350-fs, 582-nm excitation flash with and without an applied electric field of  $10^6$  V/cm are shown in Figure 3. Plots showing the time course of the bleaching of the 543-nm  $Q_x$  transition for  $H_L^{\bullet-}$  with and without the field are shown in Figure 4. The ground-state absorption spectrum and the cumulative average of all the transient absorption spectra taken between 0 and 20 ps with and without the field are shown in Figure 5. It is evident that there is a small but measurable slowing of the net rate of formation of  $H_L^{\bullet-}$  upon application of the field, but there is no evidence for additional bleaching near 530 nm which would indicate formation of  $H_M^{\bullet-}$ . (It would be desirable to measure and analyze the  $H_L^{\bullet-}$  formation curve for times much longer than 20 ps. However, the state  $P^{*+}H_L^{\bullet-}$  decays with a time constant of about 100 ps at 77 K due to the electron-transfer reaction that forms  $P^{*+}Q_A^{\bullet-}$ , rendering such a measurement difficult to interpret due to the likely electric field effects on this subsequent reaction.)

The value of  $\Delta F/F$  is found to be relatively insensitive to temperature in an applied electric field of  $7 \times 10^5$  V/cm, changing by less than 15% between 24 and 220 K (data not shown).

**Data Analysis.** The data points in Figures 4A–C are the absorbance changes,  $\Delta A$ , averaged over three different wavelength regions, referenced to the featureless, unchanging (both in time and in the presence of an electric field) transient absorption



**Figure 5.** (A) Ground-state absorption spectrum of *Rb. sphaeroides* RCs in PVA at 77 K. (B) Average of the field-on (solid lines) and field-off (dashed lines) transient absorption spectra obtained between 0 and 18 ps. (The spectra used in the average were not separated from each other by a constant time interval, i.e., the number of spectra per picosecond used in the average is not a constant; see the kinetic traces in Figure 4.) The origin of the apparent slight shift and narrowing of the bleaching in the presence of the field is not known, but it is too large to be accounted for by the usual Stark effect on the absorption in this region.<sup>31</sup>

between 500 and 530 nm. Ideally, the difference decay curves (field on minus field off) as a function of field could be used to determine the orientationally averaged distribution of rate constants.<sup>42</sup> The procedure in ref 42 could not be used here because it requires a very high signal-to-noise ratio out to tens of zero-field  $1/e$  times over a range of field strengths. The observed effects on the initial step are very small even at the highest fields used. Therefore, various strategies, all imperfect, have been used to establish whether there is an effect of the field on the rate of electron transfer and, if so, to estimate the upper limit of its magnitude.

First, both the field-off and field-on kinetic curves were fit to single-exponential functions. The quality of the fits to the field-on data are worse than for the field-off data, and the time constants given by the fits to the field-on curves are consistently larger. This is consistent both with a net slowing of the initial electron-transfer reaction and with a distribution of rate constants in the presence of the field. Second, in order to obtain curves that more adequately describe the field-on kinetics and that also can be used both as a guide to the eye and to estimate the magnitude of the integrated change in the kinetics, the field-on curves were fit to a sum of two exponentials [ $\Delta A(t) = B_1 \exp(-t/\tau_1) + B_2 \exp(-t/\tau_2) + \Delta A(\infty)$ ]. We stress that this procedure is used only as a convenient way to compare the field-on and field-off decay curves and to estimate the magnitude of the field-induced change in the kinetics and that the two time constants of the fits to the field-on decays have no direct physical significance. The single-exponential fits of the zero-field curves were used to define the  $\Delta A(\infty)$  value of the decay (the absorbance change produced in the probe region when all the initially excited RCs have reached the state  $P^{*+}H_L^{\bullet-}$ ) for the field-on curves. This is equivalent to assuming that there is no appreciable reduction in the net quantum yield of  $P^{*+}H_L^{\bullet-}$  formation in the presence of the field, but only a change in rates (see below). The data are consistent with this assumption but only allow us to state that the quantum yield of  $P^{*+}H_L^{\bullet-}$  is not reduced by more than about 10%. The assumption of identical  $\Delta A(\infty)$  points for field off and field on is required to make a quantitative comparison between the field effect on the kinetics and the observed value of  $\Delta F/F$ . It is physically reasonable based on the relatively small observed change in the decay curve, the absence of any evidence of  $H_M^{\bullet-}$  formation, and the fact that the electron-transfer reaction at zero applied field is likely several orders of magnitude faster than other competing pathways. In other words, in the absence of other field effects,  $k_{et}$  would have to be reduced by about a factor of  $10^3$  in the field for a substantial fraction of the RCs in the sample for there to be a significant

reduction in the quantum yield of  $P^{*+}H_L^{-}$  formation. For example, the observed 40% increase in the fluorescence intensity at  $10^6$  V/cm corresponds to an expected change in the quantum yield of  $P^{*+}H_L^{-}$  formation of less than 0.1%. The fits of the experimental field-on and field-off kinetic curves are integrated from zero to infinity to estimate the net change in the initial reaction for comparison with the experimental value of  $\Delta F/F$ . Third, an alternative approach that does not require fitting the field-on kinetics to any particular functional form was also applied. Assuming that in the presence of the field the formation of  $P^{*+}H_L^{-}$  is essentially complete after 20 ps, the curves can be approximately integrated by summing the  $y$  values of the points weighted by the appropriate interval along the  $x$ -axis. (The baseline for the integration is defined by the single-exponential fits of the zero-field decay curves.)

These procedures performed on the data in Figure 4, as well as on the raw  $\Delta A$  data in these wavelength regions without referencing to the 500–530-nm background region, yielded very similar results. The two-exponential fitting procedure gave relative increases in the integrated  $\Delta A$  curves which range from 0.65 to 0.87 with an average value of 0.75. The weighted summation approach gave values ranging from 0.46 to 1.0 with an average value of 0.71. The sign of the field effect on the kinetics (a net slowing) is clearly consistent with the observed increase in the fluorescence, and the magnitude is reasonably close to the value of 0.4 expected from the interpretation of the fluorescence increase in terms of a field-induced change in  $k_{et}$ . Agreement to within a factor of 2 is satisfactory for the purpose of comparison of both the kinetic and fluorescence results with the various models discussed in the following.

## Discussion

**Field Effect on the Franck–Condon Factor.** Most treatments of the initial electron-transfer reaction in the RC start with an expression of the form<sup>45</sup>

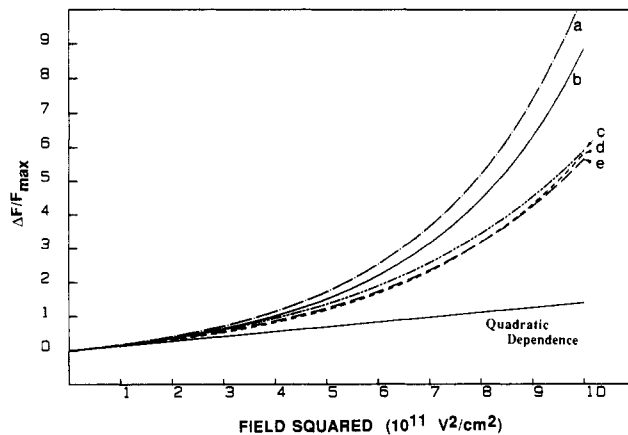
$$k_{et} = (4\pi/h)|V_{et}|^2 FC \quad (1)$$

where  $V_{et}$  is the electronic coupling matrix element and FC is the nuclear factor or Franck–Condon weighted density-of-states term. The effect of an electric field on FC is determined by the relative change in the energies of the initial and final states, which is determined primarily by the interaction of the large product state dipole moment,  $\mu(P^{*+}H_L^{-})$ , with the field. As illustrated in Figure 2, the field shifts the potential surface of the  $P^{*+}H_L^{-}$  state vertically by an amount that depends on the orientation of its dipole in the field. We assume that the field does not affect the reorganization energy at 77 K.

Standard treatments of the Franck–Condon factors include dielectric continuum models which predict a Gaussian dependence of  $k_{et}$  on the driving force,  $\Delta G_{et}$  (e.g., semiclassical Marcus theory), and more elaborate quantum mechanical multimode models which predict a roughly Gaussian dependence in the normal region and a flatter dependence in the inverted region.<sup>45</sup> The Gaussian dependence of  $k_{et}$  on  $\Delta G_{et}$  given by semiclassical Marcus theory or the dependence given by the single-mode quantum mechanical treatment of Bixon and Jortner<sup>38</sup> (who used a mode energy of  $h\nu = 100$   $\text{cm}^{-1}$  and a reorganization energy of  $\lambda = 2000$   $\text{cm}^{-1}$ ) both result in a significantly superquadratic dependence of the net change in the rate of electron transfer (and consequently of  $\Delta F/F$  also) on the applied field strength between  $10^5$  and  $10^6$  V/cm. This is illustrated in Figure 6 and is in conflict with the experimental  $\Delta F/F$  data which follows a quadratic dependence over this range. The origin of a superquadratic field dependence is straightforward to see in the case of the Marcus theory expression in which the rate constant in the presence of a field (assuming for the moment that  $V_{et}$  is field independent) is

$$k_{et}(F) \propto \exp[-(\lambda + \Delta G_{et} - \Delta\mu_{et} \cdot F)^2 / 4\lambda kT] \quad (2)$$

where  $\Delta\mu_{et}$  is the dipole moment difference between the elec-



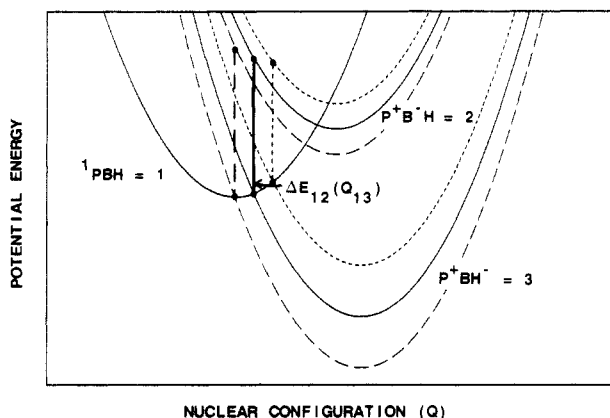
**Figure 6.** Calculated dependence of  $\Delta F/F_{max}$  on the square of the applied field strength for fields between 0 and  $10^6$  V/cm. The lower solid line shows a hypothetical quadratic dependence on field based on the low field values of the other curves. Curve a is calculated by using a semiclassical Marcus theory expression for FC with  $\lambda = -\Delta G_{et}(F_{int}=0) = 2000$   $\text{cm}^{-1}$  at 77 K and with  $V_{et}$  assumed to be independent of field. The other curves are calculated by using a single-mode quantum mechanical expression for FC with the dependence of  $V_{et}$  on  $\Delta E_{12}(Q_{13})$  as given in eq 2. For curves b and d,  $Q_{13}$  is assumed to be field independent, and  $\Delta E_{12}(Q_{13}, F_{int})$  is treated as in ref 38 and eq 5 with  $|\mu(2)| = 51$  D (curve b) or  $|\mu(2)| = 0$  D (curve d). For curves c and e,  $Q_{13}$  is explicitly field dependent, and  $\Delta E_{12}(Q_{13}, F_{int})$  depends on the energies of both the mediating and final states, as in eq 3, with  $|\mu(2)| = 51$  D (curve e) and  $|\mu(2)| = 0$  D (curve c). The calculations are performed using the following zero-field parameters:  $\lambda_{12} = 400$   $\text{cm}^{-1}$ ,  $\Delta G_{12} = 800$   $\text{cm}^{-1}$ ,  $\lambda_{13} = 2000$   $\text{cm}^{-1}$ ,  $\Delta G_{13} = -2000$   $\text{cm}^{-1}$ ,  $|\mu(3)| = 82$  D,  $k_F/k_{et}(F_{int}=0) = 10^{-3}$ , the experimental angle  $\chi$  equal to  $90^\circ$ , the angle between  $\mathbf{p}$  and  $\mu(2)$  equal to  $49^\circ$ , the angle between  $\mathbf{p}$  and  $\mu(3)$  equal to  $58^\circ$ , and the angle between  $\mu(2)$  and  $\mu(3)$  equal to  $37^\circ$ .

tron-transfer final and initial states. ( $|\Delta\mu_{et}|$  is about 50 and 80 D for the  ${}^1PBL \rightarrow P^{*+}B_L^{-}$  and the  ${}^1PH_L \rightarrow P^{*+}H_L^{-}$  reactions, respectively.<sup>60</sup>) For fields approaching  $10^6$  V/cm, the exponent is large, and an expansion of  $k_{et}(F)$  in powers of the field requires terms that depend on powers of  $F$  greater than 2. Terms depending on  $F^4$ ,  $F^6$ , etc., lead to a field dependence that is greater than quadratic (terms which depend on  $F$ ,  $F^3$ , etc., average to zero for an isotropic sample). The quantum mechanical treatment using a single low-frequency mode ( $h\nu \sim 100$   $\text{cm}^{-1}$ ) predicts a similar superquadratic field dependence. These treatments further predict that the electric field effect on the initial reaction will be strongly temperature dependent using a field of around  $10^6$  V/cm,<sup>38</sup> also in conflict with the experimental observations. The discrepancy between the calculated results obtained by using these models and the experimental results suggests that a much less steep dependence of FC (and  $\Delta G_{et}$ ) on the applied field may need to be considered (see below).

**Electric Field Effects on the Electronic Coupling: Superexchange.** The electronic coupling matrix element  $V_{et}$  depends on the electronic overlap of the orbitals on the donor ( ${}^1P$ ) and acceptor ( $H_L$ ).  $V_{et}$  can depend on electric field through a number of mechanisms, such as if the initial and final states have different polarizabilities. However, this is likely to lead to only a small effect on the electron-transfer rate compared to the effect due to the change in the energies of very dipolar states discussed above, though definitive experiments need to be developed to prove this point.

An interesting situation arises if the electronic coupling between the donor and acceptor is mediated by a third state, e.g., by superexchange. For convenience in considering this point we use the following notation (see Figure 7): state 1  $\equiv$   ${}^1PBH$ ; state 2  $\equiv$  mediating state (e.g.,  $P^{*+}B^{-}H$  or  ${}^1BH$ ); state 3  $\equiv$   $P^{*+}BH^{-}$  (the subscript L is dropped for clarity). In such a model the electronic coupling between the initial and final states of the electron-transfer reaction is enhanced by virtue of electronic coupling between the initial and mediating state,  $V_{12}$ , and between the mediating and final state,  $V_{23}$ . The overall electronic coupling,  $V_{et}$ , also depends on the energy difference,  $\Delta E_{12}(Q_{13})$ , between

(45) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265–322.



**Figure 7.** Schematic illustration of the relevant potential energy curves as a function of nuclear configuration for a superexchange mechanism for the initial electron-transfer reaction in the RC. State 1  $\equiv$   ${}^1\text{PBH}$ , state 2  $\equiv$  mediating state, e.g.,  $\text{P}^{+\bullet}\text{B}^-\text{H}$  or  $\text{P}^1\text{BH}$ , state 3  $\equiv$   $\text{P}^{+\bullet}\text{BH}^-$ . The solid lines are zero-field curves for the initial, mediating, and final states. Potential energy curves in the presence of an electric field which is aligned (long dashed) or opposed (short dashed) to the permanent dipole moment of the final state are also shown. For the purpose of illustration, it is assumed that the dipole moment of the initial state is negligible and that the dipole moment of the mediating state is half as large as that of the final state and in the same direction.  $Q_{13}$  is the value of  $Q$  (nuclear configuration) at which the curves for the initial and final states cross along the relevant reaction coordinate, and  $\Delta E_{12}(Q_{13})$  is the vertical energy difference between curves 1 and 2 at this value of  $Q_{13}$ . Note that moving the curve for state 3 vertically relative to that of state 1 (a change in  $\Delta G_{\text{et}}$ ) changes  $Q_{13}$  and thus  $\Delta E_{12}(Q_{13})$ , even if the curve for state 2 remains fixed (as would be the case if state 2 is relatively nonpolar, e.g., if state 2 is  $\text{P}^1\text{BH}$ ). The qualitative features of the figure are expected to be generally applicable to long-distance electron-transfer reactions between neutral molecules.

the initial state and the mediating state at the nuclear configuration of the crossing of the potential energy surfaces of the initial and final state,  $Q_{13}$ . (See Figure 7;  $\Delta E_{12}(Q_{13})$  is not the energy difference between the states at their individual equilibrium nuclear configurations.) A perturbation theory treatment gives<sup>15,26</sup>

$$V_{\text{et}} = \frac{V_{12}V_{23}}{\Delta E_{12}(Q_{13})} \quad (3)$$

where

$$V_{12} = \langle 1|H|2\rangle$$

$$V_{23} = \langle 2|H|3\rangle$$

$$\Delta E_{12}(Q_{13}) = E_2(Q_{13}) - E_1(Q_{13}) \gg V_{12}$$

$H_i$  is the interaction Hamiltonian which couples the states.

An electric field can affect  $V_{\text{et}}$  by changing  $\Delta E_{12}(Q_{13})$  in two ways (we assume that  $V_{12}$  and  $V_{23}$  are field independent for the reasons given earlier for the relative field independence of  $V_{\text{et}}$  in the absence of a mediating state): (i) the energy of the mediating state is changed by the field (this is especially important if the mediating state is very dipolar); (ii) a change in  $\Delta G_{\text{et}}$  (discussed in the last section in the context of its effect on FC) also results in a change in  $Q_{13}$ .<sup>46</sup>

If the field effect on  $V_{\text{et}}$  is modeled solely in terms of (i), then

$$\Delta E_{12}(Q_{13}, \mathbf{F}_{\text{int}}) = \Delta E_{12}(Q_{13}, \mathbf{F}_{\text{int}} = 0) - \mu(2) \cdot \mathbf{F}_{\text{int}} \quad (4)$$

and eq 3 becomes

$$V_{\text{et}} = V_{12}V_{23} / [\Delta E_{12}(Q_{13}, \mathbf{F}_{\text{int}} = 0) - \mu(2) \cdot \mathbf{F}_{\text{int}}] \quad (5)$$

where  $\Delta E_{12}(Q_{13}, \mathbf{F}_{\text{int}} = 0)$  is the zero-field value. This is the approach used by Bixon and Jortner<sup>38</sup> with  $\text{P}^{+\bullet}\text{B}^-$  as the mediating state. For reasonable values of the parameters involved, this approach overestimates the field effect on the fluorescence and the electron-transfer kinetics by about an order of magnitude or

more (see below). We note that the good agreement between the kinetic results presented in this paper and the fluorescence data demonstrates that the discrepancy between the experimental results and the calculations in ref 38 as well as those presented below cannot be explained by contamination of the fluorescence.<sup>61</sup>

Considering now mechanism ii above,  $\Delta E_{12}(Q_{13})$  changes if  $Q_{13}$  changes because the initial- and mediating-state potential energy surfaces are not flat. As a result,  $\Delta E_{12}(Q_{13})$  can be field dependent due to a dipolar final state, even if the mediating state is nonpolar (i.e., both  $V_{\text{et}}$  and FC depend on  $\Delta G_{\text{et}}$ ). Specifically, assuming that the potential energy curves for the initial, mediating, and final states are harmonic and have the same shape, with the shape independent of field (the Condon approximation), then in the presence of the electric field perturbation,  $\mathbf{F}_{\text{int}}$

$$\Delta E_{12}(Q_{13}, \mathbf{F}_{\text{int}}) = \Delta E_{12}(Q_{13}, \mathbf{F}_{\text{int}} = 0) - \mu(2) \cdot \mathbf{F}_{\text{int}} + (\lambda_{12}/\lambda_{13})^{1/2} [\mu(3) \cdot \mathbf{F}_{\text{int}}] \quad (6)$$

where  $\lambda_{ij}$  is the reorganization energy between states  $i$  and  $j$  and  $\mu(i)$  is the permanent electric dipole moment of state  $i$ . The model calculations summarized below show that if  $\text{P}^{+\bullet}\text{B}_L^-$  is the mediating state, then the additional effect of the field on  $V_{\text{et}}$  via the change in  $Q_{13}$  (the third term in eq 6) can mitigate the effect of the field on  $V_{\text{et}}$  through the  $\mu(2) \cdot \mathbf{F}_{\text{int}}$  term. This can be readily seen by comparing the vertical bars in Figure 7 whose length is  $\Delta E_{12}(Q_{13})$ .

Previous detailed considerations of the superexchange mechanism have focused on a role for  $\text{P}^{+\bullet}\text{B}_L^-$  as the mediating state. We also consider the possibility that the mediating state is a nonpolar excited state such as  $\text{P}^1\text{B}_L$ . ( ${}^1\text{B}_L$  is the lowest energy singlet electronic excited state of  $\text{B}_L$ .) Whereas there is no direct information on the energy of the state  $\text{P}^{+\bullet}\text{B}_L^-$  (a major difficulty in a quantitative assessment of any mechanism involving  $\text{P}^{+\bullet}\text{B}_L^-$ ), the absorption spectrum shows that the state  $\text{P}^1\text{B}_L$  in *Rb. sphaeroides* is only about 1100  $\text{cm}^{-1}$  above  ${}^1\text{P}$ . In this case, effect i is unimportant because the mediating state is essentially nonpolar, but effect ii can still cause  $\Delta E_{12}(Q_{13})$ , and thus  $V_{\text{et}}$ , to be field dependent. If the mediating state is  $\text{P}^1\text{B}_L$ , the effects due to (i) and (ii) are not expected to counteract each other, and one has the somewhat counterintuitive possibility that larger electric field effects may result with a nonpolar rather than dipolar mediating state. (Whether the effects are larger or smaller depends on the specific values of the zero-field parameters; see Table I.) A potential problem with a superexchange model based on  $\text{P}^1\text{B}_L$  is that the couplings between  ${}^1\text{P}$  and  $\text{P}^1\text{B}_L$  and between  $\text{P}^1\text{B}_L$  and  $\text{P}^{+\bullet}\text{H}_L^-$  are given by two-electron-exchange integrals, which generally are expected to be smaller than the one-electron matrix elements in the  $\text{P}^{+\bullet}\text{B}_L^-$  mediated model. However, because  $V_{\text{et}}$  also depends on the energy of the mediating state (eq 3), the overall coupling could be larger for coupling mediated by  $\text{P}^1\text{B}_L$  if the energy of  $\text{P}^{+\bullet}\text{B}_L^-$  is considerably higher.

**Comparison with Model Calculations.** To quantitatively compare the predictions of the models outlined above, we have performed calculations of the effects of electric fields both on the fluorescence quantum yield<sup>18,37</sup> and on the kinetics of the initial electron-transfer reaction reported here. The field dependence of FC follows the dependence on  $\Delta G_{\text{et}}$  of the particular model with  $\Delta G_{\text{et}}(\mathbf{F}_{\text{int}}) = \Delta G_{\text{et}}(\mathbf{F}_{\text{int}} = 0) - \Delta\mu_{\text{et}} \cdot \mathbf{F}_{\text{int}}$ , with  $\Delta\mu_{\text{et}}$  approximated by  $\mu(\text{P}^{+\bullet}\text{H}_L^-)$  with  $|\mu(\text{P}^{+\bullet}\text{H}_L^-)| = 82 \text{ D}$ .<sup>60</sup> The observed fluorescence quantum yield for an isotropic sample is given by

$$\Phi_{\text{F}}(\mathbf{F}_{\text{int}}) \propto \int \int \int (\mathbf{e} \cdot \mathbf{p})^2 \{k_{\text{F}} / [k_{\text{F}} + k_{\text{nr}} + k_{\text{et}}(\mathbf{F}_{\text{int}})]\} \sin \theta \, d\theta \, d\phi \, d\psi \quad (7)$$

where  $\mathbf{e}$  is a unit vector in the polarization direction of the analyzing polarizer,  $\mathbf{p}$  is a unit vector in the direction of the fluorescence transition dipole moment,  $k_{\text{F}}$  is the radiative rate constant,<sup>61</sup>  $k_{\text{nr}}$  is the sum of the rate constants for all other nonradiative process, and  $k_{\text{et}}(\mathbf{F}_{\text{int}})$  is the electric-field-dependent electron-transfer rate constant. We have defined  $\Delta F/F = \{(\Phi_{\text{F}}(\mathbf{F}_{\text{int}}) - \Phi_{\text{F}}(\mathbf{F}_{\text{int}} = 0)) / \Phi_{\text{F}}(\mathbf{F}_{\text{int}} = 0)\} = \{\Phi_{\text{F}}(\mathbf{F}_{\text{int}}) / \Phi_{\text{F}}(\mathbf{F}_{\text{int}} = 0)\} - 1$ . It is  $\Delta F/F$  which is observed to increase quadratically with field, not  $\{\Phi_{\text{F}}(\mathbf{F}_{\text{int}}) / \Phi_{\text{F}}(\mathbf{F}_{\text{int}} = 0)\}$ .

(46) Mikkelsen, K. V.; Ulstrup, J.; Zakaraya, M. G. *J. Am. Chem. Soc.* **1989**, *111*, 1315-1319.

**TABLE I: Calculated Values of  $\Delta F/F$  or the Relative Change in the Integrated Kinetics<sup>a</sup> for an Electric Field of  $10^6$  V/cm Calculated Using Eq 7 or 8 with Different Models<sup>b</sup> for the Field Dependence of FC and  $V_{et}$ <sup>c</sup>**

$\Delta G_{12}$	$\lambda_{12}$	$\mu(2)$	$\Delta G_{13}$	model <sup>b</sup>	$\Delta F/F^a$
400	800	51	-2000	A	5.66
		51		B	8.86
		0		A	5.92
800	800	0	B	5.83	
		51	A	5.64	
		51	B	7.85	
800	1400	0	A	5.47	
		51	B	5.83	
		0	A	5.45	
400	1400	51	-2000	A	5.43
		51	B	7.15	
		0	A	5.43	
800	800	51	-2000	B	5.83
		0	A	5.44	
		0	B	7.55	
800	800	0	A	5.67	
		51	B	5.83	
		0	A	24.35	
800	800	51	-1600	B	48.91
		0	A	7.60	
		0	B	30.27	
800	800	51	-2400	A	11.06
		51	B	5.21	
		0	A	16.69	
		0	B	9.22	
			-2000	SCM	10.42
			-1600	SCM	38.16
			-2400	SCM	38.16

<sup>a</sup>This also corresponds to the calculated value of the relative change in the total integrated area (integration from  $t = 0$  to  $\infty$ ) under the kinetic curves for the initial electron-transfer reaction

$$\Delta F/F = \left[ \int \Delta A_{on}(t) dt - \int \Delta A_{off}(t) dt \right] / \left[ \int \Delta A_{off}(t) dt \right]$$

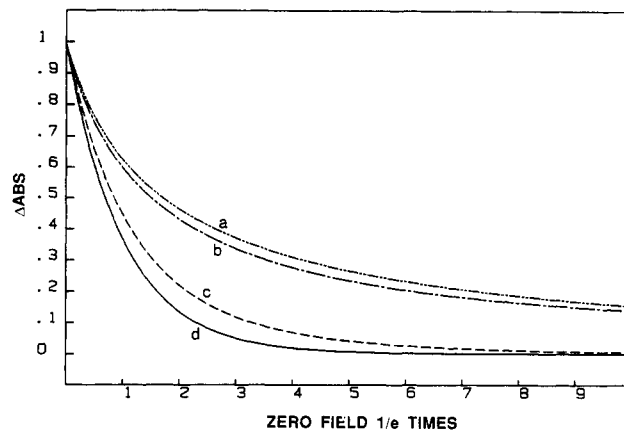
where  $\Delta A_{on}(t)$  and  $\Delta A_{off}(t)$  are the transient absorption curves with the field on or off, respectively. <sup>b</sup>Models A and B both use the single-mode quantum mechanical expression for FC with  $\lambda_{13} = 2000$   $\text{cm}^{-1}$  and  $h\nu = 100$   $\text{cm}^{-1}$  at 77 K. For models A and B, the field dependence of  $V_{et}$  on  $\Delta E_{12}(Q_{13})$  is given by eq 3. For model A,  $Q_{13}$  is explicitly field dependent and  $\Delta E_{12}(Q_{13}, F_{int})$  depends on the energies of both the mediating and final states, as given in eq 6. For model B,  $Q_{13}$  is assumed to be independent of field as in ref 38, and  $\Delta E_{12}(Q_{13}, F_{int})$  is given by eq 4. Model SCM uses the semiclassical Marcus theory expression for FC with  $\lambda_{13} = 2000$   $\text{cm}^{-1}$  at 77 K, and the value of  $V_{et}$  is assumed to be field independent. <sup>c</sup>The following parameter values are used for all calculations:  $|\mu(3)| = 82$  D,  $k_F/k_{et}(F_{int}=0) = 10^{-3}$ , the experimental angle  $\chi$  between the polarization direction of the analyzing polarizer and the applied electric field is  $90^\circ$ , the angle between the fluorescence transition dipole moment and the permanent electric dipole of state 2 and state 3 is  $49^\circ$  and  $58^\circ$ , respectively, and the angle between the permanent dipoles of states 2 and 3 is  $37^\circ$ . The internal angles are estimated from the *Rps. viridis* X-ray coordinates<sup>1-3</sup> assuming that state 2 is  $P^{*+}B_L^{-}$  and state 3 is  $P^{*+}H_L^{-}$ .<sup>60</sup>

The absorption change as a function of the time after excitation,  $\Delta A(t)$ , for an isotropic sample (excited isotropically) in the presence of an electric field is given by

$$\Delta A(t) \propto \int \int \int (\mathbf{e} \cdot \mathbf{p})^2 \{ k_{et}(F_{int}) / [k_F + k_{nr} + k_{et}(F_{int})] \} \exp[-k_{et}(F_{int}) \cdot t] \sin \theta d\theta d\phi d\psi \quad (8)$$

The terms are defined as above except that in this case  $\mathbf{p}$  is in the direction of the transition dipole moment for the transition used to follow the reaction and  $\mathbf{e}$  is a unit vector in the direction of the electric vector of the probing light. The term  $k_{et}(F_{int}) / [k_F + k_{nr} + k_{et}(F_{int})]$  is a measure of the initial electron-transfer quantum yield in the field which can be different from the zero-field value due to the competition between the field-independent radiative and nonradiative pathways<sup>61</sup> and the field-dependent electron-transfer reaction.

At the present time there is no direct information on the shapes of the potential energy surfaces or the energy of states such as  $P^{*+}B_L^{-}$ , so we follow the lead of Bixon and Jortner<sup>38</sup> and take



**Figure 8.** Simulations of the initial electron-transfer kinetics in the presence of an electric field of  $10^6$  V/cm at 77 K. Curve d is the zero-field decay curve. Curve c is the field-on curve calculated by using the rate versus free energy relation obtained from a complete modeling of all the fluorescence electric field effect data which is consistent with the observed 40% increase in the fluorescence yield in an applied field of  $10^6$  V/cm<sup>47</sup> (see Figure 9). Curves a and b are calculated by using a superexchange model with the dependence of FC on  $\Delta G_{et}$  given by the single-mode quantum mechanical treatment ( $h\nu = 100$   $\text{cm}^{-1}$ ,  $\lambda = -\Delta G_{et}$  ( $F_{int}=0$ ) = 2000  $\text{cm}^{-1}$ ). The effect of the field on the electronic coupling for these two curves depends either on the energy of the mediating state alone as in eq 5 (curve b) or on both the energies of the mediating and final states as in eq 3 (curve a). These curves are calculated by using reasonable values of the various zero-field parameters ( $\lambda_{12} = 400$   $\text{cm}^{-1}$ ,  $\Delta G_{12} = 800$   $\text{cm}^{-1}$ ,  $|\mu_2| = 51$  D,  $\lambda_{13} = 2000$   $\text{cm}^{-1}$ ,  $\Delta G_{13} = -2000$   $\text{cm}^{-1}$ ,  $|\mu_3| = 82$  D). The values of the other parameters used in the calculations are the experimental angle  $\chi$  between the direction of the electric field and the polarization direction of the probing light equal to  $90^\circ$ ,  $k_F/k_{et}$  ( $F_{int}=0$ ) =  $10^{-3}$ , the angle between the  $Q_x$  transition dipole moment for  $H_L$  and  $\mu(P^{*+}B_L^{-})$  equal to  $26^\circ$  and between the same transition dipole and  $\mu(P^{*+}H_L^{-})$  equal to  $51^\circ$ , with the angle between  $\mu(P^{*+}B_L^{-})$  and  $\mu(P^{*+}H_L^{-})$  equal to  $37^\circ$ .

physically reasonable values. Initially, we take  $\Delta G_{12} = 400$   $\text{cm}^{-1}$ ,  $\lambda_{12} = 800$   $\text{cm}^{-1}$ ,  $\Delta G_{13} = -2000$   $\text{cm}^{-1}$ , and  $\lambda_{13} = 2000$   $\text{cm}^{-1}$ . The experimental angle  $\chi$  between the electric vector of the light and the direction of the applied electric field in both the fluorescence and transient absorption experiments is equal to  $90^\circ$ , the final-state dipole is set equal to  $|\mu(P^{*+}H_L^{-})| = 82$  D, and the relative directions of the state dipoles and transition dipoles are estimated from the X-ray structure of *Rps. viridis*.<sup>1-3,60</sup> (See the next section for the consequences of a two-step model in which  $P^{*+}B_L^{-}$  is the initially formed transient state.) For the superexchange treatments outlined above, this set of parameters and a field of  $10^6$  V/cm at 77 K were used to calculate a value of  $\Delta F/F$ , which is also the calculated relative field effect on the initial electron-transfer kinetics (see footnote a, Table I). Using the dependence of  $V_{et}$  on field given by eq 4, the predicted value is 8.9 if the mediating state is  $P^{*+}B^{-}$  and 5.8 if the mediating state is  $P^1B$ . ( $|\mu(P^1B)|$  is assumed to be 0 D for the purposes of the calculations.) Using the dependence of  $V_{et}$  on field due to the change in  $\Delta E_{12}(Q_{13})$  as given in eq 6,  $\Delta F/F$  is predicted to be 5.7 if the mediating state is  $P^{*+}B^{-}$  and 5.9 if the mediating state is  $P^1B$ . The calculated results for other sets of parameters are shown in Table I. It is clear that all of the calculated values are significantly larger than the observed value of  $\Delta F/F = 0.4$  and the observed value of about 0.7 for the relative field effect on the initial electron-transfer kinetics.

Examples of the predicted field-on  $\Delta A$  decay curves obtained from the above superexchange models are shown in Figure 8. When these calculated kinetic curves are fit to a sum of two exponentials over the region between 0 and 10 zero-field  $1/e$  times (i.e., they are treated as in one of the approaches to the analysis of the experimental data), then the relative change in the integrated area of the curves (integrating between zero and infinity) is calculated to be in the range 3–4.<sup>63</sup> These calculated curves are very different from the lower dashed line in Figure 8 (curve c) which is consistent with the experimental data in Figure 4,<sup>47</sup>

and effects of this magnitude would be readily measurable.

**Sequential Mechanism.** On the basis of the results of subpicosecond transient absorption experiments performed at room temperature, Zinth and co-workers<sup>13</sup> have recently concluded that the state  $P^{*+}B_L^{-}$  is formed as a discrete intermediate prior to the formation of  $P^{*+}H_L^{-}$ . From the analysis of the kinetic data, they estimate that  $P^{*+}B_L^{-}$  is formed with a rate constant of about  $(3 \text{ ps})^{-1}$  followed by a more rapid charge shift reaction to form  $P^{*+}H_L^{-}$  with a rate constant of about  $(1 \text{ ps})^{-1}$ . This conclusion is contrary to that drawn from experiments performed in other laboratories.<sup>7-12,18</sup> In the following we sketch some of the implications of the electric field effect measurements described in the present paper on this issue.

Any analysis of this problem depends on knowledge of the energies of  $P^{*+}B_L^{-}$  and  $P^{*+}H_L^{-}$  and the shapes of their potential surfaces. Although the limiting energies of  $^1P^{48}$  and  $^3P^{49}$  are well-characterized by direct spectroscopic measurements, the energy of  $P^{*+}B_L^{-}$  is unknown. The energy of  $P^{*+}H_L^{-}$  is likely to be between 170<sup>35</sup> and 260 meV<sup>36</sup> below that of  $^1P$  at room temperature. In the following we use the value of about 170 meV (about 1400  $\text{cm}^{-1}$ ) for the energy difference between  $^1P$  and the unrelaxed  $P^{*+}H_L^{-}$  state.<sup>50,51</sup>

Because the rate of formation of  $P^{*+}H_L^{-}$  is observed to be relatively insensitive to temperature,<sup>7,10,12</sup> a two-step, sequential mechanism involving  $P^{*+}B_L^{-}$  requires that this state be lower in energy than  $^1PBH$ , by an amount between about 100 and 1200  $\text{cm}^{-1}$ . [This zero-field free energy difference is denoted  $\Delta G(^1P \rightarrow P^{*+}B_L^{-}; F_{\text{int}}=0)$ .] We can examine the consequences for the sequential mechanism of applying an electric field by stepping the unknown zero-field value of  $\Delta G(^1P \rightarrow P^{*+}B_L^{-})$  through a possible range of values. For  $\Delta G(^1P \rightarrow P^{*+}B_L^{-}; F_{\text{int}}=0) = -500 \text{ cm}^{-1}$ , in the presence of a field of  $10^6 \text{ V/cm}$ , about 20% of the RCs in the sample will have a value of  $\Delta G(^1P \rightarrow P^{*+}B_L^{-})$  which is positive; i.e., the hypothetical  $^1PB_L \rightarrow P^{*+}B_L^{-}$  electron-transfer reaction will be *endergonic* in the presence of the field for a significant fraction of the sample. (This estimate is obtained assuming  $\mu(P^{*+}B_L^{-}) = 51 \text{ D}$ , neglecting the dipole moment of  $^1P$  and taking  $f = 1.0$ .) If  $f$  is somewhat greater than 1.0 (as is physically reasonable<sup>59</sup>), then a *larger* fraction of the sample will have a positive  $\Delta G$ ; likewise, if  $-\Delta G(^1P \rightarrow P^{*+}B_L^{-}; F_{\text{int}}=0) < 500 \text{ cm}^{-1}$ , the fraction will be larger. This has two consequences: first, the field effect on the initial electron-transfer rate is expected to be extremely large, and second,  $\Delta F/F$  is expected to be very strongly temperature dependent,<sup>38</sup> both contrary to the experimental results.

If  $\Delta G(^1P \rightarrow P^{*+}B_L^{-}; F_{\text{int}}=0)$  is more negative than 700  $\text{cm}^{-1}$ , electric field effects on the second, hypothetical  $P^{*+}B_L^{-}H_L \rightarrow P^{*+}B_LH_L^{-}$  electron-transfer reaction must be considered because, in this case, the driving force for this reaction will be quite small ( $< 700 \text{ cm}^{-1}$ ). This second reaction is *also* expected to be very sensitive to an applied electric field because the change in dipole moment,  $|\mu(P^{*+}H_L^{-}) - \mu(P^{*+}B_L^{-})|$ , is about 50 D<sup>59</sup>, and the zero-field free energy difference between these states is constrained to be small. If a two-step mechanism,  $^1PBH \rightarrow P^{*+}B_L^{-}H_L \rightarrow P^{*+}B_LH_L^{-}$ , is appropriate, it is also possible that in the presence of the field the rate for the first step could become larger than for the second step for some of the RCs, thus changing the rate-determining step for formation of  $P^{*+}B_LH_L^{-}$ . This could be checked experimentally by comparing the rate of decay of the state  $^1PBH$  (via the stimulated emission from  $^1P$ ) and the rate of formation of  $P^{*+}B_LH_L^{-}$  in the presence of a large applied electric field. If the second step,  $P^{*+}B_L^{-}H_L \rightarrow P^{*+}B_LH_L^{-}$ , is significantly field dependent, then the correspondence between the field effect on the fluorescence (which in this case would depend on the rate

of the  $^1PBH \rightarrow P^{*+}B_L^{-}H_L$  reaction) and the kinetics of  $P^{*+}H_L^{-}$  formation (which depend on the rates of both reactions) is not expected to hold. As described above, this correspondence is demonstrated experimentally to within about a factor of 2.

From these considerations and the energetic constraints imposed by the value of  $-\Delta G(^1PBH \rightarrow P^{*+}B_LH_L^{-}; F_{\text{int}}=0) \sim 1400 \text{ cm}^{-1}$ , the effect of the field on the net rate of  $P^{*+}H_L^{-}$  formation within the two-step model is expected to be smallest if both  $-\Delta G(^1PBH \rightarrow P^{*+}B_L^{-}H_L; F_{\text{int}}=0)$  and  $-\Delta G(P^{*+}B_L^{-}H_L \rightarrow P^{*+}B_LH_L^{-}; F_{\text{int}}=0)$  are about 700  $\text{cm}^{-1}$ . By use of this set of values and, for the purpose of comparison, the semiclassical Marcus theory expression for  $k_{\text{et}}$  (with  $\lambda = -\Delta G = 700 \text{ cm}^{-1}$  and  $|\Delta\mu| = 50 \text{ D}$  for each of the two steps), the discrepancy between the experimental observations and the calculated results is large; however, it is not significantly worse than it is for a single-step model with  $\lambda = -\Delta G = 2000 \text{ cm}^{-1}$  and  $|\Delta\mu| = 80 \text{ D}$ , where the agreement is also poor (see Table I). The discrepancy becomes significantly greater in the two-step model if either of the zero-field  $-\Delta G$  values are less than 700  $\text{cm}^{-1}$  or if  $\lambda \neq \Delta G$  for either step. Thus, the experimental results described in this paper render the two-step mechanism unlikely and, at the very least, place very severe constraints on the energetics in a two-step model.

**Comparison with Other Experimental Results.** Popovic and co-workers have reported measurements of electric field effects on the quantum yield of  $P^{*+}Q_A^{-}$  formation, denoted  $\Phi(P^{*+}Q_A^{-})$ , for RCs oriented in Langmuir-Blodgett films.<sup>52</sup> A very large effect was observed on this quantum yield, and this was interpreted in terms of a change in the rate of the initial,  $^1PH_L \rightarrow P^{*+}H_L^{-}$  reaction.<sup>53</sup> This interpretation is inconsistent with the results reported in this paper. Furthermore, a number of issues, which we now consider, make quantitative comparisons between our experiments and the interpretation in ref 53 difficult.

The value of  $\Phi(P^{*+}Q_A^{-})$  depends on the rate of the  $^1PH \rightarrow P^{*+}H_L^{-}$  reaction as well as on several subsequent competing reactions (e.g.,  $P^{*+}H_L^{-}Q_A \rightarrow P^{*+}H_LQ_A^{-}$ ,  $P^{*+}H_L^{-} \rightarrow PH_L$ ,  $P^{*+}H_L^{-} \rightarrow ^3PH_L$ , and reactions involving  $B_L$  if these are important). Thus, a change in  $\Phi(P^{*+}Q_A^{-})$  could be due to electric field effects on processes subsequent to the initial step. Any attempt to relate a field-induced change in  $\Phi(P^{*+}Q_A^{-})$  to one in  $k_{\text{et}}$  depends critically on the zero-field value of  $\Phi_{\text{et}} = k_{\text{et}}/(k_{\text{et}} + k_d)$ , where  $\Phi_{\text{et}}$  is the quantum yield of the initial electron-transfer step and  $k_d$  is the combined rate of all other decay processes besides electron transfer. The experimental value in the literature for  $\Phi(P^{*+}Q_A^{-})$  is  $1.02 \pm 0.04$ ;<sup>54</sup> however, Moser and co-workers chose a zero-field value of  $\Phi_{\text{et}} = 0.98$ <sup>53</sup> for their analysis. The experimental uncertainty in the measurement of  $\Phi(P^{*+}Q_A^{-})$ , though small in absolute terms, is large in terms of what is relevant for relating the change in  $\Phi(P^{*+}Q_A^{-})$  to a change in  $k_{\text{et}}$ . For example, if the value of  $\Phi_{\text{et}}$  used in the analysis is varied between 0.98 and 0.999, the change in  $k_{\text{et}}$  consistent with the experimentally measured field-induced change in  $\Phi(P^{*+}Q_A^{-})$  is found to vary by more than an order of magnitude. Therefore, the field-induced change in  $k_{\text{et}}$  cannot be quantitatively determined from the change in  $\Phi(P^{*+}Q_A^{-})$ , even if we assume (see last paragraph) that the observed change in  $\Phi(P^{*+}Q_A^{-})$  is due solely to a change in  $k_{\text{et}}$ .

There is also concern about the calibration of the applied electric field in the Langmuir-Blodgett films since this requires a precise measurement of the film thickness. It was reported<sup>53</sup> that the thickness was determined by measuring the sample capacitance and using a bulk dielectric constant of 3, even though the samples were reported to be hydrated. If the dielectric constant were greater, as seems reasonable for a hydrated sample, then the actual sample thickness would be larger, and the applied field,  $F_{\text{ext}}$ , will be smaller than believed. (This is not an issue for the thicker polymer film samples used in this paper where the sample thickness can be determined directly.) Finally, Moser and co-workers treat

(47) Lockhart, D. J.; Hammes, S.; Franzen, S.; Boxer, S. G. Submitted for publication.

(48) Woodbury, N. W.; Parson, W. W. *Biochim. Biophys. Acta* **1986**, *850*, 197-210.

(49) Takiff, L.; Boxer, S. G. *Biochim. Biophys. Acta* **1988**, *932*, 325-334.

(50) Woodbury, N. W.; Parson, W. W. *Biochim. Biophys. Acta* **1984**, *767*, 345-361.

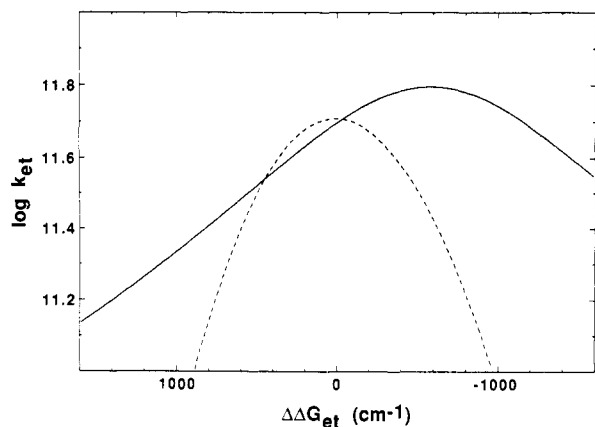
(51) Goldstein, R. A.; Boxer, S. G. *Biochim. Biophys. Acta* **1989**, *977*, 70-77, 78-86.

(52) Popovic, Z. D.; Kovacs, G. J.; Vincett, P. S.; Alegria, G.; Dutton, P. L. *Biochem. Biophys. Acta* **1986**, *851*, 38-48.

(53) Moser, C. C.; Alegria, G.; Gunner, M. R.; Dutton, P. L. *Isr. J. Chem.* **1988**, *28*, 133-139.

(54) Wraight, C. A.; Clayton, R. K. *Biochim. Biophys. Acta* **1973**, *333*, 246-260.





**Figure 9.** (—) A plot of the dependence of  $\log k_{et}$  on  $\Delta\Delta G_{et}$  (the change in the value of  $\Delta G_{et}$  due to the presence of the applied electric field) which is consistent with all of the experimental electric field effect data assuming that the primary effect of the field is due to a change in  $\Delta G_{et}$  by an amount  $-\Delta\mu_{et}\cdot F$ , with  $|\Delta\mu_{et}| = 80$  D. This curve is shown for the purpose of illustration only and for comparison with more standard treatments (---); it is not necessarily unique (see ref 47 for details). (-.-) The dependence of  $\log k_{et}$  on  $\Delta\Delta G_{et}$  calculated by using the single-mode, quantum mechanical treatment of Jortner et al.<sup>38</sup> with  $h\nu = 100$   $\text{cm}^{-1}$  and  $\lambda = -\Delta G_{et}(F=0) = 2000$   $\text{cm}^{-1}$  at 77 K, with the electronic coupling  $V_{et} = 25.7$   $\text{cm}^{-1}$  ( $V_{et}$  field independent). A similar dependence is obtained by using the semiclassical Marcus theory expression for  $k_{et}$  with  $\lambda = -\Delta G_{et}(F=0) = 2000$   $\text{cm}^{-1}$  at 77 K.

the local field correction<sup>59</sup> as the dielectric constant of the bulk Langmuir–Blodgett film (assumed to be 3) divided by the dielectric constant of the RC protein.<sup>53</sup> This approach is without theoretical justification and is contrary to standard treatments. Each of these issues makes it difficult to quantitatively compare the results presented in the present paper and those in ref 53.

It is interesting to consider the expected magnitude of the electric field effect for RCs oriented in a Langmuir–Blodgett film. Figure 9 shows a  $k_{et}$  vs  $\Delta G_{et}$  curve which is consistent with the data in the present paper, with information from the X-ray structure and with constraints imposed by the fluorescence electric field effect amplitude, line shape, and angle dependence.<sup>47</sup> Assuming that  $k_{et}$  leads to formation of  $P^{*+}H_L^{-}$ , for  $F_{ext} = 1 \times 10^6$  V/cm a change in  $k_{et}$  of less than a factor of 2 is predicted for RCs whose local  $C_2$  axis is oriented parallel or antiparallel to the field direction. It would be very desirable to measure  $k_{et}$  in such oriented samples; however, the effects are not expected to be very large because the dipoles of the initially formed states are not parallel to the  $C_2$  axis, and the dependence of  $k_{et}$  on field (Figure 9) is quite weak.

**Electron Transfer along the M Branch.** As shown in Figure 5, there is no evidence from the transient absorption spectra obtained in the presence of an applied electric field of  $10^6$  V/cm at 77 K for electron transfer to  $H_M$ , as would be indicated by a bleaching at around 530 nm. There are several possible interpretations for this interesting negative result, among them the following. (1) The  $P^{*+}B_M^{-}$  or  $P^{*+}H_M^{-}$  states are so much higher in energy than the corresponding L-side states at zero field that, even for the most favorable orientations of the dipoles relative to the field, their energies are not low enough to be relevant. (2) The distances along the L side are less than along the M side by enough so that the electronic coupling for  $P^{*+}B_M^{-}$ -mediated electron transfer to  $H_M$  is too weak for electron transfer to  $H_M$  to compete with transfer to  $H_L$  even under favorable energetic conditions. (3) The electronic asymmetry of the state  $^1P$  is great, resulting in the situation described in (2). (4) States of the type  $P^{*+}B^{-}$  are not the key mediators of the electronic coupling between  $^1P$  and  $P^{*+}H^{-}$ . The results of the experiments described in this paper alone cannot distinguish between these possibilities; however, we can conclude that the unidirectionality of the initial electron transfer is not due to small (within 200 meV) energetic differences between dipolar states involving the chromophores on the M and L sides.

## Summary

Application of an electric field of  $10^6$  V/cm at 77 K slightly reduces the net rate of the initial electron-transfer process,  $^1P \rightarrow P^{*+}H_L^{-}$ , consistent with the observed increase in the yield of competing prompt fluorescence from  $^1P$ .<sup>18,37</sup> The magnitude of the predicted electric field effect calculated by using common treatments of the dependence of  $k_{et}$  on  $\Delta G_{et}$  is larger by about an order of magnitude or more than is experimentally observed. The inclusion of the effect on  $V_{et}$  due to a field-dependent value of  $Q_{13}$  can reduce the calculated magnitude of the field effect, but likely not enough to bring it into line with experiment. It appears that semiclassical and single-mode quantum theories predict too large a field effect because these theories predict too steep a dependence of FC on  $\Delta G_{et}$ . The predicted effects of a field on the initial electron-transfer reaction thus tend to be too large regardless of the specific mechanism being modeled or the specific treatment of the field dependence of  $V_{et}$ . It appears that, at the very least, a treatment of the initial electron-transfer reaction which considers coupling to a more physically realistic distribution of low- and high-frequency modes<sup>42,55</sup> leading to a flatter dependence of  $k_{et}$  on  $\Delta G_{et}$ <sup>47,56–58</sup> is now warranted by the apparent inability of the simplified treatments to predict the experimentally observed electric field effects.

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(55) Gunner, M. R.; Dutton, P. L. *J. Am. Chem. Soc.* **1989**, *11*, 3400.

(56) Jortner, J. *J. Chem. Phys.* **1976**, *64*, 4860.

(57) Sarai, A. *Biochim. Biophys. Acta* **1980**, *589*, 71.

(58) Kakitani, T.; Kakitani, H. *Biochim. Biophys. Acta* **1981**, *635*, 498.

(59) Because  $F_{int} = fF_{ext}$ , when the applied field  $F_{ext} = 0$ ,  $F_{int} = 0$ . The notation  $F_{int} = 0$  is used to describe the situation in which the external field-induced perturbation is zero because no field is applied. Internal electric fields are always present in the RC (or any sample) at zero applied field due to the distribution of charges in the material; their effects are incorporated in the properties measured in the absence of an applied field such as the zero-field kinetics. We assume that  $f$  is a scalar quantity.

(60) For the purposes of calculating the magnitudes and directions of the permanent electric dipole moments of the charge-separated states, we have assumed that the charge distributions in the anion and cation radicals can be approximated by point charges located at the geometric centers of the macrocycles. (The geometric center of dimeric P is defined as the point midway between the centers of the overlapping rings I of the monomeric bacteriochlorophylls which comprise P.) Note that it is the electron density in the ions on the picosecond time scale, not at equilibrium, which is relevant. Small shifts in charge density will have negligible effects on the calculations presented in this paper. A central assumption is that whole charges are separated, i.e., that the cationic or anionic intermediates do not lose or gain a proton on the time scale of the electron-transfer reaction.

(61)  $\Delta F/F$  has been found to be similar for many samples using RCs from different preparations as well as RCs prepared in different laboratories. The fluorescence measurements have also been repeated independently in another laboratory, and the results obtained are in quantitative agreement with the original results (Michel-Beyerle, M. E. Personal communication).  $\Delta F/F$  for *Rps. viridis* RCs is also similar (Lockhart, D. J.; Boxer, S. G. Unpublished results).

(62) The RC fluorescence radiative rate constant,  $k_F$ , is assumed to be field independent, as is determined to be the case for purified, monomeric bacteriochlorophyll *a* and bacteriopeophytin *a* by the absence of a significant change in the net fluorescence intensity for these molecules in an applied electric field at 77 K.<sup>36</sup> Furthermore, there is very little, if any, change in the oscillator strength for the  $Q_y$  absorption transition of P in the presence of an electric field at 77 K, as determined by the line shape of the absorption Stark effect spectrum.<sup>31–33</sup> If the emitting state in the RC is the same as the one formed upon absorption into the  $Q_y$  band of P ( $^1P$ ), then  $k_F$ , which is proportional to the oscillator strength, is relatively independent of applied electric field.

(63) Note that this value is not equal to the calculated values of  $\Delta F/F$  in Table I because the calculated field-on curves are approximated by fitting only between 0 and 10 zero-field  $1/e$  times, as had to be done with the experimental data due to the onset of the subsequent  $H_L^{-} \rightarrow Q_A$  reaction; however, these calculated field-on curves are not within 1% of their long time limiting value until well past 30  $1/e$  times. The calculated curves are well fit to a sum of two exponentials between 0 and 10 zero-field  $1/e$  times, but the function that fits over this limited region does not fit well everywhere past this point because these calculated curves reach their limiting values very slowly.