Measurement of Longitudinal Relaxation Times of Free Radicals by Low Field Pulsed Dynamic Nuclear Polarization

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Introduction

PEDRI (or Overhauser MRI) allows the spatial localization of stable free radicals in large animals [1]. It is based on dynamic nuclear polarization (DNP): the EPR resonance of the free radical of interest is irradiated during the acquisition of an NMR image, resulting in an enhancement of the NMR signal in regions containing free radical. For the optimization of in vivo PEDRI experiments it is important to know the transverse (T2e) and longitudinal (T1e) relaxation times of the free radicals. In fact, the CW DNP saturation factor is determined by the ratio of the applied RF B1 field amplitude and (T1e*T2e). The relaxation times of free radicals are usually measured with CW-EPR using progressive saturation techniques [2]. However, at low field and with large lossy samples the accurate EPR measurement of T1e and T2e is limited by the available maximum RF power, non-linearity of the RF amplifier, the low efficiency of the RF resonator, and drift of the RF resonator tuning/matching at high power.

Pulsed DNP (πDNP) was proposed, at very high field (5T), for high-resolution solid-state NMR spectroscopy of bio-molecules [3]. In πDNP a train of 180° pulses is applied instead of the more usual CW irradiation. Recently, low field πDNP was used in PEDRI to decrease the average EPR irradiating power with large lossy samples [4].

In this work, we report the use of low field (10 mT) πDNP for measuring the longitudinal relaxation time (T1e) of stable free radicals in solution.

Methods

A theoretical model for the calculation of the πDNP enhancement at 10 mT has been proposed [5]. It was shown that, if TEPR>>T1p and Tr≥3T1e, the maximum πDNP enhancement, Ap, is given by:

$$Ap \propto [\exp((1 - \alpha)R1p\ tr) - 1] / [(1 - \alpha)(\exp(R1p\ tr) - 1] \quad (1)$$

where: α=R1e/R1p; tr and TEPR are the pulse repetition and duration times of the EPR train, respectively; R1p is the proton spin relaxation rate (=1/T1p, typically 1 Hz); R1e is the electron spin relaxation rate (=1/T1e, typically 1 MHz). From Eq. (1) we observe that, assuming that R1p is known, R1e can be derived from the measured πDNP enhancement as a function of the EPR pulse repetition time by a best-fitting procedure.

The πDNP apparatus used in the present work comprised a 4-coil electromagnet, generating a field of 10 mT, a 280 MHz RF source, a pulse generator, an RF power amplifier, capable of delivering 60 W between 2 and 400 MHz, a two-way directional coupler, a loop-gap EPR resonator (diameter 3.8 cm, length 3 cm), tuned to 280 MHz and with dead time of about 1 μs, an NMR transmit/receiver solenoid (70 turns, diameter 7.5 cm, length 7 cm), tuned to 425 kHz, and an NMR acquisition/data storage system. The pulse sequence used for πDNP experiments comprises a train of EPR inverting pulses (width 500 ns, tr=2 to 20 μs) applied for a time TEPR >>300 ms. The proton FID signal is detected immediately after the end of the train of EPR pulses. The enhancement is calculated as Ap=(Iz-Io)/Io, where Io and Iz are the FID amplitude without and with EPR irradiation, respectively. Aqueous solutions (1 mM) of perdeuterated TEMPOL and a novel triarylmethyl (TAM) radical [6] were used. The TAM radical was generously donated by Nycomed Innovation, Malmo, Sweden. The samples were de-oxygenated with a flux of nitrogen to reduce line broadening due to paramagnetic relaxation.

Results

Typical proton FIDs detected using the TAM sample and with a train of EPR pulses (TEPR>>300 ms, repetition time of 6 μs) showed a πDNP enhancement of about -20. The measured πDNP enhancement versus tr is reported in Fig. 1. We note that as the repetition time is reduced the DNP enhancement increases. This is in agreement with the theoretical calculation obtained from Eq. (1).

With tr=2 μs the measured maximum enhancement was -60. Shorter repetition times (<2 μs) were not used because of the EPR resonator ring down time and relaxation effects during the EPR pulses. However, a very good agreement between experiment and theory (Eq. (1)) was obtained for repetition times in the range 2 to 20 μs. In Fig.1, the solid line shows the theoretical πDNP enhancement calculated according to Eq. (1) with T1p=1 s and T1e=1.1 μs. Similar results were obtained with the PD-TEMPOL and the fitting procedure gave T1e=0.33 μs. These values of the electron spin relaxation time, for PD-TEMPOL and TAM, are in good agreement with previous CW-EPR data at 280 MHz obtained with very small samples [7].

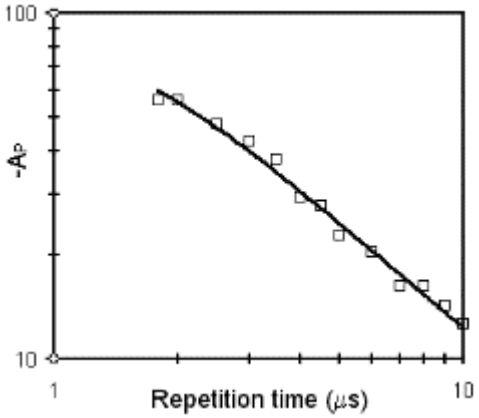

Figure 1: DNP enhancement versus repetition time (tr) of π pulses in EPR irradiation train. Sample was 1mM aqueous solution of TAM radical. The solid line is the fit obtained using Eq. (1).

Conclusions

We have shown that the low-field πDNP technique allows measurement of the electronic longitudinal relaxation time of free radicals. The method requires knowledge of the proton relaxation rate in the presence of the free radical and the measurement of the Overhauser enhancement as a function of the EPR inversion pulse train repetition time. Since the πDNP technique does not require saturation of the electron spin transitions (as with standard CW-EPR techniques), it should give a more accurate *in vitro* and *in vivo* estimation of the longitudinal relaxation time of free radicals.

References

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