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Nuclear relaxation effects in Davies ENDOR variants

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Abstract

A recent article by Yang and Hoffman [T.-C. Yang, B.M. Hoffman, J. Magn. Reson., 181 (2006) 280] presents a 'Davies/Hahn ENDOR multi-sequence' in which the α and β peaks of an electron-nuclear double resonance (ENDOR) spectrum can be distinguished. This represents one instance of a family of ENDOR sequences which have no initial microwave inversion pulse, and which can reveal information about nuclear relaxation rates and the signs of hyperfine coupling constants. Here we discuss the more general set of such sequences, which we refer to as *Saturated Pulsed ENDOR*, and show how signal sensitivity can be optimised within the context of this new technique. Through simulations, we compare its performance to other techniques based on Davies ENDOR, and experimentally illustrate its properties using the non-heme Fe enzyme anthranilate dioxygenase AntDO. Finally, we suggest a protocol for extracting both the magnitude and sign of the hyperfine tensor using a combination of ENDOR techniques.

Keywords: ENDOR; Nuclear relaxation; Anthranilate dioxygenase; Hyperfine tensor

1. Introduction

Electron-nuclear double resonance (ENDOR) represents a family of techniques in which a combination of radiofrequency (rf) and microwave (mw) excitation is used to study nuclear spins which are coupled to a paramagnetic species [1,2]. A recent paper by Yang and Hoffman [3] discusses the effects of rapidly repeating an ENDOR sequence at a rate commensurate with the relevant spin relaxation times, building on the work of Epel et al. [4]. It is shown how a steady-state ENDOR signal is obtained by repeating a sequence which is similar to the Davies ENDOR method but without the preparatory π mw pulse.

A typical ENDOR spectrum representing a nucleus with spin $I = 1/2$ coupled to an electron spin $S = 1/2$ consists of a pair of peaks with equal intensity at frequencies ν_α and ν_β, associated with the $m_S = +1/2$ (α) and $m_S = -1/2$ (β) electron spin projections. The main advantage of the approach of Yang and Hoffman is the distinguishability of these α and β ENDOR peaks, as their method yields intensities of different magnitude or of different sign. A prerequisite for asymmetry is that the temperature, T, must be around or below the Zeeman temperature $h\nu_{\mathrm{mw}}/k_{\mathrm{B}}$ (e.g. 1.7 K for a spectrometer frequency of $\nu_{\mathrm{mw}} = 35$ GHz), along with the condition of a suitable combination of relaxation rates of the electron and nuclear spin. The observation of asymmetry in peak intensity permits the determination of the sign of the hyperfine coupling constant and the simplification of a potentially busy spectrum. Furthermore, the intensity difference of the two peaks diminishes as the repetition time is increased, providing a way to measure nuclear spin relaxation processes.

Under similar, but less general, circumstances, the standard Davies ENDOR experiment also yields peaks of unequal intensity, giving access to the sign of the hyperfine coupling constant and to nuclear spin relaxation times [5].

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An alternative method for measuring nuclear spin relaxation via another modified Davies ENDOR sequence is described in [6].

In this work, we compare the method proposed by Yang and Hoffman to other Davies ENDOR variants with respect to their ability to yield asymmetric spectra. The first section summarises the underlying theory. In the following sections, simulations are discussed and an experimental comparison is given. Finally, we draw conclusions about the benefits of the various techniques.

2. Theory

The standard Davies ENDOR sequence is shown in Fig. 1A. It consists of a selective mw and a selective rf π pulse followed by a non-selective two-pulse echo sequence. In the sequence in Fig. 1B, an additional selective rf π pulse is added *after* the echo detection ([6]). This essentially undoes the nuclear spin polarisation created by the first rf pulse and increases the overall intensity of the ENDOR spectrum compared to the standard sequence, thus allowing higher repetition rates. We shall denote this the *Tidy* Davies sequence. In the sequence in Fig. 1C, the initial preparation π pulse is removed, and instead the sequence is repeated sufficiently rapidly so as to drive the electron spin into saturation. This sequence is a generalisation of the one used by Yang and Hoffman (where $t_{\mathrm{mix}} \approx 0$) ([3]). As they first noted, the mw π pulse is not necessary to observe an ENDOR effect, since the subsequent two-pulse sequence can act both as an electron polarisation detector and generator. We shall denote this the *Saturated Pulsed* ENDOR sequence, or SP-ENDOR. In contrast to the standard and *Tidy* sequences, the mw $\pi/2$ pulse in SP-ENDOR must be a selective pulse, as it assumes in addition the function of the selective π pulse used in the other variants. This distinction is, of course, unimportant when a single microwave channel is employed, as the preparation π-pulse of a Davies ENDOR sequence must be selective.

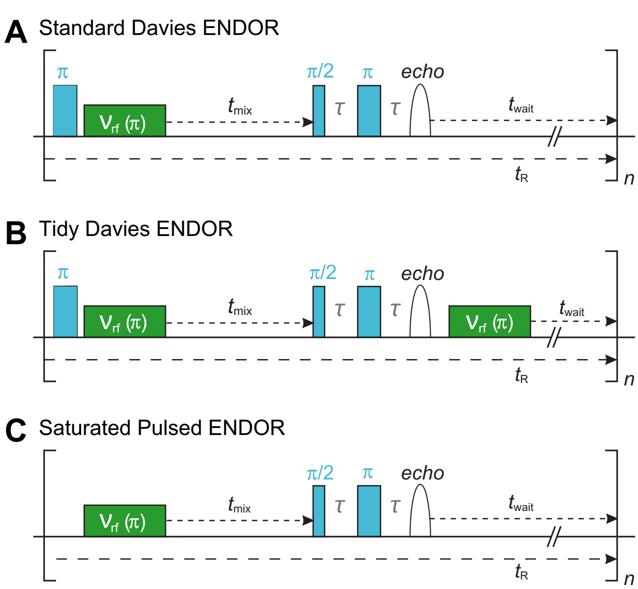

Fig. 1. Davies ENDOR sequence and modifications. (A) Standard Davies sequence, (B) *Tidy* Davies ENDOR: rf π pulse applied after the echo, (C) *Saturated Pulsed* ENDOR sequence: initial mw π pulse omitted. n is the number of repetitions of the sequence in brackets before the radiofrequency is changed.

For all sequences, the mixing time t_{mix} denotes the free-evolution time *before* the two-pulse echo sequence, and the wait time t_{wait} indicates the free-evolution time *after* the two-pulse sequence. The sequence in square brackets is repeated n times. All other inter-pulse delays and all pulses are assumed to be of negligible duration compared to t_{mix} and t_{wait}. The repetition time $t_{\mathrm{R}} \approx t_{\mathrm{mix}} + t_{\mathrm{wait}}$ is the elapsed time between the first pulse in one sequence and the first pulse in the subsequent one.

An ENDOR spectrum of an $I = 1/2$ nucleus coupled to an $S = 1/2$ electron spin consists of two lines with frequencies ν_α and ν_β and intensities I_α and I_β. For an isotropic hyperfine coupling a_{iso}, the frequencies are

$$\nu_\alpha = | \nu_{\mathrm{I}} + a_{\mathrm{iso}}/2 |, \quad \nu_\beta = | \nu_{\mathrm{I}} - a_{\mathrm{iso}}/2 |, \tag{1}$$

where $\nu_I (= -g_n \beta_n B_0/h)$ is the nuclear Larmor frequency. The intensities are determined by several factors. The nuclear transition matrix elements for the two lines are identical, and the factor mainly responsible for asymmetry is the difference in polarisations of the two nuclear transitions. Effects like the hyperfine enhancement and experimental imperfections of the rf excitation can also render the two intensities unequal. However, these effects are often negligible in high-field ENDOR.

To best describe the intensity features of an ENDOR experiment for our purpose, we introduce two parameters. The first describes the intensity of the strongest peak in the spectrum

$$I = \max\left(| I_\alpha |, | I_\beta |\right). \tag{2}$$

The second one quantifies the asymmetry of the ENDOR spectrum

$$A = \frac{I_\alpha - I_\beta}{2I}. \tag{3}$$

For symmetric spectra, the asymmetry parameter gives $A = 0$. $A > 0$ means that the α peak is stronger, whereas $A < 0$ indicates a stronger β peak. The greatest asymmetry is represented by $A = \pm 1$.

I_α and I_β depend on the initial equilibrium polarisation, which in turn is a function of the temperature T and the spectrometer frequency ν_{mw}. In addition, I_α and I_β depend on t_{wait} and t_{mix} as well as on the electron and nuclear spin–lattice relaxation rates $T_{1\mathrm{e}}^{-1}$ and $T_{1\mathrm{n}}^{-1}$ and the electron-nuclear cross relaxation rate $T_{1\mathrm{x}}^{-1}$. In paramagnetic systems, nuclei usually relax via coupling to the local electron spin, or else to neighbouring electron spins ([5,7]). The direct nuclear spin–lattice relaxation rate can thus be neglected, so that we can assume $T_{1\mathrm{n}}^{-1} \approx 0$.

In an ENDOR experiment where the objective is to measure principal values and tilt angles of hyperfine and quadrupole tensors, I should be maximised and A minimised. In