Applied Magnetic Resonance
Nuclear Coherences in Photosynthetic Reaction Centers

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Abstract

Transient electron paramagnetic resonance is used to study the secondary radical pair in plant photosystem I. Nuclear coherences are observed in the transverse magnetization at lower temperatures following light excitation. Comparative studies of deuterated and deuterated ^{15}N -substituted cyanobacteria *S. lividus* indicate assignment of these coherences to nitrogen nuclei in the primary donor and deuterons in the secondary acceptor. The modulation amplitude of a deuteron matrix line, as a function of the microwave power, reveals a distinct resonance behavior. The maximum amplitude is obtained when the Rabi frequency equals the nuclear Zeeman frequency.

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1. Introduction

Charge separation in plant photosystem I (PSI) is initiated by photoexcitation of the primary chlorophyll donor P_{700} [1]. An electron is transferred from the excited singlet state of P_{700} to a quinone acceptor, A_1 [2], through an intervening chlorophyll acceptor, A_0 . At room temperature the radical pair state $P_{700}^+A_1^-$ decays in about 200 ns by electron transfer to the next acceptor, an iron-sulfur center (FeS) [3, 4]:

At low temperatures, the lifetime of $P_{700}^+A_1^-$ is about 150 μs , and it decays primarily by charge recombination [1].

Although crystals of the PSI reaction center complex have been obtained, its X-ray structure is not known with great detail [5]. Thus, a number of studies have been directed at modeling the polarized electron paramagnetic resonance (EPR) spectra of the correlated radical pair, $P_{700}^+A_1^-$, in order to obtain structural information [6, 7]. Recently, we have presented a high time resolution transient EPR study of $P_{700}^+A_1^-$ formed by photoinduced charge separation at room temperature [8]. The time evolution of the transverse magnetization was evaluated for various static magnetic fields. Manifestations of electron zero quantum coherence were observed at early times after laser pulse excitation. Analyzing the fast initial oscillations we have been able to propose the geometry of $P_{700}^+A_1^-$ in the charge separated state [9].

Here we describe an additional coherence phenomenon [10]. At low temperatures coherent oscillations of lower frequency are observed. By comparing the EPR signals from deuterated (^{14}N) and deuterated ^{15}N -enriched cyanobacteria we have identified these oscillations as nuclear coherences [10]. Model calculations, based on a density matrix approach, support this interpretation [10], which may also apply to recent observations in bacterial reaction centers [11, 12]. In the following we present additional experimental data for this new coherence phenomenon. The results are discussed in the context of other coherence phenomena in EPR such as electron spin echo envelope modulation (ESEEM) and coherent Raman beats (CRBs).

2. Materials and Methods

Freeze-dried, whole cells of deuterated (99.7 %) and deuterated ^{15}N -substituted (95 %) *S. lividus* cyanobacteria were suspended in deuterated Tris buffer (pH = 7.5). About 5 μl of the sample was used to fill a quartz tube (2 mm inner diameter) located in the symmetry axis of the microwave resonator. The temperature of the samples was controlled by using a helium flow cryostat (Oxford model CF-935) and was stable to ± 0.1 K.

The basic concept of the EPR experiment is similar to that described previously [13]. A modified X-band spectrometer (Bruker, model ER-200 D) was used, equipped with a fast microwave preamplifier (30 dB; 1.8 dB noise figure) and a broad-band video amplifier (band width 200 Hz to 200 MHz). The sample was irradiated in a home-built split-ring resonator with 2.5 ns pulses of a Nd:YAG laser (Spectra Physics, model GCR-16S, 532 nm, 5 mJ per

pulse) at a repetition rate of 10 Hz. The split-ring resonator exhibits a high filling factor at low Q (unloaded $Q \approx 500$) and provides an easy means of sample irradiation.

The time-dependent EPR signal was digitized in a transient recorder (LeCroy, model 9450 digital oscilloscope) at a rate of 2.5 ns per 12-bit sample. The time resolution of the experimental setup is in the 10 ns range. Typically, 512 transients were accumulated at off-resonance conditions and subtracted from those on resonance to get rid of the laser background signal.

3. Results

In our EPR experiments, the sample is irradiated with a short laser pulse and the magnetization detected in the presence of a weak microwave magnetic field. Generally, a complete data set consists of transient EPR signals taken at equidistant magnetic field points covering the total spectral width. This yields a two-dimensional variation of the signal intensity with respect to both the magnetic field and time axis. Transient spectra can be extracted from such a plot at any fixed time after the laser pulse as slices parallel to the magnetic field axis. Likewise, the time evolution of the transverse magnetization may be obtained for any given field as a slice along the time axis.

Transient spectra, observed 200 ns after the laser pulse for deuterated (full line) and deuterated ^{15}N -substituted *S. lividus* (dotted line), are shown in Fig. 1. The spectra were obtained with a microwave frequency of $\omega/2\pi = 9.7896$ GHz (X-band), a microwave field of $B_1 = 0.062$ mT, and $T = 70$ K. Note that a positive signal indicates absorptive (a) and a negative emis-

sive (e) spin polarization. Apparently, the two lineshapes are scarcely distinguishable within the experimental error.

In Fig. 2 (left column) we compare the time evolution of the transverse magnetization for deuterated (full lines) and deuterated ^{15}N -substituted PSI preparations (dotted lines), measured at six different magnetic fields (A-G in Fig. 1). All time profiles refer to the same microwave field of $B_1 = 0.026$ mT. In all cases, slow oscillations with frequencies of 1-2 MHz can be seen. In addition, fast oscillations with frequencies ≥ 10 MHz are observed. Basically, the fast oscillations represent zero quantum precessions of the electrons, associated with the spin-correlated generation of the radical pair in a singlet state [9]. Because of hyperfine interactions and transverse spin relaxation, rapid averaging of these oscillations occurs. Consequently, manifestation of zero quantum coherence is restricted to single sharp peaks observed immediately after the laser pulse.

As noted previously [10], the slow persisting oscillations cannot all be assigned to Torrey precessions with a frequency of

$$\nu_1 = \frac{1}{2} (g_1 + g_2) \beta B_1 / \hbar,$$

where g_i , β and B_1 are the isotropic g-factor of radical i, the Bohr magneton and the microwave field, respectively. Rather, there are additional modulations of the transverse magnetization. This is clearly seen in the power spectra (right column), obtained by Fourier transformation of the time profiles. Only the peak marked with an arrow indicates Torrey oscillations. The remaining coherences exhibit frequencies between 0.2 and 2.5 MHz. In principle, frequencies in this range are possible for zero quantum precessions. However, the extremely slow damping of this signal modulation (see left column) contradicts the fast

damping of the electron zero quantum precessions. We therefore assign the slow oscillations to nuclear coherences, initiated by the laser pulse.

Notice that the nuclear modulation pattern varies across the powder lineshape, as expected for a weakly coupled pair of different radicals. Interestingly, the same pattern is observed at the field positions A-C for both the deuterated ^{14}N and ^{15}N sample. Thus, nitrogens are not the source of modulation at these field positions. In contrast, at the positions D-G, the observed time profiles and power spectra are different for the different samples. The change in the modulation pattern indicates that the modulations are due to the nitrogen nuclei, ^{14}N and ^{15}N , respectively.

Fig. 3 depicts experimental time profiles (left column) and corresponding power spectra (right column), evaluated at position A of the transient spectrum. They refer to the deuterated ^{15}N sample and five different microwave magnetic fields. Note the intense peak at 2.25 MHz (shaded area), which corresponds closely to the free deuteron (^2H) precession frequency,

$$\nu_D = g_D \beta_N B_0 / h,$$

in the static magnetic field of $B_0 = 348.6$ mT, thus indicating a matrix ENDOR line. Here g_D and β_N denote the ^2H g-factor and the nuclear magneton, respectively.

Let us now examine the B_1 -dependence of the peak intensities. One sees that the Torrey peak (arrows) broadens with increasing B_1 and for $B_1 \geq 0.060$ mT disappears in the base line. In contrast, the intensity of the ^2H matrix line, first increases and then decreases with growing B_1 . Empirically, the peak intensity can be fitted to a Gaussian

$$I(\nu_1) = N \exp \left[-(\nu_D - \nu_1)^2 / 2\Delta^2 \right]$$

with a line-width parameter of $\Delta = 0.5$ MHz. Thus, the modulation amplitude reveals

a distinct resonance behavior. The maximum value of $I(\nu_1)$ is obtained when the Rabi frequency ν_1 matches the nuclear Zeeman frequency ν_D ,

$$\nu_1 \approx \nu_D.$$

Similar behavior has been observed in CRB EPR of a $S = 1/2$ spin system [14].

Fig. 4 depicts time profiles (left column) and corresponding power spectra (right column), evaluated at the high field emissive part of the spectrum (position F in Fig. 1). The data refer to the deuterated ^{15}N sample and four different microwave magnetic fields. From the power spectra we determine modulation frequencies of 1.8, 2.2 and 2.6 MHz. These values are in line with values assigned to ^{15}N ENDOR frequencies in PSI preparations [15, 16]. Up to now, however, the ^{15}N hyperfine parameters of P_{700}^+ are not known with any great detail. In particular, the total number of ^{15}N nuclei involved in coupling is still open to discussion. Additional studies of light initiated nuclear coherences may provide the desired information.

4. Discussion

Nuclear modulations have been detected in the transverse magnetization of light-induced radical pairs in photosynthetic reaction centers. This observation is intimately related to other coherence phenomena in EPR such as CRBs [17, 18] and ESEEM [19, 20]. Note, however, that in the present case, nuclear spin coherence is initiated simply by a short laser pulse and does not require preparation by pulsed microwaves. This avoids artifacts introduced by the microwave pulses. Moreover, it greatly enhances the detection sensitivity due to optical spin polarization.

Light initiation of nuclear coherences can be understood in terms of the non-adiabatic change of the spin Hamiltonian at the instant of the laser pulse [21]. At time zero the nuclei suddenly experience a local magnetic field due to the electron spins in addition to the constant external field. As a consequence, the quantization axis of the nuclei changes abruptly to the new effective field. Thus, the light pulse initiates a coherent time evolution of the nuclear spin ensemble governed by the new effective field. This nuclear coherence is then transferred to observable electron coherence by means of the continuous microwave magnetic field, similar to the CRB technique.

Because of the longer decay times, CRB detection is distinguished by a higher spectral resolution. Moreover, the complete time evolution of the nuclear coherence is obtained in a single-shot experiment, in contrast to standard ESEEM schemes, where acquisition of hundreds of time sequenced echoes is required. Up to now, however, the coherence transfer, effected by the continuous microwave field, is not completely understood. In fact, different explanations have been given in the literature [17, 22]. Clearly, a comprehensive model also has to account for the observed B_1 -dependence of the modulation amplitude described above. Theoretical studies along these lines, emphasizing light-induced radical pairs, are currently in progress [23].

As noted above, the frequencies of the modulations are equal to differences between nuclear spin levels and thus correspond to ENDOR frequencies. For correlated radical pairs, the ENDOR frequencies of a particular nucleus not only depend on the corresponding hyperfine parameters, but also the spin-spin coupling [24]. Model calculations for $P_{700}^+A_1^-$, based on a density matrix approach [10], indicate that the extraction of hyperfine tensor components

might be possible even in the case of powder samples, provided the spin-spin coupling is known. Thus, thorough investigations of the ^{15}N modulation patterns can ultimately provide detailed information on the electronic structure of the primary donor, which is essential for a better understanding of the primary events of plant photosynthesis. Similarly, the observed ^2H matrix ENDOR line may be used to characterize recently discovered water channels inside the reaction center protein [25].

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Figure Captions

Fig. 1. Transient EPR spectra of the light-induced radical pairs $P_{700}^+A_1^-$ in PSI of deuterated (full line) and deuterated ^{15}N -substituted *S. lividus* (dotted line). The signal intensity was averaged in the time window 200-220 ns after laser excitation. Positive and negative signals indicate absorptive (a) and emissive (e) polarizations, respectively. Microwave frequency: $\omega/2\pi = 9.7896$ GHz. Microwave field: $B_1 = 0.062$ mT. Temperature: $T = 70$ K. A-G indicate field positions discussed in the text.

Fig. 2. Time evolution of the transverse magnetization of the light-induced radical pairs $P_{700}^+A_1^-$ in PSI of deuterated (full lines) and deuterated ^{15}N -substituted *S. lividus* (dotted lines). The transients were taken at six different field positions (A-G in Fig. 1) and refer to a constant microwave magnetic field of $B_1 = 0.026$ mT. Microwave frequency: $\omega/2\pi = 9.7896$ GHz. Temperature: $T = 70$ K. *Left column:* Time profiles. *Right column:* Power spectra. Torrey oscillations are indicated by an arrow.

Fig. 3. Time evolution of the transverse magnetization of the light-induced radical pairs $P_{700}^+A_1^-$ in PSI of deuterated ^{15}N -substituted *S. lividus*. The transients were taken at field position A (see Fig. 1) and refer to five different microwave magnetic fields. Microwave frequency: $\omega/2\pi = 9.7896$ GHz. Temperature: $T = 70$ K. *Left column:* Time profiles. *Right column:* Power spectra. Torrey oscillations are indicated by an arrow. The shaded area

denotes a deuteron matrix ENDOR line.

Fig. 4. Time evolution of the transverse magnetization of the light-induced radical pairs $P_{700}^+A_1^-$ in PSI of deuterated ^{15}N -substituted *S. lividus*. The transients were taken at field position F (see Fig. 1) and refer to four different microwave magnetic fields. Microwave frequency: $\omega/2\pi = 9.7896$ GHz. Temperature: $T = 70$ K. *Left column:* Time profiles. *Right column:* Power spectra. Torrey oscillations are indicated by an arrow.