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Stable Lanthanide Luminescence Agents Highly Emissive in Aqueous Solution: Multidentate 2-Hydroxyisophthalamide Complexes of Sm^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+}

Stéphane Petoud,[†] Seth M. Cohen,[†] Jean-Claude G. Bünzli,[‡] and Kenneth N. Raymond*,[†]

Department of Chemistry, University of California, Berkeley, California 94720-1460, and Institute of Molecular and Biological Chemistry, Swiss Federal Institute of Technology, CH-1015 Lausanne, Switzerland

Received August 14, 2003; E-mail: raymond@socrates.berkeley.edu

Applications such as high throughput assays (drug development, genomic screening, and clinical diagnostic assays) or fluorescence microscopy require highly sensitive detection of low concentration analytes, with many analyses performed in a short period of time and without preliminary purification of the sample.[1−6] Fluorescence detection is ideal for such applications. The unique electronic properties of lanthanide cations (long luminescence lifetimes, very sharp emission bands and, a large energy gap between the absorption and emission bands of the complexes)[7] make them ideal as specific reporters. Long lifetimes enable temporal discrimination through time-resolved measurements in a complex mixture. This gives accurate and highly sensitive quantification of specific targets by elimination of background fluorescence. Consequently, homogeneous, time-resolved fluoroimmunoassays which primarily incorporate Eu^{3+} complexes as donors have been marketed.[2,8,9] However, the sensitivity of these agents is limited by the low efficiency of the ligand-to-lanthanide energy conversion or by insufficient protection of the lanthanide cation from the aqueous environment (coordination of water leads to nonradiative decay of the lanthanide excited state).[10,11] To make efficient luminescent probes, the lanthanide complex must be both sufficiently bright and stable in very dilute aqueous solution, a challenging combination.[1,9,12]

We report here a new generation of lanthanide complexes based on 2-hydroxyisophthalamide chelating units. Two ligands, depicted in Figure 1, have been evaluated: the macrobicycle $H_3\mathbf{L^1}$ (whose synthesis was described previously)[13] and the octadentate ligand $H_4\mathbf{L^2}$. Both form luminescent and highly stable Ln complexes (Ln = Sm, Eu, Tb, Dy) suitable for practical applications under physiological conditions.

Two molecules of $H_3\mathbf{L^1}$ or one of $H_4\mathbf{L^2}$ react with $Ln(NO_3)_3$ or $LnCl_3$ (Ln = Sm, Eu, Tb, Dy) to give the complexes $[Ln(H_2\mathbf{L^1})_2)]$-Br and $[Ln(H_2\mathbf{L^2})]Br$, respectively (bridging N atoms are protonated). Single crystals of $[Eu(H_2\mathbf{L^1})_2)]Br$ were obtained by slow diffusion of acetone into a solution of the complex in a mixture of DMF, water, and MeOH. Two ligands (Figure 1) are wrapped around the metal such that four amide and four phenolate oxygen atoms of two of the three 2-hydroxyisophthalamide units coordinate the Eu^{3+} to give a slightly distorted square-antiprism.

Several lanthanide cations emitting in the visible (Sm^{3+}, Dy^{3+}, Tb^{3+}, and Eu^{3+}) can be efficiently sensitized when bound to ligands $H_3\mathbf{L^1}$ and $H_4\mathbf{L^2}$. As shown in Figure 2, the strong luminescence of these complexes in buffered solution can be readily seen with the naked eye. An efficient ligand → Ln^{3+} energy transfer is evidenced by the emission solution of the $[Ln(H_2\mathbf{L_1})_2]^+$ complexes with Ln = Sm, Eu, Tb, Dy: the emission from the ligand vanishes when the corresponding lanthanide is added to the ligand solution; an indication of efficient ligand-to-lanthanide energy transfer. Figure 3 shows that the emission spectra of the different lanthanide complexes have sharp emission bands with little overlap, enabling their ready discrimination.

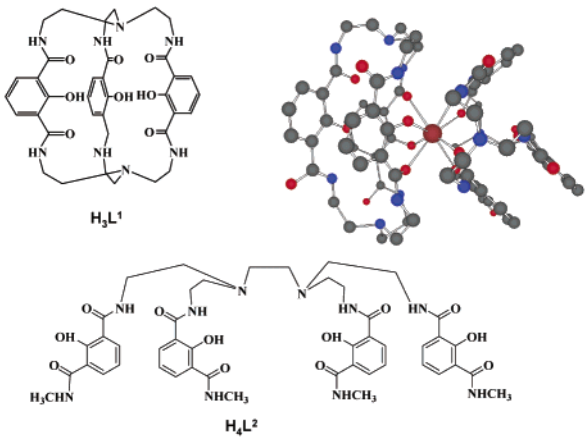

Figure 1. (Left) The ligand structures and (upper right) the single-crystal structure of $[Eu(H_2\mathbf{L^1})_2]^+$.

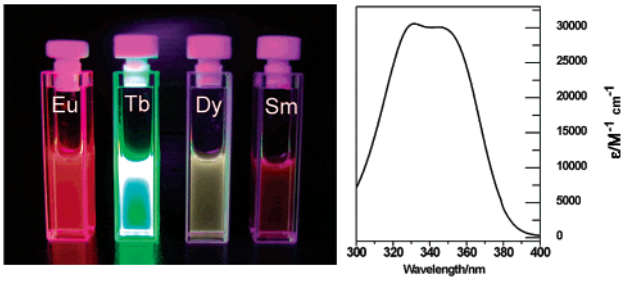

Figure 2. (Left) Picture of the emission of aqueous solutions (0.01 M TRIS buffer (10^{-4} M), pH = 8.5) of the $[Ln(H_2\mathbf{L^1})_2)]^+$ complexes illuminated by a standard laboratory UV lamp (λ_{exc} = 354 nm). (Right) Absorption spectrum of $[Tb(H_2\mathbf{L^1})_2]^+$.

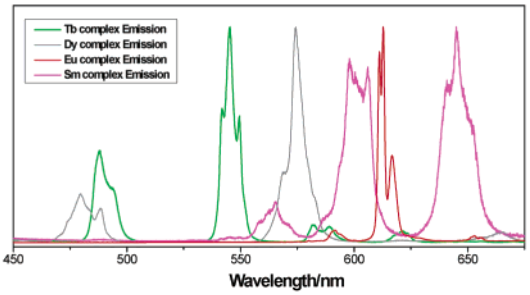

Figure 3. Normalized emission spectra of $[Ln(H_2\mathbf{L^1})_2]^+$ with Ln = Sm, Eu, Tb, Dy. All solutions $2{\cdot}10^{-5}$ M in TRIS buffer (0.01M, pH = 8.5) and λ_{exc} = 350 nm.

[†] University of California.
[‡] Institute of Molecular and Biological Chemistry, Swiss Federal Institute of Technology.

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Table 1. Quantum Yields (Φ) in Millipore Water

compound	λ_{exc}/nm	ϵ/M^{-1}cm^{-1}	Φ
Tb$\mathbf{L}^1{}_2$	354	28,482	0.61
Tb$\mathbf{L}^2$	350	26,811	0.59
Eu$\mathbf{L}^1{}_2$	361	23,496	0.06
Sm$\mathbf{L}^1{}_2$	347	30,028	0.01
Dy$\mathbf{L}^1{}_2$	347	30,028	0.03

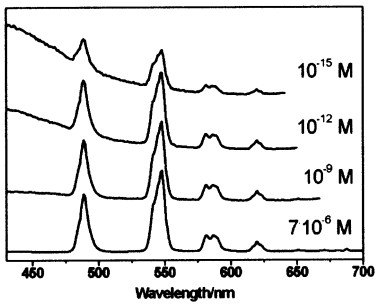

Figure 4. Emission spectra of $[Tb(HL^2)]^+$ at different concentrations in phosphate buffer (0.01 M), pH = 7.4, λ_{exc} = 347 nm.

The ligand-to-lanthanide energy transfer is confirmed by the quantum yield values. The quantum yields of $[Tb(H_2\mathbf{L_1})_2]^+$ and $[Tb(H_2\mathbf{L^2})]]^+$ are very high (Φ = 0.61 and 0.59, respectively). While other high quantum yields have been reported,[14] these are the brightest practical lanthanide complexes (high absorbance and quantum yield, sufficiently stable in aqueous solution to be used at nanomolar concentration; the luminescence is obtained without the help of external agents such as fluoride or micelles, which preclude water coordination). Compared to the cryptate Eu(bpy·bpy·bpy) (Φ = 0.02),[15] a widely used luminescent probe in commercial homogeneous time-resolved fluoroimmunoassays based on fluorescence resonance energy transfer (FRET),[2,8] the quantum yield value for the Tb^{3+} complexes imply an increase in the sensitivity of such assays by a factor 30. Although smaller by 1 order of magnitude, the quantum yield of the Eu complex is still 3 times as large as that of the (bpy·bpy·bpy) cryptate.

The quantum yields of $[Tb(H_2\mathbf{L}^1)_2]^+$ and $[Tb(H\mathbf{L}^2)]^+$ are very similar (Table 1). This shows that, despite significant differences in ligand geometry, both complexes have the same photophysical properties. Quantum yields (Φ) were determined relative to quinine sulfate[16] and are reported with an experimental error of 15%.

The stability of the complex formed with $H_4\mathbf{L^2}$ in physiological condition was evaluated by high dilution experiments, where the lanthanide complex concentration was decreased in phosphate buffer solution at pH = 7.4. At concentrations as low as 10^{-15} M the sharp luminescence signal arising from the luminescent lanthanide complex can still be observed when the solution is excited at 350 nm (Figure 4). This indicates that most of the lanthanide complex persists, since only the metal complex emits. Micromolar solutions in TRIS or phosphate buffer have been stored for more than two years without changes in the luminescence spectrum or intensity. This stability is consistent with detailed solution thermodynamic studies of the complexes[17] and indicates that they could be used in concentration ranges suitable for application such as homogeneous time-resolved fluoroimmunoassays (10^{-9}−10^{-10} M).[2,18]

The 2-hydroxyisophthalamide group is a very good ligand for Ln^{3+} cations and also provides excellent sensitization of the Tb^{3+} luminescence through a particularly efficient ligand-to-lanthanide energy transfer process. The complexes are highly soluble and stable in water at physiological pH. These properties, combined with the ease of synthesis and high water solubility, make these or related compounds excellent candidates for use in bioanalytical applications.

This molecular structure provides efficient sensitization of four lanthanide cations (Sm^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+}). The very low overlap between the emission of the four lanthanide cations (Figure 3) enables accurate discrimination between these four species and their potential use in multiplex assays.

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Supporting Information Available: X-ray crystallographic file in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Mathis, G. *J. Biomol. Screen* **1999**, *4*, 309−313.
(2) Hemmila, I. *J. Alloys Compd.* **1995**, *225*, 480−485.
(3) Beeby, A.; Botchway, S. W.; Clarkson, I. M.; Faulkner, S.; Parker, A. W.; Parker, D.; Williams, J. A. G. *J. Photochem. Photobiol., B* **2000**, *57*, 83−89.
(4) Vereb, G.; Jares-Erijman, E.; Selvin, P. R.; Jovin, T. M. *Biophys. J.* **1998**, *74*, 2210−2222.
(5) Devlin, J. P. *High Throughput Screening: The Discovery of Bioactive Substances*; M. Dekker: New York, 1997.
(6) Hemmila, I.; Webb, S. *Drug Discovery Today* **1997**, *2*, 373−381.
(7) Bünzli, J.-C. G. In *Lanthanide Probes in Life, Chemical and Earth Sciences*; Bünzli, J.-C. G., Choppin, G. R., Eds.; Elsevier: Amsterdam, 1989; p 219.
(8) Mathis, G. *Clin. Chem.* **1993**, *39*, 1953−1959.
(9) Saha, A. K.; Kross, K.; Kloszewski, E. D.; Upson, D. A.; Toner, J. L.; Snow, R. A.; Black, C. D. V.; Desai, V. C. *J. Am. Chem. Soc.* **1993**, *115*, 11032−11033.
(10) Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc., Perkin Trans. 2* **1999**, 493−503.
(11) Horrocks, W. D., Jr.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384−392.
(12) Selvin, P. R. *Nat. Struct. Biol.* **2000**, *7*, 730−734.
(13) Cohen, S. M.; Petoud, S.; Raymond, K. N. *Inorg. Chem.* **1999**, *38*, 4522−4529.
(14) Brunet, E.; Juanes, O.; R., S.; Rodriguez-Ubis, J.-C. *Photochem. Photobiol. Sci.* **2003**, *1*, 613−618.
(15) Alpha, B.; Balzani, V.; Lehn, J.-M.; Perathoner, S.; Sabbatini, N. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 1266.
(16) Meech, S. R.; Phillips, D. C. *J. Photochem.* **1983**, *23*, 193.
(17) Petoud, S.; Xu, J.; Cohen, S. M.; Melchior, M.; Bünzli, J. C.; Raymond, K. N.
(18) Mathis, G. *Clin. Chem.* **1995**, *41*, 1391−1397.

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