



W-band PELDOR with 1 kW microwave power: Molecular geometry, flexibility and exchange coupling

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ARTICLE INFO

Article history:

Received 10 November 2011

Revised 9 January 2012

Available online 8 February 2012

Keywords:

PELDOR

DEER

Nitroxide spin labels

Orientation selective PELDOR

Exchange coupling

ABSTRACT

A technique that is increasingly being used to determine the structure and conformational flexibility of biomacromolecules is Pulsed Electron–Electron Double Resonance (PELDOR or DEER), an Electron Paramagnetic Resonance (EPR) based technique. At X-band frequencies (9.5 GHz), PELDOR is capable of precisely measuring distances in the range of 1.5–8 nm between paramagnetic centres but the orientation selectivity is weak. In contrast, working at higher frequencies increases the orientation selection but usually at the expense of decreased microwave power and PELDOR modulation depth. Here it is shown that a home-built high-power pulsed W-band EPR spectrometer (HiPER) with a large instantaneous bandwidth enables one to achieve PELDOR data with a high degree of orientation selectivity and large modulation depths. We demonstrate a measurement methodology that gives a set of PELDOR time traces that yield highly constrained data sets. Simulating the resulting time traces provides a deeper insight into the conformational flexibility and exchange coupling of three bisnitroxide model systems. These measurements provide strong evidence that W-band PELDOR may prove to be an accurate and quantitative tool in assessing the relative orientations of nitroxide spin labels and to correlate those orientations to the underlying biological structure and dynamics.

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1. Introduction

Pulsed Electron–Electron Double Resonance (PELDOR or DEER) [1,2] is increasingly being used to determine structures and conformational changes of biologically relevant macromolecules. Using intrinsic paramagnetic centres or site specifically incorporated spin labels into proteins or nucleic acids PELDOR is used to measure the distance between spin centres and to relate distance distributions to conformational states [3–5]. In addition to measuring distances between spin labelled sites, PELDOR can also be used to measure the relative orientation of spin centres [6,7]. Whilst most standard spin labels such as MTSSL are very flexible and known to give a very broad distribution of orientations, new spin labels are now being developed that have less flexibility. Using such rigid spin labels or spin labelling sites with restricted spin label dynamics, orientation-sensitive PELDOR measurements have been demonstrated at X-band frequencies where they have been used to obtain information on relative orientations of spin labels and dynamics. Examples include DNAs spin labelled with the rigid spin label Ç

[8–10], the spin labelled potassium ion channel [11] and copper nitroxide biradical model systems [12,13]. The recent development of the so-called RX nitroxide spin label with two linkers [14,15] also gives hope that in the future protein systems may be spin labelled in a rigid way. Performing PELDOR measurements at 94 GHz (W-band) [7,16–18] or 180 GHz (G-band) [6,10,19] gives a much higher orientation resolution since the *g*-tensors become better resolved, but at the same time the PELDOR modulation depth decreases as a result of less microwave power being available with the spectrometers used. An exception is the W-band PELDOR study by the lab of Goldfarb where a modulation depth of ~20% was achieved with 1 W of power [20]. A deep modulation is important since the dipolar information is contained in the modulation rather than the absolute echo height. All the high-field/high-frequency studies mentioned above also used relatively high *Q* single-mode cavities to improve sensitivity, which limited the available instantaneous bandwidth, restricting the frequency separation between inversion and detection pulses. To invert and detect a full set of orthogonal orientations in a PELDOR experiment requires an effective bandwidth of 250–300 MHz, which previously required lowering the *Q*-value of the cavity substantially with a significant loss of sensitivity. One exception is the promising approach described by Bennati et al. where a bimodal cavity was used to provide separate

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tunable cavity resonances for both inversion and detection pulses, although sensitivity was still limited by the available power [16].

In this work we show that these technical limitations can be overcome and high concentration sensitivity measurements can be made over large bandwidths at 94 GHz. Measurements were made on three bisnitroxide model systems (Fig. 1) using a home-built W-band EPR spectrometer with 1 kW pulse microwave power and a flat 1 GHz instantaneous bandwidth (HiPER) [21] obtaining a detailed picture of their molecular geometry, flexibility and exchange coupling.

2. Results and discussions

Continuous wave-EPR (CW-EPR) spectra were recorded of all three bisnitroxides on HiPER at 110 K (Fig. 2). All three compounds show a typical cw W-band nitroxide spectrum with no discernible splittings due to dipolar- or exchange couplings. Simulating all three spectra yielded g -tensor values, ^{14}N hyperfine coupling values and linewidths that were used for the simulations of the PELDOR time traces (see Table 2).

In the standard terminology for nitroxide orientations in a magnetic field the nitroxide is oriented in the x -direction when the NO-bond is parallel to the applied magnetic field B_0 , in the y -direction when B_0 is orthogonal to the x -direction but in the plane of the five-membered ring system and in the z -direction when B_0 is orthogonal to the ring plane (Fig. 3, inset).

At W-band frequencies the g -tensors are sufficiently well resolved that it becomes possible to selectively excite nitroxides with specific orientations. Thus, in an orientation selective PELDOR experiment one excites the “A” spins of nitroxides oriented in a particular direction via the detection pulse sequence and then monitors the effect of inverting the “B” spins of nitroxides oriented in the same or an orthogonal direction. The overall modulation depth is determined by the fraction of B spins that were excited by the inversion pulse. The modulation frequency and its damping contains information on both the distribution of distances between the spins and the distribution in orientations of the interspin vector with respect to B_0 . In the study described here, we use six separate PELDOR measurements to correlate A and B spins with each other that are oriented in either the x -, y - or z -directions with respect to the magnetic field. Thus, we describe six inversion/detection PELDOR correlation measurements in the XX , YY , ZZ , YX , YZ and ZX directions, where for example ZX means that we invert the spins oriented in the z -direction and detect spins oriented in the x -direction. It is our experience that this measurement methodology gives a highly constrained data set that can be obtained in about 3 h with the described samples and instrumentation.

An echo detected field swept spectrum of each bisnitroxide was used to guide the positioning of the detection and inversion pulses (Fig. 3). Since HiPER works in reflection mode (without a cavity), it is not limited by a cavity band-width and thus allows one to position inversion and detection pulses at any position on the nitroxide

spectrum. This enables one to monitor not only XX , YY and ZZ correlations but also the cross-correlations XY , XZ and YZ with large frequency offsets between inversion and detection, e.g. 210 MHz for XZ .

For each of these six inversion/detection combinations a PELDOR time trace was recorded for each of the three biradicals (Fig. 4a, d, and g). The high sensitivity and high microwave power of the HiPER spectrometer allowed the acquisition of each time trace with an average signal to noise (S/N) ratio of 100 and modulation depths as large as 40% in about 30 min. This modulation depth is close to the 50% obtained at X-band [13,22] and considerably larger than the largest modulation depth of 6% for W-band PELDOR on a power-upgraded Bruker Elexsys 680 spectrometer using either a single or dual mode cavity [7,16] and still larger than the modulation of 20% achieved by Goldfarb et al. [20]. Although, it should be kept in mind that the observed PELDOR modulation depth depends also on the relative orientation and degree of correlation between the coupled spin centres. A quantitative analysis of the three sets of time traces are detailed below.

2.1. Biradical 1

The set of PELDOR time traces acquired for **1** show a high degree of orientation selection in terms of modulation frequency and modulation depth (Fig. 4a). Fourier transforming the PELDOR time traces gives a clear qualitative picture of the orientations of the nitroxides relative to the vector connecting the spin centres. The XX experiment shows the largest selection of the parallel dipolar component ($\theta = 0^\circ$) while the ZZ experiment shows the largest perpendicular dipolar component ($\theta = 90^\circ$). This implies that the orientation of the g_x tensor component is largely parallel and the g_{zz} component perpendicular to the molecular backbone/dipolar distance vector. This necessarily means that the orientation of the g_x tensor component on one nitroxide (spin A) (Fig. 3, inset) is largely perpendicular to the g_{zz} tensor component of the other nitroxide within the same molecule (spin B). This is supported by the very small modulation in the ZX time trace.

To obtain a more quantitative picture of the mutual orientation of the nitroxide spin labels and the inherent conformational flexibility of the molecule the PELDOR time traces were simulated with a home-written Matlab® program based on an approach published by the Prisner lab [23]. In a first step, a geometry optimised structure of **1** was obtained from density functional theory (DFT) calculations. The lengths of the molecular linker and nitroxide moieties obtained from that structure were then used as initial geometric values to construct a vector model where each nitroxide including the ester groups and the connecting bridge were represented by three independent vectors (Fig. 5a).

The two nitroxides groups in **1** were allowed to rotate freely around the phenolic bond with the N–O bond (g_{xx} tensor component) tracing out a cone with a mean opening of 25° and the g_{zz} and g_y tensor components having a random position in the

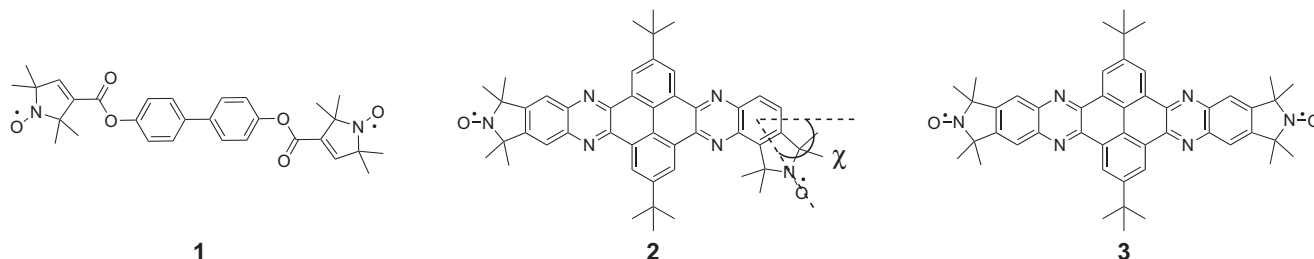


Fig. 1. Structure of nitroxide biradicals **1–3**. For **2**, χ denotes the angle between the nitroxide g_x components. Their synthesis is described elsewhere [13,22].

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