ChemComm

Selective signalling of zinc ions by modulation of terbium luminescence†

Ofer Reany,[a] Thorfinnur Gunnlaugsson[ab] and David Parker*[a]

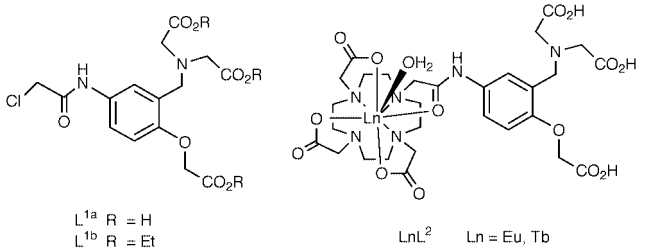
[a] *Department of Chemistry, University of Durham, South Road, Durham, UK DH1 3LE. E-mail: david.parker@durham.ac.uk*

[b] *Department of Chemistry, Trinity College Dublin, Dublin 2, Eire*

Received (in Cambridge, UK) 10th January 2000, Accepted 16th February 2000, Published on the Web, 2nd March 2000

Luminescence enhancement of terbium emission accompanies zinc ion binding at pH 7.4 with an apparent dissociation constant of 0.6 μM in a competitive ionic background.

Responsive luminescent lanthanide complexes have been defined recently in which the concentration variation of certain analytes has been signalled by changes in emission intensity,[1] lifetime[1,2] or polarisation.[3] For this purpose, kinetically robust complexes of Eu and Tb have been studied in particular and systems that respond to changes in pH,[4] pO_2[2,5] and pX[6] in water have been reported. We set out to prepare analogous complexes which respond sensitively and selectively to changes in metal ion concentration, in competitive aqueous media at physiological pH. The signalling of zinc ion concentration is of particular interest in this respect. Although total [Zn^{2+}] have long been established to be *ca.* 12 μM in serum,[7] there is considerable interest in defining the concentration of 'available' Zn^{2+}—both in the extracellular and intracellular environments. Different approaches have been adopted,[8,9] of which the most advanced involve the use of fluorescent 8-tosylamide-quinoline derivatives.[10,11] In seeking to prepare a suitable complex, the requirement for selectivity over Na^+, K^+ and more particularly Mg^{2+} (*ca.* 0.9 mM) and Ca^{2+} (1.2 mM extracellular; < 0.1 μM intracellular) was evident. Accordingly, we resolved to prepare the ligand L^{1a} and explore the complexation behaviour of the Eu and Tb complexes in the macrocyclic derivative L^2, as model systems for selective zinc binding.



Stepwise alkylation of *o*-aminomethylphenol ($BrCH_2CO_2Et$, 5% KI, Na_2HPO_4; *O*-alkylation with $BrCH_2CO_2Et$, KI, K_2CO_3) followed by mild nitration (HNO_3–HOAc; −20 °C) yielded an intermediate nitro-ester which was reduced (H_2/Pd–C/MeOH) and acylated with chloroacetyl chloride (Et_3N, DEE) to yield the triethyl ester L^{1b}. Basic hydrolysis of L^{1b} (pH 12, 20 °C, aq. NaOH) afforded the amino acid L^{1a} which was purified by ion-exchange chromatography. Reaction of L^{1b} with 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (MeCN, K_2CO_3) followed by treatment with CF_3CO_2H–CH_2Cl_2 and complexation with $Ln(NO_3)_3{\cdot}5H_2O$ (Ln = Eu, Tb; pH 5.5; 20 °C) gave the neutral lanthanide complexes; successive treatment with aqueous sodium hydroxide and then strong-acid cation exchange resin afforded the desired lanthanide complexes, [LnL^2].[12] Measurements of the rate constants for decay of the Eu or Tb excited state were made in H_2O and D_2O in the absence and presence of 10 mM $ZnCl_2$. Values for [EuL^2] (k_{H_2O} = 1.61, k_{D_2O} = 0.45 ms^{-1}; *q* (Ln hydration state) = 1.0[13]) were unchanged in the presence of zinc and data for [TbL^2] (k_{H_2O} = 0.55, k_{D_2O} = 0.29; *q* = 1.0) were similarly unaffected by addition of $ZnCl_2$ or $CaCl_2$.

Addition of $ZnCl_2$ to L^{1a} or [LnL^2] was monitored by changes in absorption, fluorescence and lanthanide emission at pH 7.4. The ligand L^{1a} absorbed at 248 nm and no change to the position and intensity of this band occurred on ion binding, suggesting that the aryl ether oxygen was not participating in ion binding. For [TbL^2], a small blue-shift was observed in absorption (λ_{max} 255 → 250 nm); in fluorescence emission two bands were observed at 440 nm and a second, half as intense at 365 nm, which may be ascribed to internal charge transfer and locally excited states, respectively. On binding zinc, the former band shifted (442 → 430 nm) and reduced in intensity, while the latter did not shift but increased in intensity. Zinc binding was also characterised by a small increase in absorbance ($\lambda_{max.}$ = 250 nm) and with a larger enhancement of lanthanide emission intensity, following excitation at the isosbestic wavelength (262 nm). Smaller changes in absorption and emission spectra were obtained following addition of $CaCl_2$ (*e.g.* < 3 nm shift in absorption $\lambda_{max.}$) and no significant variations in absorption wavelength were observed following $MgCl_2$ addition, consistent with reduced or insignificant aryl ether oxygen donation, in these cases. The enhancement of Tb (and Eu) emission intensity (26 and 42%, respectively) upon Zn^{2+} binding is likely to be related to suppression of a photo-induced electron transfer from the benzylic nitrogen to the intermediate aryl singlet excited state.[1,2] In addition for [EuL^2], the metal based emission and ligand based fluorescence intensity was *ca.* 20 times lower than for [TbL^2], owing to quenching of the intermediate singlet state by the Eu^{3+} ion.

Such spectral changes allowed titrations to be carried out, monitoring absorption and luminescence intensity variations as a function of added metal salt concentration. The data obtained reveal a consistent selectivity pattern (Table 1), with Zn^{2+} bound more strongly than Ca^{2+} or Mg^{2+} ions. Two other general features emerged from this analysis: metal ion complexation of the linking amide carbonyl enhanced the ion-binding affinity of the potentially pentadentate ligand and a slightly higher apparent affinity was also found in the excited state for the lanthanide complexes, *i.e.* when observing ligand or lanthanide emission intensity variations. The lanthanide ion may serve to promote intramolecular charge transfer involving conjugation of the ether oxygen lone pair in the ground and excited states, *via* a through-ring electron withdrawing effect. This may also lead to a better orientation of the oxygen lone pair, in those cases (*e.g.* Zn^{2+} binding) where chelation of the benzylic N and the aryl ether O occurs. Such behaviour allowed zinc concentrations to be monitored in the sub-micromolar range, even in a simulated extracellular environment, *i.e.* the presence of 0.9 mM $MgCl_2$, 1.26 mM $CaCl_2$, 140 mM NaCl and 4 mM KCl, with an apparent dissociation constant of 0.6 μmol (pH 7.4, 295 K). The observed zinc ion sensitivity and selectivity with [TbL^2] augur well for the development of practicable signalling

† Electronic supplementary information (ESI) available: representative examples of spectral changes and of data analysis. See http://www.rsc.org/suppdata/cc/b0/b000283f/

DOI: 10.1039/b000283f

Table 1 Affinity constants (log β_{ML}) for cation binding, based on modulation[c] of absorption (S_0) or lanthanide (Ln*) luminescence[d] (295 K, 10 mM HEPES, 20 mM NaCl, 115 mM KCl, pH 7.4[a])

Ligand/complex (parameter)	Zn^{2+}	Ca^{2+}	Mg^{2+}
$L^1(S_0)$	5.04	3.91	2.1
$[TbL^2](S_0)$	5.48	3.84	2.0
$[TbL^2]$(Tb*)	6.35[b]	4.03	2.5
$[EuL^2](S_0)$	5.38	3.90	2.1
$[EuL^2]$(Eu*)	5.99	4.06	1.9

[a] Potentiometric titration of L^1 (298 K, I = 0.1 M NMe_4NO_3) revealed a pK_a for N-protonation of 7.35, echoed by the pH variation of ligand absorption; for binding constant measurements, the pH was adjusted to 7.4 by addition of 'Tris' base and was also monitored at the end of the titration. [b] In a simulated extracellular environment (Na, K, Mg, Ca), the apparent binding affinity for $[TbL^2]$, based on Tb emission intensity changes, was 6.22. [c] Binding constants were obtained by iterative least-squares fitting to a 1:1 model and showed reasonably good agreement to Hill plot analysis. [d] Excitation of the Ln complexes was effected at the isosbestic wavelength (λ_{exc} = 262 nm), while absorption spectral changes were monitored at 240 or 250 nm (294 nm for L^1). Excitation was also performed in the tail at 320 nm and emission variation as a function of $[M^{2+}]$ gave slightly higher binding affinities, *e.g.* for Ca^{2+}; 4.37 with $[TbL^2]$ and 4.67 with $[EuL^2]$ with a luminescence enhancement of 20%.

systems, in which longer wavelength excitation is of course required and where changes in the form of the lanthanide emission are desirable.

We thank BBSRC for support.

Notes and references

1 A. P. de Silva, D. B. Fox, A. J. M. Huxley, N. D. McClenaghan and J. Roiron, *Coord. Chem. Rev.*, 1999, **185–186**, 297; for examples of Na/K perturbing Ln luminescence in methanol: A. P. de Silva, H. Q. N. Gunaratne, T. E. Rice and S. Stewart, *Chem. Commun.*, 1997, 1891.
2 D. Parker, K. Senanayake and J. A. G. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1998, 2129.
3 R. S. Dickins, T. Gunnlaugsson, D. Parker and R. D. Peacock, *Chem. Commun.*, 1998, 1643.
4 T. Gunnlaugsson and D. Parker, *Chem. Commun.*, 1998, 511; T. Gunnlaugsson, D. Parker and D. A. McDonaill, *Chem. Commun.*, 2000, 93.
5 D. Parker and J. A. G. Williams, *Chem. Commun.*, 1998, 245.
6 D. Parker, K. Senanayake and J. A. G. Williams, *Chem. Commun.*, 1997, 1777.
7 M. M. Parker, F. L. Humaller and D. J. Mahler, *Clin. Chem.*, 1967, **13**, 40.
8 J. D. Stewart, V. A. Roberts, M. W. Crowder, E. D. Getzoff and S. J. Benkovic, *J. Am. Chem. Soc.*, 1994, **116**, 415; A. Godwin and J. M. Berg, *J. Am. Chem. Soc.*, 1996, **118**, 6514; P. S. Eis and J. R. Lakowicz, *Biochemistry*, 1993, **32**, 7981; B. Imperiali and G. K. Walkup, *J. Am. Chem. Soc.*, 1997, **119**, 3443.
9 F. Barigeletti, L. Flamigni, G. Calogero, L. Hammerström, J.-P. Sauvage and J.-P. Collin, *Chem. Commun.*, 1998, 2333.
10 P. D. Zalewski, I. J. Forbes and W. H. Betts, *Biochem. J.*, 1993, **296**, 403; P. Coyle, P. D. Zalewski, J. C. Philcox, I. J. Forbes, A. D. Ward, S. F. Lincoln, I. Mahadevan and A. M. Rofe, *Biochem. J.*, 1994, **303**, 781.
11 K. M. Hendrickson, T. Rodopoulos, P.-A. Pilfet, I. Mahadevan, S. F. Lincoln, A. D. Word, T. Kurucsev, P. A. Duckworth, I. J. Forbes, P. D. Zalewski and W. H. Betts, *J. Chem. Soc., Dalton Trans.*, 1997, 3879.
12 Complexes gave accurate masses (ESMS$^-$), ^{1}H NMR and micro-analytical data in accord with the proposed structures: *e.g.* $[EuL^2]$: *m/z* (ESMS$^-$) 847.1660, ($C_{29}H_{39}EuN_6O_{14}$ −H) requires 847.1659. L^{1a}: Found: C, 39.2, H, 6.34; N, 12.3. Calc.(as bisammonium salt dihydrate): C, 39.3; H, 5.93; N, 12.2%. $[TbL^2]$: *m/z* (ESMS$^-$) 853.1692; ($C_{29}H_{39}TbN_6O_{14}$ –H) requires 853.1699.
13 A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams and M. Woods, *J. Chem. Soc., Perkin Trans. 2.*, 1999, 493.

Communication b000283f