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INTRODUCTION.

Water proton relaxation rates of paramagnetic solutions are governed by inner sphere and outer sphere 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) and the exchange rate of water between the first coordination sphere and bulk water ($\tau_w$). 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 ($\tau_R$) can be easily calculated from $R_1$ measurements. The aim of this work was thus to use $^2$H NMR relaxation rates of specifically labeled ligands and of their diamagnetic lanthanide (III) complexes to evaluate the $\tau_R$ in aqueous solutions. Several known contrast agents (Gd-DTPA, Gd-DOTA, Gd-DTPBMA, Gd-EOB-DTPA) as well as a new complex 1-benzylidenetriaminediaceatocarboxylate gadolinium (III) (Gd-BzDTPA) were studied. Interactions with seric proteins were also investigated through $^2$H transverse and longitudinal relaxation rates.

METHODS

Bz-DTPA was synthesized according to Brechbühl et al.'s procedure. The organic ligands DTPA, DOTA, DTPA-BMA, EOB-DTPA and Bz-DTPA were deuterated on carboxylic acid (or amide) $\alpha$-carbons using $^2$H in basic solutions (K$_2$CO$_3$). $^3$H 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 ($\Delta$D$_2^O=15\%$). Deuterium depleted water was used for $^2$H NMR measurements. Seric solutions (Kontrologen L, Behring) were prepared with deuterium depleted water. $T_1$ of $^2$H was measured using the IRFT sequence and a 3 parameters exponential fitting procedure. $\tau_w$ was estimated from $^{17}$O transverse relaxation rates of water in the different gadolinium complex solutions using linewidth measurements. 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 EOB-DTPA and Bz-DTPA was estimated to 1.9 and 2.1 respectively from water $^{17}$O chemical shift measurements performed on Dy complexes. $\tau_w$ at 310 K obtained from $R_2$ measurements of $^{17}$O of Gd-EOB-DTPA solution was $9 \times 10^{-4}$ s and thus in good agreement with the reported value for Gd-DTPA (1.05 $\times 10^{-7}$ s), whereas $\tau_w$ of Gd-BzDTPA was longer (3.1 $\times 10^{-7}$ s).

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

<table>
<thead>
<tr>
<th></th>
<th>Ligands</th>
<th>La complex</th>
<th>Gd complex</th>
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<tbody>
<tr>
<td>DTPA</td>
<td>55±7</td>
<td>58±7</td>
<td>56 (b)</td>
</tr>
<tr>
<td>DTPBMA</td>
<td>58±8</td>
<td>66±8</td>
<td>67 (b)</td>
</tr>
<tr>
<td>DOTA</td>
<td>62±8</td>
<td>71±8</td>
<td>53 (b)</td>
</tr>
<tr>
<td>EOB-DTPA</td>
<td>65±8</td>
<td>66±8</td>
<td>61 (c)</td>
</tr>
<tr>
<td>Bz-DTPA</td>
<td>63±8</td>
<td>64±8</td>
<td>57 (c)</td>
</tr>
</tbody>
</table>

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

In seric solution, $^2$H 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 the seric proteins. Stokes Einstein law predicts that $\tau_R$ is roughly proportional to molecular volume. The $\tau_R$ of a ligand bound to a macromolecule like albumin can thus be estimated at $\approx 1.10^4$ s, so that $\omega$R$_1$ is >1. Since the extreme narrowing condition is no longer valid, R$_1$ is not ideally sensitive to protein binding. On the contrary, the P$_V$ variation would be more appropriate. In seric solution, linewidth increases of DTPA, DOTA and DTPBMA are <8 Hz, whereas the resonances of the more lipophilic EOB-DTPA and Bz-DTPA are markedly broadened (>25 Hz) due to their interaction with macromolecules.

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

REFERENCES