Modulation of the Prototropic Exchange Rate at the Water Molecule Coordinated to a Gd^{III} Ion

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The aim of this work is to assess whether it is possible to control the rate of the prototropic exchange in a class of DTPA bis-amide Gd^{III} complexes.

In the last decade much attention has been devoted to the understanding of the determinants of the water-exchange process in Gd^{III} complexes because their successful application as MRI contrast agents is related to the occurrence of a suitable exchange lifetime (τ_M) of the coordinated water.[1−4] The latter parameter inversely affects the inner-sphere relaxation term (R_{1p}^{is}) and is often the main determinant of the overall relaxivity of a paramagnetic Gd^{III} complex. The R_{1p}^{is} contribution is given by Equation (1):

$$R_{1p}^{is} = \frac{q[C]}{55.5(T_{1M} + \tau_M)} \quad (1)$$

where [*C*] is the molar concentration of the paramagnetic agent, *q* is the number of coordinated water molecules and T_{1M} is the relaxation time of their protons. Thus, as long as τ_M is shorter than T_{1M} the paramagnetic relaxation effect can be efficiently transferred to the bulk water solvent.

Bis-amide derivatives of Gd-DTPA (DTPA = diethylenetriamine pentaacetic acid) display relatively long exchange lifetimes of the coordinated water,[5−8] therefore their relaxivity is usually smaller than expected. However, such a drawback has been exploited in order to assess the rate of prototropic exchange at the coordinated water molecule. The latter process is pH-dependent and becomes significantly high either at acidic or basic pH, to overcome the "quenching" effect of the slow exchanging water, thus allowing the system to raise the relaxivity expected on the basis of its T_{1M} value.

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Thus, τ_M in Equation (1) can be replaced by Equation (2):

$$\tau_M = \left[\left(\tau_M^0\right)^{-1} + \left(k_{ex} \frac{k_W}{[H_3O^+]} \right) \right]^{-1} \quad (2)$$

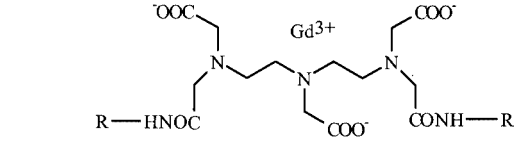
where k_{ex} is the prototropic exchange rate. Although the prototropic exchange is either acid or base catalysed, its assessment, in the case of bis-amide derivatives of Gd-DTPA, is preferentially pursued under basic conditions because the limited thermodynamic stability of these complexes may cause some release of Gd^{III} ions upon protonation of the acetic arms at acidic pH. Typically the determination of k_{ex} is then carried out by fitting the pH-dependent curve of the relaxivity in the pH range between 3 and 10.

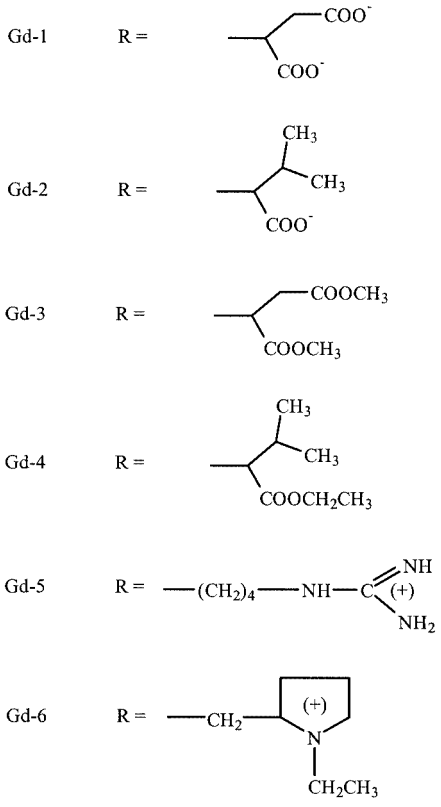
The aim of this work is to assess whether it is possible to control the rate of the prototropic exchange in this class of Gd^{III} complexes.

Six novel bis-amide derivatives of Gd-DTPA have been synthesised (Scheme 1). These ligands were obtained according to the previously reported procedure[9] based on the reaction of DTPA bis-anhydride with the amine group of the entering R substituent. Compounds **1** and **2** were obtained by hydrolyzing the ester functionalities of **3** and **4**, respectively.

The corresponding Gd^{III} complexes were obtained by reacting equimolar amounts of any given ligand and $GdCl_3$ at pH 6. The eventual excess of uncomplexed Gd^{III} ions was precipitated as the hydroxide as the pH of the solution was brought to basic values, and separated by ultracentrifugation.

At neutral pH and 298 K, the relaxivity (measured at 20 MHz) shown by all six complexes is very similar (ca. 5 ± 0.4 $mM^{-1}s^{-1}$). In all cases the relaxivity increases as the temperature is increased. This is an unambiguous symptom of the occurrence of a slow water-exchange that "quenches"





Scheme 1. Schematic representation of the structures of the six bisamide DTPA derivatives

the attainable relaxivity in analogy with previous observations in a number of related Gd-bisamide DTPA systems.[10−12] ^{17}O-R_{2p} measurements at variable temperature yield accurate values for the exchange rate of the coordinated water (Table 1) applying a well-established fitting procedure to the theoretical values obtained from the Swift−Connick equation.[13]

Table 1. Relaxometric and dynamic prameters for Gd^{III} complexes

Complexes	r_{1p} (mM^{-1} s^{-1}) at pH 7	τ_M^O (μs)[a]	T_{1M} (s)[b]	k_{ex} (M^{-1} s^{-1})
Gd-1	5	2.36	3.8×10^{-6}	4.6×10^{8}
Gd-2	5.3	1.98	3.6×10^{-6}	2.7×10^{9}
Gd-3	4.6	2.56	5.2×10^{-6}	7.5×10^{9}
Gd-4	5.1	2.22	3.8×10^{-6}	4.2×10^{9}
Gd-5	5.3	2.2	3.95×10^{-6}	1.2×10^{10}
Gd-6	5.3	2.66	3.1×10^{-6}	8.6×10^{10}

[a] Values calculated from the fitting of ^{17}O NMR profiles (pH 7 and 298 K). [b] Values calculated from the fitting of NMRD profiles recorded at basic pH.

The proton relaxivity measurements, in the pH range from 6 to 12, display the expected increase as the prototropic exchange-rate becomes fast enough to remove the limit of the slow-exchange of the coordinated water. It has been very interesting to observe that the increase of r_{1p} takes place at significantly different pH values; for instance, pH 10 for **Gd-1** and pH 7 for **Gd-6** (Figure 1). The observed behaviour clearly reflects the occurrence of different prototropic exchange rates as a function of the R substituents. The accurate determination of the prototropic exchange rates was performed by fitting the observed pH-dependent relaxivity curves according to Equation (1) modified as in Equation (2) and using T_{1M} values obtained from NMRD profiles measured at pH 12 (Table 1) and $\tau \cong \tau_M^O$ values calculated from ^{17}O NMR profiles.

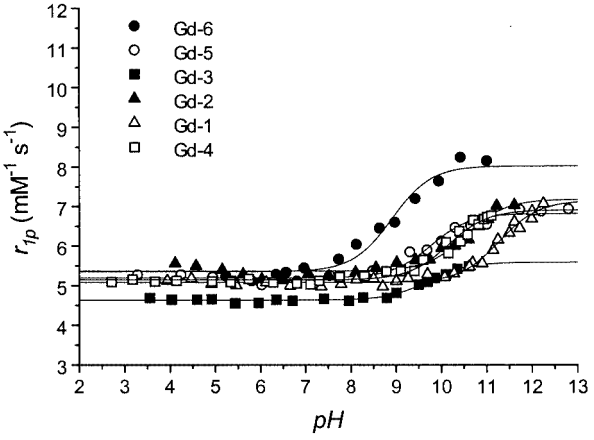


Figure 1. Effect of $[OH^-]$ catalysis on the prototropic exchange as assessed from the increase of r_{1p} as a function of pH. The continuous lines represent the theoretical values calculated according to Equations (1) and (2) for the evaluation of R_{1p}^{is}. R_{1p} is the sum of R_{1p}^{is} + R_{1p}^{os} (outer sphere) and R_{1d}. R_{1p}^{os} was obtained by fitting NMRD profiles. R_{1d} was set equal to 0.38 s^{-1}

Although not proven, we discarded any significant contribution from the exchange of amide protons.[14] When, in related complexes, saturation transfer experiments were carried out by irradiating the NH moieties in Eu^{III} complexes, k_{ex} values were found to be ca. 8×10^5 mM^{-1} s^{-1} around pH 10. Such values do not have significant effects on the R_{1p}^{is} of Gd^{III} complexes [Equation (1): T_{1M} calculated for NH protons, at a distance from the metal ion of 4.2 Å, is 3.6×10^{-5}].

From these data one draws the conclusion that the base catalysis of the prototropic exchange takes place in this series of related complexes with remarkable differences, being fastest for **Gd-6** and slowest for **Gd-1** (Figure 2). The observed behaviour is easily rationalised in terms of the accessibility of OH^- ions to the coordinated water molecule. The positively charged substituents in **Gd-5** and **Gd-6** promote the access of the negatively charged hydroxyl ions to the water coordination site. On the other hand the negatively charged carboxylate moieties in **Gd-1** and **Gd-2** hamper the approach of the hydroxyl groups, thus causing an overall decrease of the prototropic exchange. On passing from **Gd-1** to **Gd-3** and from **Gd-2** to **Gd-4**, the esterifi-

cation of these substituents removes the residual negative charge, with a consequent increase of the base catalysis of the prototropic exchange.

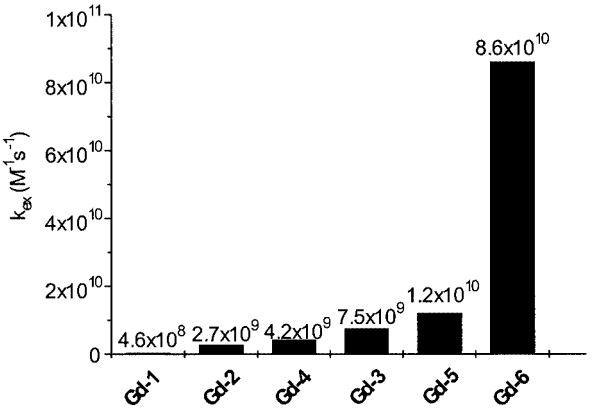


Figure 2. Prototropic exchange rates at the coordinated water molecule in Gd^{III} complexes of a series of bis-amide DTPA ligands

These observations unambiguously indicate that it is possible to control the rate of the prototropic exchange by introducing suitable substituents on the surface of the ligand coordination cage of the Gd^{III} ion. As far as OH^- catalysis is concerned it is apparent that the main determinant is the overall residual charge.

In principle, one may expect that the prototropic exchange rate is also affected by the solution structure of the investigated complex. Actually, the lanthanide(III) complexes of bis-amide DTPA ligands are present in solutions as mixtures of four pairs of interconverting isomers[15−17] and it is likely that the nature of the R substituents could affect the isomer distribution. However, if this were the case, the results reported here indicate that such effects are not of primary importance in the determination of OH^- catalysis of the prototropic exchange of the coordinated water.

Experimental Section

Synthesis of 1−4: Ligands **1**−**4** were prepared by adding DTPA-bisanhydride (1 mmol) in portions to a stirring solution of distilled DMF (15 cm^3) containing 2 mmol of HCl·Asp, HCl·Val, $Asp(OMe)_2$·HCl, or ValOEt·HCl. The reaction mixture was heated for 36 h at 80 °C, the solution was allowed to cool and the solvent was removed under vacuum to result in an oil that solidified upon addition of acetone/diethyl ether (30:70; 300 cm^3). The white solid was dried under reduced pressure over $CaCl_2$.

Synthesis of 5: Diethylenetriamine-*N*,*N*′′-bis(4-guanidinobutyl)-*N*,*N*′,*N*′′-triacetic acid (5) was prepared by adding DTPA-bisanhydride (4.2 g, 0.012 mol) in portions to a stirring solution of distilled DMF (70 cm^3) containing agmatine (3.27 g, 0.025 mol; previously purified from the sulfate by extraction with 1-butanol from water at pH 13). The reaction mixture was heated for 4 h at 50 °C, and 20 h at room temperature. The solution was then allowed to cool and the solvent was removed under vacuum to result in an oil that solidified on addition of acetone/diethyl ether (30:70; 50 cm^3). The white solid was dried under reduced pressure over $CaCl_2$.

Synthesis of 6: Diethylenetriamine-*N*,*N*′′-bis(2-aminomethylethylpyrrolidine)-*N*,*N*′,*N*′′-triacetic acid (6) was prepared by adding DTPA-bisanhydride (0.582 g 0.002 mol) in portions to a stirring solution of distilled DMF (10 cm^3) containing 2-aminomethylethylpyrrolidine (0.418 g, 0.003 mol). The reaction mixture was heated for 24 h at 50 °C, the solution was allowed to cool and the solvent was removed under vacuum to give an oil that solidified on addition of acetone/diethyl ether (30:70; 50 cm^3). The white solid was dried under reduced pressure over $CaCl_2$.

Synthesis of Gd^{III} Complexes: The general procedure for the synthesis of the gadolinium complexes consists of the addition of equimolar amounts of the corresponding ligand (2 mmol) and $GdCl_3·6H_2O$ (2 mmol) in deionized water (20 cm^3), followed by adjustment of the pH to 6.0 by adding KOH.

The longitudinal water proton relaxation rate was measured by using a Stelar Spinmaster (Mede, Pavia, Italy) spectrometer operating at 20 MHz, by means of the standard inversion-recovery technique (16 experiments, 2 scans). A typical 90° pulse width was 3.5 μs and the reproducibility of the T_1 data was ±0.5%. The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper-constantan thermocouple (uncertainty ±0.1 °C).

Variable temperature ^{17}O NMR measurements were recorded at 2.1 T on a JEOL EX-90 spectrometer, equipped with a 5 mm probe, by using a D_2O external lock. Experimental settings: spectral width 10000 Hz, 90° pulse (7 μs), acquisition time 10 ms, 1000 scans and no sample spinning. Aqueous solutions containing 2.6% of ^{17}O isotope (Yeda, Israel) were used. The observed transverse relaxation rates (R^O_{2obs}) were calculated from the signal width at half-height ($\Delta\nu_{1/2}$): $R^O_{2obs} = \pi\,\Delta\nu_{1/2}$.

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