

## THE EFFECT OF LARGE MAGNETIC FIELDS AND THE $g$ -FACTOR DIFFERENCE ON THE TRIPLET POPULATION IN PHOTOSYNTHETIC REACTION CENTERS

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The triplet population in reduced photosynthetic reaction centers is found to increase on application of large magnetic fields (1.5–14 kG). We conclude that the triplet is formed by charge recombination of spin-correlated radical ions with different  $g$  factors. An appropriate theory for the field dependence gives the value  $\Delta g = 0.001$ .

### 1. Introduction

The initial photochemical event in photosynthesis following photoexcitation is the separation of charge to form a moderately stable cation–anion radical pair. For the best understood case of the purple photosynthetic bacterium *Rhodospseudomonas sphaeroides* R-26 mutant, from which highly purified reaction centers are prepared [1], the essential components are believed to be those shown in fig. 1 [2,3]. P is the electron donor, believed to be a pair of bacteriochlorophylls (BChl) characterized by ESR and ENDOR of  $P^{\dot{+}}$  [4,5]; I is an intermediate electron acceptor, believed to be bacteriopheophytin (BChl, where 2H replace Mg, BPheo) [6]; Q is ubiquinone, the secondary acceptor;  $Fe^{2+}$  is a high-spin Fe(II) whose precise function and chemical environment remain obscure [7], and  $X^-$  is used to denote  $Q^- Fe^{II}$ .

The rates characterizing many of the possible elementary reactions are labeled in fig. 1. At the physiological redox potential all decay processes,  $k_S$ ,  $\omega$ ,  $k_T$ ,  $k_{-f}$  and  $k_{fI} + k_r$  (fluorescence and radiationless decay), could compete with the second electron transfer,  $[P^{\dot{+}} I^- X^-] \rightarrow [P^{\dot{+}} IX^-]$ , not shown in fig. 1. However, the quantum yield of charge separation to form  $[P^{\dot{+}} IX^-]$  is known to be nearly 100% under these conditions [8]. As the secondary electron transfer requires  $\approx 150$ – $250$  ps [2,3], the other decay rates must be substantially slower. The mechanism responsible for such slow decay rates, especially  $k_S$ , is of great interest in understanding the origin of the remarkable efficiency of photosynthetic charge separation.

At low redox potential Q is already in the reduced  $Q^-$  state and the secondary electron transfer described above is not possible. Under these conditions the decay processes of the primary radical pair  $[P^{\dot{+}} I^-]$  may be studied. We have investigated the field dependence of the triplet population,  $[^3P]$ , as a probe of the rates  $k_S$ ,  $k_T$  and the singlet–triplet radical pair mixing frequency,  $\omega$ . Our results support the mechanism outlined in fig. 1 and the identities of P and I.

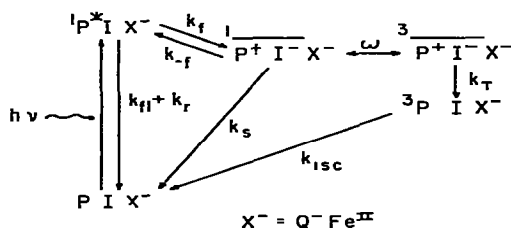


Fig. 1. Kinetic scheme for the primary intermediates in bacterial photosynthesis. Horizontal bars are used to denote spin correlated radical pairs.

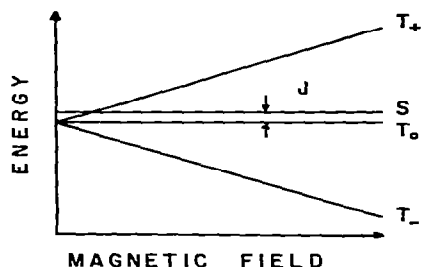


Fig. 2. Schematic energy level diagram for a weakly interacting radical pair as a function of applied magnetic field strength (no dipolar coupling).

The time evolution of the radical pair spin multiplicity from initial pure singlet character depends on the relative energies of singlet and triplet radical pair states and interactions capable of mixing these states. A possible radical pair energy level diagram is shown in fig. 2. For a small exchange interaction ( $J$ ), it is seen that the singlet is close in energy to all triplet sublevels at low magnetic field, but is close only to the  $T_0$  level at high field. Thus, for *any* interaction which mixes singlet and triplet radical pair states, the triplet yield will decrease upon application of a field. This picture is supported by the monotonic reduction of the triplet yield detected optically when the field is raised from 0 to 1000 G [9–11], and by the unusual spin polarization of the ESR spectrum of  $^3P$ , evidenced by the predominance of population in the  $T_0$  magnetic sublevel at 3 kG [12].

Similar effects of magnetic fields have been observed for solution phase radical ion pair reactions and interpreted in terms of nuclear hyperfine induced S–T mixing [13,14]. Isotope effects on the triplet yield which are in quantitative agreement with theory have provided proof of this mechanism [15,16]. This success has led to the assertion that the hyperfine mechanism is also operative in the photosynthetic reaction center [17,18]. This may very well be the case, though a recent report indicates that deuteration has no effect on the triplet yield or its field dependence, which would not be compatible with this simple mechanism [19].

Regardless of the origin of the field dependence at low field, we expect that the  $g$ -factor difference between  $P^+$  and  $I^-$ , if non-zero, should become an important source of mixing between S and  $T_0$  radical pair states at high field [20]. We then expect that the triplet population in reaction centers will increase with field as  $\Delta g\beta H$  increases, as long as  $\omega$  in fig. 1 does not greatly exceed  $k_S$  or  $k_T$ . We have observed an increase in the triplet population at high magnetic field, providing independent evidence for the validity of the radical pair mechanism and an estimate for  $\Delta g$  of 0.0010.

## 2. Experimental

Reaction centers from the R-26 mutant of *R. sphaeroides* are prepared by well established methods [1] and reduced under nitrogen with sodium dithionite. Reduced reaction centers in the concentration range 15–25  $\mu M$  are placed in a variable temperature optical dewar (1.5 mm pathlength) which is mounted as illustrated in fig. 3 in the gap of an ESR magnet.

The 752 nm beam from a Kr-ion laser is chopped at 800 Hz and pumps the reaction centers, while the triplet population is probed with a collinear 514 nm beam from an Ar-ion laser. A filter between the sample and photodiode completely excludes red light. The lifetime of the radical pair is less than  $10^{-3}$  that of the triplet and the extinction coefficients at 514 nm are comparable [21], so interfering light-modulated absorption due to the radical pair can be ignored. The output of the photodiode is divided by the output of a photodiode measuring the green beam, and the ratio fed into a lock-in amplifier whose output is digitized in a Nicolet 1180 computer, which also controls the field scan. The output of the photodiode and the intensities of both beams are completely independent of magnetic field strength, as shown by measuring either chopped beam alone. The magnetic field is calibrated with a Varian proton gaussmeter.

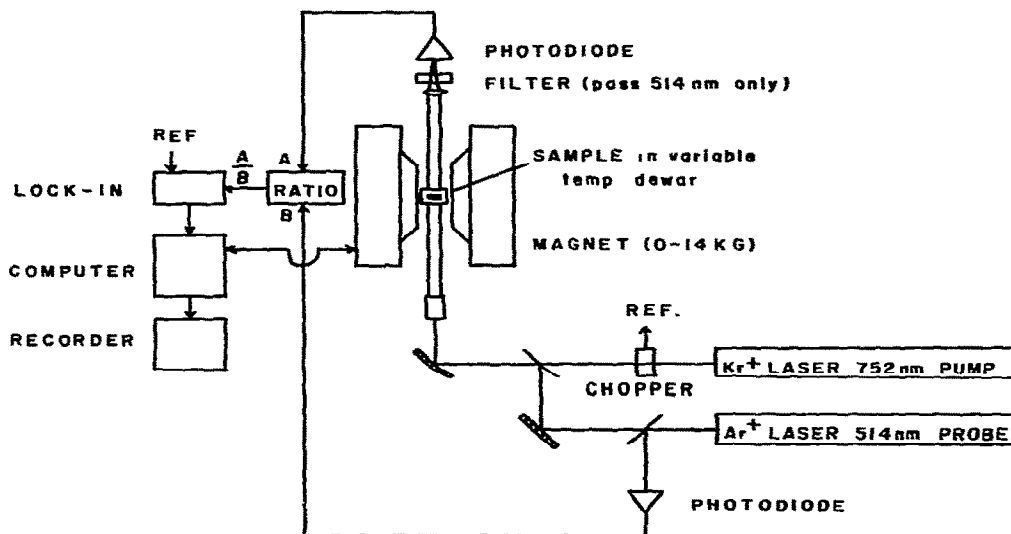


Fig 3 Experimental apparatus for measurement of steady-state triplet transmission.

### 3. Results

The field dependence of the negative of the light-induced change in sample transmission measured at 514 nm is shown in fig. 4, relative to its value at zero magnetic field. These values are proportional to the triplet population because the triplet-induced change in transmittance is on the order of 0.1% of the total transmittance. In order to avoid complications in the analysis of the population changes, we require negligible depopulation of the ground state. The triplet signal saturates with increasing pump power, showing a -7% deviation from linearity with  $0.2 \text{ W/cm}^2$  incident on the sample. To avoid depopulation we operate in the  $0.07 \text{ W/cm}^2$  region which is linear. The relative population as a function of field is the same whether the pump beam is chopped at 300 or 900 Hz. This is expected because the decay half-time of  $^3\text{P}$  is approximately  $120 \mu\text{s}$  at 150 K [21]. Thus the triplet population is at steady state over most of the illumination period in our experiment.

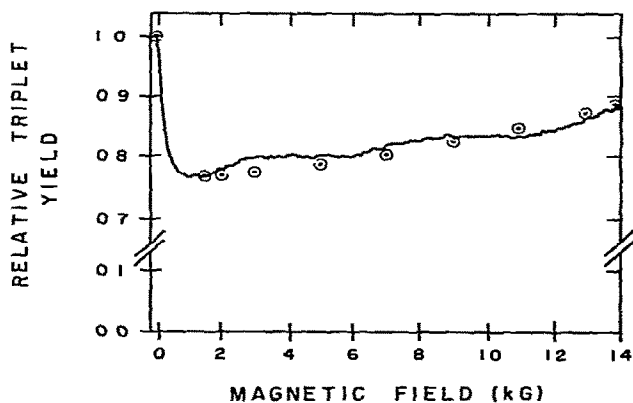


Fig. 4. —: magnetic field dependence of the triplet population in reaction centers relative to that at zero field (150 K).  $\odot \odot \odot$ : representative points from the theoretical curve for the triplet yield generated from eq. (5) at high field and eq. (6) at low field. Parameters discussed in text.

The following experimental ratios are obtained from the data in fig. 4:  $[^3\text{P}(H=0)]/[^3\text{P}(H=1.5 \text{ kG})] = 1.292 \pm 0.035$  and  $[^3\text{P}(H=14 \text{ kG})]/[^3\text{P}(H=1.5 \text{ kG})] = 1.145 \pm 0.022$  (mean value  $\pm$  s.d. of 4 data sets)

#### 4. Discussion

We find that the triplet population decreases on application of a small magnetic field (fig. 4), as has been observed by a number of investigators [9–11]. This reduction is explained by a loss of mixing of the singlet with the  $T_+$  and  $T_-$  states of the radical pair intermediate as  $H$  increases (fig. 2) [14,18]. However, we also find that the triplet population *increases* again as the field is further increased. As discussed qualitatively in section 1, this is the expected effect if the  $g$  factors for the radicals in the pair are different and the rate of singlet–triplet mixing,  $\omega$ , does not greatly exceed that of singlet or triplet radical pair decay,  $k_S$  or  $k_T$  (fig. 1).  $\Delta g$  effects have been observed on the product yields of a few solution phase thermal reactions, providing precedent for our hypothesis [22–24].

In order to calculate  $\Delta g$  we develop a theory below for its effect on the triplet yield. This theory is appropriate if the triplet population is proportional to the triplet yield, that is if  $k_{\text{isc}}$  (fig. 1) is independent of field strength. The high-field sublevels of  $^3\text{P}$  are linear combinations of the zero-field eigenstates, where the coefficients are independent of field strength for fields exceeding the zero-field splittings (0.3 kG [12]). The particular admixture of zero-field states depends only on the orientation of  $^3\text{P}$  relative to the field. Because the zero-field decay rate constants are not expected to be magnetic field dependent, a field dependence for  $k_{\text{isc}}$  could only arise if the spin–lattice relaxation rates among the high-field sublevels depend on field. If this is the case the signal amplitude should be sensitive to the orientation of the molecule in the field and photoselection is expected to affect the field dependence of the signal intensity. We observe no effect upon depolarizing the red beam, supporting our claim that the measured triplet population is proportional to the triplet yield. This analysis will be tested further by comparing time-resolved experiments at high field with the present steady-state method (note the agreement is good at low field [9–11]).

We use the following spin-hamiltonian for the radical pair

$$\mathcal{H} = \left( g\beta H + \sum_i A_{i,2} I_{i,2} \right) \cdot \mathbf{S} + J \mathbf{S}_1 \cdot \mathbf{S}_2 + \left( \Delta g \beta H + \sum_i A_{i,1} I_{i,1} - \sum_i A_{i,2} I_{i,2} \right) \cdot \mathbf{S}_1, \quad (1)$$

$$\mathbf{S} = \mathbf{S}_1 + \mathbf{S}_2, \quad g = g_2, \quad \Delta g = g_1 - g_2.$$

$\mathbf{S}_1$  and  $\mathbf{S}_2$  are the electron spin operators for each member of the radical pair,  $g_1$  and  $g_2$  are the isotropic  $g$  factors for spin 1 and 2, respectively,  $A_{ij}$  and  $I_{ij}$  are the isotropic hyperfine coupling constants and nuclear spin operators, respectively, for nucleus  $i$  on radical  $j$  interacting with the electron on this radical. We have omitted all anisotropic terms including the electron–electron dipole interaction and all interactions with other spins (e.g.  $X^-$ ).

A number of theoretical analyses have been presented which treat the effects of small magnetic fields ( $< 1$  kG) on the triplet yield in reaction centers [17,18]. Quite properly, these treatments make the approximation that  $\Delta g \beta H = 0$  at low field; however, this approximation is clearly invalid at high fields. In light of our results, the case of  $H = \infty$  in these papers should be taken as  $H \approx 1.5$  kG. Haberkorn and Michel-Beyerle [18] have introduced a one-proton model to provide an analytical solution to the problem at zero field. This provides a great simplification by replacing the large number of hyperfine interactions experienced by the electrons with a single hyperfine interaction. The one-proton model fails at high field because it predicts a minimum in the yield for that field at which  $\Delta g \beta H$  exactly cancels the single hyperfine. This is not observed experimentally and is clearly an artifact for a multi-nuclear system. For this reason we have treated the general problem of an arbitrary number of hyperfine interactions at high field.

Because the Zeeman energy is much greater than either the hyperfine or exchange energies, the singlet state does not mix with the  $T_+$  or  $T_-$  states. Therefore the  $I_x S_x$  and  $I_y S_y$  terms in the hamiltonian may be ignored, and there can be no mixing between nuclear states. Thus at high field the problem reduces to finding the triplet yield

for each nuclear state, and then summing over nuclear states.

$$\phi_T = \frac{1}{N} \sum_{k=1}^N \int_0^{\infty} k_T \text{Tr}[P^T \rho_k(t)] dt, \quad (2)$$

where  $\phi_T$  is the triplet yield,  $N$  the total number of nuclear states,  $P^T$  the triplet projection operator and  $\rho_k$  the density operator for the  $k$ th nuclear state. The density operator is found from the stochastic Liouville differential equation

$$d\rho_k(t)/dt = (-i/\hbar) [\mathcal{H}, \rho_k(t)]_- - \frac{1}{2} k_S [P^S, \rho_k(t)]_+ - \frac{1}{2} k_T [P^T, \rho_k(t)]_+, \quad (3)$$

where  $P^S$  is the singlet projection operator. Relaxation terms have been omitted and are not expected to affect  $\rho_k(t)$  during the radical pair lifetime (10–25 ns). The initial condition

$$\rho_k(t=0) = P^S / \text{Tr}(P^S) \quad (4)$$

gives unit probability that the radical pair is in the singlet state at  $t=0$ . Following the formalism of Haberkorn and Michel-Beyerle [18], eq. (2) gives:

$$\phi_T(H) = \frac{1}{N} \sum_{k=1}^N \frac{k_T(k_S + k_T)(Z_k/\hbar)^2}{[(Z_k/\hbar)^2 + 4k_S k_T](k_S + k_T)^2 + 16k_S k_T (J/\hbar)^2}, \quad (5)$$

where

$$Z_k = 2 \left( \Delta g \beta H + \sum_i A_{i1} m_{i1}^k - \sum_i A_{i2} m_{i2}^k \right),$$

and  $m_{ij}^k$  is the axial quantum number for nucleus  $i$  on radical  $j$  in the  $k$ th nuclear state. Eq. (5) is a rigorous solution for the triplet yield for the spin-hamiltonian of eq. (1) at high field. Given the large number of nuclear hyperfine interactions in these extended  $\pi$ -radical ions, we can approximate the summation over discrete nuclear states with an integral over a gaussian distribution for  $Z$  centered at  $2\Delta g \beta H$  with a second moment given by  $A^2$ .

Though correct at high field, the foregoing analysis is inappropriate at zero field. Therefore we use the one-proton model, which is a reasonable approximation for  $H=0$ , to calculate  $\phi_T(H=0)$  using the same  $A$  as shown above for the single hyperfine interaction [18]

$$\phi_T(H=0) = \frac{1}{N} \sum_{k=1}^N \frac{3k_T(k_S + k_T)(A/\hbar)^2}{[3(A/\hbar)^2 + 4k_S k_T](k_S + k_T)^2 + 16k_S k_T (J - \frac{1}{2}A)^2/\hbar^2}. \quad (6)$$

The triplet yield at 150 K and  $H=0$  is conservatively estimated to be between 0.3 and 0.8 from the data of Parson et al. [21]. Even with this large uncertainty possible fits to the data in fig. 4 are greatly restricted to the following ranges of parameters:  $5 \times 10^5 < k_S/A < 3 \times 10^6 \text{ s}^{-1} \text{ G}^{-1}$ ,  $2 \times 10^6 < k_T/A < 1.3 \times 10^7 \text{ s}^{-1} \text{ G}^{-1}$ ,  $4.5 \times 10^{-5} < \Delta g/A < 8 \times 10^{-5} \text{ G}^{-1}$ ,  $-0.5 < J/A < 0.1$ . Fixing any parameter considerably narrows the ranges of the others. Much narrower ranges could be specified if the triplet yield were known accurately or if the radical pair decay kinetics measured optically could be rigorously related to the decay rates,  $k_S$  and  $k_T$ , in fig. 1.

The ESR spectrum of  $P^{\ddagger}$  prepared photochemically is well characterized and is a gaussian with p-p width 9 G (note that  $g$ -factor anisotropy makes a very minor contribution to the linewidth of  $P^{\ddagger}$  [26], and the width includes any small contribution from hyperfine anisotropy). Less is known about the ESR spectrum of  $I^-$ , but a species which may be the precursor to  $^3P$  can be trapped by selective reduction of  $P^{\ddagger}$  in  $[P^{\ddagger} \Gamma^- X^{2-}]$ , and has a p-p width of 13 G [27]. These linewidths give  $A = (9^2 + 13^2)^{1/2} = 16 \text{ G}$ . Thus  $\Delta g$  is between 0.0007 and 0.0013, which should be compared with  $\Delta g = 0.0009$  obtained from the reported  $g$  values for  $P^{\ddagger}$  ( $g = 2.0026$ ) [26] and the species trapped by reduction of  $P^{\ddagger}$  ( $g = 2.0035$ ) [27]. Taking  $A = 16 \text{ G}$ ,  $J = 0 \text{ G}$ ,  $k_S = 3.2 \times 10^7 \text{ s}^{-1}$ ,  $k_T = 1.2 \times 10^8 \text{ s}^{-1}$

and  $\Delta g = 0.0010$ , eqs. (5) and (6) generate the curve with open circles in fig. 4. The calculated yield at  $H=0$  is 0.61.

In summary, we have provided direct experimental evidence that  $^3\text{P}$  in reaction centers has its origin in a radical pair reaction and that the radicals which precede  $^3\text{P}$  have different  $g$  factors. A quantitative analysis yields a value for  $\Delta g$  which is consistent with the  $g$  values for radicals which have been trapped in reaction centers and supports the hypothesis that these radicals lie along the pathway to  $^3\text{P}$ . It is evident from fig 1 that data at very high field will be most desirable because  $\omega$  becomes very rapid and the radical pair states can be considered an equilibrium system drained by  $k_S$  and  $k_T$ . At that field, the yield becomes independent of field and the curvature imposes strong limits on the values of the rates and magnetic parameters. Experiments at 50 kG, time-resolved experiments and the effects of removing Q and  $\text{Fe}^{2+}$  are being pursued

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