



0040-4039(94)01784-0

Synthesis and Luminescence Properties of Europium(III) and Terbium(III) Complexes of Aminopolycarboxylic Acid Ligands Containing 3-Aroylcoumarin

Juan Carlos Rodríguez-Ubis*, María Teresa Alonso and Ernesto Brunet*

Departamento de Química, C-I. Facultad de Ciencias. Universidad Autónoma de Madrid, 28049-Madrid, Spain
Fax 34 1 397 3966

Abstract: Three differently substituted 3-aroylcoumarins, $L_{1,3}$, with tetraacid chelating subunits anchored at the 3-benzoyl group were synthesized, and their complexes with Eu^{3+} and Tb^{3+} formed as new potential bioaffinity-assay markers. Some spectral and luminescence properties of the complexes in water and methanol are reported.

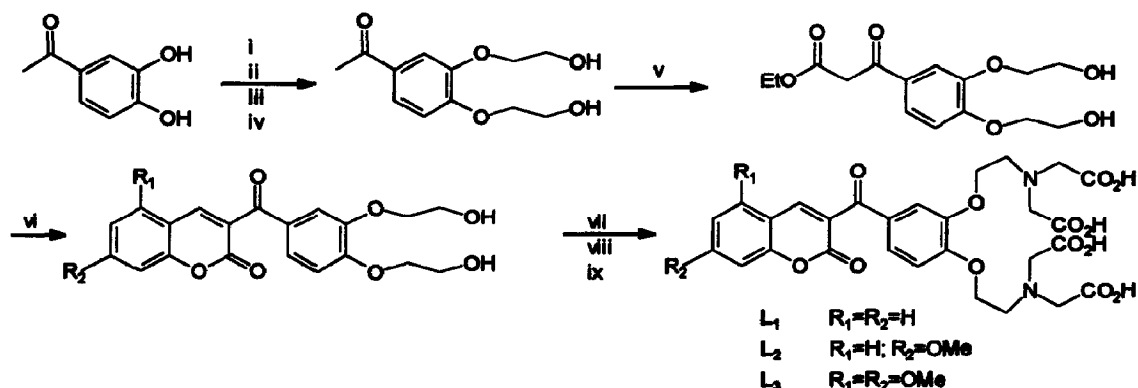
The luminescence of lanthanide (III) compounds is widely applied in various fields,¹ in particular the analysis of Ln^{3+} ions and fluoroimmunoassay.² The ions Eu^{3+} and Tb^{3+} are efficient emitters when they form complexes with ligands bearing chromophoric groups. In such complexes the luminescence of the metal is obtained by the *antenna effect*,³ which is defined as a light conversion process via an energy absorption-transfer-emission sequence involving distinct absorbing (ligand) and emitting (metal ion) species.

High emission quantum yield, high kinetic stability and good water solubility are essential requirements for luminescent lanthanide complexes if they are to be used as labels in immunoassay. In recent years, a considerable research effort has been directed towards two main classes of compounds, *i.e.* photoactive macrocyclic ligands⁴ and aminopolycarboxylic acids.⁵ Apart from efficiently absorbing incident light, photoactive macrocyclic ligands shield to a high extent the metal ion from its interaction with solvent molecules, which is the main cause for nonradiative decay of the luminescent level. However, some of the studied complexes showed poor stability in water solution, a serious shortcoming in their use as luminescent labels.⁶ On the other hand, aminopolycarboxylic acids form quite strong complexes with metal ions. Therefore, the inclusion of iminodiacetic subunits in chromophoric components gives rise to a very promising type of structures to be used as labels in immunological and DNA hybridization assays.⁷

We have previously reported on the luminescence properties of polyacid chelates derived from 2,6-Bis(*N*-pyrazolyl)pyridine,⁸ whose Eu^{3+} and Tb^{3+} complexes were highly stable and showed excellent photophysical properties. Somewhat more recently, we have described a new class of photoactive macrocycles containing the 3-aroylcoumarin nucleus and the preliminary studies suggested that they are useful as triplet sensitizers for lanthanide luminescence.⁹

In this paper we report the synthesis and preliminary luminescence study of Eu^{3+} and Tb^{3+} complexes of new polyacids derived from 3-aroylcoumarins, compounds where the excellent complex stabilities provided by iminodiacetic subunits and the sensitization power of coumarins are combined.

Scheme I shows our synthetic approach. The key intermediate was the β -ketoester (product of step v), whose condensation with differently substituted salicylaldehydes afforded coumarins in high yields (step vi). The β -ketoester was prepared from 3,4-dihydroxyacetophenone in high yield by the protection-deprotection sequence indicated in steps i-iv.



Scheme I

Reagents and conditions: i) Methyl 2-bromoacetate K_2CO_3 /acetone; ii) Ethylene glycol, amberlyst-15/triethyl orthoformate (90°C); iii) LAH/THF (0°C); iv) Amberlyst-15/acetone; v) Diethyl carbonate/potassium *tert*-butoxyde; vi) Salicylaldehyde, 4-methoxy- or 4,6-dimethoxy-salicylaldehyde, piperidine/EtOH; vii) Methanesulfonyl chloride, Et_3N/CH_2Cl_2 ; viii) Di-*tert*-Butyl iminodiacetate, $NaI/Na_2CO_3/CH_3CN$ (110°C); ix) TFA/ CH_2Cl_2 .

The tetraacid ligands $L_{1,3}$ were prepared by substitution of the mesylated coumarin with di-*tert*-butyl iminodiacetate (steps vii-viii).¹⁰ The *tert*-butyl esters were hydrolyzed quite easily to the corresponding tetraacids with trifluoroacetic acid (step ix). The use of other esters or different hydrolysis methods afforded complex mixtures of carboxylate salts/esters.

The Eu^{3+} and Tb^{3+} complexes were prepared from ligands $L_{1,3}$ in three different ways: a) the addition of aqueous or methanolic solutions of $EuCl_3$ or $TbCl_3$ on the tetraacids dissolved in water or methanol, respectively; b) the addition of the corresponding aqueous solution of lanthanide chloride to the tetraacids dissolved in NaOH (pH=9.8); c) crystalline, analytically pure complexes were obtained by treatment of the ligands with Eu_2O_3 and Tb_4O_7 . The absorption, excitation and emission spectra of each complex were very similar regardless of the method a-c used for its preparation and did not change even after several weeks.

The UV spectra of all the studied complexes were very similar to those of the parent ligands, suggesting that the coordinated metal produced low or no perturbation of the chromophore which does not participate in the coordination. The observation of an intense Eu^{3+} and Tb^{3+} luminescence at concentrations as low as 10^{-7} M indicated that an energy transfer from the ligand to the metal ion takes place (Figure 1).

The intensity of emission was somewhat lower in water compared to methanol, presumably as a consequence of the quenching effect of the water molecules in the coordination sphere of the metal ion. This was confirmed by the higher lifetime values measured in methanol solution, *i.e.* for $\text{Eu} \llcorner \text{L}_1$ $\tau(\text{H}_2\text{O}) = 0.52$ ms vs. $\tau(\text{MeOH}) = 0.92$ ms.

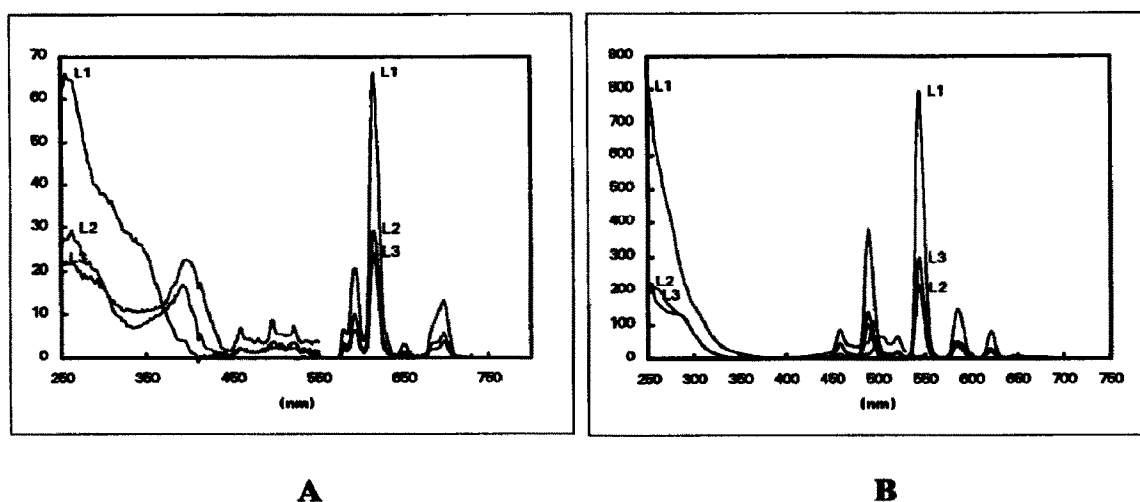


Figure 1. Luminescence excitation and emission spectra shown by lanthanide complexes of compounds L_{1-3} (see Scheme I) in water: **A**, Eu^{3+} complexes; **B**, Tb^{3+} complexes.

It can be seen in Figure 1 that Eu^{3+} and Tb^{3+} complexes of the same ligand showed different excitation spectra. In the case of Eu^{3+} complexes, in addition to a broad excitation band between 250 and 300 nm (Figure 1A), a low energy band was observed which was red-shifted as the substitution of the coumarin increased [$\lambda_{\text{ex}} = 343$ nm for L_1 ; $\lambda_{\text{ex}} = 392$ nm for L_2 and $\lambda_{\text{ex}} = 398$ nm for L_3]. In contrast, the three Tb^{3+} complexes displayed similar spectra with maxima at around 252 nm (see Figure 1B).

The aforementioned substitution-dependent bands of Eu^{3+} complexes were tentatively assigned to a ligand-to-metal charge-transfer (CT) process. We based this assignment on the fact that the corresponding Tb^{3+} complexes, where the metal ion is extremely difficult to reduce, did not show such excitation bands. However, for most systems studied in solution, the nonradiative relaxation pathways of these CT states do not pass through the $^5\text{D}_1$ excited-state manifold of Eu^{3+} and, therefore, do not lead to sensitized $^5\text{D}_0 \rightarrow ^7\text{F}_1$ emission.¹¹ A possible explanation for the high intensity observed in our complexes for these bands (see Figure 1A) could be that, in our case, most of the energy placed in a CT state finds its way to the $^5\text{D}_0$ emitting level.

Research is in progress to ascertain the precise nature of the photophysical processes involved in these complexes. Also the determination of the stability constants of these new chelates as well as the preparation of similar ligands with polyaminocarboxylates attached to the coumarin nucleus is under way.

Acknowledgment. Financial support from *Ministerio de Educación y Ciencia* (DGICYT) under grants PB90-0176 and PB93-0264 is gratefully acknowledged.

References and Notes

- 1 Bünzli, J.-C.G. in *Lanthanide Probes in Life, Chemical and Earth Sciences*, chapter 7, Bünzli, J.-C.G. and Chopin, G.R. eds., Elsevier, Amsterdam 1989.
- 2 Burroughs, S.E.; Eisenman, G.; Horrocks Jr., W.De W. *Biophys. Chem.* 1992, 42, 249. Chartier, A.; Fox, C.G.; Georges, J. *Analysis* 1992, 20, 269. Soini, E.; Lovgren, T. *CRC Crit. Rev. Anal. Chem.* 1987, 18, 105. Soini, E.; Hemmilä, Y.; Dahlen, P. *Ann. Biol. Clin.* 1990, 48, 567. Lovgren, T.; Hemmilä, Y.; Peterson, K.; Halonen, P. in *Alternative Immunoassays*, chapter 12, Collins, W.P. ed., Wiley, London 1985. Prat, O.; López, E.; Mathis, G. *Anal. Biochem.* 1991, 195, 283. Diamandis, E.P.; Christopoulos, T.K. *Anal. Chem.* 1990, 62, 1149A.
- 3 Sabbatini, N.; Perathoner, S.; Balzani, V.; Alpha, B.; Lehn, J.-M. in *Supramolecular Photochemistry*, p 187-206, Balzani, V. ed., Riedel, D., Amsterdam 1987.
- 4 Sabbatini, N.; Guardigli, M.; Lehn, J.M. *Coord. Chem. Rev.* 1993, 123, 201 and references cited therein.
- 5 Toner, J.L. in *Inclusion Phenomena and Molecular Recognition*, p 185-197, Atwood, J. ed. Plenum Press, New York 1990.
- 6 Balzani, V.; Berghmans, E.; Lehn, J.M.; Sabbatini, N.; Terörde, R.; Ziessel, R. *Helv. Chim. Acta* 1990, 73, 2083. Balzani, V.; Lehn, J.M.; Loosdrecht, J. van de; Mecati, A.; Sabbatini, N.; Ziessel, R. *Angew. Chem., Int. Ed. Engl.* 1991, 30, 190.
- 7 Mukkala, V.-M.; Kankare, J.J.; *Helv. Chim. Acta* 1992, 75, 1578. Mukkala, V.-M.; Kankare, J.J.; Takalo, H. *Helv. Chim. Acta* 1993, 76, 893. Mukkala, V.-M.; Sund, C.; Kwiatkowski, M.; Pasanen, P.; Högberg, M.; Kankare, J.J.; Takalo, H. *Helv. Chim. Acta* 1993, 76, 1621. Mukkala, V.-M.; Helenius, N.; Hemmilä, I.; Kankare, J.J.; Takalo, H. *Helv. Chim. Acta* 1993, 76, 1361. Lis, S.; Konarski, J.; Hnatejko, Z.; Elbanowski, M. *J. Photochem. Photobiol. A. Chem.* 1994, 79, 25.
- 8 Remuñán, M.J.; Román, H.; Alonso, M.T.; Rodríguez-Ubis, J.C. *J. Chem. Soc., Perkin Trans. 2* 1993, 1099
- 9 Alonso, M.T.; Brunet, E.; Hernández, C.; Rodríguez-Ubis, J.C. *Tetrahedron Lett.* 1993, 34, 7465.
- 10 Compounds L_{1,3} gave correct elemental analysis and ¹H- and ¹³C-nmr spectra.
- 11 Richardson, F.S. *Chem. Rev.* 1982, 82, 541.

(Received in UK 22 July 1994; revised 2 September 1994; accepted 9 September 1994)