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Self-assembly between dicarboxylate ions and binuclear europium complexes: moving to water—pH dependence and effects of buffers

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Abstract

Binuclear lanthanide complexes consisting of two lanthanide binding domains in kinetically stable DO3A binding pockets linked by a 3-functionalized *meta*-xylyl bridge form stable 1:1 adducts with isophthalate and dinicotinate in water. The influence of buffer, pH and ligand structure on the binding of dinicotinate has been investigated.

The nature of the *f*-electron configurations of the trivalent lanthanide ions results in long lived, high intensity luminescence and strong paramagnetic perturbation of proton resonances as well as highly reduced relaxation times in NMR. Bioassays based on lanthanide chemistry, where the long lived lanthanide centred emission allows for separation of fluorescence background and signal, exploit self-assembly on both the immunology side and to develop the highly emissive lanthanide species that supply the readout for the assay.¹ Lanthanide luminescence is also used in optical imaging,^{2–5} where time-gating methodologies can be applied to reduce background noise and detection limit.⁶ Gadolinium complexes have been extensively used as MRI contrast agents,^{7–10} while bimodal lanthanide based imaging agents have been demonstrated that combine approaches to imaging.¹¹

Self-assembled helicates, relying on thermodynamic stability, have been prepared and studied in great detail.^{5, 12–14} We and others have exploited the formation of ternary complexes between kinetically stable lanthanide complexes and simple carboxylates to probe energy transfer,¹⁵ and as a fast and effective means of screening chromophores for use as sensitizers of lanthanide emission.^{16, 17}

$$K = \frac{a_{Eu_2.1:5}}{a_{Eu_2.1} \cdot a_5} = \frac{\gamma [Eu_2.1:5]}{[Eu_2.1] \cdot \gamma [5]} = \frac{[Eu_2.1:5]}{[Eu_2.1] \cdot [5]} \quad \text{eq.1}$$

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†Electronic Supplementary Information (ESI) available: Dynafit script example, all titration curves, time-resolved intensity profiles with exponential fits and the synthesis of new ligands and complexes are available as supporting information. See DOI: 10.1039/b000000x/

Self-complementary lanthanide complexes of DO3A with benzoate pendent arms exhibit pH-dependent self-association that modulates the luminescence intensity of the lanthanide emission by a combination of water displacement and an increase in of sensitisation efficiency.¹⁸ Such modulation has been used as the basis for lanthanide luminescence sensors,¹⁹⁻²³ a number of which work in aqueous and biological media.^{3, 24-26}

The binding pocket of the binuclear complexes Eu₂.**1-3** consists of the seven-coordinate europium centres and the flat aromatic bridge (Scheme 1). The coordination number of europium is nine, which offers the possibility of both mono- and bi-dentate binding of carboxylates to each europium centre. We have recently shown that isophthalate ions are ideally suited to bind in this binding pocket (Scheme 2), in a study where the association of α, α' -bis(Eu.DO3A)-5-amino-*m*-xylene (Eu₂.**2**) and isophthalate (**5**) was investigated. Isophthalate is not water-soluble, and we were obliged to use the terbium complex Tb.**4** to study assembly processes with Eu₂.**2** in water.²⁷

In this work, we explore the binding of dinicotinate (**6**), the simplest water-soluble analogue of isophthalate, with three binuclear lanthanide complexes, and show that small changes in structure and solvation can have a profound impact upon binding. Figure 1 shows the changes in the europium centred luminescence upon titrating bis(Eu.DO3A)-3,5-dimethylpyridine (Eu₂.**1**) with **6** in TRIS buffer, maintaining [Eu₂.**1**] constant. These dramatic changes, make it evident that a binding event does occur.[§] The association constant *K* (eq. 1) was determined by using Dynafit²⁸ to fit the changes in intensity of each band and the hypersensitivity of the ⁵D₀ → ⁶F₂ transition at 617 nm; a value of 15,100 M⁻¹ was found.

In an attempt to understand the role of the bridging unit in defining the binding constant in TRIS buffer at pH = 9, the substituent on the bridge linking the macrocycles together was varied, using 3,5-dimethyl pyridine (Eu₂.**1**), and two substituted *m*-xylyl bridges bearing amino and nitro substituents (Eu₂.**2** and Eu₂.**3** respectively).²⁹ The association constants of the ternary complexes formed with dinicotinate are shown in Table 1.

Stern-Volmer plots were used to show that collisional quenching perturbs the binding curves for Eu₂.**2** to the point where no binding constant could be determined. For the other complexes, the association constant is of similar magnitude. It must be noted that the luminescence lifetime (τ) does not change significantly upon binding of dinicotinate, indicating that the quenching of the excited state of europium is similar for the hydrated and the ligated europium centre.

The four different binding pockets were studied further by determining the number of inner-sphere O-H oscillators³⁰ (*q*) for each lanthanide centre at pH = 9. The luminescence lifetimes in water are similar ($\tau \approx 0.6$ ms) for all three europium complexes (Table 2); but in D₂O the lifetime of Eu₂.**2** is significantly shorter than the other complexes, as a consequence of much reduced hydration at the lanthanide centres in Eu₂.**2**. Each lanthanide ion in the

[§]Binding constants are commonly determined by absorption measurements: in this case, the profound changes to the luminescence spectra facilitate study of the binding event, as hypersensitive effects in the weak lanthanide absorption spectrum are hard to measure.

complexes of Eu₂.1 and Eu₂.3 at pH = 9, on average is coordinated to a single water molecule, while Eu₂.2 exhibits $q = 0.5$. At this pH, all the complexes clearly have a coordination number of only 8; and it seems probable that the similar lifetimes of the ternary complexes with dinicotinate may arise because binding to a guest occurs without solvent displacement.

The equilibrium shown in Scheme 2 is highly simplified as specific solvation and the 8th and 9th ligands on the europium centres are ignored. In aqueous solution there are two water molecules bound to the lanthanide centres in Eu₂.3. The amino and pyridine heteroatoms will be hydrogen-bonded, and such interactions must be disrupted by the attraction of the carboxylates to the residual charge on the europium centres and the release of water from the hydrophobic face of the *m*-xylyl bridge before the guest can bind in the binding pocket. These processes will all be pH dependent and will also be influenced by the presence of other dissolved species.

To probe the effect of the medium on the equilibrium, the association constant of Eu₂.1:6 was established in TRIS buffer, HEPES buffer and PBS at pH = 7, 8 and 9 (Table 3). Interestingly, *K* is of a comparable order of magnitude in HEPES and TRIS, but is three times larger in PBS. As PBS is a competitive buffer, with binding of phosphate to the europium centres, the opposite observation was expected. If the pH in TRIS buffer is raised to 10.5 the same magnitude of *K* can be achieved. This value should be treated with caution: measurements at higher pH (> 9) are outside the buffering region of both TRIS and HEPES, while only PBS contains enough ions for the assumption of constant ionic strength to be valid. Furthermore, the activity coefficients in equation 1 cancel out as a species with two negative charges is present on both sides of the equilibrium. The variations in association constant with buffer suggest that specific interactions are more important in defining the association of dinicotinate and Eu₂.1 than pH and ionic strength.

The luminescence lifetime of free Eu₂.1 increases with pH in all buffer systems, but does not change significantly after titration with 6 (except in the case of HEPES buffer at pH 7 and 8, where a small increase is observed). Upon binding of dinicotinate a small increase or no change in lifetime is observed, suggesting once again that the *q* is constant throughout titration with 6.

Conclusions

Binuclear europium complexes containing appropriate bridging units bind dinicotinate strongly in aqueous solution. The capability of a buffer to coordinate to a single lanthanide centre does not appear to influence the binding of dinicotinate in the systems studied, as the highest association constants were obtained in phosphate buffered saline. Indeed the reduced binding constants observed in other buffers may indicate competitive binding of buffers normally regarded as non-coordinating.

The complexity of the systems affords considerable scope for further study; at this point no prediction can be made about the magnitude of association constant of a given binuclear lanthanide binding pocket and dicarboxylate dianion guest. Detailed information of the

specific solvation in the system studied and on the solvation around both lanthanide-binding domains is needed.

Selective and strong binding of non-endogenous molecules such as isophthalate ions by binuclear lanthanide complexes is the first step on the road to controlled formation of functional lanthanide containing assemblies *in vivo*, mediated by 3,5-aryldicarboxylates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Notes and references

1. Hemmilä, IA. Applications of fluorescence in immunoassays. Wiley; New York, Chichester: 1991.
2. New EJ, Congreve A, Parker D. Chem. Sci. 2010; 1:111–118.
3. Montgomery CP, Murray BS, New EJ, Pal R, Parker D. Acc. Chem. Res. 2009; 42:925–937. [PubMed: 19191558]
4. Faulkner S, Natrajan LS, Perry WS, Sykes D. Dalton Trans. 2009:3890–3899. [PubMed: 19440586]
5. Bunzli JCG, Piguet C. Chem. Soc. Rev. 2005; 34:1048–1077. [PubMed: 16284671]
6. Faulkner, S.; Matthews, JL. Comprehensive Coordination Chemistry. 2nd Edition. Ward, e. M. D., editor. Vol. 9. Elsevier; 2004.
7. Terreno E, Castelli DD, Viale A, Aime S. Chem. Rev. 2010; 110:3019–3042. [PubMed: 20415475]
8. Bottrill M, Kwok L, Long NJ. Chem. Soc. Rev. 2006; 35:557–571. [PubMed: 16729149]
9. Werner EJ, Avedano S, Botta M, Hay BP, Moore EG, Aime S, Raymond KN. J. Am. Chem. Soc. 2007; 129:1870–1871. [PubMed: 17260995]
10. Woods M, Woessner DE, Sherry AD. Chem. Soc. Rev. 2006; 35:500. [PubMed: 16729144]
11. Uppal R, Ciesienki KL, Chonde DB, Loving GS, Caravan P. J. Am. Chem. Soc. 2012; 134:10799–10802. [PubMed: 22698259]
12. Lemonnier JF, Guenee L, Beuchat C, Wesolowski TA, Mukherjee P, Waldeck DH, Gogick KA, Petoud S, Piguet C. J. Am. Chem. Soc. 2011; 133:16219–16234. [PubMed: 21882836]
13. Bunzli JCG. Acc. Chem. Res. 2006; 39:53–61. [PubMed: 16411740]
14. Piguet C, Bunzli J-CG. Chem. Soc. Rev. 1999; 28:347–358.
15. Faulkner S, Burton-Pye BP, Khan T, Martin LR, Wray SD, Skabara PJ. Chem. Commun. 2002:1668–1669. [PubMed: 12196941]
16. Pope SJA, Burton-Pye BP, Berridge R, Khan T, Skabara PJ, Faulkner S. Dalton Trans. 2006:2907–2912. [PubMed: 16751900]
17. Pope SJA, Coe BJ, Faulkner S, Bichenkova EV, Yu X, Douglas KT. J. Am. Chem. Soc. 2004; 126:9490–9491. [PubMed: 15291523]
18. Faulkner S, Burton-Pye BP. Chem. Commun. 2005:259–261. [PubMed: 15724205]
19. Massue J, Quinn SJ, Gunnlaugsson T. J. Am. Chem. Soc. 2008; 130:6900–6901. [PubMed: 18461933]
20. Gunnlaugsson, T.; Parker, D.; Williams, JAG. Advances in Fluorescence Sensing Technology. Vol. IV. SPIE; 1999.
21. dos Santos CMG, Harte AJ, Quinn SJ, Gunnlaugsson T. Coord. Chem. Rev. 2008; 252:2512.

22. Nwe K, Andolina CM, Huang CH, Morrow JR. *Bioconj. Chem.* 2009; 20:1375.
23. Andolina CM, Morrow JR. *Eur. J. Inorg. Chem.* 2011:154.
24. Mishra A, Gottschalk S, Engelmann J, Parker D. *Chem. Sci.* 2012; 3:131–135.
25. Smith DG, McMahon BK, Pal R, Parker D. *Chem. Commun.* 2012; 48:8520–8522.
26. Butler SJ, Parker D. *Chem. Soc. Rev.* 2012 DOI: 10.1039/C1032CS35144G.
27. Tilney JA, Sorensen TJ, Burton-Pye BP, Faulkner S. *Dalton Trans.* 2011; 40:12063–12066. [PubMed: 21850295]
28. Kuzmic P. *Anal. Biochem.* 1996; 237:260. [PubMed: 8660575]
29. Placidi MP, Natrajan LS, Sykes D, Kenwright AM, Faulkner S. *Helv. Chim. Acta.* 2009; 92:2427–2438.
30. Beeby A, Clarkson IM, Dickins RS, Faulkner S, Parker D, Royle L, de Sousa AS, Williams JAG, Woods M. *J Chem Soc Perk T.* 1999; 2:493–503.

Figure 1.

Titration of a 10^{-5} M solution of $\text{Eu}_1.1$ with **6** in TRIS at pH = 9, with 263 nm excitation.

Top: Uncorrected total emission spectra. Bottom left: binding curve based on emission intensity. Bottom right: binding curve generated from the ratio of the intensity of the band corresponding to the hypersensitive $J=2$ to the major and the other major bands in the spectrum.

Scheme 1.

Binuclear kinetically stable europium (III) complexes and dicarboxylate ions investigated in this study.

Scheme 2.
The equilibrium governing the self-assembly

Table 1

Association constants determined for the europium complexes of **1-4** and dinicotinate in TRIS at pH = 9.

Complex ^a	K Intensity [M ⁻¹]	τ_{start}^c [ms]	τ_{end}^c [ms]
Eu ₂ . 1:6	15,100 [14,991-15,247]	0.70	0.69
Eu ₂ . 2:6	^b	0.50	0.51
Eu ₂ . 3:6	16,900 [15,416-18,501]	0.80	0.70

^a [Eu₂.X] = $1.5 \cdot 10^{-5}$ M; [TRIS] = 0.1 M.

^b An association constant could not be determined as the collisional quenching exactly cancelled the increase in emission intensity.

Table 2Lifetimes in H₂O and D₂O and q for the europium complexes.

		Eu₂-1	Eu₂-2	Eu₂-3
τ/ms^a	H ₂ O	0.56	0.68	0.63
	D ₂ O	1.55	1.24	1.67
$q^{a,b}$		1.1	0.5	0.9

^a Standard error 10 %.^b q, the number of inner sphere solvent molecules coordinated to the lanthanide centre, was calculated using the equation³⁰: $q = A(\tau_{\text{H}_2\text{O}}^{-1} - \tau_{\text{D}_2\text{O}}^{-1} - B)$; with $A = 1.2 \text{ ms}$ and $B = 0.25 \text{ ms}^{-1}$.^e Determined at pH = 9.

Table 3

Binding constants for Eu₂.**1:6** and lifetimes for the free Eu₂ and the complexes. The structures and p*K_a* of the buffers used are shown.

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>TRIS p<i>K_a</i> 8.06</p> </div> <div style="text-align: center;"> <p>HEPES p<i>K_a</i> 7.48</p> </div> <div style="text-align: center;"> <p>PBS p<i>K_a</i> 7.20</p> </div> </div>				
Buffer ^a	pH	<i>K</i> [M ⁻¹]	τ _{start} ^b [ms]	τ _{end} ^b [ms]
TRIS	7	15,800 [15,601-15,995]	0.42	0.52
TRIS	8	16,000 [15,656-16119]	0.52	0.61
TRIS	9	15,100 [14,991-15,247]	0.69	0.70
TRIS	10.5	49,500 [49,067-49,946]	0.85	0.80
HEPES	7	-	0.37	0.79
HEPES	8	18,200 [17,401-18,993]	0.49	0.72
HEPES	9	18,400 [18,172-18,670]	0.76	0.79
PBS	7	39,000 [38,267-39,807]	0.57	0.57
PBS	8	35,200 [34,721-35,629]	0.64	0.58
PBS	9	44,700 [44,031-45,325]	0.84	0.78

^a [Eu₂.**1**] = 5 · 10⁻⁵ M; [TRIS] = 0.1 M; [HEPES] = 0.1 M.

^b Biexponential decay, amplitude weighted average lifetime given. Standard error 10 %