Selective excitation in pulsed EPR of a spin-correlated triplet-radical pair

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Abstract

Experiments are described in which a low-amplitude microwave pulse excites only one out of three allowed transitions of the quinone radical (Q_A^-) in a spin-correlated triplet-radical pair $^3PQ_A^-$ of the bacterial photosynthetic reaction center. A second high-amplitude pulse produces a FID whose temporal shape is strongly modulated with frequencies determined by electron–electron dipolar interaction in the pair. The FID is detected in both the in-phase and the out-of-phase channels. The out-of-phase FID is a result of switching off the magnetic dipolar interaction between ^3P and Q_A^- due to decay of ^3P during the time interval between the two pulses. Refocusing of FID by an additional non-selective pulse allows a dead-time free measurement of this modulation. The influence of the dead-time problem on the distance determination is discussed.
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1. Introduction

Out-of-phase electron spin echo (ESE) spectroscopy [1–3] has become an efficient tool in investigation of spin-correlated radical pairs. The echo signal is modulated with a dipolar frequency (ESE envelope modulation, or ESEEM). Its Fourier transform gives a Pake doublet, which provides an important structural information on the photosynthetic reaction centers [4,5].

Selective microwave excitation in many cases is a useful complement to the common ESE experiment. A pulse sequence for this experiment normally consists of a weak and long selective pulse that burns a narrow hole in the inhomogeneously broadened EPR spectrum. Then a short and strong pulse is applied which non-selectively excites the EPR spectrum and creates a free induction decay (FID) signal [6,7]. The FID for systems

in thermal equilibrium appears in the in-phase channel. Its temporal shape reflects the interaction of the excited spin with neighboring nuclei [6,7] or electrons [8], which produces modulation and/or additional decay of FID. Detection of FID modulation has an obvious advantage over common ESEEM because FID may be obtained using a single shot. This often results in an increase of the signal-to-noise ratio.

When selective excitation is applied to a spin-correlated radical pair (SCRP) [9], an out-of-phase FID appears which is modulated with the electron–electron dipolar frequency. From this frequency the distance between paramagnetic partners in the pair may be calculated. The decay of the signal with variation of the interpulse delay gives an insight into the mechanism of spin relaxation. These studies were done on the $P^+Q_A^-$ pair in the bacterial reaction center (RCs) [9] and on the $P^+A_1^-$ pair in the photosystem I [10] (P is the primary electron donor, Q_A and A_1 are the quinone electron acceptors).

Recently, a new mechanism of the ESE formation in photosynthetic triplet-radical pairs (TRP) $^3PQ_A^-$ was discovered. In a three-pulse stimulated ESE sequence

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the out-of-phase echo for a ^{3}PQ$_A^-$ pair in bacterial RCs appears [11], as a result of switching off the magnetic dipolar interaction with ^{3}P due to its decay during the interval between the second and the third microwave pulses. ESE of the remaining Q$_A^-$ is modulated with dipolar frequency when the time interval τ between the first and the second pulse is varied.

In the present work the selective excitation experiment [9] is applied to the ^{3}PQ$_A^-$ TRP. A strongly modulated FID of Q$_A^-$ was found in both the in-phase and the out-of-phase channels. Theoretical description of this phenomenon is presented. It is shown also that FID can be refocused by an additional non-selective π-pulse that allows a dead-time free measurement of the FID shape.

2. Theory

The spin-Hamiltonian of the TRP in the rotating frame may be written as

$$H = (\omega_A - \Omega)S_{Az} + \omega_d S_{Az}S_{Bz} + H_B, \qquad (1)$$

where $\boldsymbol{S_A}$ is the spin A operator (radical, Q$_A^-$), ω_A is the Larmor frequency of the spin A, Ω is the microwave frequency, $\omega_d = \gamma^2\hbar/r^3(1 - 3\cos^2\theta)$ is the strength of triplet-radical dipolar interaction in the frequency units (γ is the electron gyromagnetic ratio, r is the distance between spins A and B in the point dipole approximation, θ is the angle between the interspin vector and the direction of the external magnetic field $\mathbf{B}_0$ (applied along the Z-axis of the laboratory frame), H_B is the spin-Hamiltonian for spin B (triplet, ^{3}P) that includes the Zeeman and the zero-field splitting (ZFS) interactions).

If the condition $|D|, |E| \gg \omega_d$ holds (D and E are the ZFS parameters), then EPR transitions of the triplet spin and of the radical spin are well separated in energy, except for a negligible minority of triplet orientations (the relative fraction of these orientations may be estimated as $2\omega_d/|D| \sim 0.005$). In our case the high-field approximation is valid for both spins ($\gamma B_0 \gg |D|, |E|$). So the eigenstates of the Hamiltonian (Eq. (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, T_{-1} are the high field eigenstates of ^{3}P and α, β refer to the spin states of isolated Q$_A^-$. Three spin A subensembles corresponding to different m ($m = -1, 0, 1$) may be considered. In our case the exact form of H_B is not important and we may use a truncated spin A Hamiltonian for the mth subensemble:

$$H_A = (\omega_A - \Omega + \omega_d m)S_{Az}. \qquad (2)$$

The initial state of TRP is assumed to be incoherent with populations $p_1, \ldots, p_6$ of the levels $\psi_1, \ldots, \psi_6$. Thus the initial state of the spin A may be described by the density matrix $\rho_m^i = n_m S_{Az}$, where n_m is the spin A polari-

zation in the mth subensemble ($n_1 = p_1 - p_2$, $n_0 = p_3 - p_4$, $n_{-1} = p_5 - p_6$). The non-equilibrium polarization of Q$_A^-$ is created during the photochemical reactions in the RC triggered by the laser flash [12,13]. We assume that the delay after flash (DAF) is sufficiently long that all coherences generated by the laser pulse decay before the first microwave pulse is applied.

Consider the evolution of the photogenerated TRP under the action of the two-pulse sequence, $\pi_s - T - (\pi/2)_{ns} - \text{FID}(t)$, where the subscripts "s" and "ns" denote selective and non-selective pulse, respectively (Fig. 1). Microwave pulses do not affect the triplet because of its large spectral width ($|D|, |E| \gg \gamma B_1$, where B_1 is the pulse amplitude). Let us assume that the non-selective pulse excites the whole EPR line of the radical and the selective pulse excites only one particular mth transition and does not affect the two others. We will consider only the evolution of those spins A that are excited by the selective pulse. For other spins A the FID decays during the spectrometer dead time.

Calculation of the FID temporal shape may be performed in a similar way as it was done in [11] for stimulated ESE. Decay of the triplet during the selective pulse action is assumed to be negligible. After the action of the selective π-pulse the spin A state becomes:

$$\rho_m(0) = -n_m S_{Az} \qquad (3)$$

(the zero time corresponds to the end of the selective pulse). Note that for any particular TRP the transition is excited, for which the resonance condition, $\omega_A =$

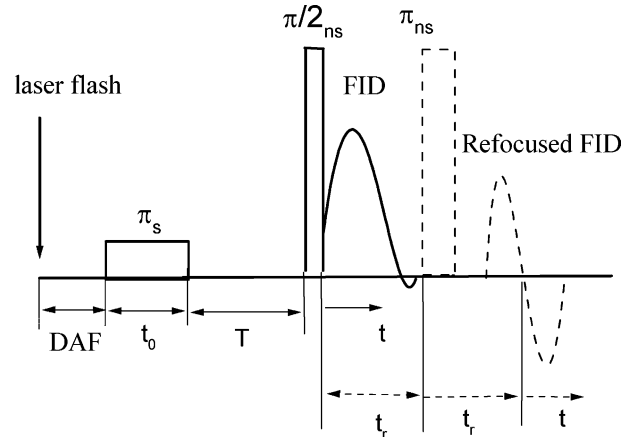Fig. 1. Pulse scheme of the experiment. The first pulse is a π low-amplitude pulse. It selectively excites only one particular transition of the radical in the triplet-radical pair, burning a narrow hole in the radical EPR spectrum. The second pulse is a $\pi/2$ non-selective high-amplitude pulse. It converts the hole into a long-lasting FID signal. For the spin-correlated TRP the hole may be shifted because of the switching off the magnetic dipolar interaction with the triplet, due to its decay between two pulses. This will result in a modulation of the FID temporal shape. The third π non-selective pulse may be applied to refocus the FID obscured within the dead time (dashed lines).

$\Omega - m\omega_d$, is held. After the action of the selective pulse a narrow hole is burned in the EPR spectrum.

During the interpulse delay T spin A is frozen along the Z-axis of the rotating frame. However, the triplet decays with the probability of $1 - \exp(-k_m T)$, where k_m is the decay rate for the mth sublevel. We assume here that $T_{1B} \gg T$, where T_{1B} is the triplet longitudinal relaxation time.

Immediately after the non-selective $\pi/2$-pulse of negligibly small duration the spin A state becomes

$$\rho_m(T) = -n_m S_{Ay}. \tag{4}$$

Each spin A subensemble is divided into two fractions, depending on whether the triplet has decayed during the T-period or not. The decay of the triplet switches off the triplet-radical dipolar interaction, which shifts the hole in the radical EPR spectrum. The coherency corresponding to the "non-decayed" fraction precesses with the same frequency while that corresponding to "decayed" fraction precesses with the frequency shifted by $m\omega_d$. Thus, at the moment t after the non-selective $\pi/2$-pulse:

$$\begin{aligned}
\rho_m(T + t) = &-n_m(\exp(-k_m T)S_{Ay} \\
&+ (1 - \exp(-k_m T))(\cos(m\omega_d t)S_{Ay} \\
&- \sin(m\omega_d t)S_{Ax})).
\end{aligned} \tag{5}$$

The mth subensemble contribution to FID is

$$\begin{aligned}
F_{y,m}(t) &= \mathrm{Tr}(S_{Ay}\rho_m(T + t)) \\
&= -n_m(\exp(-k_m T) + (1 - \exp(-k_m T)) \\
&\quad \times \cos(m\omega_d t))
\end{aligned} \tag{6}$$

for the in-phase channel and

$$\begin{aligned}
F_{x,m}(t) &= \mathrm{Tr}(S_{Ax}\rho_m(T + t)) \\
&= -n_m(1 - \exp(-k_m T))\sin(m\omega_d t)
\end{aligned} \tag{7}$$

for the out-of-phase channel.

We consider that all three spin A transitions corresponding to different m (belonging to different TRPs) are excited with equal probability due to the large inhomogeneous EPR linewidth, ΔB, of the radical ($\gamma \Delta B \gg \omega_d$). Taking into account that for high magnetic fields $k_m = k_{-m}$ [14], the summation over m gives the total FID intensities for the in-phase and the out-of-phase channels, respectively:

$$\begin{aligned}
F_y(t) &= \sum_{m=-1}^{1} F_{y,m}(t) \\
&= -n_0 - (n_1 + n_{-1})\exp(-k_1 T) - (n_1 + n_{-1})(1 \\
&\quad - \exp(-k_1 T))\cos(\omega_d t),
\end{aligned} \tag{8}$$

$$\begin{aligned}
F_x(t) &= \sum_{m=-1}^{1} F_{x,m}(t) \\
&= (n_1 - n_{-1})(1 - \exp(-k_1 T))\sin(\omega_d t).
\end{aligned} \tag{9}$$

Eqs. (8) and (9) predict that FID temporal shape must be similar to the τ-dependence of stimulated ESE in TRP (see Eqs. (9) and (10) in [11]).

Here we did not take into account the electron–nuclear modulation of FID [6]. For a SCRP it was found to be negligible [3,15]. Influence of a spin A relaxation is also neglected.

An additional non-selective short π-pulse applied at the time moment t_r after the second pulse reverses the coherence precession (Fig. 1, dashed lines). If we assume that triplet does not decay during the refocusing ($k_m t_r \ll 1$) the system may be treated as a two-level system. Then it is easy to calculate that

$$\rho_m(T + 2t_r + t) = -\rho_m(T + t), \tag{10}$$

which means that FID is refocused. The intensities of the refocused FID are

$$R_y(t) = Tr(S_{Ay} \sum_{m=-1}^{1} \rho_m(T + 2t_r + t)) = -F_y(t) \tag{11}$$

for the in-phase channel, and

$$R_x(t) = Tr(S_{Ax} \sum_{m=-1}^{1} \rho_m(T + 2t_r + t)) = -F_x(t) \tag{12}$$

for the out-of-phase channel, where $F_y(t)$ and $F_x(t)$ are given by Eqs. (8) and (9).

Note that the complete FID refocusing is possible because the non-selective pulses do not excite the broad ^3P EPR line and therefore do not change the spin interactions in the pair. This is particularly true for the out-of-phase component of the signal, which is created by those TRPs where triplet actually decays before the FID refocusing.

Eqs. (11) and (12) are valid for $t > -t_r$. As $F_y(t)$ is an even function, the in-phase refocused FID is two "normal" FIDs back-to-back. $F_x(t)$ is an odd function, so the out-of-phase refocused FID is two "normal" FIDs, inverted with respect to the coordinate origin ($t = 0$).

In this three-pulse sequence other unwanted contributions to the signal may arise. First, there is a FID after the last non-selective pulse that appears due to imperfect setting of its rotation angle to the nominal π value. This contribution may be suppressed by the phase cycling for which the results of experiments with this pulse taken at two opposite phases must be added. Second, there is a primary echo generated by the non-selective $\pi/2$ and π pulses at the time $t = 0$. It may be removed by subtraction of the results for the same experiment with the selective pulse switched off.

Due to the decay of the spin A coherence with the phase memory time T_{2A} the refocused FID is weaker than "normal" FID by the factor of $\exp(-2t_r/T_{2A})$, that restricts the range of the acceptable t_r values.

3. Experiment

The samples of bacterial photosynthetic RCs of *Rhodobacter (Rb.) sphaeroides* R26 with prereduced Q_A were the same as described in [11]. The paramagnetic Fe^{2+} ion was replaced by diamagnetic Zn^{2+} (Zn-RCs samples) [16] to avoid fast relaxation induced by the iron ion. The samples contained 60–70% (v/v) glycerol and were placed in quartz tubes with inner diameter 3 mm. Final optical density of the samples in the primary donor absorption band was ca. 15–20 cm^{-1}.

Pulsed EPR experiments 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. As the ESE signal of Q_A^- in the dark is expected to be in-phase, the phase of the microwave pulses was adjusted employing this signal.

The duration of the selective pulse t_0 was 2 μs in a two-pulse experiment and 1 μs in a three-pulse FID refocusing experiment. The amplitude of these pulses was adjusted to obtain a maximal FID intensity without affecting its temporal shape (making so the magnetization flip angle close to π). The duration of the non-se-

lective π/2 and π pulses was 12 and 24 ns, respectively. These pulses were of equal intensity, their amplitude was adjusted to obtain a maximal amplitude of a two-pulse echo from the Q_A^- in the dark (so B_1 is around 7 G). The dead time caused by the resonator ringing was 100 ns. The constant magnetic field B_0 was set to the maximum of the Q_A^- dark EPR line. The FID temporal shapes were recorded on a built-in SpecJet oscilloscope with quadrature detection and with a 4 ns time step.

As a light source for sample irradiation inside the ESE cavity we used a Continuum Surelite I laser. The excitation wavelength was 532 nm. The repetition rate of the laser flashes of ca. 4 ns duration was 10 Hz. The microwave pulses were delayed after the laser flash by 1 μs. The temperature was 4.2 K in all experiments.

4. Results and discussion

Fig. 2 shows the FID temporal shape obtained in the out-of-phase (solid line) and in the in-phase (dashed line) channels at $T = 10$ μs. The in-phase signal is strongly influenced by electron–nuclear modulation
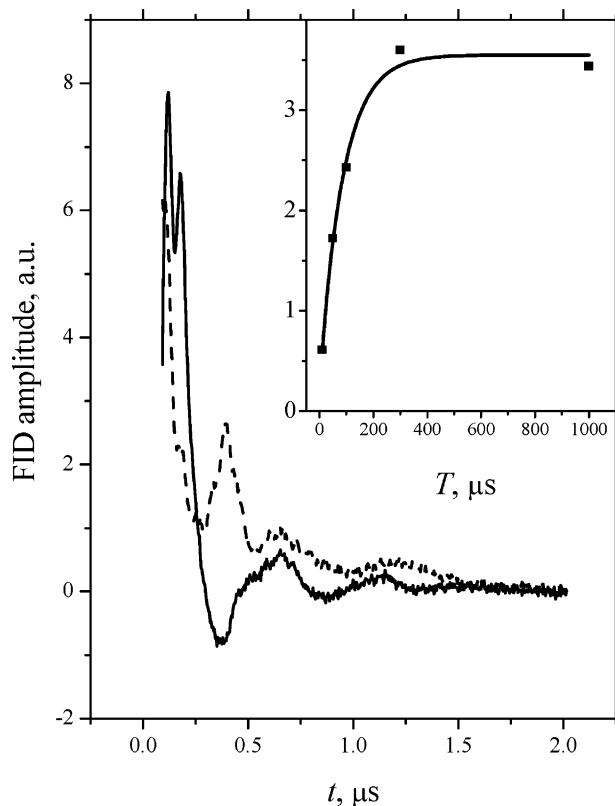

Fig. 2. Out-of-phase (solid line) and in-phase (dashed line) components of the FID appearing after second non-selective pulse (see Fig. 1). The interpulse delay T is 10 μs, the selective pulse duration t_0 is 2 μs. Insert: T-dependence of the FID, taken at $t = 200$ ns, fitted by a single exponential. Temperature is 4.2 K, number of accumulations is 5120.
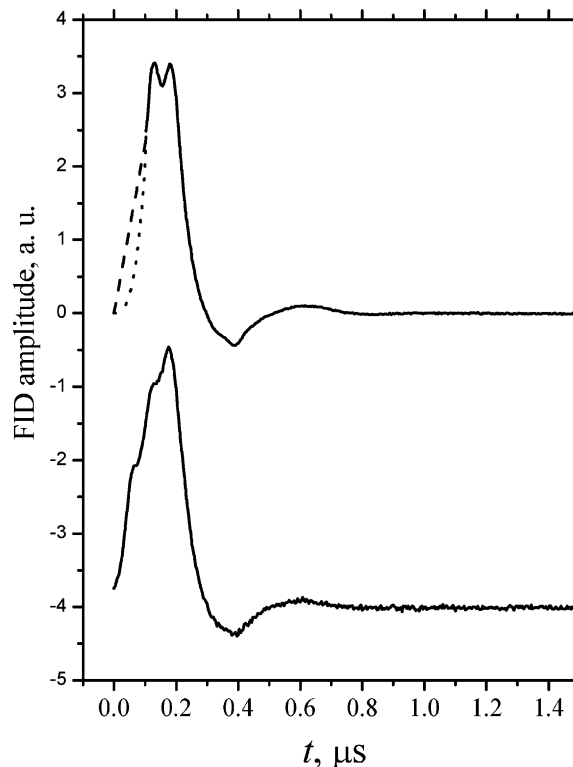

Fig. 3. The upper trace: out-of-phase FID after the second pulse, for $T = 300$ μs, $t_0 = 1$ μs. Number of accumulations is 200. The dashed and dotted lines present a linear and a cubic interpolation, respectively, within the dead time. The lower trace: the right-side part of refocused out-of-phase FID after the third pulse (see Fig. 1), with the same T and t_0, and $t_r = 1$ μs. Number of accumulations is 2000. The lower trace is shifted downward to avoid superposition with the upper one. The signals are scaled to the same amplitude. Temperature is 4.2 K.

induced by the interaction with neighboring protons and nitrogens. For this reason it is not further analyzed. The contribution of the nuclear modulation in the out-of-phase signals is known to be greatly suppressed [3,15]. The out-of-phase FID component is very similar to that obtained in the stimulated echo experiment on the same $^3PQ_A^-$ TRP (see Fig. 2 in [11]). Both these curves are in turn similar with well known two-pulse out-of-phase ESEEM for $P^+Q_A^-$ radical pair [3,4,15].

The insert in Fig. 2 shows the *T*-dependence of the out-of-phase FID for $t = 200$ ns. Its approximation by a simple exponential gives the characteristic rise-time of $87\,\mu s$. This value is close to the inverse mean rate of the physical decay of the triplet, $120\,\mu s$ [17]. This confirms that out-of-phase FID is created by the proposed mechanism. A small difference between these time constants may be explained by a distribution of triplet decay rates due to their orientation dependence. Another possible source of the discrepancy may be a decrease of the FID intensity due to longitudinal relaxation of the Q_A^- spin and its spectral diffusion, which occurs on the millisecond timescale at helium temperature [9].

The upper trace in Fig. 3 shows the out-of-phase FID obtained in the same experiment, with $T = 300\,\mu s$ and $t_0 = 1\,\mu s$. This value of *T* corresponds to the maximal FID intensity (see insert in Fig. 2). This large value of *T* in turn leads to FID damping (cf. Fig. 2) that may be explained by spectral diffusion during the *T*-period. Indeed, spectral diffusion broadens the hole burned by the selective pulse.

The lower trace in Fig. 3 presents the right-side part $(t > 0)$ of the refocused out-of-phase FID obtained in the three-pulse experiment (see Fig. 1). The *T* and t_0 values are the same as those for upper trace ("normal" FID), and $t_r = 1\,\mu s$. The number of averaged scans for refocused FID in Fig. 3 is 10 times larger than for "normal" FID. The signals are scaled to the same amplitude. In spite for the much longer acquisition time, the signal-to-noise ratio for the refocused FID is lower than that for the "normal" one. This may be explained by influence of phase relaxation during refocusing.

For $t < 100$ ns the primary FID is obscured by dead time and only the refocused FID is detected. For $t > 100$ ns the temporal shape for both FIDs is similar, except for some minor difference at the beginning. As Eq. (9) predicts that at $t = 0$ signal is equal to zero, one may use an interpolation within the dead time [3]. The dashed and dotted lines at the upper trace of Fig. 3 ("normal" FID) present simple linear and cubic (signal $\propto t^3$) signal interpolations. Fig. 4 presents a sine Fourier transform of three types of FIDs shown in Fig. 3. The result is the well known in out-of-phase spectroscopy Pake doublet, antisymmetric due to spin polarization.

One can see that difference between three lineshapes in Fig. 4 is not large. This result has a simple mathe-
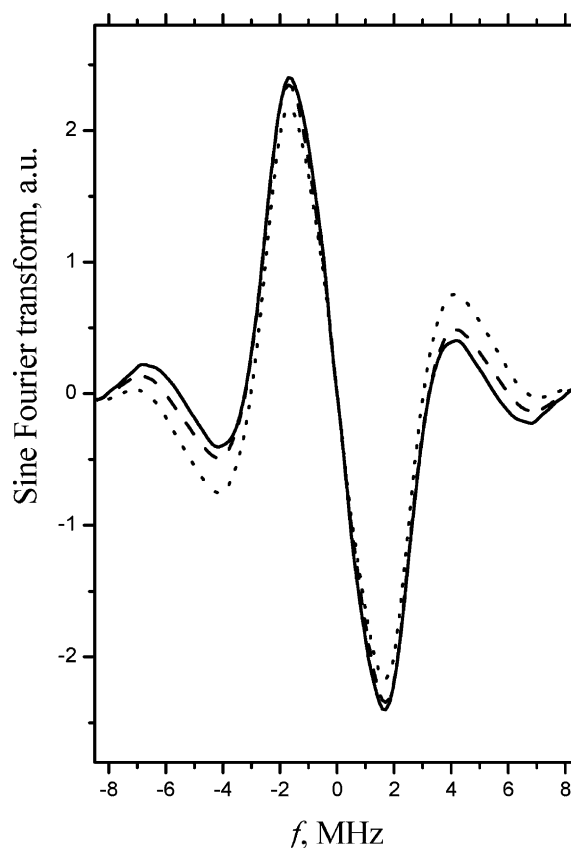

Fig. 4. Sine Fourier transform of the time traces shown in Fig. 3. The curve designation corresponds to that given in Fig. 3 within the dead time ($t < 100$ ns).

matical ground: minor differences at short times in the time domain may influence only at large frequencies in the frequency domain. We may conclude that the simple interpolation within the dead time that is normally used in out-of-phase ESE spectroscopy [3,4] provides a good result.

Some difference near $t \sim 100$ ns between two time traces seen in Fig. 3 probably is related with the non-zero pulse duration which may smooth down the oscillations in the time domain (the more pulses, the smoother oscillations).

5. Summary

The selective hole burning experiment carried out on the $^3PQ_A^-$ triplet-radical pair in the bacterial photosynthetic reaction center reveals a FID signal after applying an additional non-selective pulse. This FID is detected in both the in-phase and the out-of-phase microwave channels. The out-of-phase FID appears as a result of the switching off the magnetic dipolar interaction with ^3P, due to its decay between the two pulses. Its temporal shape is determined by dipolar modulation and is similar to that recently obtained using an out-of-phase

stimulated ESEEM [11]. Detection of FID modulation therefore provides an alternative way to perform investigation of dipolar coupling in the TRP. Also, spectral diffusion in EPR of TRP may be directly studied. FID may be recorded at a single shot, which may be useful in some applications. Refocusing of FID by an additional non-selective pulse allows a dead-time free measurement of dipolar modulation. The results of this work confirms the validity of the previously employed approach in which signal is restored within the dead time using a simple interpolation.

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