Talanta, Vol. 41, No. 1, pp. 93–98, 1994
Printed in Great Britain.

0039-9140/94 $6.00 + 0.00

AROMATIC POLYAMINOCARBOXYLATE LIGANDS FOR ENERGY TRANSFER LUMINESCENCE MEASUREMENTS OF LANTHANIDE IONS IN AQUEOUS SOLUTIONS

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(*Received* 16 *March* 1993. *Revised* 20 *May* 1993. *Accepted* 28 *May* 1993)

Summary—The promising ligand candidates for the energy transfer luminescence measurements of lanthanide (Ln) chelates on aqueous matrices are first proposed. The ligands are; 2[(2-amino-5-methylphenoxy)methyl]-6-methoxy-8-aminoquinoline-*N,N,N′,N′*-tetraacetate (Quin 2), 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetate (BAPTA), and 1,2-bis(2-amino-5-fluoro-phenoxy)ethane-*N,N,N′,N′*-tetraacetate (F-BAPTA). The Ln-chelates of these aromatic polyaminocarboxylates show the sensitized emission which results from efficient ligand-centered light absorption, and the interesting selectivity is seen; BAPTA and F-BAPTA form the luminescent chelates only with Tb(III) and Dy(III) ions, whereas the emission from Sm(III) and Eu(III) ions is greatly sensitized with Quin 2. The sufficient emission intensity can be obtained even in slightly alkaline aqueous solutions without any addition of surfactants or organic solvents. These octadentate ligands are fairly capable of shielding central Ln ions from quenching by surrounding water molecules. The luminescence enhancement factors are 1600 for Tb(III) ion with BAPTA (em. 544 nm) and 1380 for Eu(III) ion with Quin 2 (em. 615 nm), respectively, being relative to their aqueous chloride solutions.

Delayed luminescence with very narrow bands characteristic of certain lanthanide(III) chelates (Ln; Sm, Eu, Tb and Dy) has enabled one to utilize the time-resolved measurements to circumvent the back ground interferences from short-lived native emission of matrices and to obtain the enhanced sensitivity by time-resolved signal accumulation.[1–7] The luminescence decay properties of Ln ions, their chelates or labels serve as valuable sources of the physicochemical information for biomolecular systems.[8–10]

Since ligand-sensitized luminescence of Ln ions was reported, some organic ligand systems and the photophysical basis for this phenomenon have been proposed.[11–16] Because most measurements are made on aqueous matrices where Ln ions themselves are very weakly luminescent, the requisites for the ligand-sensitized luminescence are outlined; (1) provision of effective light absorbing moieties with a molar absorption coefficient, ε greater than 10^3/M/cm, (2) efficient energy transfer (via ligand S_1 or T_1 levels) to the acceptor Ln excited levels, and (3) protection of the excited energy levels from quenching by neighboring water molecules. In other words, such ligands that have π electron conjugated chromophores, are desirable to provide stable water-soluble chelates in which coordination number of Ln ions, 8 or more must be fulfilled to expel water molecules from the inner coordination sphere.

The approaches to the ligand-system designing have extensively been made; thenoyltrifluoroacetone (tta)/trioctylphosphine oxide (topo)/Triton X-100,[17]tta/1,10-phenanthroline (phen)/Triton X-100,[18] 2-naphthoyltrifluoroacetone/topo/Triton X-100,[2] diethylene triaminepentaacetic acid/4-aminosalicylic acid,[4,19] 15-crown-5/benzoic acid/ethyl acetate,[20] macrocyclic ligands incorporating 2,2′-bipyridine units,[21] and 2,9-dicarboxy-phen.[22] In some cases, synergistic agents, phen or topo are essential to replace remaining aqua ligands, and surfactant micelles have been employed to solubilize the chelates in aqueous solution protecting from the quenching due to collision with water. More recently, many kinds of chelators for Eu and Tb ions which concurrently act as enzyme substrates have been examined for fluorimetric immunoassays.[23]

Our work has been conducted to develop simpler ligand systems for the energy transfer luminescence of Ln ions in aqueous media, which form the practical basis not only for the

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detection chemistry in immunoassays but for the selective determination of Ln ions in environmental or biological samples.[24] In this paper are reported the attractive fluorescence properties of Ln chelates with 'intracellular calcium reagents', Quin 2 (2[(2-amino-5-methylphenoxy)methyl]-6-methoxy-8-aminoquinoline-*N,N,N′,N′*-tetraacetic acid), BAPTA (1,2-bis-(2-aminophenoxy)ethane-*N, N, N′, N′*-tetra-acetic acid) and difluoro-BAPTA (Scheme 1). These polyaminocarboxylates fairly satisfy the above mentioned requirements; they potentially behave as the water-soluble octadentate ligands with N,O-donor functions having phenyl or quinolyl groups which absorb near UV light.

These commercially available reagents, Quin 2, BAPTA, and F-BAPTA have been intensively employed as a fluorescence probe and buffering reagents, respectively, for Ca(II) ion in biological systems.[25–27] The reactions of the ligands with Ln ions have been reported only in terms of Ca-competitive fluorescence quenching kinetics,[28,29] and of complex formation equilibria,[30] however, no description of the energy transfer luminescence has so far been present.

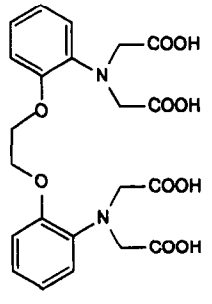
EXPERIMENTAL

Apparatus

The luminescence measurements were made with a Hitachi model 850 spectrofluorimeter with the exciting source of a 150 W Xenon lamp and the band pass was 5 nm for both excitation and emission monochrometers. The long pass filters (290 nm or 350 nm) were inserted at the entrance of the emission monochrometer to cut off scattered excitation beam. The absorption spectra were recorded on a Hitachi model U-3200 double monochrometer spectrophotometer. A Horiba M-8s pH meter was used.

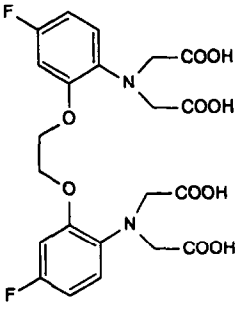
Reagents and solutions

Each stock solution (0.01*M*) of Sm, Gd, Eu, and Dy ions was prepared by dissolving the trichlorides (99.5% purity) in doubly distilled water with a few drops of concentrated HCl except for Tb ion which was prepared from Tb_4O_7 (99.5% purity). The standardization of these Ln ion solutions was carried out with the EDTA titrations to a xylenol orange end point. The reagents, Quin 2 and BAPTA, both tetrapotassium salts 'for intracellular Ca' were received from Dojindo Lab. (Kumamoto,



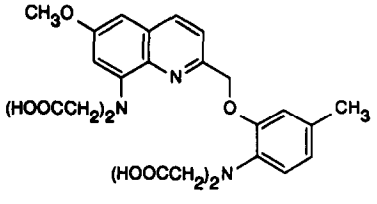
BAPTA

1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid



F-BAPTA

1,2-bis(2-amino-5-fluorophenoxy)ethane-N,N,N',N'-tetraacetic acid



QUIN 2

2[(2-amino-5-methylphenoxy)methyl]-6-methoxy-8-aminoquinoline-N,N,N',N'-tetraacetic acid

Scheme 1. Structural formulae of the ligands.

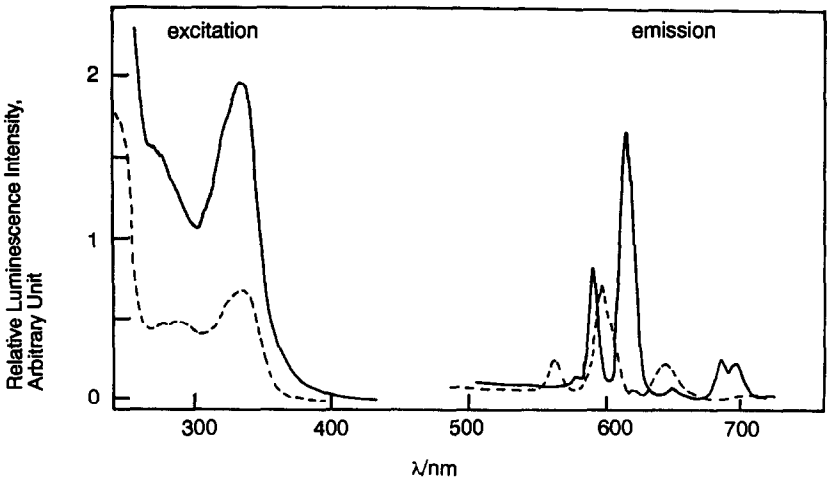


Fig. 1. Uncorrected luminescence spectra of Quin 2 chelates with Eu(III) (—) and Sm(III) (– – –) ions in aqueous solution at pH 8. Eu(III), $2.00 \times 10^{-5}M$; Sm(III), $2.03 \times 10^{-5}M$; Quin 2, $2.4 \times 10^{-5}M$; Tris–HCl buffer, $0.01M$. Eu–Quin 2, λ_{ex} 340 nm, λ_{em} 610 nm; Sm–Quin 2, λ_{ex} 335 nm, λ_{em} 600 nm.

Japan). Difluoro-BAPTA (F-BAPTA, tetrapotassium salt) was used as received from Molecular Probes Inc. (Eugene, OR, U.S.A.). Each aqueous solution of the reagents ($10^{-3}M$) was stored in a refrigerator. Buffer solutions ($0.1M$) at pH 5.0 and 8.0 were prepared from acetic acid–sodium hydroxide and tris(hydroxymethyl)aminomethane (Tris)–hydrochloric acid solutions, respectively.

Procedure

Unless otherwise noted, to an acidic solution containing Ln ion the reagent solution and a pH buffer solution (pH 5.0 or 8.0) were added to give the approximately equimolar solutions of the reagents and Ln ion, *e.g.* $4 \times 10^{-5}M$. In the calibration studies, the prescribed amount of the ligand were added, $2 \times 10^{-5}M$, in sufficient excess for varying low Ln concentrations. The experiments were carried out at ambient room temperature of 20 ± 3°C. Basically all the luminescence spectra reported in this paper are uncorrected for the photomultiplier response and for the light source.

RESULTS AND DISCUSSION

The structural formulae and abbreviations of the reagents employed in this work are given in Scheme 1. These reagents potentially act as octadentate ligands with N,O donor-set to form relatively stable complexes with calcium ion. The stability constants of the Ln(III)-BAPTA chelates were reported to be 10^{10}–10^{11} which are a factor of 10^3 greater than that of the Ca(II) chelate.[30] Although the detailed equilibrium data for the Ln(III)–Quin 2 chelates are not yet known, the stability constants of the Ln(III)–Quin 2 chelates are probably a similar order of magnitude to those for the BAPTA chelates just as is the case for Ca(II) systems.[25] Presumably, the stability of the F-BAPTA chelates are decreased because of weakened basicity of the donors caused by attaching fluorine atoms on the phenyl rings.[27]

Judging from the last deprotonation constant, $pKa_4 = 5.95$ of BAPTA,[30] only the dissociation degree in 1% of the Ln chelates can occur even in the $10^{-6}M$ range at pH 8. In addition, there were no signs of the hydroxide precipitation under the formation conditions as can be predicted from the solubility product data,[31] $K_{sp} = [Ln^{3+}][OH^-]^3$ *ca.* 10^{-24}. The luminescence intensities for Tb(III)–BAPTA and Eu(III)–Quin 2 systems were actually constant in the pH range of 5–10.

The uncorrected luminescence spectra of the Ln(III) chelates in aqueous solutions are shown in Figs 1 and 2. In order to avoid the possible photochemical degradation, the Ln(III) chelates were excited at their longest absorption bands at the sacrifice of the greater emission intensities on excitation at wavelengths shorter than 250 nm.

Each emission pattern in the range of 450–750 nm (ascribed to the *f–f* transitions) seems to be identical with those so far reported for various Ln(III) chelates (or ions),[11–23] whereas the excitation spectra are actually matched with their chelate absorption bands. The spectral change of Tb–BAPTA system is shown in Fig. 3. The broad-band fluorescence centered at 370 nm

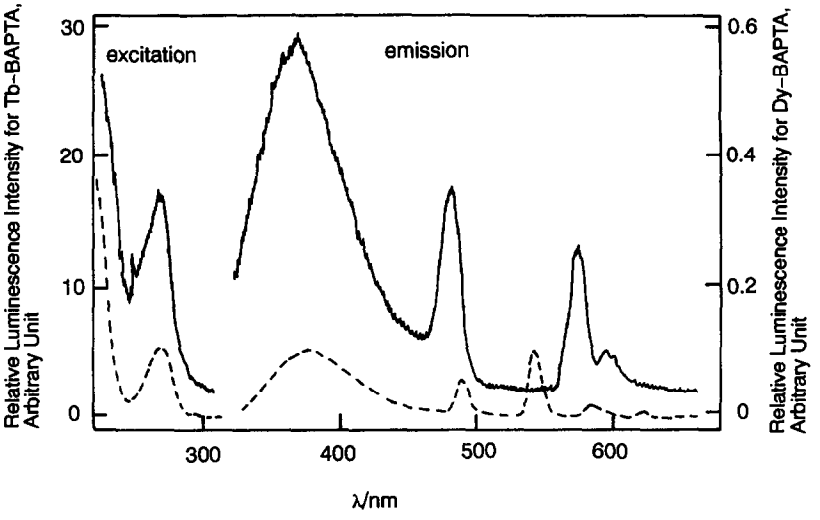


Fig. 2. Luminescence spectra (uncorrected) for Dy(III) (—) and Tb(III)–BAPTA (– – –) systems in aqueous solution at pH 8. Tb(III), $1.98 \times 10^{-5}M$; Dy(III), $1.95 \times 10^{-5}M$; BAPTA, $2.0 \times 10^{-5}M$; Tris–HCl, $0.01M$. Tb–BAPTA, λ_{ex} 271 nm, λ_{em} 544 nm; Dy–BAPTA, λ_{ex} 270 nm, λ_{em} 482 nm.

from free BAPTA is fairly quenched on addition of Tb^{3+} ion with the concomitantly increased intensities due to the *f–f* emission (490, 544 and 585 nm). Also the native fluorescence around 500 nm from Quin 2 is also effectively decreased in the presence of Eu^{3+} ion. Hence, the emission is doubtless provoked by the ligand-centered photon absorption with the ensuing energy transfer to the radiative Ln ions. Gadolinium ion seems to give no luminescent chelates with the ligands tested here probably because the excited states of Gd ion mismatch with the ligand energy levels.[15]

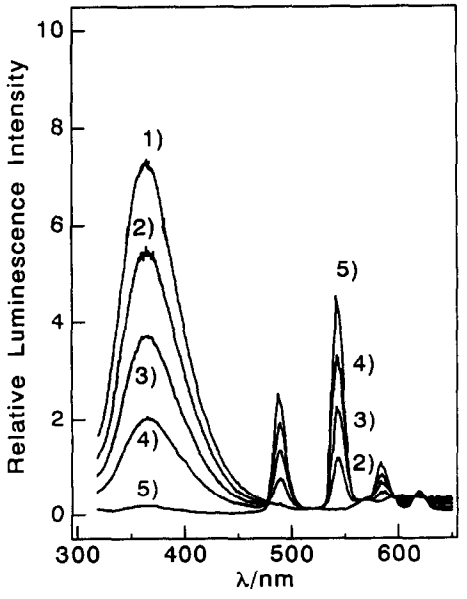


Fig. 3. Emission spectral change as the dependence of the molar ratio of $[Tb^{3+}]$/[BAPTA]. BAPTA, $5.0 \times 10^{-6}M$; pH 5.0 (acetate $0.01M$). λ_{ex} 258 nm, with a cut-off filter at 290 nm. $[Tb^{3+}]$/[BAPTA]; (1) 0.0, (2) 0.198, (3) 0.396, (4) 0.594, (5) 0.991.

The several spectroscopic data are summarized in Table 1. The ε values of the chelates are great enough $>3 \times 10^3$/M/cm which are at least by a factor of 1000–2000 larger than those of aqua Ln ions. Indeed, the enhancement factors relative to the chloride solutions are 1600 for Tb(III)–BAPTA at 544 nm and 1380 in Eu(III)–Quin 2 system at 615 nm, respectively. These facts suggest that the central Ln ions are liable to accept the photoexcited energy from each 'antenna' moiety and are well shielded from vicinal water molecules by the ligands because the emission is well observed in neat aqueous buffer solutions even without any addition of surfactants or organic solvents.

A detection limit on a 3σ basis for the Tb(III)–BAPTA chelates was $2 \times 10^{-8}M$ (3.2 ppb as Tb ion) in the presence of an excess of the reagent, $2 \times 10^{-5}M$, when the photomultiplier gain was set high (ex. 267 nm. em. 544 nm, 5 nm band pass). On the other hand, of practical importance is the excitation maximum at 334 nm for the Quin 2 chelates of Eu(III) and Sm(III) ions; this coincides with pulsed nitrogen laser radiation at 337.1 nm which is most often employed in the time-resolved measurements.

The possible interferences caused by the back ground emission from the reagents are most probably avoidable with a time-resolved scheme because of the short-lived S_1–S_0 radiative

Table 1. Several spectroscopic data of the Ln chelates

Reagent [λ_{ex}/nm]		Ln(III) ion [λ_{em}/nm]			
		Sm [599]	Eu [615]	Tb [544]	Dy [576]
Quin 2					
[334]	$\varepsilon/(10^3/M/cm)$*	4.64	4.09		
	RLI†	4.24×10^2	1.38×10^3		
BAPTA					
[270]	$\varepsilon/(10^3/M/cm)$*			3.30	3.72
	RLI†			4.85×10^3	1.53×10^3
F-BAPTA					
[272]	$\varepsilon/(10^3/M/cm)$*			4.30	3.94
	RLI†			3.63×10^3	1.71×10^3
	Free ion‡				
	RLI†		1.00	3.03	0.191
	[$\lambda_{ex}/\lambda_{em}$]		[394/615]	[264/544]	[350/573]

*Molar absorption coefficient at the excitation wavelength.
†Relative luminescence intensity (arbitrary unit) of the $1 \times 10^{-5}M$ solution normalized for that of the $EuCl_3$ solution.
‡For the chloride solutions.

transition; indeed the fluorescence lifetime of Quin 2 was reported to be as long as 1.5 nsec in an aqueous buffer solution at pH 7.3,[32] while in our preliminary lifetime measurement for Eu(III)–Quin 2 system the value of *ca.* 60 μsec was obtained using a pulsed Xenon light source.

It is also interesting that, as can be seen from Table 1, BAPTA and F-BAPTA give the luminescent chelates with Dy(III) and Tb(III) ions, in contrast the emission from Sm(III) and Eu(III) ions is greatly sensitized by Quin 2, in spite of the non-selective reactivities of those ligands towards the Ln ions. Therefore, these luminescence selectivities are the mechanistic problems closely related to the nature of the excited states and the energy transfer processes. The reliable photophysical data such as quantum efficiencies, lifetimes, and phosphorescence measurements (T_1 energy levels) for the ligand systems will be compiled and the time-resolved scheme for the sensitive detection will be reported promptly. In addition, this luminescence selectivity seems to be practically useful for Eu and Tb not only to immunoassay purpose but to environmental one because of distinctively low abundances of these elements among Ln series.

In conclusion, the choice of aromatic octadentate ligands of polyaminocarboxylate type is quite attractive for the Ln energy transfer luminescence, which satisfies two major requirements; to provide efficient 'antenna' groups and a donor set as suffices for 8-fold coordination of Ln ions. The ε values of the Ln chelates studied in this work unfortunately seem to be rather small. Chromophore systems which give the greater ε, $>10^4$/M/cm should be contemplated. In addition, the bifunctional analogues which have covalently-attaching groups to biological molecules (*e.g.* proteins) are thought to be readily derived from these ligand structures.[10] Systematic survey of ligand systems pointing in these directions is now under way.

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