Fluorescence Lifetime Measurements of Morin-Metal Ion Complexes

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(Received June 8, 1977)

Fluorescence lifetimes of morin complexes of Be(II), Zn(II), Al(III), Ga(III), In(III), Th(IV), Zr(IV), and Sn(IV) extracted into isopentyl alcohol from the acid solution at various pH values were investigated. The result indicates that the Al(III), Ga(III), and In(III) complexes change their compositions according to the change of pH. More detailed investigations by the use of mole ratio method suggest that Al(III), Ga(III), and In(III) form complexes having two different mole ratios of 1 to 1 and 1 to 2 (metal ion: morin). Fluorescence lifetimes were changed according to the changes of the composition of the complex and the differences of the metal ion.

Morin (3,5,7,2′,4′-pentahydroxyflavone) has been widely used as an analytical reagent for fluorometric determinations.

It reacts with Be(II), Zn(II), Al(III), Ga(III), In(III), Sn(IV), Th(IV), Zr(IV), and so on to form fluorescent complexes. The Al(III),[1] Zr(IV),[2] and Be(II)[3] complexes exhibit especially intense green fluorescence, and morin has been utilized very often for the determination of trace amounts of these metal ions. The fluorescence system resulting from the reaction between Al(III) and morin has been applied also to the determination of inorganic ions such as F^- by the use of its quenching ability.[4]

Katyal[5] discussed summarily the analytical utilities of flavonoid compounds including morin. The compositions of complexes formed between morin and metal ions have been investigated by several workers. Biryuk and Rabitskaya[6] reported that In(III)-morin complex with the mole ratio of 1 : 1 is formed at pH 3.6. Recently Katyal and Prakash[7] reported that Al(III) and Ga(III) give the morin complexes with the same composition as the In(III) complex at pH 3.6. In the case of Th(IV) complex, two different results were obtained by Fletcher *et al.*[8] and by Blank *et al.*[9]: that is, 1 : 2 and 1 : 3 complexes, respectively.

In the present paper, we report the new results that complexes with two different mole ratios are formed in Al(III)-, Ga(III)-, In(III)-, and Be(II)-morin systems, while Zn(II), Th(IV), Zr(IV), and Sn(IV) react with morin to give the complexes with a single mole ratio by the measurement of the fluorescence lifetime of these complexes. Although these complexes showed very similar fluorescence spectra, explicitly different fluorescence lifetimes were determined.

Experimental

Reagents and Procedure. Aluminium(III) and gallium(III) stock solutions were obtained by dissolving each metal in concentrated hydrochloric acid. An indium(III) solution was prepared by dissolving indium(III) hydroxide in boiling hydrochloric acid. Other metal ion solutions were prepared by dissolving each salt in hydrochloric acid. Working solutions of each metal ion were prepared by diluting the stock solutions with distilled water to about 10 mM.

Morin of high purity was purchased from Merck. Solutions of morin were prepared by dissolving the solid material in methanol. Spectral grade methanol and high purity isopentyl alcohol were purchased from Wako Junyaku, Co., Ltd. All the reactions of complexation were done in hydrochloric acid solutions at various pH values, and the complexes obtained were almost extracted into isopentyl alcohol, as described elsewhere.[10] After the extraction solutions were degassed by bubbling with high purity nitrogen for 5 min, fluorescence and excitation spectra and fluorescence lifetimes were measured.

Apparatus. Fluorescene and excitation spectra were measured with a Hitachi MPF-4 fluorometer. Fluorescence lifetimes were measured by the use of a home-made time-correlated single photon counting apparatus.[11] Although the time-resolution is 0.1 ns, the exciting light pulse has a time-duration of 1.6 ns. Therefore, the values of fluorescence lifetimes shorter than 2.0 ns were corrected by the deconvolution method.

Results and Discussion

Aluminium(III)-Morin Complex. Figure 1 shows fluorescence and excitation spectra of the aluminium-morin complex in isopentyl alcohol. The complex was formed in hydrochloric acid solutions at pH 1.0 and 3.0. Morin itself does not fluoresce in acid solutions. As is seen in this figure, the maximum fluorescence wavelengths are 495 nm at pH 1.0 and 503 nm at pH 3.0.

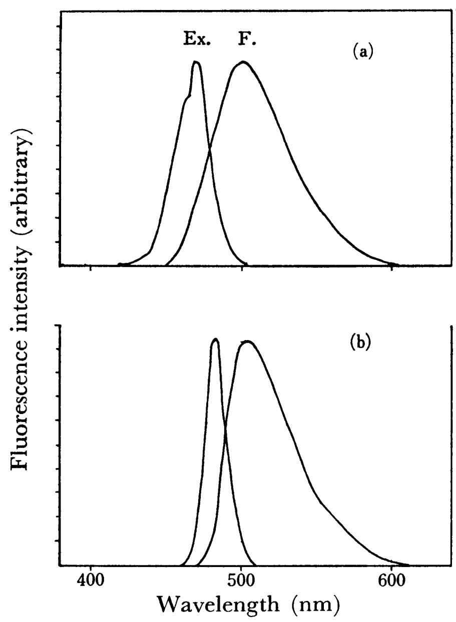

Fig. 1. Fluorescence and excitation spectra of Al(III)-morin complex in isopentyl alcohol; (a) pH 1.0 and (b) pH 3.0.

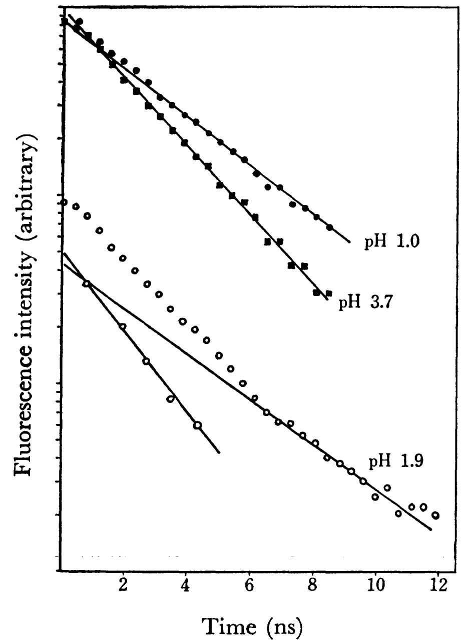

Fig. 2. Semilogarithmic plots of the decay time of the fluorescence of Al(III)–morin complex.

There is little difference between the fluorescence spectra at pH 1.0 and 3.0, while the excitation spectra are very different.

Figure 2 shows a semilogarithmic plot of the fluorescence intensity *vs.* time in order to obtain the fluorescence lifetime of the aluminium complex. As shown in Fig. 2, the decay time of fluorescence of the complex extracted at pH 1.9 has two different exponential features. On the contrary, the plots for those extracted both at pH 1.0 and 3.7 give single exponential decay times. Moreover, the component with the longer lifetime in the two fluorescence decays at pH 1.9 agrees with that at pH 1.0, and the component with the shorter lifetime with that at pH 3.7, within experimental errors. The value of the longer lifetime component is (3.3 ± 0.1) ns and that of the shorter lifetime component is (2.0 ± 0.3) ns. These results suggest that the composition of aluminium-morin complex changes near pH 2. The composition of aluminium to morin was estimated by the mole ratio method. The result is shown in Fig. 3,

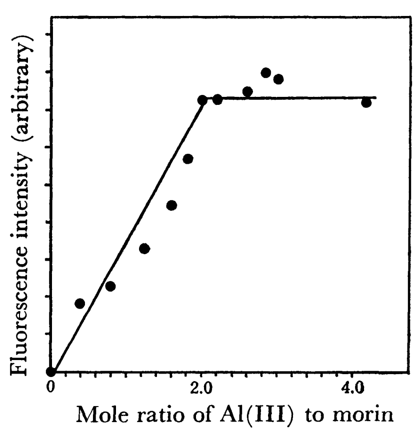

Fig. 3. The mole ratio method for Al(III)–morin complex formed at pH 1.3.

which confirms the aluminium-morin ratio of 1 : 2 for the complex formed at pH 1.0. The composition of complex at pH 3.0 was also investigated and the result obtained was in good agreement with that in the literature.[7] The structure of the complex has not been established yet, but the metal ion is assumed to be substituted for the hydrogen of the hydroxyl group at the 3- or 5-position.[10]

Gallium(III)- and Indium(III)-Morin Complexes. Figures 4 and 5 show fluorescence spectra of gallium–

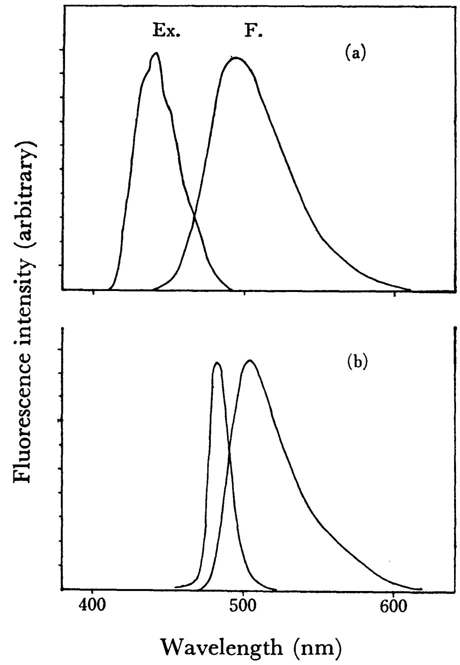

Fig. 4. Fluorescence and excitation spectra of Ga(III)–morin complex in isopentyl alcohol; (a) pH 1.0 and (b) pH 3.0.

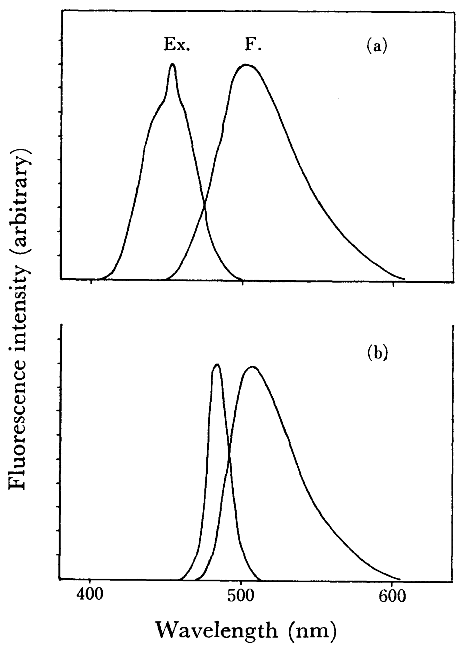

Fig. 5. Fluorescence and excitation spectra of In(III)–morin complex in isopentyl alcohol; (a) pH 1.3 and (b) pH 3.6.

and indium-morin complexes and their excitation spectra at two different pH values. The decay curve of the fluorescence showed a single exponent at each pH value. The results of the fluorescence lifetime measurement were that the longer lifetime component of the gallium complex is (2.7±0.1) ns and the shorter one is (1.6±0.4) ns. As for the indium complex, the longer lifetime component is (4.6±0.1) ns and the shorter one is (3.5±0.1) ns. Dependencies of the shape and wavelength of fluorescence and excitation spectra on pH were in good agreement with those in the case of the aluminium complex. However, the fluorescences of the gallium and indium complexes were unstable in comparison with that of the aluminium complex. Therefore, no reliable composition of these complexes has been determined by the mole ratio method. However, the same conclusion about the composition as that obtained in the aluminium complex is predictable, because aluminium, gallium, and indium belong to the same group IIIb.

Beryllium(II)-Morin Complex. Beryllium ion reacted with morin to form a stable complex in both acid and basic solutions. If forms a complex with the mole ratio of 1:2 (beryllium: morin) in hydrochloric acid at pH 2.0 and its fluorescence lifetime was estimated to be (2.1±0.1) ns. Some differences in fluorescence and excitation spectra can be seen between the complexes obtained at pH>3.4 and at pH 2.0. The fluorescence lifetime obtained at pH 4.3 is (4.8±0.3) ns, although the mole ratio has not been determined because of the poorness of experimental reproducibility.

Thorium(IV)-, Zirconium(IV)-, Tin(IV)-, and Zinc-(IV)-Morin Complexes. These complexes obtained in acid media are considered to have a single mole ratio, since the fluorescence lifetime values showed no change in the pH range of 1–5. As for the thorium and zirconium complexes, the mole ratio of metal ions to morin was estimated to be 1:2. However, the mole ratio of tin- and zinc-morin complexes has not been determined, because the complexes were unstable.

The fluorescence lifetime values obtained in this work are summarized in Table 1.

TABLE 1. FLUORESCENCE PROPERTIES OF METAL ION-MORIN COMPLEXES IN ISOPENTYL ALCOHOL

Metal ion	Mole ratio (metal ion: morin)	F_{max} (nm)	E_x (nm)	Fluoresence lifetime (ns)
Al(III)	1:2	495	438	3.3±0.1
	1:1	503	483	2.0±0.3
Ga(III)	1:2	500	468	2.7±0.1
	1:1	503	483	1.6±0.4
In(III)	1:2	500	452	4.6±0.1
	1:1	506	483	3.5±0.1
Be(II)	1:2	500	438	2.1±0.1
	1:1 (?)	508	451	4.8±0.3
Zn(II)	—	502	468	2.1±0.3
Th(IV)	1:2	513	483	2.6±0.3
Zr(IV)	1:2	508	440	4.6±0.3
Sn(IV)	—	—	—	2.3±0.4

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