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Effect of Counterion Condensation on the Fluorescence Quenching of Phenanthrene-Labeled Sodium Salt of Poly(acrylic acid-*co*-acrylamide) in Salt-Free Solution

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ABSTRACT: A small mole fraction of phenanthrene (Phen) moieties were covalently incorporated into copolymers of sodium acrylate and acrylamide varying Manning's dimensionless charge density parameter ξ ($0.67 < \xi < 2.8$). In aqueous solution with no added salt (except for quenchers), fluorescence of the Phen moieties was efficiently quenched by Tl^+ ions at very low quencher concentrations. The quenching efficiency markedly increased with increasing ξ, which was interpreted as due to counterion condensation. A kinetic model of mixed static and dynamic quenching in conjunction with Manning's condensation theory was proposed. In this model, fluorescence was postulated to be statically quenched by condensed Tl^+ ions existing in the active sphere according to the Perrin formulation and also dynamically quenched by atmospheric Tl^+ ions according to the Stern–Volmer formulation. The quenching data were satisfactorily fit to the kinetic model. The fraction of the static quenching decreased sharply with decreasing ξ. It was estimated that 34% of the condensed Tl^+ ions existed in the active sphere for the polymer with $\xi=2.8$, while only 1.2% were estimated to be in the active sphere for $\xi=1.5$.

Introduction

The effect of polyelectrolyte on chemical reactions involving ionic species has extensively been studied by many investigators since the early studies of Morawetz et al.[1] and Ise et al.[2-4] Considerable enhancement or retardation of reaction rates is generally observed due to electrostatic attraction or repulsion of ionic reactants by polymer charge.

The ionic reactants are distributed in a variety of ways between the polyelectrolyte molecular surface and the bulk phase, depending on the strength of the electrostatic interactions. Manning's counterion condensation theory,[5] though it is a simple theory with only one nonadjustable parameter, can explain fairly satisfactorily the rate-accelerating and rate-retarding effects of polyelectrolytes in some chemical reactions[4,6] as well as thermodynamic and interfacial properties of polyelectrolytes such as counterion activity coefficients[7] and counterion diffusion coefficients.[8] The effects of counterions on the acid–base equilibrium of a pH-sensitive merocyanine dye covalently attached to a polyanion[9,10] were also reasonably interpreted in terms of Manning's theory.[11]

Polyelectrolytes in aqueous solution are characterized by a dimensionless charge density parameter ξ given by

$$\xi=e^2/\epsilon kTB \qquad (1)$$

where e is the protonic charge, ϵ is the dielectric constant of the solvent, k is the Boltzmann constant, T is the Kelvin temperature, and b is the average axial spacing between charged groups on the polyelectrolyte.[5]

In Manning's theory,[5] the critical charge density parameter is defined as $\xi_c=N^{-1}$, where N is the counterion valence. When $\xi > \xi_c$, counterions are condensed until the effective charge density parameter equals ξ_c. When $\xi < \xi_c$, on the other hand, the counterions are completely dissociated.

Fluorescence quenching of charged chromophores by ionic quenchers in the presence of polyelectrolytes in aqueous solution was first reported by Taha and Morawetz.[12,13] They showed that the quenching of quinine sulfate by Fe^{2+} and Ag^+ and the quenching of uranyl ions by Fe^{2+} were enhanced in the presence of poly(vinylsulfonic acid) at low polyion concentrations.[12,13] Meisel and Matheson[14] also reported a similar effect of polyelectrolytes on the quenching of the tris(2,2′-bipyridine)ruthenium(II) emission by low concentrations of Cu^{2+}.

More recently, fluorescence quenching of chromophores covalently attached to polyelectrolytes by ionic quenchers has been investigated by several groups. Chu and Thomas[15] prepared a copolymer of methacrylic acid and a small mole fraction of 1-pyreneacrylic acid and showed that the fluorescence of the pyrene residues was efficiently quenched by Tl^+ and Cu^{2+} when the polymer was in a dissociated form at a higher pH. Similar phenomena were reported by Webber et al.[16] for a copolymer of methacrylic acid and a small mole fraction of vinyldiphenylanthracene. They showed that the effect of pH on the fluorescence quenching of the diphenylanthracene residues was much larger for cationic quenchers such as methylviologen (MV^{2+}) and Cu^{2+} than for neutral quenchers.[16] We previously prepared a copolymer of 2-acrylamido-2-methylpropanesulfonic acid and 9-vinylphenanthrene and showed that fluorescence emitted by the polyanion-bound phenanthrene (Phen) residues was efficiently quenched by a low concentration of MV^{2+} in aqueous solution.[17,18] In all these studies,[15-18] apparent second-order equivalent rate constants for the fluorescence quenching by the counterionic quenchers were estimated to be on the order of 10^{10}–10^{12} M^{-1} s^{-1} from the initial slopes of the Stern–Volmer plots. However, no detailed mechanism nor kinetic model to fully explain these efficient counterionic quenchings has so far been reported.

The purpose of the present study is to elucidate the effect of counterion condensation on the quenching of polyion-bound fluorophores by counterionic quenchers in salt-free aqueous solution and to verify the applicability of Manning's theory to the kinetic analysis of the quenching by counterions. In this work, we incorporated a small mole fraction of Phen moieties into the copolymers of sodium acrylate and acrylamide with varying molar ratios (Scheme I), ranging in charge density ξ from 0.67 to 2.8. In these polymers, the Phen moieties are forced to experience microenvironments close to the backbone because of their covalent bonding. As counterionic

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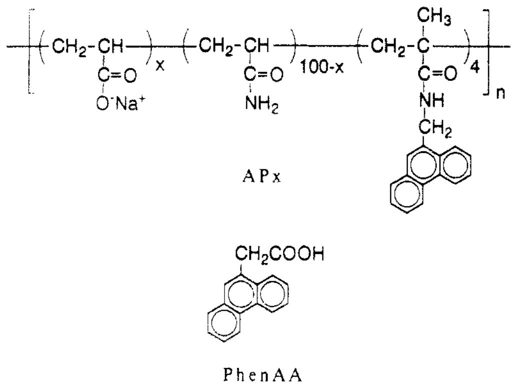
Scheme I



quencher, we chose Tl^+ because fluorescence quenching by Tl^+ occurs only through close contact with a fluorophore due to a "heavy atom effect",[19] which precludes long-range quenching due to electron transfer.

Experimental Section

***N*-(9-Phenanthrylmethyl)methacrylamide (PhenMAm).** In a cooled autoclave were placed 4.29 g (20.8 mmol) of phenanthrene-9-carboxaldehyde, 0.2 g of activated Raney nickel, 100 mL of cooled *N*,*N*-dimethylformamide (DMF), and 30 mL of liquid ammonia. Hydrogen gas was introduced into the autoclave until the pressure reached 80 kg/cm^3 at room temperature. The autoclave was then heated at 75 °C, at which temperature the hydrogen pressure was about 92 kg/cm^3. The reaction mixture was magnetically stirred for 2.5 h. After the reaction, the mixture was filtered to remove the catalyst and was evaporated under reduced pressure. The residue was treated with 200 mL of aqueous 1 N HCl at ca. 80 °C to extract the product in the hydrochloride form. This extraction procedure was repeated twice. Upon cooling, 9-phenanthrylmethylamine hydrochloride crystallized from the extracting solution. The crystals were then dissolved in hot water, and a small amount of aqueous 1 N NaOH was added to yield free 9-phenanthrylmethylamine as white needles. The yield was 37.3% (1.61 g), mp 104–106 °C: ^{1}H-NMR (DMSO-d_6) δ 4.3 (m, 2H), 7.6–8.3 (m, 7H), 8.8–9.0 (m, 2H). Anal. Calcd for $C_{15}H_{13}N$: C, 86.92; H, 6.32; N, 6.76. Found: C, 86.38; H, 6.28; N, 6.54.

To a stirred solution of 1.17 g (5.64 mmol) of 9-phenanthrylmethylamine and 3.90 g (38.0 mmol) of triethylamine in 250 mL of tetrahydrofuran (THF) was added dropwise 5.90 g (56.0 mmol) of methacryloyl chloride in 50 mL of THF over a period od 10 min at room temperature. After stirring for 6 h, the reaction mixture was filtered to remove triethylamine hydrochloride, and the solvent was evaporated. The crude product was then dissolved in 300 mL of chloroform. The solution was washed successively with 1 N HCl, saturated NaCl, 1 M $NaHCO_3$, and saturated NaCl aqueous solutions. The organic layer was dried with anhydrous $MgSO_4$ overnight, and chloroform was evaporated. The residue was recrystallized from ligroin to give 1.0 g (64.4%) of PhenMAm as white needles, mp 137–138 °C: ^{1}H-NMR ($CDCl_3$) δ 2.0 (s, 3H), 4.9 (t, 2H), 5.4 (s, 1H), 5.8 (s, 1H), 7.6–8.3 (m, 7H), 8.6 (m, 1H), 8.8–9.0 (m, 2H). Anal. Calcd for $C_{19}H_{17}NO$: C, 82.88; H, 6.22; N, 5.09. Found: C, 82.64; H, 6.19; N, 5.08.

9-Phenanthreneacetic Acid (PhenAA). To 100 mL of anhydrous ether containing 20.0 g (0.823 mol) of dried magnesium ribbons and a catalytic amount of methyl iodide was added 213 g (0.829 mol) of 9-bromophenanthrene over a period of 90 min under stirring at room temperature. The reaction mixture was refluxed for 8 h and then diluted with 450 mL of ether. The resulting Grignard reagent was added dropwise into a 200-mL solution of dry benzene containing 251 g (3.20 mol) of acetyl chloride over a period of 2.5 h. The mixture was stirred for 5 h at room temperature and refluxed for an additional 10 h. After diluting with 100 mL of benzene, the reaction mixture was treated with 1 N HCl and washed with water and aqueous 1 M $NaHCO_3$. The organic layer was dried with $MgSO_4$, and the solvent was evaporated. The residue was distilled under reduced pressure, and a fraction was collected at 175–180 °C (0.8 mmHg) to obtain 73.3 g (40.4%) of 9-acetylphenanthrene as yellowish-orange needles, mp 70–73 °C: ^{1}H-NMR ($CDCl_3$) δ 2.7 (s, 3H), 7.4–8.8 (m, 9H).

A mixture of 113 g (1.30 mol) of morpholine, 56.6 g (0.257 mol) of 9-acetylphenanthrene, and 23.8 g (0.742 mol) of sulfur was refluxed for 16 h. The mixture was then dissolved in 400 mL of acetic acid. To this solution 500 mL of 1 N HCl was added dropwise over a period of 3 h at 100 °C, and the reaction mixture was refluxed for 19 h. The mixture was poured into 2 L of pure water. Precipitates were collected and dissolved in aqueous 1 N NaOH followed by the addition of 1 N HCl to precipitate the crude product. Recrystallization from benzene yielded pure PhenAA as white needles, mp 225–226 °C: ^{1}H-NMR (DMSO-d_6) δ 4.1 (s, 2H), 7.5–8.1 (m, 7H), 8.7–8.9 (m, 2H). Anal. Calcd for $C_{16}H_{12}O_2$: C, 81.34; H, 5.12. Found: C, 81.19; H, 5.06.

Terpolymers. The terpolymers of acrylic acid (AA), acrylamide (AAm), and PhenMAm were prepared by radical polymerization in the presence of 2,2′-azobis(isobutyronitrile) (AIBN) in DMF. An ampule containing AA, AAm, PhenMAm, and 0.5 mol % (on the basis of the total monomers) of AIBN was outgassed by five freeze–pump–thaw cycles on a vacuum line and sealed off. The polymerization was carried out at 60 °C for 1 h. The reaction mixture was then poured into a large excess of ether to precipitate the resulting polymers. The polymers were purified three times by reprecipitation from a DMF solution into an excess of ether, allowed to air-dry, and dissolved in water. After adjusting the pH to 8.5 by adding dilute aqueous NaOH, the solution was dialyzed against pure water for 7 days. The polymers were recovered by freeze-drying. The compositions of the terpolymers were calculated on the basis of the N/C ratio and absorbance due to the Phen residue in the UV–vis spectra.

Other Materials. Analytical grade thallium nitrate was used without further purification. Water was doubly distilled and deionized by passing through an ion-exchange column.

Measurements. Fluorescence spectra were recorded on a Shimadzu RF-502A spectrophotometer. Sample solutions were prepared by volumetric dilution of stock solutions of the polymers and thallium nitrate in pure water. The diluted polymer and the quencher solutions were mixed in a predetermined ratio. The pH of the sample solutions was adjusted by adding aqueous NaOH or HCl with a micropipet. All the fluorescence measurements were performed at room temperature by excitation of the Phen residues at 297 nm. Prior to measurements, the sample solutions were deaerated by bubbling with argon for 30 min.

Results and Discussion

The AA–AAm copolymers tethered with a small mole fraction of the Phen moieties are abbreviated in the text as AP*x*, *x* indicating the mole percent of AA units on the basis of the total AA and AAm units in the copolymers (Scheme I). The loading amount of the Phen moieties on the polymers was limited to as low as 4 mol %.

Previously, we found that the copolymerization of AA and AAm is practically azeotropic in DMF at 60 °C.[10] We also ascertained that the copolymerizations of PhenMAm–AA and PhenMAm–AAm are random in DMF at 60 °C. Therefore, the compositions of the terpolymers of AA, AAm, and PhenMAm are virtually equal to the monomer feed compositions. The polymerization data are shown in Table I. Since the AA and AAm units are randomly distributed along the polymer chain,[10,11] the average charge density parameter ξ can be calculated from eq 1 by using the average axial spacing between charged groups.[10] The value of b=2.55 Å is ordinarily used for vinyl polymers carrying an ionic charge on each monomeric unit. Therefore, the value of ξ=2.8 is commonly used for a fully extended vinylic polyion carrying a charge on each monomer residue in aqueous solution at room temperature. The values of b for the polymers listed in Table I were calculated from the compositions of the polymers.

Figure 1 compares fluorescence spectra of the monomer model PhenAA (Scheme I) and those of AP100 in the

Table I. Free Radical Terpolymerizations of AA, AAm, and PhenMAm in DMF at 60 °C in the Presence of AIBN[a]

	feed monomers				terpolymer		
polymer	AA (mmol)	AAm (mmol)	PhenMAm (mmol)	conv (%)	x^b	PhenMAm (mol %)	ξ
AP100	64.0	0	2.65	24.9	100	4	2.8
AP90	57.6	6.41	2.61	38.5	90	4	2.5
AP71	44.8	19.2	2.65	16.2	71	4	2.0
AP52	31.9	32.0	2.47	36.1	52	4	1.5
AP27[c]	5.16	20.5	1.00	27.0	27	4	0.67

[a] AIBN, 0.5 mol % based on the total monomers. DMF, 50 mL. Polymerization time, 1 h. [b] Mol % AA units on the basis of the total AA and AAm units (Scheme I). [c] DMF, 13 mL.

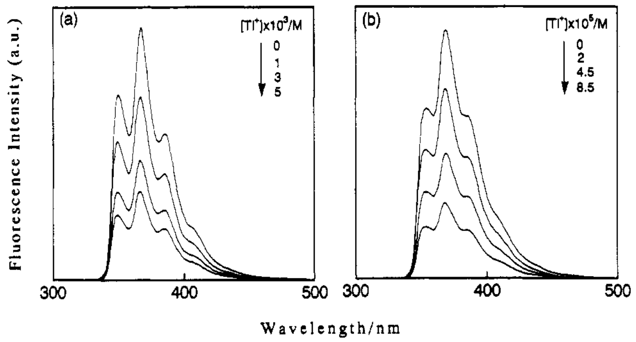


Figure 1. Fluorescence spectra of PhenAA (a) and AP100 (b) in aqueous solution at pH 8.5 in the presence of varying concentrations of Tl^+ ions: $[Phen]_0=2.6 \times 10^{-5}$ M.

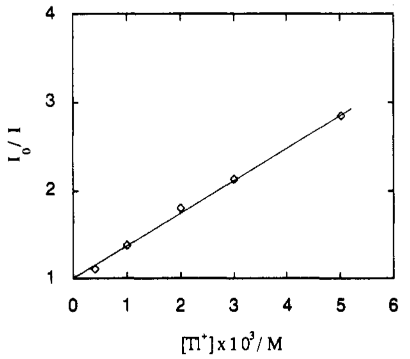


Figure 2. Stern–Volmer plot for the fluorescence quenching of PhenAA by Tl^+ ions in aqueous solution at pH 8.5: $[Phen]_0=2.6 \times 10^{-5}$ M.

presence of varying concentrations of $TlNO_3$ in aqueous solution at pH 8.5. The fluorescence emitted by the Phen moieties in the polymer was quenched much more efficiently than that of the monomer model; i.e., 2 orders of magnitude lower concentrations of Tl^+ ions were enough for the polymer to quench its fluorescence to the same extent as the monomer model.

As shown in Figure 2, the Stern–Volmer plot for the monomer model was linear. The Stern–Volmer constant was estimated from the slope to be $K_{sv}=3.7 \times 10^2$ M^{-1}. Using a lifetime of 40 ns for the monomer model in the absence of the quencher, the second-order rate constant for the quenching was estimated to be $k_q=9.3 \times 10^9$ M^{-1} s^{-1}. This indicates that the fluorescence quenching of PhenAA by Tl^+ is completely dynamic and occurs at the diffusion-limiting rate.

Figure 3 compares Stern–Volmer plots for the fluorescence quenching of AP100 with Tl^+ ions at various pHs. The fluorescence quenching was strongly dependent on pH in the neutral and acidic pH region. The quenching became less efficient as the pH was lowered. When pH > 8, the carboxyl groups in the polymer are fully ionized, and the quenching efficiency was maximized. Therefore, all the quenching experiments for the polymers with varying ξ were carried out at pH 8.5. An upward curvature with a high quenching efficiency was observed in the Stern–Volmer plot for fully ionized AP100, suggesting static quenching by Tl^+ condensed on the polymer.

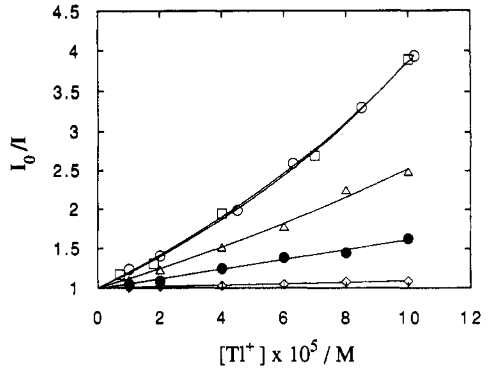


Figure 3. Stern–Volmer plots for the fluorescence quenching of AP100 by Tl^+ ions in aqueous solution at pHs 8.5 (□), 8.0 (○), 7.0 (△), 6.0 (●), and 5.0 (◇): $[Phen]=2.6 \times 10^{-5}$ M.

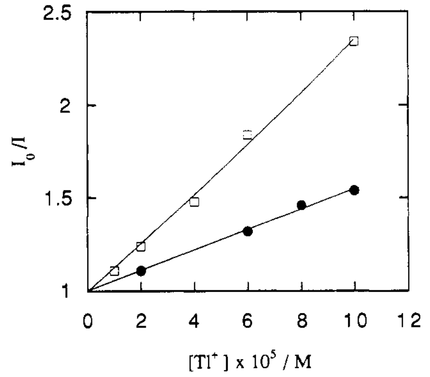


Figure 4. Stern–Volmer plots for the fluorescence quenching of AP52 (□) and AP27 (●) by Tl^+ ions in aqueous solution at pH 8.5: $[Phen]_0=2.6 \times 10^{-5}$ M. The solid line for AP52 represents the best-fit curve from use of eq 7 with the parameters listed in Table II.

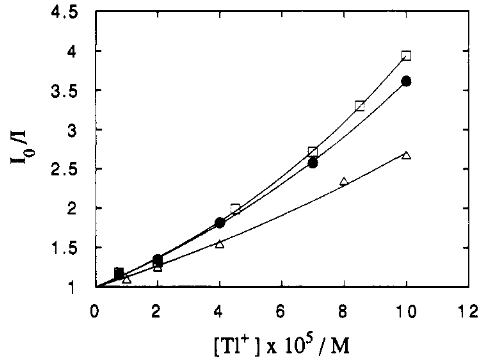


Figure 5. Stern–Volmer plots for the fluorescence quenching of AP100 (□), AP90 (●), and AP71 (△) by Tl^+ ions in aqueous solution at pH 8.5. $[Phen]_0=2.6 \times 10^{-5}$ M. The solid lines represent the best-fit curves from use of eq 7 with the parameters listed in Table II.

Figure 4 shows the Stern–Volmer plots for the polymers with lower charge densities. With AP27 the slope was almost the same as that of partially ionized AP100 at pH 6.0 shown in Figure 3. The plots for AP52 showed a slight upward curvature and resembled those of partially ionized AP100 at pH 7.0 shown in Figure 3. These observations suggest that the mechanism and the efficiency of the fluorescence quenching by Tl^+ are different for polymers with different charge densities.

The Stern–Volmer plots for the polymers with higher charge densities are compared in Figure 5. With increasing charge density of the polymer, the Stern–Volmer plot showed an increasing tendency for upward curvature and increasing quenching efficiency.

Manning's theory[5] predicts that for $\xi > 1$ monovalent countercations condense onto a polyanion until the fraction $1 - \xi^{-1}$ of charge is neutralized. The remaining polymer charge is compensated by atmospheric counterions through Debye–Hückel interactions. In the case of AP100 (ξ=2.8) at pH 8.5, for example, 64% of the polymer charge is compensated by condensed Na^+ ions in a quencher-free solution. When Tl^+ ions are added, an exchange of the condensed Na^+ ions with the added Tl^+ ions may occur, while 64% of the polymer charge remains to be neutralized.

We now assume that the singlet-excited Phen residues undergo mixed static and dynamic quenching, i.e., static quenching by the condensed Tl^+ ions and dynamic quenching by atmospheric Tl^+ ions which are concentrated in the vicinity of the polymer backbone by Debye–Hückel interactions. If the exchange of the condensed and free counterions is slow compared to the life of the singlet-excited state of the Phen moieties, a mix of the static and dynamic quenching can be expressed by a combination of the Perrin and Stern–Volmer formulations as[20]

$$I_0/I=(1+K_{sv,a}[Tl^+]_{DH})\exp(n) \qquad (2)$$

where I_0 and I are fluorescence intensities in the absence and presence of the quencher, $K_{sv,a}$ is the apparent dynamic quenching constant, $[Tl^+]_{DH}$ is the concentration of the atmospheric Tl^+ ions, and n is the average number of condensed Tl^+ ions in the active sphere. The number n can be given by

$$n=\alpha[Tl^+]_C/[\mathrm{Phen}]_0 \qquad (3)$$

where $[Tl^+]_C$ is the "macroscopic" concentration of condensed Tl^+ ions, $[\mathrm{Phen}]_0$ is the concentration of Phen residues, and α is the fraction of condensed Tl^+ ions existing in the active sphere. In the Perrin model, the active sphere is such a volume around a fluorophore that a quencher present within this volume quenches the fluorophore with unity efficiency, while quenchers outside the volume do not. If there is no particular preference for Tl^+ and Na^+ ions in the counterion condensation, $[Tl^+]_C$ can be given by

$$[Tl^+]_C=\{[Tl^+]_0/([Tl^+]_0+[Na^+]_0)\}[COO^-]_0(1-\xi^{-1}) \qquad (4)$$

where $[Tl^+]_0$, $[Na^+]_0$, and $[COO^-]_0$ are the total concentrations of Tl^+, Na^+, and carboxylate residues in the polymer, respectively. When $[Tl^+]_0 \ll [Na^+]_0$ in salt-free aqueous solution where $[Na^+]_0=[COO^-]_0$, $[Tl^+]_C$ and $[Tl^+]_{DH}$ can be given by

$$[Tl^+]_C=(1-\xi^{-1})[Tl^+]_0 \qquad (5)$$

and

$$[Tl^+]_{DH}=\xi^{-1}[Tl^+]_0 \qquad (6)$$

respectively. Consequently, the I_0/I ratio is given by

$$I_0/I=(1+K_{sv,a}\xi^{-1}[Tl^+]_0)\exp\{\alpha(1-\xi^{-1})[Tl^+]_0/[\mathrm{Phen}]_0\} \qquad (7)$$

As shown in Figure 4, AP27 (ξ=0.67) exhibited a linear Stern–Volmer plot. With AP27, no counterion condensation occurs because $\xi < 1$, and all Tl^+ ions are atmospheric. Therefore, all fluorescence quenching occurs via the dynamic process. On the other hand, in the case of AP52 (ξ=1.5) a portion of Tl^+ ions are condensed on the polymer because $\xi > 1$, and the quenching of AP52 may occur via a mix of dynamic and static processes. The quenching experiments in the present study were performed with a 2.6×10^{-5} M concentration of the Phen residues. Therefore, from the copolymer composition, $[COO^-]_0$ for AP52 can be calculated to be 3.4×10^{-4}M. The concentration range of Tl^+ employed for the quenching experiments is sufficiently low as compared to $[COO^-]_0$, and therefore the quenching data may follow eq 7. In fact, the solid line for AP52 shown in Figure 4 is the best-fit curve using eq 7 with parameters K_{sv}=2.0×10^4 M^{-1} and α=0.012. This, however, implies that the quenching is mostly dynamic and the contribution of the static quenching is very small.

Table II. Quenching Parameters Determined by Best Fits from Use of Equation 7

polymer	ξ	$K_{sv,a}$ (10^4 M^{-1})	α[a]
AP100	2.8	2.0	0.34
AP90	2.5	2.0	0.31
AP71	2.0	2.0	0.16
AP52	1.5	2.0	0.012
AP27	0.67	0.57[b]	0

[a] The fraction of condensed Tl^+ ions existing in the active sphere (see text). [b] Determined from the slope of the Stern-Volmer plot shown in Figure 4.

The dynamic term $(1+K_{sv,a}\xi^{-1}[Tl^+]_0)$ in eq 7 may be written as $(1+K_{sv}[Tl^+]_{DH,s})$ by using the local concentration of atmospheric Tl^+ ions close to the polymer backbone ($[Tl^+]_{DH,s}$) and the ordinary Stern–Volmer constant (K_{sv}). The local concentration of the Tl^+ ions can be given by

$$[Tl^+]_{DH,s}=[Tl^+]_{DH}\exp(\varphi_s/kT) \qquad (8)$$

where φ_s is the surface potential of the polymer. Thus, the apparent dynamic quenching constant is dependent on the surface potential according to

$$K_{sv,a}=K_{sv}\exp(\varphi_s/kT) \qquad (9)$$

According to Manning's theory, monovalent counterions condense onto a polyion of $\xi > 1$ until the effective charge density parameter is lowered to unity. This means that, after the condensation, the surface potentials of the polymers should be indpendent of the original ξ values. Therefore, in the context of Manning's theory, the $K_{sv,a}$ values should be the same for the polymers with $\xi > 1$. Accordingly, the value of $K_{sv,a}$ for AP52 obtained by best-fitting may be applicable to the other polymers with $\xi > 1$.

The experimental data for the three polymers with larger ξ were compared with the computed curves based on eq 7 in Figure 5. The solid lines drawn through the data plots in the figure are the theoretical curves optimally fitted by varying the parameter α. The value of $K_{sv,a}$ was constrained to be 2×10^4 M^{-1}. The concentration range of Tl^+ ions for this experiment is sufficiently low as compared to $[COO^-]_0$ ($[COO^-]_0$=4.6×10^{-4}, 5.9×10^{-4}, and 6.5×10^{-4} M for AP71, AP90, and AP100, respectively). Good agreement between the experiment and theory implies that the quenching events are reasonably explained by mixed Perrin and Stern–Volmer kinetics based on Manning's theory. The parameters that yielded best fits for the computations are listed in Table II. It should be noted that the value of α decreased sharply as ξ decreased. For AP100, 34% of the condensed Tl^+ ions are present in the active sphere, while for AP52, whose charge density is about half that of AP100, only 1.2% are in the active sphere. This large difference in the contribution of the static quenching may be due, in part, to the fact that "heavy atom quenching" by Tl^+ ions occurs only when a fluorophore and the quencher are in close contact.

In the present kinetic model, we assumed that the exchange of the condensed and free counterions was slow as compared to the life of the singlet-excited state of the Phen moieties. Taha and Morawetz[13] first pointed out that the counterion exchange rate relative to the lifetime of an excited chromophore was important in the interpretation of fluorescence quenching by counterions. They showed that in the quenching of uranyl ion fluorescence by Fe^{2+} in the presence of poly(vinylsulfonic acid), the counterion exchange was negligible over the lifetime of the excited uranyl ion (1.5×10^{-6} s).[13] The unperturbed lifetime of the Phen moieties in the present study was estimated to be 4×10^{-8} s, which is orders of magnitude shorter than relaxation times of counterion distribution in the radial direction around a rodlike polyion (10^{-7} s at the shortest)[21] and also counterion residence times calculated on the basis of the cell model of a rodlike polyion.[22] These considerations led us to assume that the counterion exchange was negligible in the time domain of the singlet lifetime of the Phen residues.

Mannings's theory considers only the charge of the counterions but does not consider any specificity for the condensation; i.e., binding free energy being entirely attributed to electrostatic forces.[5] On the basis of Manning's theory we assumed no particular preference for the condensation of Na^+ and Tl^+ ions on the AA–AAm copolymers. However, counterions can be "site bound" due to specific interactions or "territorially bound" due to solely electrostatic interactions. In fact, it has been reported that monovalent counterions interact selectively with poly(carboxylic acid)s.[23-25] For example, Rinaudo and Milas[23] showed an affinity order of $Li^+ > Na^+ > K^+ > Cs^+$ for (carboxymethyl)cellulose based on potentiometric titration data. Therefore, it is important to clarify whether the AA–AAm copolymers have a different affinity for Na^+ and Tl^+. Equation 4 assumes that the ratio of the condensed Tl^+ and Na^+ ions is given by the ratio of the total concentrations of these ions. If there is any specificity, eq 4 is not valid in its present form and it should be modified by introducing a specificity parameter. To test the validity of eq 4 and the applicability of eq 4 to salt-containing solutions, we are now investigating the effects of added salts not only with sodium salts but also with other monovalent and divalent metal salts.

Conclusions

Fluorescence of Phen moieties covalently attached to polyanions with varying ξ was efficiently quenched by Tl^+ ions in salt-free aqueous solution. The fluorescence quenching was satisfactorily interpreted by a kinetic model of mixed static and dynamic quenching based on Manning's condensation theory. In this model, fluorescence was assumed to be statically quenched by condensed Tl^+ ions according to the Perrin kinetics and also dynamically quenched by atmospheric Tl^+ ions according to the Stern–Volmer kinetics. The fraction of the static quenching decreased sharply with decreasing dimensionless charge density parameter ξ. When $\xi=2.8$, 34% of the condensed Tl^+ ions were estimated to exist in the active sphere, while the ratio drastically decreased to 1.2% when the charge density decreased to $\xi=1.5$.

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