RECOMBINATION DYNAMICS IN BACTERIAL PHOTOSYNTHETIC REACTION CENTERS

A. Ogrodnik, H. W. Krüger, H. Orthuber, R. Haberkorn, and M. E. Michel-Beyerle
Institut für Physikalische und Theoretische Chemie, Technische Universität München, D-8046 Garching, Federal Republic of Germany

H. Scheer
Botanisches Institut der Universität München, D-8 München 19, Federal Republic of Germany

ABSTRACT The time dependence of magnetic field effects on light absorption by triplet-state and radical ions in quinone-depleted reaction centers of *Rhodopseudomonas sphaeroides* strain R-26 has been investigated. Measurements on the time scale of the hyperfine interaction in the radical pair $[(BChl)_2^{\dot{+}} \ldots BPh^{\dot{-}})]$ provided kinetic data characterizing the recombination process. The results have been interpreted in terms of a recently proposed model that assumes an intermediate electron acceptor (close site) between the bacteriochlorophyll "special pair" $(BChl)_2$ and the bacteriopheophytin BPh (distant site). Recombination is assumed to proceed through this intermediate acceptor. The experiments led to effective recombination rates for the singlet and triplet channel: $k_S^{eff} = 3.9 \cdot 10^7\ s^{-1}$ and $k_T^{eff} = 7.4 \cdot 10^8\ s^{-1}$. These correspond to recombination rates $k_S = 1 \cdot 10^{10}\ s^{-1}$ and $k_T = 7.1 \cdot 10^{11}\ s^{-1}$ in the close configuration. The upper bound of the effective spin dephasing rate $k_2^{eff} \approx 1 \cdot 10^9\ s^{-1}$ is identical with the rate of the electron hopping between the distant site of zero spin exchange interaction and the close site of large interaction. Interpretation of data for the case of direct recombination yields the recombination rates, spin dephasing rate, and exchange interaction in a straightforward way.

INTRODUCTION

Kinetic information on primary reactions in reaction centers of photosynthetic bacteria has been derived from time-resolved optical spectroscopy. Picosecond studies (1) indicate that the pigment P870 in its first excited singlet state transfers an electron to one of the two bacteriopheophytin molecules within 10 ps. The species P870 has been identified with two aggregated bacteriochlorophyll molecules, the "special pair" $(BChl)_2$ (2*a*, 2*b*). Upon back transfer of this electron, the triplet state of $(BChl)_2$ is formed in nanoseconds (3). The detection of the unusual spin polarization in ESR (2*a*, 4) and the magnetic field dependent yield (5–8) of these triplets is best explained by the "radical pair" model of chemically induced spin polarization (9).

The radical pair, created in an overall singlet state, can change its spin multiplicity by hyperfine interaction (HFI) of the unpaired electrons in the two radicals.

$$\begin{tikzcd} {}^1[(BChl)_2^{\dot{+}} \cdots Bph^{\dot{-}}] \arrow[d] \arrow[r, leftrightarrow, "HFI", "(H)"'] & {}^3[(BChl)_2^{\dot{+}} \cdots BPh^{\dot{-}}] \arrow[d] \\ \text{SINGLET PRODUCTS} & \text{TRIPLET PRODUCT} \end{tikzcd}$$

An external magnetic field *H* removes the quasi degeneracy between the singlet and two of the three triplet sublevels of the pair state, inhibiting the singlet-triplet mixing. Thus, a magnetic field influences the pathway of recombination, i.e., the yields of recombination products in singlet and triplet states.

A necessary condition for such a magnetic field effect to occur is a sufficiently small spin-exchange interaction; that is, the overlap of the wave functions of the two radicals has to be negligible. The discrepancy between the small exchange interaction in the radical pair and a fast, one-step formation rate exceeding $10^{11}\ s^{-1}$ has been explained in different ways (10–12). The explanation proposed in reference 10 suggests at least two sites for the electron or the hole, a close and a distant one, with hopping of the electron between sites, and a zero-exchange interaction in the distant configuration. The idea that the electron transfer between $(BChl)_2$ and BPh proceeds by two steps has been suggested also on the basis of chemically induced dynamic electron polarization (CIDEP) measurements (13). It is further supported by photodichroism studies (14, 15) and recent picosecond (16, 17) and nanosecond (18) time-resolved spectroscopy.

In this paper we investigate the magnetic field effect on the recombination dynamics of $(BChl)_2^{\dot{+}}$ and $BPh^{\dot{-}}$ in quinone-depleted reaction centers isolated from *Rhodopseudomonas sphaeroides* R-26. The measurements, performed on the time scale of the spin precession (between 3 and 15 ns), provide information on the recombination rates

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in the singlet and triplet channels, on the exchange interaction, and on spin dephasing processes such as back hopping of the electron from the $\mathrm{BPh}^{\overline{\cdot}}$ to the intermediate electron acceptor. Information on the rate of spin dephasing is of special interest with respect to recent experiments (18) that have been interpreted in terms of a fast hopping from $\mathrm{BPh}^{\overline{\cdot}}$ to a bacteriochlorophyll monomer as the intermediate electron acceptor.

EXPERIMENTAL PROCEDURES

Sample

The crude reaction centers from *Rps. sphaeroides* R-26, obtained after the ammonium sulfate fractionation (19), were dialyzed and adsorbed on a DEAE-cellulose (DE 52) column. They were then washed with lauryl-dimethyl-aminoxide (LDAO) (4%) and *o*-phenanthroline(10 nM) according to the method of Okamura et al. (20). After a final washing with 1% LDAO the reaction centers were desorbed with Tris buffer containing 0.75 M NaCl. They were then concentrated and desalted by membrane filtration. The ratio of ubiquinone to reaction centers (21) was below 5%, and no light-induced bleaching (<2%) was observed around 870 nm. Upon reconstitution with ubiquinone, the photobleaching at 870 nm was fully restored. Small samples sufficient for one experiment were stored at −20°C in tris-buffer containing 0.1% LDAO. The concentration of reaction centers was 250 μM. All experiments were performed at room temperature (298 K).

Apparatus

Laser light pulses (1.2 ns full width half maximum [FWHM] and 80 μJ) were obtained from a nitrogen laser-pumped dye laser system (Lamda Physik, Göttingen, FRG). Emission from coumarin-153 was tunable at wavelengths between 520 and 560 nm. Stable single-pulse operation of the nitrogen laser has been achieved by previous ionizing of the incoming nitrogen gas in an electric glow discharge.

Two beams of weak intensity were split off the main excitation beam by a plane-parallel glass plate (Fig. 1). Thus, by different optical delays the two pulses probe the absorption before and after excitation. Care was taken to achieve total spatial coincidence of the probe and reference beams in the sample volume, reducing errors due to scattering inhomogeneities. Overlap of the transverse excitation beam and the probing beam was optimized by monitoring the strong bleaching of the absorbance of a suitable dye solution (erythrosine).

The detection system, consisting of a vacuum photodiode (D) and a PDP (Digital Equipment Corp., Maynard, MA) 11/04-controlled transient digitizer system (Tektronix Inc., Beaverton, OR, model 2221), has a band width of 500 MHz. If a series of pulses was simply averaged in the digitizer's "average" mode, the small absorbance changes caused by the excitation were buried under noise from various sources such as jitter of the trigger, intensity fluctuations, digitizing artifacts, and slow fluctuations in the sample (schlieren). Thus, the difference absorption ΔA was directly calculated from the maxima of each pair of successive pulses with the excitation switched on and off by turns. Pulses beyond a preset energy interval or pulses of odd shapes were rejected. After this discrimination, averaging over 150 shots was carried out to yield a resolution in ΔA of better than 0.001 at a fixed delay. This high resolution in ΔA is essential for the detection of small changes in the concentration of triplets and/or radicals, because a triplet yield of 1% corresponds to $\Delta A = 1.75\ 10^{-3}$.

Absolute errors in the difference absorption at different delay times due to variations in beam geometry and the above-mentioned fluctuations were <0.01. Careful handling of controller memory was necessary to achieve real-time data processing at a repetition rate of 2 Hz.

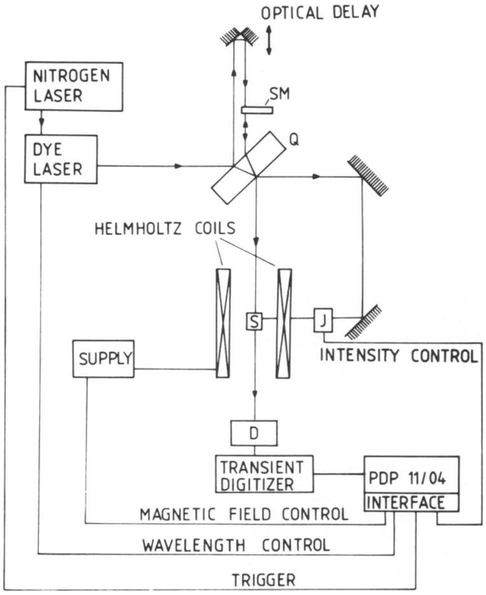

FIGURE 1 Apparatus. By means of the beam splitter (*Q*) the emission from the dye laser is split into: *a*, a reference beam reflected from the rear surface of the plane-parallel quartz plate (*Q*) and from the semitransparent mirror (*SM*) into itself, passing the sample (*S*) before excitation; *b*, an excitation beam transmitting (*Q*) and intensity controlled by a shutter and a continuously variable optical density filter (*J*). The excitation pulse is delayed by 2 ns with respect to the reference beam; *c*, a probe beam reflected from the front surface of (*Q*) with a variable delay and reflected into the reference beam by a triple prism. This beam probes alternatively the absorption of the excited and the dark sample.

EXPERIMENTAL RESULTS

The transient absorption change was measured as a function of time in the wavelength region between 520 and 560 nm. After 3 ns the typical spectrum of the radical ions, $(\mathrm{BChl})_2^{\dot{+}}$ and $\mathrm{BPh}^{\overline{\cdot}}$, referred to as P^{F} in the literature (3, 22), is observed. This spectrum is characterized by a prominent negative difference absorbance with minimum at 543 nm and two isosbestic points, one at 533.5 nm and the other at 550 nm. Due to the increasing contribution of triplet absorption with almost constant extinction coefficient in the region 530–560 nm and the concomitant decay of P^{F}, the minimum at 543 nm decreases in depth with increasing time of probing as discussed in detail in reference 8.

The extinction data for P^{F} as determined in our experiments are consistent with the ones reported in the literature (22, 3). The absolute triplet yield at zero magnetic field, $\phi_{\mathrm{T}}(0)$, and at various magnetic field strengths, $\phi_{\mathrm{T}}(H)$, was monitored at one of the wavelengths λ_0 at which the absorbance of P^{F} is the same as that of the ground state, e.g., at $\lambda_0 = 533.5$ nm. At this wavelength the contribution of P^{F} to the difference absorption is zero.

The value of λ_0 has been derived from the difference absorption measured at early times, at 3 ns after excitation, by extrapolating λ_0 from the wavelengths, λ_{H-0} and

TABLE I

Time	Radical Pairs	Triplets	Singlets
(ns)	%	%	%
3	88	4	8
5	79	9	12
7	65	17	18
15	32	32	36

Experimental yield of radical pairs, triplet and singlet products at different times.

$\lambda_{H=300\,\mathrm{Oe}}$, at which the total difference absorption approaches zero, in the absence and the presence of a saturating magnetic field. At these wavelengths, the positive contribution of the small triplet yield to the difference absorption is compensated by the negative difference absorption of the P^F state. At such early times of the spin precession, it is certainly justified to assume a maximum magnetic field effect of 3:1 on the triplet yield and on the loss of radicals. This assumption and the condition $\phi_{(P^F)} \gg \phi_T$ yields

$$\lambda_0 = \lambda_{H=0} + {}^3\!/\!_2(\lambda_{H=0} - \lambda_{H=300\,\mathrm{Oe}}).$$

The difference extinction coefficient for the triplet-triplet absorption of $^3(\mathrm{BChl})_2^*$ was taken from reference 3: $\epsilon = 6.9 \cdot 10^{-3}\ \mu\mathrm{M}^{-1}\mathrm{cm}^{-1}$ in the wavelength region 530–560 nm.

In Table I the concentrations of radical pairs and triplets measured at different times are compiled. The concentration of the singlet product states, $(\mathrm{BChl})_2$ and BPh, is calculated as the difference between the concentrations of radical pairs and triplets. At the short probing time of 3 ns only 12% of the initially formed radical pairs have decayed, and, due to the early stage of the spin precession, very few of these have recombined in the triplet channel. The magnetic field effect measured on the triplet yield is shown in Fig. 2 for different times of probing. The essential features of Fig. 2 (as also listed in Table II) show that saturating magnetic fields reduce the triplet yield by a factor of 0.33, the theoretical limit, at short times of probing, and by a factor of 0.43 at the longest time of 15 ns. Moreover, both the field strength required for saturation and the half-width, $H_{1/2}$, decrease with increasing delay of probing.

Because the wavelengths of excitation and probing are the same, it is necessary to measure the transient difference spectra at saturating light intensities. Because nonlinear effects (23) have been discussed for picosecond forward electron transfer, we measured the intensity dependence of

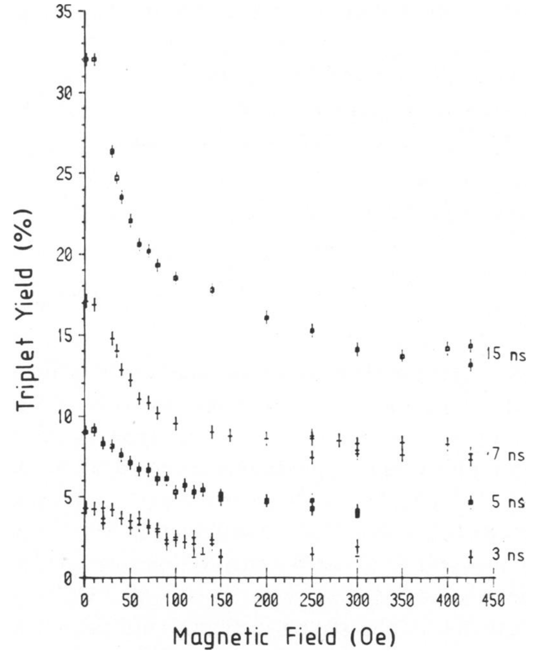

FIGURE 2 Magnetic field dependence of the triplet yield in quinone-depleted reaction centers of *Rps. sphaeroides* R-26, at different delay times between excitation and probing measured at room temperature. A triplet yield of 1% corresponds to a difference absorption ΔA of $1.75 \cdot 10^{-3}$ for the reaction center preparation under investigation (250 μM, 1 mm optical path, $\Delta\epsilon = 6.9 \cdot 10^{-3}\ \mu\mathrm{M}^{-1}\mathrm{cm}^{-1}$ [23]). Saturating light intensity.

TABLE II

Observable	Experiment	Theory $\overline{\mathrm{A}}$ = 13.23 Oe Effective nuclei	Theory $\overline{\mathrm{A}}$ = 13.23 Oe Effective nuclei	Theory $\overline{\mathrm{A}}$ = 15.61 Oe Effective nuclei
		1	9	1
Time = 3 ns	%	%	%	%
ϕ_T	4	3	3.7	4
$\phi_T(0)/\phi_T(\infty)$	3	2, 98	2, 95	2, 93
$H_{1/2}$	80	77	77	76
Time = 5 ns				
$\phi_T(0)$	9	8, 5	10, 3	11
$\phi_T(0)/\phi_T(\infty)$	2, 3	2, 87	2, 87	2, 82
$H_{1/2}$	60	50	50	50
Time = 7 ns				
$\phi_T(0)$	17	14, 9	17, 8	19
$\phi_T(0)/\phi_T(\infty)$	2, 3	2, 8	2, 79	2, 71
$H_{1/2}$	41	40, 5	41	40
Time = 15 ns				
$\phi_T(0)$	32	34, 9	37, 9	39, 9
$\phi_T(0)/\phi_T(\infty)$	2, 3	2, 44	2, 48	2, 28
$H_{1/2}$	33	30	32	31

Triplet yield in zero magnetic filed, $\phi_T(0)$, magnetic modulation of the triplet yield, $\phi_T(0)/\phi_T(\infty)$, and half-width $H_{1/2}$ thereof at different times. Comparison of experiment and theory: model I (1 effective nucleus) and model II (9 effective nuclei). The values $\overline{A}$ = 15.67 Oe = $4.4 \cdot 10^7\,\mathrm{s}^{-7}$ represent the hypothetical case of total localization of the positive charge at one bacteriochlorophyll.

the triplet yield and of the magnetic field effect. There was no evidence for any nonlinear behavior up to the highest excitation intensity, which corresponded to three photons absorbed per reaction center. This seems to be reasonable, taking into account the long time scale of nanoseconds over which the photons arrive.

THEORETICAL TREATMENT

We shall discuss the results in the framework of the recently proposed model (10) for radical pair recombination in photosynthetic reaction centers. In this model, it is assumed that the radical pair occurs in two configurations:

(*a*) A close one, in which the radicals exhibit a strong exchange interaction and can recombine rapidly to form singlet states of $(\mathrm{BChl})_2$ and BPh, or the lowest triplet state of $(\mathrm{BChl})_2$. The anionic radical in the close site may be, for example, a BChl monomer (18).

(*b*) A distant one, corresponding to $[(\mathrm{BChl})_2^{\dot{+}} \ldots \mathrm{BPh}^{\dot{-}}]$ in which the electron spins are far apart so that exchange interaction and recombination are negligible, and hyperfine interaction is of predominant influence on the spin motion.

Transitions between the distant and the close configurations may occur by hopping of the electron or the hole. If one adopts BChl as the intermediate electron acceptor (18), it would be the electron that hops between the distant BPh and the close BChl.

Assuming that the HFI develops in the distant configuration, the equation of motion of the spins, the stochastic Liouville equation for the density matrix ρ, is isomorphous to that of a simple radical pair (10) with only a distant configuration:[1]

$$\dot{\rho} = -i[\hat{H}, \rho] - k_S^{\mathrm{eff}} \hat{P}^S \rho \hat{P}^S - k_T^{\mathrm{eff}} \hat{P}^T \rho \hat{P}^T + k_2^{\mathrm{eff}} (\hat{P}^T \rho \hat{P}^S + \hat{P}^S \rho \hat{P}^T). \quad (1)$$

Here $\hat{P}^S$ and $\hat{P}^T = \mathbb{1} - \hat{P}^S$ are operators projecting on the singlet and triplet spin-subspaces, respectively. While in a one-site model the actual values of the rates and of the exchange interaction are the same as the effective values, the two-site model yields the following equations:

$$k_S^{\mathrm{eff}} = k_S k_D/(k_C + k_S) \quad (2a)$$

$$k_T^{\mathrm{eff}} = k_T k_D/(k_C + k_T) \quad (2b)$$

$$J^{\mathrm{eff}} = J k_C k_D/([J^2 + (k_C + k_2)^2] \quad (2c)$$

$$k_2^{\mathrm{eff}} = k_D[J^2 + k_2(k_C + k_2)]/[J^2 + (k_C + k_2)^2]. \quad (2d)$$

The effective first-order rate constants in the reaction terms in Eq. 1, k_S^{eff} and k_T^{eff}, reflect the formation of singlet and triplet products. These effective rate constants are modified by the forward electron transfer rate k_C from the close site C to the distant site D, and by the corresponding back-hopping rate k_D:

$$\begin{tikzcd} & & \text{singlet products} \\ D \arrow[r, shift left, "k_D"] & C \arrow[l, shift left, "k_C"] \arrow[ur, "k_S"] \arrow[dr, "k_T"'] & \\ & & \text{triplet product} \end{tikzcd}$$

The effective dephasing rate k_2^{eff} (Eqs. 1, 2d) and also the effective exchange interaction J^{eff} in the spin Hamiltonian (Eq. 3) are influenced by the exchange interaction J in the close configuration, by the rates k_C and k_D, and by a T_2-type spin relaxation rate k_2. In the presently used formulation of the stochastic Liouville equations (Eq. 2.6 and 2.7 of reference 10), k_2 has a contribution of $(k_S + k_T)/2$ from the recombination rates and additional contributions from direct spin dephasing interactions such as spin-spin interaction processes with iron. The rate k_S represents both recombination into the electronic ground states of $(\mathrm{BChl})_2$ and BPh, as well as into the excited singlet state of $(\mathrm{BChl})_2$ (24, 25). The effective dephasing rate k_2^{eff} accounts for the disappearance of the off-diagonal elements in the singlet-triplet representation of ρ in Eq. 1, including the contribution of the hopping rates that lead to a further broadening of the half-width of the magnetic field effect on the triplet yield.

It should be emphasized that the effective rates and exchange interaction as given in Eq. 2 are not simply converted into the corresponding parameters for direct one-step recombination by simply allowing k_D to approach zero; back hopping of the electron from D to C is essential for recombination in the two-site model.

A somewhat simplified expression for the spin Hamiltonian used in the radical pair theory (9) is

$$\hat{H} = g\beta H(S_1^Z + S_2^Z) + \sum_i A_i \vec{S}_1 \vec{I}_i + \sum_j A_j \vec{S}_2 \vec{I}_j - J^{\mathrm{eff}} \vec{S}_1 \vec{S}_2 \quad (3)$$

where $\vec{S}_1$ and $\vec{S}_2$ are the two unpaired electron spins, and $\vec{I}_i$, $\vec{I}_j$ and A_i, A_j are the nuclear spins and the isotropic HFI at the distant site, respectively. To a first approximation, the neglect of anisotropic contributions to the HFI is justified because the sample has been excited with unpolarized light. The HFI is practically unaffected by the hopping of the electron spin between the two configurations C and D: the contribution of the close site to the HFI can be neglected due to the strong exchange interaction at this site. Additionally, because of the short period of time site C is occupied as compared to site D, the averaging effect of hopping on the HFI is negligible.

In contrast to hopping between sites of equally small exchange interaction and equally long residence times as occurs at crystal surfaces (26) and in solution (27), in our model we assume hopping between the site D of small and the site C of large exchange interaction with a long

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[1] Throughout the paper, $\hat{H}$, A, J and the rates are given in units of s^{-1}.

residence time of the spin at the distant site D as compared with the close site C.

For simplicity, we first investigated a model (model I) with only one nucleus ($I = \frac{1}{2}$) and an average HFI

$$\bar{A} = \sqrt{\frac{4}{3} \sum_{i}^{(1)+(2)} \vec{I}_i(\vec{I}_i + 1)A_i^2}. \tag{4}$$

With model I, the total Hamiltonian can be block diagonalized. The contributions from $F_z = \pm\frac{3}{2}$, F_z being the sum of the z-components of nuclear and electron spins commuting with $\hat{H}$ and $\hat{P}^S$, are vanishing because of zero matrix elements $\langle T_+\alpha|\hat{P}^S|T_+\alpha\rangle$ and $\langle T_-\beta|\hat{P}^S|T_-\beta\rangle$. The case $F_z = \pm\frac{1}{2}$ yields two $\hat{H}$-matrices with bases $|S\alpha\rangle$, $|T_0\alpha\rangle$, $|T_+\beta\rangle$ and $|S\beta\rangle$, $|T_0\beta\rangle$, $|T_-\alpha\rangle$; α,β are nuclear states and S,T are the electronic states.

To solve the stochastic Liouville equation, a 9 × 9 supermatrix $\mathcal{A}$ is constructed in the Liouville space

$$\begin{aligned}\mathcal{A} = -i\,(\hat{H} \otimes \mathbb{1} - \mathbb{1} \otimes \hat{H}) - k_S^{\text{eff}}\hat{P}^S \otimes \hat{P}^S \\ - k_T^{\text{eff}}\,\hat{P}^T \otimes \hat{P}^T - k_2^{\text{eff}}\,[\hat{P}^S \otimes \hat{P}^T + \hat{P}^T \otimes \hat{P}^S),\end{aligned} \tag{5}$$

where $\mathbb{1}$ is the unity matrix and where $\otimes$ denotes the Kronecker product. The system of linear differential equations can be solved by usual techniques and leads to the formal solution: $\rho(t) = e^{\mathcal{A}t}\rho(0)$.

The triplet probability and, therefore, the triplet yield $\phi_T(t)$ can be simply expressed by the transformed density matrix:

$$\tilde{\rho}(t) = V^{-1}\rho V = V^{-1}e^{-\lambda_i}VV^{-1}\rho(0)\,V \tag{6}$$

$$\phi_T(t) = \int_0^t \mathrm{d}t'\, Tr(\tilde{\rho}(t')\tilde{\hat{P}}^T) \tag{7}$$

V and λ_i are the eigenvectors and eigenvalues of $\mathcal{A}$; the initial condition is $\rho(0) = \hat{P}^S/Tr\hat{P}^S$, where Tr stands for trace.

With the experimentally determined quantities, $\phi_T(0)$, $\phi_T(0)/\phi_T(\infty)$ and $H_{1/2}$ (Fig. 2 and Table II) a least-square fit was performed. Calculating the average hyperfine interaction $\bar{A} = 3.7 \cdot 10^7\,\text{s}^{-1}$ 13.23 Oe (28, 29) from Eq. 4 and restricting k_2^{eff} to $k_2^{\text{eff}} \geqq (k_S^{\text{eff}} + k_T^{\text{eff}})/2$ (11), we obtain the following set of parameters: $k_S^{\text{eff}} = 3.9 \cdot 10^7\,s^{-1}$; $k_T^{\text{eff}} = 7.4 \cdot 10^8\,\text{s}^{-1}$; $J^{\text{eff}} = 8.4 \cdot 10^6\,\text{s}^{-1}$.

The magnetic field dependencies of the triplet yield plotted in Fig. 3 are based on these data. Without any theoretical treatment, the ratio $k_S^{\text{eff}}/k_T^{\text{eff}} < 1$ can be directly derived from the opposite signs of the magnetic field effects on the triplet yield and on the concentration of radical pairs (7, 8).

In Table II, observed and calculated data are directly compared. The tolerance with respect to k_S^{eff} and k_T^{eff} is 10%. J^{eff} can be varied within the limits of $-9 \cdot 10^6\,\text{s}^{-1} < J^{\text{eff}} < +1.4 \cdot 10^7\,\text{s}^{-1}$. Simulation of experimental quantities is rather insensitive to the sign of J^{eff} when J^{eff} is close to zero. At larger absolute values of J^{eff}, the triplet yield for negative J^{eff} is calculated to decrease quicker than for positive ones. Because the half-width of the magnetic field effect is the most accurate experimental quantity and also the most sensitive to variations of the rate constants, we have tested the influence of k_2^{eff} on the half-width $H_{1/2}$ as shown in Fig. 4. From this plot and the experimental value of $H_{1/2}$, one gets an upper bound for the effective spin dephasing rate, $k_2^{\text{eff}} \leqq 1 \cdot 10^9\,\text{s}^{-1}$.

On the basis of the set of parameters given above, the temporal evolution of singlet and triplet products has been calculated as shown in Fig. 5. The plot illustrates that at early times compared to the time scale of HFI ($t < 10$ ns)

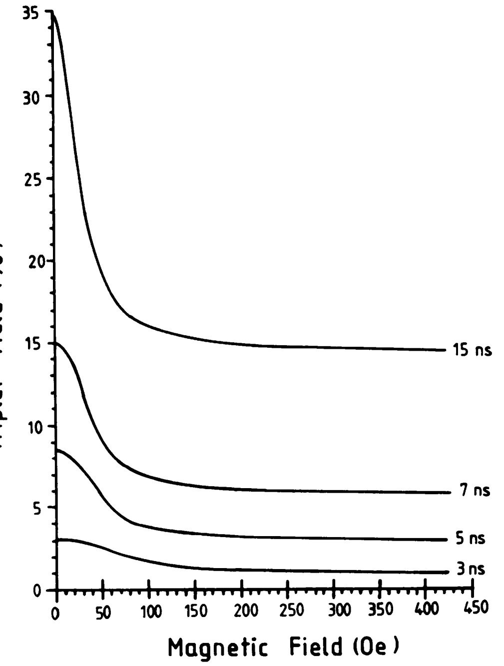

FIGURE 3 Magnetic field dependence of the triplet yield at different times calculated for one effective nucleus by least-square fitting of quantities derived from Fig. 2: $\phi_T(0)$, $\phi_T(0)/\phi_T(\infty)$ and the half-width of the magnetic field effect.

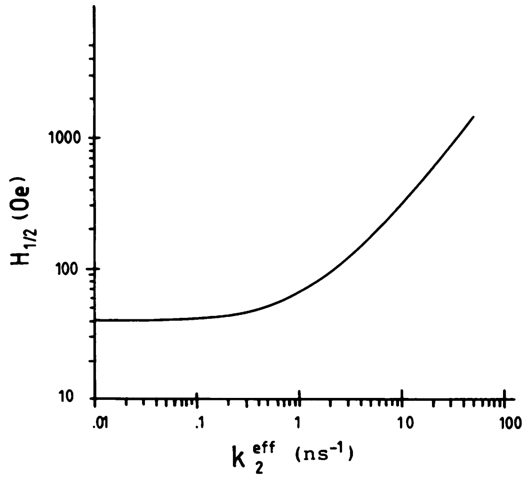

FIGURE 4 Dependence of the half-width of the magnetic field effect on the spin dephasing rate k_2^{eff} for a probing time of 7 ns, one effective nucleus, $k_S^{\text{eff}} = 0.039\,\text{ns}^{-1}$, $k_T^{\text{eff}} = 0.74\,\text{ns}^{-1}$ and $J^{\text{eff}} = 3$ Oe.

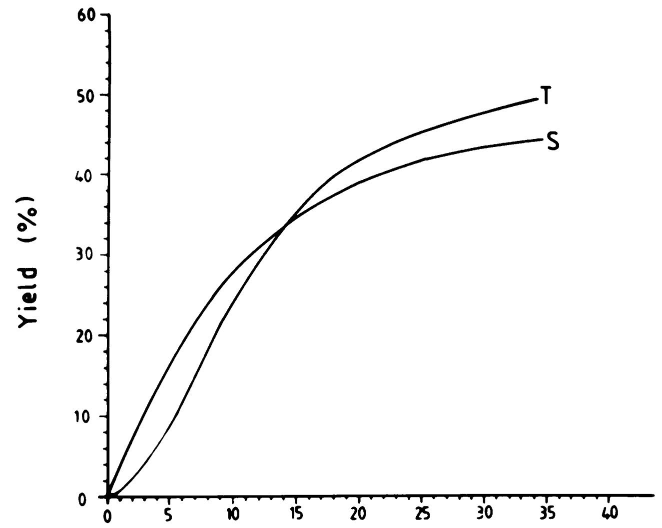

FIGURE 5 Time dependence of the singlet and triplet yield calculated with the parameters k_S^{eff}, k_T^{eff} and J^{eff} as in Fig. 4, and $k_2^{\text{eff}} = (k_S^{\text{eff}} + k_T^{\text{eff}})/2$. *T*, triplet yield; *S*, singlet yield.

the formation of singlets predominates even though $k_S^{\text{eff}} < k_T^{\text{eff}}$.

This tendency is reversed at long times. The extension of this calculation to infinitely long times (e.g., 500 ns) leads to a triplet yield of 53%. The behavior of the temporal evolution of singlet and triplet product yield shown in Fig. 5 agrees with the directly measured values given in Table I. Such a triplet yield for quinone-depleted reaction centers exceeds the yield that has been measured in chemically reduced samples (3, 8) by more than a factor of two. The dependence of the triplet yield on the state of the quinone supports the view that excess charge present in the reaction center affects electron transfer dynamics (8, 30).

To test the sensitivity of the calculation to the number of equivalent nuclei, we have developed a program[2] that takes advantage of the equivalence factoring method (31). This allows us to investigate a more realistic model (model II) with nine nuclei (4 protons of $(\text{BChl})_2^+$, $a_H = 1.14 \cdot 10^7$ s^{-1}, 4 protons and 1 nitrogen nucleus of BPh^-, $a_H = 1.21 \cdot 10^7\ \text{s}^{-1}$ and $a_N = 1.01 \cdot 10^7\ \text{s}^{-1}$). The result for this model II is given in Table II. The number of effective nuclei does not affect the half-width, and the effect on the triplet yield is only ~10%. This indicates that the rate constants are large enough to justify the use of the simplified model I with one effective nucleus for the fitting of the experimental data.

In recent electron nuclear double resonance (ENDOR) and triple resonance experiments[3] performed at room temperature in the liquid phase, the hyperfine couplings of oxidized P870 have been found to be somewhat larger than the low temperature values used in our model calculations. These findings indicate that at room temperature the special pair assumption—as also reflected in the formula $(\text{BChl})_2^{\ddagger}$—might have to be modified in the sense of a stronger localization of spin density on one of the two bacteriochlorophylls. To test the sensitivity of our fit to the value of the average HFI of the pair, we allowed the hyperfine couplings of $(\text{BChl})_2^{\ddagger}$ to increase by a factor of $\sqrt{2}$, corresponding to an increase of $\overline{A}$ by 18%, i.e., from 3.7 $\cdot\ 10^7\ \text{s}^{-1}$ to $4.4 \cdot 10^7\ \text{s}^{-1}$. This is the maximum possible increase, and would be obtained only if the spin density were localized completely on a single bacteriochlorophyll; in fact, the hyperfine couplings are still reduced as compared with the monomeric bacteriochlorophyll cation.[3]

Again restricting k_2^{eff} to $k_2^{\text{eff}} \geqq (k_S^{\text{eff}} + k_T^{\text{eff}})/2$, and performing a least-square fit of the observed data with the increased $\overline{A} = 4.4 \cdot 10^7\ \text{s}^{-1}$, we obtain the following set of parameters: $k_S^{\text{eff}} = 5.6 \cdot 10^7\ \text{s}^{-1}$; $k_T^{\text{eff}} = 7.3 \cdot 10^8\ \text{s}^{-1}$ and $J^{\text{eff}} = 8 \cdot 10^6\ \text{s}^{-1}$. The corresponding values for the triplet yield, the magnetic field modulation thereof, and the half-width are given in Table II. Comparison of experimental and theoretical data shows that theoretical predictions based on this larger value of $\overline{A}$ could just as well account for the experimental findings. An increased hyperfine interaction $\overline{A}$ induces a faster evolution of triplet probability at early times (32). This is compensated by a decrease of the ratio $k_T^{\text{eff}}/k_S^{\text{eff}}$.

DISCUSSION AND CONCLUSIONS

Independent of various experimental handicaps such as insufficient time resolution, high light intensities, and/or considerable excess energy with respect to the $(S_0 \rightarrow S_1)$ 0–0 transition of $(\text{BChl})_2$, all picosecond measurements (1, 16, 17) agree on times ≤10 ps for electron uptake by bacteriopheophytin, BPh. In particular, electron transfer to BPh from an intermediate electron acceptor that may be a bacteriochlorophyll monomer has been reported to take place within 4 ps (16–18). This corresponds to $k_C = 2.5 \cdot 10^{11}\ \text{s}^{-1}$ if we identify the intermediate acceptor with the close site of our model and BPh with the distant site. With this value of k_C and our values of k_S^{eff}, k_T^{eff}, and J^{eff}, the rate k_D for back-hopping of the electron from the distant to the close site can be estimated.

The upper bound for k_D is estimated from the experimental value J^{eff} using Eq. 2c

$$k_D \leq (J/k_C + k_C/J)J^{\text{eff}}, \qquad (8)$$

assuming the dephasing rate k_2 is negligible compared with k_C. This upper limit is lowest if the exchange interaction J at the close site is equal to the forward electron transfer rate k_C. Because $k_T^{\text{eff}} \gg k_S^{\text{eff}}$, Eq. 2*b* gives a lower bound for

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[2] Krüger, H.-W., and R. Haberkorn. Manuscript in preparation.

[3] Lendzian, F., W. Lubitz, H. Scheer, C. Bubenzer, and K. Möbius. In vivo liquid solution ENDOR and triple resonance of bacterial photosynthetic reaction centers of *Rps. sphaeroides*, R-26. Submitted for publication.

$k_D > k_T^{\text{eff}}$. Together with the experimental value, $|J^{\text{eff}}| < 1.4 \cdot 10^7\ \text{s}^{-1}$, this restricts J to $|J| < 4.8 \cdot 10^9\ \text{s}^{-1}$ ($-2.0 \cdot 10^{-5}$ eV) or to $|J| > 1.3 \cdot 10^{13}\ \text{s}^{-1}$ ($=5.5 \cdot 10^{-2}$ eV). Assuming that $|J|$ does not exceed 0.4 eV, an upper limit estimated from the $S_1 \rightarrow T_0$ gap of $(\text{BChl})_2$ (18), $k_D \leq 5.4 \cdot 10^9\ \text{s}^{-1}$. In the limit of small values of $|J|$, there is no lower bound besides the fact that the assumption of $|J|$ being larger than the hyperfine coupling parameters (corresponding to $3.6 \cdot 10^7\ \text{s}^{-1} = 1.5 \cdot 10^{-7}$ eV) is implicit in our model.

A more precise estimate of k_D is given by Eq. 2d yielding $k_2^{\text{eff}} = K_D(1 - k_C/2|J|)$ which is valid for any value of k_2. Values of $|J| > 1.3 \cdot 10^{13}\ \text{s}^{-1}$ result in $k_2^{\text{eff}} \approx k_D \approx 1 \cdot 10^9\ \text{s}^{-1}$ (see also Fig. 4).

Throughout this treatment, we have neglected the possible dependence of the hopping rate k_D on the energy splitting of the close configuration in the presence of a large exchange interaction J. To check on this problem, we derive the relative average probability to find the distant configuration D in a triplet state from our data using

$$\bar{p}_{T,r} = \frac{k_S^{\text{eff}} \cdot \phi_{T,r}}{k_S^{\text{eff}} \cdot \phi_{T,r} + k_T^{\text{eff}} \cdot \phi_{S,r}} .$$

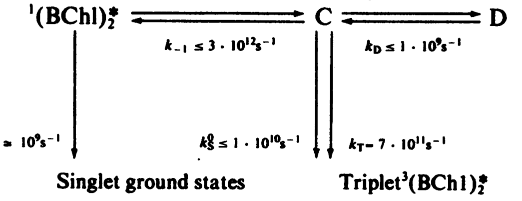
With the relative triplet yield $\phi_{T,r} = \phi_T/(\phi_T + \phi_S) = 53\%$, the triplet probability evolves only to a mean value of $\bar{p}_{T,r} = 5.6\%$ before the spin motion is disturbed by back hopping and recombination into a triplet product. Thus, the contribution of back hopping and recombination rates in the triplet state to the dephasing rate k_2^{eff} is negligible and is not expected to influence the level broadening effect of the hopping rate in the singlet phased distant configuration D. This means that in the limit of $p_{T,r} \ll 1$ our theoretical treatment of the magnetic field effect and its time dependence appears to be adequate.

The interpretation of the effective rates in Eq. 2*a*, *c*, *d* remains unaffected when k_D is set equal to the hopping rate between singlet states in the close and distant configuration. The effective triplet rate k_T^{eff} represents the overall recombination rate within the triplet channel. k_T^{eff} represents the triplet recombination rate k_T from the close site, multiplied by the branching ratio given by the competing channels involving this site.

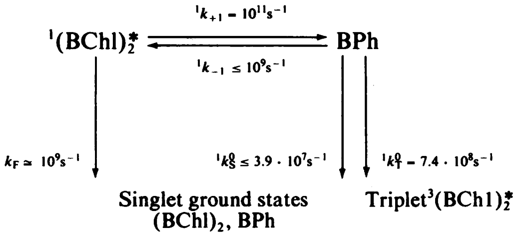
The higher value of $k_D \approx 10^{11}\ \text{s}^{-1}$ proposed recently (18) for reaction centers containing reduced quinone would result in an extreme broadening of the line width of the magnetic field effect, as illustrated in Fig. 4. The origin of this effect is lifetime broadening of the triplet and singlet levels as discussed in earlier work (7). Broadened energy levels require a larger magnetic field to remove the quasi degeneracy of singlet and triplet ($m = \pm 1$) states. Moreover, such a high value of k_D would reduce the triplet yield below observability because the time evolution of the triplet pair state would be stopped by fast hopping at very early stages of HFI.

Our estimate of $k_D \simeq 1 \cdot 10^9\ \text{s}^{-1}$ implies the recombination rates in the close site, $k_T = 7 \cdot 10^{11}\ \text{s}^{-1}$ and $k_S = 1 \cdot 10^{10}\ \text{s}^{-1}$, both competing with k_C. The smaller value of k_S as compared to k_T can be rationalized in terms of different exothermicities (33–35) of the two rates, as long as k_S represents the highly exothermic recombination into the electronic ground states of $(\text{BChl})_2$ and BPh, i.e., $k_S = k_S^0$ in the kinetic scheme below. The rate k_S could as well reflect the population of the ground-state species via $^1(\text{BChl})_2^*$: $k_S^{\ddagger} = k_{-1}k_F/k_{+1}$. If k_S is completely attributed to this channel, $^1(\text{BChl})_2^*$ would be formed with the rate $k_{-1} \simeq 3 \cdot 10^{12}\ \text{s}^{-1}$. This estimate is based on $k_{+1} \simeq 3 \cdot 10^{11}\ \text{s}^{-1}$ (16, 17) for the forward electron transfer from $^1(\text{BChl})_2^*$ to the intermediate acceptor and $k_F \simeq 10^9\ \text{s}^{-1}$ for the radiative decay rate of $^1(\text{BChl})_2^*$. Because there have been several reports on magnetic field effects on fluorescence signals in chromatophores (36) and reaction center preparations (37), the contribution of $k_S^{\ddagger}$ to k_S cannot be neglected, and high values for k_{-1} might have to be envisaged. In case $k_{-1} = 3 \cdot 10^{12}\ \text{s}^{-1}$, the singlet excited state $^1(\text{BChl})_2^*$ and the radical pair in the close configuration must be almost isoenergetic. The increasing yield of triplets upon lowering the temperature (38) would then be predominantly caused by the temperature dependence of thermally activated hopping in the singlet state from the distant to the close site. Recombination in the triplet state would be less sensitive to temperature. As the time of back hopping into the close configuration is delayed, the probability of finding the radical pair in the triplet state has significantly increased.

This discussion of the results within the frame of our two-site model (10) is represented by the following kinetic scheme with data for the forward electron transfer steps taken from reference 18:



However, the success of this scheme in accounting for the observations does not prove the validity of our two-site model. The results could also be interpreted in the frame of a one-site model with direct back transfer of the electron from BPh^- to $(\text{BChl})_2^+$. In this case the overall recombination rates ^1k_S and ^1k_T equal the effective rates k_S^{eff} and k_T^{eff} in the two-site model, $^1k_S = 3.9\ 10^7\text{s}^{-1}$ and $^1k_T = 7.4\ 10^8\text{s}^{-1}$. The rate ^1k_S represents both singlet channels leading to ground state singlet products, $^1k_S = {^1k_S^0} + {^1k^*}$ with $^1k^* = {^1k_{-1}}k_F/{^1k_{+1}}$, $^1k_{-1}$ denoting the back transfer of the electron into the excited singlet state $^1(\text{BChl})_2^*$ and $^1k_{+1}$ the formation rate of BPh^-. Because $^1k_{+1}/k_F \simeq 100$, back



transfer of the electron with the rate $^1k_{-1}$ is again leading to the radical pair state. Therefore $^1k_{-1}$ is expected to affect the line width of the magnetic field effect in a similar way as the hopping rate k_D in the two-site model. Our results restrict $^1k_{-1}$ as they did k_D to $^1k_{-1} \simeq 1 \cdot 10^9\ s^{-1}$. With this value we obtain $^1k^* \simeq 10^7\ s^{-1}$ as an upper limit. Consequently, not more than 25% of radical pairs recombining in the singlet channel are allowed to recombine via the excited singlet state $^1(BChl)_2^*$. It is interesting to compare these rates with the ones recently estimated (39) from the temperature dependence of the bacteriochlorophyll fluorescence and reaction center triplet yield in whole cells of *Rps. sphaeroides,* strain 2.4.1. with the ubiquinone being chemically reduced, $^1k_{-1} = (4–8) \cdot 10^9s^{-1}$, $^1k_S^0 = (0.8 - 1.2 \cdot 10^8s^{-1}$, $k_T = (3 - 5) \cdot 10^8s^{-1}$. The discrepancy between these rates and ours for the one-site model is not surprising if one considers the assumptions used in the estimate and especially the different experimental conditions that are compared.

The effective rates determined in this paper can be internally standardized by introducing a well-defined decay channel for the electron on the bacteriopheophytin anion competing with the effective rates and interfering with the coherent spin motion. This can be achieved by reconstitution of reaction centers with anthraquinones of different redox properties (40).

Discrimination between direct recombination and recombination via an intermediate close state can be attempted by temperature-dependent measurements of the magnetic field effect on both the delayed fluorescence and the time-dependent absorption of radical ions and triplet states.

In conclusion, it should be emphasized that unambiguous kinetic data can only be derived from the magnetic field effect measured on the time scale of the state-mixing interaction, i.e., the nanosecond time region in the present case of HFI as predominant mechanism. At longer times, contributions to the half-width from either the rates or the exchange interaction cannot be discriminated because both a larger exchange interaction as well as increasing rates result in a lower triplet yield and concomitant broadening of the half-width (25, 32).

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