Fluorescence Intensity Changes for Anthrylazacrown Ethers by Paramagnetic Metal Cations

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Three anthrylazacrown ethers in which the anthracene fluorophore π system is separated from the electron donor atoms by one methylene group were synthesized, and their photophysical study was accomplished. These fluorescent compounds showed a maximum fluorescence intensity at pH=5 in aqueous solutions and a decrease in fluorescence intensity upon binding of paramagnetic metal cations ($Mn^{2+}(d^5)$, $Co^{2+}(d^7)$, $Cu^{2+}(d^9)$). The decrease in fluorescence intensity may be attributed to the paramagnetic effect of metal cations to deactivate the excited state by the nonradiative quenching process. The benzylic nitrogen was found to play an important role in changing fluorescence intensity. From the observed linear Stern-Volmer plot and the fluorescence lifetime independence of the presence of metal ions, it was inferred that the chelation enhanced fluorescence quenching (CHEQ) mechanism in the system is a ground state static quenching process. Enhanced fluorescence was also observed when an excess Na^+ ion was added to the quenched aqueous solution, and it was attributed to cation displacement of a complexed fluorescence quencher.

Introduction

The fluorescent chemosensor is a device that senses analyte with fluorescent signal transduction, and it has received considerable attention in recent years as the potential of this process has been realized in many practical applications.[1] The development of chemosensors with high sensitivity and selectivity for analytes such as metal ions in solution is an appealing goal. However, most such chemosensors reported onto date make use of direct complexation of metal ions with aromatic heterocyclic ligands and, therefore, their potential and flexibility in the design of metal-selective fluoroionophore is considerably limited.[2] In 1977, Sousa reported the synthesis of the naphthalene-crown ether compound where the aromatic π system was separated from the crown ether containing electron-donating atoms by one methylene group.[3] He found that complexation of alkali metal chloride salts in 95% ethanol at 77 K caused a decrease in fluorescence quantum yield and attributed it to a heavy atom effect and the increase of the rate constant for intersystem crossing of S_1 to T_1. Subsequent reports by de Silva and Czarnik, however, built on Sousa's premise that complexation of metal ions to crown ethers had changed the emission spectrum of attached fluorophores, described an enhancement of the fluorescence quantum yield of anthracene-azacrown ether by the addition of alkali metal and zinc ions.[4] The fluorescence of aromatic hydrocarbon is known to be quenched by formation of exciplexes between the aromatic ring and the nitrogen lone pairs, and they attributed the enhancement of fluorescence quantum yield to the inhibition of intramolecular exciplex formation by complexation of metal ions to the nitrogen nonbonding electron pairs. While such conjugate fluorescent chemosensors with azacrown have considerable advantages in the design of

selective fluoroionophores,[5-12] little was known about how metal ion complexed azacrown ether to affect the emission changes of covalently attached fluorophores. In this paper we report the results obtained on three compounds in Figure 1. We describe the synthesis, the quenching constants obtained by the Stern-Volmer equation, and the photophysical study in the absence and the presence of protons, as well as of light- and heavy-metal cations.

Experimental Section

Instruments. Fluorescence emission spectra were recorded on a Perkin-Elmer LS5013 spectrofluorometer, and Mass spectra were obtained by direct sample introduction into a Jeol JMS-DX303 spectrometer. Nuclear magnetic resonance spectra were obtained on a Gemini-300 (300 MHz) spectrometer. Chemical shifts were reported in ppm downfield from TMS. Infrared spectra were recorded on a Jasco IR-E300 spectrophotometer. Melting points were determined on a Connecting & Maintanance melting point apparatus and were not corrected. Column chromatography was carried out on a column packed with Silica Gel 60 (70-230 mesh, Merck).

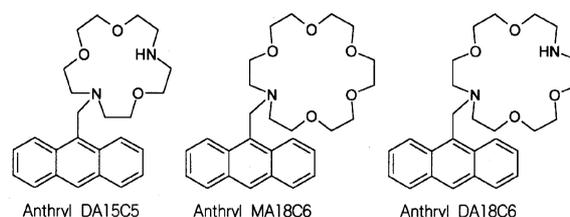**Fluorescence lifetime measurement.** The fluorescence decay was measured by using the time-correlated single

Anthryl DA15C5   Anthryl MA18C6   Anthryl DA18C6

Figure 1. Structures of the synthesized Anthrylazacrown ethers.

photon counting (TCSPC) technique. For all measurements, the polarization of the excitation laser was rotated to 90°, and the contributions of rotational diffusions to the signal were checked. But within our experimental uncertainty, we could not detect any difference in the fluorescence decay profiles for either of the polarization directions. The TCSPC technique is described only briefly here. The excitation laser is a mode-locked argon ion laser (Coherent Innova 200) pumping a dual jet dye laser (Coherent 700). The cavity-dumped dye laser beam with a Coherent 7220 Cavity Dumper has a 2-ps pulse width and average power of 20 mW with Rh6G as a gain dye and DODCI as a saturable absorber at 3.8 MHz rate. The laser pulse was focused by using a 7-cm focal length lens and its intensity was adjusted to below 10 W/cm^2 at the focused point. The emission signal was collected at 90° to the excitation laser beam by two 10-cm and 15-cm focal length lenses, focused onto a 20-cm monochromator (Jovin-Yvon H20), and detected with a photomultiplier tube (Hamamatsu model R2809). The signal was amplified by a wide band amplifier (Philip Scientific), sent to a Quad-constant fraction discriminator (Tennelec), a time to amplitude converter (Tennelec), a counter (Ortec), and a multichannel analyzer(Tennelec/Nucleus), and was stored in a computer.

Materials. 1,4,10-Trioxa-7,13-diazacyclopentadecane (DA15C5), 1,4,7,10,13-pentaoxa-16-azacyclooctadecane (MA18C6), and 9-chloromethylanthracene and metal salts were purchased from Aldrich, Kanto and Junsei and were used without further purification. 1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane (DA18C6) was synthesized using the previously reported method.[13] The organic solvents were purified by distillation over dehydrating reagents just before use. The water was deionized and distilled in glass and degassed by sonication at reduced pressure.[14]

1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane (DA18C6).[13] *N,N*-dibenzyl-7,16-diaza-18-crown-6 (1.7 g, 3.84 mmol), 10% Pd/C catalyst (0.8 g), and absolute EtOH (20 mL) were shaken in a Parr 3900 hydrogenation apparatus at 6 psi H$_2$ pressure and 25 °C for 24 h. The mixture was filtered and concentrated *in vacuo* to yield, after recrystallization from hexane, 0.65 g (65%) of a white solid: mp. 114-115 °C; ^{1}H NMR δ 2.3 (s, NH), 2.7-2.9 (t, NCH$_2$), 3.5-3.7 (q, OCH$_2$)

Anthryl DA15C5.[15] A solution of DA15C5 (0.131 g, 0.5 mmol) in benzene (15 mL) was slowly added to a stirred solution of 9-chloromethylanthracene (0.023 g, 0.1 mmol), and triethylamine (0.22 mL) in benzene (15 mL). The mixture was heated at reflux for 72 h. The reaction mixture was cooled, filtered, and extracted with water. The organic layer was separated and concentrated *in vacuo*. Column chromatography (EtOAc/hexane) gave 23% of the title compound as a yellow solid: mp. 94-96 °C; ^{1}H NMR (CDCl$_3$) δ 2.0 (s, NH), 2.8-3.0 (t, NCH$_2$), 3.4-3.7 (q, OCH$_2$), 4.5 (s, ArCH$_2$), 7.3-8.6 (m, Ar); IR (KBr, cm^{-1}) 3400, 3050, 2860, 1560, 1500, 1450, 1240, 1120; UV/Vis (MeOH) λ_{max} = 254 nm; Mass m/z 408 (M$^+$).

Anthryl MA18C6.   The synthesis was carried out using the method mentioned above. Column chromatography gave 40% of the title compound as a yellow solid: mp. 87-89 °C;

^{1}H NMR δ 2.8-2.9 (t, NCH$_2$), 3.5-3.7 (q, OCH$_2$), 4.5 (s, ArCH$_2$), 7.3-8.6 (m, Ar); IR (KBr, cm^{-1}) 3052, 2880, 1580, 1460, 1440, 1260, 1120; UV/Vis (MeOH) λ_{max} = 254 nm; Mass m/z 454 (M$^+$).

Anthryl DA18C6.   The synthesis was carried out using the method mentioned above. Column chromatography gave 29% of the title compound as a yellow solid: mp. 188-190 °C; ^{1}H NMR δ 2.1 (s, NH), 2.8-2.9 (t, NCH$_2$), 3.5-3.7 (q, OCH$_2$), 4.5 (s, ArCH$_2$), 7.3-8.6 (m, Ar); IR (KBr, cm^{-1}) 3380, 3020, 2860, 1580, 1460, 1440, 1251, 1108; UV/Vis (MeOH) λ_{max} = 254 nm; Mass m/z 453 (M$^+$).

Results and Discussion

The absorption and fluorescence spectra were measured for three anthrylazacrown ethers and 9-chloromethylanthracene in MeOH. Figure 2 shows the fluorescence spectra of four solutions at 5×10^{-5} M, excited by 254 nm. All four solutions show a maximum absorption at 254 nm, and a maximum fluorescence at 410 nm. The fluorescence spectrum shows a fluorescence quenching. The fluorescence quenching efficiency of three anthrylazacrown ethers is not much dependent on the ring size or the presence of other nitrogen atom in azacrown ether chelate. The results indicate that the fluorescence of fluorophore (anthracene) is quenched by the nonbonding electron of the benzylic nitrogen atom participating in nonradiative quenching, such as intersystem crossing.[16]

The pH dependence of fluorescence quenching was examined for three anthrylazacrown ethers. Figure 3 shows the fluorescence intensity of three anthrylazacrown ethers at 5×10^{-5} M, as a function of pH, showing a maximum at pH=5. The excitation wavelength was 254 nm. This fluorescence dependence on pH can be explained by using the intramolecular amine quenching mechanism that had been described previously.[15] Protonation of an amine group in fluorophore-amine conjugates results in the elimination of photoinduced electron transfer.[17] De Silva *et al.* explained the
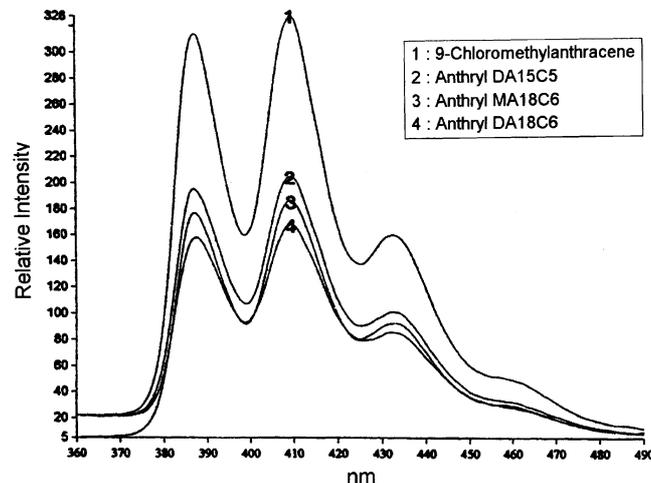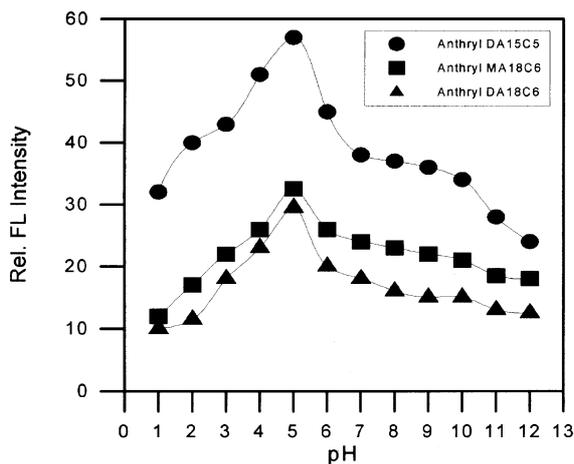
Figure 2. Relative fluorescence intensity changes for Anthrylazacrown ethers in MeOH solution.

Figure 3. pH-fluorescence profiles for 0.1 μM solutions of Anthrylazacrown ethers. Excitation was at 254 nm; Emission was measured at the emission maximum centered near 412 nm. pHs were maintained using the following solutions; 0.2 M HCl (pH 1 and 2), 0.2 M Sodium acetate (pH 3, 4, 5 and 6), 0.2 M Tris (pH 7, 8 and 9), 0.2 M NaHCO₃ (pH 10 and 11), 0.2 M NaOH (pH 12).

pH effect on the fluorescence of anthrylamine using this mechanism.[18] The result that all three anthrylazacrown ethers show a maximum intensity at the same pH indicates that the protonation at the benzylic nitrogen accounts for a majority of the observed intramolecular quenching, which is consistent with the fluorescence quenching results; and the pKa of amine is approximately 5. It is also expected that protonation of the anthracene ring may contribute to this effect; the pKa of ground-state anthracene is 3.3.

In the presence of 14 metal ion nitrites, the fluorescence intensity of three anthrylazacrown ethers was measured at pH=9.2. The fluorescence intensity at 0.2, 10, and 100 μM are displayed in Table 1, showing that only three ions, Mn^{2+},

Co^{2+}, Cu^{2+}, quench the fluorescence. This result can be explained by the paramagnetic properties of these three ions, all having the unpaired d electrons which can effectively quench both singlets and triplets of anthrylazacrown ethers. Specifically, Cu^{2+} and Mn^{2+} are weaker quenchers than Co^{2+} because of the stability of the half-filled shell of Mn^{2+} and the existence of only one unpaired electron of Cu^{2+}.

The Stern-Volmer plot for all three ions/three anthrylazacrown ethers, shown in Figure 4, fits a linear function, indicating that only one type of quenching occurs. In order to distinguish static and dynamic quenching, the fluorescence lifetime was measured by using the time-correlated single photon counting (TCSPC) technique. Figure 5 shows a TCSPC spectrum of 5.0×10^{-5} M Anthryl DA15C5, and shows that the decay profile was not influenced by the presence of the three ions. For other anthrylazacrown ethers, the almost same decay time and same independence of the metal ion concentration were also observed. Therefore, it is inferred that the contribution of dynamic quenching process could be negligible. Furthermore, the absorbance in absorption spectrum is influenced by the presence of the metal ion, proving more evidence for the ground-state complex formation. The slope of the Stern-Volmer plot yields the formation constants listed in Table 2. The quenching ions form the complexes in their ground state of anthrylazacrown ethers through the electron-donating nitrogen site; thus the charge-transfer interactions with the excited singlet state result in the increase of the quenching decay rate. Three ions show similar formation constants with three anthrylazacrown ethers, indicating that the electron-donating nitrogen site is the nitrogen near to the fluorophore (anthracene). The Co^{2+} ion can form a stable ground-state complex due to its strong paramagnetic property.

As excess Na^+, K^+, and Cd^{2+} ions were added to the

Table 1. Relative fluorescence intensities of Anthrylazacrown ethers in the presence of various metal nitrate[†]

Metal	Anthryl DA15C5			Anthryl MA18C6			Anthryl DA18C6			remark
	0.2 μM	10 μM	1000 μM	0.2 μM	10 μM	1000 μM	0.2 μM	10 μM	1000 μM	
free	108.2			87.8			43.7			
Na(I)	106.6	105.2	114.8	88.8	91.7	92.9	43.1	41.6	41.7	no change
K (I)	108.9	105.7	109.7	85.7	89.7	85.7	42.9	40.3	39.8	no change
Ca(II)	106.2	110.5	114.2	93.1	92.4	93.3	41.3	42.4	40.6	no change
Sr(II)	97.4	110.3	106.7	86.6	88.1	89.8	42.7	40.7	39.4	no change
Ba(II)	106.8	114.7	101.7	85.8	88.3	86.5	41.9	40.5	42.8	no change
Co(II)	107.8	95.3	0.3	87.3	78.5	0.3	41.4	35.6	0.2	decrease
Mn(II)	107.9	101.7	32.5	85.4	81.4	24.5	42.5	39.7	13.1	decrease
Ni (II)	104.5	107.4	96.4	89.0	85.0	82.6	42.9	40.4	35.9	no change
Cu(II)	102.7	106.4	8.9	88.8	84.4	7.7	41.7	38.9	3.5	decrease
Ag(I)	109.0	110.6	111.6	89.8	88.6	87.4	41.5	41.7	40.0	no change
Zn(II)	108.6	106.4	114.9	87.9	88.3	89.6	40.7	40.4	45.3	no change
Cd(II)	108.7	113.1	118.8	90.4	92.7	100.1	41.1	40.9	42.1	no change
Hg(II)	104.6	108.3	94.7	89.7	89.1	72.4	40.9	41.9	35.9	precipitate[‡]
Pb(II)	114.1	103.5	41.2	94.1	89.3	44.7	40.8	39.9	23.4	precipitate[‡]

[†]All solution were 0.1 mM of anthrylazacrown ethers, and were buffered with 0.2 M Tris buffer (pH 9.20). Excitation was at 254 nm; Emission was measured at the emission maximum centered near 412 nm. [‡]Hg(II) and Pb(II) were slightly soluble and formed precipitate in pH 9.2 solution.
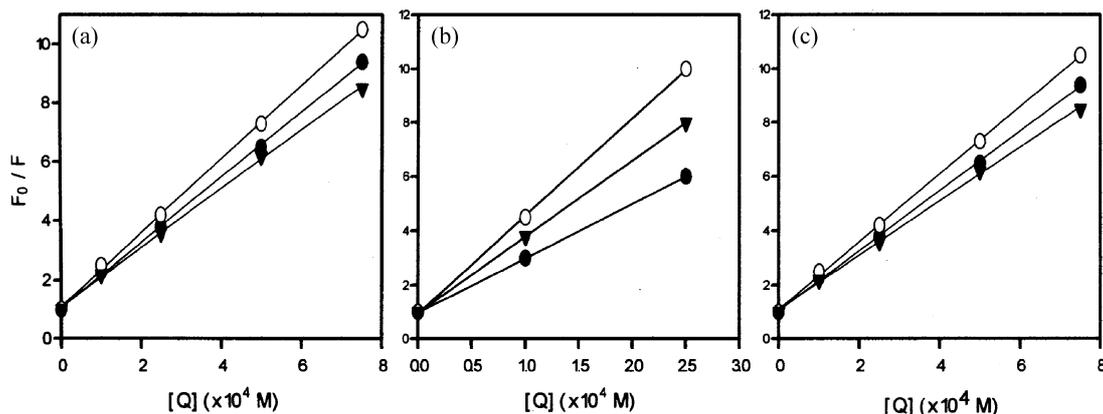
Figure 4. Stern-Volmer plotting for anthrylazacrown ethers in quenching with (a) Mn(II), (b) Co(II), and (c) Cu(II). (● : Anthryl DA15C5, ○ : Anthryl MA18C6, ▼ : Anthryl DA18C6)
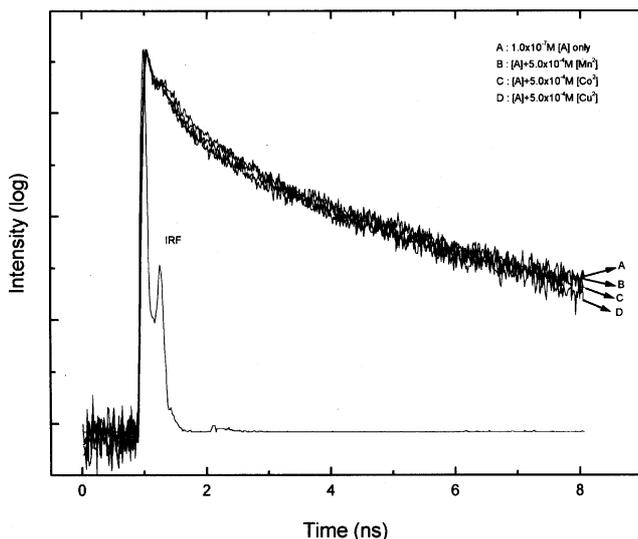

Figure 5. TCSPC spectrum of aqueous 10^{-7} M of Anthryl DA15C5 at pH=5 without and with the metal ions Mn^{2+}, Co^{2+}, and Cu^{2+}. The concentration of metal ion is 5.0×10^{-5} M. The excitation wavelength is 300 nm, and the detection wavelength is 410 nm. The notation IRF is the instrumental response function.

Table 2. Quenching constants (K_Q) obtained from Stern-Volmer plot

	Anthryl DA15C5			Anthryl MA18C6			Anthryl DA18C6		
	Mn (II)	Co (II)	Cu (II)	Mn (II)	Co (II)	Cu (II)	Mn (II)	Co (II)	Cu (II)
log K_Q	3.82	4.22	3.67	3.85	4.32	3.72	3.79	4.26	3.68

quenched solutions, only the Na^+ ion enhanced the fluorescence intensity. Figure 6 shows the enhancing effect of the Na^+ ion for the 5.0×10^{-4} M of Mn^{2+}, Co^{2+}, and Cu^{2+} solutions. Fages *et al.* reported that if the binding interaction is not strong enough, there is no effect on the fluorescence of the conjugated fluorophore.[19] The Na^+ ion, a metal ion of interest that is not a quencher but is complexed effectively, displaced a quencher ion complexed by the crown ether[20] and, as a result, the fluorescence was recovered.
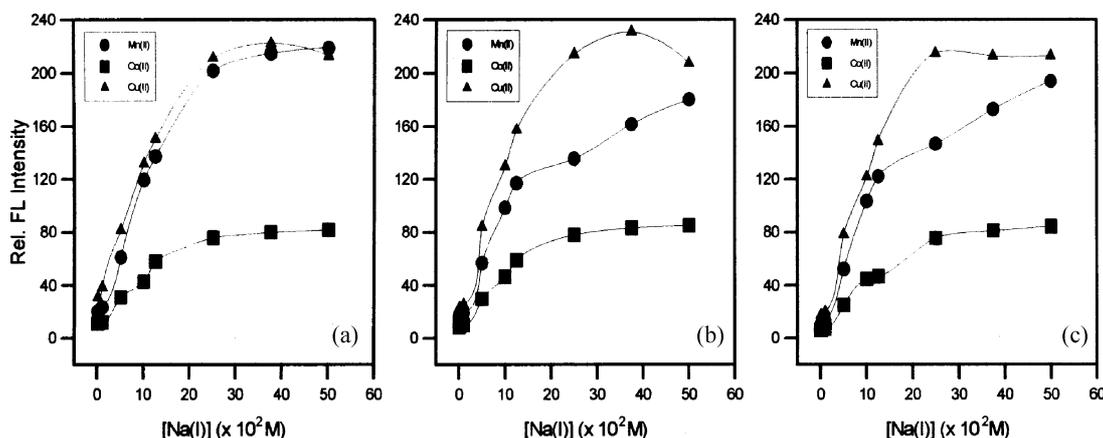
Figure 6. Enhanced fluorescence intensities in quenched anthrylazacrown ethers by Mn(II), Co(II) and Cu(II) with the concentration of added sodium nitrate; (a) Anthryl DA15C5, (b) Anthryl MA18C6, (c) Anthryl DA18C6. All solution were mixed with [anthrylazacrown ethers]=1.0×10^{-7} M and [M]=5.0×10^{-4} M. (M=Mn(II), Co(II), Cu(II)). Excitation was at 254 nm and emission maximum centered near 412 nm.

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