Sensitization of Europium(III) Luminescence by DTPA Derivatives

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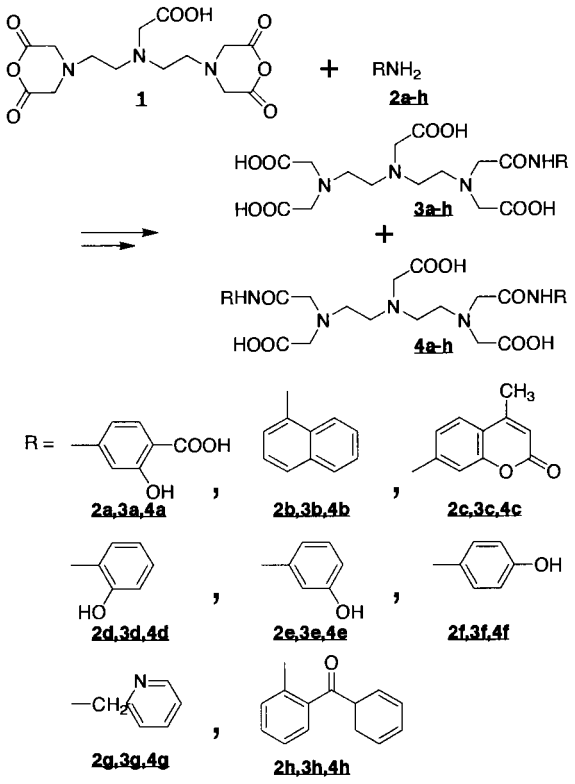
Novel ligands, diethylenetriaminepentaacetic acid bearing several aromatic amines, were synthesized and their chelates with a europium ion were prepared. The lanthanide luminescence of the chelates of 1-aminonaphthalene and 7-amino-4-methylcoumarin was largely enhanced by the energy transfer from the ligand to the europium ion.

Lanthanide chelates have unique luminescent properties such as a millisecond lifetime and a spiked emission. They have been used as a probe in biological applications.[1] Especially, they give very high sensitivity by use of a time-resolved fluorescence detection to eliminate short-lived fluorescence background signals.[2] The disadvantages of lanthanide chelates relative to organic fluorophores are their low quantum yields in an aqueous system. For overcoming this problem, several chelates were studied up to date.[3-6] These chelates possess two functional units, a chelating moiety which coordinates a lanthanide ion and an organic chromophore which absorbs and sensitizes light. Polyaminocarboxylic acids such as DTPA, ethylenediaminetetraacetic acid (EDTA), and triethylenetetraminehexaacetic acid (TTHA) form stable complexes with lanthanide ions even at low concentrations. Thus, they are suitable as a chelating moiety but their lanthanide chelates have very low fluorescence intensity. In a previous study, Bailey et al.[7] reported that a conjugate of diethylenetriaminepentaacetic acid (DTPA) and *p*-aminosalicylate enhanced lanthanide luminescence. Recently, Selvin et al.[8] reported that a conjugate of 7-amino-4-methyl-2(1*H*)-quinolinone and polyaminocarboxylic acid has been shown to be useful for the enhancement of lanthanide luminescence and as a luminescence donor of a luminescence resonance energy transfer experiment. In this paper, we report the synthesis of conjugates of DTPA with several aromatic amines and the luminescence property of their lanthanide chelates compared with that of *p*-aminosalicylate-DTPA chelate. We found that the conjugate containing 7-amino-4-methylcoumarin showed strong fluorescence intensity which could be applied for the probe.



Scheme. Synthesis of DTPA derivatives.

First, we carried out a preliminary experiment to gain information on the luminescence intensity of the lanthanide chelates of the conjugates of DTPA and several aromatic amines as a light-absorbing chromophore. Diethylenetriaminepentaacetic dianhydride (DTPA anhydride) was allowed to react with 1 equiv of several amines. *p*-Aminosalicylic acid, *o*-aminophenol, *m*-aminophenol, *p*-aminophenol, 2-(aminoethyl)-pyridine, 2-aminobenzophenone, 1-aminonaphthalene, and 7-amino-4-methylcoumarin were used as amines (Scheme). These synthesized ligands were used for chelating with a europium ion, and the fluorescence spectra of the chelates were measured in aqueous solution at 260 nm of excitation wavelength. 1-Aminonaphthalene derivative and 7-amino-4-methylcoumarin derivatives showed large enhancement of europium

Table 1. Electronic absorption data of the ligands and their chelates

Compound	λ_{max}(ε/mol^{-1}cm^{-1} x 10^{-3})	
	ligand	Eu^{3+} chelate[a]
3a	301.8(5.39)	303.0(5.02)
	262.8(12.4)	262.4(12.4)
	212.8(18.2)	213.2(18.4)
4a	302.0(8.75)	304.0(8.13)
	262.2(20.0)	261.4(18.2)
	211.4(31.2)	209.2(32.3)
3b	281.2(5.55)	281.2(5.3)
	222.0(49.1)	222.2(49.0)
4b	282.4(9.35)	283.2(8.18)
	221.2(77.9)	221.4(65.4)
3c	325.4(14.3)	322.8(13. 0)
		294.4(10.2)
	210.0(25.5)	211.8(24.2)
4c	323.4(17,3)	316.6(12.1)
		286.2(12.2)
	207.6(33.0)	211.2(27.8)

Solvent; 10 mM Tris-HCl (pH 8.0).
[a]Eu^{3+} chelate was prepared by the mixing of ligand and $EuCl_3$ (1:1, mol/mol).

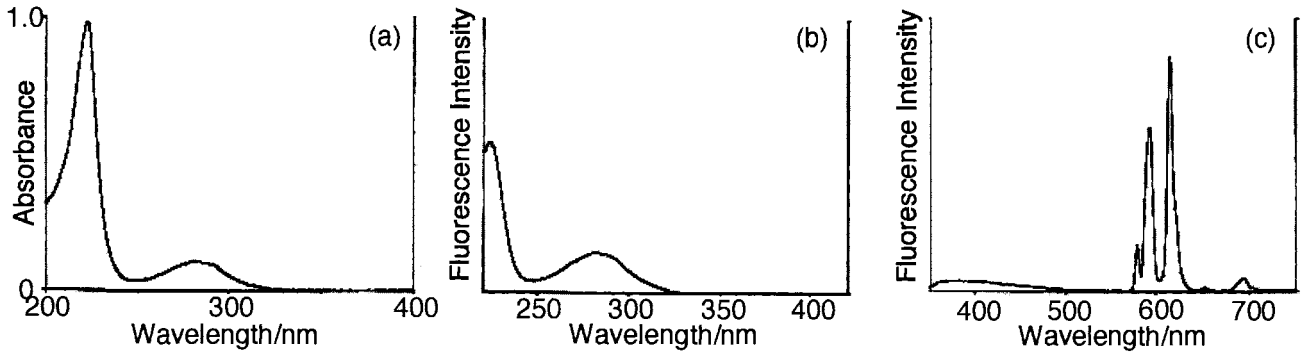


Figure 1. Electronic (a), excitation (b), and emission (c) spectra of [Eu^{3+} **3b**] in 10 mM Tris-HCl (pH 8.0). (a) [Eu^{3+} **3b**]=0.02 mM. (b) [Eu^{3+} **3b**]=0.01 mM. Emission wavelength: 615 nm. (c) [Eu^{3+} **3b**]=0.01 mM. Excitation wavelength: 281 nm. Emission filter: UV-35.

luminescence (618 nm). Thus, these two ligands were isolated and their properties were investigated in detail. A *p*-aminosalicylic acid derivative was also isolated and examined as a reference. These ligands derived from **2a-c** were purified by silica-gel column chromatography to give two conjugates, monoamides (**3a-c**) and bisamides (**4a-c**) in the ratio of about 2:1 from each reaction. The yields were 69% for **3a**, 31% for **4a**, 36% for **3b**, 19% for **4b**, 46% for **3c**, and 29% for **4c**. Each compound was characterized by ESI-MS and ^{1}H NMR spectroscopy. The formation of the chelates with a europium ion was confirmed by ESI-MS. In this analysis, each chelate shows a unique spectrum corresponding to the isotopic abundance of europium (^{151}Eu:^{153}Eu = 47.8:52.2), to indicate the formation of the chelates.

The electronic absorption spectra of the ligands and their chelates are summarized in Table 1. The europium chelates containing *p*-aminosalicylate or 1-aminonaphthalene showed very similar spectra with the corresponding ligands, indicating that the europium ion did not give rise to perturbation of a chromophore in the ground state. On the other hand, the spectra of the chelate containing 7-amino-4-methylcoumarin were slightly different from that of the corresponding ligand, which suggests the coumarin moiety interacts with the europium ion.

Luminescence of the chelates was observed with a large enhancement of the europium luminescence compared with the europium chelate of DTPA. The emission of the chelates mainly arises from transition from $^5D_0$ to $^7F_2$ (615 nm) and $^5D_0$ to $^7F_1$ (594 nm). The luminescence data are shown in Table 2. The emission intensity of these chelates at 615 nm under excitation at λ_{max} was in the following order: 7-amino-4-methylcoumarin-, 1-aminonaphthalene- and *p*-aminosalicylate-containing chelates. Monoamide derivatives showed a slightly larger or almost the same emission intensity compared with the bisamide derivatives in the case of 1-aminonaphthalene- and *p*-aminosalicylate-containing chelates, while the bisamide derivative showed larger emission intensity than the monoamide derivative in the case of 7-amino-4-methylcoumarin-containing chelate. The emission intensity of the europium chelate reached 1000 times for **3c** and 1800 times for **4c** compared with that for DTPA. This enhancement is caused by an energy transfer from the ligand to the metal. Figure 1 shows the electronic spectrum, excitation spectrum, and emission spectrum of [Eu^{3+} **3b**]. The excitation spectrum was similar to the electronic spectrum, suggesting that the energy transfer takes place. Other chelates also showed similar excitation spectra to the electric spectra (data not shown). This energy transfer process likely proceeds via an intersystem crossing from the first excited singlet state to the triplet state of the ligand followed by energy transfer to the europium ion. The synthesized chelates in this study will have great potential for biological application. Thus, work on the derivatization and the immobilization of the chelate on biological molecules such as nucleic acids and proteins is in progress.

Table 2. Luminescence emission data of the chelates

Compound	Ex Wavelength /nm	I(615 nm)	Relative intensity [a] (615 nm)
Eu^{3+}-**3a**	262	0.559	3.308
Eu^{3+}-**4a**	261	0.416	2.521
Eu^{3+}-**3b**	281	10.46	15.38[c]
Eu^{3+}-**4b**	283	12.01	41.70[c]
Eu^{3+}-**3c**	323	84.92	976.1
Eu^{3+}-**4c**[b]	317	190.6	1833

Condition: Sample conc., 0.01 mM; solvent, 10 mM Tris-HCl (pH 8.0); Temp., 20°C; 1 cm cuvette
[a]Relative to fluorescence intensity of Eu-DTPA. [b]Slightly cloudy solution. [c]Corrected value for the removal of scattering.

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