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Potential Contrast Agents for Magnetic Resonance Imaging

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Abstract. The development of *magneto-pharmaceuticals* plays an important role in the extension of nuclear magnetic resonance into diagnostic medicine. That is the reason why fundamental investigations leading to new insights into NMR contrast agents are presently being considered. The synthesis and the proton relaxation rates of some new contrast agents are presented. The high values of R_1 and R_2 relaxivities of the compounds studied by us are promising for various and novel applications.

1. Introduction

The extension of NMR to *in vivo* tissue characterization, including both imaging and spectroscopy of metabolites, has brought new chemistry into diagnostic medicine [1]. The contrast agents are an integral part of this trend. These imaging agents are heavily used today to enhance the contrast in magnetic resonance imaging (MRI) in specific ways and hence to increase their information content. The time-consuming nature of the MRI procedure and the related expense make it important to extract the maximum amount of useful information from each scan.

Typical contrast agents are comprised of a paramagnetic ion (typically Mn^{2+} or Gd^{3+}) bound by a chelate [2–4], which itself perhaps is bound to a protein or another polymer. Superparamagnetic particles represent another class of MRI contrast agents [5, 6] that is usually referred to as T_2 or T_2^* contrast agents, as opposed to T_1 agents such as paramagnetic chelates. All these agents increase contrast in magnetic resonance imaging by preferential deposition in selected tissues where they increase the relaxation rates of nearby water protons.

The dependence of the ^1H NMR image intensity on tissue relaxation times is inherent in the basic principles of pulse NMR. Tissues with short T_1 values generally yield greater image intensity than those with longer values since the steady-state magnetization along the z axis is greater in the tissue with fastest

relaxation. On the other hand, short T_2 values are always associated with lower signal intensity since T_2 processes diminish the net transverse magnetization available for detection. Under conditions normally employed, the dominant effect of the superparamagnetic particles in NMR imaging is to decrease the signal intensity of the tissue containing the agent. Following the administration of these agents, the image is strongly T_2-weighted and shows a dramatic loss of signal [7] from the tissue containing the agents. But the agent-free tumours [8] produce high signal intensity due to their long T_2, generating the desired contrast.

New compounds are presently undergoing extensive evaluation as contrast agents in MRI. The development of new compounds as agent for NMR imaging embraces a wide range of disciplines from radiology to chemical physics. The purpose of this contribution is to present the design and synthesis of some new contrast agents, their tissue-specific action and the quantitative understanding of their effect on proton nuclear relaxation behavior in solution.

2. Methods

Complexes of paramagnetic lanthanide ions as gadolinium methylene diphosphonate (Gd-MDP), gadolinium iminodiacetate (Gd-IDA), dysprosium iminodiacetate (Dy-IDA), Dy-DTPA and Gd-DTPA (DTPA-diethylenetriamine pentaacetic acid) were obtained from respective citrates and afterwards purified by recrystallization. Citrates of gadolinium and dysprosium were prepared starting from the respective metal (99.9% purity, purchased from Chemical Corp, Sun Valley) by first transforming them to chlorides and then to citrates. The silver nitrate was added to establish the purity level, for the control of complete elimination of chlorine ion. Furthermore, the products were purified by recrystallization. We have synthesized and measured the relaxivities of Dy-DTPA and Gd-DTPA to check the accuracy of our results concerning the new compounds.

The manganese *para*-aminobenzoate (Mn-PAB) was prepared from *para*-aminobenzoate acid and manganese sulphate taken in stoichiometric ratio by the procedure described below. The mixture obtained from 480 g of *para*-aminobenzoate acid dissolved in concentrated ethanol (ca. 96% concentration) at minimum dilution and 5 g manganese sulphate in same conditions was poured into a beaker and stirred to achieve a saturated solution. The mixture was vigorously stirred at room temperature for 1 hour. The saturated solution was diluted by adding twice its volume of distilled water (i.e., at 200 ml ethanol required 400 ml distilled water). The immediate formation of a precipitate was observed. The solution was filtered. Then, the precipitate was washed off the filter with a 1 : 1 mixture of ethanol and distilled water and finally dried.

The compounds ($5Fe_2O_3 + 3Gd_2O_3$)-dextran and ($5Fe_2O_3 + 3Dy_2O_3$)-dextran were prepared by the microemulsion method using a water and toluene system. The starting materials $FeCl_3/GdCl_3$ and $FeCl_3/DyCl_3$ in molar ratio of 5 : 3 were con-

verted to the corresponding oxides by treating them in a system of 420 ml of water and 120 ml of toluene on a water bath for about 10–12 hours. We used an oxides/dextran molar ratio of 1 : 1. The molecular weight of dextran was 40000.

Dysprosium phosphate was prepared from the respective oxide and phosphoric acid. The source of Dy_2O_3 (99.9% purity) was E. Merck, Darmstadt. The size of dysprosium phosphate particles was in the range from 40 to 300 Å. The ($5Fe_2O_3$ + $3Gd_2O_3$) and ($5Fe_2O_3$ + $3Dy_2O_3$) cores mean diameters were about 45–70 Å, whereas the median diameter of the dextran-stabilized particles was distributed between 800 and 1200 Å. These dimensions were estimated by X-ray diffraction and by transmission electron microscopy (TEM) methods.

Measurements of the longitudinal and transverse relaxation rates T_1^{-1} and T_2^{-1}, respectively, of the metal complexes and the superparamagnetic particles have been carried out on ^1H in aqueous solutions as a function of molar concentrations, with the exception of the water-insoluble manganese *para*-aminobenzoate and dysprosium phosphate that were maintained in carboxymethylcellulose solution. All measurements have been made at room temperature (about 25°C) at a proton Larmor frequency $\nu_0 = 90$ MHz.

The pulsed NMR spectrometer utilized was a commercial Bruker SXP4/100 spectrometer. Transverse relaxation rates were measured by the Carr-Purcell method, while longitudinal relaxation rates were measured using the inversion recovery pulse sequence, $180°-\tau-90°$. All data exhibited single-exponential behavior.

The $T_{1,2}^{-1}$ values were obtained by fitting the experimental data with the expression

$$Y_i(t_i) = A + B\exp(t_i/T_{1,2}) ,$$

t_i being the times at which the magnetization values Y_i were measured. The fitting error was about 1% and the accuracy for the longitudinal relaxation rates was about 2–3% while the accuracy for the transverse relaxation rate was about 5–7%.

R_1 and R_2 relaxivities, in $mM^{-1}s^{-1}$ were determined from the least-squares determination of the slopes of plots $1/T_{1,2}$ versus molar concentration of the compound, using at least five independent measurements at several concentrations between 0 and 2 mM.

3. Results and Discussion

The measured R_1 and R_2 relaxivities of compounds studied in aqueous and carboxymethylcellulose solutions are shown in Table 1. The proton relaxation rates (Figs. 1 and 2) are linearly dependent on the concentration of the compounds studied. This certifies the absence of solute-solute interactions [9]. The R_1 relaxivity

Table 1. The R_1 and R_2 relaxivities for the studied compounds.

Compounds	R_1 ($mM^{-1}s^{-1}$)	R_2 ($mM^{-1}s^{-1}$)
Gd-MDP	23.37	65.81
Dy-IDA	3.49	9.75
Gd-IDA	124.12	293.88
Mn-PAB	4.89	49.01
($5Fe_2O_3$ + $3Gd_2O_3$)-dextran	61.78	201.39
($5Fe_2O_3$ + $3Dy_2O_3$)-dextran	24.87	87.52
$DyPO_4$	12.81	26.77
Dy-DTPA	0.79	1.61
Dy-DTPA and blood	0.42	93.72
Gd-DTPA	5.55	10.88

of Gd-DTPA is similar to those obtained by others [2, 20], which confirms the accuracy of our results.

It is obvious that the relaxivities of gadolinium iminodiacetate (Gd-IDA) and of the compound ($5Fe_2O_3$ + $3Gd_2O_3$)-dextran are rather high, but the mechanisms involved in either compound are quite different. In the case of paramagnetic complexes, a positive susceptibility is necessary, but not sufficient for effective relaxation enhancement. The magnitude of relaxation enhancement depends also on proximity of nuclear and electronic spins and on the correlation times. A possible explanation of the remarkably high values of the R_1 and R_2 relaxivities of the gadolinium iminodiacetate (Gd-IDA) could be the presence of at least one

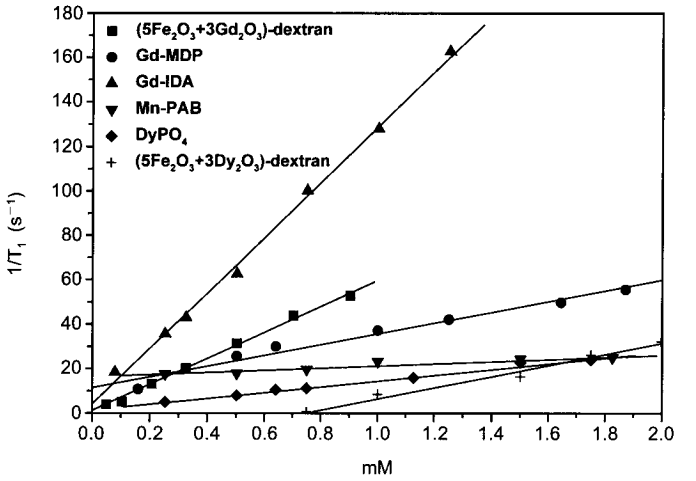

Fig. 1. Proton longitudinal relaxation rates at 2.11 T and 25°C as a function of concentration for different compounds.

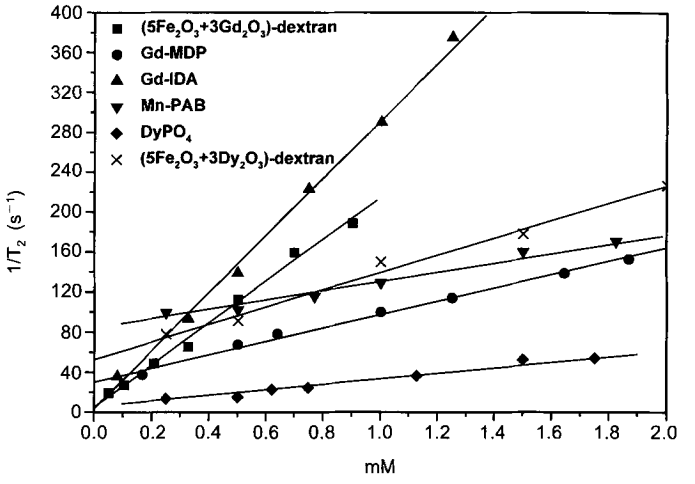

Fig. 2. Proton transverse relaxation rates at 2.11 T and 25°C as a function of concentration for different compounds.

coordinated water molecule and the delocalizing of nonspherical unpaired electron density of the gadolinium (III) ion somewhat closer to the protons on coordinated water molecules. The possible hydrogen bonds between the protons of the coordinated water molecules and the iminodiacetate can emphasize these facts. The lower relaxivities of the Dy-IDA could be explained by the shorter T_{1e} (longitudinal electron spin relaxation time) value and an increase in transient zero-field splitting of the spin levels [10]. While Dy(III) should be sensitive to these field effects, Gd (III) may not.

The r^{-6} dependence in dipolar interactions presents the opportunity to increase relaxivity by chemically inducing an orientation of bound water molecules such that the protons are closer to the metal centre, i.e., to unpaired spin density. The high values of R_1 and R_2 relaxivities of gadolinium methylene diphosphonate (Gd-MDP) can be justified by tilting of the plane of a bound water molecule with respect to the metal-oxygen vector which decreases r and increases relaxivity. Generally, such tilting can reduce the metal-proton distance about 0.2–0.4 Å [11].

The dipole-dipole term, affected critically by r^{-6} can be offset by unpaired electron spin density at the nucleus, a scalar (or "contact") effect. That is, quantum mechanics predicts a probability that the electron and nucleus coincide in space, dominating relaxation. In situations where such an effect is significant, as with manganese chelates [12], T_2 relaxation enhancement can exceed T_1 enhancement. This is the case as the manganese *para*-aminobenzoate, Mn-PAB.

Solvent relaxation in the presence of superparamagnetic particles mainly differs from that in the presence of paramagnetic solutes due to much greater weighting of the magnetic moment contribution. Compared to paramagnetic solutes,

superparamagnetic particles exhibit an increased effective magnetic moment, decreased freedom of molecular motion, and decreased water ^1H exchange.

The much greater effective magnetic moment dominates all the possible factors discussed previously. It creates greater field heterogeneity what characteristically results in shortening of T_2 for a given T_1 and more than what can be achieved in the presence of paramagnetic solutes. Of course, the role of diffusion cannot be neglected that usually modulated these phenomena [13, 14].

For particles with small domain sizes (below 100 Å), the dipolar interaction between the superparamagnetic core and surrounding solvent proton results in increasing both, longitudinal and transverse relaxation rates [15]. In addition, the susceptibility difference between the superparamagnetic core and surrounding medium generates strong magnetic field gradients, particularly around the periphery of each inclusion [16]. Diffusion in the presence of magnetic field gradients surely reduces T_2 [13]. In fact, smaller particles are adequately described by the microscopic outer-sphere theory [17].

For the studied superparamagnetic particles ($5Fe_2O_3 + 3Gd_2O_3$)-dextran, ($5Fe_2O_3 + 3Dy_2O_3$)-dextran and $DyPO_4$ the ratio R_2/R_1 ranges from 2.1 to 3.51. The higher values obtained for the R_1 relaxivity of iron oxide-gadolinium oxide-dextran and iron oxide-dysprosium oxide-dextran complexes could also be explained by an increase in the rotational tumbling time τ_R. Debye-Stokes theory predicts that for a spherical molecule [18] τ_R is directly proportional to the viscosity of medium, η, and the third power of the molecule radius.

The stabilization in dextran of ($5Fe_2O_3 + 3Gd_2O_3$) and ($5Fe_2O_3 + 3Dy_2O_3$) nanoparticles increases their size and mass causing anisotropic motion and becoming an additional factor of importance in relaxation. This fact should also apply in other situations of contrast enhancement. For example, although Dy-DTPA relaxes strongly through dipole-dipole interactions with water within the hydration sphere of each ion, it can also introduce susceptibility effects that may account for its greater efficiency in blood relaxation than can be accounted for by considering only exchange-mediated dipolar processes [19]. The ratio R_2 (on blood)/R_2 (on water solution) obtained by us for Dy-DTPA was about 58. The magnetic anisotropy of dysprosium iminodiacetate (Dy-IDA), ($5Fe_2O_3 + 3Dy_2O_3$)-dextran and $DyPO_4$, as well as the high magnetic moment of the Dy (III) ion (10 Bohr magnetons) should produce similar susceptibility effects.

4. Conclusions

Several new contrast agents such as gadolinium methylene diphosphonate (Gd-MDP), gadolinium iminodiacetate (Gd-IDA), dysprosium iminodiacetate (Dy-IDA), manganese *para*-aminobenzoate (Mn-PAB), dysprosium phosphate, iron oxide-gadolinium oxide-dextran and iron oxide-dysprosium oxide-dextran complexes were prepared and their R_1 and R_2 relaxivities were measured. The relaxivities

of almost all compounds studied by us are much higher than those of conventional contrast agents [2–6, 20, 21]. The efficiency of these compounds as contrast agents depends on a number of factors like particle size and composition and is the combined result of more than one type of relaxation processes.

The potential use of these new contrast agents as target agents is great. Gadolinium methylene diphosphonate will probably compensate the lack of *magneto-pharmaceutical* substances for tendons and cartilages, manganese *para*-aminobenzoate can be used as T_2 contrast agent for liver and spleen, and gadolinium iminodiacetate heavily labelled human serum albumin as an intravascular contrast agent.

The possible applications of particulate agents include improved visualization of the liver, gastrointestinal tract, and genitourinary tract as well as targeting and detection of small tumours or other cells with unique surface receptors. The relatively low T_1 effect of superparamagnetic particles would be useful in the assessment of the vascularization of hypoperfused or infarcted organs and for studying transient flow effects. A potential problem with all particles is long-term retention by the reticuloendothelial system [22]. Most studies concerning these agents have not addressed to this practical issue.

In comparing the development of NMR agents with that of inorganic radiopharmaceuticals, it is apparent that the former will require a great deal more in characterization due to the complexities inherent in NMR relaxation phenomena and requirements for higher dose. Though the approval of NMR agents for human use may be more difficult to obtain, the promise of *in vivo* clinical studies of anatomy, physiology and metabolism will certainly stimulate the development of this research area.

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