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Complexation Equilibria and Fluorescent Properties of Chelating Reagents Derived from Ethylene Glycol Bis(β-aminoethylether)-N,N,N',N'-tetraacetic Acid

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The complexation equilibria of divalent and lanthanoid metal ions with 1,2-bis(*o*-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) and 2-[(2-amino-5-methylphenoxy)methyl]-6-methoxy-8-aminoquinoline-N,N,N',N'-tetraacetic acid (quin2) were studied by pH titration. In lanthanoids, BAPTA shows a maximum stability for Pr^{3+}, whereas quin2 has higher affinities for heavier ones. These trends are discussed in terms of the rigidity and donor atoms of these ligands. Intense fluorescence was observed for quin2 complexes of Group-2 and -3 elements. Among the lanthanoids, only Sc, Y, La, Gd, and Lu enhance the fluorescence at around 380 nm.

1,2-Bis(*o*-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) and 2-[(2-amino-5-methylphenoxy)-methyl]-6-methoxy-8-aminoquinoline-N,N,N',N'-tetraacetic acid (quin2) have been proposed as fluorescent Ca^{2+} indicators for intracellular measurements.[1] These have been superseded by second-generation materials, such as indo-1, fura-2, and rhod-2.[2,3] All the above ligands are derivatives of ethylene glycol bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA); ether oxygen and amino nitrogen atoms connected by ethylene groups are replaced by aromatic ones. This modification affords a rapid change in the fluorescent properties upon the reaction with Ca^{2+} at physiological pH, while keeping a high selectivity against Mg^{2+}. The complexation equilibria,[1—5] fluorescent properties,[1—3,6] and an X-ray crystal structure[7] have been studied intensively for Ca complexes, but not for the others.[8,9]

In a previous paper, calcium (3.4 μg dm^{-3}) in brine (5 mol dm^{-3}) containing large amounts of magnesium was determined by the use of quin2 in a flow injection-fluorometric system.[10] These reagents are also expected to be promising for lanthanoid ions, because of their chemical similarity to alkaline earth metal ions. In this study, the complexation equilibria of BAPTA and quin2 with both lanthanoids and divalent metal ions were studied as well as the fluorescent properties of the resulting complexes, in order to seek further use of these reagents.

Experimental

Reagents. BAPTA was prepared and isolated as H_4L by modifying the method of Tsien.[1] The solution was prepared by neutralizing a suspended solution with a known amount of an alkali solution. Quin2 was supplied from Dojindo Laboratories (Kumamoto, Japan) as tetra potassium salt, which contained 8—9 water molecules, as determined by pH titration. All of metal solutions were prepared by dissolving the nitrates or oxides in dilute nitric acid.

Measurement. All the potentiometric and spectroscopic measurements were performed at 25.0±0.1°C. A solution containing 1.00×10^{-2} mol dm^{-3} nitric acid and 9.00×10^{-2} mol dm^{-3} potassium nitrate was used as a $-\log[H^+]$ standard.

Fluorescence spectra were recorded on a JASCO spectrofluorometer (FP-770) with a 10-nm bandwidth on both excitation and emission. The emission spectra were recorded with excitation wavelengths (λ_{ex}) of 267 nm for BAPTA complexes and 334 nm for quin2 complexes. Excitation at 230 nm was also examined for both reagents so as to enhance the light absorption; however, no essential differences other than the fluorescence intensities were found between the results at two excitation wavelengths. The excitation spectra were measured using the emission wavelengths (λ_{em}) listed in Table 4. These fluorescence spectra were usually not corrected for the instrumental response. The fluorescence intensity ($\lambda_{ex}=366$ nm; $\lambda_{em}=452$ nm) of 2×10^{-6} mol dm^{-3} quinine sulfate in 0.1 mol dm^{-3} sulfuric acid solution was fixed at 2.0, and the intensities of the sample solutions were expressed relative to this value. For selected complexes, the quantum yield was determined by a comparison with a reference solution of quinine bisulfate.

Results and Discussion

Protonation Equilibria. The protonation constants of BAPTA and quin2 were determined by pH titration at 25 °C and an ionic strength of 0.1 mol dm^{-3}. Potassium nitrate was adopted as a supporting electrolyte for quin2, whereas various nitrates were examined for BAPTA in order to explore the interaction between this ligand and monovalent cations. At $-\log[H^+]<2.6$, BAPTA formed white precipitates of the tetra-protonated species (H_4L), the solubility of which was 10^{-4} mol dm^{-3}. On the other hand, quin2 was soluble at least for $-\log[H^+]>1.6$.

With a decrease in $-\log[H^+]$, the average number of protons bound to the ligand approached a limiting value of 4 for BAPTA, whereas it exceeded 4 for quin2. This indicates the formation of a penta-protonated species, which makes quin2 soluble in an acidic medium. The protonation constants were calculated by the conventional method (Table 1).

The protonation constants of BAPTA and quin2 are similar to each other. The basicities of the nitrogen atoms of these ligands (K_1 and K_2) are appreciably smaller than those of EGTA.[11] The ratio of K_1 to K_2 for both BAPTA and EGTA are close to $10^{0.6}$, which is expected for two completely independent and equivalent

Table 1. Stepwise Protonation Constants of BAPTA and quin2 at 25 °C and an Ionic Strength of 0.1 $\mathrm{mol\,dm^{-3}}$ [a)]

Ligand	$\log K_1$	$\log K_2$	$\log K_3$	$\log K_4$	$\log K_5$	Supporting electrolyte
BAPTA	6.01	5.61	3.6	2.7		$(CH_3)_4NNO_3$
	6.04	5.57	3.7	2.6		$LiNO_3$
	5.95	5.60	3.6	2.6		$NaNO_3$
	5.95	5.44	3.48	2.67		KNO_3
	6.02	5.64	3.8	2.7		$RbNO_3$
	6.07	5.70	3.9	2.7		$CsNO_3$
quin2	6.25	5.30	3.59	2.67	2.0	KNO_3
EGTA[b)]	9.54	8.93	2.73	2.08		KNO_3

a) The precision of the constants was ±0.02 for $\log K_1$ and $\log K_2$, ±0.04 for $\log K_3$ and $\log K_4$ of BAPTA and quin2 in KNO_3 medium, and ±0.1 for the others.
b) Ref. 11.

protonation sites. The ratio is larger for quin2 ($10^{1.0}$) in accordance with its unsymmetric structure. The protonation constants of BAPTA are practically the same in 0.1 $\mathrm{mol\,dm^{-3}}$ $(CH_3)_4NNO_3$, $LiNO_3$, $RbNO_3$, and $CsNO_3$, but are smaller in 0.1 $\mathrm{mol\,dm^{-3}}$ KNO_3 and $NaNO_3$ because of the interaction between K^+ or Na^+ ions and the fully deprotonated species of BAPTA.

Complexation Equilibria. The complexation equilibria of metal ions with BAPTA and quin2 were studied at 25 °C and 0.1 $\mathrm{mol\,dm^{-3}}$ KNO_3 by pH-titration ($C_M = 1$—2 $\mathrm{mmol\,dm^{-3}}$; $C_L/C_M = 1.1$—1.5; $-\log[H^+] = 2$—7) and potentiometry with ion-selective electrodes. In the pH-titration of Cu–quin2 and Zn–quin2 systems, metal complexes precipitated at $pH < 3$. In Co-quin2 and Ni-quin2 systems, although a third species had to be considered in addition to ML and MHL, it could not be identified as being MH_2L or M_2L. Whichever was assumed to be the third species, the stability constants obtained for ML and MHL were not affected.

Potentiometry with ion-selective electrodes and metal exchange reactions,[12)] which affords the ratio in the stability constants between two metal complexes, was effective only in several systems. The ratio obtained by this method ($10^{4.52}$ for Pb–Ca–BAPTA, $10^{5.44}$ for Cd–Ca–BAPTA, and $10^{2.84}$ for Cd–Zn–BAPTA) were in good agreement with those calculated from the stability constants obtained by pH-titration. In other systems, the formation of binuclear complexes interfered with the evaluation.

The stability constants of complexes with divalent metal ions are summarized in Table 2. The constants of Ca^{2+} complexes agree with those previously reported,[1)] with the experimental difference being taken into account. In accordance with the decrease in ligand basicity, the stabilities of BAPTA and quin2 complexes are appreciably lowered compared with those of EGTA.[13)] The stability constants for complexes with BAPTA and quin2 are substantially the same for Ca^{2+}, Sr^{2+}, Ba^{2+}, and Cd^{2+}, but appreciably different for metal ions, such as Co^{2+} and Ni^{2+}. In Cu–BAPTA, a binuclear species Cu_2L was found, as in the case of Cu–EGTA.[14,15)]

The stability constants of lanthanoid complexes are summarized in Table 3. The $\log K_{ML}$ values for BAPTA are in good agreement with those by Hseu and Liu[8)] for lighter lanthanoid metals, but not for heavier ones. They used potentiometry with a mercury electrode in conjunction with metal-exchange reactions, which is not suitable for systems giving rise to binuclear complexed species. Figure 1 gives the $\log K_{ML}$ values plotted against the atomic number. Quin2 as well as EGTA shows a regular increase in stability across the series.[13)] BAPTA, on the other hand, presents a novel stability sequence having the maximum at Pr^{3+}. This stability trend is rather similar to those of macrocyclic polyethers or macrocyclic aminopolycarboxylic acids. The stabilities of 18-crown-6 complexes have a maximum at Ce^{3+} in methanol,[16)] and monotonously decrease in propylene carbonate;[17,18)] 1,7-diaza-4,10,13-trioxacyclopentadecane-*N*,*N'*-diacetic acid (K21DA) has maximum stability at Eu^{3+};[19)] 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane-*N*,*N'*-diacetic acid (K22DA) shows lower stabilities for heavier lanthanoids.[20)]

Figure 2 gives the logarithmic stability constants of BAPTA complexes plotted against those of EGTA complexes for metal ions of ionic radii larger than 0.95 Å. Circles for La^{3+} (1.160, an ionic radius in Å),[21)] Ce^{3+} (1.143), and Pr^{3+} (1.126) are close to the line drawn

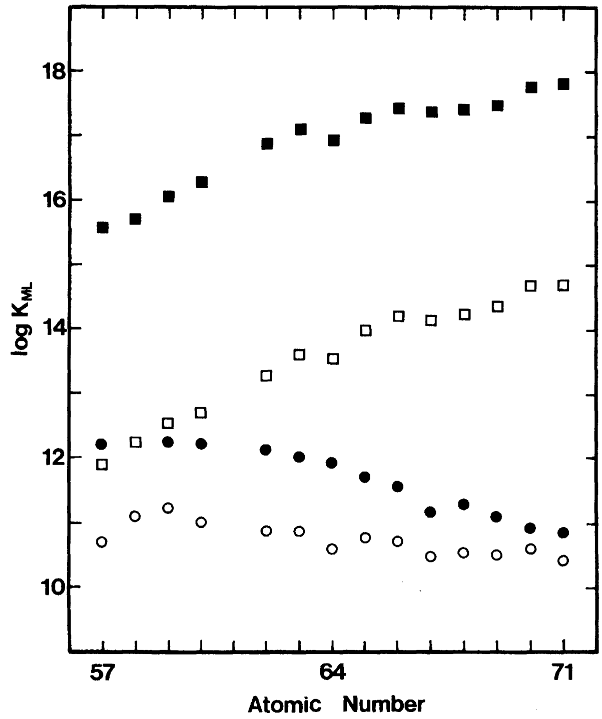

Fig. 1. Plot of the logarithmic stability constants of lanthanoid complexes vs. atomic number. 25 °C, 0.1 $\mathrm{mol\,dm^{-3}}$ KNO_3. Ligand: ○, BAPTA; □, quin2; ●, K22DA (Ref. 20); ■, EGTA (Ref. 13).

Table 2. Stability Constants of BAPTA and quin2 Complexes with Divalent Metal Ions at 25 °C and 0.1 mol dm^{-3} KNO$_3$$^{a)}$

Metal	BAPTA log K_{ML}	BAPTA log K^H_{MHL}	BAPTA log $K^H_{MH_2L}$ /log $K^M_{M_2L}$	quin2 log K_{ML}	quin2 log K^H_{MHL}	quin2 log $K^H_{MH_2L}$ /log $K^M_{M_2L}$
Ca^{2+}	6.78	3.3		7.28	3.03	
Sr^{2+}	5.13			5.17	4.29	
Ba^{2+}	5.75			5.29		
Mn^{2+}	8.72	3.05		9.91	3.43	
Co^{2+}	8.67	3.25		10.27	4.22	2.1/5.3
Ni^{2+}	7.76	4.24	2.5/	11.62	4.63	2.2/7.1
Cu^{2+}	11.7	3.65	/5.0		Precipitation	
Zn^{2+}	9.33	3.32			Precipitation	
Cd^{2+}	12.2	2.70		12.26	3.16	1.7/
Pb^{2+}	11.3	3.52	2.2/	12.24	3.45	2.6/

a) K^H_{MHL}=[MHL]/[ML][H$^+$]; $K^H_{MH_2L}$=[MH$_2$L]/[MHL][H$^+$]; $K^M_{M_2L}$=[M$_2$L]/[ML][M].

Table 3. Stability Constants of BAPTA and quin2 Complexes with Lanthanoid Ions at 25 °C and 0.1 mol dm^{-3} KNO$_3$

Metal	BAPTA log K_{ML}	BAPTA log K^H_{MHL}	quin2 log K_{ML}	quin2 log K^H_{MHL}
La^{3+}	10.70±0.01	2.2	11.89±0.02	1.8
Ce^{3+}	11.10±0.03	2.0	12.26±0.02	1.8
Pr^{3+}	11.23±0.03	1.7	12.53±0.04	1.5
Nd^{3+}	11.01±0.02	1.9	12.69±0.07	—
Pm^{3+}				
Sm^{3+}	10.87±0.02	2.1	13.28±0.05	—
Eu^{3+}	10.87±0.03	—	13.6 ±0.1	—
Gd^{3+}	10.6 ±0.1	—	13.54±0.05	—
Tb^{3+}	10.78±0.03	—	14.04±0.06	—
Dy^{3+}	10.73±0.03	—	14.2 ±0.1	—
Ho^{3+}	10.49±0.06	—	14.13±0.1	—
Er^{3+}	10.54±0.07	—	14.23±0.05	—
Tm^{3+}	10.51±0.01	—	14.35±0.08	—
Yb^{3+}	10.61±0.05	—	14.68±0.02	—
Lu^{3+}	10.42±0.08	—	14.69±0.04	—

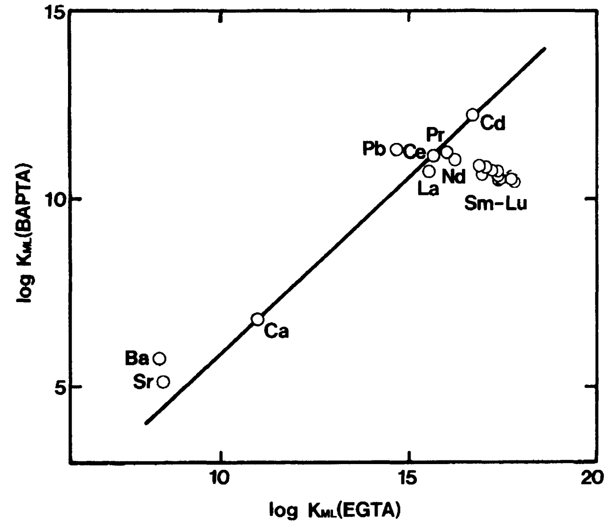

Fig. 2. Plot of the logarithimic stability constants of BAPTA complexes vs. those of EGTA complexes. The straight line is drawn so as to pass through the circles for Ca and Cd.

through those of Ca^{2+} (1.12) and Cd^{2+} (1.10) with similar ionic radii. The circles for lanthanoids smaller than Nd^{3+} (1.109) deviate downward along with a decrease in the ionic radius, whereas the circles for larger ions, such as Sr^{2+} (1.26), Pb^{2+} (1.29), and Ba^{2+} (1.42), deviate upward.

The X-ray crystal structures were determined for 5-F–BAPTA (a fluorine derivative of BAPTA) and EGTA complexes of Ca^{2+}.[7,22] Although both act as octadentate ligands, 5-F–BAPTA has, owing to its structural rigidity, much higher symmetry than EGTA in the complexes. Two iminodiacetate moieties of the EGTA complex have different configurations close to *fac* and *mer* in the cases of octahedral complexes, whereas those of the 5-F–BAPTA complex having a configuration similar to *fac*. Although the mean Ca–carboxylate O distance is slightly shorter for 5-F–BAPTA (2.34) than for EGTA (2.38), the Ca–N (2.65 for 5-F–BAPTA; 2.61 for EGTA), and especially the Ca–etheric O (2.67 for 5-F–BAPTA; 2.49 for EGTA), is more elongated for 5-F–BAPTA than for EGTA. The Ca ion in the 5-F–BAPTA complex is located close to the carboxylates and distant from the others in the cavity formed by the eight donor atoms.

While no X-ray structures have been reported for lanthanoid complexes with these ligands, the solution structures of the complexes of mid-lanthanoids, such as Eu, Gd, and Tb, have been studied by laser-induced luminescence and NMR spectroscopy.[23,24] The EGTA complexes of these metal ions have complete binding of eight donor atoms of the ligand with a single hydrated water, and undergo an equilibrium process of wrapping and unwrapping.

From these findings, it is concluded that the largest cavity size possible for BAPTA and EGTA is rather close to the ionic radius of Sr^{2+} or Pb^{2+}. EGTA can modify the cavity size through a conformational change to an appreciable extent without much energy loss, and

Table 4. Ligand-inherent Fluorescence of quin2 Complexes

Metal	λ_{em}	I_{rel}	λ_{em}	I_{rel}	Metal	λ_{em}	I_{rel}	λ_{em}	I_{rel}
None	381	0.004	491	0.15	La^{3+}	382	0.14	515	0.18
Mg^{2+}	381	0.004	495	0.20	Ce^{3+}	381	0.001	—	—
Ca^{2+}	380	0.025	498	2.0	Pr^{3+}	381	0.014	—	—
Sr^{2+}	381	0.011	500	1.2	Nd^{3+}	382	0.038	—	—
Ba^{2+}	381	0.013	500	1.2	Sm^{3+}	381	0.015	—	—
Zn^{2+}	381	0.018	514	0.13	Eu^{3+}	—	—	—	—
Cd^{2+}	381	0.021	509	0.44	Gd^{3+}	387	0.12	—	—
Hg^{2+}	381	0.005	506	0.008	Tb^{3+}	383	0.062	—	—
Pb^{2+}	381	0.007	501	0.015	Dy^{3+}	382	0.053	—	—
Al^{3+}	381	0.83	476	0.18	Ho^{3+}	382	0.035	—	—
Ga^{3+}	428	0.29	—	—	Er^{3+}	382	0.030	—	—
In^{3+}	471	0.64	—	—	Tm^{3+}	381	0.019	—	—
Sc^{3+}	407	1.4	—	—	Yb^{3+}	382	0.030	—	—
Y^{3+}	388	0.37	520	0.22	Lu^{3+}	398	0.58	508	0.18

Complex: 2×10^{-5} mol dm^{-3}; buffer 1×10^{-3} mol dm^{-3} (pH 7); $\lambda_{ex}=334$ nm.

the stability trend for lanthanoids is mainly governed by the charge density on a metal ion. In the case of BAPTA, however, a similar mode of change in the cavity size, which is restricted because of its structural rigidity, is possible only down to Pr^{3+}; smaller ions cannot fit well so as to have a lower stability. The restriction of a conformational change by cyclization gives a similar selectivity.

Although quin2 has a more rigid structure, the stability trend is similar to that of EGTA. Quin2 has an unsymmetric structure; that is, quinoline nitrogen has a much higher coordinating ability than does the etheric oxygen. Lanthanoids preferentially interact with a moiety containing quinoline nitrogen, and the remaining part would adjust to any decrease in the cavity size.

Absorption and Fluorescence Spectra. The L^{4-} species of BAPTA has an absorption maximum at 253 nm ($\varepsilon=16600$), while the complexes absorb maximally at 275 nm ($\varepsilon=4000$—7000), irrespective of the central metal ion; only the Cu complex has a characteristic absorption at 398 nm ($\varepsilon=2000$). On the other hand, the absorption maxima of L^{4-} of quin2 at 261 ($\varepsilon=28000$) and 350 nm ($\varepsilon=4000$) do not change appreciably in either the position or intensity upon complexation with metal ions.

The fluorescence spectra were recorded for 2×10^{-5} mol dm^{-3} BAPTA and quin2 solutions in the presence and absence of metal ions using an ethylenediamine buffer at pH 7.0 (Fig. 3, Tables 4 and 5). The L^{4-} species of BAPTA has a fluorescence maximum at 373 nm, which does not shift upon complexation with metal ions. Alkaline earth metal ions slightly enhance the fluorescence intensity, whereas the others reduce it.

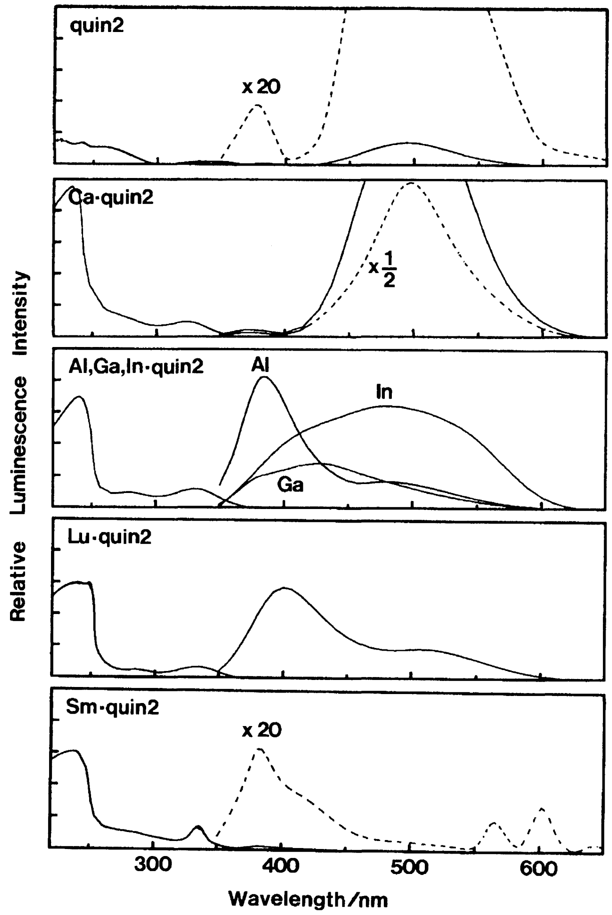

Fig. 3. Uncorrected excitation and emission spectra of quin2 and its complexes. $C_L=C_M=2\times10^{-5}$ mol dm^{-3}. pH = 7.0 (ethylenediamine buffer). λ_{em}/nm=491 (quin2); 498 (Ca); 381 (Al); 428 (Ga); 471 (In); 508 (Lu); 381 (Sm). $\lambda_{ex}=334$ nm. The dotted curves are reduced or enlarged by the factor given in each figure.

Table 5. Sensitized Luminescence

Complex	λ_{em}	I_{rel}	Complex	λ_{em}	I_{rel}
Tb–BAPTA	495	0.30	Sm–quin2	565	0.011
	549	0.50		601	0.014
	591	0.078		648	0.001
	625	0.020	Eu–quin2	595	0.011
Dy–BAPTA	486	0.022		618	0.015
	589	0.022			

Conditions were the same as those in Table 4.

The fluorescence spectrum of quin2 (L^{4-}) shows a small peak at 381 nm and a large one at 491 nm with a peak-height ratio of 1 : 10 (Fig. 3). The reported quantum yields, 0.029 and 0.025,[1,5] are larger than our value of 0.015. When EDTA was added in order to eliminate any possible interference by metal ions, it slightly decreased to 0.011. Extra care must be taken in determining the quantum yield of neat reagents.

The equilibrium constants obtained above guarantee the formation of more than a 90% ML-type complex of quin2 under the above-mentioned conditions. Only Ba and Sr complexes are in equilibrium with appreciable amounts of metal ions and free quin2. The molar ratio methods for Group-13 elements, for which complexation equilibria were not studied, demonstrated the unique presence of ML for In and the presence of stepwise equilibria for Al and Ga, as found for Mg.[1)] Thus, measurements for the equimolar mixture do not always reflect the fluorescence properties of an ML-type complex for Mg, Sr, Ba, Al, and Ga, but do reflect for the others.

Alkaline earth metal ions, Zn^{2+} and Cd^{2+} do not change the peak position and height ratio of two peaks. The intensities at around 500 nm are enhanced in the following order: $Ca \gg Sr, Ba > Cd > Zn$. The quantum yield for the Ca complex (0.13) was in agreement with those reported previously, 0.146 and 0.15.[1,6)] The group-13 elements show varying fluorescence spectra; the main peak shifts to a longer wavelength in the order $Al < Ga < In$, and the intensities decrease in the order $Al > In > Ga$.

The fluorescence characteristics of Group-3 metal complexes are classified into two: (1) Sc, Y, La, Gd, and Lu, (2) other elements. For (1), the fluorescence intensity at around 400 nm is extremely enhanced ($Sc > Lu > Y > La > Gd$), and that at around 500 nm is unchanged. For (2), the peak around 400 nm is only slightly increased, whereas that around 500 nm is eliminated. The quantum yields for the peak at around 380 nm were 0.01 for La, 0.046 for Lu, 0.002 for Sm, and 0.008 for Dy. Such selectivity for the lanthanoids has been observed and discussed regarding some other reagents.[25—28)]

A change in the fluorescence intensity during the course of the measurement was observed for Group-2 and -3 elements, Sc, Y, La, and Lu. For example, the emission at 515 nm of the La–quin2 complex increased twice within 20 min with $\lambda_{ex} = 230$ nm and 2.5 times in 10 min with $\lambda_{ex} = 334$ nm. The UV spectra indicated the decomposition of quin2. No further efforts, however, were made to identify the product of this reaction.

Sensitized luminescence[29,30)] was also observed for Tb–BAPTA, Dy–BAPTA, Sm–quin2, and Eu–quin2 complexes (Fig. 3, Table 5). Both BAPTA and quin2 complexes have relatively large absorption in the ultraviolet region. The energy is effectively transferred from the ligand to the metal, thus giving the metal ion-characteristic luminescence. Radiationless deactivation is reduced by the wrapping of metal ions with these ligands. The $^5D_4$–$^7F_5$ emission of Tb–BAPTA is especially strong and promising as a luminescent probe.

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References

1) R. Y. Tsien, *Biochemistry*, **19**, 2396 (1980).

2) G. Grynkiewicz, M. Poenie, and R. Y. Tsien, *J. Biol. Chem.*, **260**, 3440 (1985).

3) A. Minta, J. P. Y. Kao, and R. Y. Tsien, *J. Biol. Chem.*, **264**, 8171 (1989).

4) S. M. Harrison and D. M. Bers, *Biochim. Biophys. Acta*, **925**, 133 (1987).

5) B. Svensson, B. Jonsson, M. Fushiki, and S. Linse, *J. Phy. Chem.*, **96**, 3135 (1992).

6) M. Guardigli and N. Sabbatini, *Chem. Phys. Lett.*, **179**, 539 (1991).

7) J. T. Gerig, P. Singh, L. A. Levy, and R. E. London, *J. Inorg. Biochem.*, **31**, 113 (1987).

8) T. -M. Hseu and K. -L. Liu, *J. Chin. Chem. Soc.*, **37**, 237 (1990).

9) A. Yuchi, M. Hirai, H. Wada, and G. Nakagawa, *Chem. Lett.*, **1990**, 773.

10) H. Wada, H. Atsumi, and G. Nakagawa, *Anal. Chim. Acta*, **261**, 275 (1992).

11) L. G. Sillen and A. E. Martell, "Stability Constants of Metal-Ion Complexes, and Supplement No. 1," The Chemical Society, London (1964 and 1971); D. D. Perrin, "Stability Constants of Metal-Ion Complexes, Part B," IUPAC, Pergamon (1979).

12) A. Yuchi, H. Wada, and G. Nakagawa, *Anal. Sci.*, **1**, 19 (1985).

13) J. L. Mackey, M. A. Hiller, and J. E. Powell, *J. Phys. Chem.*, **66**, 311 (1962).

14) K. Ohzeki, M. Saruhashi, and T. Kambara, *Bull. Chem. Soc. Jpn.*, **53**, 2548 (1980).

15) C. K. Schauer and O. P. Anderson, *Acta Crystallogr., Sect. C*, **44**, 981 (1988).

16) R. M. Izatt, J. D. Lamb, and J. J. Christensen, *J. Am. Chem. Soc.*, **99**, 8344 (1977).

17) M. -C. Almasio, F. Arnaud-Neu, and M. -J. Schwing-Weill, *Helv. Chim. Acta*, **66**, 1296 (1983).

18) J. -C. G. Bunzli and F. Pilloud, *Inorg. Chem.*, **28**, 2638 (1989).

19) C. A. Chang and M. E. Rowland, *Inorg. Chem.*, **22**, 3866 (1983).

20) C. A. Chang and V. O. Ochaya, *Inorg. Chem.*, **25**, 355 (1986).

21) R. D. Shannon, *Acta Crystallogr., Sect. A*, **32**, 751 (1976).

22) C. K. Schauer and O. P. Anderson, *J. Am. Chem. Soc.*, **109**, 3646 (1987).

23) R. C. Holz, S. L. Klakamp, C. A. Chang, and W. D. Harrocks, *Inorg. Chem.*, **29**, 2651 (1990).

24) C. A. Chang, H. G. Brittain, J. Telser, and M. F. Tweedle, *Inorg. Chem.*, **29**, 4468 (1990).

25) A. Fuernandez-Gutierrez and A. Munoz de la Pena, "Molecular Luminescence Spectroscopy. Methods and Application: Part I," ed by S. G. Schulman, Wiley, New York (1985), pp. 371—546.

26) B. Budesinsky and T. S. West, *Anal. Chim. Acta*, **42**, 455 (1968).

27) N. S. Poluektov, R. S. Lauer, and O. F. Gaidarzhi, *Zh. Anal. Khim.*, **26**, 898 (1971).

28) K. Soroka, R. S. Vithanage, D. A. Phillips, B. Walker, and P. K. Dasgupta, *Anal. Chem.*, **59**, 629 (1987).

29) W. D. Harrocks and D. R. Sudnick, *Acc. Chem. Res.*, **14**, 384 (1981).

30) F. S. Richardson, *Chem. Rev.*, **82**, 541 (1982).