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MAGNETIC FIELD DEPENDENCE OF RADICAL-PAIR DECAY KINETICS AND MOLECULAR TRIPLET QUANTUM YIELD IN QUINONE-DEPLETED REACTION CENTERS

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Radical-pair decay kinetics and molecular triplet quantum yields at various magnetic fields are reported for quinone-depleted reaction centers from the photosynthetic bacterium *Rhodopseudomonas sphaeroides* R26. The radical-pair decay is observed by picosecond absorption spectroscopy to be a single exponential to within the experimental uncertainty at all fields. The decay time increases from 13 ns at zero field to 17 ns at 1 kG, and decreases to 9 ns at 50 kG. The orientation averaged quantum yield of formation of the molecular triplet of the primary electron donor, ^{3}P, drops to 47% of its zero-field value at 1 kG and rises to 126% at 50 kG. Combined analysis of these data gives a singlet radical-pair decay rate constant of $5 \cdot 10^7$ s^{-1}, a lower limit for the triplet radical-pair decay rate constant of $1 \cdot 10^8$ s^{-1} and a lower limit for the quantum yield of radical-pair decay by the triplet channel of 38% at zero field. The upper limit of the quantum yield of ^{3}P formation at zero field is measured to be 32%. In order to explain this apparent discrepancy, decay of the radical pair by the triplet channel must lead to some rapid ground state formation as well as some ^{3}P formation. It is proposed that the triplet radical pair decays to a triplet charge-transfer state which is strongly coupled to the ground state by spin-orbit interactions. Several possibilities for this charge-transfer state are discussed.

Introduction

Reaction centers are the pigment-protein complexes responsible for photoinduced charge separation in photosynthetic organisms [1–7]. One way to study the interactions in the reaction center is to interrupt the normal electron flow by removing or prereducing one or more of the electron carriers [8,39]. We present here both the decay kinetics of the primary radical ion pair and the quantum yield of the triplet state of the primary electron donor at three magnetic fields after pulsed excitation of reaction centers isolated from *Rhodopseudomonas sphaeroides* R26 and depleted of the secondary electron acceptor, ubiquinone. These data are shown to be incompatible with the model currently accepted for decay of the primary radical pair when electron transfer to the quinones is blocked. Our data support a modified model similar to one proposed by Schenck et al. [10] to explain results obtained with reaction centers containing singly reduced ubiquinone. We discuss what such a modification may imply about the nature of the early intermediates in the charge separation process.

The primary electron donor (P) in reaction

Abbreviations: P, primary electron donor; BChl, bacteriochlorophyll *a*; ^{1}P, excited singlet state of P; ^{3}P, excited triplet state of P; I, electron-acceptor complex; BPh, bacteriopheophytin *a*; LDAO, lauryldimethylamine oxide; RYDMR, reaction yield detected magnetic resonance; S, singlet; T, triplet.

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centers from *Rps. sphaeroides* R-26 is thought to be a dimer involving two bacteriochlorophyll *a* (BChl) molecules [1], although the physical properties of the donor remain the topic of much discussion [11]. The current view [2–4,6] of the primary charge-separation process is that photoexcitation of P to the excited singlet state (^{1}P) leads to the formation of the singlet radical pair, ^{1}(P$^+$I$^-$), within 10 ps of excitation. This event is followed by transfer of an electron from I$^-$ to Q in about 200 ps at room temperature. The electron-acceptor complex, I, appears to involve both bacteriopheophytin *a* (BPh) and BChl. On the basis of the temperature dependence of the absorption changes near 800 nm at low redox potential, Shuvalov and Parson [12] suggested that ^{1}(P$^+$I$^-$) may be an equilibrium mixture of ^{1}(P$^+$BChl$^-$) and ^{1}(P$^+$BPh$^-$). Picosecond measurements have been taken to indicate [13] that BChl is an electron acceptor prior to BPh in the charge-separation process.

With Q removed or reduced prior to excitation, electron transfer from I$^-$ to Q is blocked and ^{1}(P$^+$I$^-$) decays by alternative pathways. The simplest current model of the decay of ^{1}(P$^+$I$^-$) in quinone-depleted reaction centers is shown in Fig. 1A. ^{1}(P$^+$I$^-$) can decay by three routes: (i) by reverse electron transfer to give the ground state, (ii) by reverse electron transfer to give the singlet excited state ^{1}P followed by deactivation to the ground state or (iii) by spin conversion to give the triplet radical pair, ^{3}(P$^+$I$^-$). Routes (i) and (ii) occur with the total rate constant k_s. Route (iii) is due to the differing hyperfine and Zeeman interactions in the two radicals, denoted as a coupling frequency, ω. ^{3}(P$^+$I$^-$) can decay to the molecular triplet state, ^{3}P, with rate constant k_T [14] or return to the singlet radical pair. The radical pair lives 10–20 ns [10]; ^{3}P lives 10–100 μs [12].

In quinone-depleted reaction centers, the quantum yield of ^{3}P decreases monotonically to approx. 50% of the zero-field yield on application of a 1 kG magnetic field. This has been ascribed to the loss of the near degeneracy of the singlet radical pair with the T_+ and T_- spin sublevels of the triplet radical pair [15,16]. The ^{3}P yield increases again on increasing the field from 1 kG to 50 kG, asymptotically approaching a value higher than the zero-field value [17]. This increase and leveling off of the triplet yield are due to the increasing difference in the Zeeman energy of the two radicals with increasing field; that is, ω increases with field. At high enough fields, the S and T$_0$ states equilibrate prior to charge recombination [17]. Microwave irradiation in the presence of an appropriate magnetic field also affects the ^{3}P yield by mixing T$_0$ with T$_+$ and T$_-$, thus disturbing the spin dynamics prior to charge recombination [18,19]. This effect is known as reaction yield detected magnetic resonance (RYDMR).

These magnetic-field effects indicate that P$^+$I$^-$ is a weakly coupled radical pair; that is, the electron-electron exchange interaction must be very small (approx. 10^{-3} cm^{-1}) [15]. In order to reconcile the rapid rate of ^{1}(P$^+$I$^-$) formation from ^{1}P with this very small exchange interaction, Haber-

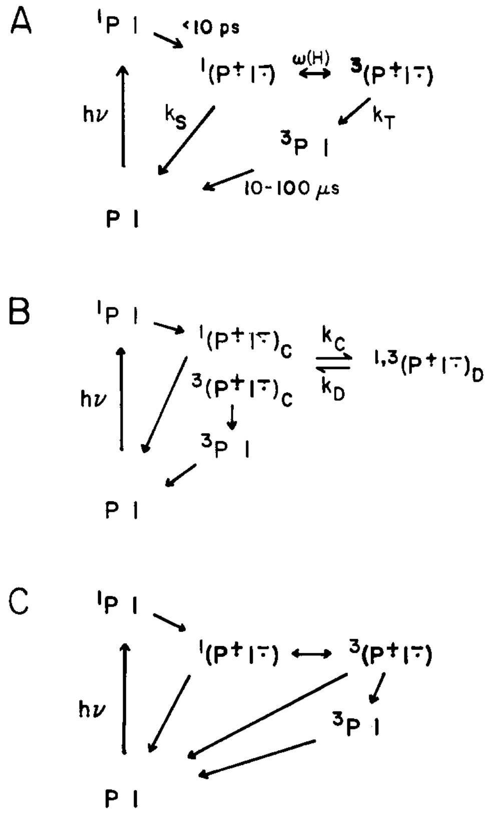

Fig. 1. Various models of the primary photochemistry in reaction centers in which electron transfer from I$^-$ to Q is blocked. (A) Simplest model. (B) Model incorporating a close and distant radical pair. Magnetic field-dependent spin conversion occurs in the radical pair labelled ^{1,3}(P$^+$I$^-$)$_D$. (C) Model incorporating decay of the triplet radical pair to the ground state.

korn et al. [20] proposed a modified model (Fig. 1B) in which one of the unpaired electrons resides on one of two sites: one site close to the other radical to explain rapid electron transfer and one site distant to explain the small observed exchange interaction. The authors introduced electron-transfer rate constants k_C and k_D for transfer from the close site to the distant site and back again, respectively. They demonstrated that the theoretical expression for the ^{3}P yield would be similar to that for the simpler model (Fig. 1A), though the physical meaning of the parameters would be somewhat different. One example of this model was considered by Ogrodnik et al. [21], who suggested that the close form of the radical pair might be $^{1,3}(P^+BChl^-)$, while the distant form might be $^{1,3}(P^+BPh^-)$.

Schenck et al. [10] provided the first indication that neither of these two models was sufficient. Their measurements of the P^+I^- lifetime and ^{3}P yield at zero field and 650 G in quinone-containing reaction centers at low redox potential (blocked) could not be reconciled with either of the models discussed above. The lifetime of P^+I^- increased too much on application of the 650 G field to be explained by the observed decrease in the absolute ^{3}P yield, even for extremely disparate values of the singlet and triplet radical-pair decay rate constants, k_S and k_T. These authors proposed an additional triplet radical-pair decay path leading rapidly back to the ground state to account for the apparently large fraction of triplet radical-pair decay which does not lead to ^{3}P formation (Fig. 1C). Thus, we are careful here to distinguish the quantum yield of ^{3}P formation from the quantum yield of radical-pair decay by the triplet channel.

Support for this additional radical-pair decay path in quinone-depleted reaction centers can be found by contrasting the reported values of k_S and k_T with the apparent high-field asymptote of the ^{3}P quantum yield. Values of k_T of $(5-10)\cdot 10^8$ s^{-1} have been suggested by analyses of the RYDMR linewidth [18] and of the magnetic field dependence (0–500 G) of the ^{3}P concentration at various times during ^{3}P formation [21]. With such a large value of k_T, k_S must be less than the reciprocal of the P^+I^- lifetime, so less than about $5\cdot 10^7$ s^{-1}. These values imply a high-field asymptote of the ^{3}P quantum yield near unity. Taking ω to be large in the model in Fig. 1A, one obtains:

$$\Phi_T(H=\infty) = k_T/(k_S+k_T) \approx 0.9$$

In contrast, data on the field dependence of the ^{3}P yield [17] suggest the asymptote to be about 0.50 at room temperature. However, were the decay of the triplet radical pair to lead to some rapid ground state recovery as well as ^{3}P formation (Fig. 1C), then the ^{3}P yield would represent only a fraction of the total decay of the triplet radical pair and a lower high-field asymptote of the ^{3}P yield than that expected from the model in Fig. 1A.

In order to test this new model further, we have performed radical-pair lifetime measurements similar to those of Schenck et al., but for quinone-depleted reaction centers at 0, 1 and 50 kG using picosecond optical techniques to obtain both near-infrared transient difference spectra and decay kinetics. We find the decay of P^+I^- to be slower at 1 kG than at zero field, but faster at 50 kG than at zero field. In addition, the ^{3}P quantum yield has been measured on the microsecond time scale by comparison with the known quantum yield of P^+I^- in quinone-containing reaction centers. These results strengthen the contention that an additional triplet radical-pair decay path exists and provide numerical estimates or constraints for the values of k_S, k_T and the quantum yield of radical-pair decay by the triplet channel.

Materials and Methods

Sample preparation

Reaction centers were purified from *Rps. sphaeroides* R26 cells by the procedure of Schenck et al. [10]. Purity was judged by the ratio, $A_{280\,nm}/A_{802\,nm} \approx 1.25$, of the protein-to-pigment absorptions at 280 nm and 802 nm, respectively, in 0.1% lauryldimethylamine oxide (LDAO), 10 mM Tris-HCl (pH 8.0) [1]. Ubiquinone was removed by binding the reaction centers to a small DEAE-cellulose column, and washing for 14 h at room temperature with 2 ml 4% LDAO/10 mM *o*-phenanthroline/10 mM Tris-HCl (pH 8.0) per ODV_{802} ($ODV_{802} = A_{802\,nm}\cdot$ pathlength (cm) $\cdot$ volume (ml)) [22]. They were then washed with several column volumes of 0.025% cholic acid/10 mM Tris-HCl pH 8.0 (buffer 1) to remove LDAO and removed from the column with 1 M NaCl in buffer 1. Not all reaction centers could be removed under these conditions. All experiments were done with those that were removed. They were dialysed against buffer 1, concentrated to $A_{802\,\text{nm}} = 40$ in a 1 cm pathlength and stored at $-50°\text{C}$. Samples for experiments were diluted with 0.1% Triton X-100, 10 mM Tris-HCl (pH 8.0) (buffer 2), deoxygenated by repeatedly evacuating and flushing a small flask containing the stirred reaction centers with argon, and transferred anaerobically to the appropriate cuvette. $A_{802\,\text{nm}}$ was about 0.8; the pathlength was 1 mm except for the low field, microsecond measurements when it was 1 cm. In some experiments, in order to remove residual oxygen, 1–5% (v/v) 200 mM $Na_2S_2O_4$/1 M Tris-HCl (pH 8.0) was added. All data were taken at room temperature.

Microsecond measurements

The flash-induced bleaching of the 868 nm absorbance on the microsecond-to-second time scales was measured with a transient absorption spectrometer employing a pulsed, frequency-doubled Nd:YAG laser (6 ns, 532 nm) and a continuous, 868 nm diode laser. Weak flashes were used to ensure a linear response. Helmholtz coils or a superconducting solenoid supplied the magnetic field [23]. In quinone-containing preparations, the 868 nm bleaching decays in 0.05–5 s with no rapid component. In quinone-depleted preparations, the bleaching decays to 2% with an approx. 40 μs time constant characteristic of ^{3}P decay [12]; the remaining 2% is due to residual quinone-containing reaction centers. The ratio of the bleaching of the 868 nm absorption per photon absorbed in quinone-depleted to that in quinone-containing reaction centers was measured 2 μs after a less than 10% saturating flash (less than 250 $\mu J/cm^2$ at 532 nm) with small corrections for (i) the approx. 2% bleaching due to quinone-containing reaction centers in the quinone-depleted sample, (ii) the lifetime of the bleaching in quinone-depleted reaction centers and (iii) the slightly different absorbances of the two samples at 532 nm (see Results).

Picosecond measurements

The dual-beam picosecond transient absorption spectrometer used here has been described [24,27]. It is based on a passively mode-locked Nd:YAG laser system that delivers 35 ps, 1064 nm, 10 mJ pulses at a 10 Hz repetition rate. Excitation flashes at the 532 nm, second harmonic were attenuated to cause approx. 75% saturation of the 870 nm absorption. 'White-light' (700–950 nm) probe pulses of approx. 35 ps duration were generated in $CHCl_3/CCl_4$ from a delayed portion of the fundamental and were expanded in the vertical direction in order to pass through excited and unexcited regions of the sample, defined by two pinholes. With a variable delay line and an additional fixed delay, times up to 25 ns after the actinic flash were probed. Care was taken to insure that the addition of the fixed delay did not change spatial overlap of the pump and probe pulses at the sample. To help achieve this, the focussing optics were set up so that at the sample the probe light was approx. 1 mm wide, while the pump light was approx. 2 mm in diameter. The electric vectors of the polarized pump and probe pulses were at 45° to each other and both at 90° to the magnetic field.

Samples were agitated every 600 flashes to bring fresh sample into the small excitation region. This prevented noticeable change of the transient difference spectrum at a given delay over a 5 h experiment. Transient spectra sometimes contained a small offset in ΔA (smaller than 0.01)

TABLE I

ORIENTATION AVERAGED RELATIVE ^{3}P YIELD AND 868 nm OBSERVATION ANISOTROPY [23] AS A FUNCTION OF MAGNETIC FIELD STRENGTH

$H(G)$	$I^{av}(H)$ [a]	$a(H)$ [a]
0	1.00	0.00
10	0.99 ± 0.01	
20	0.92 ± 0.01	
40	0.75 ± 0.01	
100	0.57 ± 0.02	
1000	0.47 ± 0.02	+0.035 ± 0.002
5000	0.48 ± 0.02	+0.015 ± 0.005
12500	0.60 ± 0.02	−0.047 ± 0.012
25000	0.86 ± 0.04	−0.120 ± 0.005
37500	1.08 ± 0.05	−0.146 ± 0.004
50000	1.26 ± 0.07	−0.150 ± 0.005

[a] Uncertainties are standard deviations of at least threee measurements.

across the 170 nm wavelength interval investigated. Therefore, decay kinetics were determined using the time dependence of the difference in ΔA between two wavelengths.

Results

It is our experience and that of others [25], that the numerical values of various properties of quinone-depleted reaction centers, such as the radical-pair lifetime and the magnetic-field effects, often vary noticeably among different preparations, although all preparations are qualitatively similar. At present it is not clear that any one preparation best represents the 'native' case. Here, we present results on one quinone-depleted preparation. We believe similar conclusions would be obtained with all quinone-depleted reaction centers, but caution against exacting numerical comparisons among preparations.

Microsecond experiments

As observed previously [23], the extent of ^{3}P formation in quinone-depleted reaction centers as probed by the 868 nm bleaching at 2 μs decreases on application of small magnetic fields (less than 1 kG) and then increases with larger fields. The orientation-averaged ^{3}P yields relative to zero field, $I^{\mathrm{av}}(H) = \Phi^{\mathrm{av}}_{^3\mathrm{P}}(H)/\Phi^{\mathrm{av}}_{^3\mathrm{P}}(H=0)$, are shown in Table I. The field at which half the low field decrease in $I^{\mathrm{av}}(H)$ is observed, $B_{1/2}$, was found to be 42 ± 2 G. Also shown in Table I is the 868 nm observation anisotropy of ^{3}P formation in viscous buffer, $a(H)$, a measure of the dependence of the ^{3}P yield on the orientation of the reaction center in the magnetic field (see Ref. 23 for details).

In order to compare the extinction coefficients of quinone-depleted and quinone-containing samples, the absorption spectra of two identical samples of about 3 μM quinone-depleted reaction centers were measured before and after addition of 1% by volume 1 mM ubiquinone-50 (Sigma Chemical Co.) in ethanol to one and 1% by volume pure ethanol to the other. Microsecond measurements showed no ^{3}P formation in the quinone-reconstituted sample. Rather a bleaching lasting seconds was seen which is characteristic of charge separation in samples containing two quinones [2]. Very minor differences between the two samples were observed in the near-infrared absorption bands. The maximum difference in absorption after reconstitution was about 5%; at 868 nm, there was no effect to within 0.5%.

The ratio of bleaching of the 868 nm absorption per photon absorbed in quinone-depleted to that in quinone-containing reaction centers was found to be 0.32 ± 0.04. This uncertainty represents the extremes obtained from many measurements with several samples both with and without the reductant $\mathrm{Na_2S_2O_4}$ added to the quinone-depleted sample. No effect of pulse intensity below 10% saturation could be detected. If one condition is met, this quantity is the absolute quantum yield of ^{3}P formation from $^1(\mathrm{P^+I^-})$, because the quantum yield of $\mathrm{P^+Q^-}$ formation from $^1(\mathrm{P^+I^-})$ in quinone-containing samples is unity [26]. That condition is that the difference of the ground state and ^{3}P extinction coefficients at 868 nm in quinone-depleted reaction centers be equal to the difference of the ground state and $\mathrm{P^+Q^-}$ extinction coefficients at 868 nm in quinone-containing reaction centers. We also assume that the quantum yield of $^1(\mathrm{P^+I^-})$ for 532 nm excitation is the same in both types of preparations. The major uncertainty in obtaining the quantum yield of ^{3}P is its unknown extinction coefficient. As the exact nature of the ground state absorption of P is not understood, there is the possibility of residual 868 nm absorption in ^{3}P. An upper limit on the quantum yield of ^{3}P can be obtained from saturation studies with quinone-containing preparations at low redox potential [8]; strong, 20 ns flashes cause about 70% as much bleaching at 865 nm on the microsecond time scale in reduced quinone-containing samples as in unreduced samples. These results provide an upper limit of about 0.46 on the quantum yield of ^{3}P in quinone-depleted reaction centers if the extinction coefficient of ^{3}P is assumed to be the same in the two types of reaction centers. This is only an upper limit because the 20 ns flash used in the measurement cited here was not long enough to insure more extensive recycling through $\mathrm{P^+I^-}$, which would be required to drive all reaction centers into the ^{3}P state. In the discussion that follows, $\Phi^{\mathrm{av}}_{^3\mathrm{P}}(H=0)$ is taken to be 0.32 ± 0.04.

Picosecond experiments

Near-infrared absorption difference spectra at

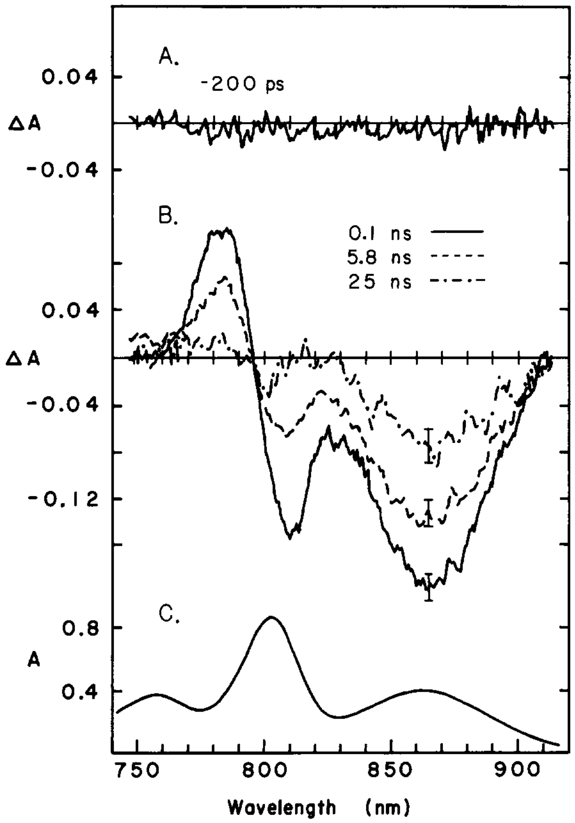

Fig. 2. Absorption difference spectra obtained at several delay times following excitation of quinone-depleted *Rps. sphaeroides* reaction centers with subsaturating 532 nm 35 ps flashes at room temperature. (A) Absorption changes obtained when the probe flash arrived at the sample before the excitation flash (zero field). The spectrum is the average of spectra obtained using 300 excitation flashes. (B) Difference spectra at delays after the excitation flash in a 50 kG magnetic field. The 0.1 and 5.8 ns spectra are each the average of spectra obtained using 600 excitation flashes, while the 25 ns spectrum is the average of spectra obtained using 1200 excitation flashes. (C) Ground state absorption spectrum (zero field).

three times after the flash are shown in Fig. 2B for quinone-depleted reaction centers in a 50 kG magnetic field. The ground-state absorption spectrum at zero field is shown in Fig. 2C for comparison. Similar difference spectra were found at zero field and at 1 kG, but the time dependence of the absorption changes were not the same, as discussed below. The nill absorbance changes observed in Fig. 2A at the negative delay, where the probe pulse arrives at the sample prior to the excitation pulse, indicate that the reaction centers have relaxed completely between flashes, so that no transient species remain in the excited volume.

The positive and negative features at 786 and 812 nm, respectively, and the zero crossing near 795 nm in the 0.1 and 5.8 ns difference spectra in Fig. 2B are characteristic of the formation of P^+I^- [12,27]. These absorption changes and those for P^+Q^- in quinone-containing samples are quite complex and not fully understood. They are probably due to a combination of an electrochromic blue shift and alterations of pigment-pigment interactions caused by oxidation of P and reduction of I, though additional possibilities have been discussed [2–5,27]. The absorption changes in the 800 nm region in Fig. 2B at a 25 ns delay are similar to those seen for ^3P by Shuvalov and Parson [12]. Thus, in our spectra as P^+I^- decays and ^3P forms, the absorption changes between 780 and 815 nm decrease in amplitude, the negative feature shifts to shorter wavelengths from 812 to near 800 nm, and the zero-crossing point shifts to the blue by about 5 nm.

The room-temperature difference spectra of P^+I^- and ^3P presented by Shuvalov and Parson [12] indicate that the difference in ΔA between the peak at 786 nm and the trough at 812 nm in the P^+I^- spectrum is at least 10-times greater than the difference in ΔA for the same wavelengths in the ^3P spectrum, when both spectra are normalized at 870 nm. We thus take the difference in ΔA at 786 and 812 nm as a measure of the P^+I^- concentration. Below, we discuss the possible effects on the interpretation of our results of any small contribution of ^3P to this ΔA difference. The negative feature at 870 nm is due to ground-state bleaching (Figs. 2B and 2C) and will be taken as a measure of the total concentration of P^+I^- and ^3P. The assumption here is that ^3P and P^+I^- both have small extinction coefficients compared to that of the ground state at 870 nm. This is essentially the same assumption as that made above in the determination of $\Phi_{^3P}(H=0)$.

Examples of the time dependence of these features are shown in Fig. 3. The absorption changes in the 800 nm and the 870 nm regions are plotted as a function of time for three magnetic fields. In both regions, the differences in the absorption changes at two wavelengths are plotted. This procedure removes any small (less than 0.01) baseline fluctuations (see Materials and Methods) and ensures maximum sensitivity to the P^+I^- decay in the 800 nm region. In each case, the circles are the experimental points for one set of measurements. There is no evidence of any rapid decay (shorter than 100 ps). The solid curves through the $(\Delta A_{786\,\mathrm{nm}} - \Delta A_{812\,\mathrm{nm}})$ data in Fig. 3A represent nonlinear least-squares fits to single exponentials with zero asymptote. The decay times determined from such fits of the data from three samples at zero field, two at 50 kG and one at 1 kG are listed in the second column of Table II, (radical-pair decay time, zero asymptote). We were unable to obtain difference spectra at a very long delay (over 25 ns) and so unable to exclude a small asymptote for the $(\Delta A_{786\,\mathrm{nm}} - \Delta A_{812\,\mathrm{nm}})$ data. The asymptote should scale with the ^{3}P yield; so, the 50 kG data would be most affected (see Table I). Using the transient absorption data of Shuvalov and Parson [12] mentioned above, the maximum asymptote is 0.1 $\Phi_{^3\mathrm{P}}(H)$. The lifetime determined with this asymptote is listed in the third column of Table II

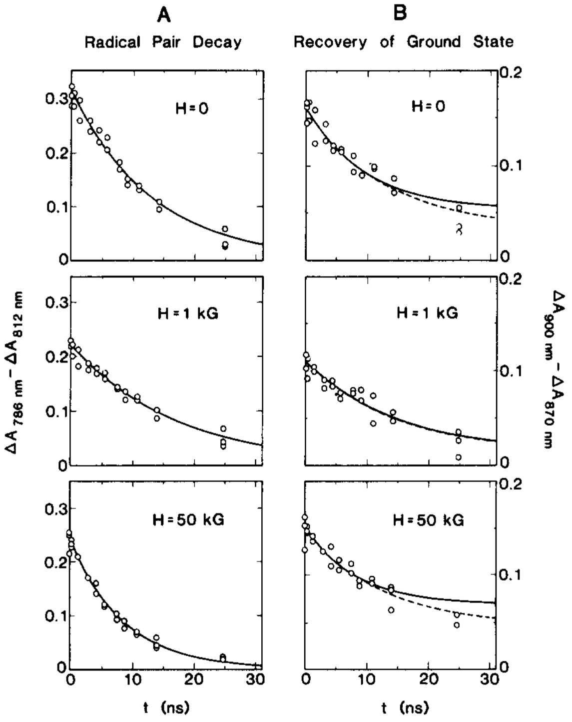

Fig. 3. (A) Plots of the radical pair decay in the 800 nm region. The solid curves are nonlinear, least-squares fits of the $(\Delta A_{786\,\mathrm{nm}} - \Delta A_{812\,\mathrm{nm}})$ data to single exponentials with zero asymptote; the lifetimes of these exponentials are 13.0, 17.3 and 8.5 ns at zero field, 1 kG and 50 kG, respectively. (B) Plots of the ground state recovery in the 870 nm region at three magnetic fields. The solid curves are fits of the $(\Delta A_{900\,\mathrm{nm}} - \Delta A_{870\,\mathrm{nm}})$ data to single exponentials with asymptotes of 32%, 15% and 46% at zero field, 1 kG and 50 kG, respectively (see Table II, text and footnote on p. 431); the lifetimes of these exponentials are 11.3, 15.2 and 8.1 ns, respectively. The dashed curves in (B) are fits in which both the lifetime and the asymptote are fit. The lifetime and asymptote (in parentheses) are 11.8 ns (23%), 15.4 ns (12%) and 12.5 ns (30%) at zero field, 1 kG and 50 kG, respectively. Average values determined from measurements on a number of samples are listed in Table II.

TABLE II

PICOSECOND MEASUREMENTS
SUMMARY OF KINETICS

The relative asymptotes (given in parentheses) are defined to be:

$$x \equiv \frac{\Delta A_{786\,\mathrm{nm}}(t=\infty) - \Delta A_{812\,\mathrm{nm}}(t=\infty)}{\Delta A_{786\,\mathrm{nm}}(t=0) - \Delta A_{812\,\mathrm{nm}}(t=0)} \quad \text{and:} \quad y \equiv \frac{\Delta A_{900\,\mathrm{nm}}(t=\infty) - \Delta A_{870\,\mathrm{nm}}(t=\infty)}{\Delta A_{900\,\mathrm{nm}}(t=0) - \Delta A_{870\,\mathrm{nm}}(t=0)}$$

$\Phi_{^3\mathrm{P}}(H=0)$ is taken to be 0.32. $I(H)$ values are taken from $I^{\mathrm{av}}(H)$ values in Table I and from the equation given in the footnote on p. 431. Errors reported are standard deviations of three sets of measurements at zero field and two sets of measurements at 50 kG. The measurements at 1 kG were not repeated.

Magnetic field (kG)	Radical-pair decay time (ns)		Ground-state recovery time (ns)	
	Zero asymptote	Non-zero asymptote ($x = 0.1\Phi^3_{\mathrm{P}}(H=0)I(H)$)	Fixed asymptote ($y = \Phi^3_{\mathrm{P}}(H=0)I(H)$)	Fit asymptote (y)
0	13.3±0.3	12.6±0.3 (0.032)	10.1±1.0 (0.32)	12.8±1.0 (0.21±0.01)
1	17.3	16.9 (0.015)	15.0 (0.15)	15.5 (0.12)
50	9.2±1.0	8.3±0.8 (0.046)	7.4±1.0 (0.46)	9.2±4.7 (0.40±0.15)

(radical-pair decay time, non-zero asymptote). The asymptotes are in parentheses; see footnote to the table. These non-zero asymptotes did not improve the fits significantly. Larger asymptotes yielded poorer fits in all cases.

Examples of the time dependence of the quantity ($\Delta A_{900\,\mathrm{nm}} - \Delta A_{870\,\mathrm{nm}}$) are shown at three magnetic fields in Fig. 3B. The solid curves are fits to single exponentials with fixed asymptotes equal to the ^{3}P yield appropriate to that field (Table I *) using $\Phi_{^3\mathrm{P}}(H = 0) = 0.32$. The ground state recovery times thus determined from several sets of data, and the asymptotes (in parentheses) are listed in the fourth column of Table II. Comparison of the solid curves with the experimental data in Fig. 3B shows that the 870 nm bleaching recovers to a lower asymptote than expected. This suggests that the absolute ^{3}P quantum yield measurement discussed earlier may be high; it certainly argues against it being too low. Thus, the value of 0.32 for $\Phi_{^3\mathrm{P}}(H = 0)$ is likely to be an upper limit. However, because of the nature of the two methods and the associated errors, the microsecond measurements should give better relative ^{3}P yields than the picosecond measurements.

The quality of the fits to the ($\Delta A_{900\,\mathrm{nm}} - \Delta A_{870\,\mathrm{nm}}$) data is improved, and the agreement with the radical pair decay times better, when the asymptote is not fixed, but rather is fit along with the ground state recovery time (dashed curves in Fig. 3B). The ground state recovery times and asymptotes (in parentheses) determined from this procedure are shown in the last column of Table II.

The agreement between the radical pair decay times and the ground state recovery times in Table II at a given magnetic field is good, considering the difficulty of the measurements; they are the same to within the error of the measurements. A clear trend in lifetime as a function of magnetic field is established. Application of a 1 kG field slows the decay but leads to a more complete decay of the 870 nm bleaching (reflecting the diminished ^{3}P yield). Increasing the field to 50 kG, accelerates the decay of all features but also leads to less complete decay of the 870 nm feature (now reflecting the increased ^{3}P yield at 50 kG). We conclude that the radical-pair decay and ground-state recovery times are the same to within the experimental uncertainty, and that the best values of the radical-pair lifetime, $\tau(H)$, are: 13 ± 1, 17 ± 1 and 9 ± 1 ns at zero field, 1 kG and 50 kG, respectively.

Discussion

Analysis of data

From Fig. 1 one deduces that the increased $\mathrm{P}^+\mathrm{I}^-$ decay time with decreased ^{3}P yield at 1 kG and the decreased decay time with increased ^{3}P yield at 50 kG imply that k_T is greater than k_S. In fact, k_T is greater than 1/(9 ns), and k_S is less than 1/(17 ns). This is in contrast to the earlier conclusion that k_S was greater than or equal to k_T drawn from the field dependence of the ^{3}P yield alone [23]. This previous conclusion was based on the apparent high-field asymptote of the ^{3}P yield (approx. 50%) and the assumption of the model in Fig. 1A that triplet radical-pair decay leads exclusively to ^{3}P formation (in which case $\Phi_{^3\mathrm{P}}(H = \infty) = k_\mathrm{T}/(k_\mathrm{S} + k_\mathrm{T})$). Thus, the present results suggest that radical-pair decay by the triplet channel does not lead exclusively to ^{3}P, and that some rapid ground state recovery occurs by this decay channel. With this addition to the model, the difference in relative magnitudes of k_S and k_T deduced from the two types of measurements can be reconciled.

In order to interpret this new data more quantitatively, we define, as others have [16], a generalized, field-dependent, radical-pair lifetime, $\tau(H)$, as the time integral of the concentration of the radical pair normalized to its initial concentration:

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* In the picosecond measurements, the electric vector of the polarized probe light was perpendicular to the field. The relevant 'observed' ^{3}P yield, here called simply $\Phi_{^3\mathrm{P}}(H)$, is approximately that appropriate for an immobilized reaction center [23]:

$$\Phi_{^3\mathrm{P}}(H) = \{1 - a(H)\}\,\Phi^{\mathrm{av}}_{^3\mathrm{P}}(H)$$

This is because the rotational correlation time for a 100 kDa complex in a buffer of 1 cp viscosity is approx. 40 ns [28], several times longer than the average time before detection of the ^{3}P in this experiment. Similarly, the relative observed yield, here called simply $I(H)$, is taken to be $\{1 - a(H)\}I^{\mathrm{av}}(H)$.

$$\tau(H) \equiv \frac{\int_0^\infty \{C_S(H,t)+C_T(H,t)\}\,dt}{C_S(H,t=0)}$$

where $C_S(H,t)$ and $C_T(H,t)$ are the concentrations of singlet and triplet radical-pairs, respectively. This lifetime has the advantage of being equal to the conventional lifetime for a single exponential decay and so may be readily equated with the measured decay times. In fact, Tang and Norris [29] have shown that with $k_T > k_S$, the decay should be very nearly single exponential. This lifetime can also be related to the yields of decay of the radical pair by the singlet and triplet channels, $\Phi_S(H)$ and $\Phi_T(H)$, respectively, and the singlet and triplet decay times, $\tau_S \equiv 1/k_S$ and $\tau_T \equiv 1/k_T$, respectively:

$$\tau(H) = \Phi_S(H)\tau_S + \Phi_T(H)\tau_T \tag{1}$$

This relation comes directly from the following expressions for the yields:

$$\Phi_S(H) = \frac{\int_0^\infty k_S C_S(H,t)\,dt}{C_S(H,t=0)}$$

$$\Phi_T(H) = \frac{\int_0^\infty k_T C_T(H,t)\,dt}{C_S(H,t=0)}$$

Finally, the total yield of radical-pair decay by both channels is unity:

$$\Phi_S(H) + \Phi_T(H) = 1 \tag{2}$$

Ideally, one would like to take advantage of Eqns. 1 and 2 to obtain expressions for τ_S and τ_T in terms of measured quantities. However, the discussion above indicates that the yield of radical pair decay by the triplet channel, $\Phi_T(H)$, appears not to be equal to the ^{3}P yield, $\Phi_{^3\mathrm{P}}(H)$. In the following analysis, we will assume $\Phi_{^3\mathrm{P}}(H)$ and $\Phi_T(H)$ to be proportional but not necessarily equal (see footnote on p. 431):

$$I(H) \equiv \Phi_{^3\mathrm{P}}(H)/\Phi_{^3\mathrm{P}}(H=0) = \Phi_T(H)/\Phi_T(H=0) \tag{3}$$

This assumption is justified if the inequality of $\Phi^{3}{}_{\mathrm{P}}(H)$ and $\Phi_T(H)$ is due to competition between two field-independent decay processes from $^3(\mathrm{P}^+\mathrm{I}^-)$. We then solve for τ_S and $(\tau_S - \tau_T)\Phi_T(H = 0)$ from Eqns. 1–3:

$$\tau_S = \frac{\tau(H_1)\mathrm{I}(H_2) - \tau(H_2)\mathrm{I}(H_1)}{\mathrm{I}(H_2) - \mathrm{I}(H_1)} \tag{4}$$

$$(\tau_S - \tau_T)\Phi_T(H=0) = \frac{\tau(H_1) - \tau(H_2)}{\mathrm{I}(H_2) - \mathrm{I}(H_1)} \tag{5}$$

These quantities are overdetermined because there are data at three fields. Various pairs of fields give the same values within the uncertainty. The values and limits of error are:

$$\tau_S = 20.6 \pm 1.6 \text{ ns}$$

and:

$$(\tau_S - \tau_T)\Phi_T(H=0) = 7.9 \pm 1.5 \text{ ns}$$

Because τ_T must be positive, we can also extract a lower limit for $\Phi_T(H = 0)$ from Eqns. 4 and 5:

$$\Phi_T(H=0) \geqslant \frac{\tau(H_1) - \tau(H_2)}{\tau(H_1)\mathrm{I}(H_2) - \tau(H_2)\mathrm{I}(H_1)} = 0.38 \pm 0.05 \tag{6}$$

This should be contrasted with $\Phi_{^3\mathrm{P}}(H = 0)$ of 0.32 ± 0.04. The lower τ(50 kG) values which would result from including a ^{3}P yield-dependent asymptote in fits to the $(\Delta A_{786\,\mathrm{nm}} - \Delta A_{812\,\mathrm{nm}})$ data (third column of Table II) would increase the values of the expressions in Eqns. 4–6. Furthermore, if, as the $(\Delta A_{900\,\mathrm{nm}} - \Delta A_{870\,\mathrm{nm}})$ data in Fig. 3B and Table II suggest, the microsecond measurement of $\Phi_{^3\mathrm{P}}(H = 0) = 0.32$ is in fact too high, then the difference between $\Phi_{^3\mathrm{P}}$ and Φ_T is further accentuated.

We next examine calculations of the triplet radical pair decay yield [23]. These calculations are based on the kinetic and magnetic parameters of the radical pair (see footnote on p. 433). We use the value of $k_S = 5 \cdot 10^7$ s^{-1} obtained above and look for values of k_T which fit the relative ^{3}P yield data subject to the constraint that:

$$(\tau_S - \tau_T)\Phi_T(H=0) = 7.9 \pm 1.5 \text{ ns}$$

We choose parameters for the difference in the hyperfine and Zeeman interactions of the radicals based on ESR data of trapped radicals in the reaction center [5]. That is, at high field ($H \geqslant 1$ kG), we choose a Gaussian distribution of the

TABLE III

FITS TO ^{3}P YIELD AND P$^+$I$^-$ DECAY DATA

[1] k_T and Δg_{iso} were varied; other parameters were fixed as described in the text and footnote on this page. No fit was possible for values of J and D left blank.

$D/g_e\beta_e$ (G)	$J/g_e\beta_e$ (G)	-15	-10	0	$+10$	$+15$	$+20$
	k_T (s^{-1})		$7\cdot10^8$	$8\cdot10^8$	$7\cdot10^8$		
0	Δg_{iso}	–	$8\cdot10^{-4}$	$8\cdot10^{-4}$	$8\cdot10^{-4}$	–	–
	$\Phi_T(H=0)$		0.45	0.47	0.48		
	k_T (s^{-1})				$4\cdot10^8$		
-50	Δg_{iso}	–	–	–	$9\cdot10^{-4}$	–	–
	$\Phi_T(H=0)$				0.46		
	k_T (s^{-1})					$3\cdot10^8$	
-75	Δg_{iso}	–	–	–	–	$9\cdot10^{-4}$	–
	$\Phi_T(H=0)$					0.41	
	k_T (s^{-1})						
-100	Δg_{iso}	–	–	–	–	–	–
	$\Phi_T(H=0)$						

difference in hyperfine energies of the two radicals of second moment $(A_{iso}/2)^2$ and a difference in the isotropic g-tensors of Δg_{iso} where [23]:

$$A_{iso}/g_e\beta_e = 16 \text{ G}$$

and:

$$\Delta g_{iso} \approx 10^{-3}$$

(g_e and β_e are the free electron g-factor and the Bohr magneton, respectively.) At low field one hyperfine interaction with a spin $\frac{1}{2}$ nucleus for each radical is chosen [30]:

$$A_{P^+}/g_e\beta_e = -9.5 \text{ G}$$

and:

$$A_{I^-}/g_e\beta_e = +13 \text{ G}$$

Fig. 4 shows one example of a fit to the relative ^{3}P yield data from the microsecond measurements [†]. Table III shows the values of k_T and Δg_{iso} which give fits to the data for various values of the exchange parameter, J (the average difference of triplet and singlet energies), and the electron dipole-electron dipole parameter, D. Values of J and D were excluded when no pair of k_T and Δg_{iso} values could be found to give a relative ^{3}P yield vs. magnetic field strength curve that fit the experimental data (Fig. 4). Only a limited range of J and D allows fits to the data; moreover, J and D have opposite signs for significant values of D; they compensate to provide a small average second moment of the singlet-triplet splitting at high field.

The values of k_T of 3–8 $\cdot$ 10^8 s^{-1} listed in Table III are in rough agreement with the values of k_T of (5–10) $\cdot$ 10^8 s^{-1} obtained by both Norris et al. [18] and Ogrodnik et al. [21]. Thus having abandoned the assumption that all triplet radical-pair decay produces ^{3}P, we have determined a k_S value of

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[†] The development of the theory until this section does not depend on more subtle details of the model chosen for the dynamics of the radical pair. Effects due to electron hopping from close to distant sites or reformation of ^{1}P have been considered by others [21], but are neglected here, as they have been by others (see, e.g., Refs. 18, 19, 25 and 29). Although this may not be a valid approximation, there is, in our opinion, insufficient quantitative information at present to allow a reasonable guess of their magnitude. As further approximations, we have neglected anisotropic hyperfine effects: $A_{ax} = 0$; D is chosen to be negative as expected for a radical pair; and non-axial electron-dipole–electron-dipole effects are neglected: $E = 0$ [23]. β_D is chosen to fit a (1 kG). We find it impossible to fit both the very negative $a(H)$ data near 50 kG and, with the same parameters, $I^{av}(H)$. We are not sure of the origin of this difficulty. Since the parameters $\Delta g_{iso} = -2\Delta g_{ax}$, $\Delta g_{rh} = 0$ and $\beta_g = 0°$ give large negative a (50 kG) values, they are used throughout. However, we have chosen to fit $I^{av}(H)$ and the curvature of $a(H)$ and not the absolute magnitude of $a(H)$ at high fields.

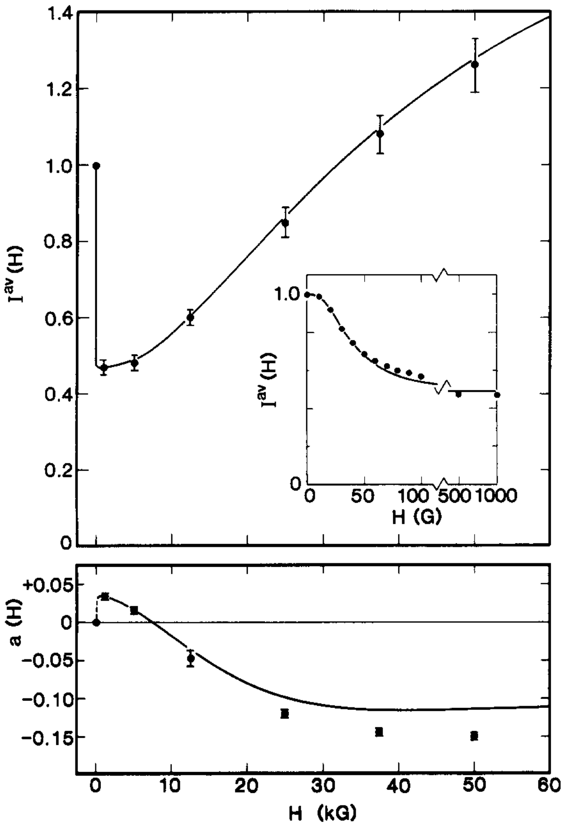

Fig. 4. Experimental relative ^{3}P yield and 868 nm observation anisotropy (●) and calculated relative ^{3}P yield and anisotropy (———) for the following parameters (see text and footnote on p. 433): $k_S = 5 \cdot 10^7$ s^{-1}; $k_T = 3 \cdot 10^8$ s^{-1}; $J/g_e\beta_e = +15$ G; $D/g_e\beta_e = -75$ G; $E = 0$; $\beta_D = 60°$. High field: $A_{iso}/g_e\beta_e = 16$ G; $A_{ax} = 0$; $\Delta g_{iso} = -2\Delta g_{ax} = -9 \cdot 10^{-4}$; $\Delta g_{rh} = 0$; $\beta_g = 0°$. Low field: $A_{P^+}/g_e\beta_e = -9.5$ G; $A_{I^-}/g_e\beta_e = 13$ G; $g_{P^+} = 2.0026$; $g_{I^-} = 2.0036$. $\Phi_T(H = 0)$ was calculated to be 0.41 for these parameters. $a(H)$ was not calculated between 0 and 1 kG (- - - - - -).

about $5 \cdot 10^7$ s^{-1} from the kinetic data and k_T of (3–8)$\cdot 10^8$ s^{-1} from the relative ^{3}P yield data. With such large values of k_T, the low-field data cannot be used to determine the magnitude of the dipolar interaction, D, unambiguously [17,30]. However, given the relatively large absolute value of the exchange interaction, $|J/g_e\beta_e| = 16 \pm 4$ G (expressed in the convention for J used here), obtained by Norris et al. [18] from the dependence of the RYDMR effect on microwave power, a relatively large absolute value of the dipolar interaction $D/g_e\beta_e \approx -75$ G is required (see Table III and Fig. 4). This value of D is in rough agreement with the value obtained by Norris et al. [18], $D/g_e\beta_e = -50 \pm 10$ G, but the required sign of J is in contrast to the conclusion of Norris et al. that $J < 0$. Their determination of the sign of J is not based on the RYDMR effect, but rather on a more complex analysis involving the magnetic field effect.

The calculated yield of radical pair decay by the triplet channel at zero field in Fig. 4, $\Phi_T(H = 0) = 0.41$, is at the low end of the range of the values in Table III, but is still substantially greater than the ^{3}P yield observed assuming the extinction coefficients of P^+Q^- and ^{3}P at 868 nm to be the same. If only a fraction of the decay actually gives ^{3}P, then the low values of the ^{3}P yield, $\Phi_{^3P}$, can be reconciled with the higher values of the yield of radical pair decay by the triplet channel, Φ_T. Our results indicate that at room temperature between 50 and 80% of the radical pairs which decay by the triplet channel actually form ^{3}P. The rest return rapidly to the ground state. This conclusion supports the model in Fig. 1C originally proposed by Schenck et al. [10].

Possible branching mechanisms

How does this fraction return to the ground state? Certain triplet biradicals formed by cycloalkanone photolyses give singlet products without prior spin conversion to the singlet biradicals [31]. De Kanter and Kaptein [32] have recently discussed the role of spin-orbit coupling in promoting these reactions. A related mechanism may be responsible for the production of the singlet ground state from a triplet radical ion pair in reaction centers. However, it is unlikely that $^3(P^+I^-)$ is the radical pair coupled by the spin-orbit interaction to the ground state. A triplet radical pair with strong interactions between the radicals is a much more likely candidate.

Spin-conserving electron transfer from a weakly coupled radical pair (e.g., $^3(P^+I^-)$) to give another state of the same spin multiplicity depends on the perturbation of the initial state by the Coulomb interactions of the electrons on one radical with the nuclei on the other [33]. On the other hand, electron transfer from a weakly-coupled radical pair to give another state of different spin multiplicity depends on perturbation by the magnetic (spin-orbit) interactions of the electrons on one radical with the nuclei on the other [34]. The Coulomb interactions are generally much larger than the spin-orbit interactions[¶]. Thus, electron transfer is not expected to result in a spin change until a triplet state is reached that is stable with respect to spin-conserving electron transfer due to Coulomb interactions. One possibility is that branching takes place in an intermediate triplet state as illustrated in Fig. 5A. Another possibility (not illustrated in Fig. 5) is that branching occurs at $^3(P^+I^-)$ leading to formation of ^3P and another triplet state that can relax rapidly to the ground state.

One possibility for the nature of either of these new states is a charge-transfer triplet state. In a charge-transfer state [36], as opposed to a weakly coupled radical-pair state, two 'radicals' are strongly coupled and the system is best described by eigenstates of the Hamiltonian including the Coulomb interactions between the electrons and nuclei of these 'radicals'; for this reason a charge-transfer state is stable with respect to those Coulomb interactions. In this model, electron transfer from $^3(P^+I^-)$ would populate quasi-stationary triplet states. Spin-orbit coupling to the ground state could be significant in a charge-transfer triplet state if the π-electron systems involved were not parallel, a condition which allows spin and orbital angular momenta to be exchanged on electron transfer. Such a state might well go undetected if it is formed more slowly than it decays.

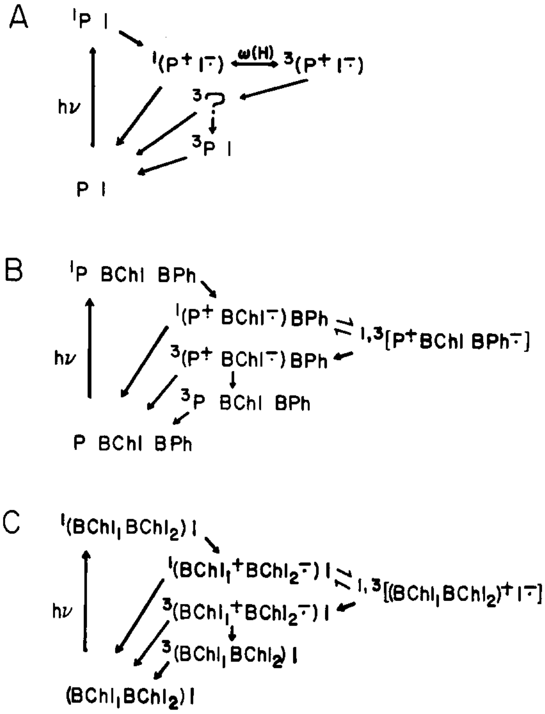

Fig. 5. (A) A model of the initial photochemistry in blocked reaction centers incorporating an unknown triplet state which is coupled by spin-orbit interactions to the ground state and by vibronic interactions to ^3P. The possibility that some or all of the branching of the triplet decay channel occurs from the weakly-coupled radical pair, $^3(P^+I^-)$ is not illustrated here, but is not ruled out by our analysis. (B) A model incorporating two electron acceptors: BChl and PBh. BChl participates in a charge-transfer state with P. (C) A model incorporating charge-transfer states of the dimeric electron donor. The energy differences between states illustrated in these figures is only for clarity of presentation. It is possible, for example, that the triplet charge-transfer state lies above the energy of the weakly-coupled radical pair. Magnetic field-dependent spin-conversion, $\omega(H)$, occurs in the radical pairs labelled $^{1,3}[P^+I^-]$.

What is the chemical nature of this charge-transfer triplet, if indeed it does exist? Current ideas about the reaction center suggest several possibilities. One possibility (Fig. 5B) is based on the proposal of Shuvalov and Parson [12] that P^+I^- is an equilibrium mixture of P^+BChl^- and P^+BPh^-. As pointed out in the introduction, it has been proposed that $^1(P^+BChl^-)$ may be an intermediate charge-transfer state en route to $^1(P^+BChlBPh^-)$. $^3(P^+BChl^-)$ could be the triplet

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[¶] The first depend on the matrix elements of $(Z^{eff}e^2/r)$ where r is, for instance, the separation of the electron on I^- from a nucleus of effective charge $Z^{eff}e$ on P^+. The spin-orbit interactions involve matrix elements of $(\hbar^2/2m_e^2c^2)(Z^{eff}e^2/r^3)$. Because the first factor, $(\hbar^2/2m_e^2c^2) = (2.7 \cdot 10^{-13}\ \mathrm{m})^2$, is very small compared with atomic dimensions, these spin-orbit matrix elements are much less than the Coulomb matrix elements. The spin-orbit interaction also requires that the participating atomic orbitals have orbital angular momentum. The commonly used spin-orbit Hamiltonian [35] is:

$$\mathcal{H}_{so} = \frac{\hbar^2 e^2}{2m_e^2c^2}\sum_{K=1}^{N}\sum_{i=1}^{n}\frac{Z_K^{eff}}{r_{iK}^3}\,\mathbf{l}_i^K\cdot\mathbf{s}_i$$

where K is an index over all nuclei and i is an index over all electrons. For both this and the Coulombic interaction, the argument being made here neglects those possible relative orientations of the radicals which give no interaction. Such orientations are very unlikely; furthermore, small relative motions within the reaction center would lead to some interaction.

charge-transfer state preceeding ^{3}P formation. It should be pointed out that the role we suggest for ^{3}(P$^+$BChl$^-$) is incompatible with certain details of the model of Shuvalov and Parson. In particular, from the temperature dependence of the ^{3}P decay rate, they suggest that the intrinsic time constant for ^{3}(P$^+$BChl$^-$) intersystem crossing to the ground state is 3 μs. In contrast, the intrinsic time constant for the intersystem crossing in the triplet-charge transfer state that we are considering must be at least two orders of magnitude faster to account for the small observed ^{3}P yield.

Another possibility is that P, considered to be a dimer of BChls [1], could itself have charge-transfer states. P$^+$I$^-$ might be best described as (BChl$_1$BChl$_2$)$^+$I$^-$ and the triplet charge-transfer state as ^{3}(BChl$_1^+$BChl$_2^-$)I, localized on the dimer (Fig. 5C). A charge-transfer state of P has been proposed to account for the spectral and kinetic results in reaction centers with both I and Q reduced prior to excitation [9]. Alternatively, P$^+$I$^-$ might correspond to a distant radical pair with the positive charge on one half of the dimer, BChl$_1^+$BChl$_2$I$^-$, and the triplet charge-transfer state might correspond to a tightly coupled 'radical pair' with the positive charge on the other half of the dimer, BChl$_1$^{3}(BChl$_2^+$I$^-$).

The ^{3}P yield increases substantially with decreasing temperature in quinone-containing reaction centers [10] and in quinone-depleted ones [37]. The singlet radical-pair decay pathway may be temperature dependent, and the branching of the triplet radical-pair decay pathway may also be temperature dependent. Both effects should contribute to the temperature dependence of the ^{3}P yield and possibly could be distinguished by the temperature dependence of the radical pair decay kinetics. Unfortunately, the strong temperature dependence of the 870 nm absorption band [38] makes molecular interpretations of other temperature-dependent effects difficult.

In conclusion, we have presented evidence that the triplet radical pair, ^{3}(P$^+$I$^-$), decays not only to the molecular triplet, ^{3}P, but also, to a significant extent, by another pathway that rapidly regenerates the ground state. We suggest this may be due to formation of a strongly coupled 'radical pair' (a charge-transfer triplet state), which experiences relatively strong spin-orbit coupling to the ground state.

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