Second Coordination Sphere Water Molecules and Relaxivity of Gadolinium(III) Complexes: Implications for MRI Contrast Agents

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Dipolar interaction between the metal ion and proximate water molecules represents an efficient mechanism for solvent relaxation in Gd^{3+} complexes currently employed as MRI contrast agents. Besides *inner sphere* (metal bound) and *outer sphere* hydration molecules, a well-defined second coordination shell may provide an additional mechanism for paramagnetic relaxation leading to a strong enhancement of the relaxivity of the complexes. Through a careful choice of hydrogen-bond-acceptor groups on the ligand we may: (1) promote the formation of a strong interaction; (2) increase the number of water molecules in the second hydration shell; (3) decrease their average distance from the paramagnetic metal center. These possibilities have been explored by considering complexes bearing phosphinate, phosphonate and carboxoamide pendant arms, by exploiting the formation of ion-pairs with cationic substrates and inclusion compounds of these adducts with β-cyclodextrin. Finally, the contribution of this relaxation mechanism to the relaxivity of the commercially available MRI contrast agents is discussed and the NMRD data reevaluated and compared with crystallographic data.

Introduction

The emergence of MRI as an important clinical tool has fostered the development of agents designed to enhance tissue differentiation.[1] These drugs, mainly complexes of Gd^{3+} and Mn^{2+}, are useful for increasing the image contrast between normal and diseased tissue and/or providing dynamic tissue function. Unlike the contrast agents (CAs) used in other clinical imaging studies, these paramagnetic complexes are not themselves directly imaged but rather enhance contrast indirectly by affecting the nuclear magnetic relaxation times of the water protons in surrounding tissues. Because Gd^{3+} has particularly favorable magnetic properties, complexes of this ion have been the primary focus for MRI contrast agent development.[2] The clinically approved CAs are stable, highly water soluble poly(aminocarboxylate) derived complexes (Gd^{3+} complexes with ligands **1–4**, Scheme 1). The requirements to design a practical, general, MRI CA can be summarized as follows:

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Mauro Botta was born in Cuneo in 1958. He studied chemistry at the University of Torino where he received the Laurea degree in 1985. After three years spent as a research assistant in the Department of Chemistry working in the group of Prof. Silvio Aime, he held a tenure of Researcher (1990–1998) at the faculty of Pharmacy of the University of Torino and was nominated associate Professor (1998) at the University of Piemonte Orientale "Amedeo Avogadro." In 1998 he was awarded the Nasini Medal of the Inorganic Chemistry Division of the Italian Chemical Society. His research has mainly focused on the application of NMR spectroscopy to investigate solution and solid state properties of metal carbonyl clusters and coordination complexes for biomedical applications.

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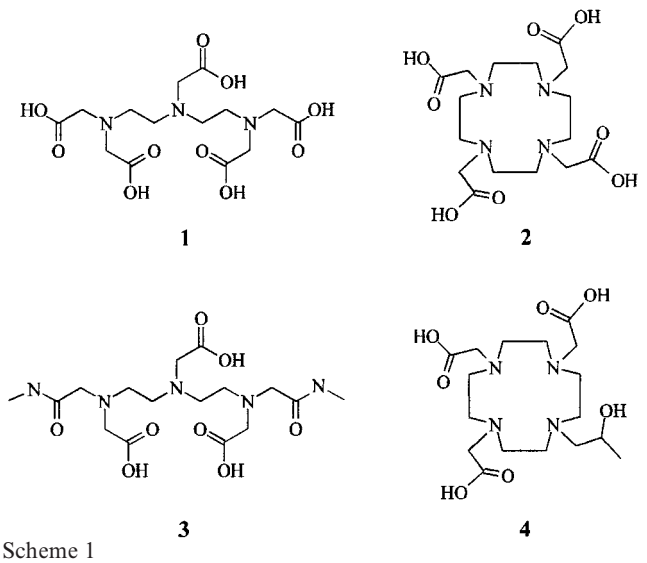


Scheme 1

(1) *High stability.* The injection of gram amounts of gadolinium into patients presents some potential toxicity problems which requires strong metal complexation by the parent ligand.

(2) *Good water solubility and low osmolality of the solutions used clinically.* Injection of relatively small volumes of a fairly concentrated solutions of the metal complex is required. Neutral complexes have been developed with the goal of lessening the discomfort from injection of high osmolality salt solutions (Gd-**3** and Gd-**4**).

(3) *High relaxivity.* The relaxivity (r_{1p}) is an intrinsic property of a paramagnetic complex that characterize its ability to enhance the nuclear magnetic relaxation rates (R_i, i = 1,2) of nearby protons and is defined as the increase in R_i per millimolar concentration of the CA.[1–3]

The dependence of the relaxivity on structural features of the paramagnetic compounds has represented an important area of research in the field of inorganic chemistry over the last ten years. These studies have made significant contributions towards a rational design of MRI CAs, opened new perspectives in the use of paramagnetic complexes as diagnostic probes and increased our knowledge of the coordination chemistry of lanthanide ions. The general properties of MRI CAs,[1b] the determinants of the relaxivity,[2] the crucial role of the water exchange rate,[4] and the perspectives for attaining high relaxivities[5] have been recently reviewed. Here I will discuss recent results that have allowed us to pinpoint a direct role of the second coordination shell water molecules in determining the relaxivity of the paramagnetic complexes, the strategies used for optimizing this contribution and the implications for the analysis of relaxation data for MRI CAs.

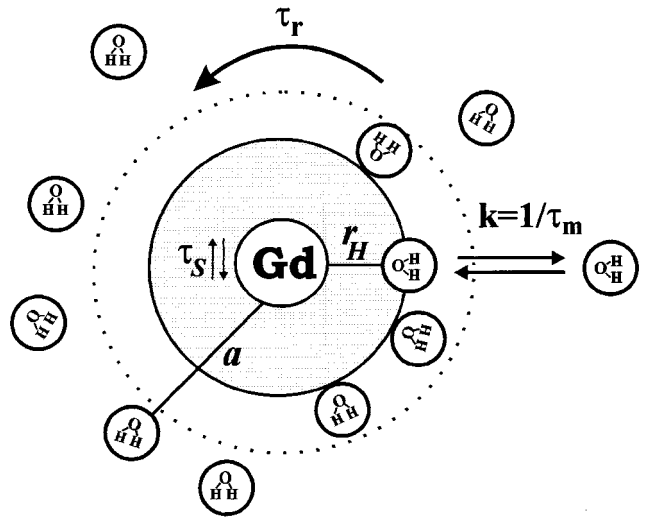


Figure 1. Schematic representation of the three types of hydration layers around a Gd^{3+} complex and the most relevant parameters of paramagnetic relaxation

Relaxivity: Contributions and Parameters

The relaxivity enhancement of water protons in aqueous solutions of Gd^{3+} complexes arises from time fluctuation of the dipolar coupling between the electron magnetic moment of the metal ion and the nuclear magnetic moment of the solvent nuclei.[6] This interaction is traditionally described with a model that considers two contributions (Figure 1): *inner sphere* (*IS*), due to water molecules present in number q in the coordination sites of the Gd^{3+} ion, and *outer sphere* (*OS*), which involves all the solvent molecules diffusing by the complex:

$$R_1^{obs} = R_{1p}^{IS} + R_{1p}^{OS} + R_1^W \quad (1)$$

where R_1^{obs} is the measured relaxation rate and R_1^W the relaxation rate of the solvent in the absence of the paramagnetic complex.

Inner Sphere

This term represents the most important contribution to r_{1p} and thus the efforts towards contrast agents of improved efficacy have been directed mainly towards its optimization. The large number of experimental data recorded over the last 15 years on small Gd^{3+} chelates have been interpreted on the basis of the Solomom-Bloembergen-Morgan approach,[7] and have provided a good understanding of the role played by the different relaxation parameters, of their mutual relationship, and of their dependence on the structural features of the complexes.[1–5,8] The relevant Equations are:

$$R_{1p}^{IS} = \frac{[M]q}{55.6}\frac{1}{T_{1M}+\tau_M} \quad (2)$$

$$\frac{1}{T_{1M}} = \frac{2}{15}\frac{\gamma_H^2 g^2 S(S+1)\beta^2}{r_H^6}\left[\frac{3\tau_{C1}}{1+\omega_H^2\tau_{C1}^2}+\frac{7\tau_{C2}}{1+\omega_S^2\tau_{C2}^2}\right] \quad (3)$$

$$\frac{1}{\tau_{Ci}} = \frac{1}{\tau_R} + \frac{1}{\tau_M} + \frac{1}{\tau_{Si}} \quad (4)$$

where M is the concentration of the CA, T_{1M} the longitudinal relaxation time of the bound water protons, S is the electron spin quantum number (7/2 for Gd^{3+}), γ_H is the proton nuclear magnetogyric ratio, β is the Bohr magneton, g is the Landè factor for the free electron, r_H is the distance between the metal ion and the bound water protons; ω_H and ω_S are the proton and electron Larmor frequencies, respectively, and τ_{Ci} (i = 1,2) are the correlation times of the modulation of the dipolar electron-proton coupling. The overall correlation times τ_{Ci} receive contributions from the reorientation of the paramagnetic species, τ_R, by the residence lifetime of the bound water protons, τ_M and by the electronic relaxation times of the metal ion, τ_S. A marked enhancement of r_{1p} at the magnetic fields currently employed clinically has been obtained primarily by slowing down the molecular tumbling (increasing τ_R) by different approaches.[9–15] However, the expected increase of r_{1p} has not been observed because of the limiting effect of the mean water residence lifetime τ_M.[2,4,11,12] A fine tuning of this parameter has emerged as a primary objective of the current research in this field, as only values around 30 ns would allow the full exploitation of the decrease of T_{1M} induced by the lengthening of τ_R.

Outer Sphere

This contribution accounts for about 40% of the relaxivity of monoaquo Gd^{3+} complexes at the imaging fields and arises from the modulation of the dipolar interaction by diffusion of the solvent molecules next to the paramagnetic center:

$$R_{1p}^{OS} = C^{OS}\left(\frac{1}{aD}\right)\left[7J(\omega_S) + 3J(\omega_H)\right] \quad (5)$$

$$J(\omega) = Re\left[\frac{1 + 1/4\left(i\omega\tau_d + \frac{\tau_d}{\tau_{Si}}\right)^{1/2}}{1 + \left(i\omega\tau_d + \frac{\tau_d}{\tau_{Sj}}\right)^{1/2} + 4/9\left(i\omega\tau_d + \frac{\tau_d}{\tau_{Sj}}\right) + 1/9\left(i\omega\tau_d + \frac{\tau_d}{\tau_{Sj}}\right)^{3/2}}\right] \quad (6)$$

$$with \quad j = 1,2; \quad \tau_d = \frac{a^2}{D}$$

where C^{OS} is a constant (5.8×10^{-13} s^{-2} M^{-1}) and the non-Lorentzian spectral density functions $J(\omega_i)$ contain the dependence on τ_S. At the magnetic fields of interest it depends primarily on the distance of closest approach a, related to the molecular dimension and charge distribution of the complex, and on the relative diffusion coefficient of solute and solvent D. It follows that r_{OS}^{1p} assumes a comparable value (above 10 MHz) for all the small gadolinium chelates of clinical interest.

Second Coordination Sphere: A Third Contribution to Relaxivity

In principle the outer sphere relaxivity can also receive contributions by another mechanism: fluctuation of the dipolar electron-nuclear coupling involving water molecules held in the second coordination shell of the paramagnetic ion by hydrogen bonding interactions with polar groups of the ligand (Figure 1). This term (2S) has to be distinguished from the traditional diffusion-controlled outer-sphere relaxivity only when the residence lifetime of the structured water in the second shell is longer than the diffusional correlation time τ_D ($\tau_D = a^2/D$). In this latter case the quantitative description of the effects on the relaxivity is analogous to that of the inner sphere water molecules (Equation 2), as clearly pointed out by Lauffer.[1b] Firm experimental evidence for the occurrence of this solvent/metal complex interaction is difficult to obtain and, in most cases, its consideration may only represent a negligible correction to the inner- and outer-sphere relaxivities in the traditional model. However, in several cases, a careful analysis of the relaxation rate of the solvent as a function of magnetic field, pH and temperature allowed the evaluation of this contribution and the extraction of more useful and correct information from the data analysis.

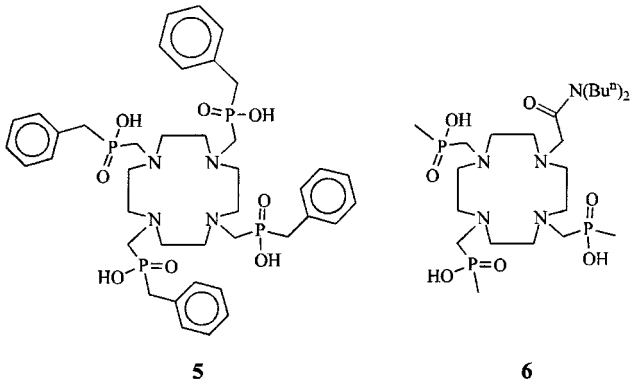
In 1981 Koenig et al. reported that replacement of the iron(III)-bound water molecule in methemoglobin by fluoride ion increases the relaxation rate drastically.[17] It was shown by variable temperature relaxivity data that the heme-iron-coordinated water molecule in met-Hb is kinetically inert as it is stabilized by a hydrogen bond with a proximate hystidine residue, and that the relaxivity enhancement upon fluoride binding can be attributed to a labile water molecule hydrogen bonded to the coordinated anion. Such structural arrangement brings a proton of this second sphere water molecule close enough to the paramagnetic center to be strongly relaxed. Recently this interpretation was confirmed by a crystallographic study that revealed the presence of a water molecule hydrogen bonded to the fluoride anion at a distance of 2.71 Å.[18] In 1984 Kushnir and Navon investigated the water relaxation-rate enhancement by Mn^{2+} bound to macromolecules and found the presence of an additional contribution to r_{1p}^{IS} which dominated the observed relaxivity at high fields and originated from outer sphere solvent molecules.[19] This additional contribution could not be explained on the basis of a spin-diffusion mechanism from the protein protons or of the usual dipolar interaction with the diffusing solvent molecules. A good quantitative account was obtained by considering water molecules bound outside the first hydration shell with an average lifetime of 0.3 ns.

The possible contribution of r_{1p}^{2S} to the overall relaxivity of small paramagnetic complexes has seldom been considered in the past. Oakes and Smith studied the proton relaxation properties of $[Mn(EDTA)(H_2O)]^{2-}$ and attributed the outer sphere contribution to the presence of

four water molecules hydrogen bonded to the four coordinated carboxylate groups.[20] The calculated Mn–H distance for these water molecules is 3.7 Å, a value that seems reasonable even though the validity of this very simple model is questionable, and it has not been checked by a magnetic field- and temperature-dependent study. More recently, Merbach et al. reported a detailed ^{17}O NMR and computer modeling study on the structure and dynamics of the second coordination shell water molecules around the Cr^{3+} hexaaqua ion.[21] In the case of this ion, which is endowed with a kinetically inert first hydration shell, where the bonding occurs through the O-atom of the water molecules, the authors were able to assess the presence of 13 water molecules in the second shell with an average lifetime of 128 ps. On the other hand no evidence was found for a well defined second shell in the case of a cation of larger ionic radius like Ln^{3+}.[22] It is worth noting that only in these latter two cases, characterized by an isotropic disposition of the ligand molecules around the metal ion, is the term "second sphere" strictly applicable. In the polyamminopolycarboxylate(-polyphosphonate) complexes considered here, the widely used definition of second sphere can be misleading as it refers exclusively to water molecules hydrogen-bonded to polar groups of the ligand present on the hydrophilic side of the metal complex.

Gd^{3+} Complexes

The lanthanide complexes with the macrocyclic tetrabenzylphosphinate ligand **5** (Scheme 2) have been characterized both in the solid state and in solution, where they are present as a single diastereoisomer with an inverted square antiprismatic coordination polyhedron.[23]



5 **6**

Scheme 2

Unlike the case of the carboxylate analogues, the higher steric bulk of the phosphinate groups prevents the access to the metal ion of the water molecules and the complexes have $q = 0$. Therefore, the relaxivity of [Gd-**5**]$^-$ does not present the complications of the inner sphere component and its NMRD (**N**uclear **M**agnetic **R**elaxation **D**ispersion, i.e. the magnetic field dependence of the relaxivity) profile is rather simple and characterized by a shape and an amplitude as predicted by the translational diffusion model of pure outer sphere relaxivity (Figure 2). Fitting of the data yields a value of 4.35 Å for the distance a of closest approach of the protons of the solvent molecules, in good agreement with the value estimated from the X-ray structure.[23]

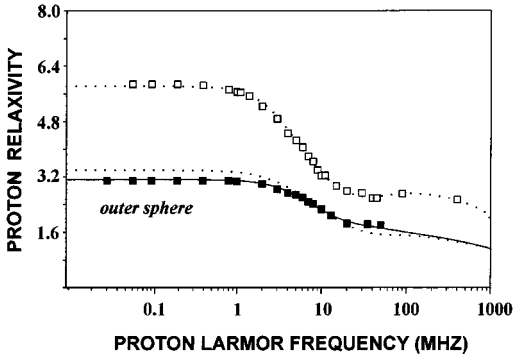


Figure 2. NMRD profiles (25 °C) of [Gd-**5**]$^-$ (filled squares) and Gd-**6** (open squares) and best-fit curves; the dotted line at the bottom is the calculated outer-sphere contribution for [Gd-**6**]$^-$

By substituting one phosphinate group with a less bulky carboxoamide **6** we obtain neutral Gd^{3+} complexes, of potential interest as low osmolality MRI CA, and furthermore we favor the access of water to the paramagnetic center by releasing part of the steric encumbrance.[24] The NMRD profile has the typical shape of $q = 1$ complexes such as [Gd-**1**]$^{2-}$, [Gd-**2**]$^-$ and Gd-**4**, but its magnitude is significantly lower. On the other hand, assuming $q = 0$ would imply for Gd-**6** either an a value shorter than the molecular radius or a dramatic change of the diffusional dynamics of its aqueous solution. The profile shows a small hump of relaxivity around 100 MHz, particularly evident at lower temperatures, which suggests a dependence of the relaxivity upon the rotational dynamics of the complex (τ_R), which substantiates the hypothesis of the presence of an "inner sphere" contribution. This increase in relaxivity observed on passing from pure outer-sphere complexes such as [Gd-**5**]$^-$ to Gd-**6** can be quantitatively explained on the basis of the presence of a tightly bound water molecule in the second coordination sphere of the metal ion at a distance of ca. 3.6 Å and with a sufficiently long lifetime. This interaction is likely to involve a hydrogen bond between the carboxoamide oxygen atom and a water molecule, which results in a metal–proton distance short enough to affect the solvent relaxation (Figure 3). Interestingly, a good correlation has been found in a series of related complexes between the r_H values derived from NMRD analysis and the average hydration state q ($0 < q < 1.3$) obtained by luminescence measurements on the Eu^{3+} derivatives.[24b]

This suggested the hypothesis that, besides the pure outer- ($q = 0$) and inner-sphere ($q = 1$) limiting cases, complexes with octadentate ligands may assume in solution any intermediate value depending on the number and nature of the hydrogen-bond acceptor groups of the ligand. In other words, this implies that fractional q values (and long r_H distances) can be correlated to the existence of a well-defined second hydration shell of the metal ion.[24b,25]

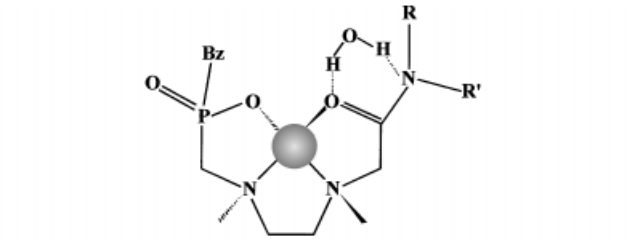
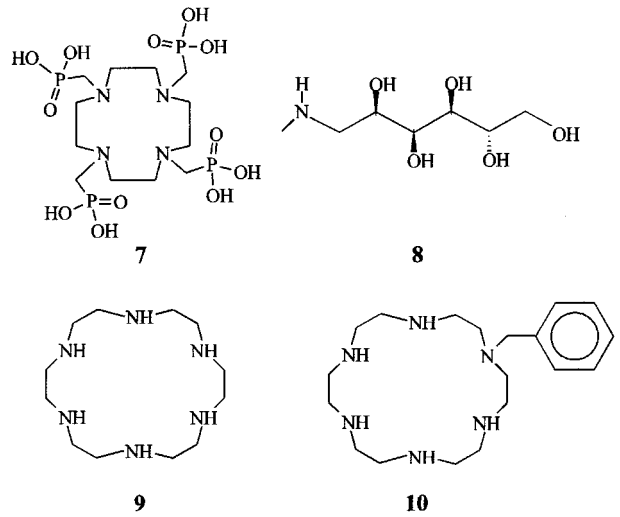


Figure 3. Schematic view of Gd-**6** showing the possible location of the second sphere water molecule

These results caused the reconsideration of the case of the anionic tetraphosphonate macrocyclic complex [Gd-**7**]$^{5-}$, whose solution structure is strictly related to that of the complexes [Ln-**5**]$^{-}$.[26] In recent years Ln^{3+} complexes of **7** (Scheme 3) have been extensively investigated as effective shift reagents for metal cations (Dy^{3+}, Tb^{3+} and Tm^{3+}), relaxation agents for MRI and probes of the charged regions of protein surfaces.



Scheme 3

The relaxation properties of [Gd-**7**]$^{5-}$ present unusual features whose interpretation has originated conflicting explanations: formation of oligomers,[27] fractional number q of coordinated waters (mixture of isomers differing in the q value),[27] one coordinated water molecule ($q = 1$) with an unusually long r_H distance.[28] The NMRD profile shows an anomalous behavior: on the basis of considerations of symmetry, rigidity and molecular dimension [Gd-**7**]$^{5-}$ is expected to show relaxivity values greater than the structurally similar [Gd-**2**]$^{-}$ over the entire frequency range. Nevertheless, the NMRD profile of [Gd-**7**]$^{5-}$ falls well below that of [Gd-**2**]$^{-}$ (but well above that of Gd-**6**) and the best-fitting analysis of the experimental data to the established theory of inner- and outer-sphere relaxivity components yielded a distance $r_H = 3.26$ Å for the inner sphere water molecule.[28] This value is significantly longer than that obtained by X-ray analysis of related macrocyclic Ln^{3+} complexes or derived from NMRD analysis and is therefore appears disputable. By considering that the phosphinate complexes of the type [Gd-**5**]$^{-}$ and Gd-**6** have $q = 0$ and that the high negative charge distributed over the eight uncoordinated oxygen atoms of the phosphonate groups may promote the formation of strong hydrogen bonds with the solvent molecules, we could interpret the relaxivity of [Gd-**7**]$^{5-}$ by considering a strong second-sphere contribution. By adopting values for a and D similar to those found for [Gd-**5**]$^{-}$ and Gd-**6**, and by allowing for the second-sphere water molecules an average distance in the range 3.3–4.0 Å, an excellent fit of the NMRD data is obtained for two solvent molecules localized at a distance of 3.89 Å. This result, although in good agreement with the available structural data for related complexes, represents only a rough estimation since there is probably an array of hydrogen-bonded solvent molecules characterized by different distances r_H and residence lifetimes τ_M. Moreover, these water molecules may present different rotational mobilities and the assumption of a single τ_R appears unlikely. What does seem reasonable is to attribute the nearly 50% increase of relaxivity of [Gd-**7**]$^{5-}$ versus [Gd-**5**]$^{-}$ to structured solvent molecules in the second coordination shell of the metal ion involved in a relatively strong bonding interaction with the oxygen atoms of the phosphonate groups. A strong support for this hypothesis was found by the measurement of the ^{17}O transverse relaxation rate (R_2^Q) as a function of temperature, a well-recognized method for obtaining information on the dynamics of the metal-bound water molecule(s). The profile of R_2^Q with temperature is typical of a $q = 0$ complex and thus confirms the absence of any *IS* water molecule for [Gd-**7**]$^{5-}$.[29] The case of [Gd-**7**]$^{5-}$ is particularly striking as it shows that a few solvent molecules held in the vicinity of the paramagnetic center through hydrogen-bonding interactions with polar groups on the ligand in a suitable geometrical arrangement provide a contribution to r_{1p} similar to that of a metal-bound water molecule. The second coordination sphere relaxation pathway depends on the same parameters as the *IS* term, and thus its efficacy is enhanced by slowing down the rotational mobility of the complex, as shown by the increase of r_{1p} by decreasing the temperature. There are some distinct features though that must be considered for optimizing r_{1p}^{2S}:

1) The value of τ_M for the currently used MRI CAs is about one or two orders of magnitude longer than the optimal value and this represents a serious obstacle to the achievement of the high relaxivity expected for dendrimeric or macromolecular derivatives and required by the new and emerging applications of MRI. Conversely, the mean lifetime of the interaction of the 2*S* water molecules, although only roughly estimated by relaxometry, is much shorter and can play a negative role in determining τ_C (Equations 3–4). Longer values of τ_M imply stronger interaction through a proper choice of the hydrogen-bond acceptor groups of the ligand and their suitable mutual spatial disposition on the Gd^{3+} complex;

2) The r_H values for *IS* water molecules show a negligible variation among Ln^{3+} chelates of different charge, coordination number and hydration state. The distance of 2*S* water molecules is a far more flexible parameter, which may assume values in the range 3.2 (*IS*) – 4.5(*OS*) Å. Because

of the $1/r_H^6$ dependence of r_{1p}, a control of this parameter, which is determined by the type and geometry of the interaction, would be of primary importance for attaining high relaxivities.

3) Unlike the *IS* case, the number of water molecules in the second shell can in principle be increased without altering the number of the donor atoms of the ligand and compromising the thermodynamic stability of the complex.

This latter possibility has been explored through the formation of ion-pair adducts between anionic Gd^{3+} chelates and positively charged organic bases. [Gd-**7**]$^{5-}$ was found to be particularly useful for this purpose: its negative charges localized on the oxygen atoms of the phosphonate groups directed outwards from the coordination cage favor multiple electrostatic interactions and formation of strong ion-pairs in solution with a variety of substrates.[29][30] It has been found that *N*-methyl-glucamine (**8**, Scheme 3) interacts with [Gd-**7**]$^{5-}$ and induces a marked relaxation enhancement of the bulk water protons that, at pH = 9 and 25 °C, is as large as 3.5 and corresponds to r_{1p} of about 15 $mM^{-1}s^{-1}$ (at 20 MHz), a value comparable to that of Gd^{3+} chelates linked to macromolecular substrates.[28] This effect was originally explained by a contribution of the exchangeable protons of the organic base maintained in the close proximity of the metal ion by the chelate-type structural organization of the substrate in the supramolecular adduct.[31] In light of the above considerations we can attribute the relaxivity gain to a high number of water molecules involved in a network of H-bonding interactions with the phosphonate groups of the complex and the hydroxyl groups of the substrate. Moreover, the coordination polyhedron of [Gd-**7**]$^{5-}$, an inverted square antiprism where the negative charges are localized about the O_4 square plane above the metal ion, suggests that the effects associated with the ion-pairing interaction could be further optimized by choosing polyammonium macrocycles of suitable size and charge as the anion receptor substrates. This hypothesis was verified in the case of exacyclen (**9**, Scheme 3). The large cavity size of **9** matches very well the square plane of [Gd-**7**]$^{5-}$ and results in a strong interaction ($K_A = 1 \times 10^4$ M; pH = 6) that is pH dependent as it depends on the protonation of both the anionic and cationic species. In the ion-pair adduct the average distance between the ring carbons of the macrocycle and the Gd^{3+} has been estimated to be 6.1 Å by ^{13}C NMR T_1 data, thus suggesting the possible presence of a number of water molecules sandwiched between the two planes, with the hydrogen atoms directed towards the paramagnetic center at a distance short enough for optimal relaxation effects (Figure 4).[29]

As pointed out above, the 2*S* relaxation mechanism is governed by the same type of parameters as *IS* and, in particular, depends strongly on rotation (τ_R). Thus, the increase of τ_R by increasing the molecular size of the complexes should provide a further relaxivity gain. This has been observed when a benzyl group is introduced into **9**. The substituted exacyclen (**10**, Scheme 3), interacts with [Gd-**7**]$^{5-}$ just as strongly but now the supramolecular adduct can be further involved in the formation of an inclusion compound

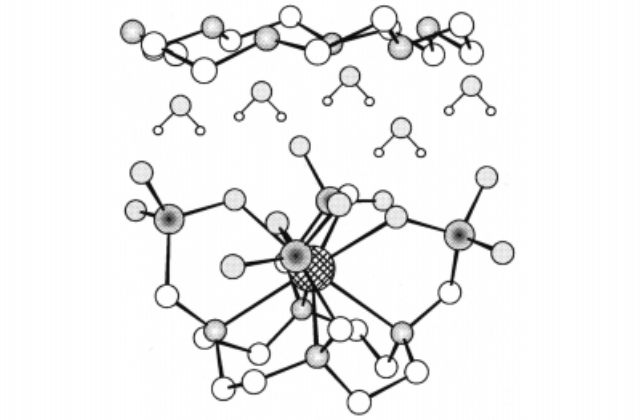


Figure 4. Model of the ion pair adduct between [Gd-**7**]$^{5-}$ and **9** showing the "sandwiched" water molecules of the second hydration shell

with β-cyclodextrin (β-CD), as shown in Figure 5. The noncovalent ternary complex presents a remarkably high relaxivity ($q = 0$!) of 18 $mM^{-1}s^{-1}$ at 20 MHz and 25 °C, which is entirely attributable to an optimized contribution of 2*S* hydration.[29]

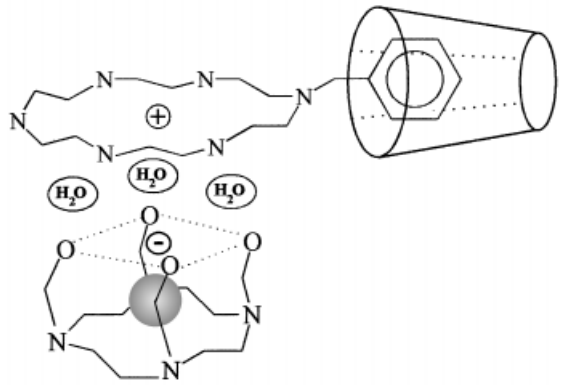
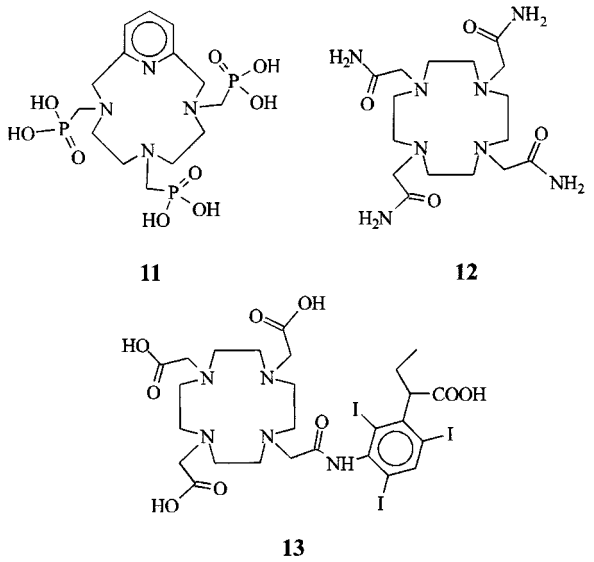


Figure 5. Schematic view of the noncovalent ternary complex between [Gd-**7**]$^{5-}$, **10** and β-cyclodextrin

From all these studies it appears evident that a very good candidate for a relaxation reagent of improved efficacy should be a complex where all the three relaxation mechanisms are operating, thus combining the properties of [Gd-**2**]$^-$ and [Gd-**7**]$^{5-}$. This interesting approach has been explored by investigating the relaxometric properties of the Gd^{3+} complex with **11**, a heptadentate macrocyclic ligand which features only three pendant arms bearing phosphonate groups (Scheme 4). As expected, the decreased denticity of the ligand allows a water molecule to occupy a coordination site of Gd^{3+}, and the relaxivity was measured to be as high as 7.7 $mM^{-1}s^{-1}$ (20 MHz, 25 °C).[32,33]

This high value of r_{1p}, about 50% higher than that of the commercial MRI CA, was shown to arise from comparable contributions of the three mechanisms: *OS*, *IS*, 2*S*. A quantitative analysis of the NMRD profile was performed under this hypothesis and an excellent fit of the data was obtained in the presence of a single *IS* water molecule at a distance of 3.06 Å from the metal ion. Moreover, in this octacoordinate complex, the exchange rate of the metal-



Scheme 4

bound water molecule is much faster than those found for enneacoordinated Gd^{3+} complexes. [Gd-**11**]$^{3-}$ represents the first example that clearly shows the feasibility and the potential of this strategy for relaxation enhancement.

Unusual, pH-dependent relaxation effects produced by second coordination shell water molecules have been observed also in the case of cationic Gd^{3+} complexes of macrocyclic tetraamide ligands such as **12** (Scheme 4).[34,35] The complexes are nine-coordinate, being bound to one water molecule, the four ring nitrogen and the amide carbonyl oxygen atoms, with a geometry corresponding to a monocapped square antiprism. At ambient pH and temperature the *IS* water exchange-lifetime, as determined by ^{17}O NMR spectroscopy, is long enough (19 μs) to strongly limit the *IS* contribution to r_{1p} and to allow a detailed study of the hydration structure and exchange dynamics. In the pH region above 7, despite the deprotonation of the bound water, which decreases the effective q value from 1 to 0.5, the relaxivity increases as a result of a base-catalyzed prototropic exchange taking place at the water coordination site. In fact, at high pH the rate of this process becomes faster than the rate of exchange of the whole water molecule, thus decreasing the effective τ_M and increasing the relaxivity. However, at pH 11, the relaxivity reaches a value (about 6 $mM^{-1}s^{-1}$) which is too high to be explained on the basis of prototropic exchange catalysis. This increase is associated with the deprotonation of the amide nitrogen (pK_a = 11.02 and 11.89) and has been shown to depend on the formation of a well-defined second hydration sphere consisting of water molecules bridging the amide group and the bound hydroxyl group through hydrogen-bond interactions (Figure 6). The profile of r_{1p} versus pH could be nicely fitted by considering two water molecules at a distance r_H = 3.6 Å.[35]

An analogous behavior was observed more recently in related macrocyclic tetra- and triamide derivatives.[36] Other examples of second coordination sphere water molecules tightly interacting with a negatively charged functional

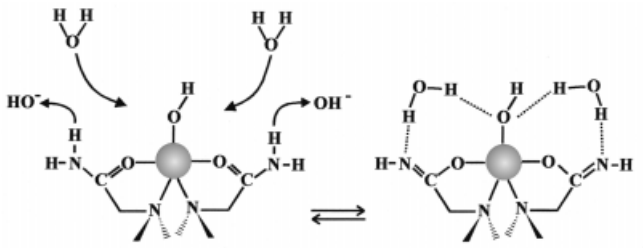


Figure 6. Deprotonation of the amide NH protons on [Gd-**12**]$^{3+}$ favors the formation of a second hydration sphere leading to a relaxivity enhancement

group of the ligand are the Gd^{3+} complexes with ligands **4** and **13** (Scheme 4). The monoaquo neutral complex [Gd-**4**] features a coordinated hydroxyl group that deprotonates at basic pH (pK_a = 11.36) with a concomitant structural rearrangement involving the loss of the coordinated water molecule. Surprisingly, this coordination equilibrium is not followed by a decrease of relaxivity which, at pH 12 (q = 0), assumes almost the same value as when measured at pH 7 (q = 1). It was concluded that the new contribution to r_{1p} measured at basic pH arises from a second coordination sphere water molecule hydrogen bonded to the alkoxide functional group whose protons were estimated to be at an average distance of 3.2 Å from Gd^{3+}.[4] Similarly, the monoamide neutral complex [Gd-**13**] presents a fairly acidic amidic proton ($pK_a \simeq 9$) and the profile of r_{1p} with pH is sensitive to this equilibrium: it passes from a value of 6.7 $mM^{-1}s^{-1}$ at pH 7 to 8.5 $mM^{-1}s^{-1}$ at pH > 10. This high relaxivity is again a consequence of the concomitant occurrence of all three relaxation mechanisms due to: one water coordinated to Gd^{3+} (*IS*) contributing about 50% of the overall relaxivity, one water molecule hydrogen bonded to the negatively charged nitrogen in the second coordination shell (2*S*, 20%), and the closely diffusing water molecules in the outer hydration sphere (*OS*, 30%).[4] It must be stressed that the relative contributions of the three mechanisms are not given as a direct result of experiments but rather derive from an analysis of the data based on a more plausible model, and therefore are to be considered as a qualitative interpretation.

Implications for MRI Contrast Agents

The relatively large body of data presented here clearly shows that 2*S* relaxation plays an important role in determining the relaxivity of aqueous solution of Gd^{3+} complexes bearing phosphonate or anionic groups next to the coordination sites of the metal ion. Can these effects be of more general importance and also partly affect the relaxivity of the currently available poly(aminocarboxylate) MRI CAs? Or can the contribution of 2*S* relaxation for these compounds be safely neglected as has so far been implicitly assumed? Some insights to correctly address these questions have recently been gained. Chen et al. have investigated the complexes of vanadyl (VO^{2+}) in order to exploit the absence of inner-sphere water molecules and analyze the re-

laxation data only in terms of *OS* and 2*S* contributions.[37–38] The rotational correlation times τ_R, one of the most important parameters for 2*S* relaxation, and τ_V were determined by ESR measurements and used to interpret the NMRD profiles. A large 2*S* contribution, predominant in the case of the DTPA complex, was found for all the compounds investigated. By making several assumptions the results from this study were then used to aid the interpretation of the NMRD profiles for the Gd^{3+} complexes with **1** and its ethoxybenzyl derivative. In the case of [Gd-**1**]$^{2-}$ the authors estimated a 2*S* contribution of roughly 25% to the overall relaxivity at the imaging fields. Though providing a qualitatively convincing interpretation of the relative role of inner-, outer- and second-sphere mechanisms to the relaxivity of the Gd^{3+}-based MRI CAs, the best-fitting procedure used yields poor values for the *IS* parameters τ_R (about two times larger than the value calculated for related complexes of similar molecular weight or estimated from ^{13}C T_1 data on diamagnetic derivatives), r_H (3.36, much longer than the values obtained from X-ray and ^{17}O NMR spectroscopic studies) and τ_M (20.9 ns, about one order of magnitude shorter than the value measured by a variable temperature ^{17}O $1/T_2$ data). We used a more direct approach to evaluate the 2*S* contribution of MRI CAs, involving a greatly reduced number of assumptions and fitting parameters, by taking advantage of a new X-ray crystal structure of [Yb-**1**]$^{2-}$ which clearly shows the three types of hydration shells of the complex.[39] In particular, a water molecule was located at a distance r_H = 3.28 Å from the ytterbium ion, strongly hydrogen bonded to the carboxylate oxygen atoms, and clearly belonging to the second coordination sphere. Six water molecules were also found in the outer coordination sphere surrounding the anionic complex, at an average r_H distance of 4.5 Å, a value in excellent agreement with that derived for the distance of closest approach *a* from NMRD analysis for the Gd^{3+} complexes with **5**, **6** and **7**. On the basis of the solid-state data we reanalyzed the NMRD profile of [Gd-**1**]$^{2-}$ on the basis of a model considering contributions from the water molecules in the three different environments (Figure 7).

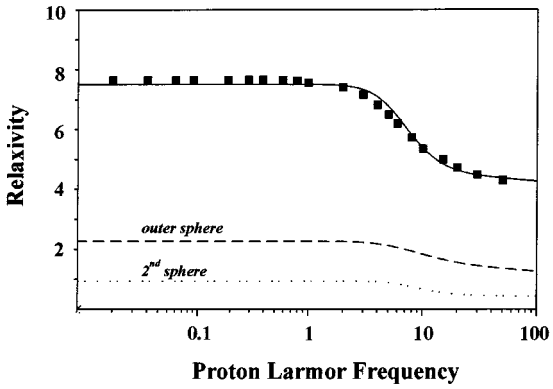


Figure 7. NMRD profile of [Gd-**1**]$^{2-}$ at 25 °C and pH = 7 showing the calculated outer- and second-sphere contributions

An excellent fitting of the relaxation data was obtained with relaxation parameters that are in very good agreement with crystallographic and NMR spectroscopic data and where: (1) the *IS* water molecule is located at 2.93 Å; (2) the 2*S* water molecule is hydrogen-bonded to the carboxylate oxygen atoms with a residence lifetime of 94 ps (at 25 °C) and at a distance of 3.28 Å, giving a contribution of about 10% to the total relaxivity at high fields; (3) the *OS* water molecules diffuse near the complex at a minimum distance of 4.5 Å.[39]

Conclusions

The qualitative picture which emerges from these data recognizes the importance of the structured water around the complexes and the potential of relaxometry to study the effects and test different hypotheses. 2*S* Solvation plays a small role in determining the relaxivity of the commonly used small poly(aminocarboxylate) Gd^{3+} chelates but its consideration is very important for a correct and more realistic interpretation of the relaxation data and for a better understanding of the structure-relaxivity relationship. Furthermore, large relaxation enhancements due to the 2*S* relaxation mechanism are possible through a careful ligand design and by exploiting strong interactions with suitable substrates (Figure 8).

Finally, the study of these effects has a direct implication in the understanding of the relaxivity changes occurring when a small Gd^{3+} chelate interacts with a macromolecule. We found that q = 0 complexes such as [Gd-**5**]$^{-}$, or its aryl methoxy-substituted derivatives, interact strongly with *bovine serum albumin* (BSA) leading to a marked relaxivity

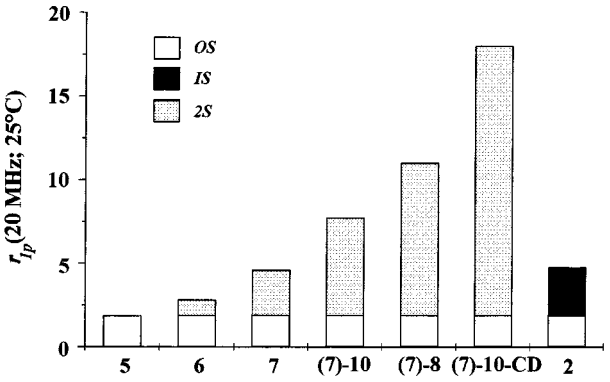


Figure 8. Plot of the relaxivity for some of the Gd^{3+} complexes discussed in this work showing the large relaxation enhancement achieved by exploiting the 2*S* mechanism

enhancement which cannot be accounted for either with an optimization of the outer sphere component of r_{1p} or with a change in the coordination number of the metal ion upon interaction with the protein.[23][40] Rather, it arises from exchangeable protons on the protein close to the interaction site of the complex and from a network of hydrogen-bonded water molecules in the second coordination sphere of Gd^{3+}. This contribution is only detected in the presence of the protein because of the reduced mobility of its well-structured hydration layer. A higher relaxation enhancement is observed in the case of the adduct of [Gd-**7**]$^{5-}$ with

BSA,[29,32] and the NMRD profile (Figure 9) is rather similar both in shape and in amplitude to those measured for $q = 1$ complexes, which shows that, even in this latter case, the 2*S* mechanism plays a major role, probably as a consequence of the limiting effect of the long τ_M value on r_{1p}^{IS}.

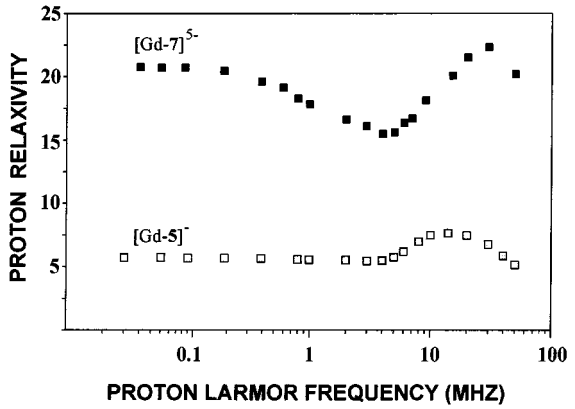


Figure 9. NMRD profiles of the non covalent adduct with BSA of [Gd-**5**]$^-$ (open squares) and [Gd-**7**]$^{5-}$ (filled squares) at 25 °C and pH = 7

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