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Notes

pH Dependence of Relaxivities and Hydration Numbers of Gadolinium(III) Complexes of Macrocyclic Amino Carboxylates

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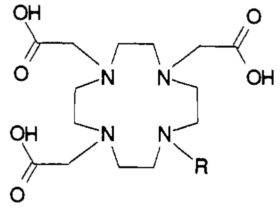
Introduction

In our previous paper,[1] we reported the pH dependence of T_1 relaxivities (R_1, the second-order rate constant that describes the catalysis of T_1 relaxation by a paramagnetic species) for Gd(III) complexes, and hydration numbers (q, the number of inner-sphere coordinated water molecules) for analogous Tb(III) complexes of the linear amino carboxylic acid ligands: EDTA, HEDTA, EGTA, DTPA, and TTHA. A plot of R_1 vs q values for all the complexes of the linear amino carboxylic acids in monomeric forms gave a straight line with an intercept of 2.2 ± 0.1 (mM s)$^{-1}$ and a slope of 1.6 ± 0.1 (mM s)$^{-1}$ per coordinated water. It was concluded that the intercept represents the outer-sphere relaxivity for these Gd(III) complexes, and the slope represents the estimated contribution of each inner-sphere water to R_1. To further examine the effect of charge and ligand structure on the relaxation behavior, we have determined the relaxivities and inner-sphere hydration numbers of Gd(III) and Tb(III) complexes of three macrocyclic amino carboxylic acids, i.e., DO3A, HP-DO3A, and DOTA (Figure 1) as a function of solution pH. An energy-transfer quenching experiment using Eu(III) and Tb(III) complexes was carried out to detect any marked tendency to form oligomers in these macrocyclic complex solutions.[2]

Experimental Section

Materials. Gadolinium, europium, and terbium salts (≥99.9%) were obtained from Alfa Inorganics and Research Chemicals and oven dried at 110 °C for at least 24 h before use. D_2O, NaOD, and DCl were purchased from Aldrich. The macrocyclic amino carboxyl ligands used in this work (DO3A, HP-DO3A, and DOTA) were prepared according to published procedures.[3]

Methods and Procedures. Relaxivity Experiment. The macrocyclic Gd complex samples used in the relaxivity experiment were prepared as in ref 1. To account for the slow kinetics of metal complex formation with the macrocyclic ligands, a back-titration method was used to standardize solutions of the three ligands. Excess Gd^{3+} (approximately 2×) was added to the buffered ligand (pH 5) and the resulting solution heated at 70 °C for 12 h. A standardized EDTA solution was used as a titrant for the excess Gd^{3+} with xylenol orange as indicator. The procedure was also repeated with reaction solutions being heated for 3 days. The same ligand concentrations were obtained. Note that in the back-titration, the EDTA complexes only the free Gd^{3+} rather than competing with DO3A for bound Gd^{3+} in the Gd(DO3A) complex. This is because Gd(DO3A) has a high dissociation kinetic barrier, though its conditional stability constant is not substantially greater than that for Gd(EDTA)$^-$.[4]



R = H DO3A
R = $CH_2CH(OH)CH_3$ **HP-DO3A**
R = CH_2COOH **DOTA**

Figure 1. Structural formulas of the three macrocyclic amino carboxyl ligands.

Spin–lattice relaxation time (T_1) measurements were obtained on an IBM PC/20 Multispec relaxometer (Bruker Ltd., Canada) operating at a fixed magnetic field of 0.47 T (20 MHz) at 40 ± 1 °C.[1] For a series of five Gd-complex concentrations (0.2–5.0 mM) at a given pH, all the T_1 values were used to obtain the relaxivity of a sample through linear least-squares fitting to eq 1, where $(1/T_1)_{obsd}$ = observed spin–lattice

$$(1/T_1)_{obsd} = (1/T)_d + R_{1,GdL}[GdL] \qquad (1)$$

relaxation rate constant (s^{-1}), $(1/T_1)_d$ = diamagnetic relaxation rate constant (s^{-1}), $R_{1,GdL}$ = spin–lattice relaxivity of GdL (mM^{-1} s^{-1}), and [GdL] = GdL concentration (mM).

An IBM BASIC program for linear least-squares fitting was used to analyze the T_1 data. The slopes of the linear $(1/T_1)_{obsd}$ vs [GdL] plots were taken as the R_1 values. The measured solvent T_1 value was ~2 s, which depended slightly on the type of buffer used.

Luminescence Experiment. Slow chelation kinetics of the macrocyclic ligands prompted us to synthesize the Tb(III) complexes prior to our luminescence water-counting experiment. The macrocyclic Tb complexes of DO3A, HP-DO3A, and DOTA were made by reacting the acid form of the ligands with Tb(III) salts in 10% molar excess in deionized H_2O at ca. 80 °C. The complexation process was monitored by the pH changes of the reaction solution. After 12 h of reflux, the majority of the unreacted Tb was removed in the form of a $Tb(OH)_3$ precipitate by raising the solution pH to ~10. Further purification of the complex product was accomplished by repetitive elutions of the neutralized reaction solution through a reversed phase C18 column (YMC, Inc., Morris Plains, NJ) run by a preparative HPLC (Rainin Instrument Co., Inc., Ridgefield, NJ) with automated sample injection and fraction collection. The mobile phase was made of deionized H_2O mixed with 1–10% CH_3CN. The purities of the Tb(III) complexes were finally assessed by analytical HPLC with fluorescence detection and elemental analysis.[5,6] The complexes were dried in an oven at 120 °C overnight before being used for the luminescence experiments.

The transient luminescence data were collected with a pulsed nitrogen laser-based luminescence apparatus.[1] Two long-pass color filters, 475 and 535 nm, were added between the 1-cm sample cuvette and the PMT. A 1-ms initial delay was used to bypass fast fluorescence emissions, if any, and to allow the PMT to restore itself from possible saturation caused by the scattered excitation light. The emission decay rate constants were obtained by least-squares fitting of the decay curves into single exponential functions.

The pH of the sample solutions was varied between 2 and 11, and the transient luminescence measurement being made at each pH reading. For the TbL samples with pH less than 4, the transient luminescence experiments were performed after thermostating the sample solutions at

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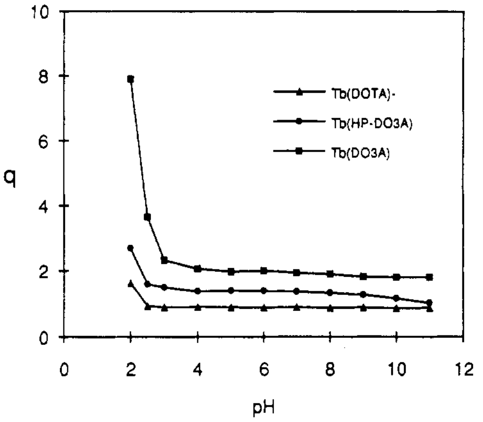

Figure 2. pH dependence of the hydration number, *q*, for the three macrocyclic amino carboxylato Tb(III) complexes, at 22 °C.

70 °C for 24 h. This was necessary for the TbL solutions to reach their equilibrium conditions because acid-assisted dissociations of these macrocyclic complexes are often kinetically slow.[4] The acidity of the D_2O solutions of TbL was also varied by adding standard NaOD or DCl. The luminescence decay rate constants of TbL in H_2O and D_2O solutions, k_{H_2O} and k_{D_2O}, at various pH/pD values were used to calculate the q values based on eq 2,[7] where *A* is a proportionality constant taken as 4.2

$$q = A(k_{H_2O} - k_{D_2O}) \qquad (2)$$

ms as determined from previous luminescence and crystallographic studies of various Tb(III) complexes.[7,8]

For intermolecular energy-transfer quenching studies, the solutions of TbL and EuL were buffered with 100 mM PIPES (pH 6), THAM (pH 8), or NH_4OH (pH 10) to ensure that the pH of each TbL/EuL mixture was the same. The Tb(III) luminescence intensities of the samples were measured by a Spex Fluorolog II spectrometer (Spex Industries Inc., Edison, NJ). Excitation wavelength was set at 495 nm and the emission spectrum scanned over the region from 530 to 575 nm. The Stern–Volmer intensity quenching constant, K_{SV}^{ϕ}, was evaluated from the emission intensities of TbL measured as a function of the concentration of EuL quencher [Q]

$$(I_o - I)/I = K_{SV}^{\phi}[Q] \qquad (3)$$

where I_0 and I are the TbL emission intensities in the absence and presence of EuL quencher, respectively.[2,9]

Results and Discussion

Luminescence Studies. Determination of Inner-Sphere Hydration Numbers. The justifications for studying the analogous Tb(III) complexes for the determination of the inner-sphere coordinated water numbers and using these numbers to represent the *q* values of the corresponding Gd(III) complexes have been discussed and referenced in our previous paper.[1]

Plotted in Figure 2 are the *q* values of the Tb(III) complexes calculated from the luminescence decay rate constants determined at various pH readings. The relative standard deviations associated with these *q* values were less than ±5%. The *q* values did not show significant variations with the solution pH in the range from 4 to 11. The average *q* values within this pH range are 1.8 ± 0.2 for Tb(DO3A), 1.3 ± 0.1 for Tb(HP-DO3A) and 1.0 ± 0.1 for Tb(DOTA)$^-$ respectively. The fractional *q* value that we observed with Tb(HP-DO3A) reflects probably additional quenching by the coordinated –OH group of the ligand and/or the effect of an equilibrium distribution of different solution species with different coordination structures. Under more acidic conditions, the measured *q* values increased with the decrease of the solution pH as a result of partial complex dissociations. It is known that the acid-assisted dissociation for macrocyclic amino carboxylate complexes is sluggish due to the slow kinetics associated with the process of the ligand structural reorganization.[4] Slow dissociation kinetics were observed in our *q* measurements: *q* values determined immediately after the adjustments of the solution pH did not show significant changes. The *q* data presented in Figure 2 between pH 2 and 4 were determined after thermostating 24 h at 70 °C to allow the sample solutions to reach equilibrium. No further significant variations in *q* value were observed with samples thermostated for longer times. Under equilibrium conditions, the dissociation of Tb(DO3A) was almost complete at pH 2, while Tb(HP-DO3A) and Tb(DOTA)$^-$ were only partially dissociated. The Tb(DOTA)$^-$ complex was most acid-resistant, as reflected by the *q* data in Figure 2. These results are consistent with the thermodynamic stability measurements of the Ln(III) complexes of the same ligands.[4]

Table I. K_{SV}^{ϕ} Values in M^{-1} from the Luminescence Quenching Studies

ligand	K_{SV}^{ϕ} pH 6	pH 8	pH 10
DO3A	120	139	222
HP-DO3A	64	107	153
DOTA	50	N/A	116

Luminescence Quenching Studies. The purpose of this study was to demonstrate that the macrocyclic Gd(III) complexes exist as monomeric species in solution. As explained in the Experimental Section, the slopes of linear plots of $(I_0 - I)/I$ vs [Eu] (i.e. [Q]) are the Stern–Volmer intensity quenching constants. These K_{SV}^{ϕ} values were determined, and they are listed in Table I for Tb(DO3A) and Tb(HP-DO3A) at pH 6, 8, and 10, and Tb(DOTA)$^-$ at pH 6 and 10.

It is known that intermolecular energy-transfer luminescence quenching in fluid solution may take place by dynamic (collisional in nature) or static (formation of an associated species between donor and acceptor) processes.[2,9,10] The two quenching mechanisms can be differentiated through a comparison of emission intensity and lifetime quenching studies. The Stern–Volmer lifetime quenching constant, K_{SV}^{τ} (obtained from luminescence lifetime measurements in a manner identical to eq 3), is a measure of dynamic quenching alone, as static quenching does not affect the observed emission lifetime.[2,9] It follows thus that, for monomeric compounds with no tendency for association in solution, the Stern–Volmer quenching constants obtained from emission intensity and lifetime measurements should be equal (i.e., $K_{SV}^{\phi} = K_{SV}^{\tau}$). Since static quenching is normally much more effective in deexcitation of the emissive donor species than is dynamic quenching, the formation of associated species in solution to any significant extent is usually marked by a large inequality in the Stern–Volmer quenching constants obtained from luminescence intensity and lifetime measurements, respectively (i.e., $K_{SV}^{\phi} \gg K_{SV}^{\tau}$).

Dynamic quenching efficiency depends mainly on the collision frequency of molecules in solution. At constant temperature and solution conditions, the variation of K_{SV}^{τ} with ligand structure is small. For the luminescence quenching systems consisting of Tb(III)/Eu(III) polyamino carboxylate complexes studied so far, we observed that $K_{SV}^{\tau} = 110 \pm 50$ M^{-1}.[2,10] We noted that almost all the K_{SV}^{ϕ} data given in Table I are in the vicinity of the above averaged K_{SV}^{τ} value, indicating that the three macrocyclic complexes showed no sign of significant static quenching over the pH range specified and hence little self-association. A slightly larger K_{SV}^{ϕ} value was observed for DO3A complex at pH 10, suggesting a limited tendency of association for the complex at

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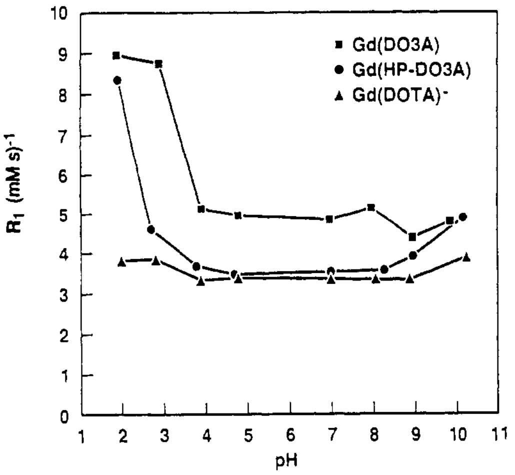

Figure 3. pH dependence of the relaxivity, R_1, for the three macrocyclic amino carboxylato Gd(III) complexes, all in 0.1 M buffers, at 20 MHz and 40 °C.

this high pH. The extent of association is small, compared for example, to that in the HEDTA complex which had a $K^{\ddagger}_{\mathrm{SV}}$ value of 500 M^{-1} at pH 10.[10] This type of molecular self-association tends to occur at high pH because the associated species involve μ-hydroxy bridges.[2,10]

Relativity Studies. The spin–lattice relaxivity values, R_1, for the three macrocyclic Gd(III) complexes at various pH values (ionic strength 0.1) are plotted in Figure 3. The relative standard deviations associated with these R_1 values are estimated to be ±5%. The relaxivity of Gd(HP-DO3A) was measured repeatedly as a control with $R_1 = 3.65 \pm 0.08$ (mM s)$^{-1}$ ($n = 10$).

Paramagnetic metal complexes have both inner-sphere and outer-sphere relaxivities.[12-14] The inner-sphere relaxivity at a fixed radio frequency and temperature depends on a number of parameters as shown in the following abbreviated form of the Solomon–Bloembergen–Morgan (SBM) equations, eq 4, where

$$R_1 \propto q(\mu_{\mathrm{eff}})^2 \tau_c / r^6 \qquad (4)$$

q is the inner-sphere hydration number, μ_{eff} is the effective magnetic moment of the metal ion, τ_c is the correlation time during which the proton nucleus experiences a constant magnetic field from the paramagnetic species, and r is the internuclear distance between the metal ion and the protons of the coordinated water molecules, which may be estimated from the Gd–O bond lengths and Gd–OH_2 coordination geometries.[15-17] Since all the amino carboxylate complexes studied have similar Gd(III)–ligand coordination bonds, r and μ_{eff} are not expected to vary substantially from complex to complex. However, q and τ_c can vary significantly,[1,11,17] leading to a range of R_1 values.

The correlation time, τ_c, of a paramagnetic metal complex reflects weighted contributions from its rotational (τ_r), electronic (τ_e), and coordinated-water exchange (τ_m) components, as described by eq 5.[12] For Gd complexes of relatively low molecular

$$1/\tau_c = 1/\tau_r + 1/\tau_e + 1/\tau_m \qquad (5)$$

weight in buffered aqueous solutions, the dominant correlation time is assumed to be τ_r at 20 MHz and 40 °C.[12,17,23] We have recently determined the τ_r values of analogous Y(III) complexes using a ^{13}C NMR method. The τ_r values of the studied complexes fell in a narrow range from 53 to 68 ps.[18] These results led us to the expectation that the inner-sphere relaxivities of the macrocyclic Gd-complexes would be governed primarily by their q values.

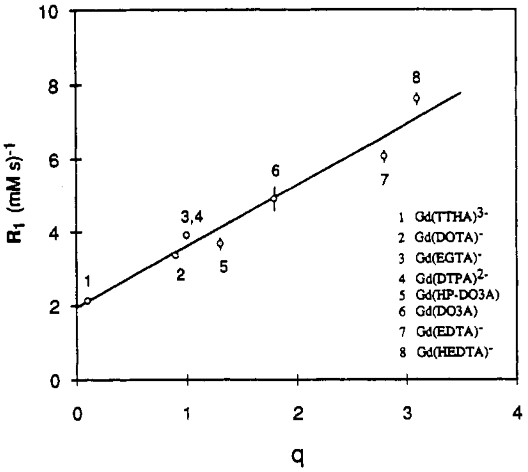

Figure 4. Linear plot of R_1 vs q for all eight amino carboxylato Gd(III) complexes including both linear and macrocyclic ligands. The R_1 and q values were obtained from data presented in Figure 2 and 3 and in ref 1 within the pH ranges over which the complexes were stable and in monomeric forms.

Data shown in Figure 3 illustrate how the relaxivities of the macrocyclic Gd complexes vary with their hydration states (q), as caused by solution pH changes. In the lower pH range (2–4), the Gd-complexes underwent partial dissociation. The observed relaxivity trend was in qualitative agreement with the q values determined under similar pH conditions (Figure 2). The dissociation of the complexes appeared to be more complete for Gd(DO3A) than for Gd(HP-DO3A) and Gd(DOTA)$^-$. In the higher pH range (4–10), over which the macrocyclic Gd complexes are stable, Gd(HP-DO3A) and Gd(DOTA)$^-$ exhibited similar relaxivities: 3.65 ± 0.08 (mM s)$^{-1}$ for Gd(HP-DO3A) and 3.38 ± 0.07 (mM s)$^{-1}$ for Gd(DOTA)$^-$, respectively. Note that these relaxivity values are about the same as those found for the linear amino carboxylate complex Gd(DTPA)$^{2-}$, 3.80 ± 0.10 (mM s)$^{-1}$. The crystal structures of the above three complexes (i.e., Gd(HP-DO3A), Gd(DOTA)$^-$, and Gd(DTPA)$^{2-}$) showed that they all have one coordinated water molecule in their inner coordination spheres.[19,20] Solution-phase transient luminescence studies using the Tb(III) analogues have now confirmed the complexes' hydration states. Studies on Eu(DOTA)$^-$ using ^{17}O NMR[21] and luminescence[22] techniques also demonstrated that $q = 1$ for this

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complex. Thus, the similar relaxivities observed with these three Gd(III) complexes suggest that they have similar outer-sphere contributions to R_1. Gd(DO3A) exhibited higher relaxivity in the pH range from 4 to 10, its average R_1 value being 4.85 ± 0.26 (mM s)$^{-1}$. This relaxivity increase is apparently the result of increased inner-sphere metal hydration, as revealed by the q value ($q = 1.8$).

A plot of R_1 vs q for all complexes in stable monomeric forms studied thus far by our group, including both linear and macrocyclic ligands of amino carboxylic acids, gives a least-squares fitted straight line with an intercept of 2.0 ± 0.3 (mM s)$^{-1}$, a slope of 1.7 ± 0.1 (mM s)$^{-1}$ per coordinated water, and a correlation coefficient of 0.98, as shown in Figure 4. The error bars associated with the data points in Figure 4 reflect the standard deviations in R_1 values obtained in the pH ranges over which the complexes were stable and in monomeric forms. The intercept and slope values of the fitted line in Figure 4 are not significantly different from those reported previously for the linear Gd(III)-complexes alone.[1] As discussed in our previous paper,[1] we may conclude from the data in Figure 4 that the outer-sphere relaxivity for the Gd(III) complexes of all the studied amino carboxylate ligands is about 2.0 ± 0.3 (mM s)$^{-1}$ and the estimated contribution of each inner-sphere water to R_1 for this series of simple mononuclear and highly hydrophilic Gd(III) amino carboxylate complexes, both linear and macrocyclic, anionic and neutral, is 1.7 ± 0.1 (mM s)$^{-1}$.[24]