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Rapid-scan coherence signals in X-band EPR spectra of semiquinones with small hyperfine splittings

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Abstract

Rapid-scan EPR signals for semiquinones with very-small well-resolved hyperfine splittings exhibit coherence signals at a time after passing through the EPR line that is proportional to the reciprocal of the hyperfine splitting. Such coherences are a general phenomenon due to constructive interference of the responses to transient excitation of spins by rapid scan of the magnetic field across equally spaced spin packets. Examples are shown for 2,3,5,6-tetramethoxy-1,4-benzosemiquinone with $a_H = 46$ mG for 12 protons and for 2,5-di-*t*-butyl-1,4-benzosemiquinone with $a_H = 59$ mG for 18 protons.

Introduction

Electron spin resonance under rapid-scan conditions has similarities with pulsed EPR [1]. The transient spin response in a rapid magnetic field scan oscillates analogous to an FID (free induction decay), but the period of the oscillation decreases as the field is scanned away from resonance. Each hyperfine component responds as predicted by the Bloch equation. When there are multiple equally-spaced hyperfine lines, there are coherences in the signal response that produce an echo-like signal. This effect can be understood as follows. Passage of the magnetic field through resonance creates an FID-like signal for each hyperfine line. Because of the spacing a_H between the hyperfine lines, the oscillation

frequencies differ by $f_{diff} = \frac{\gamma a_H}{2\pi}$. Each frequency increases as the field moves further off

resonance. At odd integer multiples of the period $T_{diff} = 1/f_{diff} = \frac{2\pi}{\gamma a_H}$ the oscillations of the individual hyperfine lines overlap constructively and thus build up to the coherence signal. At integral multiples of $T_{diff}/2$ the FIDs destructively interfere and produce no signal. The primary hyperfine coherence signal occurs at time T_{diff} [2]. Larger hyperfine splittings result

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in coherences closer to the main peak, and if the main peak is large enough, may not be resolved. Because the frequency of these hyperfine coherences is much higher than that in the main peaks, the Q of the resonator must be sufficiently low to provide adequate bandwidth for their detection.

The rapid-scan coherence phenomenon for nuclear spins was observed early in the development of NMR [3]. Béné and coworkers observed a 'beat' pattern between the rapid scan oscillations following the NMR signals for two lines with slightly different chemical shifts [4]. Andrew cited those results and also calculated a beat pattern due to rapid scan oscillations on the signals for two samples located at slightly different field strengths [3]. Rapid-scan coherence signals have now been observed in the X-band EPR spectra for 2,3,5,6-tetramethoxy-1,4-benzo-semiquinone (TMBSQ) and 2,5-di-*t*-butyl-1,4-benzo-semiquinone (DTBSQ). This paper is the first report of this coherence phenomena in rapid-scan EPR. Analogous FID coherences have been observed in EPR saturation recovery experiments for semiquinones [2] and nitroxides [5].

Materials and Method

Sample preparation

Semiquinone radicals were generated as in [6]. DTBSQ was prepared by mixing equal volumes of air-saturated solutions of 2,5-di-*t*-butyl hydroquinone (5 mM) and KOH (5 mM) in ethanol. TMBSQ was prepared by mixing 2:1 volume ratio of 25 mM KOH and 5 mM tetrafluoro-1,4-benzoquinone in methanol.

EPR spectra were measured for alcohol solutions of the semiquinones in sample tubes of various diameters and lengths of sample solutions to demonstrate the sensitivity of the rapid-scan coherence to the homogeneity of the scanned magnetic field. When needed to ensure that the resonator Q would not excessively filter the signal response, as for a sample in a 2.9 mm o.d. tube, additional alcohol was added to a surrounding 4 mm o.d. tube to lower the Q .

Oxygen removal

As reported previously [6, 7] high-yield generation of long-lived semiquinones involves attention to details of mixing solutions initially in air, followed promptly by purging with N₂. After mixing the reagents listed above, the vapor space in the tube was purged vigorously with N₂, after which N₂ was gently bubbled into the solution through thin Teflon tubing immersed in the sample. The vapor space above the solutions was then purged

continuously with N₂ during the spectroscopy. Under these conditions, samples were useful for several hours, permitting multiple rapid-scan measurements on the same sample.

EPR spectroscopy

CW spectra were recorded on a Bruker EMX spectrometer. Rapid-scan signals were obtained on a Bruker E500T X-band spectrometer with a Bruker Flexline ER4118X-MD5 dielectric resonator as described in [8]. Rapid magnetic field scans were generated with scan drivers similar to those described previously [9, 10]. Signals were acquired with a Bruker SpecJetIII fast digitizer. The rapid-scan signals were collected at several different scan frequencies and scan widths to observe the effect of the scan rate on the coherence signal. To demonstrate the generality of the phenomenon both linear (triangular) and sinusoidal scans were performed. In the linear response region of a power saturation curve for the main signal the ratio of the amplitude of the coherence signal to the main signal is approximately independent of microwave power.

Microwave power is transmitted to the resonator via a coax that terminates in an antenna near the dielectric resonator. The metal of the antenna could result in eddy currents at the sample that impact the uniformity of the scanned field. This effect was probed by moving a small sample vertically in the resonator. Rapid-scan spectra at several positions in the resonator are shown in the Supplementary Material.

Resonator Q was measured using an HP Model No. 8719D, Network Analyzer. For DTBSQ in ethanol the Q was 320 for a 5 mm long sample in a 4 mm o.d. tube; ~500 or 1500 for a 3 mm long sample in a 4 mm od tube centered in the resonator or near the top of the resonator, respectively; and ~2500 for a 2 mm long sample in a 2.9 mm o.d. tube inside a 4 mm tube that also contains ethanol to lower the Q , with the sample positioned near the top of the resonator. For TMBSQ in methanol the Q was 99 for a 3 mm long sample in a 4 mm tube.

Slow-scan absorption spectra were obtained by deconvolution of the rapid-scan responses [11, 12], in which the time axis is converted to a magnetic field axis.

Results and Discussion

The rapid-scan coherence phenomenon for TMBSQ is illustrated in Fig. 1 for a sinusoidal scan with 7.7 kHz scan frequency and 10 G scan width. TMBSQ has four equivalent methoxy groups with a coupling constant of 46 mG (4.6 μ T) for the 12 equivalent protons. When the main magnetic field is selected such that resonance is at the position of maximum slope of the sinusoid, the field scan rate is 242 kGs⁻¹ (24.2 Ts⁻¹). The spin packet line width is about 36 mG, so the magnetic field traverses each spin packet in less than 150 ns. The spin response to the short time on resonance is equivalent to an FID after a microwave pulse at constant field, since the rapid-scan experiment “turns on” the microwave field to the spin packet for a very short time. An FID occurs for each of the 13 hyperfine lines that result from the 12 equivalent methoxy protons. These FIDs constructively interfere at 7.7 μ s after the passage through resonance, which is the value of T_{diff} calculated for the 46 mG hyperfine coupling. The spacing of lines within the coherence pattern, after conversion of units from time to gauss, is equal to the 46 mG hyperfine splitting. After deconvolution of

the rapid-scan response, the direct-detected spectrum was differentiated; the resolved hyperfine splittings are observed in the derivative spectra (Fig. 1). The comparable high-resolution CW spectrum of this semiquinone is in Fig. S1. The resolution of the hyperfine splitting is similar for the rapid scan (Fig. 1C) and CW spectra (Fig. S1).

The rapid-scan coherence is further illustrated for DTBSQ, which has a 3-line pattern (1:2:1) due to 2.14 G coupling to two equivalent protons on the ring. The 18 equivalent t-butyl protons further split each of these three lines into 19 lines with 59 mG coupling. This hyperfine splitting is resolved in the conventional first-derivative CW EPR spectrum recorded with low microwave power and modulation amplitude (Fig. S2). The CW spectra were simulated using a t-butyl hyperfine coupling of 59 mG and line width of 56 mG.

When a short sample was used, the rapid-scan response of DTBSQ showed coherence patterns at $T_{\text{diff}} = 6 \mu\text{s}$ after passage through each of the EPR lines (Fig. 2). T_{diff} for DTBSQ is smaller than for TMBSQ because a_{H} is larger (59 mG vs. 46 mG). As predicted, the position of the coherence relative to the peaks in the rapid-scan signal depended on the magnetic field scan rate (Fig. S4A,B) because the scaling between time and magnetic field changes with scan rate. When the y-axis scale was expanded there was a hint of the second coherence signal at $2 T_{\text{diff}} = 12 \mu\text{s}$ after passage through resonance (Fig. 2). The hyperfine splittings in the derivative of the deconvolved rapid-scan signal (Fig. 2C) agreed well with the hyperfine in the CW EPR spectrum (Fig. S3). The scan rates in Figures 1, S3, and S4A,B are slow enough that there is a hint of the characteristic rapid-scan oscillations on the trailing edges of the signals. When the scan frequency was increased to 41.6 KHz, several cycles of rapid-scan oscillations were observed, and the amplitude of the coherence signal decreased to the point that it was difficult to distinguish from noise (Fig. S4C).

The rapid-scan EPR signal for DTBSQ was simulated using numerical solution of the Bloch equations for all 57 (3×19) hyperfine lines individually and the results summed after binomial weighting of the hyperfine lines. The offset from resonance for each of the lines was 2.14, 0 or -2.14 G for the three main lines with steps of 59 mG for each of the t-butyl hyperfine lines. The magnetic field, B_0 , was scanned linearly from -6 G to $+6$ G at a frequency of 5.1 kHz. B_1 was set at a low value (50 mG) to avoid saturation. The sample relaxation times were $T_1 = 8 \mu\text{s}$ and $T_2 = 3 \mu\text{s}$ [6]. The m_x - and m_y -components of the computed rapid-scan signal were combined into a complex function for deconvolution to obtain the slow-scan spectrum. The lines in these semiquinone spectra are homogeneously broadened. The linewidths are determined by T_2 , so the amplitudes of the coherence peaks are determined by T_2 [3]. For inhomogeneously broadened lines the amplitudes of the coherence peaks would be determined by T_2^* .

The rapid-scan simulation assuming ideal experimental conditions (Fig. 3), including uniform magnetic field over the sample, shows the coherence signals at T_{diff} . The second coherence at $2 T_{\text{diff}}$ also is clearly defined. A hint of this second coherence pattern is visible in the inset in Fig. 2A. The amplitudes of the rapid-scan coherences in the simulation are larger than in the experimental data (Fig. 2). Simulations showed that the decreased amplitudes for the rapid-scan coherence signals after all three hyperfine peaks is due to

attenuation of the higher frequency components of the data by the limited bandwidth of the resonator with $Q \sim 2500$.

Some of the rapid-scan signals (e.g., Fig. S4) did not exhibit coherence patterns after all three lines when wide scans were recorded, but did exhibit coherence patterns after each of the three lines when recorded separately. This was eventually traced to inhomogeneities in the scanned magnetic fields, probably due to eddy currents, over samples that were more than a few mm long. This effect is larger when the scan is farther from the center, especially when going away from the center; which explains why the coherence pattern was more intense for narrow scans of individual lines than for the third hyperfine line in wider scans. Some examples of the effect of sample location relative to the resonator structure are shown in Fig. S5, S6, and S7.

The simulations were extended with a linear distribution of scan widths to estimate the effect of inhomogeneity of the scanned magnetic field. The calculations showed that a 2% variation in scan width attenuates the amplitude of the coherence signal, and nearly eliminates the coherence signal after the third line in the spectrum (Fig. 4). This attenuation of the amplitude of the rapid-scan coherence signals to differing extents for the three proton hyperfine lines is similar to the experimental observations with long samples (Fig. S4).

The coherence phenomena were observed with both linear (triangular) and sinusoidal scans of various rates, using more than one model of scan driver. The simulations demonstrate that although the FID-like behavior and rapid-scan coherences are a general phenomenon, the coherences will be observable only in cases in which there are well-resolved closely-spaced hyperfine splittings. In most EPR samples the lines encompass multiple partially overlapping spin packets with multiple hyperfine couplings, and are unlikely to result in rapid-scan coherences. Similarly, magnetic field variations over the sample disrupt the phase in hyperfine couplings, and suppress the coherences. If the magnetic field inhomogeneity is due to eddy currents induced by the rapid field scans, there will be time and spatial dependence of the field effects of the eddy currents. Consequently, the use of a sample such as the DTBSQ radical provides an extremely sensitive measure of the magnetic field homogeneity over the sample, and hence will be a useful quality control tool for high-resolution rapid-scan EPR.

Conclusion

The coherence described here was observed for semiquinones with well-resolved hyperfine splittings. Since each molecule has a single set of nuclear spin states, different hyperfine lines in the spectra originate from a different subset of molecules. The rapid-scan coherence could also occur for two different radicals with slightly different g values or two radicals in different magnetic fields created by a gradient. Even when rapid-scan spectra are obtained under conditions that do not clearly reveal the hyperfine, the time between resonance and observation of the coherence signal identifies the magnitude of the hyperfine splitting. For large hyperfine splittings, the reciprocal of the hyperfine splitting is so small that the coherence is difficult to observe. Field homogeneity and eddy currents are important factors that impact the observation of the coherence pattern, as shown by the simulations. The

exquisite sensitivity of the intensity of the coherence signals to magnetic field homogeneity suggests the use of these signals as an important quality control criterion for a rapid-scan resonator and scan coil system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Eaton, SS.; Quine, RW.; Tseitlin, M.; Mitchell, DG.; Rinard, GA.; Eaton, GR. Rapid Scan Electron Paramagnetic Resonance in. In: Misra, SK., editor. Multifrequency Electron Paramagnetic Resonance: Data and Techniques. Wiley; 2014. p. 3-67.
2. Kathirvelu V, Sato H, Quine RW, Rinard GA, Eaton SS, Eaton GR. EPR Free Induction Decay Coherence Observed after a Single-Pulse in Saturation Recovery Experiments for Samples with Resolved Multi-line CW Spectra. *Appl. Magn. Reson.* 2007; 32:269–281.
3. Andrew, ER. Nuclear Magnetic Resonance. Cambridge, U. K.: Cambridge University Press; 1955. p. 129-136.
4. Béné GJ, Denis PM, Extermann RC. Battements de battements. *Physica.* 1951; 17:308–309.
5. Nielsen RD, Canaan S, Gladden JA, Gelb MH, Mailer C, Robinson BH. Comparing continuous wave progressive saturation EPR and time domain saturation recovery EPR over the entire motional range of nitroxide spin labels. *J. Magn. Reson.* 2004; 169:129–163. [PubMed: 15183364]
6. Elajaili HB, Biller JR, Eaton SS, Eaton GR. Frequency Dependence of Electron Spin-lattice Relaxation for Semiquinones. *Alcohol Solutions J. Magn. Reson.* 2014; 247:81–87. [PubMed: 25261741]
7. Kathirvelu V, Sato H, Eaton SS, Eaton GR. Electron Spin Relaxation Rates for Semiquinones between 25 and 295 K in Glass-Forming Solvents. *J. Magn. Reson.* 2009; 198:111–120. [PubMed: 19223213]
8. Mitchell DG, Tseitlin M, Quine RW, Meyer V, Newton ME, Schnegg A, George B, Eaton SS, Eaton GR. X-Band Rapid-scan EPR of Samples with Long Electron Relaxation Times: A Comparison of Continuous Wave, Pulse, and Rapid-scan EPR. *Mol. Phys.* 2013; 111:2664–2673.
9. Quine RW, Czechowski T, Eaton GR. A Linear Magnetic Field Scan Driver. *Conc. Magn. Reson., Magn. Reson. Engineer.* 2009; 35B:44–58.
10. Quine RW, Mitchell DG, Eaton SS, Eaton GR. A Resonated Coil Driver for Rapid Scan EPR. *Conc. Magn. Reson., Magn. Reson. Engineer.* 2012; 41B:95–110.
11. Joshi JP, Ballard JR, Rinard GA, Quine RW, Eaton SS, Eaton GR. Rapid-Scan EPR with Triangular Scans and Fourier Deconvolution to Recover the Slow-Scan Spectrum. *J. Magn. Reson.* 2005; 175:44–51. [PubMed: 15949747]
12. Tseitlin M, Rinard GA, Quine RW, Eaton SS, Eaton GR. Deconvolution of Sinusoidal Rapid EPR Scans. *J. Magn. Reson.* 2011; 208:279–283. [PubMed: 21163677]

Research Highlights

- Rapid scan EPR produces FID-like responses from each hyperfine line.
- Selected semiquinones have closely-spaced well-resolved hyperfine splittings.
- Constructive interference of the transient responses produces coherence signals.
- A highly-homogeneous scan field is required to observe the rapid-scan coherence.

Figure 1.

X-band sinusoidal rapid-scan signals for TMBSQ recorded with a scan frequency of 7.7 kHz, a 10 G scan width, and a center field of 3438 G. (A) A full cycle (up-field scan followed by down-field scan) rapid scan response. The inset in part (A) shows the coherence signal with the x axis expanded by a factor of 10. (B) Sum of the up- and down scans after deconvolution; and (C) the first derivative of the absorption spectrum obtained by numerical differentiation of the spectrum in (B). Note that the rapid-scan response is acquired with a time axis, and only after deconvolution becomes a spectrum with a magnetic field axis.

Figure 2.

X-band rapid-scan signals for DTBSQ recorded using a linear scan with scan frequency of 5.1 KHz, a 12 G scan width, and center field of 3438 G. A small (2 mm long) sample in a 2.9 mm o.d. EPR tube was carefully positioned at a location of minimum eddy current effects on the spectrum. Part (A) is an up-field scan. The insert in part (A) shows the second coherence signal with the y axis amplitude expanded by a factor of 40. The up-field and down-field scans were deconvolved and combined to yield the absorption spectrum in B.

Figure 3.

Simulation of the rapid-scan signal for DTBSQ calculated with a linear scan frequency of 5.1 kHz and other parameters described in the text, assuming a uniform scan field over the sample. The simulations do not include the frequency limiting effect of the resonator bandwidth and thus, represent ideal conditions.

Figure 4. Simulation of the rapid-scan signal for DTBSQ calculated with a linear scan frequency of 7.7 kHz and other parameters as described in the text, after introducing 2% variation in scan width.