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High Field Dynamic Nuclear Polarization with High-Spin Transition Metal Ions

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Abstract

We report the dynamic nuclear polarization of ¹H spins in magic angle spinning spectra recorded at 5 T and 84K via the solid effect using Mn²⁺ and Gd³⁺ complexes as polarizing agents. We show that the magnitude of the enhancements can be directly related to the effective linewidth of the central ($M_S = -1/2 \rightarrow +1/2$) EPR transition. Using a Gd³⁺ complex with a narrow central transition EPR linewidth of 29 MHz, a maximum enhancement of ~13 is observed, which is comparable to previous results on the narrow linewidth trityl radical.

Applications of magic angle spinning (MAS) nuclear magnetic resonance (NMR) are often limited by the low Boltzmann polarization of nuclear spins and therefore the low inherent sensitivity. Thus, in samples such as large biomolecules or compounds with a low abundance of magnetic nuclei, the experimental acquisition time can be prohibitively long. The same is true even when uniformly ¹³C/¹⁵N labeled samples are employed and multi-dimensional techniques are required to perform assignments and measure ¹³C-¹⁵N and ¹³C-¹³C distances and torsion angles^{1–4}. In such cases high frequency dynamic nuclear polarization (DNP) can be used to significantly boost the sensitivity of MAS NMR by transferring the relatively large polarization of electron spins to the nuclei^{5–10}. Accordingly, DNP has been applied successfully to the investigation of functional membrane proteins in their native lipid environment^{11,12} and amyloid fibrils^{13–16} without compromising spectral resolution¹⁷. These experiments would not be feasible in a reasonable amount of time with conventional MAS NMR.

The polarizing agents used in contemporary DNP experiments have primarily been persistent, $g \approx 2$ organic radicals (e.g. trityl, BDPA, nitroxide-based mono- and biradicals)^{18–20}. However, in principle paramagnetic metal ions can also be used as a source of polarization and offer at least two potential advantages. First, many metalloproteins contain paramagnetic metals or can be doped with a paramagnetic substitute, thus providing an intrinsic source of polarization that could lead to efficient enhancement of protein resonances. Second, they offer the possibility of locally polarizing the nuclei adjacent to the metal and could yield site specific structural details for the active sites of metalloproteins. This local character of the polarization could, in addition, provide important details about the DNP process. Besides that, many inorganic samples often contain or can be doped with

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Supporting Information Available. Supporting Information includes sample preparation and experimental as well as instrumental details. This information is available free of charge via the internet at <http://pubs.acs.org>.

paramagnetic metal ions. This opens the possibility of applying DNP to a variety of materials science related problems. For these reasons we initiated investigations of DNP enhancements with high-spin transition metal ions and report the initial results for Gd^{3+} and Mn^{2+} in this communication.

The two metals were used in the form of complexes with the octadentate chelating ligands 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and diethylene triamine pentaacetic acid (DTPA) shown in Fig. 1. These ligands form extremely stable complexes and are used as magnetic resonance imaging (MRI) contrast agents in clinical applications. Furthermore, Gd^{3+} complexes with DOTA and DTPA exhibit significantly different electric field gradients at the metal site²¹ allowing us to investigate the influence of the magnitude of the zero-field splittings (ZFS) on the efficiency of the DNP process. Experimental details on both the DNP/NMR and EPR experiments are available in the Supporting Information.

The DNP mechanism that governs the polarization transfer in the compounds used in this study is the solid effect (SE)²². This is a two-spin process in which microwave irradiation at $\omega_{mw} = \omega_{0S} \pm \omega_{0I}$ (where γ_{0S} and γ_{0I} are the electron and nuclear Larmor frequencies, respectively) excites forbidden electron–nucleus transitions that become partially allowed due to mixing of adjacent states. The Hamiltonian relevant to this system is

$$H = \omega_{0S} S_z - \omega_{0I} I_z + C S_z I_z + C^* S_z I_-$$

where $C = (-3/2)(\gamma_S \gamma_I / r^3) \sin \theta \cos \theta e^{-i\phi}$ is the usual term in the Van Vleck form of the electron–nuclear dipole Hamiltonian and S and I are spin operators for electrons and nuclei, respectively²³. Double- or zero-quantum transitions that are partially allowed as a result of state mixing by the $S_z I_{\pm}$ terms are excited at $\omega_{mw} = \omega_{0S} \pm \omega_{0I}$, and transfers polarization from the electron to the nuclear spins. The transition probabilities scale as ω_{0I}^{-2} with magnetic field^{24,25}. A requirement for a large solid effect enhancement is that both the homogeneous (δ) and the inhomogeneous (Δ) EPR linewidths of the paramagnetic center are smaller than the Larmor frequency (ω_{0I}) of the nucleus to be polarized ($\delta, \Delta \ll 2\omega_{0I}$). If this criterion is not satisfied, then the zero- and double-quantum transitions will broaden or overlap, leading to cancellation of positive and negative enhancements. The remaining net enhancement is due to a differential solid effect (DSE). In addition, large γB_I 's are known to increase SE enhancements. For example, in experiments to be reported elsewhere we show that $\epsilon \approx 100$ is observed using trityl as a polarizing agent, together with high microwave field strengths available in a microwave resonator.²⁶ Despite these limitations significant enhancements of ~ 15 are observed using even moderate γB_I 's and $B_0 = 5$ T.²⁵

140 GHz EPR spectra of all three complexes in the form of glassy solutions are shown in Fig. 2. As can be seen in the case of Gd^{3+} ($S=7/2$), each EPR signal consists of a narrow resonance arising from the central ($m_S = -1/2 \rightarrow +1/2$) transition, and a broad resonance resulting from the single-quantum transitions ($7/2 \rightarrow 5/2 \rightarrow 3/2$, etc.) that are broadened by the zero-field splitting (ZFS). Although neither ligand significantly alters the g -value of Gd^{3+} ($g = 1.9918$), the linewidth of the central EPR lines differ by more than a factor of 5 (29 MHz FWHM for GdDOTA and 150 MHz for GdDTPA). This difference is due to the significantly different ZFS constants of $D = 0.57$ GHz, $E = 0$ and $D = 1.44$ GHz, $E = 0.39$ GHz for GdDOTA and GdDTPA, respectively.²¹ Though the central transition is not affected by the ZFS to first order, residual second-order effects scaling with D^2/ω_0 lead to non-vanishing coupling effects which govern the linewidth of the central transition.²⁷ Therefore, other broadening mechanisms such as g -strain, hyperfine coupling to ^{155}Gd and ^{157}Gd (which both occur with a natural abundance of about 15 %) and ^{14}N as well as

homogeneous broadening can be considered as being small compared to the second-order ZFS for both Gd^{3+} complexes. Although this line broadening mechanism is not present in $S = 1/2$ systems, the SE experiments reported to date using carbon centered radicals in aqueous solutions have utilized a minimum linewidth of 55 MHz observed with trityl²⁸, which is still significantly broader than the GdDOTA line.

The EPR spectrum of MnDOTA shows the typical sextet pattern centered at $g = 2.0014$ due to hyperfine coupling to ^{55}Mn ($I = 5/2$) with a hyperfine coupling constant of $A = 254$ MHz. Again, only the central transition of the high-spin ($S = 5/2$) system yields narrow EPR lines whereas the higher spin transitions are broadened by the ZFS and contribute to the spectrum as a broad component visible in the spectrum. The FWHM of each central line is about 25 MHz. Despite the narrow linewidth, we expect a reduced enhancement because the $S=1/2$ transition is dispersed by the $I=5/2$ nuclear hyperfine coupling.

In a high-spin system ($S= 5/2$ or $7/2$) the selective polarization of the $m_S = -1/2 \rightarrow +1/2$ transition is lower compared to an $S = 1/2$ spin. For the $S= 7/2$ Gd^{3+} system, the relative polarization (population difference in the $m_S = -1/2 \rightarrow +1/2$ subspace divided by sum population of all m_S states) is $1/4$ of that of an $S = 1/2$ system, and for Mn^{2+} ($S = 5/2$) it is $1/3$. However, in the case of Mn^{2+} the selection of only one line of the hyperfine sextet leads to an effective reduction of the selective relative polarization to only $1/18$. For both the Gd^{3+} and Mn^{2+} complexes a concentration of 10 mM in 60:30:10 % (v/v) d₈-glycerol/D₂O/H₂O yielded the maximum enhancements and was chosen for all experiments.

The field dependent enhancement profiles of the Gd^{3+} compounds (shown in Fig. 3) exhibit the shape characteristic of the SE. In the case of GdDOTA the positive and the negative enhancement peaks are well separated, since the overall EPR linewidth of the central transition (29 MHz) is much smaller than the ^1H Larmor frequency (213 MHz). In contrast GdDTPA approaches the condition of the DSE because the EPR linewidth is comparable to the ^1H Larmor frequency (150 MHz vs. 213 MHz). The maximum enhancements obtained at 6 W microwave power^{29–31} for GdDTPA and GdDOTA are 3.5 and 12.8, respectively. Thus, because of the linewidth at this field, GdDTPA is not an attractive polarizing agent. A similar Gd^{3+} linewidth was present in a recent study of polarization transfer in DNP experiments. However, only the decay of the EPR signals, rather than the nuclear polarization, were examined in that study³².

In contrast, for GdDOTA the $\epsilon=12.8$ which is comparable to the performance of conventional trityl polarizing agents where $\epsilon \approx 15$. This result is quite surprising considering that the population difference in the $m_S = -1/2 \rightarrow +1/2$ subspace of the high-spin system is only about $1/4$ of the polarization found in an $S = 1/2$ system. A possible reason for the relatively good DNP performance might be the x4 larger transition moment of the $S = 7/2$ central transition compared to low-spin ($S = 1/2$) radical centers³³ which corresponds to a 16-fold increase in effective microwave power. However, this neglects the contribution of the higher m_S transitions to paramagnetic relaxation. Additionally, the complex relationship between electronic relaxation, nuclear relaxation, hyperfine coupling as well as double- and zero-quantum transition moments and the respective influence on DNP efficiency remains to be elucidated theoretically and experimentally.

The field dependent enhancement profile of MnDOTA illustrated in Fig. 4 has a more complicated structure. It reflects the overlap of two hyperfine sextets with amplitudes of opposite sign that are shifted by twice the ^1H Larmor frequency with respect to one another. Due to the narrow linewidth of the SE transitions and the mismatch between the ^1H Larmor frequency and the hyperfine coupling (212 vs. 254 MHz), all twelve DNP peaks are clearly resolved. However, the maximum enhancement observed at the second outermost peak on

the high-field side is only ~1.9 at 5 W microwave power. We ascribe this small enhancement to the distribution of the DNP conditions over all six hyperfine lines which leads to an even smaller fraction (1/18) of spins involved in the DNP process as compared to Gd^{3+} in which ~80% of the nuclear spins lack a hyperfine coupling. In addition, the lower spin state of Mn^{2+} ($S = 5/2$), the lower transition moment of the central EPR transition (3 times larger than that for $S = 1/2$) might result in a slightly lower performance compared to Gd^{3+} .

To quantitatively compare the relative performances of the different polarizing agents we evaluate the area under the field dependent polarization profiles (enhancement profile 1). We emphasize, that the shape and width of the enhancement profile peak is neither influenced by nor does it resemble the linewidth of the enhanced ^1H NMR signal. It is rather primarily a reflection of the linewidth of the EPR central transition; in fact the field-swept DNP profile can be considered as an EPR spectrum being indirectly detected via the ^1H enhancement. Thus, the integral over the DNP peak should allow us to compare the different paramagnetic species independently of their EPR central transitions' linewidth. The intended integration is straightforward in the case of both Gd^{3+} compounds since we can integrate over the complete positive enhancement peak. In contrast, the full integration is not possible in the case of the Mn^{2+} complex due to overlap of the hyperfine lines of the positive and negative DNP peaks. Therefore we only integrate over the two high-field peaks that are clearly resolved and well separated from any negative DNP condition and multiply the result by 3. The results of this simple analysis are summarized in Table 1. Despite the very different maximum enhancements obtained from the three compounds, the integrated enhancements, which represent excitation of all transitions yielding positive enhancement, are very similar. GdDTPA performs ~30 % less efficiently than the corresponding DOTA complex which might be caused by the onset of DSE condition. Although there should be little overlap of double- and zero-quantum transitions at the respective peaks' centers, there will be some cancellation effects especially closer to the enhancement profile's center. However, since the use of a monochromatic microwave source does not allow us to utilize this integral enhancement, these results show that the effect of spectral dilution due to ZFS or hyperfine coupling dominates the actual enhancement obtained under experimental DNP conditions. Considering that the magnitude of the second-order ZFS which broadens the central transition scales inversely as the external magnetic field, the performance of these compounds at yet higher fields might potentially outperform organic radicals like trityl, whose EPR resonances are mostly broadened by g -anisotropy under these conditions.²⁸

The dynamics of polarization build-up do not differ significantly among the three compounds investigated as can be seen in Fig. 5. The polarization build-up times are listed in Table 1 and are comparable to build-up times using biradical polarizing agents.¹⁷ The fact that deviations of the dynamics are negligible is not surprising. Generally, differences in the build-up times might be attributed to differences in relaxation times of the paramagnetic center and the nuclei, variations in effective sample concentrations, among other things, as well as the actual rate of polarization transfer from the electron spin to the surrounding nuclei and the subsequent spreading of the polarization to the bulk via ^1H - ^1H spin diffusion. The fact that the build-up and relaxation properties are very similar for all three compounds furthermore supports our findings of the DNP performance being mostly influenced by the actual width of the DNP transitions which are in turn closely related to the EPR linewidths.

We have shown that dynamic nuclear polarization via the solid effect is possible with high-spin transition metal compounds as polarizing agents. Given that the EPR linewidth is narrow and there is no spectral dilution by strong hyperfine coupling to the metal nucleus, DNP performance comparable to that obtained with the well-established trityl radical can be achieved. However, if significant broadening of the central transition by second-order ZFS

or strong hyperfine coupling to the metal nucleus is present, the enhancement is strongly compromised. The depletion of the enhancement is not caused by alteration of the actual DNP process but rather simply due to more selective excitation of electron spins participating in DNP. In this context we suggest that the higher effective transition moment of the high-spin system in combination with reduced line broadening caused by second-order ZFS might lead to improved performance of these compounds even at higher fields (e.g. 9 T and above).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Chemical structures of the (protonated) octadentate chelating ligands DOTA and DTPA.

Figure 2.

EPR spectra of the complexes under investigation recorded at 139.5 GHz. Spectra were recorded using a Hahn echo sequence. The solution (1 mM in 60:40 % (v/v) glycerol/H₂O) was contained in a quartz capillary of 0.5 mm o.d. at a temperature of 20 K.

Figure 3.

Field dependent DNP enhancement profiles of GdDOTA and GdDTPA recorded at 84 K with a microwave frequency of 139.65 GHz and a microwave power of 6 W. $\omega_r/2\pi = 5$ kHz. Data points were obtained by directly detecting the ¹H polarization via a Bloch decay.

Figure 4. Field dependent DNP enhancement profiles of MnDOTA recorded at 86 K using a microwave frequency of 139.65 GHz and a microwave power of 5 W. For further details see Fig. 2.

Figure 5.

(a)–(c) Comparison of the mw-on and -off signals detected after transfer of the ^1H polarization to ^{13}C (in 1 M ^{13}C -urea) via cross-polarization. Both Gd^{3+} complexes were measured at 84 K and 6 W microwave power; MnDOTA was measured at 86 K and 5 W. (d) Buildup of polarization for the DNP enhanced signals under the same experimental conditions used for the enhancement measurements.

Table 1

Experimental DNP parameters.

Sample	Enhancement	Integral enhancement	Build-up time (s)
GdDOTA ^a	12.8	0.98	6.6
GdDTPA ^a	3.5	0.70	5.2
MnDOTA ^b	1.9	0.89	5.6

^a at 6 W microwave power,^b at 5 W microwave power; for further details see text.