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Syntheses and Complexing Behavior of New Fluorescent Reagents for Alkaline Earth Metal Ions

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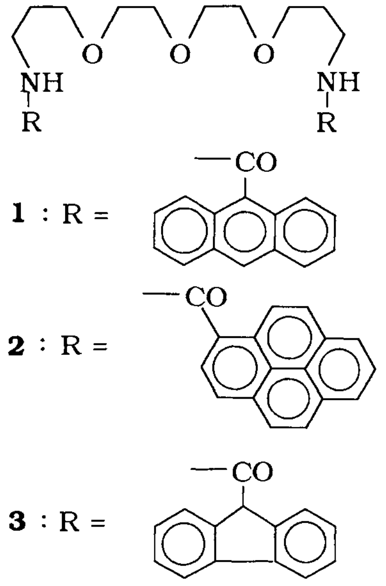
A new fluorescent reagent (**1**) which has two anthracene moieties at both terminals of linear polyether was synthesized, and the complexation behavior of **1** with alkaline earth metal ions was investigated. At the complexation with the metal ions, the fluorescence spectrum of **1** changed from that of monomer to dimer of anthracene. **1** formed both 1:1 and 1:2 complexes with alkaline earth metal ions, and the complex formation was selective to Ca^{2+}.

Many kinds of crown ether type macrocyclic compounds have been synthesized, and have been used for analytical application such as chemical sensors,1) stational phases of chromatography,2) and spectrophotometries.3) However, the application for fluorimetry was scarce. In our previous paper, some fluorescent reagents for alkali and alkaline earth metal ions were reported.4) However, the spectral change of the fluorescence were based on a change of an excitation efficiency at a fluorescent substituent by a dissociation of the phenol moiety of the reagent. In the present communication, we chose a linear polyether as an analogue of a crown ether, and synthesized a new fluorescent reagent **1** which has fluorescent functional groups at both terminals of the polyether. The mechanism of the change of fluorescence spectra of these reagents are completely different from previous one.4)

The compound **1** was synthesized as follows: The anthracene-9-carboxylic acid was converted to an acid-chloride by thionyl chloride. The acid-chloride was reacted with diethylene glycol bis(3-aminopropyl)ether to yield the diamide compound (**1**). The product was separated through a silica gel column and purified by recrystallization.5) Measurements of fluorescence spectra were carried out in acetonitrile solution of the reagent (5×10^{-5} M (1 M= 1 mol·dm^{-3})) at 25 °C, and alkaline or alkaline earth metal perchlorate was added to the solution for the complex formation experiments. To prevent a non-linearity of the fluorescence intensity, an isosbestic point (350 nm) of the absorption spectra was chosen as an excitation wave length.6) The concentration of the counter anion (ClO_4^-) was maintained to be 0.1 M by addition of tetramethylammonium perchlorate in the solution.



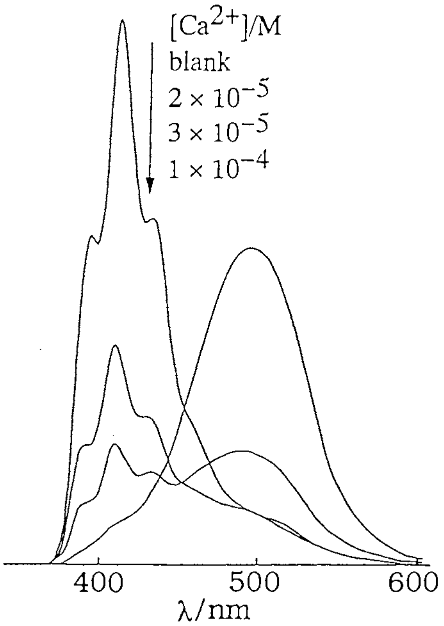


Fig. 1. Fluorescence spectra (uncorrected) of **1** and its Ca^{2+} complex, as excited at 350 nm. [**1**]= 1 × 10^{-5} M in acetonitrile at 25 °C.

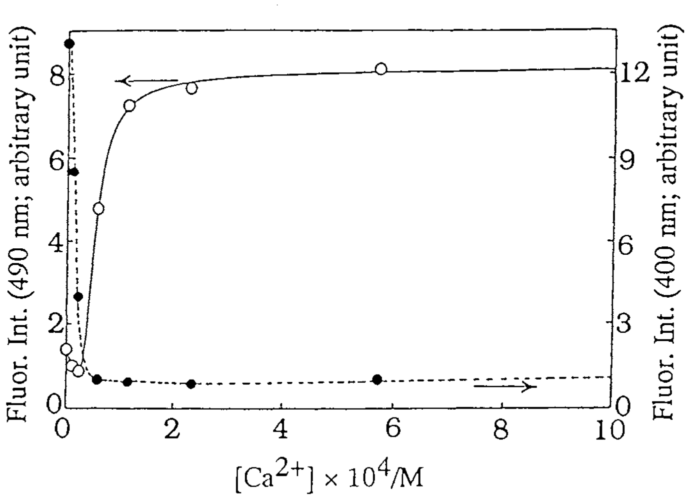


Fig. 2. Dependence of fluorescence intensities on the concentration of Ca^{2+}. Conditions were the same as Fig. 1.

The compound **1** formed a complex with Ca^{2+} ion. The shape and the peak of the fluorescence spectrum markedly changed from three relatively sharp peaks at around 400 nm to a broad peak at 490 nm on the complexation (Fig. 1). The peak at around 400 nm of uncomplexed species and the broad peak at 490 nm of complexed species could be attributed to an anthracene monomer and a dimer, respectively.[7] In the presence of Sr^{2+} or Ba^{2+}, the same spectral changes were observed. The compound **2** and **3**,[5] the analogues of **1** were also synthesized and tested. However, the spectrum of **2** or **3** did not changed by the addition of any metal salts. The fluorescence spectrum of **2** was already that of dimer pyrene , and this shows the strong interaction of pyrene units before complexation. On the other hands, **3** shows only monomer type fluorescence spectra in the absence and presence of calcium perchlorate. This may be caused by little interaction between the fluorene units. In the case of alkali metal ions or ammonium ion, only small spectral changes were observed for **1**–**3**. This suggests that the complexation abilities of these reagents to alkali metal ions or ammonium ion are small.

To study the complexation behavior of **1** with alkaline earth metal ions quantitatively, the measurements of the fluorescence were carried out in detail. The fluorescence spectra of **1** in the presence of several concentration of Ca^{2+} were also shown in Fig. 1. Change of the spectra was not straightforward, and divided into two steps. In the first step, the peak intensity of the anthracene monomer (400 nm) decreased by the addition of Ca^{2+} until the amount reached a half molar of **1**. Then, a peak of the dimer (490 nm) appeared on the further addition of Ca salt, and the intensity increased with Ca^{2+} concentration (Fig. 2). These phenomena suggests that , at first, Ca^{2+} ion formed 1:2 complex (metal:ligand) with **1** when **1** is excess to Ca^{2+}, and that the 1:2 complex changed to a 1:1 complex by addition of excess Ca^{2+}. These reaction schemes is summarized in eq. 1 and 2.

$$M + L \overset{K_1}{\rightleftharpoons} ML \qquad (1) \qquad\qquad K_1 = \frac{[ML]}{[M][L]} \qquad (3)$$

$$ML + L \overset{K_2}{\rightleftharpoons} ML_2 \qquad (2) \qquad\qquad K_2 = \frac{[ML_2]}{[ML][L]} \qquad (4)$$

$$I_t = I_1[L] + I_2[ML] + I_3[ML_2] \qquad (5)$$

I_t: total fluorescence intensity, I_1: fluorescence intensity coefficient of L, I_2: fluorescence intensity coefficient of ML, I_3: fluorescence intensity coefficient of ML_2, L: ligand **1**, M: metal ion.

From this complexation model as described in eq. 1 and 2, non-linear least square curve fitting method (NLSQ) based on Marqart Method was adapted for reproducing the experimental data of fluorescence intensity at 490 nm. In this method, total intensity of the fluorescence was given by eq. 5. The coefficient I_3 is assumed to be zero, because NLSQ usually concluded negative values for I_3, inconsistently. The best fit curve for Ca^{2+} is superimposed in Fig. 2 as a solid line. The complex formation constants (K_1 and K_2) of **1** were obtained for several alkaline earth metal ions by this method, and were listed in Table 1.

As shown in Table 1, the order of the formation constant (K_1) of **1** for 1:1 complex is $Ca^{2+} > Sr^{2+} > Ba^{2+} > Mg^{2+}$. Although K_2 for 1:2 complex is $Mg^{2+} > Sr^{2+} > Ca^{2+} > Ba^{2+}$, **1** formed the most stable complex with Ca^{2+} as comparing with $K_1 \times K_2$ values. This may be due to the suitable enclosure on Ca^{2+} by the oxygen atoms of polyether and carbonyl moieties like a 18-crown-6 molecule. This suitable conformation of **1** to Ca^{2+} causes a dimer-like interaction between two anthracene units. This was also observed in a change of absorption spectra (Fig. 3). On the other hand, the anthracene units of free **1** existed as a monomer.

Table 1. Complex formation constants

Metal ion	$K_1 \times 10^{-5}/M^{-1}$	$K_2 \times 10^{-5}/M^{-1}$
Mg^{+2}	1.3	5.7
Ca^{+2}	18.3	1.9
Sr^{+2}	4.9	2.2
Ba^{+2}	1.6	1.7

Solvent: acetonitrile at 25 °C. [**1**]= 5×10^{-5} M.

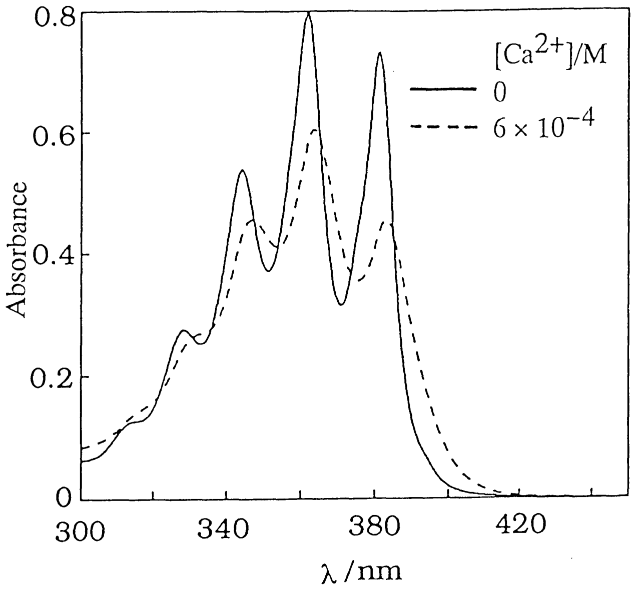


Fig. 3. Absorption spectra of **1** and its Ca^{2+} complex. Conditions were the same as Fig. 1.

It should be mentioned that the value of K_1 and K_2 were laying on the same order, and the 1:2 complexes are quite easy to form. This results could be explained as follows: The 1:1 complex of **1** and M^{2+} is not surrounded by the polyether moiety of **1** completely, and still affords to interact with another **1** molecule. Further interaction between anthracene moieties of two **1** molecules is also expected. This interaction caused a formation of anthracene tri- or tetramer, and diminished the fluorescence from the 1:2 complex. This coincides with the fact that I_2 for 1:2 complex was almost zero.

References

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5) N,N'-(4,7,10-trioxatridecane-1,13-diyl)bis(anthracene-9-carbonamide) (**1**): Yield 42.8%, mp 120-122 °C, ^{1}H NMR ($CDCl_3$) δ= 1.82-1.88 (4H, m; C-CH_2-C), 2.62 (4H, t, J=4.5 Hz; O-CH_2-), 2.82 (4H, t, J=4.5 Hz; O-CH_2-), 3.33 (4H, t, J=5.9 Hz; O-CH_2-), 3.70 (4H, q, J=6.0 Hz; N-CH_2-), 6.77 (2H, t, J=6.0 Hz; N-H), 7.39-7.47 (8H, m; aromatic 2,3,6,7-H), 7.92 (4H, d, J=8.1 Hz; aromatic 1,8- or 2,4-H), 8.00 (4H, d, J=9.3 Hz; aromatic 2,4- or 1,8-H), 8.37 (2H, s; aromatic 10-H). Found: C, 75.90; H, 6.54; N, 4.33%. Calcd for $C_{40}H_{40}N_2O_5$: C, 76.41; H, 6.41; N, 4.46%.

N,N'-(4,7,10-trioxatridecane-1,13-diyl)bis(1-pyrenecarbonamide) (**2**): Yield 5.3%, mp 100-102 °C, ^{1}H NMR ($CDCl_3$) δ= 1.75-1.81 (4H, m; C-CH_2-C), 3.01 (4H, t, J=2.7 Hz; O-CH_2-), 3.05 (4H, t, J=2.7 Hz; O-CH_2-), 3.32 (4H, t, J=5.9 Hz; O-CH_2-), 3.59 (4H, q, J=5.9 Hz; N-CH_2-), 6.90 (2H, t, J=6.0 Hz; N-H), 7.92-8.04 (12H, m; aromatic), 8.14 (4H, t, J=6.4 Hz; aromatic), 8.49 (2H, d, J= 9.3 Hz; aromatic). Found: C, 78.08; H, 5.94; N, 4.14%. Calcd for $C_{44}H_{40}N_2O_5$: C, 78.08; H, 5.96; N, 4.14%;

N,N'-(4,7,10-trioxatridecane-1,13-diyl)bis(9-fluorenecarbonamide) (**3**): Yield 18.4%, mp 181 °C, ^{1}H NMR ($CDCl_3$) δ= 1.56-1.60 (4H, m; C-CH_2-C), 3.19-3.31 (16H, m; -CH_2-), 4.76 (2H, s; -CH=), 5.76 (2H, t, J=6.0 Hz; N-H), 7.33 (4H, t, J=7.5 Hz; aromatic), 7.41 (4H, t, J=7.3 Hz; aromatic), 7.67 (4H, d, J= 6.8 Hz; aromatic), 7.76 (4H, d, J=6.8 Hz; aromatic). Found: C, 75.47; H, 6.67; N, 4.63%. Calcd for $C_{38}H_{40}N_2O_5$: C, 74.95; H, 6.71; N, 4.58%.

6)Fluorescence intensity in consideration of absorption of excitation light by inner filter effect is given as follows;

$$\text{Fluor. Int.} = C_i \cdot \Phi_i \cdot I_0 \cdot (1 - 10^{-\text{Abs}}) / (2.303 \cdot \text{Abs}).$$

Here, C_i, Φ_i, I_0, and Abs are a concentration of fluorescent species i, the quantum efficiency of fluorescence, excitation light intensity, and absorbance of the solution, respectively. At an isosbestic point, since the parameters except for C_i are constant, the fluorescence intensity is linear to C_i.

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