

^1H and ^{17}O -NMR relaxometric investigations of paramagnetic contrast agents for MRI. Clues for higher relaxivities

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Abstract

The analysis of ^1H - and ^{17}O -NMR relaxometric data allows us to get a better insight into the understanding of the structural and dynamic factors responsible for the relaxivity of a given paramagnetic system. High relaxivities are obtained in the presence of a long molecular reorientational time and fast exchange of the water molecule(s) coordinated to the paramagnetic metal ion. Long molecular reorientational times are pursued either through the formation of covalent conjugates between a paramagnetic complex and a macromolecular substrate or through the formation of non-covalent adducts between suitably functionalized

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complexes and endogenous (e.g. serum albumin) or exogenous (e.g. poly- β -cyclodextrin) substrates. Within the class of covalent conjugates, it has been shown that the use of a DOTA-like chelate bearing the squaric acid as linking moiety leads to an improved relaxivity with respect to analogous systems obtained through reactions involving the bifunctional DTPA bisanhydride. As far as the exchange of the coordinated water in Gd(III) chelates is concerned, it depends on the energy difference between the ground ennea-coordinated state and the activated octa-coordinated state. In the presence of bulky substituents, the ground state is destabilized with a consequent increase of the exchange rate. An elongation of the exchange lifetime can occur upon the interaction with serum albumin. This behaviour may result in a decrease of the attainable relaxivity. Finally, it has been shown that Mn(II) chelates may represent a viable alternative to Gd(III) complexes. In fact, in spite of the lower effective magnetic moment, the non-covalent adducts between Mn(II) chelates and albumin display very high relaxivities. This result has been accounted for in terms of the very short exchange lifetime of the Mn(II) coordinated water molecule. © 1999 Elsevier Science S.A. All rights reserved.

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1. Introduction

The excellent soft tissue discrimination has made Magnetic Resonance Imaging (MRI) the modality of choice in modern medical diagnosis [1,2]. The contrast in an MR image is the result of a complex interplay of numerous factors, including the longitudinal (T_1) and transverse (T_2) relaxation times, proton density of the imaged tissues and instrumental parameters. MR image contrast can be further enhanced by the administration of suitable MRI contrast agents (CA).

Unlike contrast agents for X-ray or γ -scintigraphy, CA for MRI are not directly visualized in the image. Their effects on the tissue contrast are the result of their ability to reduce the tissue relaxation times. Gd(III) polyaminocarboxylate complexes were recognized early on as good candidates to act as CA for MRI as they display three basic properties: (i) the Gd(III) ion with its seven unpaired electrons possesses a high magnetic moment and a relatively long electronic relaxation time; (ii) polyaminopolycarboxylic ligands can efficiently wrap the lanthanide ion to form highly stable complexes which prevent the loss of harmful Gd(III) ions and (iii) the complexes are highly water-soluble allowing their administration under the form of concentrated solutions.

The ability to enhance proton relaxation rates in the tissue where they distribute is usually evaluated first 'in vitro' by the determination of their relaxivity (r_{1p}) which refers to the relaxation enhancement promoted by a given complex at a 1 mM concentration.

2. Determinants of the relaxivity of paramagnetic complexes

A number of parameters are responsible for the relaxation enhancement of water protons promoted by a paramagnetic complex. The observed relaxivity is often considered as the sum of two contributions [3]:

$$R_{1p}^H = R_{1p}^{His} + R_{1p}^{Hos} \quad (1)$$

where R_{1p}^{His} represents the contribution due to the dipolar interaction between unpaired electrons and water protons in the inner coordination sphere of the metal ion exchanging with the bulk water and R_{1p}^{Hos} is the contribution of the solvent molecules diffusing in close proximity to the paramagnetic center. For Gd(III) complexes the inner sphere relaxation rate is described in terms of the following set of equations [4]:

$$R_{1p}^{His} = \frac{[C] q}{55.56(T_{1M}^H + \tau_M)} \quad (2)$$

$$(T_{1M}^H)^{-1} \propto \frac{D^{is}}{r^6} J(\omega_I, \omega_S, \tau_{ci}) \quad (3)$$

$$\tau_{ci}^{-1} = \tau_M^{-1} + \tau_R^{-1} + T_{IE}^{-1} \quad (4)$$

$$T_{IE}^{-1} \propto K_i \Delta^2 J(\omega_S, \tau_V) \quad (5)$$

where $[C]$ is the molar concentration of the paramagnetic complex, q is the number of water molecules coordinated to the metal ion, τ_M is their mean residence lifetime, T_{1M} is their longitudinal relaxation time, r is the proton-Gd distance, τ_{ci} ($i = 1, 2$) are the overall correlation times for the dipolar nucleus–electron interaction, τ_R is the reorientational correlation time of the H–Gd position vector, T_{IE} is the averaged relaxation time of the Gd(III) unpaired electrons, D^{is} and K_i are constant terms related to the nuclear and electron relaxation mechanism, Δ^2 is the square of the transient zero field splitting (ZFS) energy, τ_V is the correlation time for the processes modulating the ZFS hamiltonian and ω_s and ω_I are the electronic and proton Larmor frequencies, respectively.

The outer-sphere relaxivity, which depends on the electronic relaxation time of the metal ion, on the distance of closest approach of solute and solvent and on the relative solute–solvent diffusion coefficients, is usually treated on the basis of the set of equations developed by Hwang and Freed [5].

The solvent proton relaxation rate has a magnetic field dependence through Eqs. (3)–(5) (and through the outer sphere term also) and thus the set of parameters involved in the relaxation process can be best obtained through a magnetic field dependent study. Experimentally, this is performed by measuring solvent longitudinal relaxation rates over a wide range of magnetic fields with a field-cycling spectrometer that rapidly switches the magnetic field strength over a range corresponding to the proton Larmor frequencies of 0.01–50 MHz. The data points represent the so-called Nuclear Magnetic Relaxation Dispersion (NMRD) profile that can be adequately fitted to yield the values of the relaxation parameters.

3. Evaluation of the exchange rate of the coordinated water

The exchange lifetime of the coordinated water molecule(s) can be obtained by analyzing the temperature dependence of the solvent water ^{17}O transverse relax-

ation rate. According to the Swift and Connick theory [6], the paramagnetic contribution R_{2p}^O to the observed relaxation rate is given by the following equation:

$$R_{2p}^O = \frac{[C]q}{55.6} (\tau_M^O)^{-1} \frac{R_{2M}^{O^2} + (\tau_M^O)^{-1} R_{2M}^O + \Delta\omega_M^{O^2}}{(R_{2M}^O + (\tau_M^O)^{-1})^2 + \Delta\omega_M^{O^2}} \quad (6)$$

where R_{2M}^O represents the ^{17}O transverse relaxation rate of the coordinated water molecule, τ_M^O its residence lifetime at the metal site and $\Delta\omega_M^O$ the chemical shift difference between the coordinated and bulk water ^{17}O -NMR resonances.

R_{2M}^O is determined as follows:

$$R_{2M}^O = \frac{1}{3} \left(\frac{A}{\hbar} \right)^2 S(S+1) \left(\tau_{E1} + \frac{\tau_{E2}}{1 + \omega_s^2 \tau_{E2}^2} \right) \quad (7)$$

and

$$\tau_{Ei}^{-1} = \tau_M^{O-1} + T_{iE}^{-1} \quad (8)$$

where S is the electronic spin quantum number (7/2 for Gd(III)), $(A/\hbar)$ is the Gd- ^{17}O scalar coupling constant, τ_{Ei} ($i = 1, 2$) represents the correlation times of the processes modulating the scalar interaction. This modulation may occur through both the longitudinal and the transverse average electronic relaxation times (T_{1E} and T_{2E}) and the mean residence lifetime (τ_M^O) of the water molecule at the paramagnetic site.

The temperature dependence of R_{2M}^O is determined by the temperature effect on τ_M^O , τ_v , and $\Delta\omega_M^O$ according to the following equations:

$$\tau_j^{-1} = \frac{(\tau_j^{-1})^{298.15} T}{298.15} \exp \left[\frac{\Delta H_j}{R} \left(\frac{1}{298.15} - \frac{1}{T} \right) \right] \quad (9)$$

$$\Delta\omega_M^O = \frac{g_e \mu_B S(S+1) B A}{3 k_B T \hbar} \quad (10)$$

where the subscript j refers to the different correlation times, ΔH_j is the activation enthalpy for the corresponding dynamic process, B is the magnetic field strength and k_B is the Boltzmann constant.

4. Routes to long τ_R values

It was recognized early on that high relaxivities at the imaging fields (0.5–1.5 T) may be observed for systems endowed with a long molecular reorientational time τ_R [7].

The occurrence of this condition is typically shown by a relaxivity peak in the high frequency region of the NMRD profile (Fig. 1).

One method of getting long τ_R values consists of forming covalent conjugates between the paramagnetic complex and slowly tumbling substrates such as albumin, polylysine, dextran and dendrimers [8,9]. The simplest route to attach a chelating moiety to such macromolecular systems is based on the ability of the bis-anhydride

DTPA to form amide and ester linkages with aminic and alcoholic functionalities available on the macromolecular surface.

The main drawback in the use of the bifunctional DTPA bis-anhydride stems from the occurrence of undesirable intra- and inter-molecular cross linking reactions. Furthermore, the diconjugation of DTPA is expected to cause a large reduction in the thermodynamic stability [10] of the metal complex as well as to display long exchange lifetime of the coordinated water (in the range of μs) which, in turn, may significantly limit the attainable relaxivity.

Since it has been recently reported that squaric esters readily react with amino groups under mild conditions [11], we have explored their use in the formation of stable linkages between Gd(III) complexes and $-\text{NH}_2$ groups on polylysine and polyornithine substrates characterized by a different chain length (Scheme 1) [12].

First, the ligand DO3ASQ was synthesized and then it was conjugated to the polyaminoacid derivative. According to the stoichiometry ratio between the number of ligand molecules and the available NH_2 groups, derivatives which differ in the number of attached chelates have been obtained. This allows easy control of the relaxation enhancement abilities of the various derivatives.

The system $(\text{GdDO3ASQ})_{30}\text{-Orn}_{114}$ (where 114 refers to the degree of polymerization of the polyaminoacid; M_w ca. 35 000 Da) shows a relaxivity at 39°C of $26.5 \text{ mM}^{-1} \text{ s}^{-1}$ which is significantly higher than those for macromolecular conjugates previously reported (for instance GdDTPA-polylysine has $r_{1p} = 13 \text{ mM}^{-1} \text{ s}^{-1}$) [8]. We think that such a behaviour may be related to the higher exchange rate of the coordinated water in GdDO3ASQ than in Gd-bisamide DTPA complexes. In fact, for the $(\text{GdDO3ASQ})_{30}\text{-Orn}_{114}$ system a τ_M value of 150 ns was obtained; this value is about one order of magnitude shorter than those observed for bifunctionalized DTPA derivatives.

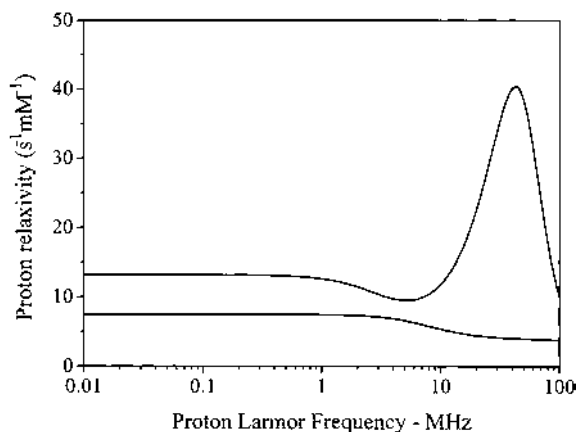
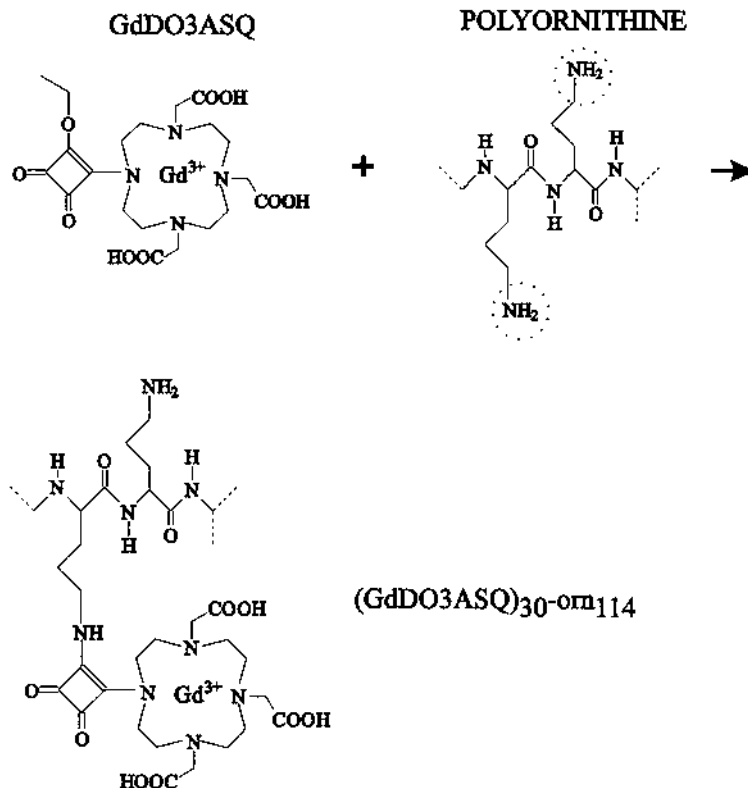


Fig. 1. Effect of the elongation of τ_R on NMRD profiles for a Gd(III) chelate: lower curve $\tau_R = 50 \text{ ps}$; upper curve $\tau_R = 50 \text{ ns}$. The other parameters used to calculate the curves are here reported: $\Delta^2 (\text{s}^{-2})/10^{19} = 5.0$, $\tau_v (\text{ps}) = 2.0$, $\tau_m (\text{ns}) = 200$, $r (\text{\AA}) = 3.0$, $q = 1$, $a (\text{\AA}) = 3.8$, $D (\text{cm}^2 \text{s}^{-1}) = 2.2 \times 10^{-5}$.



Scheme 1. Novel paramagnetic macromolecular complexes derived from the linkage of a macrocyclic Gd(III) complex to polyaminoacids through a squaric acid molecule.

An alternative route to get long τ_R values may be pursued by forming host-guest non-covalent interactions between suitably functionalized complexes and slowly tumbling substrates (Fig. 2). Over the last few years we have investigated the non-covalent interactions between a variety of substituted derivatives of GdDTPA^{2-} and GdDOTA^- with human serum albumin (HSA). We found that different binding sites may be available on the protein characterized by binding constants which cover a range of $\log K_A$ from 2 to 5 depending on both the number and the structure of the recognition synthons and the number of the negative charges on the paramagnetic chelate. The relaxivity of these macromolecular adducts are up to five times higher with respect to the free complex, on going from 15 to $53.0 \text{ mM}^{-1} \text{ s}^{-1}$ (20 MHz and 25°C) (Fig. 3). As in the case of covalently bound conjugates, the observed relaxation enhancements appear to be strongly dependent upon the exchange rate of water molecules in the inner coordination sphere. The occurrence of the condition $T_{1M} < \tau_M$ may be easily verified by looking at the behaviour of r_{1p} as a function of temperature. An increase of the relaxivity as the temperature increases is an indication that the attainable relaxivity of the

system (mainly determined by T_{IE} and τ_{R}) is, to some extent, ‘quenched’ by too slow an exchange rate of the coordinated water.

5. The issue of long τ_{M} values in Gd(III) chelates

The understanding that the exchange lifetime τ_{M} may be long enough to represent a limiting factor to the attainable relaxation enhancement promoted by Gd(III) complexes is rather recent. Actually, this drawback is an intimate consequence of the dissociative exchange mechanism occurring in ennea-coordinated system. In fact, when this mechanism is operating, the exchange rate is determined by the difference in energy between the ennea-coordinated ground state and the octa-coordinated transition state. On DTPA- and DOTA-like structures, the introduction of bulky substituents induces some strain in the coordination cage which results in a decrease in stability of the ground state with respect to the parent complexes and, in turn, in a shortening of τ_{M} . Of course, within the two main classes of Gd(III) complexes with $q=1$ (DTPA- and DOTA-derivatives), the overall residual charge is very important. In fact τ_{M} increases approximately by one order of magnitude on passing from the negatively charged GdDTPA^{2-} [13] to the neutral GdDTPA-BMA [14] and by another order of magnitude to the tripositively charged GdDTMA^{3+} [15] (DTMA = DOTA tetramethylamide). In the latter system the exchange rate of the coordinated water is so slow that the resonance of the coordinated water can be observed in the high resolution ^1H spectrum of the corresponding Eu(III) complex [16]. Moreover, since EuDTMA^{3+} is present as a pair of diastereoisomers (as occurs for the parent DOTA complex) [17] it has been possible to show that there is a large difference in the exchange rate of the coordinated water molecule in the two isomers. This finding is of high relevance to the design of more efficient contrast agents.

In principle, one may easily calculate from Eqs. (2)–(5) the optimum value of τ_{M} in order to attain the higher relaxivity in slowly moving systems. It appears that

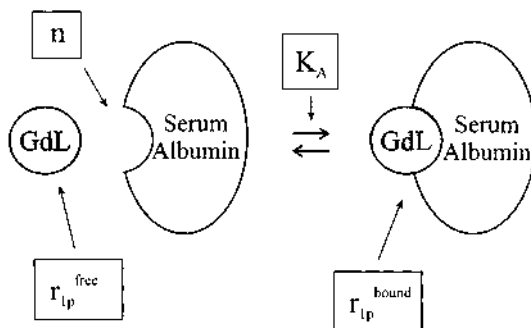


Fig. 2. Schematic representation of the non-covalent interaction between a Gd(III) chelate (GdL) and serum albumin (n is the number of interaction sites, K_{A} is the association equilibrium constant and $r_{\text{lp}}^{\text{free}}$ and $r_{\text{lp}}^{\text{bound}}$ refer to the relaxivity of the free and bound chelate, respectively).

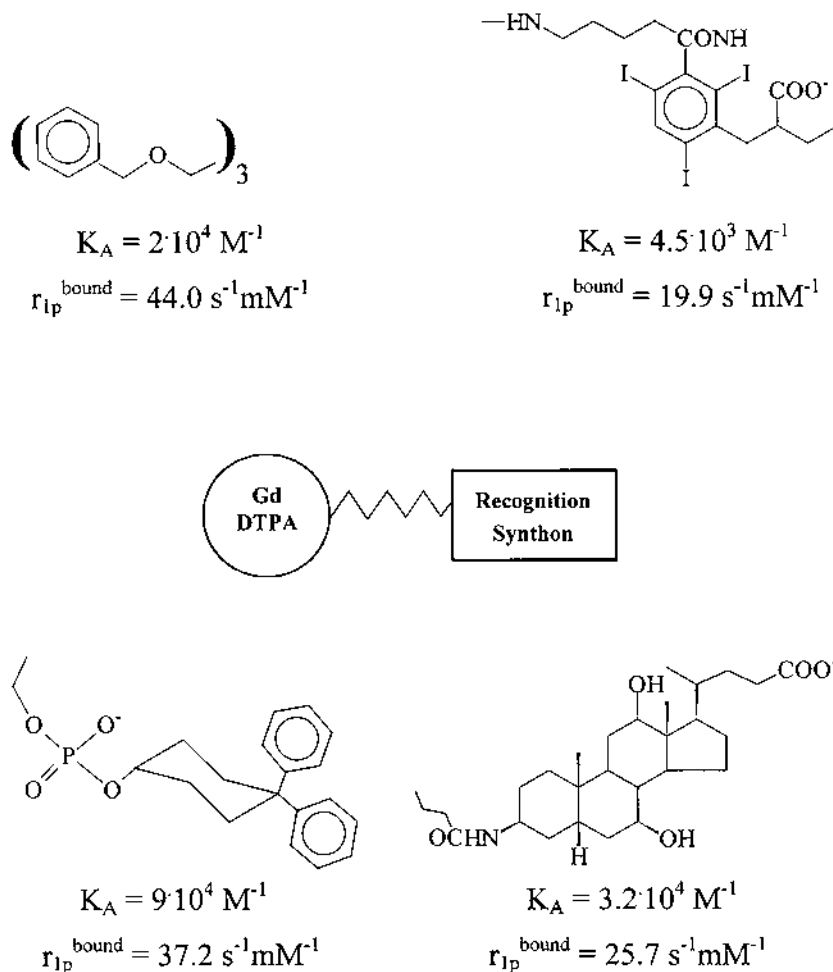
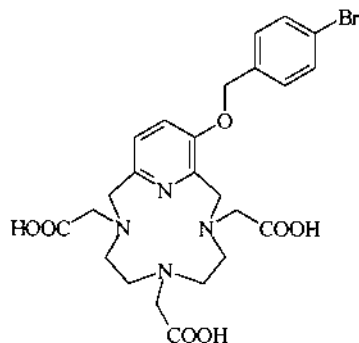


Fig. 3. K_A and r_{1p}^{bound} values of some non-covalent adducts between HSA and GdDTPA-like chelates functionalized with suitable recognition synthons (25°C, 20 MHz).

optimum values are in the range of 10–30 ns [18] and, in the presence of long electronic relaxation times, this would allow the attainment for Gd(III) chelates of relaxivities higher than $100 \text{ mM}^{-1} \text{ s}^{-1}$. Values close to these figures have been measured in the case of GdEGTA[−] [19]. However, as anticipated above, it may happen that a further elongation of the water residence lifetime may result in the formation of the adduct with HSA as a consequence of a steric constrain at the water coordination site caused by the interaction mode of the complex with the protein surface [20]. This drawback should be overcome by developing a suitable CA endowed with a rigid spacer in order to separate, as much as possible, the recognition synthon from the paramagnetic chelate.



Scheme 2. 12-Membered, pyridine containing, macrocyclic triacetate ligand (PCTA) with a *p*-bromobenzyloxy substituent on the pyridine ring.

6. Other routes to enhanced relaxivities: interaction with poly- β -cyclodextrin

In this paragraph we deal with a novel route to get MRI CA based on the formation of non-covalent adducts with macromolecular systems, which are represented by exogenous materials administered together with the paramagnetic complex. To this purpose a 12-membered, pyridine containing, macrocyclic triacetate ligand (PCTA) with a *p*-bromobenzyloxy substituent on the pyridine ring has been synthesized (Scheme 2) [21]. Its Gd(III) complex shows a relaxivity of $8.25 \text{ mM}^{-1} \text{ s}^{-1}$ at 20 MHz and 25°C . The presence of the hydrophobic *p*-bromobenzyloxy substituent promotes the formation of a tight inclusion complex with β -cyclodextrin (β -CD) ($K_A = 800 \text{ M}^{-1}$) whose relaxivity is 2.1 times higher than that of the free

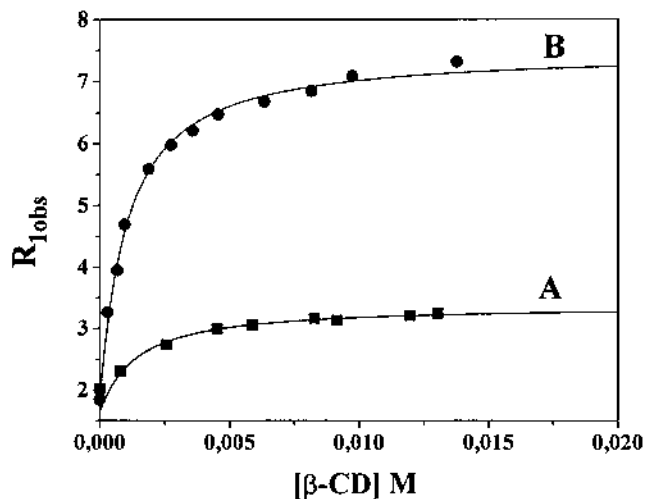


Fig. 4. Longitudinal water proton relaxation rate of a 0.2 mM Gd-PCTA-*p*-bromobenzyloxy solution as a function of the β -cyclodextrin ($\blacksquare$) and poly- β -cyclodextrin ($\bullet$) concentration (20 MHz, 25°C).

complex (Fig. 4A). The observed relaxation enhancement is not huge because the molecular weight of the adduct is only twice as larger with respect to the free chelate. A simple extension of this approach led us to consider a poly- β -CD derivative as a substrate for the formation of larger inclusion complexes with the paramagnetic chelate. Poly- β -cyclodextrins are systems containing several β -CD units linked through the condensation of C6 hydroxyls systems of different β -CD units. The poly- β -CD used in this work is a mixture of oligomers containing from three to nine units. The average binding strength for the paramagnetic inclusion compounds is slightly higher ($K_A = 1100 \text{ M}^{-1}$) than that observed for the monomeric β -CD. Being that the interaction with HSA is significantly weaker, one concludes that, following the simultaneous administration of the Gd(III) complex and poly- β -CD in blood, the paramagnetic complex is mostly present in the form of the inclusion compound. The observed relaxation enhancement for the poly- β -CD/Gd(III) chelate is 4.1 with respect to the free complex (4B).

The analysis of the NMRD profile for the free chelate and for the two inclusion compounds with β -CD and poly- β -CD shows that the differences of the relaxivity for the three systems are entirely due to changes in the reorientational molecular correlation time τ_R . Since the other relaxation parameters do not change upon the interaction with the cyclodextrin systems, one may speculate on the maximum relaxivity is attainable once the proper τ_R for the macromolecular adduct is available. Simple calculations show that τ_R could reach the value of 30 ns before τ_M starts to limit the attainable relaxivity. Under these conditions a relaxivity value of about $140 \text{ mM}^{-1} \text{ s}^{-1}$ (at 25°C) would have been expected! It does not seem impossible to synthesize a polymeric β -cyclodextrin derivative endowed with such a τ_R value.

7. Can Mn(II) chelates represent a viable alternative to Gd(III) complexes?

Until now, only one Mn(II) chelate (MnDPDP, Mangafodipir[®], Nycomed) has been approved for clinical use as contrast agent for MRI [22]. It has been proposed as an hepatobiliary CA and its effect is due to the release of the Mn(II) ion which is taken up by the hepatocytes. In principle, Mn(II) complexes endowed with high thermodynamic stability may operate relaxation catalysis in an analogous way to Gd(III) chelates. In this context, we have been interested firstly to assess whether Mn(II) chelates with $q = 1$ display water exchange rates of the coordinated water faster than those observed in the related Gd(III) complexes.

To this purpose, ^1H - and ^{17}O -NMR relaxometric properties of Mn(II)EDTA have been considered. The proton relaxivity of this complex with $q = 1$ is $3.3 \text{ mM}^{-1} \text{ s}^{-1}$ [7a], i.e. ca. 70% of the value reported for Gd(III)DTPA at the same temperature (298 K) and frequency (20 MHz) which is the most used among the commercially available CA for MRI. From the analysis of the temperature dependence of the water ^{17}O - R_{2p} , a τ_m value of 2.3 ns [23] has been obtained at ambient temperature. This finding appears highly encouraging as an such exchange lifetime is two orders of magnitude smaller than those found for the corresponding Gd(III)

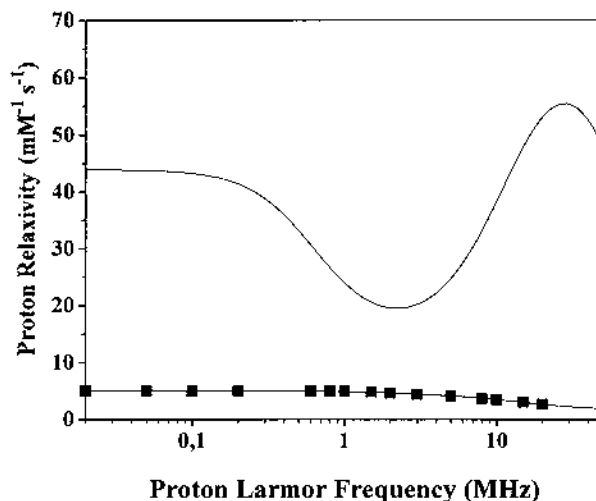


Fig. 5. The filled squared represents the r_{1p} values obtained for a solution containing 1 mM Mn(II)EDTA. This curve has been fitted through Eqs. (1)–(5) [23]. The upper curve reports a simulated NMRD profile of a macromolecular adduct based on the relaxation parameters found for Mn(II)EDTA but with a molecular reorientational time typical of the serum protein ($\tau_r = 3.0 \times 10^{-8}$ s).

complexes with $q = 1$ (e.g. 300 ns [24] for Gd(III)DTPA). A simulation (Eqs. (2)–(5)) has then been carried out by using the relaxation parameters obtained from the analyses of the NMRD [25] profile and of the VT ^{17}O – R_{2p} [23] curve of Mn(II)EDTA but introducing a τ_r value (3×10^{-8} s) typical of the slowly moving macromolecules. The resulting profile (Fig. 5) should correspond to that expected for a paramagnetic adduct between a suitably functionalized Mn(II)EDTA derivative and a protein like HSA. A relaxivity peak of $55 \text{ mM}^{-1} \text{ s}^{-1}$ occurs at ca. 30 MHz, i.e. in the range of the highest values reported up to now for macromolecular adducts between Gd(III) chelates (with $q = 1$) and HSA.

Thus, in spite of the lower effective magnetic moment, Mn(II) complexes appear to compete quite well for high relaxivities with Gd(III) complexes and it is worth pursuing the synthesis of suitably functionalized ligands with the ability to promote the binding of the Mn(II) chelate to HSA. Indeed preliminary results on Mn(II)EDTA-like complexes are in good agreement with these expectations.

8. Conclusions

Detailed ^1H - and ^{17}O -NMR relaxometric investigations provide a deep understanding of the determinants involved in the relaxation processes of solutions containing paramagnetic complexes. This allows the design of novel CA endowed with a higher efficacy in operating the relaxation catalysis of water protons. The availability of improved CA allows a decrease in the dose commonly administered in an MRI assay. Furthermore, CA based on clusters of paramagnetic chelates on

a macromolecular matrix may be exploited in the targetting of selected tissues and organs.

The huge deal of data reported in recent years on the relaxivity of paramagnetic chelates provides the chemist with the tools to control key-parameters like τ_R and τ_M . Very high relaxivities ($> 100 \text{ mM}^{-1} \text{ s}^{-1}$) still await a better understanding of the relationships between solution structure and dynamics of paramagnetic chelates and their electronic relaxation times.

By now, it is noteworthy that the search for enhanced relaxivities based on systems endowed with slow tumbling rates has provided novel CA which may be particularly useful in the development of Magnetic Resonance Angiographic (MRA) applications. In fact, such macromolecular adducts (either covalent or non-covalent) display longer plasmatic half-life, a basic requisite for a good CA for MRA.

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References

- [1] D.D. Stark, W.G. Bradley (Eds.), *Magnetic Resonance Imaging*, The V.C. Mosby Company, St. Louis, 1988.
- [2] P.A. Rinck (Ed.), *Magnetic Resonance in Medicine*, Blackwell Scientific Publications, Oxford, 1993.
- [3] S.H. Koenig, R.D. Brown III, *Prog. NMR Spectrosc.* 22 (1990) 487.
- [4] L. Banci, I. Bertini, C. Luchinat, *Nuclear and Electron Relaxation*, VCH, Weinheim, 1991.
- [5] L.P. Hwang, J.H. Freed, *J. Chem. Phys.* 63 (1975) 118.
- [6] T.J. Swift, R.E.J. Connick, *J. Chem. Phys.* 37 (1962) 307.
- [7] (a) R.B. Lauffer, *Chem. Rev.* 87 (1987) 901. (b) J.A. Peters, J. Huskens, D.J. Raber, *Prog. NMR Spectrosc.* 28 (1996) 283.
- [8] R.C. Brasch, *Magn. Reson. Med.* 22 (1991) 282.
- [9] E.C. Wiener, M.W. Brechbiel, H. Brothers, R.L. Magin, O.A. Gansow, D.A. Tomalia, P.C. Lauterbur, *Magn. Reson. Med.* 1 (1994) 1.
- [10] A.D. Sherry, W.P. Cacheris, K. Kah-Tiong, *Magn. Reson. Med.* 8 (1988) 180.
- [11] L.F. Tietze, C. Schroter, S. Gabius, U. Brinck, A. Goerlach-Graw, H.J. Gabius, *Bioconjugate Chem.* 21 (1991) 48.
- [12] S. Aime, M. Botta, S. Geninatti Crich, G.B. Giovenzana, G. Palmisano, M. Sisti, *Bioconjugate Chem.* (1999), in press.
- [13] K. Micksei, L. Helm, E. Brücher, A.E. Merbach, *Inorg. Chem.* 31 (1993) 1011.
- [14] G. González, D.H. Powell, V. Tissières, A.E. Merbach, *J. Phys. Chem.* 98 (1994) 53.

- [15] S. Aime, A. Barge, M. Botta, D. Parker, A.S. De Sousa, *J. Am. Chem. Soc.* 119 (1997) 4767.
- [16] S. Aime, A. Barge, M. Botta, A.S. De Sousa, D. Parker, *Angew. Chem.* 37 (1998) 2673.
- [17] S. Aime, M. Botta, G. Ermondi, *Inorg. Chem.* 31 (1992) 4291.
- [18] S. Aime, M. Botta, M. Fasano, E. Terreno, *Chem. Soc. Rev.* 27 (1998) 19.
- [19] S. Aime, A. Barge, A. Borel, M. Botta, S. Chemerisov, A.E. Merbach, U. Müller, D. Pubanz, *Inorg. Chem.* 36 (1997) 5104.
- [20] S. Aime, M. Botta, S. Geninatti Crich, G.B. Giovenzana, R. Pagliarin, M. Piccinini, M. Sisti, E. Terreno, *J. Biol. Inorg. Chem.* 2 (1997) 470.
- [21] S. Aime, M. Botta, L. Frullano, S. Geninatti Crich, G.B. Giovenzana, R. Pagliarin, G. Palmisano, M. Sisti, *Chemistry Eur. J.* (1999), in press.
- [22] A. Graul, P.A. Leesson, J. Castaner, *Drugs Future* 22 (1997) 974.
- [23] M.S. Zetter, M.W. Grant, E.J. Wood, H.W. Dodgen, J.P. Hunt, *Inorg. Chem.* 11 (1972) 2701.
- [24] D.H. Powell, O.M. Ni Dhubhghaill, D. Pubanz, L. Helm, Y.S. Lebedev, W. Schlaepfer, A. Merbach, *J. Am. Chem. Soc.* 118 (1996) 9333.
- [25] S.H. Koenig, C. Baglin, R.D. Brown III, *Magn. Reson. Med.* 1 (1984) 496.