Dependence of the relaxivity and luminescence of gadolinium and europium amino-acid complexes on hydrogencarbonate and pH

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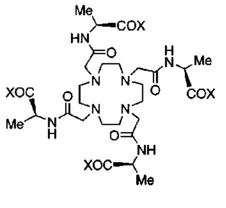
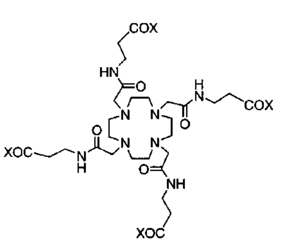
Reversible binding of hydrogencarbonate to a chiral di-aqua lanthanide complex occurs in the pH range 6.5–8.5, limiting the measured relaxivity in the gadolinium complex and enhancing the metal-based emission in the europium analogue.

The behaviour of well defined lanthanide complexes is currently being explored in which the metal-based luminescence (Eu, Tb, Yb)[1] or relaxivity (Gd)[2,3] is a function of a defined biochemical parameter or set of variables, such as pH, $p(O_2)$ or anion concentration. Modulation of the luminescence or relaxivity of a given lanthanide complex may occur *via* ligand- or metal-based processes. Deprotonation of water molecules bound to the metal centre offers an effective means of reducing the relaxivity of cationic gadolinium complexes and pH-dependent behaviour has been demonstrated.[3] For luminescent complexes of europium and terbium, the removal of proximate OH oscillators increases both the lifetime and intensity of the metal-based emission,[4] as intramolecular vibrational quenching of the excited $^5D_0$ or $^5D_4$ state is significantly reduced.[5] Recent preliminary reports have suggested that the binding of hydrogencarbonate to a tri- or di-aqua metal centre may modulate the luminescence[4] (Eu, Tb) and the relaxivity[6] (Gd) of lanthanide complexes under ambient conditions. Such behaviour offers a means of studying both the concentration of HCO_3^- in solution and the pH in the ambient range *via* the H_2CO_3/HCO_3^- equilibrium (effective $pK_a = 6.16$, $I = 0.1$ mol dm^{-3}, 298 K).

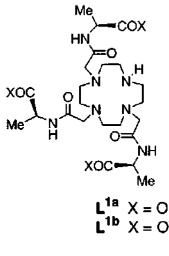
With this in mind, a heptadentate ligand L^{1a} and two model octadentate analogues L^{2a} and L^{3a} have been prepared, and the luminescence and relaxivity of their Eu and Gd complexes studied as a function of pH in the presence or absence of HCO_3^-. Reaction of (*S*)-ethyl-*N*-2-chloroethanoylalanate with 1,4,7,10-tetraazacyclododecane (MeCN, Cs_2CO_3, 65 °C, 18 h, 1% KI) yielded the tetra-ester L^{2a}, and a similar reaction with the β-alanine analogue gave L^{3a} in 55% yield, after purification by column chromatography on neutral alumina. Under milder alkylation conditions (MeCN, $NaHCO_3$, 4 Å sieves, 48 h, 20 °C), the tri-ester L^{1a} was formed and purified on silica gel (CH_2Cl_2, 0→3% Pr^i_2NH). Reaction of the anhydrous $Ln(CF_3SO_3)_3$ salt with each of these ligands in boiling dry MeCN afforded the corresponding cationic esters, and controlled hydrolysis of the ester groups (20 °C, 24 h, 0.02 M NaOH) followed by cation exchange chromatography (Dowex 50 W H^+, eluent 0.5 M aq. ammonia) yielded the carboxylic acid complexes $[LnL^{2b}]^-NH_4^+$, $[LnL^{3b}]^-NH_4^+$ and $[LnL^{1b}]$. The complex $[EuL^{2a}]^{3+}$ crystallised as the hydrated hexafluorophosphate salt from aqueous solution and the crystal structure† revealed a regular mono-capped square-antiprismatic geometry about the europium ion. The NC–CN and NC–CO torsion angles averaged −58.9 and +28.6°, respectively consistent with a Δ(λλλλ) absolute configuration, as observed previously for related tetra-amides with an *S*-configuration at the stereogenic carbon centre.[7] Europium–ligand oxygen bond lengths averaged 2.405 Å [Eu–N(av) 2.642 Å] and the Eu–OH_2 bond length was 2.429(4) Å.



L^{2a} X = OEt
L^{2b} X = O$^-$



L^{3a} X = OEt
L^{3b} X = O$^-$



L^{1a} X = OEt
L^{1b} X = O$^-$

The kinetic stability of the gadolinium complexes was assessed by measuring the change in the water proton relaxation rate as a function of time in 2.5 M HNO_3 at 298 K. The half-lives for dissociation under these conditions were 639 h $[GdL^{2b}]^-$, 304 h $[GdL^{3b}]^-$ and 0.2 h $[GdL^{1b}]^-$. Such high stability to acid-catalysed dissociation has been observed previously for tetra-amide Gd complexes[9] and may be compared to a value of 4.5 h for $[Gd(dota)]^-$ (dota = 1,4,7,10-tetraazacyclododecane tetracetate). The corresponding europium complexes were stable in aqueous solution at 20 °C in the pH range 3–10 for several weeks.

Variable temperature ^{17}O NMR experiments with the Gd complexes of L^{1b}, L^{2b} and L^{3b} allowed the mean lifetime for water exchange to be estimated. At 298 K and pH 7, τ_m values of 8.31, 19.0 and 1.24 μs were measured for $[GdL^{2b}]^-$, $[GdL^{3b}]^-$ and $[GdL^{1b}]$ respectively which are sufficiently slow to limit the overall relaxivity of the two $q = 1$ complexes[9] [eqn. (1); $\tau_m > T_{1m}$, where R_{1p}^{is} is the inner sphere contribution to the

$$R_{1p}^{is} = \frac{C_{tot}q}{55.6\,(T_{1m} + \tau_m)} \tag{1}$$

measured relaxivity, τ_m is the mean water exchange rate, q is the number of bound water molecules, C_{tot} is the complex concentration (mM) and T_{1m} is the longitudinal relaxation time of the coordinated water protons]. Thus at 20 MHz, 298 K and pH 6, the measured proton relaxivity was 2.86 mM^{-1} s^{-1} for $[GdL^{2b}]^-$, 2.31 mM^{-1} s^{-1} ($[GdL^{3b}]^-$) and 7.21 mM^{-1} s^{-1} for $[GdL^{1b}]$. The pH dependence of the relaxivity of $[GdL^{2b}]^-$ and $[GdL^{3b}]^-$ (20 MHz, 298 K) was similar. In the pH range 5–8, the overall relaxivity is dominated by the outer sphere contribution, and at lower pH successive protonation of the carboxylate groups (pH 4.5–2.5) slightly enhanced the relaxiv-

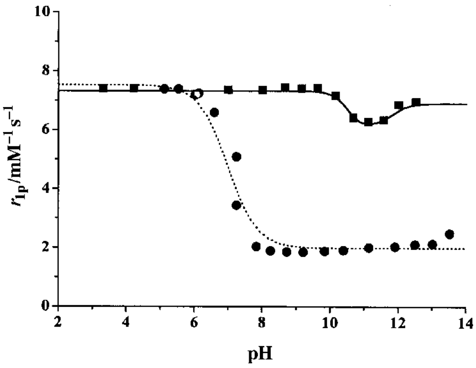


Fig. 1 pH Dependence of the relaxivity of [GdL^{1b}] (1 mM, 20 MHz, 298 K) in degassed aqueous solution (■), and in a saturated aqueous solution of $NaHCO_3$ (●). The solid and dotted lines show the fits to the experimental data.[11]

ity associated with prototropic exchange. At pH > 9, base-catalysed prototropic exchange occurs, involving the co-ordinated water molecule (pK_a = 9.3, measured potentiometrically, 298 K, I = 0.1 M NMe_4NO_3); at pH > 11, fast exchange of the amide NH protons is associated with even higher overall relaxivity values owing to an enhanced interaction with second-sphere waters, as suggested for related tetra-amide complexes.[8,11]

The relaxivity behaviour of [GdL^{1b}] differed markedly: in degassed solution (*i.e.* in the absence of dissolved CO_2 and [HCO_3^-]) the relaxivity in the pH range 2→10 was very similar to that observed for other q = 2 complexes . In aerated solution, and more clearly in a saturated aqueous solution of $NaHCO_3$, the relaxivity was very low in the pH range 8→12 (1.90 mM^{-1} s^{-1}), typical of a purely 'outer-sphere' contribution. As the pH tends towards 6, the [HCO_3^-] falls and the relaxivity increased markedly (Fig. 1).

With the corresponding europium complex, [EuL^{1b}], the rate constant for decay of the Eu luminescence was measured at pH/D = 3.80 to be 2.94 (H_2O) and 1.23 ms^{-1} (D_2O), and at a pH/D of 8.6 in the presence of a 40-fold excess of $NaHCO_3$ k_{H_2O} = 2.27, k_{D_2O} = 1.33 ms^{-1}. Such values are consistent with a reduction in the number of directly bound water molecules (q) and q values of 1.49 (pH 3.8) and 0.55 (pH 8.6) may be estimated.[5] The intensity (and lifetime) of the Eu-emission was only pH dependent in the presence of HCO_3^- and the intensity of the hypersensitive ΔJ = 2 band (λ_{em} = 618 nm) decreased by a factor of 9 as the pH fell to 6.5 (1 mM [EuL^{3b}], 40 mM $NaHCO_3$). A pH titration (Fig. 2) in a simulated background of physiological anions highlights the reduction in intensity of this band with pH. The ^{1}H NMR spectrum of [EuL^{1b}] at pD 3.8 (293 K) revealed a pattern of resonances characteristic of a square-antiprismatic coordination environment,[7,8,10] with the most shifted ring axial protons resonating at δ 24.9, 17.3, 14.8 and 12.0. At pD = 8.6, in the presence of a 40-fold excess of $NaHCO_3$, the spectrum changed dramatically and the four ring axial protons resonated at δ 11.0, 9.2, 7.7 and 6.2. In the absence of added $NaHCO_3$, no significant spectral changes occurred over the pD range 3–8.

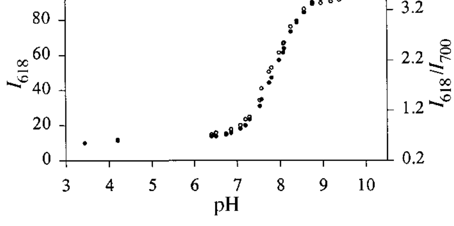
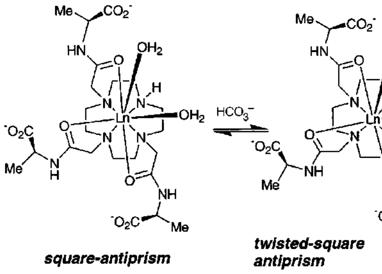


Fig. 2 pH Dependence of the europium luminescence of [EuL^{1b}] [1 mM, 298 K, 30 mM added $NaHCO_3$, 0.1 M NaCl, 2.3 mM lactate, 0.13 mM citrate, 0.9 mM hydrogenphosphate; λ_{em} = 618 nm (ΔJ = 2), and with the *ratio* of ΔJ = 2/ΔJ = 4 bands (open circles)].



Scheme 1

Taken together the solution behaviour is consistent with reversible chelation of HCO_3^- at the lanthanide centre, displacing the bound water molecules. In the chelated ternary complex, the lanthanide ion adopts a twisted square-antiprismatic structure with a reduced helicity about the metal centre[4] (Scheme 1). The behaviour observed with these model complexes augurs well for the development of practicable relaxation and luminescent complexes that respond to pH and $pHCO_3$ changes in biological fluids under physiological conditions.

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Notes and references

† *Crystal data* for $C_{36}H_{70}EuF_{18}N_8O_{14.5}P_3$, M = 1433.87, orthorhombic, space group $P2_12_12_1$, a = 12.5731(4), b = 15.4085(5), c = 29.7563(10) Å, U = 5764.8(3) Å^3, D_c = 1.652 g cm^{-3}, λ(Mo-Kα) 0.71073 Å, Z = 4, μ = 1.294 mm^{-1}. Data were collected on a SMART at 150(2) K. Refinement of 786 parameters by full matrix least squares on F^2 (SHELX 96) converged at R = 0.049, wR_2 = 0.094 for 13218 reflections with $I > 2\sigma(I)$. CCDC 182/1240. See http://www.rsc.org/suppdata/cc/1999/1047/ for crystallographic files in .cif format.

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11 Parameters for the fitting of the relaxivity/pH profiles, using T_{1m} data from NMRD profiles (0→100 MHz) and τ_m data from VT-^{17}O NMR measurements, gave, for example [Fig. 3, (○)]: q = 2, T_{1m} = 5 × 10^{-6} s, τ_m = 1.24 10^{-6} s, R^{os} = 1.99 ± 0.17 s^{-1} with an effective dissociation constant associated with bound HCO_3^- protonation of 6.99 ±0.05. Full details of the model used to analyse the pH dependence of related tetra-amide complexes are being published elsewhere.[9]

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