

# Effects of Applied Electric Fields on the Quantum Yields of the Initial Electron-Transfer Steps in Bacterial Photosynthesis. 1. Quantum Yield Failure

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The effect of an applied electric field has been measured for the quantum yield of formation of the  $P^+H^-$ ,  $P^+Q_A^-$ , and  $^3P$  states of nonoriented *Rb. sphaeroides* reaction centers at 80 K in poly(vinyl alcohol) (PVA) films and in glycerol–buffer glasses. Both the shape and magnitude of the field dependence are very similar for all three observables in both media where the data overlap. This result suggests that the reduction of the quantum yield occurs at a very early step in the charge separation process. More than 50% reduction in the quantum yield is observed at fields of  $2.5 \times 10^6$  V/cm in PVA films. Possible mechanisms include field-dependent nonradiative decay of  $^1P$ , a field-dependent rate of formation or decay of  $P^+B^-$  if  $P^+B^-$  is important, and field-dependent formation or decay of an unrelaxed form of  $P^+H^-$ . Small electric field effects are observed on the recombination rate of the radical pair  $P^+H^-$  and the magnetic field dependence of the  $^3P$  quantum yield. This is likely because those orientational subpopulations of reaction centers which survive to become  $P^+H^-$  and  $^3P$  in an electric field are those which did not experience quantum yield failure at an early step. The field-induced effects provide a basis for a possible mechanism to regulate photosynthetic charge separation by transmembrane potentials.

## Introduction

The mechanism of the initial charge separation steps in bacterial photosynthesis is a fascinating example of long-distance electron transfer in an organized molecular assembly. These first electron-transfer reactions are among the fastest reactions known and show remarkably little dependence on temperature.<sup>1</sup> The positions of the redox-active components are known from the X-ray structure of bacterial reaction centers (RCs).<sup>2–5</sup> A reaction scheme involving these components is shown in Figure 1. P is the primary electron donor, a closely interacting pair of bacteriochlorophyll molecules whose singlet excited state  $^1P$  initiates the electron-transfer events. B is a bacteriochlorophyll monomer which may serve as the initial electron acceptor. Evidence for formation of  $P^+B^-$  from  $^1P$  comes mainly from transient absorption spectroscopy,<sup>6</sup> however, there is much disagreement on the data and its interpretation.<sup>7,8</sup> There is no experimental information on the energy of this state, so it is not included in Figure 1. There is general agreement that a monomeric bacteriopheophytin H is reduced within a few picoseconds.<sup>9,10</sup> The electron on  $H^-$  moves on to quinone  $Q_A$  within several hundred picoseconds,<sup>1</sup> and this  $P^+Q_A^-$  state lives for many milliseconds.<sup>11</sup> The overall quantum yield for  $^1P \rightarrow P^+Q_A^-$  is close to unity.<sup>12</sup> If  $Q_A$  is prerduced or removed,  $P^+H^-$  decays by charge recombination either to the ground state or to the triplet state of P,  $^3P$ .<sup>13</sup>

The reaction scheme in Figure 1 includes approximate values for the rates and the current best estimates for the free energies of each state at room temperature.<sup>14–16</sup> Formation of the  $P^+H^-$  state involves only a very small free energy change. This puts severe constraints on the two-step mechanism of  $P^+H^-$  formation via  $P^+B^-$  because the free energy change for formation of  $P^+B^-$  from  $^1P$  must be smaller still. Direct, one-step formation of  $P^+H^-$  from  $^1P$  involves movement of an electron over an edge-to-edge distance of about 10 Å in only a few picoseconds, and if this mechanism is correct, the  $P^+B^-$  or  $^1B$  state almost certainly enhances the electronic coupling between  $^1P$  and  $P^+H^-$ .<sup>17–20</sup> Irrespective of which mechanism or combination of mechanisms proves to be correct, the phenomenology of these early steps in bacterial photosynthesis offers a serious challenge to most theories of electron transfer.<sup>17,18</sup>

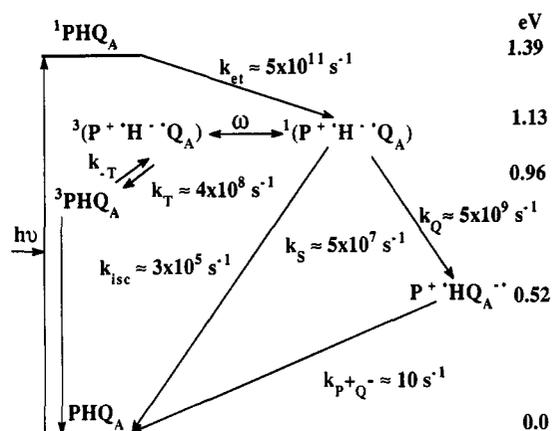


Figure 1. Approximate kinetics and energetics of the initial charge separation steps in *Rb. sphaeroides* RCs.<sup>14–16</sup> The kinetics are for low temperature, whereas the energetics are for room temperature. If  $Q_A$  is removed or prerduced, the  $k_Q$  pathway is eliminated, and the  $P^+H^-$  intermediate lives for tens of nanoseconds. During this time, the spin multiplicity of the  $P^+H^-$  radical-ion pair can evolve to the triplet state with frequency  $\omega$ , so that decay of the  $P^+H^-$  state branches to either the singlet ground state or  $^3P$  state depending on the values of  $\omega$ ,  $k_S$ , and  $k_T$ .  $^3P$  decays either by intersystem crossing ( $k_{isc}$ ) or activated electron transfer to re-form  $P^+H^-$ .

External electric fields provide a useful perturbation of the reaction dynamics in the RC because all of the intermediates are dipolar states.<sup>21</sup> Furthermore, studies in applied electric fields *in vitro* may shed light on the role of the transmembrane potential in the regulation of charge separation steps *in vivo*. Taking as a first approximation the center-to-center distances among components to estimate the magnitudes of these electric dipoles, one obtains 50 D for  $P^+B^-$ , 80 D for  $P^+H^-$ , and 130 D for  $P^+Q_A^-$ . The  $^1P$  and ground state of P may also have dipole moments; however, it is likely that these are small relative to the fully charge-separated intermediates.<sup>22</sup> As described below, it is possible to apply electric fields as large as about 3 MV/cm to RCs embedded in polymer films at low temperature; consequently, the energies of the states illustrated in Figure 1 can be changed enormously. For calibration, a 50-D dipole, such as the  $P^+B^-$  dipole, aligned with a field of 1 MV/cm has its energy changed by 100 meV. In general, the rate of an electron-transfer reaction is expected

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to be sensitive to a change in the driving force,<sup>54</sup> so applied fields should affect the rates of each step, and the branching ratio among competing steps, and may open up new reaction pathways which were inaccessible in the absence of a field. Because most of the reactions in Figure 1 are approximately optimally exothermic, the application of a field is expected to slow the reaction for most RC orientations, leading to a reduction in the quantum yield for the forward steps. In the following, the quantum yields for formation of the P<sup>+</sup>H<sup>-</sup>, <sup>3</sup>P, and P<sup>+</sup>Q<sub>A</sub><sup>-</sup> states in nonoriented RCs are examined in very large electric fields, along with the decay rate of the P<sup>+</sup>H<sup>-</sup>. A preliminary report of these results was presented elsewhere.<sup>23,24</sup> The decay of the P<sup>+</sup>Q<sub>A</sub><sup>-</sup> state in an applied field following its formation in the absence of a field under similar conditions has been reported in detail earlier.<sup>25</sup> In part 2 of this series,<sup>26</sup> a field-induced anisotropy technique is developed and applied to provide further constraints on possible mechanisms.

## Materials and Methods

**Samples.** Reaction centers from *Rb. sphaeroides* R-26 were obtained by standard methods.<sup>11</sup> RCs were prepared which contained Q<sub>A</sub> only (for P<sup>+</sup>Q<sub>A</sub><sup>-</sup> quantum yield experiments) or no quinones (for the <sup>3</sup>P or P<sup>+</sup>H<sup>-</sup> quantum yield experiments) using the method of Okamura et al.<sup>27</sup>

Two types of poly(vinyl alcohol) (PVA) film samples were prepared differing primarily in the thickness. (1) Thin films (3–10 μm, *A*<sub>802</sub> ≈ 0.2 OD) were prepared by spin-coating RCs in buffer (10 mM Tris, pH 8.0, 0.025% lauryldimethylamine oxide, LDAO) and 18% (w/v) PVA onto indium tin oxide (ITO)-coated (1700 Å) glass slides at ca. 4000 rpm using a photoresist spinner (Headway Research). The thickness was measured using a Dektak thickness measuring system (0.1-μm accuracy). The second electrode was applied to the film by evaporation of Al (3000 Å). These samples were attached to the copper cold finger of a closed cycle helium refrigerator using thermally conductive, electrically insulating varnish on the Al-coated side of the film. The films are in vacuum (0.04 mTorr). The unpolarized probe beam passed through the sample at the Brewster angle to the normal and reflected off the back Al layer. (2) Thick films (25–100 μm, *A*<sub>802</sub> ~ 1.5 OD) were prepared by pouring solutions of RCs in the same buffer with less than 12% PVA onto glass slides. The thickness was measured with a precision caliper. These films were pressed between ITO-coated glass slides with superglue. These samples were plunged into liquid nitrogen, and electric field effects were measured in transmission with the probe beam normal to the sample surface.

Some studies were also performed on frozen glycerol–buffer glasses (50% (v/v) composition). In this case the sample is contained in a cell consisting of two ITO-coated glass slides held apart by a 30-μm Teflon spacer, and these samples were immersed in liquid nitrogen and probed at normal incidence. These samples are useful for making comparisons with the PVA samples under more native conditions of hydration and salt concentration; however, it is not possible to obtain such high applied fields (<1.1 MV/cm), so only limited comparisons are possible.

**Measurements.** The samples were excited using the frequency-doubled output of a Nd:YAG laser with 9-ns pulse width and typically 1.5 mJ/cm<sup>2</sup> per pulse photon flux. The repetition rate of the laser flash was adapted for the decay time of the species being probed. The change in absorption of the sample was probed using a CW beam. The probe light at 870 nm was either light from a conventional source (tungsten–halogen or high-pressure Xe lamp filtered using glass filters or dispersed using a 0.25-m monochromator, 4.9-nm fwhm) or a Ti–sapphire laser pumped by an Ar ion laser (line width 1 cm<sup>-1</sup>). The Ti–sapphire probe is highly collimated and can be used to spatially filter fluorescence which can obscure the signal on the nanosecond time scale when 532-nm excitation is used. Si detectors, whose amplifier time

response was optimized for the time scale of the signal of interest, were used to measure the change in absorption. For the fastest time response, a detector with 4-ns rise and fall time of local design was used.<sup>28</sup> The output of the photodiodes was digitized using a Tektronix digital oscilloscope (DSA602) with a fastest time base of 1 ns per channel under computer control.

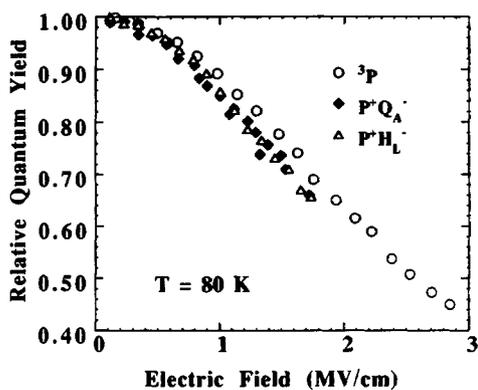
Voltage pulses were generated using a Trek 10/10 high-voltage amplifier with input from a digital delay pulse generator under computer control. The timing of the voltage pulses relative to the excitation flash and probe is a critical variable in the following experiments. The rise time of the applied field depends on the resistance and capacitance of the sample and the slew rate of the high-voltage amplifier. For the samples used in these experiments, the rise and fall time of the field, measured either by an analog output from Trek 10/10 or by the effect on the absorption spectrum (see below), was typically 10–30 μs. The timing of the application and the laser flash can be varied precisely, so the field can be at its maximum value and constant during the short excitation flash, it can be applied before the flash, or it can be gated on during the decay of long-lived intermediates. The external field, *F*, is obtained by dividing the measured applied voltage by the sample thickness.

A significant complication is that the applied field very strongly affects the absorption of P in the near-infrared (the electrochromic or Stark effect) as has been well documented elsewhere.<sup>29,30</sup> Therefore, as the absorption of P recovers following excitation and for that fraction of P which is not excited using subsaturating excitation flashes, the change in absorption due to the Stark effect, especially at the highest applied fields, may be as large as that due to the population changes caused by the excitation flash. For the P<sup>+</sup>Q<sub>A</sub><sup>-</sup> state, which decays with a time constant of tens of milliseconds at 80 K and whose decay rate at this temperature is only very weakly dependent on an applied field,<sup>25</sup> the Stark effect on the absorption can be cleanly separated from the field effect on the quantum yield of formation of P<sup>+</sup>Q<sub>A</sub><sup>-</sup> by having the field on during the formation of P<sup>+</sup>Q<sub>A</sub><sup>-</sup> but off during its decay. For the case of the P<sup>+</sup>H<sup>-</sup> quantum yield and decay, it is not possible to turn the field on and off fast enough to separate the field effect on these processes. These issues are discussed in detail below for each type of measurement.

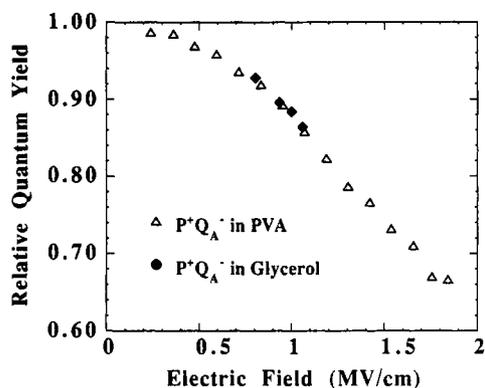
With these caveats in mind, the relative change in the P Q<sub>A</sub> ground-state absorption,  $\Delta A(F, t=0) / \Delta A(F=0, t=0)$ , is a measure of the relative quantum yield of formation,  $\Phi(F) / \Phi(F=0)$ , of the state measured in applied field *F* on the appropriate time scale and sample (Q<sub>A</sub>-depleted for P<sup>+</sup>H<sup>-</sup> and <sup>3</sup>P formation and Q<sub>A</sub>-containing for P<sup>+</sup>Q<sub>A</sub><sup>-</sup> formation). Time *t* = 0 is the initial amplitude for the state of interest prior to significant decay of that state. The relative quantum yield can also be determined within 0.5% accuracy by comparing the integrated area of the decay, so long as the field effect on the decay is found to be small or the field is off during the decay.

## Results

**P<sup>+</sup>Q<sub>A</sub><sup>-</sup> Quantum Yield Failure.** The P<sup>+</sup>Q<sub>A</sub><sup>-</sup> decay has a time constant of ~40 s<sup>-1</sup> in a PVA film at 80 K. The field pulse occurs on a time scale which is 3 orders of magnitude shorter, so P<sup>+</sup>Q<sub>A</sub><sup>-</sup> can be formed with the field on and its yield can be detected with the field off. The field effect on the relative P<sup>+</sup>Q<sub>A</sub><sup>-</sup> quantum yield in PVA is shown in Figure 2. It is observed that the P<sup>+</sup>Q<sub>A</sub><sup>-</sup> quantum yield drops substantially relative to its value at zero field. This loss of quantum yield is called quantum yield failure (QYF). Figure 3 compares the effects in PVA and in a glycerol–buffer glass. Over the field range where data in both sample types can be obtained, the relative quantum yield failure is identical within the signal-to-noise ratio. This comparison is important because some of the properties of RCs in PVA have been found to be different from those in a frozen glycerol–buffer glass.<sup>22,24,33</sup>



**Figure 2.** Effect of an applied electric field on the quantum yield for formation of  $P^+H^-$ ,  ${}^3P$ , and  $P^+Q_A^-$  at 80 K. The relative amplitudes of  ${}^3P$  and  $P^+H^-$  were measured in  $Q_A$ -depleted samples, and the relative amplitude of  $P^+Q_A^-$  was measured in  $Q_A$ -containing samples. Due to the time scale of the measurement, it is easiest to obtain high signal-to-noise ratio data of  ${}^3P$ .

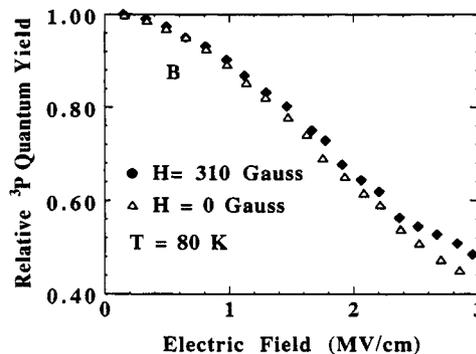
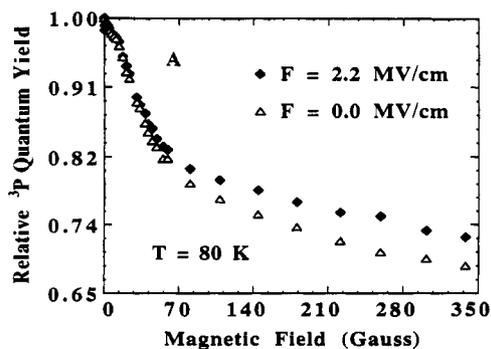


**Figure 3.** Comparison of the electric field effect on the reduction of the relative amplitude of  $P^+Q_A^-$  in PVA film (triangles) at 80 K and 50% (v/v) glycerol/buffer glass (solid diamonds) at 77 K.

**${}^3P$  Quantum Yield Failure.** The  ${}^3P$  decay has a time constant of  $\sim 140 \mu s$  in PVA at 80 K; therefore, the Stark effect on the absorption of P when the electric field pulse is on decays about 5 times faster than  ${}^3P$  population. The Stark effect transient,  $\Delta A_{\text{Stark}}(F, t)$ , can be removed from the data by the following procedure. The field-induced transient absorbance change,  $\Delta A(F, t)$  is given as

$$\Delta A(F, t) = \Delta A_{\text{cor}}(F, t) + \{C_1[1 - \Delta A(F, t)/\Delta A(F, 0)] + C_2\}\Delta A_{\text{Stark}}(F, t) \quad (1)$$

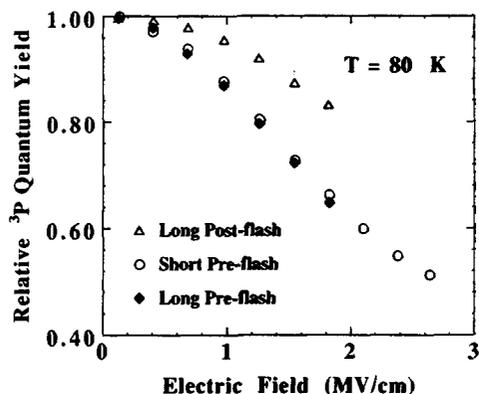
The first term in eq 1,  $\Delta A_{\text{cor}}(F, t)$ , is the absorbance change after correcting for Stark effects. Two kinds of Stark effect, transient and static, contribute to  $\Delta A(F, t)$ . The transient Stark effect,  $C_1[1 - \Delta A(F, t)/\Delta A(F, 0)]\Delta A_{\text{Stark}}(F, t)$ , comes from those RCs which have returned to the ground state P through the  ${}^3P$  decay. Here, the term  $[1 - \Delta A(F, t)/\Delta A(F, 0)]$  is the normalized fraction of P at time  $t$ . The static Stark effect,  $C_2\Delta A_{\text{Stark}}(F, t)$ , is related to that fraction of RCs which is not excited by the laser.  $\Delta A(F, t)$  and  $\Delta A_{\text{Stark}}(F, t)$  are obtained independently by applying the electric field to the sample with the excitation laser on and off, respectively. The fractional coefficients  $C_1$  and  $C_2$  are determined by a least-squares fitting procedure. This correction yields the true shape of the  ${}^3P$  decay curve in the presence of a 30- $\mu s$  electric field pulse. The field effect on the relative quantum yield of  ${}^3P$  in PVA films corrected for the Stark effect is shown in Figure 2. Since the pulsed field can be turned off in 10  $\mu s$  after excitation, the QYF of  ${}^3P$  can also be obtained from that part of the  ${}^3P$  decay curve after the field is turned off: the results agree with the method described above to within 1% error, validating the correction method.



**Figure 4.** Combined electric and magnetic field effects. (A) Effect of an applied electric field of 2.2 MV/cm on the magnetic field dependence of the  ${}^3P$  quantum yield monitored at 870 nm at 80 K. (B) Effect of an applied magnetic field of 30 G on the electric field dependence of the  ${}^3P$  quantum yield monitored at 870 nm at 80 K.

The  ${}^3P$  quantum yield depends on applied magnetic fields,<sup>13</sup> and the magnitude and shape of the magnetic field effect is a sensitive probe of the competition between  $k_S$ ,  $\omega$ , and  $k_T$  (see Figure 1). It is therefore interesting to consider the combined effect of magnetic and electric fields on the yield of  ${}^3P$ . The magnetic field dependence of the  ${}^3P$  quantum yield at 80 K is shown in Figure 4A in the absence and presence of a large applied electric field (2.2 MV/cm). There is a 4% decrease of the  ${}^3P$  quantum yield  $\Phi_T$  in an electric field; however, the magnetic field at which the change in the quantum yield is 50% of its full change between 0 and 1 kG (also called the  $B_{1/2}$  value) is the same in the absence and presence of an electric field of 2.5 MV/cm within the error of the measurement. At all electric field strengths  $B_{1/2} = 44 \pm 5$  G, in agreement with the reported value at zero electric field.<sup>13</sup> The small increase of the relative  ${}^3P$  yield at the highest electric field may be due to a weak dependence of  $k_S$ ,  $k_T$ , the magnetic exchange coupling constant  $J$  in the radical pair, or some other magnetic parameter on electric field. Conversely, we have measured the electric field dependence of the relative  ${}^3P$  quantum yield as a function of an applied magnetic field at 80 K as shown in Figure 4B. The effect of the magnetic field on the QYF of the  ${}^3P$  state is less than 4% at a magnetic field of 300 G.

We demonstrated several years ago that  ${}^3P$  decays in part by thermally activated re-formation of  ${}^3(P^+H^-)$ , leading to a temperature- and magnetic field-dependent decay pathway which competes with  $k_{\text{isc}}$  (Figure 1).<sup>34</sup> Application of an electric field shifts the energy of the dipolar  ${}^3(P^+H^-)$  level; thus, even at low temperature, it may be possible that some orientations of RCs in the  ${}^3P$  state could decay by a field-activated pathway. That is, application of a field, even after  ${}^3P$  is formed, can lead to an enhanced decay of  ${}^3P$  for the orientational subpopulation of  ${}^3(P^+H^-)$  that shifts closer in energy to  ${}^3P$ . To estimate the contribution from a possible field-induced activated recombination pathway, an approximately 1-ms pulsed field was used to obtain the  ${}^3P$  quantum yield in two experimental configurations: the field pulse was turned on either 20  $\mu s$  before the laser flash (preflash) or 1  $\mu s$  after the laser flash (postflash), then remaining



**Figure 5.** Comparison of the preflash and postflash electric field effect on the  $^3\text{P}$  quantum yield monitored at 870 nm at 80 K. Triangles are the 1-ms pulsed field turned on 30  $\mu\text{s}$  after the laser flash; circles and solid diamonds are the 30- $\mu\text{s}$  and 1-ms pulsed field turned on synchronized with the laser flash, respectively.

on for the entire  $^3\text{P}$  decay. The relative  $^3\text{P}$  quantum yield in both experimental configurations is plotted in Figure 5. The preflash data are nearly identical with the data obtained with a 30- $\mu\text{s}$  pulsed field. The postflash amplitude, which is likely due to a field-induced activated recombination of  $^3\text{P}$ , is about a factor of 2 smaller than that of the preflash amplitude. Further results and discussion of the effects on the  $^3\text{P}$  decay are deferred to a separate communication.<sup>35</sup>

**$\text{P}^+\text{H}^-$  Quantum Yield Failure and Decay.** The  $\text{P}^+\text{H}^-$  radical pair decay has a time constant of  $\sim 20$  ns at 80 K which is 3 orders of magnitude faster than the 30- $\mu\text{s}$  field pulse. Therefore, the field is constant during the time required for measuring the  $\text{P}^+\text{H}^-$  amplitude and decay. The correction for the Stark effect in this case must be based on the difference in the population of absorbers at the frequency of the probe monitored before and after the field application. When this is done, eq 1 can be applied. The effect of the correction is not very large. Similar results for the relative initial amplitude of  $\text{P}^+\text{H}^-$  radical pair transient were obtained when monitored in the Soret bandshift region at 420 nm where the electrochromic effect is very small,<sup>29b</sup> or at 870 nm where the electrochromic effect is large, even when no correction for the Stark effect is applied. The effect of the applied electric field on the relative  $\text{P}^+\text{H}^-$  quantum yield is shown in Figure 2. The decay time of the radical pair  $\text{P}^+\text{H}^-$ ,  $\tau_{\text{RP}}$ , was found to decrease somewhat from 21 ns at zero applied field to 16 ns at a field of 1.7 MV/cm.

## Discussion

**Where Quantum Yield Failure Occurs.** Because the relative reductions of the quantum yields of  $\text{P}^+\text{H}^-$ ,  $^3\text{P}$ , and  $\text{P}^+\text{Q}_\text{A}^-$  upon application of an electric field are observed to be the same within the overall experimental error of about 3% at all values of the applied field (Figure 2), a common precursor of all three is most likely the field-sensitive branch. Before evaluating possible branch steps in detail in light of other experimental information, a few comments are in order on this basic conclusion and how each observable contributes.

An understanding of the apparent absence of quantum yield failure of the  $\text{P}^+\text{H}^- \rightarrow \text{P}^+\text{Q}_\text{A}^-$  (rate constant  $k_\text{Q}$ ) step in the applied electric field depends on the extent of quantum yield failure in the earlier step(s) and the direction of the difference dipole for charge transfer of the second step relative to that of the earlier step(s). For example, for the process  $\text{P}^+\text{H}^- \rightarrow \text{P}^+\text{Q}_\text{A}^-$ , the difference dipole moment is  $\Delta\mu_\text{Q} = \mu(\text{P}^+\text{Q}_\text{A}^-) - \mu(\text{P}^+\text{H}^-)$ . Since the projection of  $\Delta\mu_\text{Q}$  along  $\Delta\mu(\text{P}^+\text{H}^-) = \mu(\text{P}^+\text{H}^-) - \mu(^1\text{P}) \sim \mu(\text{P}^+\text{H}^-)$  is large (i.e., they are nearly parallel), if quantum yield failure is due to the  $\text{P}^+\text{H}^-$  electric dipole, this will tend to mitigate the further effect which would be observed on the second step. This is because the most-affected orientational subpopulations,

those for which the difference dipole moment responsible for quantum yield failure,  $\Delta\mu_\text{QYF}$ , is approximately parallel or antiparallel to the field, do not survive the initial step. It is likely that  $k_\text{Q}$  is field-sensitive;<sup>36</sup> however, because QYF occurs prior to  $k_\text{Q}$ , we are only able to measure that orientational subpopulation which survives, and for this residual oriented subpopulation the field effect is expected to be small as  $\Delta\mu_\text{Q}$  is roughly perpendicular to the field. The state  $\text{P}^+\text{Q}_\text{A}^-$  is formed too rapidly in ubiquinone-containing RCs to contemplate the desired experiment in which the electric field is gated on after the state  $\text{P}^+\text{H}^-$  is formed but prior to the formation of  $\text{P}^+\text{Q}_\text{A}^-$ . Thus, there is currently no experimental approach to determine the magnitude of the QYF for the second step ( $\text{P}^+\text{H}^- \rightarrow \text{P}^+\text{Q}_\text{A}^-$ ) if all of the isotropic dipolar orientations of  $\text{P}^+\text{H}^-$  were present and could go on to form  $\text{P}^+\text{Q}_\text{A}^-$  in a field. This theme is repeated for other processes below. Obviously, the degree to which QYF on an earlier step reduces the effect on a later step depends on the orientation of the dipole(s) which is responsible for QYF.

A similar argument with interesting consequences applies to the formation of  $^3\text{P}$  from  $^3(\text{P}^+\text{H}^-)$  via the process  $k_\text{T}$  (Figure 1). For  $\text{P}^+\text{H}^- \rightarrow ^3\text{P}$ , the difference dipole is  $-\Delta\mu(\text{P}^+\text{H}^-)$ , where the dipole moment of  $^3\text{P}$  is taken to be very small compared to that of  $\text{P}^+\text{H}^-$ . If we consider only the effect of the applied field on the energy of the  $\text{P}^+\text{H}^-$  state, then for RC orientations where this dipole is aligned with the field, the energy of the state will be lowered, becoming closer to  $^3\text{P}$  and further separated from  $^1\text{P}$  (again neglecting the effect of the field on the energy of  $^1\text{P}$  as its dipole moment is much smaller than for  $\text{P}^+\text{H}^-$ ). Because the energy difference between  $^3(\text{P}^+\text{H}^-)$  and  $^3\text{P}$  is quite small at zero field,<sup>16,34</sup> at fields in excess of 1 MV/cm a substantial orientational subpopulation of  $^3(\text{P}^+\text{H}^-)$  will be at lower energy than  $^3\text{P}$  and will therefore be incapable of forming  $^3\text{P}$  ( $k_\text{T} = 0$ ). Therefore, as with  $\text{P}^+\text{Q}_\text{A}^-$  formation from  $\text{P}^+\text{H}^-$  via  $k_\text{Q}$  discussed above, one might expect a large  $^3\text{P}$  quantum yield failure on the second ( $k_\text{T}$ ) step in the postflash configuration (Figure 5). However, the relative effect of the field on the quantum yield of  $^3\text{P}$  at both 1-ms and 30- $\mu\text{s}$  pulsed fields in the preflash configuration (Figure 5), and  $\text{P}^+\text{Q}_\text{A}^-$  (Figure 2) is observed to be identical. In other words, the most-affected orientational subpopulations for the  $^3\text{P}$  field-induced activated recombination do not survive the initial step. The clear implication is that the branching step must be at or preceding formation of  $\text{P}^+\text{H}^-$ . It would be very interesting to form an isotropic distribution of  $\text{P}^+\text{H}^-$  in the absence of an electric field and then turn the field on (say 1 ns after exciting the sample) to measure the field effect on the  $^3\text{P}$  quantum yield. This is not yet technically feasible.

As discussed in the Experimental and Results sections, measurement of the field effect on the  $\text{P}^+\text{H}^-$  quantum yield is compromised by two difficulties: (i) the time resolution of our apparatus is adequate, but not ideal, for measuring the  $\text{P}^+\text{H}^-$  quantum yield, and (ii) it is not possible to switch off the field during the observation of the  $\text{P}^+\text{H}^-$  state, which requires correcting for the Stark effect. The former is avoidable by having improved time resolution, and this has been reported recently.<sup>37</sup> At the present time, given the resistance and capacitance of the samples and the slew-rate limits of high-voltage amplifiers, there is no simple way to obtain electric field pulses shorter than nanoseconds.

The competition between singlet radical pair decay  $k_\text{S}$  and singlet-triplet mixing  $\omega$  in a  $\text{Q}_\text{A}^-$ -depleted sample is a sensitive function of magnetic field.<sup>38,39</sup> If the rate constant  $k_\text{S}$  were significantly electric field dependent, the effect on the  $^3\text{P}$  quantum yield in an electric field would be expected to differ from the effect on the formation of the  $^1(\text{P}^+\text{H}^-)$  state. The shape of the magnetic field effect on the  $^3\text{P}$  quantum yield at low magnetic field is sensitive to the value of  $k_\text{T}$ .<sup>32,38,39</sup> Because  $k_\text{T}$  should depend on an electric field, the absence of an electric field effect on the shape of the magnetic field dependence implies that  $k_\text{T}$  is

not greatly changed for the population of RCs that survive the initial charge separation step in an electric field to form  ${}^3\text{P}$ . A similar argument would apply to delayed (recombination) fluorescence from  ${}^1(\text{P}^+\text{H}^-) \rightarrow {}^1\text{P}$ : the orientational subpopulation of  ${}^1(\text{P}^+\text{H}^-)$  which is most affected by the field experiences quantum yield failure and therefore is not available to undergo appreciable field-induced repopulation of  ${}^1\text{P}$ . Thus, the expected very strong dependence of delayed fluorescence on an applied electric field<sup>40,41</sup> will likely not be observed because of QYF.

**Possible Mechanisms for Quantum Yield Failure.** It is apparent from these data that an electric field-dependent branching pathway is present at an early stage following photoexcitation. Several possibilities for the branching step can be proposed (these are not mutually exclusive): (i) field-dependent, nonradiative decay of  ${}^1\text{P}$ , which might, for example, depend on charge-transfer states within the special pair, sometimes denoted  $\text{P}^+\text{P}^-$ ; (ii) field dependence of the  ${}^1\text{P} \rightarrow \text{P}^+\text{B}^-$  step if this is the primary charge separation step (note that even if this step is not important in the absence of a field it may become important upon application of a field for appropriately oriented subpopulations); (iii) field-dependent enhancement of the decay of the  $\text{P}^+\text{B}^-$  state to the ground state; (iv) field dependence of the  ${}^1\text{P} \rightarrow \text{P}^+\text{H}^-$  step if this is the primary charge separation step; (v) field-dependent enhancement of the decay of  $\text{P}^+\text{H}^-$  immediately after its formation, but prior to further charge separation to  $\text{Q}_\text{A}$ , for example, involving an unrelaxed form of  $\text{P}^+\text{H}^-$ ;<sup>38,42</sup> (vi) opening of new pathways involving the nonfunctional branch of chromophores (the M or B branch). In the following we consider these possibilities at a qualitative level. In part 2<sup>26</sup> an experimental approach is developed based on the notion that because these possibilities depend on the  $\text{P}^+\text{P}^-$ ,  $\text{P}^+\text{B}^-$ , or  $\text{P}^+\text{H}^-$  electric dipole moment orientations, they can be distinguished, in principle, by a measurement of the anisotropy of the quantum yield failure in an applied electric field.

The observation that the reduction in quantum yield is greater than 50% at fields of 2.5 MV/cm or higher has an immediate implication. If only a single state is affected by the field, then shifting the energy of its dipole both up and down in the field must cause a reduction of the quantum yield. Alternatively, more than one state may be involved in QYF, as discussed in detail in part 2.<sup>26</sup> In principle, even for an isotropic sample, at a sufficiently high electric field, nearly 100% QYF is possible.

The quantum yield for the initial electron-transfer step from  ${}^1\text{P}$  can be expressed as

$$\Phi(\text{F}) = \frac{k_{\text{et}}(\text{F})}{k_{\text{f}}(\text{F}) + k_{\text{nr}}(\text{F}) + k_{\text{et}}(\text{F})} \quad (2)$$

where  $k_{\text{et}}(\text{F})$  is the rate constant for the electron-transfer process  ${}^1\text{PH} \rightarrow \text{P}^+\text{X}^-$  (where X is likely B or H),  $k_{\text{f}}(\text{F})$  is the fluorescence rate constant which is likely to be only weakly dependent on electric field,<sup>24</sup> and  $k_{\text{nr}}(\text{F})$  is the nonradiative rate of internal conversion of  ${}^1\text{P}$ .

Some information is available on the value of  $k_{\text{nr}}$  at zero field from RCs where H is absent<sup>43</sup> or reduced<sup>44,45</sup> or where  $k_{\text{et}}$  is very slow.<sup>46</sup> These data suggest that  $k_{\text{nr}}(\text{F}=0) \approx 10^{10} \text{ s}^{-1} \gg k_{\text{f}}(\text{F}=0)$ . In order for a change in the nonradiative rate to be responsible for the QYF, it would need to increase by 2 orders of magnitude for all orientations of the  $\text{P}^+\text{H}^-$  dipole moment, because  $k_{\text{et}}(\text{F}=0) \approx 10^{12} \text{ s}^{-1}$ . Such a change would be readily detected by transient absorption spectroscopy; however, measurements of the primary charge separation rate showed only a very small change in kinetics.<sup>20</sup> It is conceivable that a small base line offset which was observed in the picosecond transient absorption experiments upon application of the field could have masked a larger effect on the kinetics;<sup>37</sup> however, the signal-to-noise ratios of the earlier<sup>20</sup> and more recent<sup>37</sup> measurements are not yet sufficient to provide a definitive answer. The steady-state fluorescence quantum yield is observed to increase on application of an electric field;<sup>40</sup> however,

the change in fluorescence is much smaller than what would be expected if the process which competes with fluorescence slowed by 2 orders of magnitude.<sup>20</sup> It is also possible that the observed enhancement of the steady-state fluorescence in an electric field<sup>40</sup> is contaminated by effects on long-lived states, i.e., that it does not reflect the field effect on the spontaneous fluorescence.<sup>41</sup> If this is the case, then the actual effect of the field on the spontaneous fluorescence is even smaller than what was observed, further strengthening the argument that the field-sensitive step is not the primary charge separation step from  ${}^1\text{P}$  in the absence of a large change in  $k_{\text{nr}}$ . Similarly, if the observed increase of the steady-state fluorescence has a significant contribution from field-induced re-formation of  ${}^1\text{P}$  from  ${}^1(\text{P}^+\text{H}^-)$ , then the actual field effect on the spontaneous fluorescence is smaller than what was observed. These issues can be settled by measuring the field effect directly on the spontaneous fluorescence.<sup>8</sup> For now, we are left with the dilemma that QYF occurs at an early stage and is substantial, while the field effect on the primary charge separation kinetics from  ${}^1\text{P}$  appears to be considerably smaller.

There are two general ways to reconcile these results. (1) The primary charge separation rate  $k_{\text{et}}(\text{F})$  is highly field dependent, but so is internal conversion  $k_{\text{nr}}(\text{F})$ . The fluorescence is quenched by  $k_{\text{nr}}(\text{F})$  even if  $k_{\text{et}}(\text{F})$  is retarded significantly in an electric field. In order to account for the observed effects on the steady-state fluorescence, it must be the case that  $k_{\text{nr}}(\text{F})$  speeds up by about 1 order of magnitude in those RCs in which the electron-transfer rate constant  $k_{\text{et}}(\text{F})$  decreases by 1 order of magnitude. (2) There is another electric field-dependent pathway or intermediate en route to  $\text{P}^+\text{H}^-$ , which shunts the electron back to  $\text{P}^+$  after it leaves  ${}^1\text{P}$ . This intermediate could be the state  $\text{P}^+\text{B}^-$  (which has not been observed directly but has been suggested as an intermediate in a two-step hopping mechanism<sup>6</sup>) or an unrelaxed form of the state  $\text{P}^+\text{H}^-$  which has strong coupling to  ${}^1\text{P}$  and possibly also to ground-state P.<sup>55</sup> There are, of course, more exotic possibilities. In part 2,<sup>26</sup> experimental data are presented which tend to favor possibility (2).

**Comparison with Other Experiments.** A reduction in the quantum yield for formation of  $\text{P}^+\text{Q}_\text{A}^-$  at 295 K in Langmuir-Blodgett films was observed by Popovic and co-workers.<sup>49</sup> These experiments were designed to obtain information on the field effect for a fully oriented sample, and surprisingly, the results are quite similar to those we have obtained with isotropic samples. Recent work<sup>50</sup> has shown that multilayers containing LDAO of the type used<sup>49</sup> tend to form nearly isotropic samples providing an explanation for the similarity. However, in the absence of information on the precise degree and axis of orientation and the sample thickness, it is not possible to make further quantitative comparisons.

More recently, a reduction in the quantum yield for formation of  $\text{P}^+\text{H}^-$  at 90 K in PVA films was detected in picosecond transient absorption by Ogrodnik et al.<sup>37</sup> These investigators observed an instantaneous 11% reduction of the  $\text{P}^+\text{H}^-$  quantum yield at 0.7 MV/cm within the time resolution of their experiment (30 ps). This result suggests that the QYF of charge transfer occurs prior the formation of the state  $\text{P}^+\text{H}^-$ , which is consistent with our conclusion. They also observed a recovery of the ground state with a decay rate of  $(900 \text{ ps})^{-1}$ , implying that there is a field-induced pathway between some intermediate state(s) and the ground state. The observed decay at 870 nm was interpreted as being due to two separate events: at times less than 900 ps, the decay is due to an intermediate-state relaxation to ground-state P (11%), while at times longer than 900 ps it is due to a field-induced  ${}^3\text{P}$  recombination (5%). This interpretation does not agree with our results (Figures 2 and 5). If there is any  ${}^3\text{P}$  field-induced activated recombination for those which survive the initial QYF, the quantum yields for formation of  $\text{P}^+\text{H}^-$ ,  ${}^3\text{P}$ , and  $\text{P}^+\text{Q}^-$  states would be different in the overlapped field range. The relative amplitudes of the preflash long and short field pulses are nearly

same as the one of the  $P^+Q^-$  and  $P^+H^-$ . This indicates that the contribution of  $^3P$  activated recombination is negligible because the most-affected orientational subpopulations do not survive the initial steps. A problem with the interpretation of the kinetics of the field-induced relative change of the ground-state bleaching of  $P$  may result because it was probed at 870 nm where the Stark effect is very large. The fraction of the time-dependent Stark effect cannot be corrected by a simple experimental subtraction. The rate of ground-state recovery will, in fact, change the Stark effect on the bleaching of  $P$  on the time scale of  $^3P$  decay. Thus, the observation that decay data measured at 870 and 545 nm are not consistent in the work of Ogrodnik et al.<sup>37</sup> could be due to the inadequate treatment of the Stark effect. The rate of decay into the loss channel at zero field was estimated to be  $(6-14 \text{ ps})^{-1}$  based on the assumption that this rate is independent of the applied field. This unreasonable assumption led these investigators to the conclusion that the quantum efficiency of RCs is only 0.67-0.85 at zero field. This is in disagreement with the measured quantum yield of approximately unity.<sup>12,51</sup>

**Possible Physiological Function of Quantum Yield Failure.** The results presented in this paper demonstrate a significant reduction in the quantum yield of charge separation in applied electric fields. If all RCs had the same orientation in the field (as for RCs in the native chromatophore membrane) and if the difference dipole responsible for QYF had a substantial projection along the field direction (the normal to the bilayer), then large effects of transmembrane potential on charge separation might be possible. Furthermore, because the native system is coupled to the antenna pigments, and there may be a field effect on both the trapping and detrapping rates, even larger effects might be observed. There is no known chemical feedback mechanism which stops charge-separation processes from occurring in the RC. The creation of long-lived charge-separated states can be potentially harmful to the function of the cell.<sup>52</sup> Such states may be created if strong light intensity drives the buildup of charged intermediates more rapidly than they can be utilized. The observation of quantum yield failure in an applied electric field at an early charge-separation step suggests that the transmembrane potential itself may be a feedback inhibitor for the RC. It has been proposed that the RC may cease to perform electron transport in a sufficiently high transmembrane potential *in vivo*.<sup>53</sup> The membrane potential is estimated to be around 150 mV under conditions where electron transport ceases. The magnitude of the electric field experienced by the RC is not known. Note that 150 mV across 50 Å corresponds to a field of 0.3 MV/cm. As shown in Figure 2, QYF for an isotropic sample is quite small at this field; however, in the membrane all RCs and therefore  $\Delta\mu_{\text{QYF}}$  have a fixed orientation relative to the transmembrane electric field. Changes in dipole moments which are most parallel to the local  $C_2$  axis will be most parallel to this transmembrane field and therefore most affected, and this could lead to a sufficient interaction to cause the substantial quantum yield failure which can inhibit the normal function RC in electron transport. Thus, it is possible that the electric field-dependent pathways discussed in this paper function as a nonphotochemical quenching mechanism which switches off the light reactions of photosynthesis when they are not needed. This speculation warrants testing in an oriented system where the field is accurately known.

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(55) There may be an interesting parallel with a mutant where the driving force for the initial electron-transfer step is reduced to only 75 meV by replacement of H<sub>L</sub> with a bacteriochlorophyll denoted β [Kirmaier, C.; Gaul, D.; DeBey, R.; Holten, D.; Schenck, C. *Science* **1991**, *251*, 922]. The initial charge-separated state P<sup>+</sup>β<sup>-</sup> can be considered as a model for an unrelaxed native P<sup>+</sup>H<sup>-</sup> state or the orientational subpopulation of P<sup>+</sup>H<sup>-</sup> which is moved closer in energy to <sup>1</sup>P in an applied electric field. P<sup>+</sup>β<sup>-</sup> was found to decay much more rapidly to the ground state than P<sup>+</sup>H<sup>-</sup>.