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Characterization of Molecular Dynamics of Small Ligands and their Paramagnetic Complexes by Multinuclear Relaxometry (H-1, H-2, O-17).

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INTRODUCTION.

Water proton relaxation rates of paramagnetic solutions are governed by innersphere and outersphere interactions and are usually analyzed by their Nuclear Magnetic Resonance Dispersion (NMRD) profiles. This step requires some a priori knowledge and some adjustment of the parameters governing the magnetic interactions. It can therefore be helpful to obtain quantitative information by alternative techniques. In this respect, ^{17}O NMR can be used to estimate the number of coordinated water molecules (q)[1] and exchange rate of water between the first coordination sphere and bulk water (τ_M).[2] The nuclear relaxation rate of a deuterium covalently bound to a carbon depends only on the quadrupolar coupling constant and on the molecular tumbling. Hence the rotational correlation time of the molecule (τ_R) can be easily calculated from R_1 measurements.[3] The aim of this work was thus to use ^2H NMR relaxation rates of specifically labelled ligands and of their diamagnetic lanthane (III) complexes to evaluate the τ_R in aqueous solutions. Several known contrast agents (Gd-DTPA, Gd-DOTA, Gd-DTPABMA, Gd-EOBDTPA) as well as a new complex 1-benzyldiethylenetriaminepentaacetate gadolinium (III) (Gd-BzDTPA) were studied. Interactions with seric proteins were also investigated through ^2H transverse and longitudinal relaxation rates.

METHODS

Bz-DTPA was synthetized according to Brechbiel et al's procedure.[4] The organic ligands DTPA, DOTA, DTPA-BMA, EOB-DTPA and Bz-DTPA were deuterated on carboxylic acid (or amide) α-carbons using D_2O in basic solutions (K_2CO_3).[5] ^2H and ^{17}O NMR spectra were obtained on a Bruker MSL 200 spectrometer (4.7 T) using a broadband probe respectively tuned at 30.7 and 27.1 MHz. No field frequency lock was used except for measurement of ^{17}O chemical shifts $(D_2O \approx 15\%)$. Deuterium depleted water was used for ^2H NMR measurements. Seric solutions (Kontrollogen L, Behring) were prepared with deuterium depleted water. T_1 of ^2H were measured using the IRFT sequence and a 3 parameters exponential fitting procedure. τ_M was estimated from ^{17}O transverse relaxation rates of water in the different gadolinium complex solutions using linewidth measurements.[2] Diamagnetic relaxation rate of ^{17}O water was obtained from a Carr-Purcell-Meiboom-Gill sequence. Samples (2 ml) were contained in 10 mm outer diameter pyrex tubes. Temperature was controlled by a BVT 1000 unit using air or nitrogen gas flow. Concentration of ligands or complexes was 50 mM except for Dy complexes for which concentrations varied from 10 to 80 mM.

RESULTS AND DISCUSSION

^{17}O NMR: The number of coordinated water molecules in lanthanide EOBDTPA and BzDTPA was estimated to 1.9 and 2.1 respectively from water ^{17}O chemical shift measurements performed on Dy complexes. τ_M at 310K obtained from R_2 measurements of ^{17}O of Gd-EOBDTPA solution was 9.10^{-8} s and thus in good agrement with the reported value for Gd-DTPA $(1.05\ 10^{-7}$ s)[4], whereas τ_M of Gd- BzDTPA was longer $(3.1\ 10^{-7}$ s).

^2H NMR: τ_R values derived from ^2H longitudinal relaxation rates (Table 1) were calculated using a quadrupolar coupling constant of 170,000 kHz. The τ_R of ligands and La^{3+} complexes are very close and in good agreement with those obtained from the analysis of ^1H NMRD profiles (Table 1).

	Ligands	La complex	Gd complex (a)
DTPA	55±7	58±7	56 (b)
DTPABMA	58±8	66±9	67 (b)
DOTA	62±8	71±9	53 (b)
EOBDTPA	65±8	66±9	61 (c)
BzDTPA	63±8	64±8	57 (c)

Table 1: τ_r (ps) of ^2H labelled ligands and La^{3+} complexes in aqueous solution (pH=7, T=37°C).
(a) values obtained from ^1H NMRD profiles. (b) q=1 (c) q=2

In seric solution, ^2H R_1 increased slightly for all labelled ligands. This relaxation enhancement may result from a viscosity or microviscosity effect and/or from interaction between the ligand and seric proteins. Stokes Einstein law predicts that τ_R is roughly proportional to molecular volume. The τ_R of a ligand bound to a macromolecule like albumin can thus be estimated at $\approx 1.10^{-8}$ s, so that $\omega\tau_R$ is >1. Since the extreme narrowing condition is no longer valid, R_1 is not ideally sensitive to protein binding. On the contrary, the R_2 variation would be more appropriate. In seric solution, linewidth increases of DTPA, DOTA and DTPABMA are < 8 Hz, whereas the resonances of the more lipophilic EOBDTPA and BzDTPA are markedly broadened (>25 Hz) due to their interaction with macromolecules.

In summary, in aqueous saline solutions, τ_R of labelled ligands or diamagnetic complexes can easily be obtained by longitudinal relaxation rates of ^2H. On the other hand, analysis of ^2H linewidths is more appropriate to get information on possible interaction between ligands and macromolecules. This technique showed that DTPA, DOTA and DTPABMA do not interact with protein, whereas EOBDTPA and BzDTPA clearly associate with seric macromolecules.

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