Highly Sensitive Energy-Transfer Luminescence of the *N,N'*-Bis(2-hydroxybenzyl)-ethylenediamine-*N,N'*-diacetatoterbium(III) Complex in Aqueous Solution

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The luminescent properties of a terbium complex with a hexadentate ligand of *N,N'*-Bis(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (HBED) having a phenolate functional group as antenna moiety, have been investigated. The complex exhibits a high emission quantum yield (ϕ = 0.222, ex. 288 nm, em. 546 nm) with a long decay lifetime (τ_{H2O} = 1.2 ms) in aqueous solution, which indicates energy-transfer type emission. The number of metal-bound water molecules in the complex was determined to be q = 1.8. The triplet energy level was estimated based on the data of its low-temperature phosphorescence. The calibration graph for Tb(III) was linear over the range from 3×10^{-11} to 1×10^{-8} mol dm^{-3}. A 3σ-blank detection limit of 3 ppt was achieved.

The sensitized luminescence of lanthanide (Ln) complex has been widely used in analytical methods for lanthanide ions as well as organic substances converted into their lanthanide complexes,[1—4] including the fluorescent labels used for time-resolved immunoassay.[5—9] These methods are based on the advantages of this luminescence, such as large Stokes shift, millisecond-range lifetime, and narrow-band emission.

Numerous ligands for Ln ions, particularly Eu and Tb ions, have been designed as probes and labels in analytical and biochemical fields: bidentate ligands of β-diketonates[6—8,10—13] and catechol analogues,[3,14] the tridentate ligand of pyridine dicarboxylic acid,[15,16] and polydentate ligands of Shiff bases,[9,17] cryptates,[18] and polyaminocarboxylates.[19—25]

These studies have revealed some requirements to obtain efficiently sensitized luminescence: (1) high molar-absorption coefficient of the photo-absorbing moieties of the ligand, which is generally called an "antenna chromophore", (2) high quantum yield, which is related to the triplet state of the ligand and the lowest radiative f-level of the Ln center, (3) removal of the coordinated waters in the first coordination sphere, where the O–H oscillators provide a path for the radiationless deexcitation of the lanthanide ion,[26] and (4) sufficiently short distance between the antenna chromophore and the center Ln metal; it is known that the efficiency of dipole–dipole energy-transfer is inversely proportional to the sixth power of distance.[27] Although some theoretical approaches have been reported, the mechanism of energy-transfer luminescence of lanthanide complexes is still at issue.

In this work, an ethylenediamine derivative, *N,N'*-bis(2-hydroxybenzyl)-ethylenediamine-*N,N'*-diacetic acid (HBED, H_4hbed) was investigated as a promising energy-transferable ligand which meets the requisites listed above. Since this reagent acts as a hexadentate ligand, the number of metal-bound water molecules is expected to be less than three for Eu and Tb ions, which generally prefer nine-fold coordination. A few ligands having phenolate as an antenna moiety were developed for the energy-transfer luminescence of Tb and Eu ions, such as calixarene derivatives[28—31] and Shiff bases.[17] Since these Ln complexes have high quantum yields, the phenolate is expected to improve the energy-transfer efficiency of EDTA-type complexes. In addition, the distance from a metal ion to a chromophore is shortened in complexes with ligands having phenolate functional groups, due to the proximity of the antenna moiety to the coordinating sites. Since the reagent, HBED, satisfies the above-mentioned requirements, its lanthanide complex is a candidate for highly efficient sensitized-luminescence.

This chelator, developed in 1967 by Martell et al.,[32] exhibits a strong coordination ability to various metal ions, particularly for the Fe(III) ion; the stability constant is markedly as large as $10^{39.68}$. Lanthanide complexes with HBED also have large stability constants, for example $10^{20.38}$ for Lu(III). This ligand was used for the determination of metal ions by capillary electrophoresis.[33,34] However, there are no reports on the detailed characteristics of the sensitized luminescence of the Ln-hbed complexes.

In this report, we first describe the spectroscopic and photophysical properties of Tb-hbed complexes in aqueous solution, including the luminescence lifetime and the phosphorescence spectra. From these data, the quantum yield, the T_1 energy level and the number of water molecules bound in the inner shell of the Tb(III)-hbed complex are estimated. Furthermore, the ultra trace determination of Tb(III) ion is also proposed.

Experimental

Apparatus. The absorption spectra were obtained with a

Hitachi model U-3200 double-monochrometor spectrophotometer. Steady-state luminescence measurements were made with a Hitachi F-4500 spectrofluorometer and the phosphorescence spectra in liquid nitrogen at 77 K were obtained with mechanical chopping light with 40 Hz frequency and a 2 millisecond delay time. In order to avoid second-ordered light, 430 and 500 nm cutoff filters were inserted at the entrance of the emission monochromater. The lifetime of the emission was measured with 200 μs resolution using an Aminco–Bowman Series 2 spectrofluorometer equipped with a 7 W pulsed xenon lamp. Deuterium oxide (D_2O) was used as a solvent for the lifetime measurements. The solution pH was measured with a Horiba M-13 pH-meter.

Reagent and Solution. Standard solutions of lanthanide ions were prepared by dissolving the chloride salts (99.9% purity) in doubly distilled water with a few drops of concentrated hydrochloric acid, except for Tm ion from Tm_2O_3 (99.9% purity). The solutions were standardized by EDTA titration with xylenol orange used as an indicator. The reagent, HBED (H_4hbed), purchased from Dojindo Lab. (Kumamoto, Japan) was dissolved in doubly distilled water with a minimum amount of sodium hydroxide. A dilute NaOD solution in D_2O (99.8% purity) was used as purchased from Merck (Darmstadt, Germany).

Typical Procedure. An aliquot of the Tb standard solution (10^{-7} mol dm^{-3}) was added to HBED solution (10^{-2} mol dm^{-3}) in a 25 ml volumetric flask. The pH value of the solution was adjusted to 11 with a 1.0 M NaOH solution (1 M = 1 mol dm^{-3}). The solution was then diluted to an appropriate volume with doubly distilled water. Spectral measurements were made for solutions containing approximately equimolar amounts of the reagent and the Tb ion. The characteristics of the monochromator and the photomultiplier were examined using Rhodamine B and a diffuser to obtain corrected excitation and emission spectra. The total luminescence quantum yields relative to that of quinine sulfate (ϕ = 0.546 in 1/2M H_2SO_4) with excitation at 288 nm were obtained in the same manner as described by Parker and Rees.[35] Demas and Crosby reported that the quantum yield of quinine sulfate in 1/2M H_2SO_4 was constant for excitation in the region 200—390 nm.[36] Phosphorescence data were obtained in liquid nitrogen at 77 K. The luminescence lifetime measurement was carried out in D_2O and NaOD. In calibration studies, a prescribed concentration of the ligand (1×10^{-7} mol dm^{-3}), which is in sufficient excess for the Ln concentrations, was used.

Results and Discussion

Luminescence Property. Among the Ln-hbed complexes tested, energy-transfer luminescence was observed for Tb, Eu, and Dy. In particular, the Tb complex exhibited strong fluorescence. The corrected excitation and emission spectra of the Tb-hbed complex are shown in Fig. 1. The peak of the excitation spectrum at 292 nm is approximately the same as that of the absorption spectrum. The emission spectrum exhibited four distinct bands peculiar to Tb (490, 546, 587, and 618 nm).

Figure 2 shows the pH dependence of the luminescence intensity for the Tb-hbed complex. Since the absorption spectrum of the luminescence active form of the Tb-hbed complex is consistent with that of fully deprotonated hbed^{4-}, the luminescent species probably exists as a deprotonated form as [Tb(hbed)]$^-$ in alkaline solution, and the no-luminescent or weaker-luminescent species probably exists as a doubly protonated phenol form, [Tb(H_2hbed)]$^+$, in neutral and acidic solution. According to the potentiometric titration study by Martell,[32] the protonation processes of Lu(III)-hbed complex ([Lu(H_2hbed)]$^+$) resulting in LuL$^-$ unusually occur in one step with an equilibrium constant of $10^{10.14}$. Kolat et al. reported proton-association constants of Ln-edta complexes.[37] Their values were in a similar range among different Ln ions. Therefore, nearly 99% of the Tb(III) complex exists as the unprotonated form at pH 6, based on an equilibrium calculation using the constant for the Lu complex. However, the luminescence intensity of the Tb complex (Fig. 2) was constant above pH 8. This may be due to the different proton-association constant of the Tb-hbed complex from that cited for the Lu complex.

Emission Decay of Tb(III)-hbed Complex. As shown

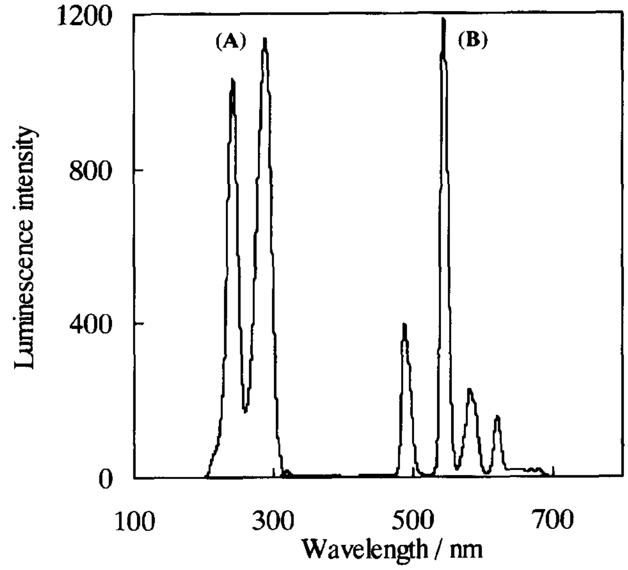

Fig. 1. Corrected luminescence spectra of Tb(III)-hbed complex in aqueous solution at pH 12. (A) Excitation spectrum (em. 546 nm); (B) Emission spectrum (ex. 288 nm). Tb(III), 1.0×10^{-6} mol dm^{-3}; HBED, 1.0×10^{-6} mol dm^{-3}. The excitation maximum at 241 nm was not used.

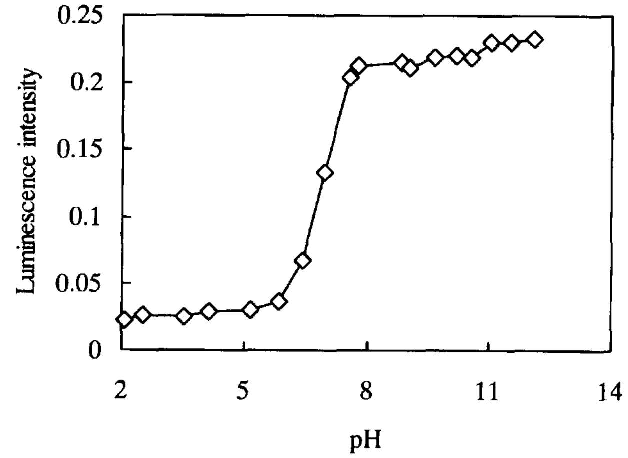

Fig. 2. Effect of pH on the Tb(III)-hbed fluorescence. Tb(III), 1.0×10^{-7} mol dm^{-3}; HBED, 2.0×10^{-7} mol dm^{-3}; ex. 288 nm, em. 546 nm.

in Fig. 3, the emission lifetimes of the Tb(III) complex in both H_2O and D_2O are in the millisecond range. The number of metal-coordinated water molecules in the complex, q, was estimated using the following relationship,[26] which was derived from the correlation between the fluorescence lifetimes and X-ray structures of crystalline complexes:

$$q = A_{\mathrm{Tb}}(\tau_{\mathrm{H2O}}^{-1} - \tau_{\mathrm{D2O}}^{-1}), \tag{1}$$

where τ_{H2O}^{-1} and τ_{D2O}^{-1} represent the reciprocals of the lifetimes (i.e. decay rate constants) in H_2O and D_2O, respectively, and the proportional coefficient, A_{Tb}, is 4.19 for Tb. The lifetime values and the number of metal-bound water molecules are listed in Table 1 along with other photophysical parameters. It should be noted that the estimated value of q for the Tb(III)-hbed complex, $q = 1.8$, is smaller than those reported for the EDTA complex, 3[26] or 2.4,[38] and CyDTA complex, 2.1.[38] These facts suggest that the cavity size and the rigidity of the ligand affect the number of water molecules in the primary coordination sphere. The smaller q value in the HBED system is advantageous to minimize the vibrational quenching by the –OH oscillator.

Quantum Yields and Triplet State. The total quantum yields of the Tb complex relative to that of quinine sulfate are given in Table 1, where the deoxygenation effect was examined with N_2 bubbling. High quantum yields, more than 0.2, were obtained. There have been only a few reports of a system for Tb having a high quantum yield ($\phi > 0.2$) in aqueous solution, for example $\phi = 0.3$ with a cyclic phosphazene derivative,[39] and 0.48 with a pyridine dicarboxylic acid derivative.[19] The reagent, HBED, is superior to those ligands in stability of its Tb complexes and selectivity for Tb being free from interferences from other Ln ions (see Calibration section). The sensitizing effect of deoxygenation most likely arises from an elimination of the collision between the triplet state of the ligand and dissolved O_2. The dioxygen quenching of the Tb luminescence indicates probable the back-energy transfer from the $^5D_4$ Tb level to the triplet state of the ligand.

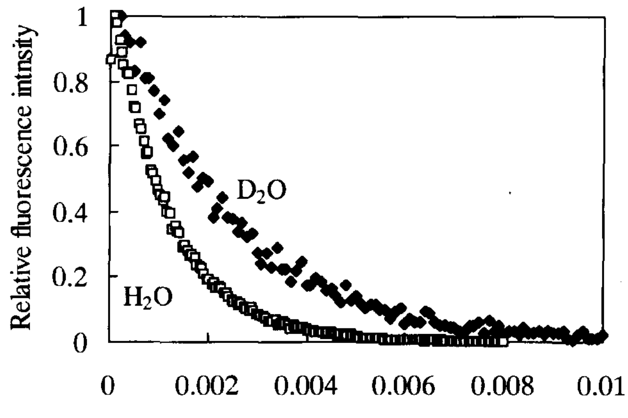

Fig. 3. Luminescence decay plots of Tb(III) complex. Tb(III), 2.5×10^{-6} mol dm^{-3}; HBED, 5.0×10^{-6} mol dm^{-3}. pH 12.16, pD 12.6; ex. 292 nm, em. 547 nm.

Table 1. Absorption and Emission Properties of Tb(III)-hbed Complex

$\varepsilon^{a)}/10^3$ M^{-1} cm^{-1}	5.17
τ/ms in H_2O	1.13
τ/ms in D_2O	2.26
q	1.8
Quantum yield (ϕ) with N_2 bubbling	0.222
Quantum yield (ϕ) without N_2 bubbling	0.206
T_1 level/cm^{-1}	<24000

λ_{ex} 288 nm; λ_{em} 546 nm. a) Molar absorptivity.

The triplet-state energy level was calculated from the phosphorescence spectra of the free ligand (hbed^{4-}) shown in Fig. 4. In the low-temperature luminescence spectra of the Tb complex, the peaks of the instinctive sensitized luminescence of the center metal f–f transition appeared without any phosphorescence from the free ligand. Hence, these facts indicate that energy transfer from the triplet state of the ligand to the center Tb ion works effectively. The phosphorescent profiles of other Ln-hbed complexes (Ln = La—Lu) were investigated to estimate the triplet-state level of the Tb-hbed complex. Those profiles were akin to that of the free ligand, centered at 405—420 nm, except for the lowered emission intensity for the Ln complexes. The triplet-state of hbed in the Tb complex is probably similar to that of the free ligand. Since the fine structure was not resolved to ascertain the 0–0 radiative transition lying at the longest-wavelength, the energy level of the lowest triplet state could not be accurately determined. It was reported that the back-energy transfer takes place when the lowest triplet energy level of the ligand was smaller than 22300 cm^{-1} for the Tb complexes.[19] As stated above, because dioxygen quenching is at work to a

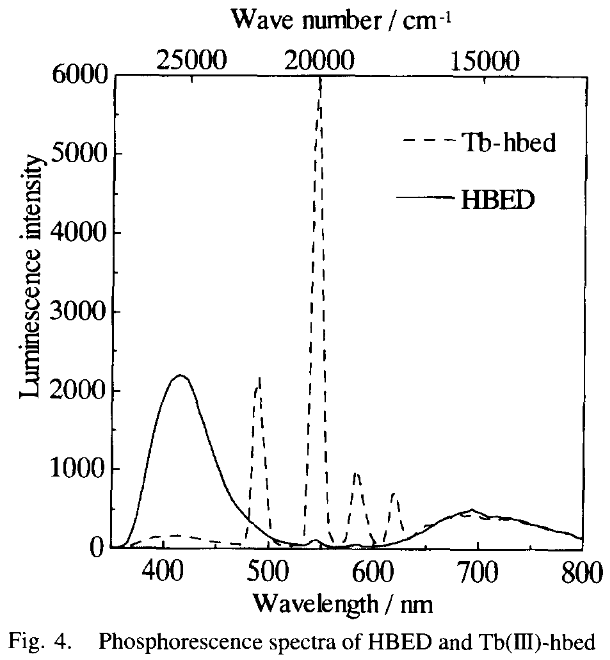

Fig. 4. Phosphorescence spectra of HBED and Tb(III)-hbed complex at 77 K. Concentration of ligand and Tb(III) are 5.0×10^{-4} mol dm^{-3}; solution pH before freezing, 12; ex. 288 nm.

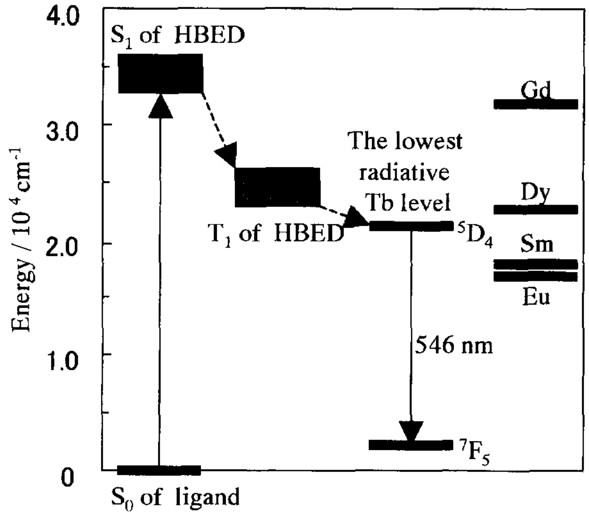

Fig. 5. Schematic energy diagram of intramolecular energy transfer for the Tb(III)-hbed complex.

small extent in this case, the triplet state most likely lies around (22000—24000) cm^{-1}. A plausible energy-level diagram is shown in Fig. 5. The results described above are consistent with the reported relationship between the back-energy transfer and the lowest triplet-energy level.[19]

Calibration of Tb. A linear correlation was found between the Tb(III) concentration and the luminescence intensities in the range from 3×10^{-11} to 1×10^{-8} mol dm^{-3}. The detection limit, defined as 3σ, was 2.1×10^{-11} mol dm^{-3} (3.3 ppt) when the photomultiplier voltage was set at 1270 V (ex. 292 nm, band pass 8 nm, em. 547 nm, bandpass 16 nm) with a 150 W xenon lamp for excitation. The excitation and emission wavelengths for the determination of Tb were different from that for the corrected spectra (ex. 288 nm, em. 546 nm), because the uncorrected apparatus was used for the calibration. In earlier works on Tb determination, the detection limits were 2.1×10^{-11} mol dm^{-3} for the thenoyltrifluoroacetone (TTA)-trioctylphosphine oxide (TOPO) system[40] and 1.1×10^{-11} mol dm^{-3} for the pyridine-2,6-dicarboxylic acid (DPA)-dodecyl sodium sulfate (SDS) system with laser excitation.[41] The proposed HBED system provides a comparable performance to these systems without any detergent or laser excitation. A hundred times other lanthanide ions gave no interference (±5%) with the determination of Tb ion at 10^{-9} mol dm^{-3}. The high sensitivity and selectivity of the proposed system are applicable to the determination of trace Tb ion.

The Tb-hbed complex reported here will be useful for applications in many fields, particularly as time-resolved luminescent labels. A study by us in this direction is now underway.

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