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Spectroscopic Studies of Competitive Binding of Thallium and Alkaline-Earth Metal Cations onto Poly(sodium acrylate-*co*-acrylamide) Tagged with Optical Probes

Yotaro Morishima,* Takeshi Sato, and Mikiharu Kamachi

Department of Macromolecular Science, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

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ABSTRACT: Competitive binding between Tl^+ and alkaline-earth metal cations (Mg^{2+}, Ca^{2+}, and Ba^{2+}) onto copolymers of sodium acrylate and acrylamide with varying charge densities ($0.94 < \xi < 2.5$) was investigated by fluorescence and absorption spectroscopy with use of phenanthrene (Phen) and a pH-sensitive merocyanine dye (Mc) as optical reporters labeled on the copolymers. Fluorescence from the Phen label on the copolymer with $\xi = 2.5$ was efficiently quenched by Tl^+ because of the condensation of Tl^+ onto the copolymer, but the quenching was strongly suppressed by the addition of the divalent cations. There are two components in the fluorescence decay of the copolymer with $\xi = 2.5$ in the presence of Tl^+: fast and slow decay components attributed to the quenching by condensed and atmospheric Tl^+ ions, respectively. The addition of the divalent cations led to a marked decrease in the component of the fast decay, while the lifetime for the fast decay remained unchanged. Conversely, the lifetime and its component for the slow decay increased with increasing concentration of added divalent cations. Both the steady-state and time-dependent fluorescence data indicate that condensed Tl^+ ions are completely replaced by the added alkaline-earth metal cations even at low concentrations (on the order of 10^{-5} M) due to their highly preferential binding on the copolymer. In contrast, for the copolymer with $\xi = 0.94$ (onto which no Tl^+ condensation occurs but the divalent cations are condensed to decrease the charge density to $\xi = 0.5$), the divalent cations showed a much smaller suppressive effect on the fluorescence quenching by Tl^+. The highly preferential binding of alkaline-earth metal cations on the copolymers was also shown by spectroscopic behavior of the Mc label. The pK_{obs} for the acid−base equilibrium for the Mc label was decreased by added divalent cations but not affected at all by added Tl^+, indicating that Tl^+ is bound onto the polyanion less intimately than are the alkaline-earth metal cations. There was no significant difference between Tl^+ and Na^+ in the effect on the pK_{obs}, although Tl^+ was shown, in a previous study, to be bound onto the polyanions in preference to Na^+; the pK_{obs} does not distinguish the bound Tl^+ and Na^+ species, but fluorescence of the Phen label does.

Introduction

Fluorescence of aromatic chromophores is quenched by Tl^+ due to a heavy atom effect[1] which requires a close contact of Tl^+ with a fluorophore. Recently, a number of investigations have focused on the use of Tl^+ as a quencher for fluorescence from aromatic chromophores labeled on polyelectrolytes with an aim of the characterization of the polyelectrolytes in aqueous solution.[2−9] Fluorescence quenching of polyanion-bound aromatic chromophores by Tl^+ is very efficient owing to polyion−counterion interactions.[2−4] In previous papers, we reported on the fluorescence quenching of the phenanthrene (Phen)-labeled copolymers of sodium acrylate (NaAA) and acrylamide (AAm) by Tl^+ in aqueous solution with[10] and without[4] added alkali metal salts and attempted to explain the efficient fluorescence quenching by Tl^+ on the basis of Manning's theory.

The counterions can exist in a variety of states and locations with respect to a polyelectrolye chain in aqueous solution depending on the type and magnitude of the interactions with the polyelectrolyte. Manning's counterion condensation theory,[11] which considers only electrostatic interactions, can explain some thermodynamic behavior of polyelectrolytes,[12,13] kinetic effects of polyelectrolytes on some chemical reactions,[14,15] and acid−base equilibria of pH-sensitive dyes tagged on polyelectrolytes.[16−18] However, in some cases, Manning's theory fails to explain the behavior of polycarboxylates in the presence of multivalent cations,[19−24] because it does not take into account any specific interactions.[11] Binding of Mg^{2+} and Ca^{2+} to dextran sulfate in the presence of various monovalent salts was studied by measuring free divalent cation activity by use of a dye spectrophotometric technique,[25−27] and the results were explained by Manning's two-variable theory.[28]

Our earlier article was concerned with the fluorescence quenching of the Phen label by Tl^+ in the presence of Li^+, Na^+, and K^+ added in excess amounts.[10] The results indicated that Tl^+ had a strong preference in the binding onto the polyanions as compared with the alkali metal cations. Counterions can be "site bound" due to specific interactions or "territorially bound" due to solely electrostatic interactions.[11] Our previous study of the fluorescence decay of the Phen label suggested that Tl^+ ions were not site bound but were territorially bound onto the polyanions.[10]

We demonstrated previously that a pH-sensitive merocyanine dye (Mc) tagged on polyanions provided a useful tool to investigate the counterion condensation.[18] Cations of Mg^{2+}, Ca^{2+}, Ba^{2+}, and Sr^{2+} added in a very small amount caused a significant decrease in pK_{obs} (negative logarithm of the apparent dissociation constant) for the acid−base equilbrium of the Mc dye labeled on a polyanion with a high charge density, whereas no such effects were recognized with added Li^+, Na^+, and K^+. These observations imply that a low concentration of divalent cations are exhaustively bound on the polyanion in preference to coexisting monovalent cations.

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In the present study, we investigated the competitive binding between Tl^+ and alkaline-earth metal cations on the copolymers of NaAA and AAm by fluorescence quenching of the Phen label tagged on the copolymers. In order to gain insight into the intimacy of the condensed Tl^+ with the polyanions, as compared with that of the divalent cations, we also investigated the effects of added Na^+, Tl^+, and Ca^{2+} on the acid−base equilibrium of the Mc label tagged on the copolymer.

Experimental Section

Materials. The Phen-labeled copolymers of NaAA and AAm were prepared by terpolymerizaton of NaAA, AAm, and *N*-(9-phenanthrylmethyl)methacrylamide as reported previously.[4] The contents of the Phen label were determined from the absorbance at 347 nm due to the 0−0 band of the Phen moiety. The molar ratios of NaAA/AAm in the copolymers were determined by titration.[10]

The Mc-labeled copolymer of NaAA and AAm was prepared by terpolymerization of NaAA, AAm, and 1-(2-(methacryloyloxy)ethyl)-4-(2-(4-hydroxyphenyl)ethenyl)pyridinium bromide according to our previous method.[18] The composition of the terpolymer was determined by the C/N ratio in elemental analysis in combination with the absorbance due to the Mc label.

Analytical grade $TlNO_3$, $Mg(NO_3)_2$, $Ca(NO_3)_2$, and $Ba(NO_3)_2$ were used without further purification. Water was doubly distilled and deionized by passing through an ion-exchange column.

Measurements. Sample solutions for the steady-state and time-dependent fluorescence measurements on the Phen-labeled NaAA−AAm copolymers were prepared by volumetric dilution of stock solutions of the copolymers, $TlNO_3$, $Mg(NO_3)_2$, $Ca(NO_3)_2$, and $Ba(NO_3)_2$ in pure water. The diluted solutions of the polymer, $TlNO_3$, and one of the alkaline-earth metal salts were mixed at a predetermined ratio. The pH of the sample solutions was adjusted to 8.5 by adding a small amount of aqueous NaOH with a micropipet. The concentration of the Phen moiety was adjusted to 1.0×10^{-5} M in all sample solutions. All the steady-state and time-dependent fluorescence measurements were performed at the constant pH of 8.5 at room temperature by excitation of the Phen label at 297 nm. The sample solutions were purged with Ar for 30 min prior to measurements.

Steady-state fluorescence spectra were recorded on a Shimadzu RF-502A spectrophotometer. For Stern−Volmer plots the peak intensities at 357 nm in the Phen fluorescence spectra were employed.

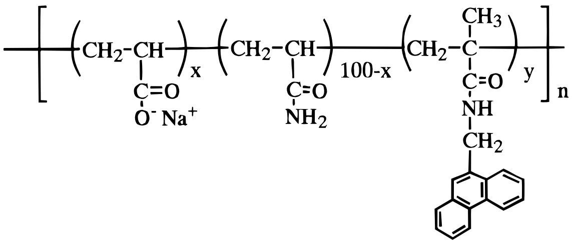
Fluorescence decays were measured by a time-correlated single-photon counting technique using a Horiba NAES 550 system. The decay curves were analyzed by conventional deconvolution techniques.

The determination of the acid−base equilibrium constants for the Mc label in the copolymer was performed by spectroscopic titration as reported previously.[18] The pH values of the sample solutions for the titration were adjusted with aqueous NaOH and aqueous HCl by use of a micropipet. The UV−visible absorption spectra due to the Mc label were measured in the pH range 8−11. The pK_{obs} values (the negative logarithm of the apparent dissociation constants) were calculated according to a previous report.[18] The pH readings were obtained on a Horiba H-7 LD pH meter with a Horiba combination electrode no. 6328. Absorption spectra were recorded on a Shimadzu UV-2100 spectrophotometer.

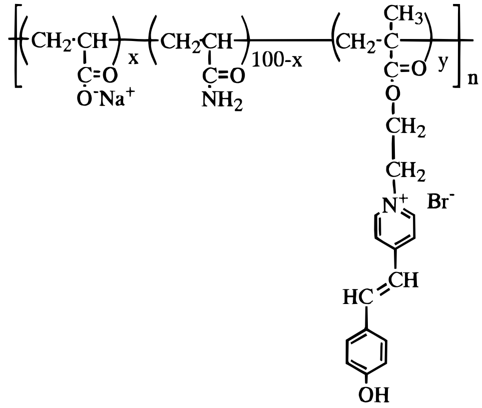
Results and Discussion

Polyelectrolytes are characterized by a dimensionless charge density parameter ξ which is defined as $\xi = e^2/\epsilon kTb$, where e is the protonic charge, ϵ the dielectric constant of the solvent, k the Boltzmann constant, T the Kelvin temperature, and b the average axial spacing between charged groups on the polyelectrolytes.[11] The Phen-labeled copolymers of NaAA and AAm employed in the present study have three different charge densities, i.e., $\xi = 2.5$, 1.5, and 0.94, corresponding to NaAA contents of 90, 55, 34 mol % on the basis of the total NaAA and AAm units in the copolymers (Scheme 1). These ξ values are calculated from the average b values based on the copolymer compositions.[17,18] In the text, the copolymers are abbreviated as AP90, AP55, and AP34, the numbers indicating the mole percent contents of the NaAA unit. The loading amounts of the Phen label on the copolymers are in the range 1.5−2.6 mol %.

Scheme 1



AP90 : x = 90mol% , y = 2.6 mol% ; $\xi = 2.5$
AP55 : x = 55mol% , y = 1.9 mol% ; $\xi = 1.5$
AP34 : x = 34mol% , y = 1.5 mol% ; $\xi = 0.94$



AMc90 : x = 90 mol% , y = 4 mol% ; $\xi = 2.5$

In Manning's theory,[11] 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 (ξ_{eff}) equals ξ_c. When $\xi < \xi_c$, on the other hand, the counterions are completely dissociated. In AP90 ($\xi = 2.5$), for example, 60% of the polymer charge is neutralized by condensed monovalent cations such that ξ_{eff} is reduced to 1, while 80% of the charge is neutralized by condensed divalent cations such that ξ_{eff} is reduced to 0.5. The remaining polymer charge is compensated by atmospheric counterions through Debye−Hückel interactions. If a salt of a divalent cation is added to a polyanion solution containing a salt of a monovalent cation, the already condensed monovalent cations are expelled by the divalent cations condensed onto the polyanion to the extent that $\xi_{eff} = 0.5$.

Previously, we reported that fluorescence from the Phen label on the copolymers with $\xi > 1$ was efficiently quenched by condensed Tl^+ showing an upward curvature in the Stern−Volmer plot, whereas the copolymers with $\xi < 1$ showed a straight line due to dynamic quenching by atmospheric Tl^+.[4,10]

Figure 1 compares the Stern−Volmer plots for fluorescence quenching by AP90 by Tl^+ in the absence and presence of various added alkaline-earth metal salts at

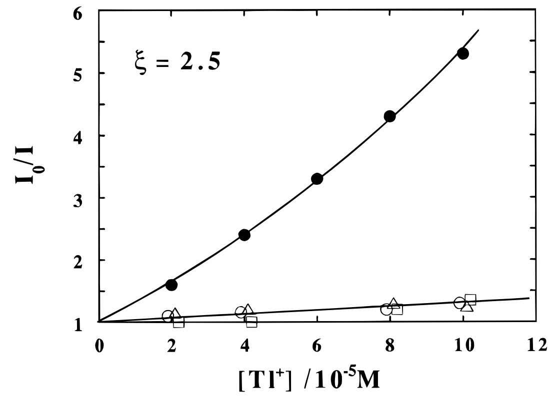


Figure 1. Stern−Volmer plots for fluorescence quenching of AP90 by Tl^+ in the absence (●) and presence of 1.76×10^{-4} M of $Mg(NO_3)_2$ (○), $Ca(NO_3)_2$ (△), and $Ba(NO_3)_2$ (□).

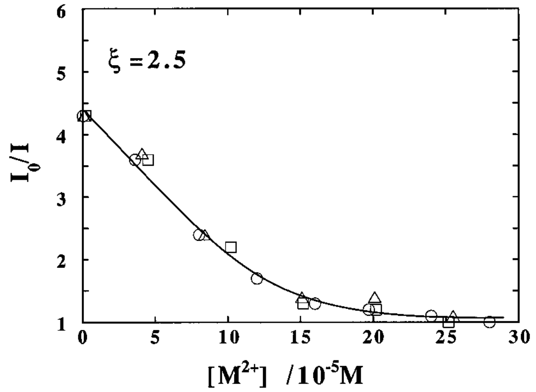


Figure 2. Plots of I_0/I at a constant concentration of 8.0×10^{-5} M Tl^+ for AP90 vs concentrations of added Mg^{2+} (○), Ca^{2+} (△), and Ba^{2+} (□) in the nitrate form. I_0 and I represent the Phen fluorescence intensities in the absence and presence of Tl^+, respectively.

a constant concentration of 1.76×10^{-4} M. As expected, the fluorescence quenching is strongly suppressed by the addition of the divalent salts because Tl^+ ions condensed on AP90 are expelled into the bulk solution by preferential condensation of the added divalent cations. In Figure 2, I_0/I values are plotted as a function of the concentration of the alkaline-earth metal cations added in the form of the nitrate salt, where I_0 and I are the fluorescence intensities in the absence and presence of 8.0×10^{-5} M Tl^+. In this experiment, the background concentration of the Na^+ counterion (from the NaAA unit) is 3.45×10^{-4} M. The I_0/I values markedly decrease even at low concentrations of the added divalent salts of the order of 10^{-5} M which is the same order of magnitude as the concentration of Tl^+ and 1 order of magnitude lower than the background concentration of the Na^+ counterion. In the concentration range (2−3) $\times 10^{-4}$ M of the added divalent cations, the I_0/I values fall down to ca. 1, which means that the fluorescence that was quenched by condensed Tl^+ almost completely recovers because the condensed Tl^+ ions are completely kicked out into the bulk phase by the divalent cations. Furthermore, the electrostatic interaction between the polymer and the atmospheric Tl^+ ions should be decreased because the polymer charge is neutralized by the condensed divalent cations to the extent that $\xi_{eff} = 0.5$. In addition, the atmospheric Tl^+ ions should be displaced with the divalent cations remaining in the bulk solution because of their higher effective charge densities. These effects should suppress collisional quenching[4,10] by atmospheric Tl^+.

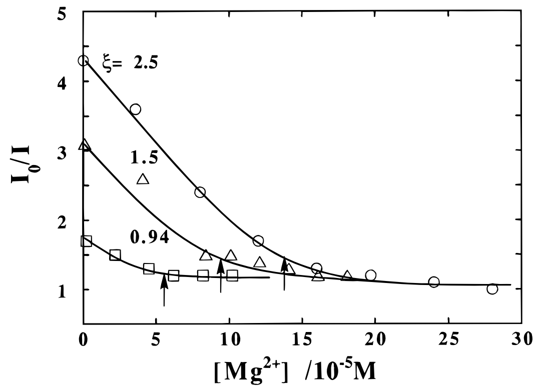


Figure 3. Plots of I_0/I at a constant concentration of 8.0×10^{-5} M Tl^+ for AP90 ($\xi = 2.5$), AP55 ($\xi = 1.5$), and AP34 ($\xi = 0.94$) vs concentration of Mg^{2+}. Arrows indicate the C_e (see text) of Mg^{2+}.

Figure 3 compares the effects of added Mg^{2+} on the fluorescence quenching at a constant concentration of 8.0×10^{-5} M Tl^+ for the polyanions with varying ξ. The addition of Mg^{2+} (from $Mg(NO_3)_2$) causes a larger decrease in I_0/I for the polyanions with larger ξ. However, the effect of added Mg^{2+} becomes less pronounced as ξ decreases. For AP90 ($\xi = 2.5$), I_0/I decreases rather sharply in the Mg^{2+} concentration region on the order of 10^{-5} M. By contrast, for AP34 ($\xi = 0.94$), on which no Tl^+ ions are condensed because $\xi < 1$, there is a slight decrease in I_0/I due to the reduced atmospheric interaction and the displacmeent of the atmospheric Tl^+ ions by the added Mg^{2+} as discussed above. It should be noted that the I_0/I values decrease sharply in the lower concentration region of added Mg^{2+} and then remain almost constant, merging into almost the same I_0/I value with a further increase in the concentration of added Mg^{2+} regardless of the ξ values of the polyanions. The concentrations of the carboxylate residues in AP90, AP55, and AP34 in these experiments were 3.45×10^{-4}, 2.88×10^{-4}, and 2.21×10^{-4} M, respectively. Manning's theory predicts that 80, 66.5, and 47% of the carboxylate groups in AP90, AP55, and AP34 are neutralized by condensed Mg^{2+} ions, respectively.

We define a concentration of Mg^{2+} that is equivalent to the concentration of the neutralized charge on the polyanion as the "equivalent concentration" (C_e).[18] The arrows indicated in Figure 3 show the C_e values for the three polymers. As can be seen from Figure 3, the I_0/I values strongly depend on the concentration of Mg^{2+} at concentrations lower than C_e and the I_0/I values merge into almost the same values for all the three polymers at Mg^{2+} concentrations higher than C_e. Namely, a transition from the strongly dependent regime to the much less dependent regime seems to occur at Mg^{2+} concentrations in the vicinity of C_e, although the transition occurs rather gradually. These observations imply that, at concentrations lower than C_e, added Mg^{2+} ions are exhaustively condensed onto the polyanions, and at C_e the fraction of $1 - (N\xi)^{-1}$ of the polymer charge is neutralized. At concentrations higher than C_e, however, an excess of Mg^{2+} ions exists in the bulk phase atmospherically interacting with the polyanions.

Figure 4 compares fluorescence decay data including the best-fit curves for AP90 in the presence of 8.0×10^{-5} M of Tl^+ and varying concentrations of added Mg^{2+}. A decay curve for a quencher-free solution without added salt is also indicated in Figure 4 for comparison, which can be reasonably fitted with a single-exponential decay

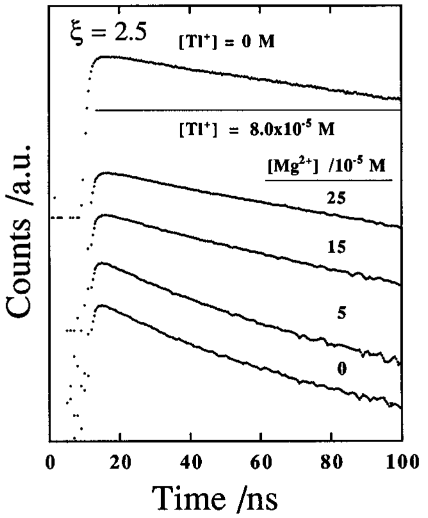


Figure 4. Fluorescence decay curves for AP90 at a constant concentration (8.0×10^{-5} M) of Tl^+ in the absence and presence of Mg^{2+} at varying concentrations. A decay curve for AP90 in a Tl^+-free solution without added salt is presented for comparison.

function with a lifetime of 36 ns. The decay becomes faster in the presence of Tl^+, which is best-fitted by a double-exponential function with shorter-lived and longer-lived fluorescence components. Taken together with steady-state fluorescence quenching data, we previously attributed the shorter-lived component to quenching by condensed Tl^+ and the longer-lived component to collisional quenching by atmospheric Tl^+.[10] When Mg^{2+} is added to an AP90 solution containing Tl^+, the fluorescence decay curve is significantly changed such that the decay becomes slow and importantly the contribution from the shorter-lived component becomes less pronounced with increasing concentration of Mg^{2+}. The same tendency was observed with other alkaline-earth metal cations.

The lifetimes of the shorter- and longer-lived components (τ_1 and τ_2, respectively) and the fractions of the shorter- and longer-lived components (α and $1 - \alpha$, respectively) at a constant concentration (8.0×10^{-5} M) of Tl^+ are plotted in Figure 5 as a function of the concentration of Mg^{2+}. The lifetimes of the shorter-lived component are confined in a narrow range of 7–8 ns independent of the concentration of added Mg^{2+}. However, the fractional contribution of the shorter-lived component sharply decreases with increasing Mg^{2+} concentration in a manner similar to the decrease in the I_0/I value shown in Figure 3. Conversely, the lifetime of the longer-lived component and its contribution increase with increasing Mg^{2+} concentration. This is an experimental manifestation that the fraction of quenching by condensed Tl^+ is decreased and, in turn, the fraction of quenching by atmospheric Tl^+ is increased as the concentration of added Mg^{2+} is increased in the low-concentration regime of $(0-2) \times 10^{-4}$ M Mg^{2+}. Namely, the condensed Tl^+ ions are expelled by the divalent cation into the bulk solution where Tl^+ ions can only interact with the polyanion atmospherically, leading to collisional quenching with less quenching efficiency. At concentrations of Mg^{2+} higher than ca. 2.5×10^{-4} M, all fluorescence quenching is essentially due to the collision of the atmospheric Tl^+ with the polymer-bound fluorophore. This is an indication that all condensed Tl^+ ions are completely expelled by Mg^{2+}.

The preferential binding of alkaline-earth metal cations on the polyanions can be shown by spectroscopic information provided by the pH-sensitive Mc label on the polyanions.[18] The Mc label shows absorption peaks at 452 and 378 nm due to the quinoid (D) and benzenoid (DH^+) forms, respectively (Scheme 2).[18] The acid–base equilibrium of the Mc label is affected by interfacial electrostatic potential exerted by the polyanion such that the equilibrium shown in Scheme 2 shifts toward left hand side. In other words, pK_{obs} increases due to the negative potential;[18] the pK_{obs} for a monomeric Mc dye is 8.52 in a salt-free aqueous solution, whereas it increases to ca. 10.1 when the Mc moiety is covalently attached to an NaAA–AAm copolymer with $\xi = 2.5$.[18]

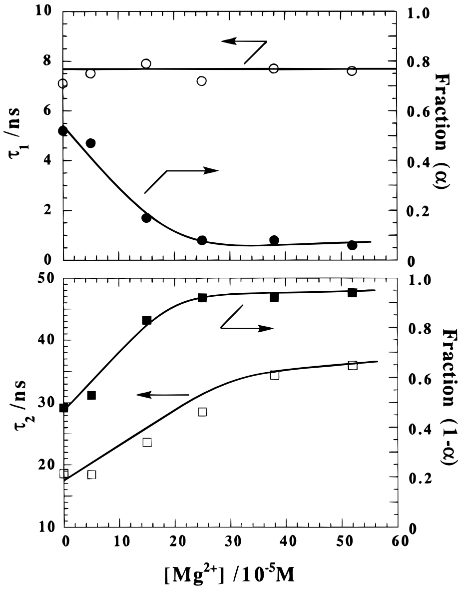
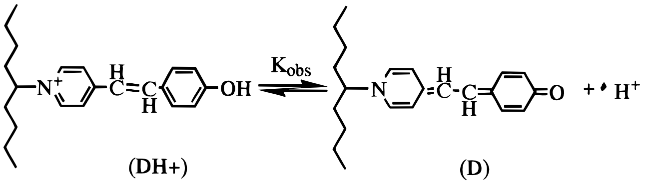


Figure 5. Lifetimes of the shorter- and longer-lived components and their fractions for AP90 at a constant concentration (8.0×10^{-5} M) of Tl^+ plotted vs concentration of added Mg^{2+}.

Scheme 2



In an earlier paper, we reported that the condensed Tl^+ ions on AP90 were not replaced by Li^+, Na^+, and K^+ ions even though they are added in excess amounts, i.e., a preferential binding of Tl^+ over the alkali metal cations.[10] This is a sharp contrast to the effects of added Mg^{2+}, Ca^{2+}, and Ba^{2+} which completely expel the condensed Tl^+.

In Figure 6 are plotted the pK_{obs} values for AMc90 ($\xi = 2.5$) (Scheme 1) as a function of the concentration of various added cations. In these experiments, nitrates of Na^+, Tl^+, and Ca^{2+} were added at varying concentrations to solutions of AMc90 at a constant concentration of 3.45×10^{-4} M NaAA unit. Before adding the salts, 60% of charge in AMc90 is already neutralized by condensed Na^+ counterions given that the background concentration of Na^+ is 3.45×10^{-4} M. We previously reported that the pK_{obs} values were not affected by added salts of Li^+, Na^+, and K^+ ions but were markedly decreased by added Mg^{2+}, Ca^{2+}, Ba^{2+}, and Sr^{2+} ions.[18] When the alkaline-earth metal cations are added, the divalent cations are condensed on AMc90 by expelling the already condensed Na^+ counterions. Divalent cations have a stronger tendency for a site binding, and

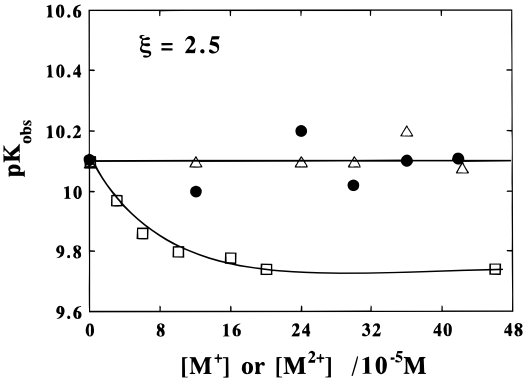


Figure 6. Plots of pK_{obs} for the acid−base equilibrium of the Mc label in AMc90 (ξ = 2.5) vs concentrations of added Na$^+$ (△), Tl$^+$ (●), and Ca^{2+} (□).

therefore they are more intimately involved with polyanions than are condensed monovalent cations.[29] In other words, the condensation layer[28] of the divalent cations is "thinner" than that of the monovalent cations. Since the AMc90 charge is neutralized within a thin layer of the condesned divalent cations (the layer should be thinner than the distance of the Mc label from the polymer main chian[18]), the Mc label experiences a reduced electrostatic potential, leading to a decrease in the pK_{obs} value.[18] In Figure 6 is shown a considerable decrease in pK_{obs} due to the effective charge neutralization by the addition of very low concentrations of Ca^{2+}. When Tl$^+$ ions are added to a solution of AMc90, the already condensed Na$^+$ counterions should be replaced by Tl$^+$ even at low concentrations of the added Tl$^+$ ions, according to our previous study.[10] However, as can be seen in Figure 6, there is no significant effect of added Tl$^+$ on pK_{obs} (although the plots are scattered). Namely, added Tl$^+$ ions appear to behave in a manner similar to added Na$^+$ in the effect on the acid−base equilibrium of the Mc label in AMc90, despite the fact that the Tl$^+$ ions are condensed onto AP90 in preference to the Na$^+$ ions, thus the already condensed Na$^+$ ions being driven out into the bulk solution.[10] Therefore, Figure 6 implies that there is no significant difference in the microscopic behavior between Tl$^+$ and Na$^+$ condensed on the polyanion; Tl$^+$ and Na$^+$ are involved much less intimately with the polyanion than Ca^{2+}.

Conclusions

Competitive binding between Tl$^+$ and alkaline-earth metal cations (Mg^{2+}, Ca^{2+}, and Ba^{2+}) onto copolymers of sodium acrylate and acrylamide with varying charge densities (0.94 < ξ < 2.5) was investigated. Both steady-state and time-dependent fluorescence of phenanthrene (Phen) tagged on the copolymer with ξ = 2.5 showed a strong preference of the divalent cations over Tl$^+$ in the binding; condensed Tl$^+$ ions were completely replaced by the added alkaline-metal cations even at low concentrations of the divalent cations on the order of 10^{-5} M.

The preferential binding of alkaline-metal cations was also shown by a pH-sensitive merocyanine (Mc) dye tagged on the copolymer with ξ = 2.5; pK_{obs} for the acid−base equilibrium for the Mc label was decreased by added divalent cations but not affected by added Tl$^+$, implying that Tl$^+$, unlike the alkaline-earth metal cations, is territorially bound onto the polyanions and not site-bound. There was no significant difference between Tl$^+$ and Na$^+$ in the effect on the pK_{obs}, although Tl$^+$ was shown, in a previous study, to be bound onto the polyanions in preference to Na$^+$.

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