

## Out-of-Phase Stimulated Electron Spin–Echo Appearing in the Evolution of Spin-Correlated Photosynthetic Triplet-Radical Pairs

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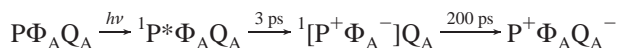
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A strong out-of-phase stimulated electron spin–echo is observed for the  $Q_A^-$  radical in the spin-correlated triplet-radical pair  ${}^3PQ_A^-$  in photosynthetic bacterial reaction centers. The formation of this echo is shown to be induced by spin polarization of  $Q_A^-$  and by decay of the triplet state. The out-of-phase and in-phase echoes show deep envelope modulation induced by electron–electron dipole interaction between the partners in the pair. The analysis of this modulation provides dipolar frequencies. The interspin distance in  ${}^3PQ_A^-$  is shown to be the same as in the radical pair  $P^+Q_A^-$ . This new type of experiment appears to be widely applicable for the study of chemical reactions and intermolecular distances in solids.

### Introduction

In bacterial photosynthesis, excitation of the reaction center (RC) triggers a sequence of fast electron-transfer reactions, which proceed along one of the two pseudosymmetric cofactor chains (A-branch).<sup>1</sup> The primary charge separation may be summarized as follows:

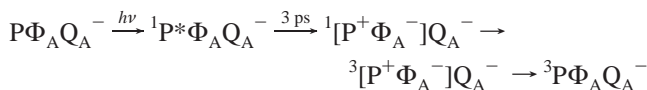
#### SCHEME 1



where P denotes the primary donor, a dimer of bacteriochlorophyll (BChl) molecules,  $\Phi_A$  is the intermediary electron acceptor, a bacteriopheophytin, and  $Q_A$  is the first quinone acceptor. The first relatively stable product of this process is the radical pair (RP) state  $P^+Q_A^-$ , which in the absence of further electron transfer decays by recombination on a millisecond time-scale.<sup>2</sup>

When forward electron-transfer past acceptor  $\Phi_A$  is blocked by prereduction of  $Q_A$ , the spin-correlated triplet-radical pairs (TRPs)  ${}^3PQ_A^-$  are formed with a yield close to unity at cryogenic temperatures.<sup>1</sup>

#### SCHEME 2



Pulsed electron paramagnetic resonance (EPR) techniques have often been used to study photoinduced triplet systems and radical pairs (for review, see refs 3–5 and references therein). Application of the simplest Hahn's pulse sequence to the study of the spin-correlated RP  $P^+Q_A^-$  shows unusual features, an out-of-phase echo<sup>6</sup> and deep envelope modulation mainly determined by the dipolar and/or exchange interactions between the unpaired electrons in the pair.<sup>7–11</sup> The out-of-phase electron

spin–echo envelope modulation (ESEEM) is nearly insensitive to electron–nuclear interactions, which contribute remarkably to the normal in-phase ESEEM.<sup>9,10</sup> A specific requirement for the formation of the out-of-phase echo in the spin-correlated radical pair is simultaneous microwave excitation of both partners of RP.

Determination of the values of dipolar and exchange interactions could reveal important structural information: the dipole–dipole interaction reflects the radical separation in the pair, while the exchange interaction is related to the overlap of the electronic wave functions, which correlates with the rate of electron transfer. Due to the  $r^{-3}$  dependence, determination of the dipolar interaction allows accurate calculation of the center-to-center distance between the two radicals.

It was shown recently that the three-pulse stimulated echo sequence is suitable for the study of dipole–dipole interaction in biradicals. This method was called relaxation-induced dipolar modulation enhancement (RIDME).<sup>12</sup> The advantage of this technique is that it *does not* require the microwave (mw) excitation of both partners in the pair. While the echo is formed by the resonant spins (spins A), the orientation of the nonresonant partner spins B may change due to longitudinal relaxation during the period between the second and the third mw pulses. This produces sudden alteration of the local dipolar field experienced by spin A and leads finally to modulation of the echo signal with the dipolar frequency, when the time interval  $\tau$  between the first and the second pulses is varied.

In the present work we applied the stimulated echo technique to study the  $Q_A^-$  radical in spin-correlated triplet-radical pair  ${}^3PQ_A^-$  (Scheme 2). The EPR spectrum of the triplet is very broad so that simultaneous excitation of both partners in the pair cannot be achieved. Therefore one would expect no electron–electron modulation in a routine two-pulse Hahn's sequence. Surprisingly, strong stimulated ESE signals with deep modulations, when the time interval  $\tau$  is varied, are observed in both in- and out-of-phase mw channels.

Theoretical explanation of this effect takes into account spin polarization of  $Q_A^-$  and  ${}^3P$ , and decay of the nonresonant  ${}^3P$  partner to the ground state. This decay switches off dipolar

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interaction in the pair when magnetization of  $Q_A^-$  is stored along the magnetic field between the second and third mw pulses. The absence of dipolar interaction when the third mw pulse is applied, and the initial spin polarization results in the shift of the precession frequency, which finally produces the out-of-phase echo. This echo is strongly modulated when  $\tau$  is varied. Also, the sudden disappearance of dipolar interaction results in modulation of the in-phase echo.

This mechanism resembles that of the two-pulse out-of-phase echo in spin-correlated radical pairs.<sup>7–11</sup> In both cases, the initial spin polarization and the sudden change of dipolar interaction in the pair are responsible for the formation of the out-of-phase echo that is modulated with dipolar frequency. The difference is that in the radical pair the change of the dipolar interaction between spins is caused by the second microwave pulse that excites the partner spin, while in the triplet-radical pair this change is induced by the decay of the partner spin.

### Experimental Section

In our study we used bacterial reaction centers of *Rhodobacter (Rb.) sphaeroides* R26 isolated as described by Feher et al.,<sup>13</sup> in which paramagnetic  $Fe^{2+}$  ion is replaced by diamagnetic  $Zn^{2+}$  ( $Zn-RCs$ )<sup>14</sup> to avoid fast relaxation induced by the iron ion. Typical EPR samples contained 60–70% (v/v) glycerol and were prepared in 3 mm i.d. quartz tubes. Final optical density of the samples was ca. 15–20  $cm^{-1}$  in the primary donor absorption band.

Two different types of sample were used. In the first one the primary quinone acceptor  $Q_A$  was chemically prerduced to form paramagnetic  $Q_A^-$ . This procedure creates conditions for  $^3P$  formation. In the second sample  $Q_A$  was not prerduced.

Electron spin–echo measurements were carried out on an Elexsys E-680X/E-580E FT EPR spectrometer equipped with a dielectric cavity (Bruker ER 4118 X-MD-5) inside an Oxford Instruments CF 935 liquid helium flow cryostat. The spectrometer dead time was 88 ns. As the EPR signal of  $Q_A^-$  in the dark is expected to be in-phase, the phase of the mw pulses was adjusted by employing this signal. ESEEM patterns were acquired using the two-pulse and stimulated echo techniques. In both experiments the echo signal was recorded as a function of  $\tau$  (time separation between the first and second pulses), with a step of 4 ns in quadrature detection mode. For the two-pulse echo experiment the pulse length was set to 8 ns for the first and 16 ns for the second pulse. The amplitude of the microwave pulses in the two-pulse experiment was adjusted to provide maximal signal amplitude of the out-of-phase echo of the light-induced RP  $P^+Q_A^-$ . For the stimulated echo experiment all pulse lengths were set to 12 ns. The amplitude of microwave pulses in the stimulated echo experiment was adjusted to provide the maximal in-phase echo amplitude of  $Q_A^-$  signal in the dark. The ESEEM time traces were acquired by integrating the signal over the whole echo shape by a pulse integrator (Bruker). Other experimental conditions are given in the figure captions.

X-band direct detection time-resolved EPR experiments were performed as described earlier<sup>15</sup> by taking into account the effect of magnetophotoselection.<sup>16</sup>

As a light source for sample irradiation inside the EPR cavity we used a Continuum Surelite I pumped OPO laser. The excitation wavelength was 532 nm for pulse EPR measurements and 900 nm for time-resolved EPR. The repetition rate of the laser flashes of ca. 4 ns duration was 10 Hz. If not stated otherwise, the mw pulses were delayed after the laser flash for 1  $\mu s$  (DAF = 1  $\mu s$ ).

### Theory

Let us consider a simple case of a two-spin system with spin A ( $S_A = 1/2$ ) excited completely by mw pulses, and spin B ( $S_B = 1$ ) which is nonresonant. The spin Hamiltonian of the weakly coupled triplet-radical pair in the high-field approximation is

$$H = \omega_A S_{Az} + \omega_d S_{Az} S_{Bz} + H_B \quad (1)$$

where  $S_A$  is the operator of spin A (radical,  $Q_A^-$ ),  $\omega_A$  is the Larmor frequency of the isolated spin A in the rotating frame,  $\omega_d = (\gamma^2 \hbar / r^3)(1 - 3 \cos^2 \theta)$  is the strength of triplet-radical dipolar interaction in frequency units ( $\gamma$  is the electron gyromagnetic ratio,  $r$  is the distance between the spins A and B in the point dipole approximation,  $\theta$  is the angle between the interspin vector and the direction of the external magnetic field  $B_0$ , the  $z$  axis of the laboratory frame),  $H_B$  is the spin B (triplet,  $^3P$ ) Hamiltonian that includes Zeeman and zero field splitting interactions. The eigenstates of Hamiltonian (1) are  $\psi_1 = T_{+1}\alpha$ ,  $\psi_2 = T_{+1}\beta$ ,  $\psi_3 = T_0\alpha$ ,  $\psi_4 = T_0\beta$ ,  $\psi_5 = T_{-1}\alpha$ ,  $\psi_6 = T_{-1}\beta$ , where  $T_{+1}$ ,  $T_0$ , and  $T_{-1}$  are the high field eigenstates of  $^3P$  and  $\alpha$  and  $\beta$  refer to the spin states of isolated  $Q_A^-$ .<sup>17</sup> Let us assume that the initial spin states  $\psi_1, \dots, \psi_6$  are occupied with populations  $p_1, \dots, p_6$  and no coherences between the states exist. For simplicity, no anisotropic hfi is included in (1). Also, in our consideration we neglect paramagnetic relaxation.

The six electron-spin states of  $^3PQ_A^-$  are well separated in energy, because of the large zero field splitting in  $^3P$  ( $D = 20.1$  mT,  $E = 3.7$  mT<sup>18</sup>), for all but a narrow range of orientations of  $^3P$  with respect to the magnetic field direction. To the first order of perturbation theory, the spin states for both spins are not mixed, so we may consider the evolution of each spin separately. Furthermore, spin B is not affected by mw pulses (see above) and is described by its  $z$ -projection  $m$ , which may be considered as a parameter. Each of the three subensembles, corresponding to  $m = +1, 0$ , and  $-1$ , may be treated as a quasi two-level system with the truncated spin A Hamiltonian:

$$H = (\omega_A + \omega_d m) S_{Az} \quad (2)$$

The role of spin B is simply reduced to the creation of the dipolar magnetic field at spin A location.

We consider the spin A evolution under the action of the stimulated echo pulse sequence,  $(\pi/2)_x - \tau - (\pi/2)_x - T - (\pi/2)_x - \tau - \text{echo}$ , accompanied with spin B decay, using the product operator formalism.<sup>19</sup> Within this formalism the initial spin A density matrix for the  $m$ th subensemble is  $\rho_m(0) = n_m S_{Az}$ , where  $m = -1, 0, +1$  and  $n_1 = p_1 - p_2$ ,  $n_0 = p_3 - p_4$ ,  $n_{-1} = p_5 - p_6$  are the polarizations of the corresponding transitions.

After the second pulse separated by interval  $\tau$  from the first one, the density matrix becomes

$$\rho_m(\tau_+) = n_m [-S_{Az} \cos((\omega_A + \omega_d m)\tau) + S_{Ax} \sin((\omega_A + \omega_d m)\tau)] \quad (3)$$

The triplet decay with rate constant  $k_m$  depends on the  $z$ -projection of spin B<sup>20</sup> and is assumed to be ineffective during the  $\tau$  period ( $k_m \tau \ll 1$ ). As stated above, we assume also that the  $T_1$  process is slow for both spins. Thus only physical decay of  $^3P$  remains important in our consideration. In the absence of relaxation, the  $z$ -projection of the spin A magnetization is conserved during the following  $T$  period. The transversal magnetization in (3) will not be refocused at the moment  $t = 2\tau + T$  and thus may be neglected. So, prior to the third mw pulse,

$$\rho_m(\tau + T) = -n_m S_{Az} \cos((\omega_A + \omega_d m)\tau) \quad (4)$$

Let us divide the spin A sub-ensemble into 2 fractions: one with statistical weight of  $1 - \exp(-k_m T)$ , for which the triplet decays during period  $T$ , and the other with statistical weight of  $\exp(-k_m T)$ , for which it does not. These fractions behave differently after the third  $(\pi/2)_x$  pulse, as their magnetizations precess now with different frequencies. At the moment of stimulated echo formation:

$$\rho_m(2\tau + T) = n_m \cos((\omega_A + \omega_d m)\tau) \{ \exp(-k_m T) [S_{Ay} \cos((\omega_A + \omega_d m)\tau) - S_{Ax} \sin((\omega_A + \omega_d m)\tau)] + (1 - \exp(-k_m T)) [S_{Ay} \cos(\omega_A \tau) - S_{Ax} \sin(\omega_A \tau)] \} \quad (5)$$

After averaging over  $\omega_A$  due to inhomogeneous broadening

$$\langle \rho_m(2\tau + T) \rangle = (n_m/2) \{ S_{Ay} [\exp(-k_m T) + (1 - \exp(-k_m T)) \cos(m\omega_d \tau)] + S_{Ax} (1 - \exp(-k_m T)) \sin(m\omega_d \tau) \} \quad (6)$$

The observable signals along the  $y$  and  $x$  axes of the rotating frame are

$$M_y = \text{Tr}(S_{Ay} \sum_m \langle \rho_m(2\tau + T) \rangle) = n_1 \exp(-k_1 T) + n_0 + n_{-1} \exp(-k_{-1} T) + [n_1(1 - \exp(-k_1 T)) + n_{-1}(1 - \exp(-k_{-1} T))] \cos(\omega_d \tau) \quad (7)$$

(in-phase echo) and

$$M_x = \text{Tr}(S_{Ax} \sum_m \langle \rho_m(2\tau + T) \rangle) = [n_1(1 - \exp(-k_1 T)) - n_{-1}(1 - \exp(-k_{-1} T))] \sin(\omega_d \tau) \quad (8)$$

(out-of-phase echo).

In the high-field limit  $k_{+1} = k_{-1}$ ,<sup>21</sup> and eqs 7 and 8 may be further simplified:

$$M_y = n_0 + (n_1 + n_{-1}) \exp(-k_1 T) + (n_1 + n_{-1})(1 - \exp(-k_1 T)) \cos(\omega_d \tau) \quad (9)$$

$$M_x = (n_1 - n_{-1})(1 - \exp(-k_1 T)) \sin(\omega_d \tau) \quad (10)$$

From eqs 8 and 10 it follows that at  $T > 0$  an out-of phase echo appears. The out-of-phase echo is strongly modulated with the dipolar frequency  $\omega_d$ . It is clear that the out-of-phase echo appears only if the polarizations of spin A in  $T_{+1}$  and  $T_{-1}$  subensembles are different and if the partner spin decay ( $k_{\pm 1} T$  is not small).

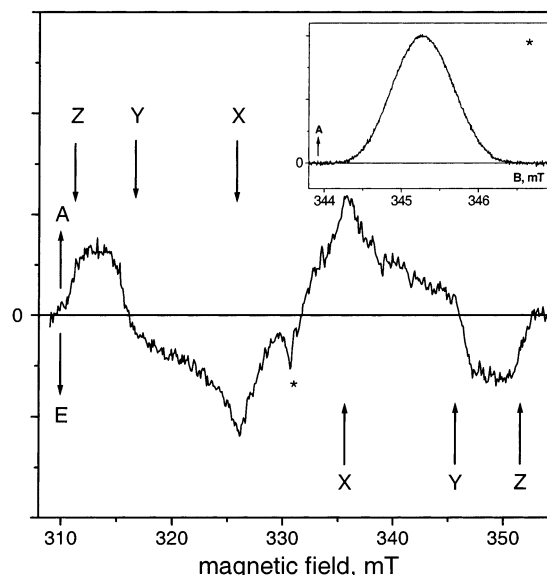
It is generally accepted that populations of the  $T_{+1}$  and  $T_{-1}$  sublevels of  $^3\text{P}$  immediately after recombination of the primary radical pair  $[\text{P}^+\Phi_A^-]$  are equal and vanishingly small. This assumption was, however, never tested explicitly. There is some evidence<sup>17,22</sup> that spin dynamics of the three-spin system  $[\text{P}^+\Phi_A^-]\text{Q}_A^-$  may result in a nonzero and different population of the  $^3\text{P}$  outer spin sublevels.

Modulation of the out-of-phase stimulated echo in the spin-correlated triplet-radical pair (eq 10) is very similar to modulation of the out-of-phase primary echo in spin-correlated RP.<sup>9-11,23</sup> In the notations of ref 23, the primary out-of-phase echo amplitude is  $M_x(2\tau) \sim \sin(2b\tau)$ , where  $2b = (\gamma^2 \hbar / r^3)(1 - 3 \cos^2 \theta)$  (the meaning of symbols is the same as in our case).

Equations 7 and 9 show that dipolar modulation appears also for the in-phase echo, if  $k_{\pm 1} T$  is not small.

## Results and Discussion

Figure 1 shows the direct detection time-resolved EPR spin-polarized signals for the  $^3\text{P}$  and  $\text{Q}_A^-$  states in prerduced Zn-



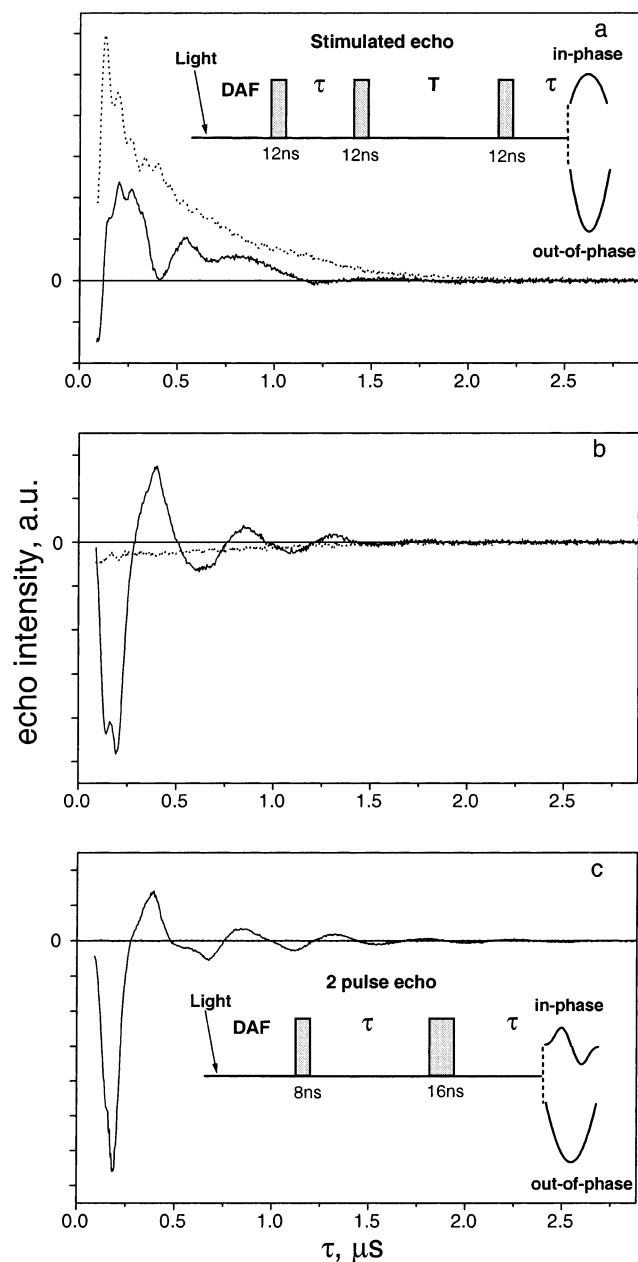
**Figure 1.** Time-resolved direct-detection EPR spectrum of prerduced Zn-RCs of *Rb. sphaeroides* R26 recorded 0.2–1.5  $\mu\text{s}$  after the excitation flash. Experimental conditions: temperature 10 K,  $\nu_{\text{mw}} = 9.714$  GHz. Labels X, Y, and Z indicate the canonical field positions of  $^3\text{P}$ . A and E stand for absorption and emission of microwaves. The  $\text{Q}_A^-$  spin-polarized signal is indicated by a star. The inset shows a two-pulse echo-detected field-swept spectrum of  $\text{Q}_A^-$  in the dark. Temperature 4.2 K,  $\nu_{\text{mw}} = 9.303$  GHz,  $\tau = 144$  ns.

RCs from *Rb. sphaeroides* R26. Without light irradiation, only a narrow (about 0.8 mT line width) signal of the stable  $\text{Q}_A^-$  radical is present (see inset in Figure 1), which becomes emissively spin-polarized<sup>1</sup> under light irradiation (the central line in Figure 1, marked by a star). The broad (ca. 40 mT) line belongs to the spin-polarized triplet of the primary donor appearing under illumination. One can see that the line width of the  $^3\text{P}$  EPR signal is much broader than that of  $\text{Q}_A^-$ . This is because of the strong spin-spin dipolar interaction between unpaired electrons of  $^3\text{P}$ . The large difference of the line widths allows nearly-selective microwave excitation of one partner in the pair,  $\text{Q}_A^-$ , without influencing the other partner,  $^3\text{P}$ .

Figure 2 demonstrates the in-phase (a) and out-of-phase (b) components of the  $\tau$ -dependence of stimulated echo signal of  $\text{Q}_A^-$  in the  $^3\text{P}\text{Q}_A^-$  pair obtained for the sample with prerduced  $\text{Q}_A$ . One observes deep modulation of the out-of-phase echo (Figure 2b). Stimulated echo experiment was performed at the field position where the in-phase signal of  $\text{Q}_A^-$  in the dark attains its maximum (see inset in Figure 1). Solid lines (Figure 2) represent experimental data recorded after light excitation, and dotted lines, those in the dark.

A very similar out-of-phase echo modulation pattern was obtained for the sample where  $\text{Q}_A$  was not prerduced, employing a two-pulse echo sequence (Figure 2c). This modulation is produced by the well-known dipolar interaction in the spin-correlated RP  $\text{P}^+\text{Q}_A^-$ .<sup>7-11</sup> In these measurements the magnetic field was set at the maximum of the out-of-phase echo signal. Note that for the prerduced sample we did not observe any two-pulse out-of-phase echo, so one may rule out an incomplete reduction of  $\text{Q}_A$  in this case.

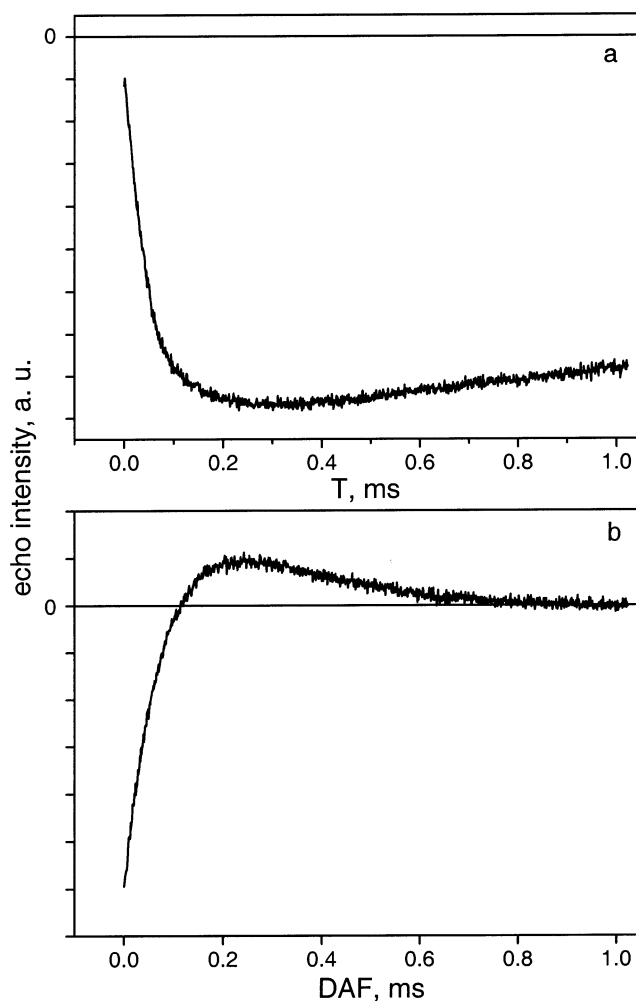
Figure 3a shows the  $T$ -dependence of the out-of-phase stimulated echo signal of the  $\text{Q}_A^-$  in spin-correlated pair  $^3\text{P}\text{Q}_A^-$ . This dependence has a characteristic rise time of  $65 \pm 5 \mu\text{s}$  and a broad maximum around 300  $\mu\text{s}$ . The two-pulse echo decay of the primary donor triplet at the field position near the Y canonical orientation (Figure 1), as the delay after flash (DAF)



**Figure 2.** In-phase (a) and out-of-phase (b) stimulated echo measured for samples with reduced  $Q_A$ , as a function of  $\tau$ . Delay between the second and third pulses  $T = 10 \mu\text{s}$ . (c) Two-pulse out-of-phase echo modulation of spin-correlated radical pair  $P^+Q_A^-$  for samples with nonreduced  $Q_A$ . The insets show the pulse sequences used in the experiments. Temperature 4.2 K. Dotted lines represent signals without illumination.

is increased, is displayed in Figure 3b. The fast decaying component has a decay time of  $81 \pm 5 \mu\text{s}$ . The change of the polarization in Figure 3b is related to overlapping of the signals arising from different orientations of RCs relative the magnetic field. The decay of  $^3P$  depends on the field position at which it is measured and at each field position this decay is a combination of various components decaying at different rates.

The appearance of the out-of-phase stimulated echo and its strong modulation (Figure 2b) is readily explained by the theory presented above. The  $Q_A^-$  spin is excited uniformly by the mw pulses. For the  $^3P$  spin the line is much broader than the excitation bandwidth ( $D \gg B_1 \approx 9 \text{ G}$ ). The condition  $k_m\tau \ll 1$  ( $m = 0, \pm 1$ ) is also fulfilled. Indeed,  $^3P$  decays on the time scale of hundreds of  $\mu\text{s}$ , while the variation in  $\tau$  is restricted to several  $\mu\text{s}$  (typical  $T_2$  of  $Q_A^-$ ).  $T_1$  of  $Q_A^-$  at liquid helium



**Figure 3.** (a) Dependence of the out-of-phase echo signal on the delay  $T$  ( $\tau = 176 \text{ ns}$ ), for samples with reduced  $Q_A$ . (b) Decay of the two-pulse echo signal ( $\tau = 136 \text{ ns}$ ) of  $^3P$ , measured near its Y canonical peak (see Figure 1), as a function of delay after flash. Temperature 4.2 K.

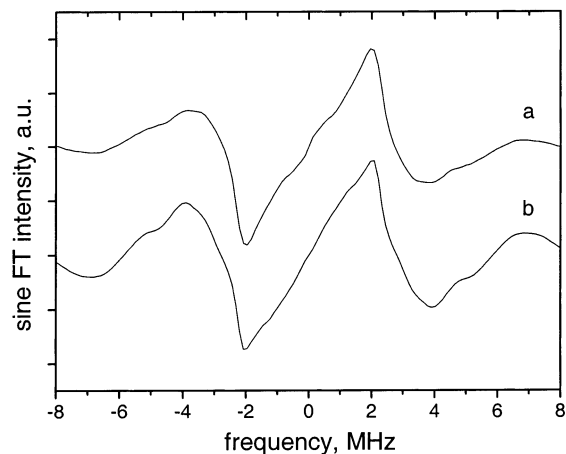
temperature measured by the inversion–recovery experiment is in the millisecond time range (data not shown). Finally, the characteristic rise time of the  $T$  dependence of the out-of-phase stimulated echo signal in the triplet-radical pair ( $65 \pm 5 \mu\text{s}$ ) is close to the characteristic decay time ( $81 \pm 5 \mu\text{s}$ ) of the fast decaying sublevels of the triplet state of  $P$ . The mismatch of the two rates may arise from stimulated echo decay and the orientation dependence of the  $^3P$  lifetime<sup>21,24</sup> and needs further investigation.

The DAF dependence of the fast component of the  $^3P$  echo signal (Figure 3b) was found to be the same in the temperature range between 4.2 and 30 K (data not shown). Since spin–lattice relaxation is expected to be temperature dependent, this allows us to assume that the decay of the  $^3P$  signal in this temperature range is determined mainly by the triplet lifetime.

The origin of the nonequilibrium polarization of  $Q_A$  is the interplay between the dipolar and exchange interactions in the three-spin system  $P^+\Phi_A^-Q_A^-$ .<sup>17,22,25</sup> When  $P^+\Phi_A^-$  undergoes charge recombination to form  $^3PQ_A^-$ , the spin sublevels separate in energy and no significant change in the polarization of  $Q_A^-$  is expected during the lifetime of  $^3P$ .<sup>17,22</sup>

Our assignment of the signal is additionally supported by the fact that the  $\tau$  dependence of the out-of-phase stimulated echo signal in the spin-correlated triplet-radical pair (Figure 2b) is very similar to that of the two-pulse echo in the spin-correlated





**Figure 4.** Sine Fourier transformation of the out-of-phase time-domain ESE traces (see Figure 2b,c) obtained for the spin-correlated triplet-radical pair  ${}^3\text{PQ}_A^-$  (a) and the radical pair  $\text{P}^+\text{Q}_A^-$  (b).

$\text{P}^+\text{Q}_A^-$  radical pair (Figure 2c). This result is not surprising, since it is known that under the conditions of our experiment, the triplet state is localized on the same molecule as  $\text{P}^+$ . Similar results on the interactions in the  ${}^3\text{PQ}_A^-$  pair were obtained by de Groot et al.<sup>26</sup> and Bosch et al.<sup>27</sup> with continuous wave (cw) EPR.

Figure 4 shows the result of sine Fourier transformations (FT) of the out-of-phase time-traces presented in Figure 2b,c. Prior to FT the signals were reconstructed within the dead time using parabolic extrapolation to zero.<sup>23</sup> Sine FT allows one to evaluate dipole–dipole interaction for the triplet radical ( ${}^3\text{PQ}_A^-$ ) and the radical ( $\text{P}^+\text{Q}_A^-$ ) pairs. We estimated the dipole–dipole interaction as  $D = -3.1 \pm 0.2$  MHz for both pairs. From the point dipole model, the dipole coupling derived from echo modulation corresponds to a distance of  $29.3 \pm 0.6$  Å, which is in agreement with the previously determined values for the  $\text{P}^+\text{Q}_A^-$  pairs ( $28.9 \pm 0.4$  Å<sup>23</sup>).

An interesting feature of the described experiment is that the modulation of the  $\text{Q}_A^-$  echo signal is induced by  ${}^3\text{P}$  but this modulation appears *after*  ${}^3\text{P}$  has decayed. In fact, the phase of the  $\text{Q}_A^-$  spin bears the memory of the dipolar interaction with the  ${}^3\text{P}$  spin, which is manifested in different precession frequencies of the  $\text{Q}_A^-$  spin during the first and the last  $\tau$  intervals of the stimulated echo pulse sequence. Finally, this leads to the modulation of the stimulated echo.

The out-of-phase echo signal of the spin-correlated triplet-radical pair  ${}^3\text{PQ}_A^-$  appears only if spin polarization of  $\text{Q}_A^-$  is nonzero and different in the  $\text{T}_{+1}$  and  $\text{T}_{-1}$  subensembles. The appearance of the out-of-phase echo in this pair indicates that the presence of chemically pre-reduced  $\text{Q}_A^-$  results in the deviation in population of the triplet states of primary donor from pure  $\text{T}_0$  population. This is the first direct evidence that  $\text{T}_{+1}$  and  $\text{T}_{-1}$  sublevels of  ${}^3\text{P}$  become differently populated after recombination of the primary radical pair [ $\text{P}^+\Phi_A^-$ ]. In refs 17 and 22 it was shown that spin dynamics of the three-spin system [ $\text{P}^+\Phi_A^-$ ] $\text{Q}_A^-$  may result in a nonzero and different population of the  ${}^3\text{P}$  outer spin sublevels. This deviation is not large enough to induce detectable changes in the  ${}^3\text{P}$  spectrum at the X-band but is sufficient to produce the effect described in this work.

## Conclusions

The described method potentially has a wide field of application. It may be applied to any system consisting of slowly relaxing spin A ( $\text{Q}_A^-$ , nitroxide spin label, other  $S = 1/2$  free radicals), coupled to a rapidly decaying (and/or relaxing)

paramagnetic partner (molecular triplet, photoinduced RP). Also, it allows studies of chemical reactions in solids with the help of an additional paramagnetic “observer”.

The method does not require complete excitation of the EPR line. This opens a perspective of its application in high-field EPR, where complete mw excitation is generally unavailable. It may lead to a new direction in the study of weakly interacting pairs for which one partner has a broad EPR spectrum.

Another advantage is the simplicity of the pulse sequence and its transparent interpretation in terms of the spin A truncated Hamiltonian.

An important property of the out-of-phase signal is that it is not affected by stable paramagnetic impurities in thermodynamic equilibrium. In addition, nuclear ESEEM is greatly suppressed in the out-of-phase echo as compared with the in-phase one. The extrapolation of the out-of-phase echo signal to  $\tau = 0$  is trivial and diminishes the dead time problem.

Modulation of the out-of-phase component of the stimulated echo reflects the magnetic interactions in the triplet-radical pair. Its analysis allows one to determine dipolar frequencies and the interspin distance in the pair. We obtained the same interspin distance for triplet-radical pair  ${}^3\text{PQ}_A^-$  as was previously found for the  $\text{P}^+\text{Q}_A^-$  radical pair in bacterial RC.

A more general treatment of the observed phenomenon, including spin B longitudinal relaxation, spin A nuclear ESEEM, and possible application of selective excitation and subsequent free induction decay (FID) detection is currently in progress.

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