Cation-Responsive Fluorescence of Dansyl-Labeled Polyanions Bearing Crown Ether Units

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Anionic polymers bearing both crown ether units and Dansyl units were prepared by radical terpolymerization of the corresponding monomers, and the effect of cations on the fluorescence properties of the polymers was studied in aqueous media. The fluorescence peaks of the polymers are observed at 500–527 nm. The fluorescence quantum yields (Φ) of the polymers were found to be 6.3×10^{-3}–4.0×10^{-1}, depending on the polymer structure. On the addition of alkali metal cations, the fluorescence intensity increases in the order LiCl < NaCl < KCl < RbCl < CsCl. The polymer which has almost-equimolar amounts of crown ether groups and –COOH groups shows the highest responsiveness to the cations added. The structure of the anionic moieties in polymers strongly affects the cation-responsive fluorescence. The mechanism for the cation-induced changes in fluorescence is discussed.

INTRODUCTION

Molecular recognition is a subject of interest because of its applications to many fields: biology, medicine, environment, and so on. In particular, the detection of metal cations such as sodium, potassium, calcium, and magnesium cations involved in biological process has received considerable attention. Among numerous methods employed, fluorescent sensors display distinct advantages in terms of sensitivity and specificity.[1]

Crown ethers are known to bind alkali and alkaline earth metal cations in their cavities.[2] Crown ethers,[3–7] mono-aza-crown ethers,[8] cryptands,[9,10] and podands[11] holding fluorescent moieties such as naphthalene, anthracene, and benzoxazine have been synthesized and the effects of cation complexation on the fluorescence and/or phosphorescence properties of the ionophores

have been studied. Recently we have reported the effect of cations on the fluorescence of the polyelectrolytes bearing crowned fluorophore units.[12–15] The cation binding ability of crown ethers is governed primarily by the relationship between cation diameter and crown ether hole size. Although the cation binding ability of crown ethers is high in organic solvents, it is very low in aqueous media because of the strong hydration of cations. The observed binding ability of crown ethers to cations in aqueous media can be strongly enhanced by anchoring the crown ether units into the backbone of polyanions, because the anionic groups such as –COO$^-$ in the polymer chain increase the concentration of cations close to the polymer-bound crown ether groups.

In this paper we report a system showing the cation-responsive fluorescence using Dansyl-labeled polyanions bearing crown ether units. The excited-state chemistry of Dansyl derivatives has been well characterized.[16] The fluorescence maximum of these derivatives exhibits a large solvatochromic shift and is relatively insensitive to oxygen.[17] The concept of the present system is as follows: the fluorescence intensity

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Table I. Polymerization Conditions[a]

Polymer	Monomer (mmol)						DMF[h] (ml)	Polymerization time (h)	Yield (wt%)
	VBCR[b]	MA[c]	AA[d]	IA[e]	DMA[f]	AMPS[g]			
1	—	8.25	—	—	0.25	—	1.0	5.0	56.0
2a	1.24	8.25	—	—	0.17	—	1.7	10.5	46.6
2b	2.95	2.96	—	—	0.17	—	0.5	5.5	24.8
2c	2.19	1.26	—	—	0.10	—	0.5	8.0	20.7
2d	2.09	0.59	—	—	0.08	—	0.4	7.0	29.7
2e	3.35	0.59	—	—	0.11	—	0.5	6.8	27.9
3	2.80	—	2.79	—	0.16	—	1.6	5.0	57.5
4	2.09	—	—	—	0.13	0.92	4.0	10.0	50.0
5	2.07	—	—	2.07	0.12	—	1.0	10.0	25.0

[a] Concentration of AIBN used as initiator was 0.3 mol% to total monomer.
[b] 4'-Vinylbenzo-18-crown-6.
[c] Methacrylic acid.
[d] Acrylic acid.
[e] Itaconic acid.
[f] N-[2-[[[5-(Dimethylamino)-1-naphthyl]sulfonyl]amino]ethyl]-2-methyl-2-propenamide.
[g] 2-Acrylamido-2-methyl-1-propanesulfonic acid. It was used as a lithium salt.
[h] N, N-Dimethylformamide.

of Dansyl units can be altered by a polarity change of the medium. Interactions between anionic groups such as $-COO^-$ and crown ether units complexed with cations in water can induce the contraction of the polymer chain in aqueous media, resulting in a change in the polarity around the Dansyl units. In the present system the fluorescence of the Dansyl units is cation sensitive because the crown ether units show selectivity in cation binding.

EXPERIMENTAL

Materials

N-[2-[[[5-(Dimethylamino)-1-naphthalenyl]sulfonyl] amino]ethyl]-2-methyl-2-propenamide (Dansyl ethyl methacrylamide) (DMA) was prepared from methacryloyl chloride and N-(2-aminoethyl)-5-(dimethylamino)-1-naphthalenesulfonamide (ADMNS) according to the literature.[18] 4'-Vinylbenzo-18-crown-6 (VBCR) was synthesized according to the method already reported.[19] Acrylic acid (AA) and methacrylic acid (MA) were distilled prior to use. Itaconic acid (IA) was recrystallized from ethanol before use. 2-Acrylamido-2-methyl-1-propanesulfonic acid (AMPS) and alkali metal chlorides were of reagent grade and used as received.

Polymerization

Polymers were prepared via radical terpolymerization or copolymerization of the corresponding monomers
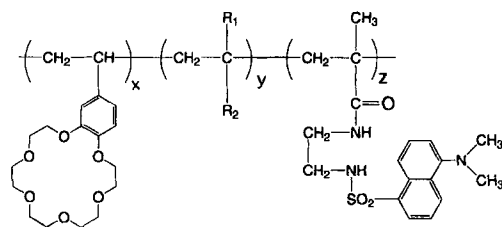
Polymer	R₁	R₂	x	y	z
1	CH₃	COOH	0.000	0.984	0.016
2a	CH₃	COOH	0.307	0.684	0.009
2b	CH₃	COOH	0.494	0.497	0.009
2c	CH₃	COOH	0.596	0.395	0.009
2d	CH₃	COOH	0.683	0.307	0.010
2e	CH₃	COOH	0.846	0.142	0.012
3	H	COOH	0.494	0.487	0.019
4	H	CONH-C-CH₂-SO₃H (CH₃)₂	0.414	0.576	0.009
5	CH₂COOH	COOH	0.450	0.536	0.014

Scheme I

using 2,2'-azobisisobutyronitrile (AIBN) as initiator. Polymerization was carried out at 55°C. Detailed polymerization conditions are given in Table I. Polymers 2a–e, 3, and 5 were purified by dissolving in 1,4-dioxane and precipitating with diethyl ether (Scheme I). Polymer 4 was purified by dissolving in a 1,4-dioxane-methanol mixture (1/9; v/v) and precipitating with diethyl ether. Polymer 1 was purified by dissolving in methanol and precipitating with diethyl ether.

Table II. Spectral Data of Model Compounds

Model compound	Solvent	ε (L/mol · cm)	
		λ = 280 nm	λ = 335 nm
PVBCR[a]	1,4-Dioxane	2765	0
PVBCR[a]	1,4-Dioxane/methanol = 1/1 (v/v)	2621	0
PVBCR[a]	Water/methanol = 3/1 (v/v)	2811	0
ADMNS[b]	1,4-Dioxane	1932	5022
ADMNS[b]	1,4-Dioxane/methanol = 1/1 (v/v)	1672	4540
ADMNS[b]	Water/methanol = 3/1 (v/v)	1679	4211
ADMNS[b]	Methanol	—[c]	3693

[a] Poly(4'-vinylbenzo-18-crown-6).
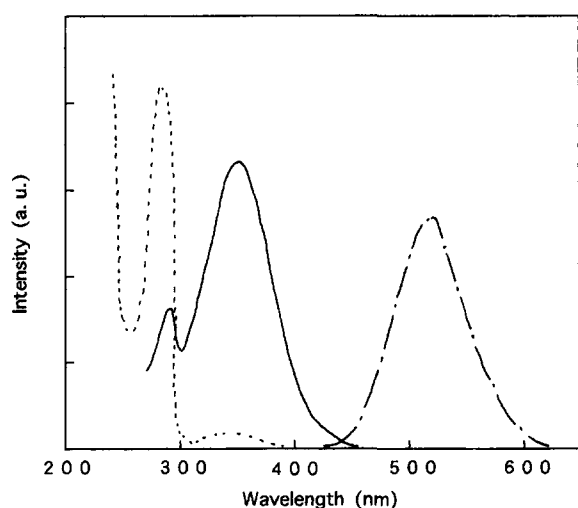[b] N-(2-Aminoethyl)-5-(dimethylamino)-1-naphthalenesulfonamide.
[c] Not measured.

Fig. 1. Absorption (- - - - - - -), fluorescence (— · —), and fluorescence excitation (——) spectra of 2b in water at 30°C. [Crown ether units] = 3.76 × 10⁻⁴M; [Dansyl units] = 6.85 × 10⁻⁶M.

The contents of crown ether units and Dansyl units in the polymers were determined by measuring the absorbance at both 335 and 280 nm. The molar extinction coefficients of the crown ether units and Dansyl units in the polymers were estimated to be equal to those of poly (4'-vinylbenzo-18-crown-6) (PVBCR) and N-(2-aminoethyl)-5-(dimethylamino)-1-naphthalenesulfonamide (ADMNS), respectively. Several types of solvents were used to make polymer solutions as follows: methanol for 1, 1,4-dioxane for 2a–e and 3, water–methanol (3/1; v/v) for 4, and a 1,4-dioxane–methanol mixture (1/1; v/v) for 5. The molar extinction coefficients of the model compounds in these solvents are listed in Table II.

Measurements

All polymers were used as lithium salts for the fluorescence measurements in water at 30°C using a Shi-

madzu RF-500 spectrofluorophotometer. Slit widths of excitation and emission were 10 and 5 nm, respectively. The wavelength of the excitation light was usually 350 nm. The absorbances of the sample solutions were usually below 0.1 at 350 nm. The quantum yields (Φ) of the fluorescence were determined by comparison of the fluorescence intensities of the polymers with the fluorescence intensity of quinine sulfate.[20]

RESULTS AND DISCUSSION

Absorption and Fluorescence Spectra of Polymers

The lithium salts of the polymers showed an absorption maximum at 280 nm and a shoulder peak at 335 nm in aqueous solutions, except polymer 1, which showed an absorption maximum at 245 nm and a shoulder peak at 335 nm. The absorption peaks of the polymers did not change on the addition of alkali metal chlorides. On excitation with 350-nm light, 2b showed fluorescence at 519 nm in water at 30°C as shown in Fig. 1. When the emission of 2b at 519 nm was monitored, the λ_{max} of the excitation spectrum was observed at 350 nm, suggesting that the fluorescence occurred from Dansyl units in the polymer. As shown in Table III the fluorescence maximum peaks of the polymers were observed at 500–527 nm, depending on the structure. The fluorescence quantum yields (Φ) were observed in the range of 6.3 × 10⁻³ to 4.0 × 10⁻¹, depending on the polymer structure. For the series of polymers 2a–e, the fluorescence peak shifted to shorter wavelengths as the fraction of crown ether units in the polymers increased. The shorter the wavelength of the fluorescence peak, the higher the Φ values. The excited-state chemistry of Dansyl derivatives has been well characterized.[21,22] The fluorescence maximum of these

Table III. Fluorescence Quantum Yields (Φ) and Emission Peaks (λ_{max}) of the Polymers in Water[a]

Polymer	Φ	λ_{max} (nm)
1	6.3×10^{-3}	527
2a	3.0×10^{-2}	521
2b	6.1×10^{-2}	519
2c	7.5×10^{-2}	516
2d	1.2×10^{-1}	513
2e	1.9×10^{-1}	510
3	7.5×10^{-2}	517
4	4.0×10^{-1}	500
5	4.7×10^{-2}	520

[a] [Dansyl units] $= 5.7 \times 10^{-6} - 1.55 \times 10^{-5}$ M.
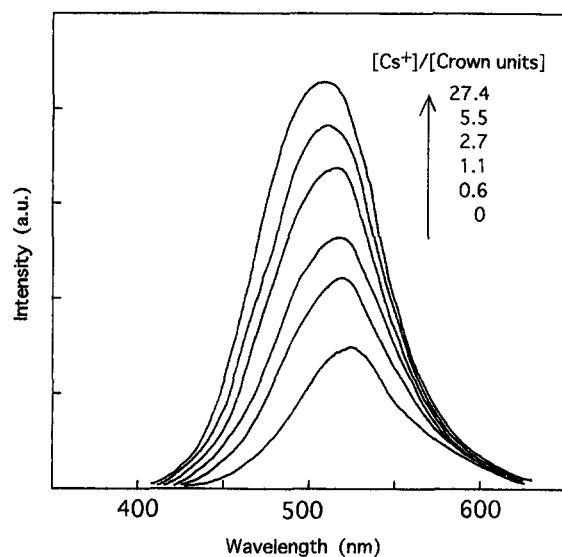
Fig. 2. Changes in fluorescence spectra of **2b** on the addition of CsCl. [Crown ether units] $= 4.52 \times 10^{-4} M$; [Dansyl units] $= 8.22 \times 10^{-6}$ M.

Fig. 3. Changes in the fluorescence intensity of **2b** on the addition of alkali metal chlorides. [Crown ether units] $= 3.76 \times 10^{-4}M$; [Dansyl units] $= 6.85 \times 10^{-6}M$. Salt added: ($\square$) LiCl; ($\bigcirc$) NaCl; ($\triangle$) KCl; ($\bullet$) RbCl; ($\blacksquare$) CsCl.

derivatives exhibits a large solvatochromic shift. The emission maximum shows a red shift with increasing polarity of the environment where Dansyl units are present. The Dansyl units in **1**, which does not include crown ether units, are suggested to be in the most polar environment among the polymers studied here. As observed for the fluorescence λ_{max} of **2a–e**, the introduction of crown ether units into the polymer probably decreases the polarity of the environment of the Dansyl units.

Effect of Cations on the Fluorescence

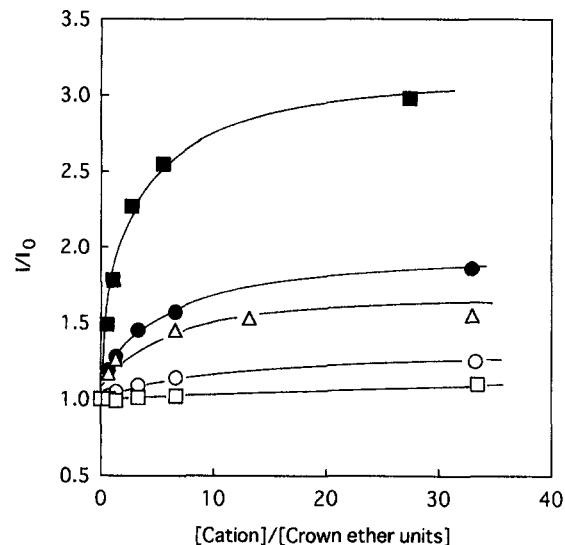The change in the fluorescence spectra of **2b** on the addition of CsCl is shown in Fig. 2. The fluorescence

intensity simply increased with increasing CsCl concentration along with the blue shift of the fluorescence peak, suggesting that the environment of the Dansyl units changed from polar to less polar. A similar change in the fluorescence spectrum was observed when alkali metal salts other than CsCl were added. Since the formation of intramolecular ion complexes between $-COO^-$ groups and cation-complexed crown ether units induces the contraction of the polymer coils, the Dansyl units which exist in the water phase can be incorporated into the less polar environment in coils. Figure 3 shows the I/I_0 ratio for **2b** on the addition of alkali metal chlorides, where I and I_0 are the fluorescence intensities in the presence and absence of salts, respectively. The I/I_0 values increased with the salts added. When compared at the same cation/crown ether unit ratio, the I/I_0 values decreased in the order $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$. This order was consistent with the order of the cation binding ability of the polymers bearing benzo-18-crown-6 moieties.[19] The formation of crown ether moiety–cation 2:1 complexes may play a part in the binding of large cations such as Cs^+ and Rb^+.[19] The fluorescence intensity of **2b** in the presence of CsCl ([CsCl]/[crown units] $= 28$) was three times higher than that in the absence of CsCl. Precipitations of the polymers were observed at [cation]/[crown ether units] ratios higher than 35. The increase in I/I_0 was very small on the addition of LiCl, which is negligibly bound to the crown ether units in the polymer. This also means that the in-
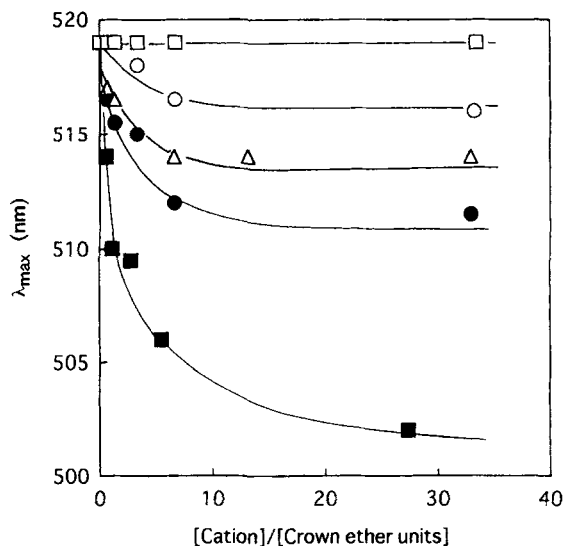
Fig. 4. Changes in the λ_{max} of the fluorescence spectra of 2b on the addition of alkali metal chlorides. [Crown ether units] = 3.76 × 10^{-4} M; [Dansyl units] = 6.85 × 10^{-6} M. Salt added: (□) LiCl; (○) NaCl; (△) KCl; (●) RbCl; (■) CsCl.
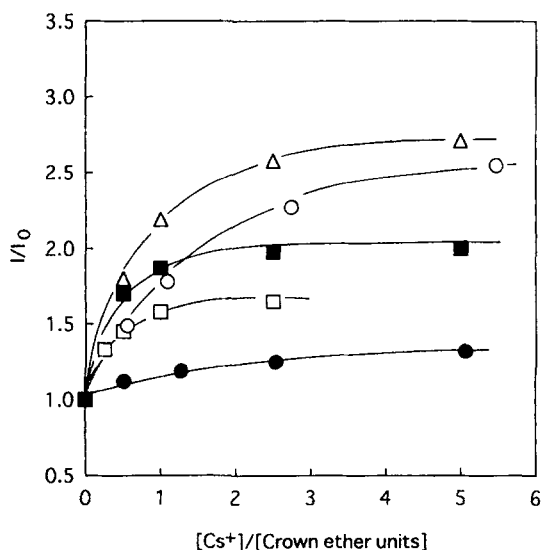

Fig. 5. Effect of the methacrylic acid unit fraction in polymers on the fluorescence intensity on the addition of CsCl. [Crown ether units] = 3.76 × 10^{-4}–4.52 × 10^{-4} M. Polymer: (●) 2a; (○) 2b; (△) 2c; (■) 2d; (□) 2e.

Fig. 6. Changes in the fluorescence intensity of 2b (○) and 1 (□) on the addition of CsCl. [–COO^{-}] = 3.76 × 10^{-4} M.

crease in the ionic strength of the aqueous solution did not cause the enhancement of the fluorescence.

As shown in Fig. 4, the λ_{max} of the fluorescence spectra of 2b shifted to shorter wavelengths on the addition of salts, and the magnitude of the shift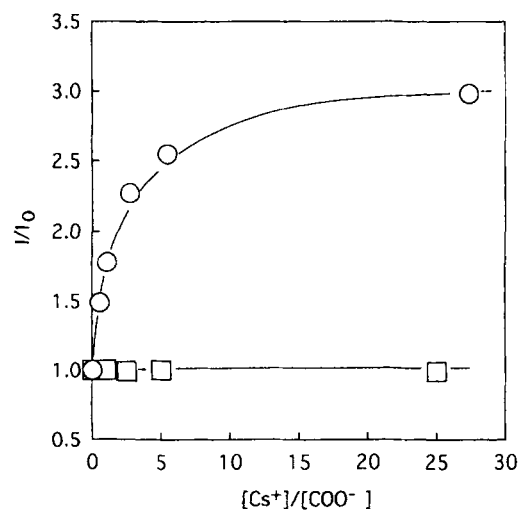 was dependent on the kind of cation added. When compared at the same cation/crown ether unit ratio, the magnitude of the blue shift of emission λ_{max} increased in the order Li^{+} < Na^{+} < K^{+} < Rb^{+} < Cs^{+}. This shows that the cations which are strongly bound to the crown ether units in the polymer induced a larger blue shift of the λ_{max}, suggesting that the environment of the Dansyl units changed from polar media to less polar media. The contraction of the polymer coil by the formation of intramolecular interaction between –COO^{-} and cation-complexed crown ether units can change the polarity around Dansyl units from polar to less polar. It was reported that the intermolecular polyion complexes between anionic polymers such as poly(acrylic acid) and polymeric crown ethers were formed and precipitated when the crown ether units were complexed with alkali metal cations.[23-25]

On the addition of CsCl, the effect of crown ether unit fraction in the polymers on the I/I_0 values was investigated (Fig. 5). The fractions of Dansyl units were 0.9–1.6 mol%. The I/I_0 values increased with increasing [Cs^{+}]/[crown units] ratios and saturation was observed at high [Cs+]/[crown unit] ratios. The polymer that has almost-equimolar fractions of crown ether and –COOH groups showed the most efficient responsiveness to Cs^{+}. The same phenomenon was observed for K^{+}. It was reported that the most effective formation of intermolecular polyion complexes between polyanions and cation-complexed polymeric crown ethers was observed when equimolar amounts of these polymers were mixed.[23,24]

Changes in the fluorescence intensity of 2b and 1 on the addition of CsCl are shown in Fig. 6. Although the I/I_0 value for 2b increased with increasing Cs^{+}/COO^{-} ratios, the I/I_0 value for 1, which has no crown ether
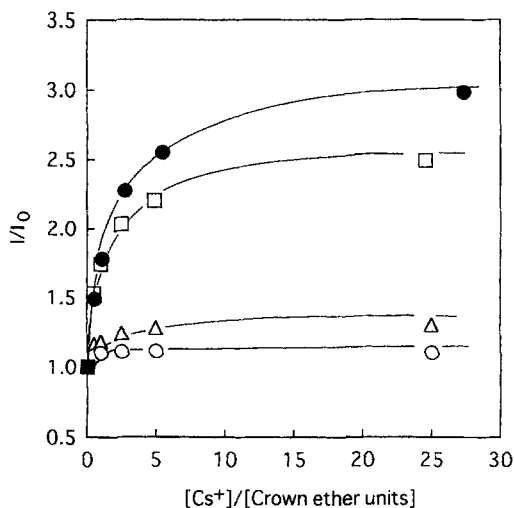
Fig. 7. Effect of the polymer structure on the fluorescence intensity on the addition of CsCl. [Crown ether units] = 3.76 × 10⁻⁴–3.83 × 10⁻⁴ M. Polymer: (●) 2b; (□) 3; (○) 4; (△) 5.

units, did not change on the addition of CsCl. This means that the crown ether units incorporated into the polymer chain are indispensable for the cation-responsive fluorescence change.

Figure 7 shows the effect of the structure of the anionic moiety on the fluorescence intensity on the addition of CsCl. The crown ether unit fractions of the polymers were 0.494–0.414. The fluorescence responsiveness of the polymers to Cs^+ increased in the order 4 < 5 < 3 < 2b. Polymers 2b and 3 showed a strong fluorescence change responding to Cs^+. As shown in Table III the fluorescence λ_{max} for 4 was observed at 500 nm. This means that Dansyl units in 4 already existed in a less polar environment compared to the other polymers studied. Polymer 5 showed poor responsiveness to cations compared to 2b. This may be due to the low ratio of crown ether units to –COO– units in the polymer because itaconic acid is a dibasic acid.

CONCLUSION

Water-soluble polymers bearing anionic groups, crown ether units, and Dansyl units were prepared by the terpolymerization of the corresponding monomers. When alkali metal cations were added to the dilute solution of the polymers, the fluorescence intensity increased along with the blue shift of the fluorescence

peak. The increase in the fluorescence intensity corresponded to the cation binding ability of polymeric crown ethers. The most efficient cation-responsive fluorescence was observed for the polymer bearing almost-equimolar fractions of crown ether units and anionic units. The cation-responsive fluorescence was affected by the structure of the anionic moiety of the polymers.

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