

Magnetic characterization of the primary state of bacterial photosynthesis

(primary radical pair/reaction yield-detected magnetic resonance/optically detected magnetic resonance/electron-electron dipolar interaction)

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ABSTRACT The results of reaction yield-detected magnetic resonance (RYDMR) experiments carried out on modified bacterial photosynthetic reaction centers (RCs) are interpreted in terms of a model that assigns the initial charge-separated radical ion-pair state, P^F , as the carrier of the spectrum. The radical pair theory, which has been invoked to explain magnetic field effects in RCs, was significantly expanded to take into consideration the electron dipole-dipole interaction. It is shown that this is the largest interaction between the components of the radical ion pair. Quantum statistical calculations are described simulating the RYDMR spectra and low-field effects in quinone-depleted RCs. The experimental data on which the simulations are based are (i) the magnitude of the field effect at 3,000 G, (ii) the field at which 0.5 of the maximal field effect is observed, (iii) the P^F population as a function of time at zero magnetic field, (iv) the RYDMR line-width for low microwave field strength, (v) the RYDMR intensity and width as a function of microwave field, and (vi) the maximum RYDMR intensity at $H_1 \approx 2|J|$. With this information it was found possible to characterize P^F in terms of four parameters, two containing structural information and two with kinetic implications. These are the dipole-dipole interaction, $D = -47 \pm 10 \times 10^{-4} \text{ cm}^{-1}$; the exchange interaction, $J = -7.5 \pm 1.9 \times 10^{-4} \text{ cm}^{-1}$; and the inverse rate constants of the decay of the radical pair states with singlet and triplet spin functions, respectively, $k_5^{-1} = 15 \pm 4 \text{ nsec}$ and $k_7^{-1} = 1.8 \pm 0.2 \text{ nsec}$. The structural and dynamic implications of these parameters are discussed.

For the last two decades, the early events of photosynthesis have been probed by magnetic techniques with the aim of gaining structural and mechanistic insights into the initial charge-separation steps. The existence of a short-lived paramagnetic radical pair state, P^F (1, 2), in bacterial reaction centers had been predicted (3) on the basis of unusual non-Boltzmann populations determined by the conventional EPR spectrum of the triplet state, P^R (4). The unique features of the EPR spectrum of the triplet state P^R were explained as arising from annihilation of charge separation within the radical ion-pair state P^F . In addition, it was suggested that the short lifetime of P^F would necessitate the application of optical detection methods to observe its magnetic resonance spectrum (3). A recent paper from these laboratories (5) reports the optically detected EPR spectrum of P^F , presumably the earliest paramagnetic state in bacterial photosynthesis. It is the purpose of this paper to report the results of a quantitative evaluation of these spectra and the associated magnetic field effects yielding structural and dynamic parameters that are of importance to the general mechanism of photosynthesis.

At this point, P^F is thought to contain the primary donor, special pair bacteriochlorophyll cation (P_{870}), and the primary acceptor, bacteriopheophytin anion (6). Most recently another bacteriochlorophyll molecule has been invoked as a bridging molecule, functioning on the picosecond time scale (7). Although this bridging molecule may be of considerable importance for the rapidity of charge separation, its presence or absence is of little relevance to the discussion of this work and will therefore be neglected. It is important to recognize that because of the small magnitude of the interactions between molecular components comprising the P^F state, there are really four nearly degenerate states describing the radical ion pair. They differ only by their spin functions and can be described in first order as one singlet state and three triplet states. In conventional optical absorption experiments, the four states are indistinguishable, and only the average properties are measured. Thus, the rise and decay times for P^F of 5 and 200 psec, respectively, obtained from optical experiments on unaltered reaction centers, (7) do not contain any information about any individual one of the four states. The decay of P^F in unaltered photosynthetic reaction centers (RCs) corresponds to the transfer of the electron to the secondary acceptor, the ubiquinone-Fe complex.

However, by eliminating the possibility of secondary electron transfer in specially prepared bacteria or bacterial RCs, the lifetime of P^F can be lengthened 100-fold (4, 7). For example, in ubiquinone-depleted RCs, where the ubiquinone-Fe secondary acceptor is no longer present so that the next electron-transfer reaction cannot occur, the P^F state still forms in ≈ 5 psec at room temperature but remains for about 10–20 nsec. Twenty nanoseconds is the time span over which charge separation exists until self-annihilation occurs. Even though a 20-nsec lifetime is 100 times longer than the lifetime measured in unmodified reaction centers, it is still too short for direct study by conventional or time-resolved EPR or electron spin-echo techniques. Although previous attempts at the magnetic resonance of P^F have failed in part because of the short lifetime, it will be shown in this paper that an even more crucial reason for these failures is the fact that P^F is just not very paramagnetic when it is initially generated. A relatively large anisotropic electron dipole-dipole interaction prevents effective mixing between singlet and triplet spin states.

The data to be evaluated in this paper were obtained by a previously described experimental technique (5) in which the yield of one of the recombination products is used to monitor the microwave transitions. The method is referred to as reaction

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Abbreviations: RYDMR, reaction yield-detected magnetic resonance; ODMR, optically detected magnetic resonance; RC, photosynthetic reaction centers.

yield-detected magnetic resonance (RYDMR) (8). Because the RYDMR method as applied here uses optical detection of magnetic resonance (ODMR), it constitutes a special form of the ODMR technique. In very general terms, P^F is created nonadiabatically with singlet spin function, and a resonant microwave field will flip an electron spin of one of the radicals comprising singlet P^F to form a triplet P^F . Charge annihilation occurs with spin conservation (9) so that triplet P^F leads to formation of metastable triplet P^R , whereas singlet P^F yields the singlet ground state. Thus, the magnetic resonance of P^F can be detected by monitoring optically the increase or decrease of the yield of the triplet state P^R when resonant microwaves are applied to P^F . The dynamics of the experiment is summarized in the energy level diagram shown in Fig. 1. As previously reported, such spectra were obtained on modified RCs (5). One of the more startling observations was that high microwave power in excess of 200 W was required to obtain a spectrum (ordinary EPR experiments require milliwatts of power). This observation led us to conclude that previous theoretical descriptions of P^F (10–17) had to be in error or were at best incomplete. The high microwave requirements indicate that one, or both, of two situations exist: (i) the P^F lifetimes must be much shorter than those measured optically or (ii) the initially formed singlet state of P^F has little mixing with the triplet branch, leading to very small transition probabilities.

Our observation of the RYDMR gives a minimum linewidth of ≈ 25 G. By assuming that lifetime broadening is the only source of linewidth and using the uncertainty principle, a lower limit of 1.8 nsec is placed on the lifetime of triplet P^F in quinone-depleted RCs. Because paramagnetic species with a 2-nsec lifetime do not require such high microwave power, we have ruled out the short lifetime as the sole reason for the difficulty in performing the experiment.

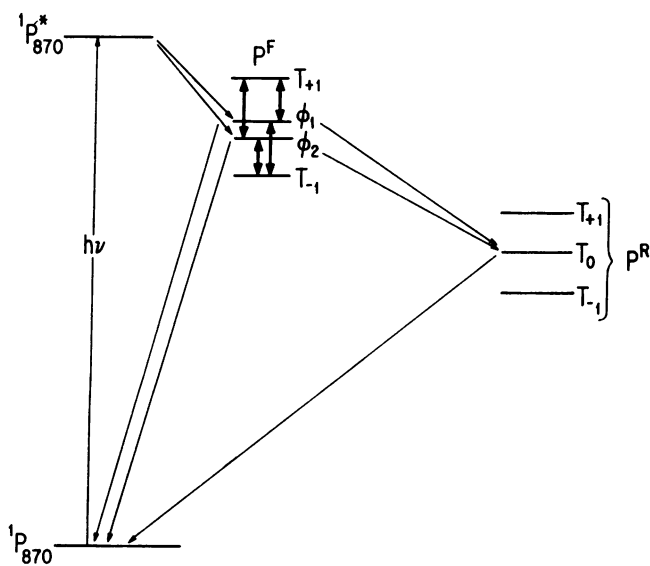


FIG. 1. Energy level diagram showing ground state and first excited singlet state of P_{870} , the four-level radical pair state P^F , and the triplet state of P_{870} known as P^R . The P^F and P^R sublevels are greatly exaggerated relative to the singlet–triplet energy gaps. The levels are labeled for a high magnetic field. Bold arrows in the P^F manifold indicate microwave transitions. Other arrows indicate the usual optical or radiationless transitions. Because the upper and lower states of P^F are essentially pure triplet, they decay only to P^R . If microwaves pump molecules from the center levels of P^F to the upper or lower levels of P^F , then more of the molecules decay to P^R and the yield of P^R increases. A similar diagram applies to zero magnetic field except that the sublevels of P^F and P^R have smaller energy gaps, and the sublevel labels are changed to reflect the zero-field eigenfunctions.

In addition, because the RYDMR linewidth is due only to a short lifetime for triplet P^F (essentially our conclusion in this paper), then, if the electron spin–spin interactions were less than the hyperfine interactions, it would logically follow that the RC P^F system would optically display a lifetime much shorter than 10–20 nsec. We note that the isotropic exchange interactions are known to be quite weak from previous work (10–17). Thus, the following reasoning can be made. If only a weak electron spin–spin interaction existed between donor and acceptor, then the local magnetic environment (e.g., nuclear magnetic moments) would mix singlet and triplet P^F states (3, 9). In other words singlet P^F would be mixed rapidly and efficiently with triplet P^F and, consequently, the decay kinetics would necessarily be heavily influenced by both singlet P^F and triplet P^F . The RYDMR intensity would require a lifetime no longer than 2 nsec if a short triplet P^F lifetime were the sole mechanism for the high microwave requirement, in contrast to the observed 12- to 20-nsec lifetime.

The only consistent explanation is to invoke a large electron spin–electron spin interaction within the radical pair so that the initial state of P^F is relatively pure. The results of this work on photosynthesis show that the initial “pure” state is predominantly singlet in nature and is the dominant state in the observed optical decay. The observed P^F must be predominantly nonparamagnetic and singlet when born and remain so for its lifetime of about 10–20 nsec. For a radical pair to remain predominantly singlet in nature, an electron spin–electron spin interaction large compared to the nuclear hyperfine interaction (i.e., the local magnetic environment of the unpaired electrons) must exist between the two components of the radical pair. As mentioned previously, experimental and theoretical calculations can rule out large isotropic exchange interactions (10–17). We obtain similar results. The only remaining source of a large interaction between the radical members of P^F is the previously neglected anisotropic electron–electron magnetic dipolar interaction. In fact, the anisotropic dipolar interaction between electrons of the radical pair state P^F turns out to be the largest spin–spin interaction of P^F , even though it has never been considered explicitly in previous work on photosynthesis (17).

Because previous studies largely have neglected this electron–electron dipolar interaction, we have calculated the time-dependent quantum mechanics for dynamic radical pairs, including this anisotropic interaction. In addition, because hyperfine interactions provide the major mixing between singlet and triplet in the radical pair state P^F , we have included these interactions in a more realistic manner than in previous calculations.

Finally, by combining theory and experiment, we have obtained the magnetic properties of the primary charge-separation state, P^F , in quinone-depleted RCs prepared from *Rhodospseudomonas spheroides* R-26 bacteria. For this characterization of P^F , we utilize two structural and two kinetic parameters. The first structural parameter is the isotropic exchange interaction, J , where $2J$ is the singlet–triplet energy gap. Positive J has the singlet lowest in energy. The magnitude of J is not only related to the distance of charge separation in the primary act but also reflects the rate of the short-circuiting back reaction. The other structural parameter is the size of the dipolar interaction, D , a more precise structural parameter than isotropic exchange, giving more reliable distances between radicals. Negative D is typical for radical pairs, in that the most nearly degenerate pair of triplet sublevels lies lowest in energy in the absence of a magnetic field. The kinetic parameters used are the rate of recombination of singlet P^F , $1/\tau_S$, and the corresponding rate from triplet P^F , $1/\tau_T$.

THEORY

This section briefly describes the theoretical calculations that have been used to interpret the experimental data and thereby provide J , D , τ_S , and τ_T . The Hamiltonian used is given by Eq. 1.

$$\begin{aligned} \mathcal{H}_0(\phi, \theta) = & g_1 \beta B_0 S_{1z} \\ & + g_2 \beta B_0 S_{2z} + S_1 \cdot \sum_{\alpha} A_{1\alpha} I_{\alpha} \\ & + S_2 \cdot \sum_{\alpha} A_{2\alpha} I_{\alpha} + J(1/2 + 2S_1 \cdot S_2) \\ & + \mathcal{H}_D - g_N \beta B_0 \sum_{\alpha} I_{\alpha z}, \\ \mathcal{H}_D = & \exp(-i\phi S_z) \exp(-i\theta S_x) D [S_z^2 - S(S+1)/3] \\ & \exp(i\theta S_x) \exp(i\phi S_z). \end{aligned} \quad [1]$$

This spin Hamiltonian does not include the decay dynamics of the radical pair. To include this, we write an effective Hamiltonian with a nonhermitian damping term Γ ,

$$\mathcal{H}_{\text{eff}} = \mathcal{H}_0(\phi, \theta) - \frac{i}{2} \Gamma, \quad [2]$$

where Γ is given by

$$\Gamma = k_S \hat{Q}_S + k_T \hat{Q}_T. \quad [3]$$

Here k_S and k_T are the singlet and triplet decay rate constants, respectively, and \hat{Q}_S and \hat{Q}_T are singlet and triplet projection operators given by

$$\hat{Q}_S = 1/4 - S_1 \cdot S_2, \quad \hat{Q}_T = 3/4 + S_1 \cdot S_2. \quad [4]$$

The time evolution of the density matrix is given by

$$\rho(t) = \exp(-i \mathcal{H}_{\text{eff}} t) \rho(0) \exp(+i \mathcal{H}_{\text{eff}}^{\dagger} t). \quad [5]$$

The triplet yield Φ_T can be evaluated by using Eq. 6

$$\Phi_T = \sum_{\text{spin angles}} \sum k_T \text{tr} \left[\hat{Q}_T \int_0^{\infty} \rho(t) dt \right], \quad [6]$$

where the summations are over all nuclear spin functions and angular distributions.

To determine the four unknown parameters, J , D , τ_S , τ_T , we fit six different types of experimental data: (i) the RYDMR baseline (the relative triplet yield at 3,200 G normalized by the zero-field triplet yield); (ii) the magnetic field at which half the magnetic field effect occurs; (iii) the P^F population as a function of time at zero magnetic field; (iv) the RYDMR linewidth for low microwave magnetic field; (v) the RYDMR width and intensity as a function of high microwave magnetic field; and (vi) the maximum RYDMR intensity for $H_1 \approx 2|J|$. The calculations of RYDMR parameters *iv* through *vi* use the high-field approximation for the electron dipolar effects. We neglect the small differences in g values for the electron Zeeman interaction and assume isotropic hyperfine interactions and isotropic g values. Both of these approximations are valid for chlorophyll cations and anions for these field strengths. We assume Gaussian distributions for the electron-nuclear hyperfine interactions, using a 9-G first derivative peak-to-peak linewidth for the cation and 16 G for the anion of the radical pair. These are the experimental linewidths for these same species when not interacting. We also make the approximation that the dipolar term E is zero because the purpose of these calculations is to demonstrate that the en-

ergy gap between singlet S and triplet T_0 or $T_{x,y,z}$ is much larger than previously suspected because of an electron-electron dipolar interaction. The largest energy gap is the most important and is controlled by D . In addition, calculations of simple radical pair models yield $|D| \geq$ approximately $8E$. We take $D < 0$, as is generally the case for radical pairs.

RESULTS

We first discuss the determination of k_S and k_T for the radical pair state P^F . The observed kinetics is approximately exponential and characterized by a lifetime of 12–20 nsec. The short lifetime is observed in fresh preparations, and the lifetime increases the more the samples age. Because a combination of k_S and k_T produces this optical decay, then it is obvious that either τ_S or τ_T must exceed 12 nsec or both must equal 12 nsec in freshly prepared samples. However, in order to explain the 25- to 30-G linewidth observed for the RYDMR with a consistent set of parameters, the triplet lifetime must be $1.8 \text{ nsec} \pm 0.2 \text{ nsec}$. For $\tau_S = \tau_T$, the calculated RYDMR intensity becomes much too large and the RYDMR linewidth becomes much too small. For the case $\tau_T > \tau_S$, the calculated results are incom-

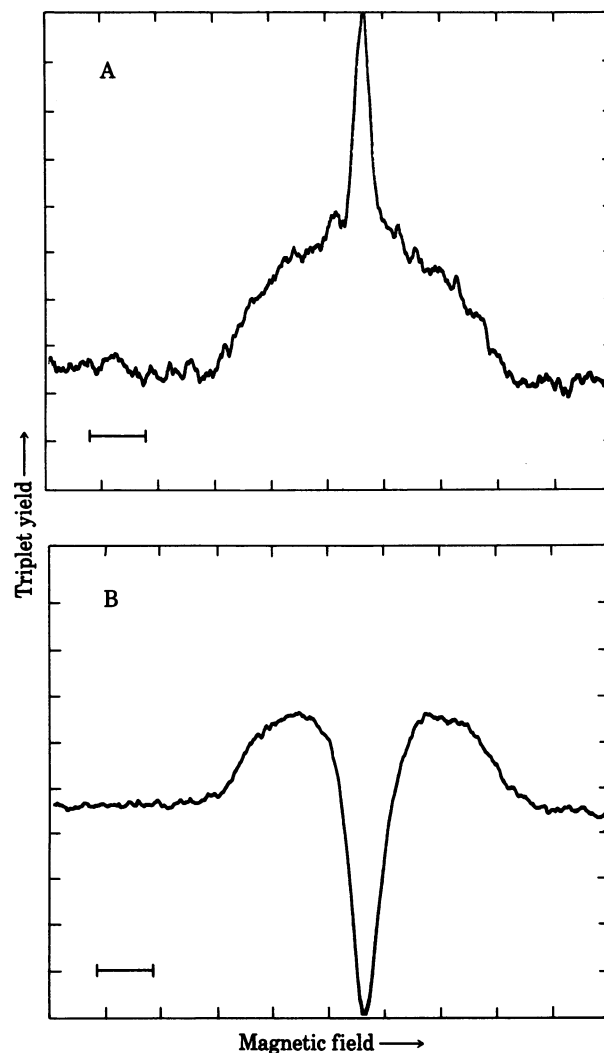


FIG. 2. Experimental RYDMR spectra of *R. spheroides* R-26 RCs depleted in ubiquinone. Spectra were obtained at room temperature. (A) Spectrum for microwave power of less than 2,000 W. (B) Spectrum for microwave power considerably greater than 2,000 W but less than 50,000 W. The 400-G wide signal is due to the resonance signal of P^R , whereas the narrow central signal is from P^F . (Bar = 100 G.)

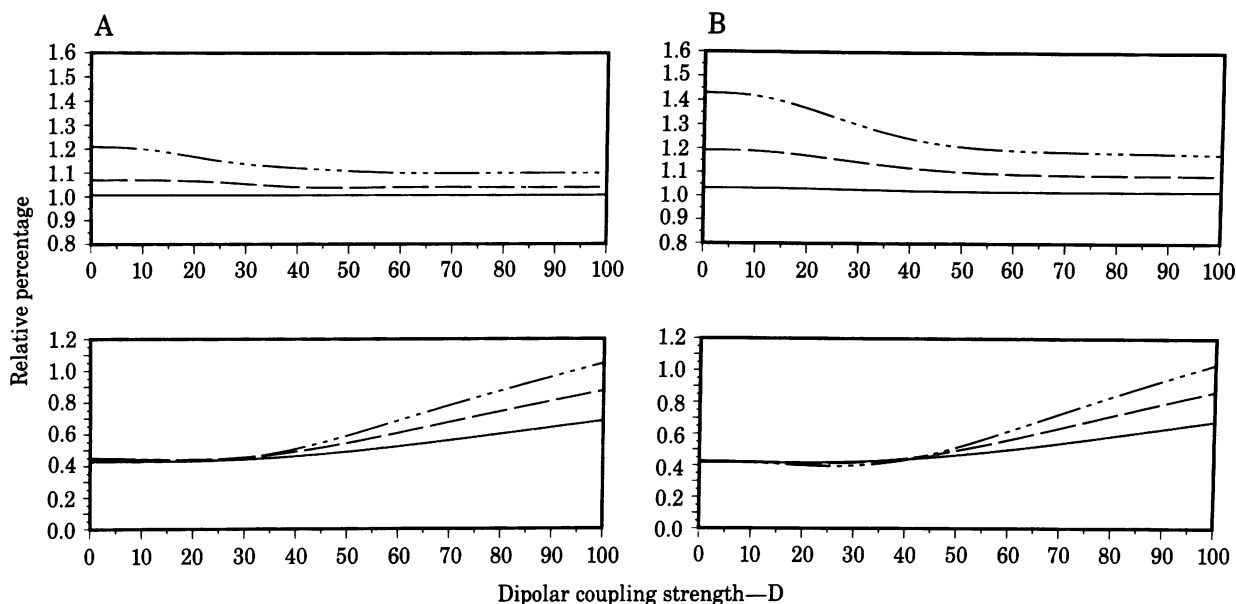


FIG. 3. Calculated RYDMR properties. (A and B upper) Maximum positive RYDMR signal vs. the dipolar coupling strength, D (in gauss). (A and B Lower) RYDMR baseline as a function of D , showing the triplet yield. —, $\tau_T = 1.0$ nsec; ---, $\tau_T = 1.5$ nsec; - · - ·, $\tau_T = 2.0$ nsec. (A) $J = -8$ G and $\tau_S = 15$ nsec. (B) $J = -10$ G and $\tau_S = 15$ nsec.

patible with the absolute yield of P^R , which we take to be between 10% and 50%. Also for this case, an obviously nonexponential decay would be observed optically for P^F . A τ_T of 1.8 nsec and a τ_S of 15 nsec give an effective calculated lifetime for all P^F of ≈ 14 nsec. These lifetime calculations will be presented elsewhere in more detail.

The determination of J is fairly straightforward. First of all, when $J = 0$, the calculated RYDMR displays only a decrease in triplet yield of P^R in the center portion of the RYDMR spectrum. Because we observe an increase in triplet yield, $J \neq 0$. Calculations of types v and vi show that the maximum RYDMR signal intensity is reached when $H_1 \approx 2J$. Fig. 2 shows typical experimental spectra in the two extremes of H_1 : (i) where the center signal is maximal and positive (A) and (ii) where the center signal is negative and minimal (B). The 400-G broad signal in Fig. 2 is relatively independent of microwave power. When the microwave pulse is delayed until after P^F has decayed but before much of P^R has decayed, only the 400-G broad signal remains, indicating this signal is the magnetic resonance spectrum of P^R . The maximum narrow signal occurs with $\approx 2,000$ W of microwave power in a high Q microwave cavity, roughly corresponding to 20 G for H_1 in the rotating frame. In other words, J is ≈ 10 G. We can determine J more accurately from the calculations of Fig. 3 A and B.

In Fig. 3 A and B, we have plotted calculations of

$$\Phi_T[H_0 = 3,200; H_1 = 0]/\Phi_T[H_0 = 0; H_1 = 0]$$

and

$$\Phi_T[H_0 = 3,200; H_1 \approx 2J]/\Phi_T[H_0 = 3,200; H_1 = 0]$$

as a function of D for $J = -8$ and -10 G and for $\tau_S = 15$ nsec

Table 1. Magnetic and kinetic parameters of P^F

Magnetic parameters		
D	$-47 \pm 10 \times 10^{-4} \text{ cm}^{-1}$	$(-50 \pm 10 \text{ G})$
J	$-7.5 \pm 1.9 \times 10^{-4} \text{ cm}^{-1}$	$(-8 \pm 2 \text{ G})$
Kinetic parameters		
τ_S	$15 \pm 4 \text{ nsec}$	
τ_T	$1.8 \pm 0.2 \text{ nsec}$	

and τ_T near 2.0 nsec. The experimental value for the RYDMR intensity is $\approx 1.08 \pm 0.02$, and

$$\Phi_T[H_0 = 3,200; H_1 = 0]/\Phi_T[H_0 = 0; H_1 = 0] = 0.56 \pm 0.04.$$

Inspection of Fig. 3 A and B reveals that a D value of -50 G and a J value of -8 G comes closest to these requirements ($D = +50$ G with $J = +8$ G gives identical results). We should mention that the experimental values can be obtained for J and D with opposite signs. However, in this case $|D|$ becomes quite large, a value too large for highly delocalized chlorophyll-like radical pairs.

As summarized in Table 1, we choose the following parameters, assuming that the sign of D is negative: $J = -8 \pm 2$ G, $D = -50 \pm 10$ G, $\tau_T = 1.8 \pm 0.2$ nsec, and $\tau_S = 15 \pm 4$ nsec. We also point out that these parameters are compatible with an absolute triplet yield of about 35% for P^R as well as the low-field data.

DISCUSSION

The data obtained from the RYDMR and field-effect experiments were interpreted within the framework of the scheme presented in Fig. 1. Inspection of the Hamiltonian, \mathcal{H}_{eff} , used for the calculations shows that, in addition to the hyperfine interaction, four parameters (D , J , k_S , and k_T) had to be adjusted to obtain a realistic simulation of the data. The magnitude of the parameters is directly related to structure and dynamics in RCs and merits some discussion. The parameter with the largest structural implications is the electron dipole-dipole interaction, D , determined to be -50 ± 10 G.

This classical interaction serves to put limits on distances and relative orientations of the components of the radical ion pair. Although it is not possible to derive a unique geometry from this one parameter, its magnitude demands that the components of the pair must be almost within the Van der Waals radius of each other. It is important to note that the omission of E in the calculations is not meant to imply axial symmetry. A more detailed discussion of possible geometries will be the subject of a different paper.

The other structural parameter, the isotropic exchange in-

teraction J , is found to be small but finite, in contrast to other interpretations that set this interaction to zero. According to theory, J is an exponentially decreasing function of distance, although the parameters for that function are not known for highly delocalized molecules. However, J is closely related to the rate of the charge-annihilating back reaction, and the small value found is in agreement with the relatively slow rate of that process (≈ 10 nsec) (4).

The remaining parameters k_S and k_T determine the kinetics of the radical pair decay. Our results show that the lifetime of the radical pair with triplet spin function is approximately an order of magnitude shorter than the singlet component. The energy gap between the triplet radical pair and the molecular triplet P^R is considerably smaller than that between the singlet radical pair and the ground state. A faster rate for the triplet decay is, therefore, in agreement with Marcus theory on electron transfer processes (18). Because for energetic reasons the initial reaction in photosynthesis is through the singlet state, rapid intersystem crossing in the radical pair state is detrimental to the overall efficiency of the creation of chemical potential. Fast forward reaction in a second electron-transfer step will help to conserve the high initial efficiency. However, a slowing down of intersystem crossing in the radical pair state by the electron-electron dipole interaction also helps to prevent the back reaction.

In summary, we believe that the data generated by RYDMR supplement the magnetic field effect data to the extent that a unique set of structural and kinetic parameters can be obtained. This is not possible with data obtained from the field effect alone because the experimental measurements describing the field effect (the shape and the magnitude of the effect) leave the problem underdetermined.

Finally we emphasize that the method of high-microwave-power ODMR/RYDMR, as we have developed it, appears useful in a much broader context than the field of bacterial photosynthesis. We anticipate applications to many other systems both in photochemistry as well as in photosynthesis.

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