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# Deuterium NMR study of the MP-2269: albumin interaction — a step forward to the dynamics of non-covalent binding

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**Abstract**

MP-2269, the Gd(III) complex of 4-pentylbicyclo[2.2.2]octane-1-carboxyl-di-L-aspartyl-lysine-derived-DTPA, is a small Gd-agent that binds non-covalently to serum albumin in vivo to assume the enhanced relaxivities associated with macromolecular agents, (due in part to increased rotational correlation time, $\tau_R$). To further explore the fundamental parameters that govern the dynamics of water proton relaxation enhancement by this prototypical albumin-binding agent, the rotational correlation time ($\tau_R$) for the deuterated La(III) analog of MP-2269 has been independently measured in the presence and absence of 4% albumin using $^2$H-NMR approaches. The diamagnetic La(III) analog of MP-2269 was deuterated at the α-position of the carbonyl groups. $^2$H-NMR studies were conducted at 7.05T (46 MHz) and 310°K on a Bruker NMR spectrometer. Spectral deconvolution permitted calculation of transverse relaxation rates, $1/T_2$, from the NMR linewidths and subsequently, $\tau_R$. The results yielded a $\tau_R$ of the albumin bound complex of ~8 ns. This value is intermediate between those earlier estimated by $^{17}$O-NMR (~1 ns) and $^1$H-NMRD (~20–50 ns) and significantly shorter than that of albumin. The $^2$H-NMR study results also indicate that the exchange between free and albumin-bound forms of the La(III) analog is slow (exchange lifetimes >1 ms). This slow exchange does not affect the water residence lifetimes ($\tau_M$ 140–280 ns). 

*Keywords:* NMR; Non-covalent binding; MP-2269; Deuterium

## 1. Introduction

The proton NMR and NMRD studies of the prototype blood pool MRI contrast agent MP-2269 (the Gd(III) complex of 4-pentylbicyclo[2,2,2]octane-1-carboxyl-di-L-aspartyl-lysine derived DTPA) which binds to serum albumin through hydrophobic interaction between the non-aromatic cyclic side chain and the protein, have been the subject of recent studies [1,2]. In these, comparisons of the NMR, EPR, and NMRD profiles with relaxation theory were carried out in order to probe the utility of theory as a guide for the design of better paramagnetic agents for MRI as earlier described [3,4].

Briefly, inner sphere contributions tend to dominate $T_1$ relaxation enhancement from macromolecular Gd-complexes at typical MRI fields. Such inner sphere contribution to macromolecular $1/T_1$ is given by: $1/T_{1p}^{is} = q[Gd^{3+}]/55.5(T_{1M} + \tau_M)$; where, $q$ is the number of coordinated inner sphere water molecules (typically $q = 1$), $T_{1M}$ is the longitudinal relaxation time of the coordinated water protons, and $\tau_M$ is their lifetime on the metal ion (typically of the order of microseconds for $Gd^{3+}$). $1/T_{1M}$ is a complicated function of the strength of the interaction, the magnitude of the applied MRI field, and the overall correlation times, $\tau_{Ci}$ ($i = 1$ or 2) ($\tau_{Ci}^{-1} = \tau_R^{-1} + \tau_{Si}^{-1} + \tau_M^{-1}$), the inverse of the sums of the reciprocals of the rotational correlation time $\tau_R$, the electronic relaxation times of the paramagnetic metal ion $\tau_{Si}$, and $\tau_M$. Attachment of $Gd^{3+}$-chelates to macromolecules generally leads to enhancement in $1/T_1$ in the 10–50 MHz field-range. This phenomenon is well understood and is associated with the long $\tau_R$ of macromolecular complexes. Hence,

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formation of a rigid macromolecular paramagnetic complex has been the design goal for obtaining high-relaxivity contrast agents [5–7]. The peak relaxivity of the covalently bonded $Gd^{3+}$-macromolecules have been limited either by lack of rigidity or by a long value of $\tau_M$. Recent innovations yielded relatively rigid paramagnetic macromolecular complexes in-vivo in the blood pool through reversible non-covalent hydrophobic interactions of small lipophilic $Gd^{3+}$-chelates with serum, leading to development of highly efficient intravascular contrast agents [8,9].

Combined $^1$H-NMRD, $^{17}$O-NMR and EPR studies of MP-2269 bound to serum albumin showed that the water residence time ($\tau_M$) is not markedly influenced by the protein binding. These studies, however, were unable to assess the time scale of the protein-to-ligand exchange interactions and also gave indirect (and disparate) values for the rotational correlation time ($\tau_R$) of the albumin-bound complex [1,2,10]. $^2$H relaxation rates of diamagnetic compounds originate predominantly from quadrupolar interactions and are thus modulated by $\tau_R$. $^2$H transverse relaxation rate of carbon-deuterium bond is described by the following equation:

$$\frac{1}{T_2} = \frac{3\pi^2}{20}\left(\frac{e^2Qq}{h}\right)^2\left[3\tau_R + \frac{5\tau_R}{1+\omega^2\tau_R^2} + \frac{2\tau_R}{1+4\omega^2\tau_R^2}\right]$$

where $e^2qQ/h$ is the quadrupolar coupling constant and $\omega$ is the angular frequency of deuterium.

Hence, binding a deuterated sample to a macromolecule results in enhanced transverse relaxation rate ($1/T_2$, $R_2$) of the bound material. If the exchange between bound and unbound compound is fast on the relaxation rates scale, a decrease in the initial concentration of the ligand at constant macromolecule concentration results in an increase of the observed $R_2$ [11,12]. An $^2$H-NMR relaxation rate study of the diamagnetic La(III)-$d_{15}$-MP-2269 was, therefore, undertaken in order to obtain an independent and non-ambiguous value of $\tau_R$ for the albumin bound MP-2269 analog.

## 2. Material and methods

Details of the synthesis and analyses of MP-2269, the tetrasodium salt of 4-pentylbicyclo[2.2.2]octane-1-carboxyl-di-L-aspartyl-lysine-derived-Gd(DTPA), have been published [8] (Fig. 1).

The La(III) analog was prepared by mixing aqueous solutions of equimolar amounts of lanthanum chloride and ligand (4-pentylbicyclo[2.2.2]octane-1-carboxyl-di-L-aspartyl-lysine-derived-DTPA). The La-complex was deuterated on the α-position of the carbonyl groups following the procedure outlined by Wheeler and Legg [13]. The deuterated La complex was dialyzed and isolated after lyophilization. The deuteration was confirmed by $^1$H-NMR spectroscopy; the product contains 15 deuterium atoms. The sodium and potassium contents were determined by flame photometry (Flame Photometer IL 943, Instrumentation Laboratory S.p.A., Milan, Italy). A solution of 4% HSA (Sigma, Bornem, Belgium) in deuterium depleted water was used for the binding study and for the $^2$H-NMR measurements performed at 7.05 T and 310 K on a Bruker AMX-300 spectrometer. $^2$H transverse relaxation rates ($1/T_2$) were derived from the linewidths ($\Delta\nu_{1/2} = 1/\pi T_2$) of the deconvoluted spectra using a computer algorithm (Linesim Bruker program). A quadrupolar coupling constant of 170 KHz was used for the calculations.

## 3. Results

Since $^2$H relaxation rates ($1/T_2$, $R_2$) of diamagnetic compounds originate predominantly from quadrupolar interactions modulated by $\tau_R$, the enhanced transverse relaxation rate of the bound material is a direct source of $\tau_R$. When the deuterated La-analog of MP-2269 was added to HSA, no marked change of the linewidth was noticed. The lack of effect on the linewidth can be explained either by an absence of binding interaction, (unreasonable since prior evidence points to association, and ongoing studies yield $K_a$ of the order of 9000 $M^{-1}$, at a minimum of two equivalent binding sites) or by a slow exchange between bound and unbound material.

The presence of a broad component in the $^2$H-NMR spectra of La(III)-$d_{15}$MP-2269 (0.25 and 0.75 mM), in aqueous albumin solutions at 310 K (Fig. 2) confirms the slow exchange hypothesis. The spectra were deconvoluted in their underlying components.

At low concentrations of the ligand (0.25 and 0.75 mM), the deuterium spectra show two narrow peaks-one from residual HDO (left hand peak), one from the unbound material (right hand peak) and a broad component due to the albumin bound ligand. Analysis of the linewidth of the broad component allows the evaluation of the $\tau_R$ of the bound ligand and the relative area

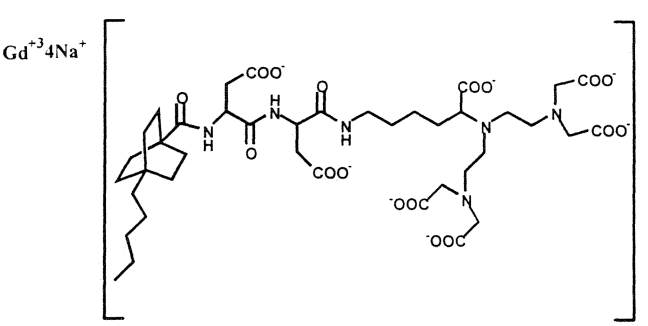


Fig. 1. Chemical structure of MP-2269.

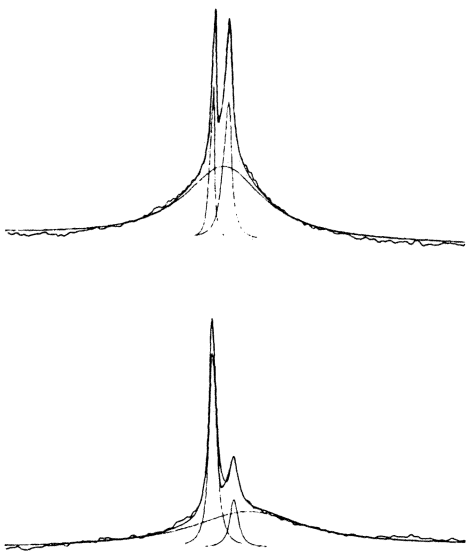

Fig. 2. $^2$H-NMR spectra of solutions containing 4% of HSA and 0.75 mM (top spectrum) or 0.25 mM (bottom spectrum) of the La-analog of MP-2269.

of the narrow and broad peaks give the amounts of each species present (Fig. 2 and Table 1). Values of $\tau_R$ for the albumin bound contrast agent around 8 ns are found (compared with ~ 140 ps in albumin free solution). The percentages of bound compounds are in very good agreement with the expected values (Table 1).

## 4. Discussion

In earlier studies [1,2,10], the $Gd^{3+}$ contributions to the measured NMR, EPR and NMRD profiles of albumin solutions were fit to theory, using values for the parameters for the analogous Gd-DTPA$^{2-}$ as initial values [3]. The assumptions needed to simplify the theoretical fits and the extent to which these carry over to other Gd-analogs and their macromolecular adducts remain to be established. Using these approaches, studies of the title compound yielded disparate $\tau_R$ values for the albumin adduct despite the firm establishment of $\tau_M$ [2]. Similar controversies exist in the current literature [11,14,15]. We have, therefore, adopted the $^2$H-NMR approach to reach $\tau_R$ values that are not subject to additional extrinsic parameters. This hopefully would form a basis for generating consistent $\tau_R$ values that can be compared, (in addition to independently-derived $\tau_M$) in assessment of the impact of various structural modifications on relaxation rates.

The $\tau_R$ value of the bound contrast agent found in this work ($\tau_R = 8$ ns) is shorter than the expected $\tau_R$ for a fully immobilized ligand ($\tau_R = 20$–$22$ ns) [16] indicating some segmental mobility of the contrast agent with respect to the protein one.

Further, the impact of the protein–ligand exchange rate on the water–metal exchange is not easily delineated from the earlier studies. The deuterium NMR approach in this study clearly demonstrated the various time regimes for this system, i.e. significantly slow protein–ligand exchanges that do not impact the metal–water exchange ($\tau_M$).

## 5. Conclusion

As compared with the other albumin binding contrast media studied so far [11,12], MP-2269 exhibits slower exchange rate between the bound and free states. These kinetic aspects which are not reflected by water $^1$H-NMR relaxometry are adequately addressed by $^2$H studies. Clearly, no relationship exists between the residence time of the coordinated water molecule and the residence time of the lanthanide complex on the protein. In the present case, MP-2269 has a residence time on the protein of the order or longer than 1 ms, whereas the exchange of the water molecule ranges between 140 and 280 ns. This study also provided the first estimate of the exchange rate between free and bound forms of this molecule (the slowest amongst albumin-binding agents of this class).

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Table 1

| | $\Delta\nu_{1/2}$ of the broad component (Hz) | $\tau_R$ (ns) | Percentage of bound compound[a] |
|---|---|---|---|
| 4% of HSA+0.75 mM of La-analog of MP-2269 | 379 | ~7 | 84 (84) |
| 4% of HSA+0.25 mM of La-analog of MP 2269 | 400 | 8–9 | 90 (93) |

[a] Values in parentheses calculated for $K_a = 9000$ M$^{-1}$ and two equivalent binding sites.

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