A Significant Fluorescence Quenching of Anthrylaminobenzocrown Ethers by Paramagnetic Metal Cations

Jeong Ho Chang,[†] Young Muk Choi, and Young-Kook Shin*

Department of Chemistry, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea
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Molecular systems that combine binding ability and photophysical properties are of great interest for designing new chemosensors and molecular devices. The process involving energy transfer in the separated large molecules is of current interest.[1] The methods applied to such studies involve the fluorescence quenching, excimer or exciplex emission, and charge transfer (CT) or photoinduced electron transfer (PET) complex emission. It has been well established that energy transfer through the fluorescence quenching proceeds via the formation of an exciplex with CT or PET character.[2]

The flow of charge or electron, either from the fluorophore to the quencher or from the quencher to the fluorophore depends upon the oxidation and reduction potential of respective species. The most commonly employed fluorescent chemosensor is one in which a fluorophore and a receptor are connected through a spacer unit. The interaction between the fluorophore and the receptor, most often through photoinduced electron transfer, leads to quenching of the fluorescence.

In the presence of the guest molecules to be sensed, the fluorophore-receptor interaction is turned off due to the binding of the guest (molecules) at the receptor site and it leads to chelation enhanced fluorescent quenching (CHEQ). In using the fluorescent chemosensor, which is consisted of 'fluorophore-spacer-receptor', one needs to suppress the interaction between the fluorophore and the quenching metal ions so as to observe fluorescence changes on the metal ion binding. Czarnik,[3] and de Silva[4] successfully developed sensor systems to show chelation enhanced fluorescence (CHEF) and CHEQ in the presence of metal ions using an anthryl-polyaza receptor that complexes the metal ions in its cavity, leading to the suppression of the quenching interaction of the metal ions with the fluorophore.

We have recently developed three PET sensors for the quenching metal ions such as Mn^{2+}, Co^{2+}, and Cu^{2+} by aza-crown ethers attached to 9-chloromethylanthracene.[5]

Although these fluoroscecent chemosensors have a structurally simple spacer, methylene group, there are a lot of advantages about how metal ion complexed azacrown ether to affect the emission changes of covalently attached fluorophores. We report here fluorescent quenching for paramagnetic metal ions on the sensor compounds which contain
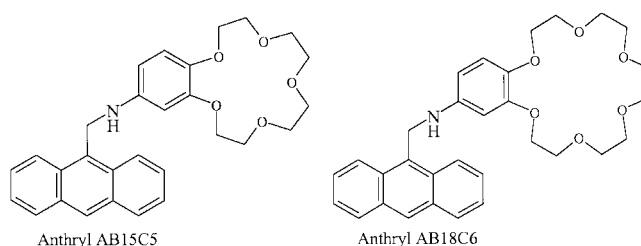
**Figure 1.** Structures of the synthesized anthrylaminobenzocrown ethers.

methylaminobenzo group as a functional spacer (Figure 1). In general, benzylic nitrogen shows a fluorescence quenching because of the interaction between the lone-pair electron of benzylic nitrogen and the fluorophore π system. Two PET sensors were designed as a model of a 'fluorophore-spacer (methylaminobenzene)-receptor'. The synthesis, the quenching constants obtained by Stern-Volmer equation, the photophysical study in the absence, and the presence of protons, as well as of light- and heavy metal ions for two PET sensors designed as a 'fluorophore-spacer-receptor' are described.

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 Germi-300 (300 MHz) spectrometer. Chemical shifts were reported in ppm downfield from TMS. Infrared and UV-Vis spectra were obtained on a Jasco IR-E300 and a Jasco V-500 spectrophotometer. Melting points were determined on a Connecting & Maintanance melting point apparatus and were not calibrated. Column chromatography was carried out on a column packed with Silica Gel 60 (70-230 mesh, Merck).

Materials. Benzo-18-crown ether, 9-chloromethylanthracene, cathecol, and metal salts were purchased from Aldrich, Fluka, and Junsei and were used without further purification. Benzo-15-crown ether, nitrobenzocrown ethers, aminobenzocrown ethers and anthrylaminobenzocrown ethers were synthesized using the previously reported methods.[6,7] The organic solvents were purified by distillation over dehydrating reagents just before use. The water was deionized and distilled in glass, and also all the sample solutions were
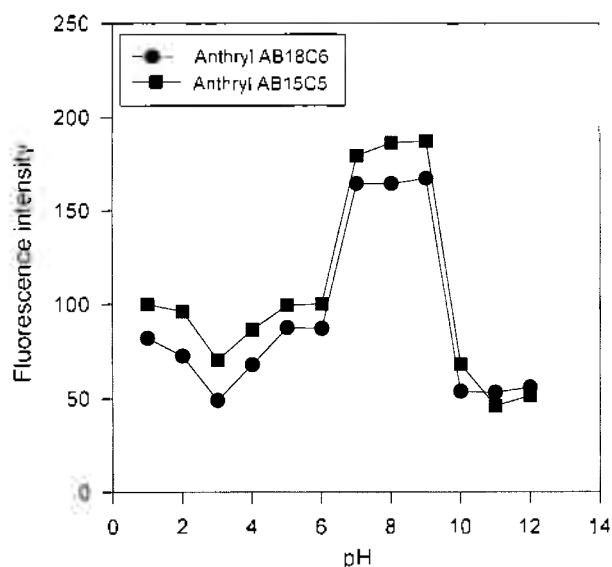
Figure 2. pH-fluorescence profiles for 0.1 μM solutions of anthryl-aminobenzo crown ethers. Excitation was at 254 nm; Emission was measured at the emission maximum centered near 412 nm. pHs were maintained using the following solutions; 0.2 M HCl (pH 1 and 2), 0.2 M Sodium acetate (pH 3, 4, 5 and 6), 0.2 M Tris (pH 7, 8 and 9), 0.2 M NaHCO₃ (pH 10 and 11), 0.2 M NaOH (pH 12).

degassed by sonication under reduced pressure.[5]

9-(4'-Aminobenzo-15-crown-5)methylanthracene (Anthryl AB15C5). A solution of 0.85 g (3 mmol) of 4'-aminobenzo-15-crown ether in benzene (100 mL) was slowly added to a stirred solution of 9-chloromethyl anthracene (0.75 g, 3.32 mmol) in benzene (100 mL). The mixture was heated at reflux for 30 hr. The reaction mixture was cooled, filtered, and extracted with water. The organic layer was separated and concentrated *in vacuo*. Column chromatography (EtOAc/hexane) gave 85% of the title compound as a yellow solid; m.p 152-156°C; ^{1}H NMR (CDCl₃) δ 1.5 (s, NH), 3.8-4.1 (m, OCH₂), 5.1 (s, ArCH₂), 6.5-8.5 (m, ArCH); IR (KBr, cm^{-1}) 3400, 3050, 2860, 1560, 1500, 1450, 1240, 1120; Mass base peak m/z 191 (relative intensity 100), parent peak m/z 473 (relative intensity 30), fragment peak m/z 150 (relative intensity 10)

9-(4'-Aminobenzo-18-crown-6)methylanthracene (Anthryl AB18C6). The same synthetic procedure was used as above. Column chromatography gave 63% of the title compound as a yellow solid; m.p 130-132°C; ^{1}H NMR (CDCl₃) δ 1.9 (s, NH), 3.7-4.1 (m, OCH₂), 5.1 (s, Ar CH₂), 6.3-8.5 (m, ArCH); IR (KBr, cm^{-1}) 3400, 3052, 2880, 1580, 1460, 1440, 1260, 1120; Mass base peak m/z 191(relative intensity 100), parent peak m/z 517 (relative intensity 20), fragment peak m/z 150(relative intensity 10)

Results and Discussion

The absorption and fluorescence spectra were measured for two anthrylamino-benzocrown ethers and 9-chloromethyl-anthracene in methanol at 5×10^{-7} M, excited by 254 nm. All three solutions show a maximum absorption at 254 nm.

and a maximum emission at 412 nm. The fluorescence spectra show a fluorescence quenching in order of 9-chloro-methylanthracence > Anthryl AB15C5 > Anthryl AB18C6 (relative fluorescence quantum yields 0.33 > 0.26 > 0.22 using anthracene ($\Phi_F = 0.3$) as a reference). The fluorescence quenching efficiency of the anthrylaminobenzo crown ethers is dependent on the ring size and the number of donor atom. The results indicate that the fluorescence of fluorophore is quenched by the nonbonding electrons of benzylic nitrogen and oxygen atoms participating in nonradiative quenching such as intersystem crossing.[8]

Figure 2 shows the fluorescence intensity of two anthryl-aminobenzocrown ethers as a function of pH at 0.1 μM, showing a maximum at pH 7-9 in Tris-buffer solution. The
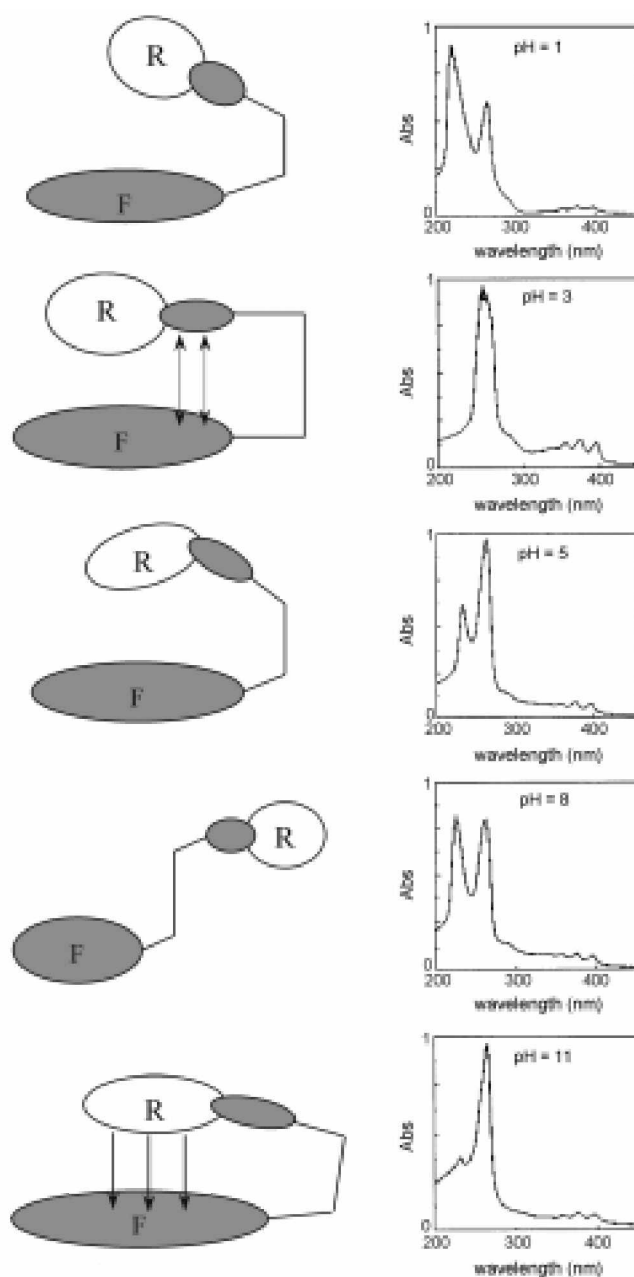
Figure 3. Conformational changes corresponding to UV-Vis spectrum in each pH (R: receptor and F: flurophore).

Notes

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excitation wavelength was 254 nm. The pH dependence of fluorescence quenching can be explained by the intra-molecular amine quenching mechanism that has been previously described.[3a] Protonation of an amine group in a spacer unit results in the elimination of photoinduced electron transfer. Fabbrizzi *et al.*[9] explained the pH effect on the fluorescence of anthrylpolyamine using this mechanism. The fact that the two anthrylaminobenzocrown ethers show same maximum intensity at the same pH would be a major reason, indicating the protonated form on the benzylic nitrogen accounts for a majority of the observed intramolecular quenching, which is consistent with the fluorescence quenching results.[10] This means that an electron transfer process from the excited anthracene moiety to the partially positive-charged amine takes place, thus competing with radiative deactivation and partially quenching fluorescence. Also, fluorescence quenching occurs from the electron-rich benzylic amine moiety in a spacer unit to a π-orbital of the photoinduced anthracene fragment, and then this quenching is known by the interaction between the lone pair electron and π-orbital of fluorophore. Figure 3 shows the schematic diagrams and the UV-Vis spectrum as a function of pH. From the corresponding fluorescence intensity in each pH range, we can infer the conformational change of ligand. In case of pH 8, where shows the highest fluorescence intensity, the two fluorophores - anthracene and benzene moiety - exist as same amount, and this shows a maximum fluorescence intensity because of no π-π* interaction.[2] On going to lower pH range (1 < pH < 5), species containing also one protonated fluorophore

begin to form. It seems that one species exist and shows a minimum fluorescence intensity owing to π-π* interaction between two fluorophores as a 'sandwitch type' in pH 3. At higher pH (pH > 10), the lone pair electrons-π interaction between crown ether and anthracene may bring a result in quenching attributed to deprotonation in pH 11. The π-π* interaction as well as lone pair electrons are known for major quenching factors. However the difference of quenching effect for the two systems at higher pH (pH > 10) could be neglected.

In the presence of metal nitrates, the fluorescence intensity of the anthrylamino-benzocrown ethers was measured at pH 9.5. Table 1 shows the fluorescence intensity at 0.1, 1, 10, 1000 μM, and only four paramagnetic metal ions, $Mn^{2+}(d^5)$, $Fe^{3+}(d^5)$, $Co^{2+}(d^7)$, and $Cu^{2+}(d^9)$ quench the fluorescence drastically at high concentration (Q > 100-1000 μM). The results show significant quenching in the presence of the paramagnetic metal ions, which is same as the previous report.[5]

Figure 4(a) shows the decrease of fluorescence intensity by the incremental addition of metal ions. Mn^{2+}, Fe^{3+}, and Cu^{2+} are weaker quencher than Co^{2+} because of stability of

Table 1. Relative fluorescence intensities of Anthrylaminobenzocrown ethers in the presence of the various metal nitrates*
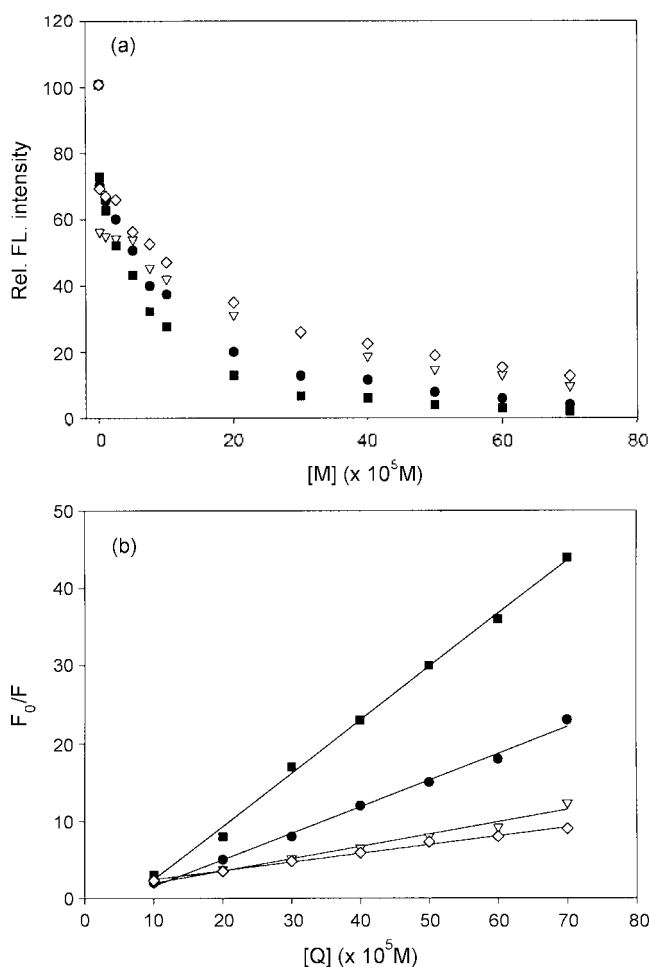
	Anthryl AB15C5				Anthryl AB18C6			
	0.1 μM	1 μM	10 μM	1000 μM	0.1 μM	1 μM	10 μM	1000 μM
Free	98.8				91.4			
Na(I)	62.6	62.4	62.2	62.1	52.6	53.1	53.2	53.0
K(I)	65.5	62.7	61.3	61.5	55.5	56.2	56.4	52.2
Rb(I)	61.7	61.7	61.5	62.0	55.3	55.5	55.8	53.0
Cs(I)	61.7	60.5	59.5	59.7	54.0	53.3	53.6	51.6
Mg(II)	62.3	60.2	60.2	61.3	53.6	55.8	56.4	54.7
Ca(II)	61.7	61.1	62.6	61.7	55.0	54.8	54.0	54.2
Sr(II)	64.0	64.0	64.2	63.8	56.6	55.5	60.2	58.8
Ba(II)	63.2	66.0	69.9	68.5	56.2	57.1	57.1	56.9
Cr(II)	54.1	54.6	54.2	54.3	55.4	55.6	51.3	50.6
Mn(II)	68.8	67.2	64.3	0.84	63.4	63.0	57.1	0.86
Fe(III)	53.9	51.5	51.5	6.2	54.9	55.5	49.6	7.3
Co(II)	66.7	68.6	60.0	0.28	62.1	62.7	55.5	0.34
Ni(II)	50.1	50.1	48.0	42.8	55.4	49.9	52.1	41.8
Cu(II)	68.2	71.1	65.5	6.30	60.8	63.5	58.1	5.60
Zn(II)	66.9	71.5	67.3	64.2	60.3	62.8	59.7	56.6
Ag(I)	70.6	68.5	68.3	66.2	63.7	61.3	63.3	59.5
Cd(II)	72.1	69.3	72.6	73.1	63.4	62.3	64.3	62.5

*All solutions were buffered with 0.2M Tris buffer at pH 9.5 and were 0.1 μM of anthrylaminobenzocrown ethers. Excitation was at 254 nm; Emission was measured at the emission maximum centered near 412 nm.

Figure 4. (a) Quenched Fluorescence intensity and (b) Stern-Volmer plot in Anthryl AB15C5 with concentration of metal ions. (■ : Co^{2+}, ● : Mn^{2+}, ▽ : Fe^{3+}, and ◇ : Cu^{2+}).

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

	Anthryl AB15C5				Anthryl AB18C6		
	Mn(II)	Fe(III)	Co(II)	Cu(II)	Mn(II)	Fe(III)	Co(II)	Cu(II)
log K_Q	4.41	4.10	4.65	4.03	4.42	4.07	4.67	4.06

the half-filled shell of d^5-metal ions (Mn^{2+}, Fe^{3+}) (Table 2) and the existence of only one unpaired electrons of Cu^{2+}.[11] Figure 4(b) shows that the Stern-Volmer plot for all four ions and the anthrylaminobenzocrown ethers agrees nicely to the linear portion of the plot of F_0/F versus [Q], indicating that fluorescence quenching is dynamic in nature in the linear part. In order to elucidate the static or dynamic quenching without measurement of fluorescence lifetime,[12] the absorption spectra were measured carefully to distinguish static and dynamic quenching. Dynamic quenching only affects the excited states of the fluorophores, and thus no change in the absorption spectra is predicted. It was found that the measured spectra were not influenced by the metal ions in the presence of metal ions.

In conclusion, we have demonstrated that two PET sensors designed show a CHEQ by paramagnetic metal ions such as $Mn^{2+}(d^5)$, $Fe^{3+}(d^5)$, $Co^{2+}(d^7)$, and $Cu^{2+}(d^9)$. This phenomenon could be attributed to the fact that the four paramagnetic metal ions have propensity to deactivate the excited state by nonradiative quenching process. From the obtained linear Stern-Volmer plot and the unchanged absorption spectrum in the presence of metal ions, it was inferred that CHEQ mechanism is the dynamic (collision) quenching process.

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