Fluorescence study of polyelectrolyte complex formation: 2. Effect of acidity of polyanions on complexation

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Poly(sodium 2-acrylamido-2-methylpropanesulphonate) and poly(sodium *p*-styrenesulphonate) containing a small amount of anthryl groups (PAMPS-A and PSS-A, respectively) were prepared. Formation of polyelectrolyte complexes (PEC) of these polyanions with a series of polycations in aqueous solution was investigated by fluorescence techniques. The result was compared with that for the PEC of anthracene-loaded poly(methacrylic acid) (PMA-A). The fluorescence intensity of PAMPS-A sharply increased when an equimolar amount of polycations were added, suggesting the formation of 'stoichiometric' PECs. The fluorescence lifetimes also increased with complexation. The fluorescence spectra of the PECs with the polycations containing phenyl groups showed broad emission, whereas the PECs without phenyl groups sharpened their spectra. These observations allowed estimation of the anthryl chromophore location in the PEC microdomains.

(Keywords: fluorescence; anthracene; polyanion)

INTRODUCTION

Luminescent probes covalently linked to polyelectrolytes are particularly attractive because they provide useful information about polymer structures in solutions. Among these probes, polycyclic aromatic hydrocarbons such as naphthalene and pyrene have been widely used as fluorescent probes to investigate associations of water-soluble polymers[1–16]. It has been demonstrated that various photophysical parameters obtained from fluorescence spectra, fluorescence lifetime, fluorescence quenching, and efficiency of non-radiative energy transfer for the pyrene chromophore reflect intra- and inter-polymer interactions[1–10]. Naphthalene[11–13], phenanthrene[14] and anthracene[15,16] have been also linked to polyelectrolytes.

We have been investigating the properties and applications of polyelectrolyte complexes (PEC), one interesting class of polymer associates, which are prepared by mixing oppositely charged polyelectrolytes[17,18]. Physical and chemical properties of these PECs consisting of hydrophilic and hydrophobic microdomains depend on many factors, e.g. charge density, properties of ionic groups (acidity, basicity, etc.) and contributions of secondary binding forces such as hydrogen bonding, van der Waals' force and hydrophobic interaction[17,18]. Because of these diversities, detailed investigations of the microdomains formed by PECs have not yet been done. However, applications of PECs have been rapidly advanced in many research fields, e.g. membranes, colloidal titration, biomedical materials, solid electrolytes, etc.[17,18].

One of our primary interests is to probe the microdomains formed by PECs in aqueous solution. In our preceding paper[19], we reported on the PEC formation of poly(methacrylic acid) containing a small amount of anthryl groups (PMA-A) with several types of polycations. It was demonstrated that the fluorescence intensity of anthracene (Anth) was very sensitive not only to PEC formation, but also to the hydrophobicity of PEC microdomains. In the present article, the fluorescence studies on PEC formation are described in more detail. The photophysical properties of the poly(sulphonate)s, poly(sodium 2-acrylamido-2-methylpropanesulphonate) and poly(sodium styrenesulphonate), containing anthryl probes (PAMPS-A and PSS-A, respectively), and their PEC formation with polycations have been investigated. The results were compared with those for the PMA-A system. It was found that there was a specific interaction between the Anth chromophore in polyanions and the aromatic groups in polycations, which enabled the estimation of the location of the Anth moieties in the PEC microdomains.

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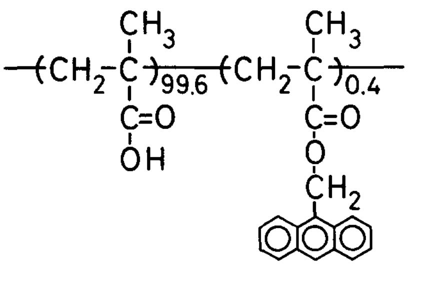
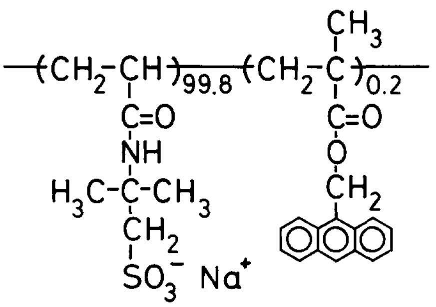
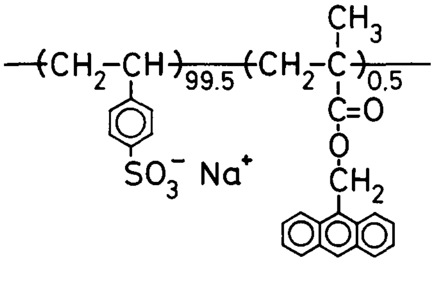


PMA-A



PAMPS-A



PSS-A

EXPERIMENTAL

Materials

9-Anthrylmethyl methacrylate (AMMA) was synthesized as reported previously[19]. 2-Acrylamido-2-methylpropanesulphonic acid (AMPS) was a gift from Nitto Chemical Industry Co. Ltd. and was neutralized with an equimolar amount of NaOH (AMPS-Na). 3-Acrylamidopropyltrimethylammonium chloride (QP) was provided by Kojin Co. Ltd. and used without further purification. Sodium *p*-styrenesulphonate (SS) (Tokyo Kasei Co.) was recrystallized from aqueous ethanol.

Polymers

The copolymers of methacrylic acid and AMMA (PMA-A) was prepared as reported previously[19].

SS was copolymerized with AMMA (0.5 mol% in the monomer feed) in the presence of 1 mol% of 2,2′-azobis(isobutyronitrile) on the basis of the total monomers in N,N-dimethylformamide (DMF)–H_2O mixture (2:1). The polymerization was achieved in a vacuum-sealed tube at 60°C for 4 h. The resulting polymer (PSS-A) was precipitated by an excess of diethyl ether and further purified by reprecipitation from dimethylsulphoxide into diethyl ether twice. The polymer was then dissolved in water and the solution was dialysed against pure water and finally lyophilized: conversion, 32%.

The copolymer of AMPS-Na and AMMA (PAMPS-A) was prepared in the same way, by using methanol as a solvent. The polymer was purified by repeated precipitations from H_2O into ethyl acetate–acetone (2:1): conversion, 4%.

Poly[(dimethyliminio)polymethylene(dimethyliminio)-methylene-1,4-phenylenemethylene dichloride]s (2X, 3X, 6X), poly[(dimethyliminio)-trimethylene(dimethyliminio)-hexamethylene dichloride] (3,6-I), poly(vinylbenzyltrimethylammonium chloride) (PVBMA) and poly(allyltrimethylammonium chloride) (QPAlAm) are those reported previously[19].

Table 1 Properties of Anth-loaded polymers

Polymer	f_{Anth} [a] (mol%)	η_{SP}/C [b] (dl g^{-1})
PMA-A	0.40	0.087
PSS-A	0.47	0.26
PAMPS-A	0.18	0.55

[a] Mole fraction of Anth group in the copolymer (mol%) determined by the optical density of the anthryl residue ($\varepsilon = 10\,600\ \mathrm{M^{-1}\,cm^{-1}}$ at 368 nm) in methanol

[b] Reduced viscosity at 0.5 g dl^{-1} in 0.2 M of aqueous KCl at 30°C

The content of AMMA in the copolymer was determined by u.v. spectroscopy. Properties of the anthracene-loaded polyanions and the polycations are summarized in *Table 1* and *Table 2*, respectively.

Measurements

Preparation of the PEC solution was described previously[19]. Fluorescence spectra were recorded on a Hitachi 650-10 S fluorescence spectrophotometer at room temperature. The excitation wavelength was 365 nm.

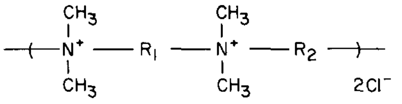
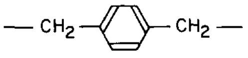
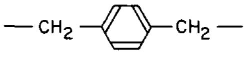
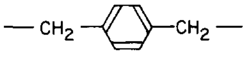
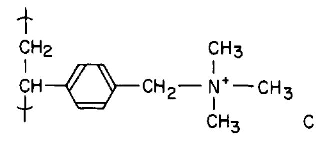
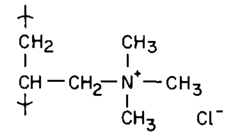
Fluorescence lifetime measurements were made on a computer-controlled single-photon counting system equipped with a 1 ns pulsed hydrogen lamp (Hitachi-Horiba, Model NAES-1100). The scattered light was cut off by using a high-pass 420 nm filter.

RESULTS AND DISCUSSION

Fluorescence spectra of Anth-loaded polyanions

Figure 1 compares the fluorescence spectra of the Anth-loaded polyanions in aqueous solutions at pH 10.

Table 2 Structures of polycations

Abbreviation	Type	R_1	R_2	η_{sp}/C^a (dl g^{-1})
	Integral-type			
2X	 $-\!\!\left(\overset{CH_3}{\underset{CH_3}{N^+}}-R_1-\overset{CH_3}{\underset{CH_3}{N^+}}-R_2-\right)\!\!-\quad 2Cl^-$	$-\!(CH_2)_2\!-$		0.22
3X		$-\!(CH_2)_3\!-$		0.23
6X		$-\!(CH_2)_6\!-$		0.072
3,6-I		$-\!(CH_2)_3\!-$	$-\!(CH_2)_6\!-$	0.082
	Pendant-type			
PVBMA				0.12
QPAIAM				0.34

^{a}Reduced viscosity at 0.5 g dl^{-1} in 0.2 M of aqueous KCl at 30°C

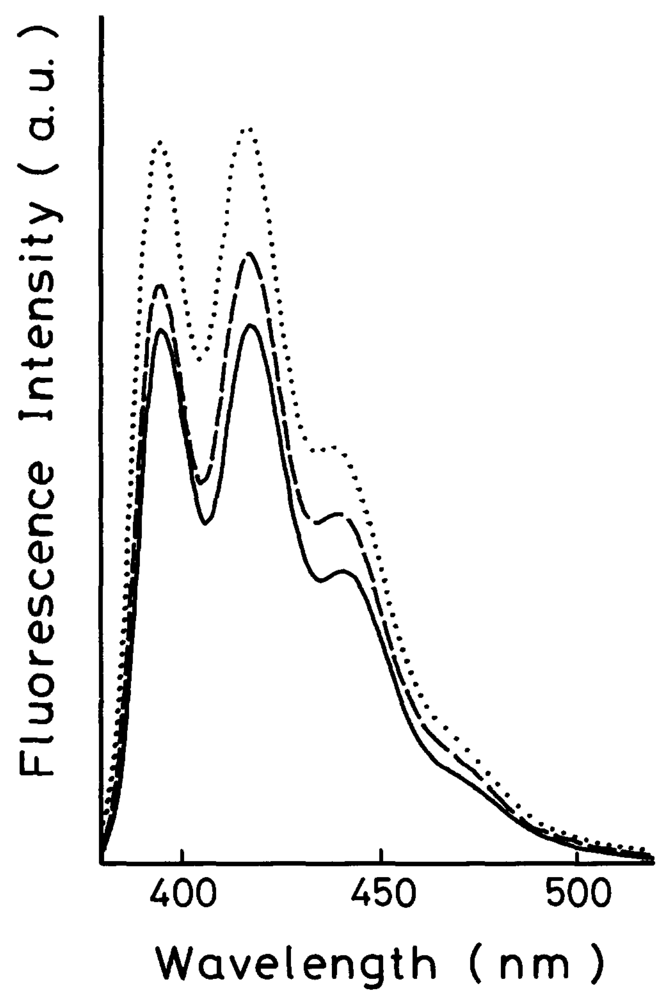


Figure 1 Fluorescence spectra of Anth-loaded polyanions in aqueous solution at pH 10: (——) PMA-A; (– – –) PAMPS-A; (······) PSS-A; excitation wavelength, 365 nm

The fluorescence intensity of PSS-A was higher than those of PMA-A and PAMPS-A, which is indicative of a more hydrophobic environment around the Anth moiety in PSS-A[19]. (In addition, the rigidity of the polymer chain partly contributes to enhance the fluorescence intensity.) The relative fluorescence intensities (I_{rel}) at the emission maxima (λ_{max}) around 420 nm are presented in *Table 3*. These parameters for PAMPS-A and PSS-A were not much affected by pH because these polymers were strong polyacids. In contrast, the I_{rel} value of PMA-A, a weak polyacid, decreased at higher pH because of its dissociation[19].

Careful inspection revealed a very slight broadening of the spectrum of PSS-A shown in *Figure 1*. Such a spectral change was observed more clearly for the polyanions complexed with polycations containing aromatic groups such as 3X (*Figure 2*). On the contrary, the spectrum of PMA-A at low pH showed sharp emission[19]. It is known that for many aromatic compounds the vibrational structures in both absorption and fluorescence spectra tend to be sharper in cyclohexane than in an aromatic solvent (benzene) or polar solvent (ethanol)[20]. In fact, the fluorescence spectrum of 9-methylanthracene was slightly sharper in n-hexane than those in benzene and ethanol (data not shown). Therefore, the broadening of the fluorescence spectrum of PSS-A suggests an interaction between the Anth and aromatic groups. For quantitative evaluation, ratios of the peak intensity at λ_{max} to the minimum intensity at *ca.* 410 nm (I_{max}/I_{min}) were calculated (*Table 3*).

The transient fluorescence data showed that the decay curves for the Anth-loaded polyanions were multiexponential. The lifetimes obtained by triple-exponential fitting of the decay curves are listed in *Table 4*. The multiexponential decay implies heterogeneous distribution of the Anth moiety in the polymer domain. It

Table 3 Photophysical parameters of Anth-loaded polyanions

	PMA-A		PAMPS-A		PSS-A	
Parameter	pH 3	pH 10	pH 3	pH 10	pH 3	pH 10
$\lambda_{max}{}^{a}$ (nm)	417	418	420	420	419	419
$I_{rel}{}^{b}$	1.23	1	1.04	1.13	1.26	1.36
$I_{max}/I_{min}{}^{c}$	1.88	1.60	1.62	1.60	1.52	1.48

[a] Wavelength at emission maximum

[b] The relative fluorescence intensity at λ_{max}, which is normalized to the intensity of PMA-A at pH 10

[c] The ratio of the peak intensity at λ_{max} (I_{max}) to the minimum intensity at *ca.* 410 nm (I_{min}) (see *Figure 1*)

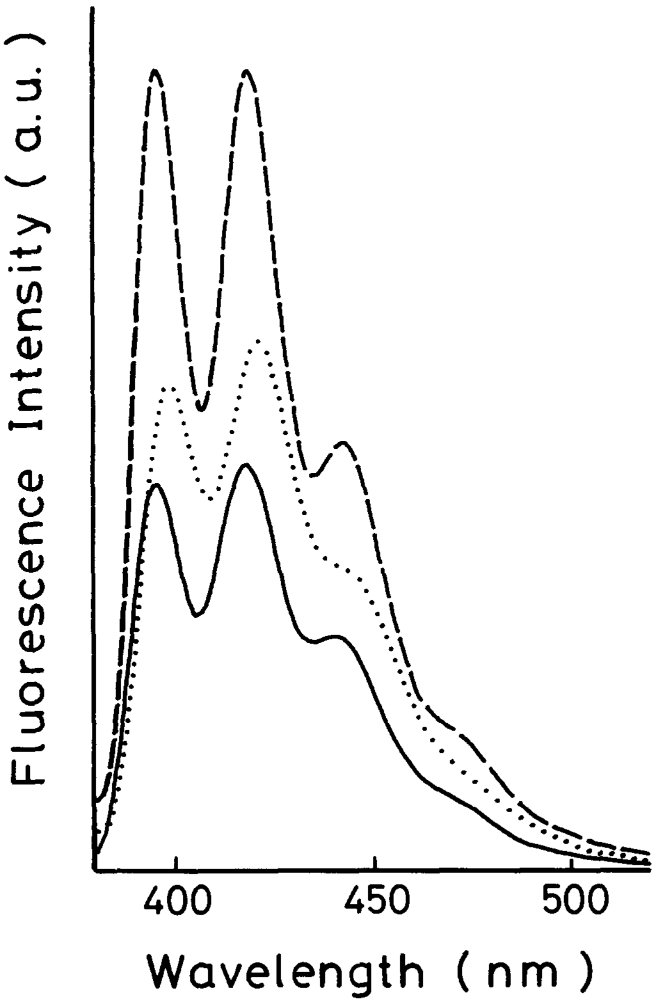


Figure 2 Fluorescence spectra of PAMPS-A in the presence of polycations in aqueous solution at pH 10: (——) without polycation; (---) PVBMA; (······) 3X; $[\text{polyanion}]_{unit} = 10^{-4}$ M; $[\text{polycation}]_{unit} = 10^{-3}$ M; excitation wavelength, 365 nm

should be noted that for PMA-A a lower pH caused the increase of both the overall lifetime and the fraction of the longest-lifetime component and that at pH 10 PAMPS-A and PSS-A had longer lifetimes than PMA-A. Similar tendencies were observed for the values of I_{rel} in *Table 3*. These results indicate that the increase of lifetime is in part due to enhanced hydrophobicity of the microenvironment. It was also found that the lifetime of PSS-A was slightly longer than that of PAMPS-A, which might reflect the effect of aromatic groups on the fluorescence decays.

Table 4 Fluorescence decay parameters for Anth-loaded polyanions in H_2O^{a}

		Lifetime[b] (ns)		
Polymer	pH	τ_1	τ_2	τ_3
PMA-A	10	0.1 (0.03)	4.2 (0.77)	11.7 (0.20)
PMA-A	3	0.5 (0.07)	5.4 (0.49)	13.6 (0.44)
PAMPS-A	10	0.5 (0.06)	4.2 (0.53)	10.9 (0.41)
PSS-A	10	0.5 (0.04)	4.5 (0.45)	11.1 (0.51)

[a] Excitation wavelength, 366 nm; $[\text{polyanion}]_{unit} = 10^{-4}$ M

[b] Numbers in parentheses are relative quantum yields

Effect of the concentration of polycations on PEC formation

In our previous study[19], it was found that the I_{rel} value of PMA-A gradually increased when integral-type polycations such as 2X and 3X were added to the PMA-A solution up to about tenfold concentration in molarity, whereas this value sharply increased when an equimolar amount of pendant-type polycations such as PVBMA and QPAlAm were added. These results have led to the conclusion that integral-type polycations tend to form PECs non-stoichiometrically with PMA-A while pendant-type polycations do so stoichiometrically.

The present study compares the effect of the acidity of polyanions on the formation of PEC. In *Figure 3* the fluorescence intensities are plotted against the unit molar concentration ratio of polycation and polyanion on a logarithmic scale ($\log C/A$) at pH 10. In contrast with the PMA-A-3X system, the I_{rel} value of PAMPS-A increased sharply at $\log C/A = -0.5$ to 0 for both the 3X and PVBMA systems, indicating that PAMPS-A forms a PEC stoichiometrically with any polycation more easily than PMA-A. These observations are consistent with the result reported by Okubo *et al.*[21] that the association constant of poly(ethylene sulphonate) with protonated polyethyleneimine determined by the conductance stopped-flow method was one order of magnitude larger than that of poly(acrylate) with 3,6-I.

Interactions of low-molecular-weight quaternary ammonium salts with anionic polyelectrolytes have already been investigated[22–25]. Turro and co-workers studied the binding of cationic fluorescent probes by polyanions and proposed a binding mechanism[24]. Namely, when an electrostatic force predominates in the association and a hydrophobic interaction additionally enhances the binding, a stoichiometric and structurally specific binding (site binding) would easily occur. On the contrary, when a preferentially hydrophobic force leads to the formation of hydrophobic associates, a stoichiometric binding would be difficult to occur. They concluded that an

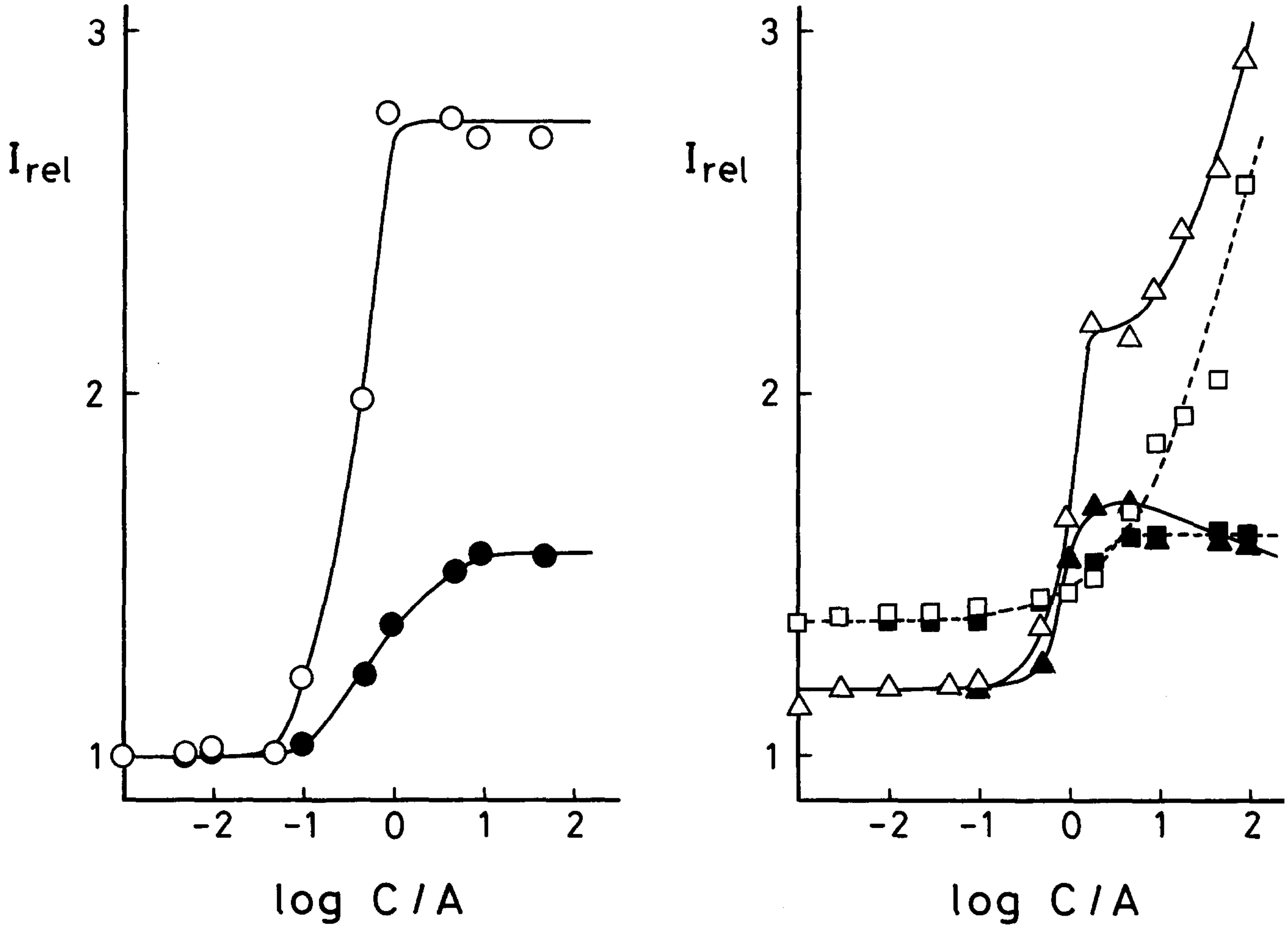


Figure 3 Relative fluorescence intensities of Anth-loaded polyanions in the presence of PVBMA and 3X in aqueous solution at pH 10: open symbol, PEC with PVBMA; closed symbol, PEC with 3X; (○, ●) PMA-A; (△, ▲) PAMPS-A; (---□---, ---■---) PSS-A; $[\text{polyanion}]_{\text{unit}} = 10^{-4}$ M; the abscissa indicates the logarithm of the concentration ratio of polyanion and polycation (mol l^{-1}); the fluorescence intensity is normalized to that of PMA-A at pH 10

electrostatic interaction made an important contribution to the binding of ammonium cations by poly(sulphonate)s (the former case) and that the interactions between ammonium cations and poly(carboxylate)s were predominantly of the hydrophobic type (the latter case). These speculations may interpret the different features of the PEC formation for our PMA-A and PAMPS-A systems shown in *Figure 3*.

Several interesting phenomena were also observed in *Figure 3*. First, the I_{rel} values of PAMPS-A and PSS-A further increased when more than 10^{-3} M of PVBMA was added to these solutions, whereas a large excess of 3X caused little change in the value of I_{rel}. It is known that polyelectrolytes with highly hydrophobic groups form intermolecular associates through hydrophobic interaction in the high-concentration range (e.g. higher than 10^{-3} M), resulting in the formation of hydrophobic microdomains[26,27]. Therefore, it is reasonable to consider that, in a higher concentration range, PVBMA, the most hydrophobic compound among the present polycations studied[19], forms intermolecular hydrophobic aggregates by itself and thus the I_{rel} values of PAMPS-A and PSS-A would be further enhanced due to association with these aggregates. On the other hand, for the PMA-A-PVBMA system such a more-enhanced fluorescence intensity after the initial increase at $\log C/A = -1$ to 0 was hardly observed in the concentration range less than 10^{-2} M of PVBMA. (However, the detailed investigation showed that the addition of more than 10^{-2} M of PVBMA slightly increased the I_{rel} value of PMA-A.) These results may be due to the difference of hydrophobicity among the polyanions. Since the I_{rel} values of polyanions at pH 10 increased in the order PSS-A > PAMPS-A > PMA-A, as shown in *Table 3*, the higher hydrophobic aggregates seemed to be formed easily for the PSS-A and PAMPS-A systems rather than for the PMA-A system.

Secondly, the I_{rel} values for the PECs of PSS-A were equal to or lower than those for the PECs of PMA-A and PAMPS-A, especially in the case of PVBMA, although PSS-A was the most hydrophobic among these polyanions (its fluorescence intensity itself was the highest). This may not be due to weaker interactions between PSS-A and the polycations than those for the PMA-A and PAMPS-A systems, but due to little change in the hydrophobicity around the Anth moiety in PSS-A with complexation, i.e. surrounding the Anth residues with phenyl groups in PSS-A may make themselves insensitive to changes in the microenvironment with complexation, while it also enhances the hydrophobicity of PSS-A itself. The values of I_{rel} for the PECs at $\log C/A = 1$ are summarized in *Table 5*.

Such a change in the microenvironment around the Anth moieties by PEC formation could also be evaluated by observation of the changes in the spectral shapes

Table 5 Effect of additives on the photophysical parameters for AM and Anth-loaded polyanions[a]

	AM[b]		PMA-A		PAMPS-A		PSS-A	
Additives	I_{rel}[c]	I_{max}/I_{min}	I_{rel}[d]	I_{max}/I_{min}	I_{rel}[d]	I_{max}/I_{min}	I_{rel}[d]	I_{max}/I_{min}
—	1	1.82	1	1.60	1.13	1.60	1.36	1.48
2X	1.00	1.82	1.19	1.33	1.22	1.43	1.45	1.35
3X	1.02	1.81	1.55	1.43	1.47	1.45	1.60	1.39
6X	1.04	1.80	1.69	1.50	1.57	1.55	1.69	1.46
3,6-I	1.04	1.82	1.80	1.66	1.53	1.65	1.74	1.49
QPAlAm	1.05	1.82	1.81	1.66	1.69	1.66	1.80	1.52
PVBMA	1.75	1.61	2.72	1.69	2.23	1.73	1.86	1.57
CTAC[e]	0.94[h]	2.12	2.14	1.89				
BTAC[f]	1.12	1.75	1.43	1.56				
PSS[g]	1.15	1.70						

[a] Measured at pH 10; $[\text{polyanion}]_{unit} = 10^{-4}$ M; $[\text{polycation}]_{unit} = 10^{-3}$ M

[b] $[\text{AM}] = 10^{-7}$ M

[c] I_{rel} is normalized to the intensity of AM

[d] I_{rel} is normalized to the intensity of PMA-A at pH 10

[e] Cetyltrimethylammonium chloride (10^{-2} M)

[f] Benzyltrimethylammonium chloride (10^{-1} M)

[g] $[\text{PSS}]_{unit} = 10^{-2}$ M

[h] The absorption spectrum was largely changed

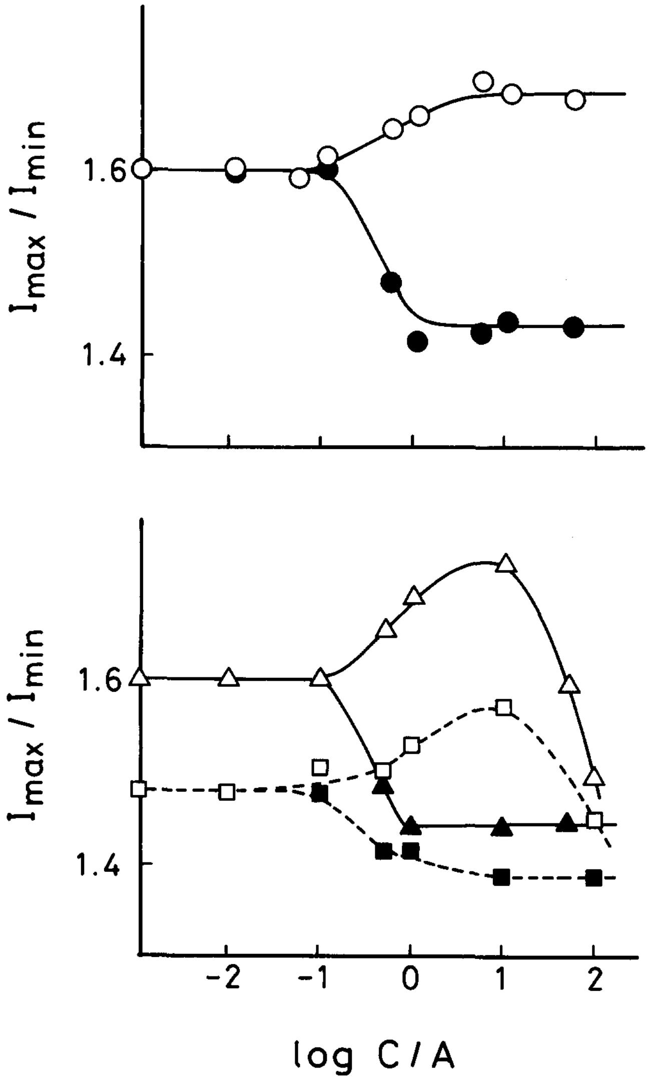


Figure 4 Dependence of the fluorescence intensity ratios (I_{max}/I_{min}) on the concentration of polycations: open symbol, PEC with PVBMA; closed symbol, PEC with 3X; (○, ●) PMA-A; (△, ▲) PAMPS-A; (---□---, ---■---) PSS-A; $[\text{polyanion}]_{unit} = 10^{-4}$ M

(*Figure 2*). The fluorescence intensity ratios (I_{max}/I_{min}) were plotted as a function of the concentration ratio of polycation and polyanion (log C/A) in *Figure 4*. For any polyanion, the intensity ratio for the 3X system decreased at log $C/A = -1$ to 0 and levelled off at log $C/A = 0$. This result suggests that the PEC formation permits access of the aromatic groups in 3X to the Anth moieties in the polyanions, facilitating an interaction between the Anth and aromatic groups. A comparison can be made of the intensity ratio among the integral-type polycations with xylylene groups (X-type): the I_{rel} values for the PECs at log $C/A = 1$ increased with the length of alkyl chains (i.e. 2X < 3X < 6X) (*Table 5*) because the hydrophobicity of the polycations themselves increased in that order[19]. However, the I_{max}/I_{min} values for the PECs, which were always smaller than those of the polyanions, also increased with the length of the alkyl chain (i.e. 2X < 3X < 6X), indicating that the interaction between the Anth and aromatic groups becomes stronger for the PECs with the X-type polycations composed of shorter alkyl chains (i.e. 2X > 3X > 6X) (*Table 5*). In contrast to the X-type polycations, the addition of QPAlAm and 3,6-I, containing no aromatic groups, slightly increased the I_{max}/I_{min} values of the polyanions.

Reference data were obtained for 9-anthracenemethanol (AM) in the presence of polyelectrolytes and low-molecular-weight ammonium salts (*Table 5*). Hydrophobic additives with aromatic groups such as PVBMA, poly(sodium *p*-styrenesulphonate) (PSS) and benzyltrimethylammonium chloride (BTAC) enhanced the I_{rel} value of AM but decreased the value of I_{max}/I_{min}. In contrast, cetyltrimethylammonium chloride (CTAC), a hydrophobic additive with no aromatic group, increased the intensity ratio. (No enhancement of the fluorescence intensity by addition of CTAC was observed because the absorption spectrum was red-shifted, leading to a decrease of absorbance at the excitation wavelength.) Such phenomena were also observed for the PMA-A-BTAC and -CTAC systems. These facts support the presence of a specific interaction between the Anth and aromatic groups. It may further be said that the

Table 6 Fluorescence decay parameters for the PECs in H_2O at pH 10[a]

		Lifetime[b] (ns)		
Polyanion	Polycation	τ_1	τ_2	τ_3
PMA-A	3X	0.1 (0.02)	5.6 (0.17)	13.6 (0.81)
PMA-A	PVBMA	0.5 (0.08)	5.0 (0.23)	12.5 (0.70)
PAMPS-A	3X	0.1 (0.07)	6.3 (0.25)	14.8 (0.68)
PAMPS-A	PVBMA	0.5 (0.09)	5.2 (0.26)	13.1 (0.65)
PAMPS-A	PVBMA (10^{-2} M)	~0.0 (0.03)	9.0 (0.37)	12.8 (0.61)
PSS-A	3X	0.1 (0.04)	4.3 (0.24)	14.3 (0.72)
PSS-A	PVBMA	~0.0 (0.02)	3.7 (0.22)	13.1 (0.76)

[a] Excitation wavelength, 366 nm; $[\text{polyanion}]_{\text{unit}} = 10^{-4}$ M; $[\text{polycation}]_{\text{unit}} = 10^{-3}$ M
[b] Numbers in parentheses are relative quantum yields

Table 7 Effect of added salt on the photophysical parameters for the PECs[a]

		PMA-A		PAMPS-A		PSS-A	
Additive	[NaCl] (M)	I_{rel}	$I_{\text{max}}/I_{\text{min}}$	I_{rel}	$I_{\text{max}}/I_{\text{min}}$	I_{rel}	$I_{\text{max}}/I_{\text{min}}$
—	0	1.0	1.60	1.14	1.61	1.35	1.48
	0.9	0.99	1.60	1.08	1.61	1.34	1.48
3X	0	1.43	1.42	1.30	1.45	1.47	1.39
	0.9	1.00	1.60	1.29	1.45	1.47	1.39
PVBMA	0	2.04	1.66	1.63	1.69	1.50	1.54
	0.9	1.04	1.59	1.96	1.80	1.45	1.55

[a] Measured at pH 10; $[\text{polymer}]_{\text{unit}} = 10^{-4}$ M

presence of hydrophobic alkyl groups around the Anth moiety sharpens the spectrum.

However, it should be noted that, despite PVBMA having aromatic groups, the $I_{\text{max}}/I_{\text{min}}$ ratios for the PECs with PVBMA increased for $\log C/A = -1$ to 1 (*Figure 4*) and that the AM-PVBMA system had a lower value than that of AM (*Table 5*). Such facts may be caused by a difference in the location of the Anth chromophore in the microdomains. For the former PEC systems, since PVBMA has bulky ionic groups in the side chains, the Anth groups attached to the polyanions would be unable to make contact with the phenyl groups in PVBMA directly. So, the value of $I_{\text{max}}/I_{\text{min}}$ increased exclusively due to the increase of hydrophobicity around the Anth groups, which was in a marked contrast to the X-type polycation systems (*Figure 4*). For the latter system, AM, a small molecule would be located close to the aromatic groups in PVBMA, decreasing the $I_{\text{max}}/I_{\text{min}}$ value. It may therefore be concluded that the Anth chromophores in the polyanions could approach the aromatic groups in the integral-type polycations more readily than those in the pendant-type polycations. It is also worth noting that the further addition of more than 10^{-3} M of PVBMA ($\log C/A \geqslant 1$) inversely decreased the $I_{\text{max}}/I_{\text{min}}$ values of PAMPS-A and PSS-A, in parallel with the further increase of the values of I_{rel} (*Figure 3*). Thus we could speculate that associations of PAMPS-A and PSS-A with the intermolecular hydrophobic aggregates of PVBMA permit the contact of the Anth chromophore with the aromatic groups in PVBMA.

PEC formation could also be confirmed by fluorescence decay measurements (*Table 6*). The lifetimes of the Anth-loaded polyanions obviously increased in the presence of polycations although the short-lifetime components still remained. The increase of lifetime is explained by both the enhanced hydrophobicity of microenvironment and the suppression of molecular motion of the probe through a PEC formation. The lifetimes for the PECs with 3X were slightly longer than those for the PECs with PVBMA, which might be caused by the interaction between the Anth chromophore and the aromatic groups in 3X. It is also interesting to note that further addition of PVBMA to the PAMPS-A solution changed the emission decay of PAMPS-A, which can be related to the increase of I_{rel} and the decrease of $I_{\text{max}}/I_{\text{min}}$.

Effect of ionic strength on PEC formation

In our preceding paper[19], it was found that the addition of more than 0.7 M of NaCl no longer enhanced the I_{rel} values for the PMA-A-PVBMA and -3X systems, indicating the dissociation of the PECs to the individual polyelectrolyte components. This is supported by the fact that the $I_{\text{max}}/I_{\text{min}}$ ratios for the PECs of PMA-A returned to their original values at high ionic strength (*Table 7*). By contrast, there was only a small effect of ionic strength on the photophysical parameters for the PAMPS-A and PSS-A systems.

These results are consistent with the fact obtained by macroscopic analyses that PECs of poly(carboxylate)s with the polycations used here are dissociated into the component polyelectrolytes at higher ionic strength for more than 0.7 M of NaCl while PECs of poly(strong acid)s and poly(strong base)s are formed stably even at high ionic strength[17,18]. It should be noted that both the I_{rel} and $I_{\text{max}}/I_{\text{min}}$ values for the PAMPS-A-PVBMA system increased on addition of NaCl. This observation suggests that the addition of salt facilitates the hydrophobic association of PAMPS-A and PVBMA, thus increasing the hydrophobicity of the PEC microdomain.

CONCLUSIONS

The spectral parameters obtained from the fluorescence intensities, spectral shapes and fluorescence lifetimes for the Anth chromophores attached to the polyelectrolytes respond well to the formation of PECs. The results are summarized as follows.

Formation of PEC results in the increase of hydrophobicity around the Anth chromophore, which mainly reflects the hydrophobicity of the polyelectrolyte components.

Poly(methacrylate) tends to form PECs non-stoichiometrically with integral-type polycations and stoichiometrically with pendant-type polycations. On the other hand, poly(sulphonate)s tend to form 'stoichiometric' PECs with any polycation.

In the PEC microdomains, the Anth chromophores in the polyanions are located closer to the aromatic groups in integral-type polycations than to those in pendant-type polycations.

Addition of hydrophobic polycations in a higher concentration to the polyanion solutions induces formation of higher aggregates, changing the spectral features of the Anth chromophore.

Consequently, the present fluorescent probe method provides useful information about both mechanisms of PEC formation and characterization of PEC microdomains.

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