Europium(III) complex-based luminescent sensing probes for multi-phosphate anions: modulating selectivity by ligand choice†

Na Shao,[a] Jianyu Jin,[a] Guilan Wang,[b] Ying Zhang,[a] Ronghua Yang*[a] and Jingli Yuan*[b]

Received (in Cambridge, UK) 11th October 2007, Accepted 11th December 2007
First published as an Advance Article on the web 10th January 2008
DOI: 10.1039/b715719c

Four tetradentate β-diketonate–Eu^{3+} complexes were developed as probes for the luminescent sensing of multi-phosphates. By using an appropriate ligand, the pyrophosphate ion (PPi) could be selectively and sensitively detected.

Luminescent probes that are capable of recognition and determination of anions are useful tools for chemistry, biology, and environmental science.[1] Recently, lanthanide complexes have been attracted as luminescent probes for sensing of some anions, such as NO_3^-,[2] Cl^-,[3] CO_3^{2-}/HCO_3^-,[4] PO_4^{3-}/HPO_4^{2-},[5] AcO^-,[6] citrate,[7] and malate,[8] owing to their unique luminescence properties. Compared with organic fluorescent dyes, luminescent lanthanide complexes show highly desirable spectral characteristics, including relatively high quantum yields, large Stokes shifts, narrow emission bands, and long luminescence lifetimes under ambient conditions.

The main signal transduction for anion recognition was the reversible binding of the lanthanide ions with the anion. The coordinative unsaturated lanthanides displayed strong affinity towards anions, resulting in the displacement of bound water molecules by anions to cause enhancement of the lanthanide luminescence.[2–8] However, improvement of selectivity is still a challenge since most of the anions have higher binding affinities with a lanthanide centre than that of water molecules. An alterative strategy is displacing the bound ligand from the lanthanide centre by the analyte.[9] Recently, Gunnlaugsson *et al.* demonstrated that a self-assembly cyclen-Eu^{3+}–β-diketonate ternary complex was useful as a luminescent sensor for anions based on displacement of the bound β-diketonate.[10] But the approach could not selectively recognize a desired target in the presence of structurally similar anions.

We found that the binding affinities of β-diketonate derivatives with Eu^{3+} ion are determined by the substituents of the ligands. The selectivity may thus be modulated through selecting an appropriate β-diketonate whose binding affinity with Eu^{3+} ion is lower than that of the envisaged analyte, but higher than those of the interferents. To test this strategy, four tetradentate β-diketone ligands $\mathbf{L^1}$, $\mathbf{L^2}$, $\mathbf{L^3}$ and $\mathbf{L^4}$ (see Fig. 1), which have different binding affinities to Eu^{3+} ion, were used as the primary ligands. We demonstrate here how decisive the choice of ligand is to achieve the expected selectivity and sensitivity for multi-phosphates sensing.

The multi-phosphorylated species such as pyrophosphate ($P_2O_7^{4-}$, PPi) and adenosine triphosphates (ATP) are the most important anions in the natural world.[11] In the past decades, a number of luminescent probes for phosphates have been developed.[12] These probes are generally designed by linking a light-emitting group to an anion receptor that employs electrostatic, hydrogen bonding, and metal–ligand interactions as attractive forces. They usually display moderate or high affinities to the analytes in organic media but low affinities in aqueous media, and thus are difficult to discriminate the structural similar anions, such as PPi and ATP, in aqueous solution.[13]

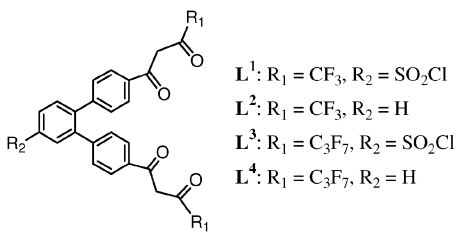
Since Eu^{3+} forms a 1 : 2 complex with the ligand (L),[14] the over-all equilibrium can be represented as following:

$$Eu^{3+} + 2L \overset{K_L}{\rightleftharpoons} [EuL_2]^{3+} \quad (1)$$

One basic requirement in our proposed approach is that the ligand **L** is coupled to the Eu^{3+} centre with an appropriate affinity constant (K_L) for the complex equilibrium. In particular, if a competitive substrate X binding with Eu^{3+} displays an affinity constant K_X,

$$Eu^{3+} + nX \overset{K_X}{\rightleftharpoons} [EuX_n]^{3+} \quad (2)$$

and the interferent X′ gives its own $K_{X'}$ value (for simpleness, the associated water molecules of the Eu^{3+} complexes in eqn (1) and (2) are omitted), the best situation for discrimination of X and X′ in the presence of large excess of X′ is expressed by the inequality $K_X > K_L \gg K_{X'}$. Under these conditions only the envisaged substrate X, but not the interferent X′ even



$\mathbf{L^1}$: $R_1 = CF_3$, $R_2 = SO_2Cl$
$\mathbf{L^2}$: $R_1 = CF_3$, $R_2 = H$
$\mathbf{L^3}$: $R_1 = C_3F_7$, $R_2 = SO_2Cl$
$\mathbf{L^4}$: $R_1 = C_3F_7$, $R_2 = H$

Fig. 1 Chemical structures of the ligands $\mathbf{L^1}$–$\mathbf{L^4}$.

[a] *Beijing National Laboratory for Molecular Sciences, College of Chemistry and Molecular Engineering, Peking University, Beijing, 100871, China. E-mail: Yangrh@pku.edu.cn; Fax: +86-10-62751708*
[b] *State Key Laboratory of Fine Chemicals, Department of Chemistry, Dalian University of Technology, Dalian, 116012, China. E-mail: jingliyuan@yahoo.com.cn; Fax: +86-411-84706293*

† Electronic supplementary information (ESI) available: Experimental procedures, some results of spectroscopic characterization, and the luminescence responses of the complexes to anions. See DOI: 10.1039/b715719c

Table 1 Photophysical parameters and cumulative binding constants (log K_L) between Eu^{3+} and the ligands $\mathbf{L^1}$–$\mathbf{L^4}$

Complex	λ_{max}/nm	ε_{max}/cm^{-1} M^{-1}	ϕ (%)	τ/μs	Binding constant
$\mathbf{L^1}$–Eu^{3+}	335	2.54×10^4	0.17	532	11.23(±0.03)
$\mathbf{L^2}$–Eu^{3+}	337	2.27×10^4	0.16	525	11.84(±0.10)
$\mathbf{L^3}$–Eu^{3+}	330	2.35×10^4	0.20	494	12.91(±0.05)
$\mathbf{L^4}$–Eu^{3+}	329	4.04×10^4	0.21	509	13.86(±0.08)

at higher concentration, can displace **L** from the Eu^{3+} complex. The complexation behaviors of four ligands $\mathbf{L^1}$–$\mathbf{L^4}$ with Eu^{3+} were studied by UV-vis and luminescence spectroscopic methods in a cetylpyridinium bromide (CPB) micellar solution at pH 7.2. The UV-vis absorption spectra of the ligands in the presence of Eu^{3+} displayed obvious blue shifts of maximum absorption wavelengths ($\Delta\lambda$ = 6–8 nm) with the increases of absorption coefficients.† Room-temperature excitation of the ligand or Eu^{3+} did not lead to any measurable luminescence. However, after the addition of each β-diketone to a Eu^{3+} solution, a typical Eu^{3+}-complex emission spectrum with a main emission peak at 612 nm ($^5D_0 \rightarrow {}^7F_2$) and several side emission peaks was observed. Table 1 summarizes the photophysical parameters and binding constants (K_L) of four Eu^{3+} complexes, of which the K_L values were estimated by a curve-fitting method.† The difference of the binding affinities of four ligands to Eu^{3+} is the basis for selective recognition of different analytes.

The Eu^{3+} complex was prepared by reacting 2 : 1 of the ligand to $EuCl_3$ in the CBP micellar solution. Then each complex was titrated with some representative substrates, including NO_3^-, CO_3^{2-}, HPO_4^{2-}, lactate, succinate, citrate, tartrate, PPi, ATP, ADP, AMP, BSA, H_2O_2, Mg^{2+} and Cu^{2+}, separately.† Fig. 2 shows the dependences of the Eu^{3+} luminescence intensity ratios at 612 nm, I_0/I, on different concentrations of the added anions. The $\mathbf{L^1}$–Eu^{3+} (Fig. 2a) could not discriminate between multi-phosphate and multi-carboxylate ions. In fact, $\mathbf{L^1}$–Eu^{3+} displays a significant luminescence quenching effect by PPi, ATP, ADP or citrate, although the luminescence emission is hardly influenced by other substrates. The situation was favorable with $\mathbf{L^2}$–Eu^{3+} (Fig. 2b), which could satisfactorily discriminate multi-phosphate ions from citrate. The luminescence intensity of $\mathbf{L^2}$–Eu^{3+} was 29.1-fold reduced by 10 μM PPi but only 1.54-fold by citrate. However, $\mathbf{L^2}$–Eu^{3+} could not discriminate between PPi and ATP. The high sensing selectivity was found by $\mathbf{L^3}$–Eu^{3+} (Fig. 2c). Titrations of $\mathbf{L^3}$–Eu^{3+} with different substrates showed only PPi could displace the ligand, accompanied by 8.2-fold luminescence decrease in the presence of 10 μM PPi. The luminescence emission of $\mathbf{L^4}$-Eu^{3+} was hardly affected by all selected substrates (Fig. 2d).

The dynamic response of the Eu^{3+} complexes toward PPi strongly depends on the ligand. For example, the dynamic luminescence quenching of $\mathbf{L^1}$–Eu^{3+} at 612 nm for PPi was observed in the range of 0.4 μM to 0.2 mM,† while that of $\mathbf{L^3}$–Eu^{3+} for PPi was 1.0 μM to 0.4 mM.† The corresponding detection limits for PPi, defined as 3σ in blank solution, were 0.02 μM and 0.1 μM, respectively. The flexibility of ligand controlling allows us to monitor the PPi concentration with an appropriate choice of the ligand, so that the sample response falls within the most sensitive response range.

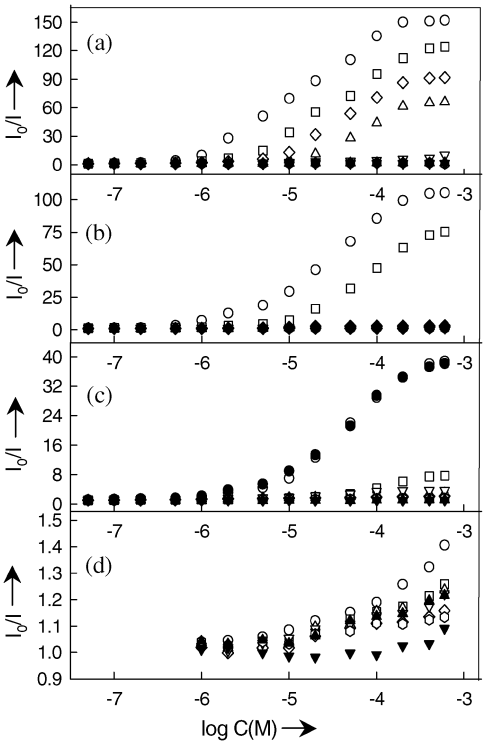


Fig. 2 Titrations of the Eu^{3+} complexes in CPB micellar solution at pH 7.2 with selected substrates: PPi (○), ATP (□), citrate (◇), ADP (△), tartrate (▽), HPO_4^{2-} (■), H_2O_2 (▲), and Cu^{2+} (▼) and titration of $\mathbf{L^3}$–Eu^{3+} with PPi (Fig. 2c, ●) in the presence of competitive substrates. (a) $\mathbf{L^1}$–Eu^{3+}, (b) $\mathbf{L^2}$–Eu^{3+}, (c) $\mathbf{L^3}$–Eu^{3+}, (d) $\mathbf{L^4}$–Eu^{3+}. [L] = 2.0 μ M, [Eu^{3+}] = 1.0 μ M, λ_{ex} = 334 nm, λ_{em} = 612 nm.

The examination of the luminescence intensity changes of the Eu^{3+} complexes as a function of anion concentration showed that the stoichiometry of the metal-to-anion complex is 1 : 2 by curve fitting analysis.† The satisfactory agreement of binding constants for the same anion by using different Eu^{3+} complexes (Table 2) also demonstrates the validity of the method. It is clear that the binding constants of PPi, ATP, ADP, and citrate with Eu^{3+} are higher than that of $\mathbf{L^1}$, which is agreement with the results of Fig. 2a. Since the binding constants of ADP and citrate with Eu^{3+} are lower than that of $\mathbf{L^2}$, the two anions cannot displace $\mathbf{L^2}$ from the Eu^{3+} complex. However, the higher binding constant of ATP causes this anion to compete with PPi for Eu^{3+} binding. The more favorable situation is the use of $\mathbf{L^3}$, whose logK_L is adequately lower than that of PPi but higher than those of other

Table 2 Cumulative binding constants (logK_X) of different anions to the L–Eu^{3+} complexes

Substrate	$\mathbf{L^1}$–Eu^{3+}	$\mathbf{L^2}$–Eu^{3+}	$\mathbf{L^3}$–Eu^{3+}	$\mathbf{L^4}$–Eu^{3+}
PPi	13.47	13.43	13.49	6.24
ATP	12.26	12.28	12.21	nd[a]
ADP	11.36	11.32	11.37	nd
Citrate	11.42	11.44	11.40	nd
Tartrate	7.32	7.44	7.29	nd
HPO_4^{2-}	5.33	5.26	5.42	nd
Other analytes	nd	nd	nd	nd

[a] Signal change was too small to be fitted to the theoretical equation.

substrates, which enables only PPi to displace $\mathbf{L^3}$ from its Eu^{3+} complex. Due to the strongest affinity of $\mathbf{L^4}$ with Eu^{3+}, no investigated substrates can displace $\mathbf{L^4}$ from its complex. The interaction of PPi with $\mathbf{L^3}$–Eu^{3+} was further characterized by ^{31}P NMR.† The ^{31}P NMR of PPi shows one single peak at −6.83 ppm, whereas that of ($\mathbf{L^3}$–Eu^{3+} + PPi) displays two well-defined signals at −6.84 and −22.66 ppm, respectively. The spectrum is almost superimposable with that of Eu^{3+} + PPi, indicating that PPi can completely displace $\mathbf{L^3}$ to form a PPi–Eu^{3+} complex.

Since the analysis of multi-phosphates in biological systems is commonly carried out in the presence of some metal ions, anions, amino acids and proteins, the competitive ligations of PPi with $\mathbf{L^3}$–Eu^{3+} in the presence of 100 mM of NaCl and KCl, 10 mM of $CaCl_2$ and $MgSO_4$, 0.1 mM of $Fe(NO_3)_3$ and $ZnCl_2$, 1.0 mM of $NaHCO_3$ and NaH_2PO_4, 0.1 mM L-glutamine, and 1.0 mg ml^{-1} BSA were chosen as examples. As shown in Fig. 2c, the response curve is almost superimposable with that obtained in the presence of PPi alone, indicating high selectivity of the method for PPi sensing.

The luminescence of the Eu^{3+} complexes in general aqueous buffers is weaker,† to ensure efficient Eu^{3+} complexation and luminescence sensing, we examined the abilities of surfactants to protect the Eu^{3+} emission from water by forming micellar systems. In several tested surfactants, CPB, Triton X-100, cetyl trimethylammonium bromide, sodium dodecyl sulfonate, and sodium dodecylsulfate, CPB was found to be most effective with an optimum concentration range of 30 to 60 μM. The effects of pH on luminescence in the absence and presence of PPi were examined using $\mathbf{L^3}$–Eu^{3+} complex in CPB micellar solution.† In the absence of PPi, the Eu^{3+} emission intensity increases as the solution's pH rises from 2.0 to 5.0, and remains constant between pH 5.0 and 8.0. After addition of 10 μM PPi, the luminescence intensities were 2.6 and 8.8-fold decreased at pH 5.2 and 9.3, respectively. Since the signal decrease at neutral pH was not distinctly smaller than that at more alkaline pH, we chose a neutral pH of 7.2 to make the assay useful for physiological studies.

The anion selectivity of the complexes toward PPi over phosphate ion enables the real-time assay of PPi hydrolysis catalyzed by pyrophosphatase (PPase), a Mg^{2+}-dependent hydrolase[15] that serves to drive reactions dependent upon PPi release by further hydrolysis to phosphate ion. To this purpose, a CPB buffer containing 1.0 μM $\mathbf{L^2}$–Eu^{3+}, 100 μM PPi and 1.0 μM $MgCl_2$, was prepared and used for the measurements of hydrolysis kinetic curves of PPi at different PPase concentrations. The Eu^{3+} luminescence intensity was monitored as a function of time.† The PPase-catalyzed PPi hydrolysis causes distinct luminescence enhancement with over reaction time until a steady value is reached. The results suggest that PPase activity could be monitored by using the $\mathbf{L^2}$–Eu^{3+}. The present method has advantage over previously reported PPase-activity assay,[15] in which the excitation and emission wavelengths are all less than 400 nm, and thus not suitable for biological application.

In summary, new luminescent sensing methods for multi-phosphates were developed by using four β-diketonate–Eu^{3+} complexes as probes. The anion recognition of these complexes is based on displacing the bound ligand by the analyte. Different affinities of the β-diketones to Eu^{3+} can be used to identify whether an analyte can be selectively detected or not. The properties of high sensitivity and selectivity of the probes suggest that they could be widely used for the luminescence detection of multi-phosphates, especially PPi, in many chemical and biological systems.

We thank the National Outstanding Youth Science Foundation of China (No. 20525518) and the National Natural Foundation of China (Nos. 20775005, 20575069) for support.

Notes and references

1 (*a*) A. Bianchi, K. Bowman-James and E. E. García-España, *Supramolecular Chemistry for Anions*, Wiley-VCH, New York, 1997; (*b*) P. D. Beer and P. A. Gale, *Angew. Chem., Int. Ed.*, 2001, **40**, 487; (*c*) R. Martinez-Manez and F. Sancenon, *Chem. Rev.*, 2003, **103**, 4419.
2 (*a*) M. Montalti, L. Prodi, N. Zaccheroni, L. Charbonnière, L. Douce and R. Ziessel, *J. Am. Chem. Soc.*, 2001, **123**, 12694; (*b*) L. J. Charbonniere, R. Ziessel, M. Montalti, L. Prodi, N. Zaccheroni, C. Boehme and G. Wipff, *J. Am. Chem. Soc.*, 2002, **124**, 7779.
3 T. Yamada, S. Shinoda and H. Tsukube, *Chem. Commun.*, 2002, 1218.
4 Y. Bretonnière, M. J. Cann, D. Parker and R. Slater, *Chem. Commun.*, 2002, 1930.
5 (*a*) P. Atkinson, Y. Bretonnière and D. Parker, *Chem. Commun.*, 2004, 438; (*b*) S. J. A. Pope, B. P. Burton-Pye, R. Berridge, T. Khan, P. J. Skabara and S. Faulkner, *Dalton Trans.*, 2006, 2907; (*c*) A. Duerkop, M. Turel, A. Lobnik and O. S. Wolfbeis, *Anal. Chim. Acta*, 2006, **555**, 292.
6 R. S. Dickins, S. A. S. Aime, B. A. Batsanov, M. Botta, J. I. Bruce, J. A. K. Howard, C. S. Love, D. Parker, R. D. Peacock and H. Puschmann, *J. Am. Chem. Soc.*, 2002, **124**, 12697.
7 D. Parker and J. H. Yu, *Chem. Commun.*, 2005, 3141.
8 J. H. Yu and D. Parker, *Eur. J. Org. Chem.*, 2005, **000**, 4249.
9 T. Gunnlaugsson, M. Glynn, G. M. Tocci, P. E. Kruger and F. M. Pfeffer, *Coord. Chem. Rev.*, 2006, **250**, 3094.
10 J. P. Leonard, C. M. G. dos Santos, S. E. Plush, T. McCabe and T. Gunnlaugsson, *Chem. Commun.*, 2007, 129.
11 W. N. Lipscombe and N. Sträter, *Chem. Rev.*, 1996, **96**, 2375.
12 (*a*) P. J. Anzenbacher, K. Jursikova and J. L. Sessler, *J. Am. Chem. Soc.*, 2000, **122**, 9350; (*b*) D. H. Lee, K. H. Lee and J. I. Hong, *Org. Lett.*, 2001, **3**, 5; (*c*) J. H. Liao, C. T. Chen and J. M. Fang, *Org. Lett.*, 2002, **4**, 561; (*d*) S. L. Tobey, B. D. Jones and E. V. Anslyn, *J. Am. Chem. Soc.*, 2003, **125**, 4026; (*e*) A. Ojida, Y. Mitooka, K. Sada and I. Hamachi, *J. Am. Chem. Soc.*, 2004, **126**, 2453; (*f*) D. Aldakov and P. A. Jr, *J. Am. Chem. Soc.*, 2004, **126**, 4752; (*g*) A. Duerkop, M. Turel, A. Lobnik and O. S. Wolfbeis, *Anal. Chim. Acta*, 2006, **555**, 292; (*h*) L. Fabbrizzi, L. Marcotte, F. Stomeo and A. Taglietti, *Angew. Chem., Int. Ed.*, 2002, **41**, 3811; (*i*) T. Gunnlaugsson, A. P. Davis, J. E. ÓBrien and M. Glynn, *Org. Lett.*, 2002, **4**, 2449; (*j*) D. H. Lee, J. H. Im, S. U. Son, Y. K. Chung and J.-I. Hong, *J. Am. Chem. Soc.*, 2003, **125**, 7752; (*k*) Y. J. Jang, E. J. Jun, Y. J. Lee, Y. S. Kim, J. S. Kim and J. Y. Yoon, *J. Org. Chem.*, 2005, **70**, 9603.
13 (*a*) D. H. Lee, S. Y. Kim and J. I. Hong, *Angew. Chem., Int. Ed.*, 2004, **43**, 4777; (*b*) H. N. Lee, Z. C. Xu, S. K. Kim, K. M. K. Swamy, Y. Kim, S.-J. Kim and J. Yoon, *J. Am. Chem. Soc.*, 2007, **129**, 3828.
14 J. L. Yuan, S. Sueda, R. Somazawa, K. Matsumoto and K. Matsumoto, *Chem. Lett.*, 2003, **32**, 492.
15 D. H. Vance and A. W. Czarnik, *J. Am. Chem. Soc.*, 1994, **116**, 9397.