Physicochemical Characterization of P760, a New Macromolecular Contrast Agent with High Relaxivity[‡]

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P760-Gd, a hydrophilic derivative of DOTA-Gd with a molecular mass of 5.6 kDa, has been characterized in various media: aqueous solution, protein-containing solution, and Zn^{2+}-containing solution. Several investigations have been carried out, including the determination of the number of inner-sphere water molecules by laser-induced luminescence of the europium complex, water ^1H nuclear magnetic relaxation rates as a function of field strength, and ^{17}O relaxometry of water of the gadolinium complex, pulsed magnetic field gradient spin echo of proton, and ^2H transverse relaxation rates of the labeled lanthanum analogue. The large proton relaxivity of P760-Gd in water results from the long rotational correlation time of the hydrated complex ($\tau_R \approx 2$ ns at 310 K). As with DOTA-Gd, no significant Zn^{2+} transmetallation process was observed by proton relaxometry. Finally, analysis of the non-covalent binding of P760-Gd to serum proteins by proton relaxometry showed that P760-Gd does not interact with human serum albumin.

Introduction

Intensive research into the development of MR Blood Pool Agents (BPAs) has now been in progress for more than 10 years. Depending on their pharmacokinetic properties, these BPAs may be divided into three different classes: Low Diffusion Agents (LDAs), Rapid Clearance Blood Pool Agents (RCBPAs), and Slow Clearance Blood Pool Agents (SCBPAs).[1−3]

P760-Gd (Guerbet, Roissy − France) (Figure 1) is an original MR Contrast Agent belonging to the Low Diffusion Agent pharmacokinetic category. Indeed, because of its intermediate molecular size, P760-Gd can diffuse through the vascular endothelium, but at a much lower rate than a non-specific agent such as DOTA-Gd, whereas its renal excretion remains totally free.[1,3−5]

Very little concerning the physicochemical properties of this DOTA-Gd derivative has been reported. Its proton relaxivity at 20 MHz and 37 °C is quite large (24.7 s^{-1} mM^{-1}),[5] in good agreement with its large molecular size. In this work, an extensive in vitro physicochemical characterization of P760-Gd was performed. The number of inner-sphere water molecules q was evaluated by laser-induced luminescence of P760-Eu. The residence time τ_M of the coordinated water molecule was estimated by analysis of the temperature dependence of the water ^{17}O transverse relaxation of P760-Gd solution, whereas the rotational correlation time τ_R was calculated from the ^2H transverse relaxation rate of deuterated P760-La. The translational diffusion coefficient D, useful for calculation of the outer-sphere contribution to the relaxation rate and for the estimation of the molecular volume, was obtained by proton NMR on P760-La. The rate and magnitude of transmetallation by Zn^{2+} ions in aqueous phosphate solutions at pH = 7.0 was evaluated by proton relaxometry.

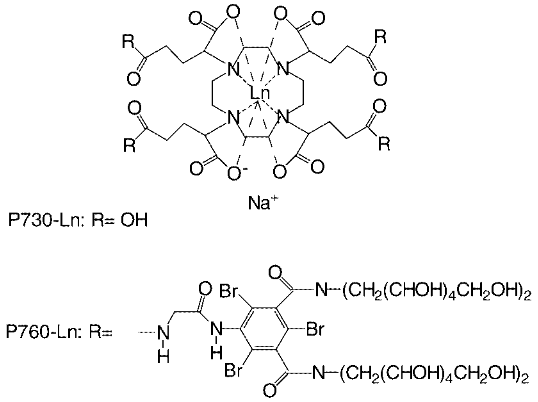

Figure 1. Chemical structures of P730 and P760

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Finally, the proton T_1 Nuclear Magnetic Relaxation Dispersion (NMRD) curve of P760-Gd in water and its longitudinal proton relaxation rate in a 4% HSA solution were analyzed. In view of the clinical context of this kind of study, most of the data are reported at 310 K, physiological body temperature.

Results and Discussion

q Determination

Horrocks and Sudnick have shown that the differences in the decay rates of Eu^{3+} or Tb^{3+} luminescence in H_2O ($1/\tau_{H_2O(ms)}$) and D_2O ($1/\tau_{D_2O(ms)}$) solutions are related to the number of water molecules in the first coordination sphere [Equation (1)].[6] Recently, Beeby et al.[7] have refined the method, by consideration of the contributions from water molecules diffusing close to the Ln^{3+} complex and of other protons, exchangeable oscillators [Equation (2)]. The measured luminescence lifetimes of P760-Eu in H_2O and D_2O were 0.63 ms and 2.32 ms, respectively. The corresponding calculated q values are 1.16 by use of Horrock's equation or 1.03 according to Beeby et al., in good agreement with the expected value of 1.

$$q = 1.05\left(\frac{1}{\tau_{H_2O}} - \frac{1}{\tau_{D_2O}}\right) \tag{1}$$

$$q_{corr} = 1.2\left(\frac{1}{\tau_{H_2O}} - \frac{1}{\tau_{D_2O}} - 0.25\right) \tag{2}$$

τ_R Determination

The ^2H relaxation rates of diamagnetic compounds are predominantly functions of the interaction between the quadrupolar moment (Q) and the electric field gradient at the nucleus (q), and hence depend on the rotational correlation time τ_R. For small molecules, the extreme narrowing condition ($\omega_I^2\tau_R^2 << 1$) is fulfilled and R_1 and R_2 are equal. For larger molecules characterized by larger values of τ_R, $\omega_I^2\tau_R^2 \geq 1$ and consequently, $R_2 >> R_1$. At the magnetic field used for the ^2H NMR measurements (B_0 = 7.05 T), this situation occurs when $\tau_R \geq 1$ ns. At 310 K, the spectrum of the monodeuterated P760-La analogue shows a broad resonance ($\Delta\nu_{1/2}$ = 218 Hz) arising from the slowly rotating deuterium nuclei. The linewidth can thus be used to measure R_2 ($R_2 = \pi\ \Delta\nu_{1/2}$) and its dependence on the temperature. Values of τ_R calculated from R_2 by use of Equation (3) and a quadrupolar coupling constant e^2qQ/h equal to 170 kHz are 2.2 ns at 310 K and 3.3 ns at 298 K. In Equation (3), I is the nuclear spin and η is the asymmetry parameter of the field gradient. This latter parameter is negligible for deuterium nuclei involved in carbon−deuterium bonds.[8]

$$\frac{1}{T_2} = \frac{3}{400}\frac{2I+3}{I^2(2I-1)}\left(1+\frac{\eta^2}{3}\right)\left(\frac{e^2Qq}{\hbar}\right)^2\left[3\tau_R + \frac{5\tau_R}{1+\omega_I{}^2\tau_R{}^2} + \frac{2\tau_R}{1+4\omega_I{}^2\tau_R{}^2}\right] \tag{3}$$

A rough estimation of the τ_R value of P760-Gd has been made on the basis of the τ_R value of DOTA-Gd at 310 K (τ_R = 59 ps)[9] and the two molecular volumes [V^m(DOTA-Gd) = 0.38 nm^3 and V^m(P760-Gd) = 11.5 nm^3 obtained from photon correlation spectroscopy data[5]] from the relationship: $\tau_R^{P760\text{-}Gd} = \tau_R^{DOTA\text{-}Gd}\cdot V^m(\text{P760-Gd})/V^m(\text{DOTA-Gd})$ derived from the Stokes−Einstein equation [see Equation (4)] and by the assumption that the microviscosity factor f_R can be set to 1 either for DOTA-Gd or for P760-Gd. Such an estimation gives a value of 1.79 ns for P760-Gd, for a viscosity (η) of 0.69 mPa·s corresponding to a 1 mM solution.

$$\tau_R = \frac{\eta f_R V_m}{kT} \tag{4}$$

As the actual viscosity of the deuterated P760-La solution (28 mM) is equal to 1.32 mPa·s at 310 K, this value can be corrected by the viscosity factor, giving a τ_R value of 3.4 ns.

These calculations are somewhere imprecise, because the above molecular volumes calculated from the diffusion constant by the Stokes−Einstein−Debye model did not take the microviscosity factor (f_T) for the translational diffusion[10] into account [Equations (5) and (6), where r and r_s are the radius of the solute and solvent molecules, respectively]. If the microviscosity factors are included in the calculations, the calculated molecular volumes of DOTA-Gd and P760-Gd are 0.78 nm^3 and 14.4 nm^3, respectively

$$D = \frac{kT}{6\pi\eta f_T r} \tag{5}$$

$$f_T{}^{-1} = \frac{3r_s}{2r} + \frac{1}{\left(1+\frac{r_s}{r}\right)} \tag{6}$$

$$f_R{}^{-1} = 6\frac{r_s}{r} + \frac{1}{\left(1+\frac{r_s}{r}\right)^3} \tag{7}$$

By including the microviscosity factor for the rotational correlation time f_R [≈ 0.4 for DOTA-Gd and ≈ 0.68 for P760-Gd calculated by Equation (7)[10]], the estimated τ_R^{310} of P760-Gd by Equations (4) and (7) are 2 ns for a viscosity of 0.69 mPa·s (corresponding to a 1 mM solution) and 3.9 ns for a viscosity of 1.32 mPa·s (corresponding to a 28 mM solution), a value somewhat larger than that obtained from deuterium relaxation measurement. The activation energy for the rotation (E_R) was derived from the temperature dependence [Equation (8)] and was found to be equal to 32.3 kJ/mol, a value larger than those already reported for smaller complexes (Figure 2).[11−13]

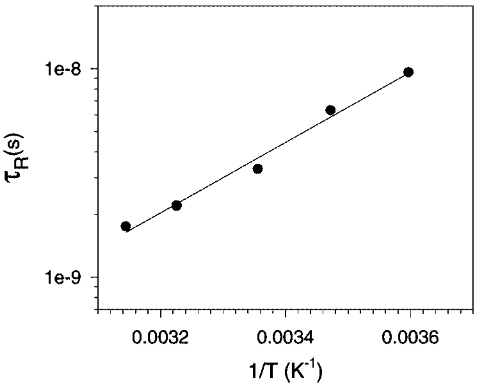

Figure 2. Temperature dependence of τ_R values obtained by deuterium NMR

$$\tau_R = \tau_R{}^{298}\, exp\left(\frac{E_R}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right) \qquad (8)$$

τ_M Determination

The rate of exchange of the water molecule in the first coordination sphere of Gd^{3+} can be determined by analysis of the temperature dependence of the paramagnetic transverse relaxation rate ($R_2 = 1/T_2$) of the ^{17}O NMR signal of water in the gadolinium complex solution.[11–13] The temperature dependence of ^{17}O R_2 from 288 to 357 K was studied.

Analysis of the experimental data may be performed by use of two different hypotheses concerning the diamagnetic contribution, and theoretical fittings were performed for both, with or without quadrupolar contribution, providing four different solutions (Table 1). In the first approach, the reduced relaxation rate $\{1/T_2^R = 55.55{\cdot}(T_2{\cdot}q{\cdot}[\text{P760-Gd}])^{-1}\}$ was calculated by assuming that the diamagnetic contribution was that of pure water (closed circles, Figure 3). The fitting of the experimental data was then performed either with a model in which the transverse relaxation rate of the bound nuclei of ^{17}O ($1/T_2^M$) results mainly from scalar interaction with the gadolinium ion, as described in ref.[11] (2nd column of Table 1), or with a second model in which the quadrupolar contribution to $1/T_2^M$ was taken into account (3rd column of Table 1).

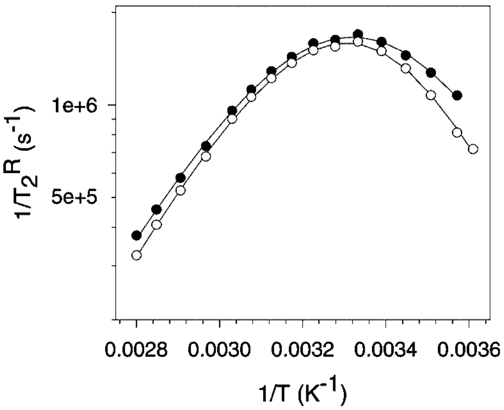

Figure 3. ^{17}O reduced transverse relaxation rate of water in P760-Gd solution (concentration: 19 mM) versus the reciprocal of the temperature; the closed circles correspond to the reduced relaxation rate calculated with the diamagnetic contribution of water, and the open circles correspond to the reduced relaxation rate calculated with the diamagnetic relaxation rate of a solution of P760-La

Both types of fittings allow for the determination of the following parameters:

(i) the hyperfine coupling constant between the oxygen-17 nucleus and the electronic spin of Gd^{3+} ($A/\hbar$),

(ii) the parameters related to the electronic relaxation times τ_{S1} and τ_{S2} (i.e., the correlation time for the modulation of the zero field splitting τ_V), the activation energy E_V for this process, and B which depends on the trace of the square of the zero field splitting tensor (Δ^2) and on the electron spin S $\{B = \Delta^2[4S(S + 1) - 3]/25\}$,

(iii) the parameters of the water exchange rate [i.e., the enthalpy ($\Delta H^{\neq}$) and the entropy ($\Delta S^{\neq}$) of activation],

(iv) when the quadrupolar contribution is taken into account, additional parameters describing this contribution are included [see Equation (3)]: the rotational correlation time τ_R and the activation energy for the rotation, which were fixed to the value estimated from the data obtained by ^2H NMR (τ_R^{298} = 3 ns, E_R = 32.3 kJ/mol), as well as a term related to the quadrupolar coupling constant and the asymmetry parameter of ^{17}O set to the value of water $[(1 + \eta^2/3)^{1/2}(e^2qQ/h) = 7.58\ \text{MHz}]$.[13]

Table 1. Parameters obtained by the fittings of the ^{17}O NMR spectroscopic data

	Without quadrupolar contribution and with water diamagnetic contribution	With quadrupolar contribution and water diamagnetic contribution	Without quadrupolar contribution and with P760-La diamagnetic contribution	With quadrupolar contribution and P760-La diamagnetic contribution[a]
τ_V^{298} [ps]	7.5±1.0	7.7±0.4	0.7±1.6	10.1±0.4 (18.2±1.6)
B [10^{20} s^{-2}]	0.77±0.06	0.84±0.04	0.69±0.10	1.02±0.08 (1.26±0.20)
E_v [kJ/mol]	5.6±12.8	5.2±4.4	12.6±7.7	11.2±5.6 (11.9±7.5)
$A/\hbar$ [10^6 rad s^{-1}]	−2.91±0.12	−2.91±0.10	−2.50±1.3	−2.82±0.02 (−2.50±0.02)
$\Delta H^{\neq}$ [kJ/mol]	42.9±0.26	43.4±0.06	44.8±0.32	47.8±0.12 (45.4±0.88)
$\Delta S^{\neq}$ [J/mol·K]	27.2±0.74	28.9±0.27	30.9±0.48	41.6±0.34 (33.1±3.42)
τ_M^{310} [ns]	101±19	98±6	137±25	118±10 (133±59)

[a] Two sets of fitted parameters giving similarly good fits are reported.

In the second approach, the change in viscosity and the possible contribution of the oxygen atoms of water molecules interacting with the hydrophilic arms of P760-Gd were included in the diamagnetic contribution by measurement of the oxygen-17 transverse relaxation rate of water in a 19 mM solution of P760-La. From these data, a second set of reduced relaxation rates $1/T_2^R$ was obtained (open circles, Figure 3). On comparing the two $1/T_2^R$ sets, we observed a decrease in the reduced transverse relaxation rate over the whole temperature range, with a more pronounced effect at low temperature (Figure 3) when the P760-La diamagnetic contribution was taken into account. The fitting parameters with P760-La diamagnetic contribution, with or without quadrupolar contribution, are listed in Table 1 (4th and 5th columns). With the first approach, the results of the fitting show that the water residence time is short (about 100 ns at 310 K) and close to that reported for the parent compound DOTA-Gd at the same temperature.[13] The use of the appropriate diamagnetic contribution (2nd approach) gives slightly larger values of τ_M (average value of 128 ns).

It should be pointed out that the fitted values of the hyperfine coupling constant τ_V and B are interrelated and that similarly good fits can be obtained with two different sets of parameters, as shown in the last column of Table 1.

Determination of the Self-Diffusion Coefficient (*D*) by ^{1}H NMR

The aim of the measurement of D was twofold. Firstly, it is useful for estimation of the molecular radius [see Equation (5)], and secondly, the value of the relative diffusion coefficient (D_{water} + D_{complex}) is one of the parameters describing the outer-sphere contribution to the relaxation rate. The measurements were performed at four temperatures (293, 298, 303, and 310 K) and 4.7 T by the gradient spin echo method with selective saturation of the water resonance on a 3.3 mM P760-La solution in water; D was calculated from the variation in the intensity of the resonance at $\delta = 3.8$ ppm as a function of the applied gradient. To avoid interference between the signal arising from residual water, the intensities were measured with gradient strengths larger than 170 Gauss/cm. The obtained values are shown in Table 2. The value obtained at 293 K is in good agreement with the previously reported value of 1.5 10^{-10} m^{2}s^{-1} obtained by photon correlation spectroscopy.[5] Consequently, the radius calculated from the Debye−Stokes theory [Equation (5)] with f_T equal to 1 is 1.53 nm, a value slightly larger than that obtained by PCS ($r = 1.4$ nm). Refinement of the calculation by use of the translational microviscosity theory gives a value of 1.64 nm [Equation (6)]. This value corresponds to a τ_R^{310} of 2.1 ns ($\eta = 0.69$ mPa·s), consistent with the previous value of 2 ns found by deuterium transverse relaxation rates experiments.

Table 2. Diffusion coefficients measured on P760-La and water in a 3.3 mM solution at different temperatures

Temperature [K]	D of P760-La [10^{-10} m^{2}s^{-1}]	D of H_2O [10^{-10} m^{2}s^{-1}] in the P760-La solution
293	1.40	18.52
298	1.62	21.01
303	1.81	23.93
310	1.98	27.28

Transmetallation by Zn^{2+} Ions

Transmetallation of gadolinium complexes by Zn^{2+} ions induces a release of Gd^{3+} ions, which form insoluble phosphate complexes in the presence of phosphate ions; these released lanthanide ions no longer contribute to the proton paramagnetic relaxation rate of the solution. Subsequently, the water proton relaxation rate decreases during the transmetallation process and its evolution can be used to monitor the phenomenon quantitatively.[14] Under identical experimental conditions ($B_0 = 0.47$ T, $T = 310$ K, 2.5 mM of gadolinium complex, 2.5 mM of Zn^{2+} ions, and phosphate buffer pH = 7), no significant change in the paramagnetic relaxation rate was reported for the macrocyclic gadolinium complexes DOTA-Gd and HPDO3A-Gd {[10-(2-hydroxypropyl)-1,4,7-tris(methoxycarbonyl)-1,4,7,10-tetraazacyclododecane]gadolinium(III)} whereas R_1^p was about 57% of its initial value for DTPA-Gd and 22% for DTPA BMA-Gd after 1400 min, showing that significant transmetallation takes place for these open-chain gadolinium complexes. A similar experiment was performed with P760-Gd, but because of the large relaxivity of this complex at 20 MHz and to the limitations of the instrument, the concentrations of the gadolinium complex and of Zn^{2+} ions had to be reduced to 0.5 mM. As would be expected for a macrocyclic complex, the results show no significant evolution of the proton paramagnetic relaxation rate over a period of 1400 min and agree with the absence of transmetallation by Zn^{2+} ions (Figure 4).

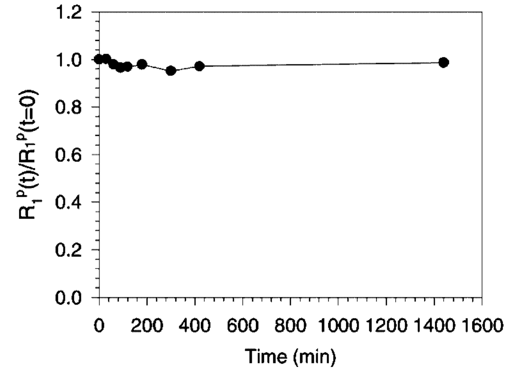

Figure 4. Evolution of the ratio $R_1^p(t)/R_1^p(t = 0)$ with time for a solution containing 0.5 mM of P760-Gd and $ZnCl_2$ at pH = 7.0 and 37 °C ($B_0 = 0.47$ T)

NMRD Fitting

The proton longitudinal relaxation rates were measured between 0.01 and 300 MHz on a 0.19 mM P760-Gd solution; the transverse relaxation rates were measured between 20 and 300 MHz. The calculated relaxivities (the relaxation rate enhancement afforded by 1 mmol/L of the gadolinium

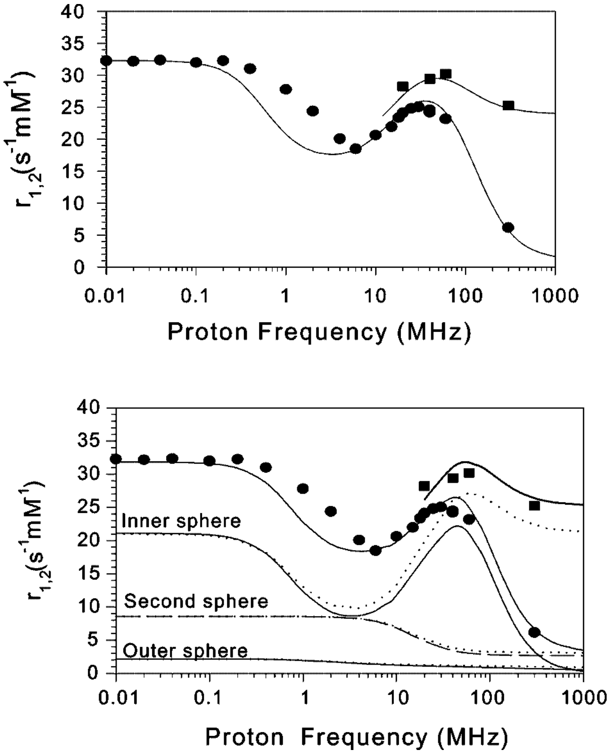

Figure 5. Proton NMRD data obtained at 310 K on a 0.19 mM solution (circles: r_1; squares: r_2; best fit); the top graph was obtained by use of the classical inner-sphere and outer-sphere theories with d = 0.36 nm, r = 0.31 nm, τ_{SO} = 740 ps, τ_V = 14 ps, τ_R = 1.8 ns, and τ_M = 320 ns; the bottom graph was obtained by use of the inner-sphere, outer-sphere, and second-sphere theory with d = 0.6 nm, r = 0.325 nm, τ_{SO} = 410 ps, τ_V = 13 ps, τ_R = 1.7 ns, τ_M = 170 ns, 25 water molecules in the second sphere at a distance of 0.4 nm and τ_{SS} = 15.6 ps

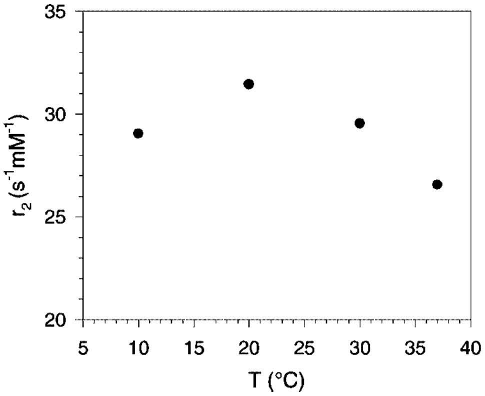

Figure 6. Proton transverse relaxivity of P760-Gd versus temperature at 7.05 T

complex) versus proton Larmor frequencies (proton NMRD curve) are shown on Figure 5.

In a first approach, ^{1}H NMRD curves were fitted according to the classical inner-sphere model described by Solomon[15] and Bloembergen[16] and to the outer-sphere model described by Freed.[17] In these fittings, q (the number of water molecules coordinated to the Gd^{3+} ion) was fixed to 1, r (the distance between the proton nuclei of the inner-sphere water molecule and the Gd^{3+}) was set to 0.31 nm, the relative diffusion constant D was set to the values obtained above by ^{1}H NMR spectroscopy (2.93 10^{-9} m^2s^{-1}), d (the distance of closest approach for the outer-sphere contribution) was set at 0.36 nm,[18,19] and the parameters describing the electronic relaxation times, τ_V and τ_{SO} (the electronic relaxation time at zero field), were optimized for the outer-sphere and the inner-sphere contributions simultaneously. Parameters τ_R and τ_M were allowed to fluctuate around the values obtained by ^{2}H and ^{17}O NMR spectroscopy. Satisfactory fits of the data could be obtained at low fields and at high fields with a τ_R value of 1.8 ns and a τ_M value of 320 ns (Figure 5, top graph). This latter value is significantly larger than that determined by ^{17}O NMR spectroscopy. The τ_{SO} and τ_V values are slightly larger than those reported for the parent compound DOTA-Gd (τ_{SO}^{310} = 480 ps and τ_V^{310} = 11 ps[13]). These discrepancies could be related to the fact that the simple model used to fit the ^{1}H NMRD curve is not appropriate to describe the behavior of slowly tumbling paramagnetic molecules[20] or to the presence of a second sphere of water molecules due to the hydrophilic nature of the P760-Gd complex. Such a second sphere, not included in this fitting of the proton NMRD data, can be taken into account (see below).

Data for r_2 were also obtained at 310 K and at magnetic fields ranging from 0.47 to 7.05 T (Figure 5). In addition, the temperature dependence of r_2 at 7.05 T was also studied (Figure 6). At this field, r_2 is predominantly determined by the inner-sphere interaction and so depends mainly on τ_R and τ_M [Equation (9)]. The data (Figure 6) suggest a situation of intermediate water exchange in the temperature range investigated with a limitation of r_2 by the water exchange.

$$r_2 \cong \frac{0.001}{55.55}\frac{1}{T_{2M}+\tau_M} \qquad (9)$$

Values of (T_{2M} + τ_M) calculated from the experimental data [Equation (9)] range between 570 and 670 ns. Consequently, values of τ_M of the order of hundreds of nanoseconds and τ_R values greater than 1.3 ns would be expected. The theoretical fitting of r_2 NMRD data by the classical inner- and outer-sphere models[15-17] and the parameters obtained by the fitting of r_1 data agree very well with the experimental data.

In a second approach, the interactions between the gadolinium ion and water molecules in its second sphere were considered.[21] The hydrogen atoms of these water molecules are assumed to be at a distance of 0.4 nm, and the distance of closest approach was set to 0.6 nm because of the size of the complex. To obtain reasonable r_1 and r_2 fittings, the distance r had to be increased to 0.325 nm. With these settings, the experimental data could be fitted as shown in Figure 5 (bottom graph). The τ_R value (1.7 ns) is similar to that obtained above, but the value of τ_M (170 ns) is closer to that obtained by ^{17}O NMR spectroscopy. The τ_{SO} (410 ps) and τ_V (13 ps) values are similar to those reported for the parent compound DOTA-Gd. In this fitting, 25 water molecules are found in the second sphere and their interaction with the paramagnetic center is modulated by a correlation time (τ_{SS}) equal to 15.6 ps.

Interaction of P760-Gd with HSA and Blood Proteins

Proton relaxation measurements were performed at 20 MHz and at 37 °C on P760-Gd solutions (0.05−1.9 mM) in 4% non-defatted HSA. No significant change in the longitudinal proton relaxation rate was observed [$R_1{}^p$(water)/ $\mathrm{R}_2{}^p$(HSA, 4%) = 1.08 for a 1mM solution of P760-Gd], showing either that P760-Gd does not interact with HSA or that there is an interaction between P760-Gd and HSA but that the rotational correlation time of the paramagnetic center is not significantly modified by the binding. Similarly, longitudinal and transverse paramagnetic relaxation rates of 0.2 mM of P760-Gd in serum were only slightly increased (increase of 6% for R_1^p and 27% for R_2^p) at 310 K and 7.05 T, confirming the results discussed above.

Conclusion

The large longitudinal and transverse proton relaxivities ($r_1 \approx 25$ s^{-1}mM^{-1} and $r_2 \approx 29.5$ s^{-1}mM^{-1} at 310 K and 0.94 T) of P760-Gd are related to its large molecular weight (5.6 kDa), and consequently to its quite large rotational correlation time ($\tau_R \approx 2$ ns at 310 K). Like its parent complex DOTA-Gd, this original complex does not undergo transmetallation by zinc(II) ions and the residence time of its coordinated water molecule is of the order of a few hundred nanoseconds at 310 K. The r_1 relaxivity of P760-Gd is one of the highest reported in the literature for a gadolinium complex without any protein binding.[20] The proton relaxivity data clearly show that the expected benefits from the τ_R increase are not completely achieved because of the too slow water-exchange rate. For such types of complex, maximum r_1 and r_2 values of the order of 35 s^{-1}mM^{-1} and 45 s^{-1}mM^{-1}, respectively, should be achievable at magnetic fields of 1.1−1.5 T, assuming a water residence time of about 20 ns.

Experimental Section

Chemical Synthesis

Synthesis of Ligand L (P730): Alkylation of cyclen with racemic dimethyl 2-bromoglutarate (K_2CO_3/CH_3CN/reflux) resulted in the formation of expected octamethyl ester. Base hydrolysis (6 M NaOH, 80 °C) afforded octacarboxylic acid ligand L (P730).[22]

Synthesis of L-Ln Complexes (Gd, Eu, La). P730-Ln: Lanthanide complexes were formed by heating of ligand L and Ln_2O_3 (Gd_2O_3, Eu_2O_3, La_2O_3) at pH = 5−6 in deionized water (80−100 °C, 1−24 h).

Synthesis of the Deuterated La Complex: Dry P730-La was heated at reflux in D_2O at pD = 3 (DCl) for 4 d. The solution was filtered and concentrated. After crystallization, the complex was isolated by filtration. Isotopic exchange assessed by mass spectrometry showed that the compound was monodeuterated on the α-carbon atom of the arm.

Synthesis of P760-Ln (Gd, Eu, La): A solution of L-Ln complex and polyhydroxylated aminotribromoisophthalic derivative R (4 equiv.) was mixed with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI). The reaction mixture was stirred at pH = 7 for 2 d (room temperature). The solution was ultrafiltered through a 3KDa membrane (FILTRON®). After removal of water by evaporation, the white solid was dried at reduced pressure.[5]

Chemicals: All commercial chemicals [$ZnCl_2$ (Merck) and Titrisol buffer pH 7.0 (Merck)] were used without further purification. Non-defatted human albumin (fraction V) was purchased from Sigma (Bornem, Belgium).

Viscosity: The viscosity of P760 Gd was measured with a Visco-Clock viscosimeter (SCHOTT − Germany) at 37 °C by the capillary standard procedure described in the European Pharmacopoeia, 3rd ed., **2001**, method number 2.2.9.

Luminescence Studies on the Eu Complexes: The Eu^{III} phosphorescence emission spectra were acquired with an LS-50B Perkin−Elmer spectrofluorimeter equipped with a pulsed Xenon lamp and a Hamamatsu R928 photomultiplier tube operating in time-resolved mode. Solutions of the Eu^{III} complex ($9.7 \cdot 10^{-3}$ M and $5.7 \cdot 10^{-3}$ M in H_2O and D_2O, respectively) were used in these experiments. Lifetimes (τ) were obtained by monitoring of the emission intensity at 616 nm resulting from pulsed excitation at 395 nm and 140 (H_2O) or 240 (D_2O) different delay times. Excitation and emission slit widths of 5 and 20 nm, respectively, were used and the gate time was 0.4 ms. The decays of the europium emitting state were fitted by use of an equation of the form $I(t) = I(0)\exp(-t/\tau)$, with the aid of a current software fitting procedure (Microsoft Excel≁). High correlation coefficients were observed in each case (typically 0.9999). The number of water molecules (q) bound to the inner coordination sphere of the europium ion was quantified by use either of the relation proposed by Horrocks and Sudnick[6] [Equation (1)], in which τ is the lifetime (in ms) of the complex in H_2O and D_2O, or of the equation reported by Beeby et al.[7] [Equation (2)].

NMR: Proton Nuclear Magnetic Relaxation Dispersion (NMRD) profiles were recorded with a Field Cycling Relaxometer (Field Cycling Systems, Oradell, New Jersey, USA) working between 0.24 mT and 1.2 T on 0.6 mL solutions contained in 10 mm o.d. tubes. Proton relaxation rates were also measured at 0.235, 0.47, 0.94, and 1.5 T with Minispec PC-110, PC-120, PC-140, and mq-60 (Bruker, Karlsruhe, Germany). The additional relaxation rates at 7.05 T were obtained with a Bruker AMX 300 (Bruker, Karlsruhe, Germany) spectrometer. ^{1}H NMRD data were fitted according to the theoretical inner-sphere model described by Solomon[15] and Bloembergen[16] and to the outer-sphere contribution described by Freed.[17] Calculations were performed with previously described software.[23,24] Transmetallation by Zn^{II} ions was evaluated from the decrease in the water proton longitudinal relaxation rate at 310 K and 20 MHz (Bruker Minispec PC 120) of buffered solutions (pH = 7, phosphate buffer) containing 0.5 mM of the gadolinium complex and 0.5 mM of Zn^{II} according to the procedure described previously.[14] ^{17}O and ^{2}H NMR spectra were recorded on 2 mL samples (10 mm o.d. tubes) with a Bruker AMX 300 spectrometer (Bruker, Karlsruhe, Germany) with a broadband probe and a Bruker BVT 2000 unit for temperature control. ^{17}O transverse relaxation times of distilled water (pH = 6.5) were measured by use of a CPMG sequence and a subsequent two-parameter fit of the data points. The 90° and 180° pulse lengths were 25 μs and

50 µs, respectively. The value for the ^{17}O T_2 of water in P760-Gd ([P760-Gd] = 19 mM, pH = 6.0−7.0) was obtained from linewidth measurement. All ^{17}O NMR spectra were proton-decoupled. The analyses of the ^{17}O data and of the transverse relaxation rates of deuterium were described elsewhere.[11] All T_1 measurements at 7.05 T (^{1}H, ^{2}H) were performed with the inversion recovery or fast inversion recovery Fourier transform technique and a subsequent three-parameter fit of the peak heights. Diffusion coefficients were measured with an Avance 200 spectrometer fitted with a thermostatted diffusion probe and the gradient spin echo sequence (25−30 gradient strengths ranging from 10 to 250 G/cm for water diffusion measurement or from 170 to 750 G/cm for P760-La diffusion measurement; gradient pulse duration: 1 ms, diffusion time: 6.05 ms, NA:1 for water or 8−32 for P760-La, TR: 8−10 s depending on the temperature). The sample (260 µL) was contained in a 5 mm o.d. tube. Thermostatting was guaranteed by the water flow inside the probe.

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