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Preferential Binding of Thallium Cations onto Poly(sodium acrylate-*co*-acrylamide) in the Presence of Alkali Metal Salts in Aqueous Solution As Studied by Fluorescence Quenching of Polymer-Bound Phenanthrene Labels

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ABSTRACT: Binding of Tl^+ ions on phenanthrene (Phen)-labeled copolymers of sodium acrylate and acrylamide with different charge densities ($\xi = 2.5$ and 0.94) was studied by fluorescence quenching in aqueous solution with added alkali metal salts. The polyanion with $\xi = 2.5$ showed very efficient quenching of Phen fluorescence with an upward curvature in a Stern−Volmer plot. The fluorescence decays, in the presence of Tl^+, consist of two components; fast and slow decays are attributed to quenching by bound and free Tl^+ ions, respectively. Both the steady-state and time-dependent fluorescences indicate that the fluorescence quenching by Tl^+ (8.0×10^{-5} M) is not affected at all by the addition of Li^+, Na^+, and K^+ ions (5.0×10^{-4} M), indicating that polymer-bound Tl^+ ions are not replaced by the added alkali metal cations. In contrast, the polymer with $\xi = 0.94$ shows much less effective quenching with a straight line in a Stern−Volmer plot. There is no fast decay component due to the quenching by bound Tl^+ in the Phen fluorescence. Unlike the case of the polymer with $\xi = 2.5$, fluorescence quenching is caused by atmospheric Tl^+. The fluorescence quenching by Tl^+ was significantly reduced by the addition of the alkali metal cations, indicating that the added alkali metal cations can displace the atmospheric Tl^+. These data show that the binding of Tl^+ onto the polyanion with a high charge density is preferential over the binding of Li^+, Na^+, and K^+ ions.

Introduction

Fluorescence quenching of fluorescent-labeled water-soluble polymers by Tl^+ provides a useful tool to study conformational and dynamic behavior of the polymers in aqueous solution.[1−9] This is particularly true for the characterization of self-organizing amphiphilic polymers labeled with aromatic chromophores.[5−9] Amphiphilic polymers may form hydrophobic microdomains in micelle-like structures in aqueous solution, where aromatic labels would be trapped. Since Tl^+ can only exist in the aqueous phase, fluorophores confined to the hydrophobic microdomains are protected from quenching by Tl^+, and thus the formation of the self-organized structures can be verified.[5−9]

Fluorescence of polyanion-bound aromatic chromophores is efficiently quenched by Tl^+ due to polyion−counterion interactions.[1−4,8] The counterions are distributed in a variety of fashions between the polyanion and the bulk phase depending on the strength of the electrostatic interactions. Manning's counterion condensation theory,[10] though it is a simple theory with only one nonadjustable parameter, can explain fairly satisfactorily thermodynamic and interfacial properties of polyelectrolytes[11,12] and effects of polyelectrolytes on some chemical reactions[13,14] and on acid−base equilibria of pH-sensitive dyes covalently attached to polyanions.[15−17] Manning's theory does not consider any specific interactions for the counterion condensation, binding free energy being entirely attributed to electrostatic interactions.[10] In our previous study, we attempted to explain fluorescence quenching of phenanthrene (Phen)-labeled copolymers of sodium acrylate (NaAA) and acrylamide (AAm) by Tl^+ in salt-free aqueous solution on the basis of Mannin's theory in conjunction with combined Perrin and Stern−Volmer models.[3] In the kinetic analysis, we assumed no particular preference in the binding of Tl^+ (as compared with Na^+ from the NaAA unit) to the copolymers, but we pointed out a possibility that Tl^+ is bound to the polyanions through a specific interaction.[3] Counterions can be "site bound" due to specific interactions or "territorially bound" due to solely electrostatic interactions. There are several papers which report selective interactions of monovalent counterions with poly(carboxylic acids).[18−20] For example, Rinaudo and Milas[18] reported an affinity order of $Li^+ > Na^+ > K^+ > Cs^+$ for carboxymethylcellulose based on potentiometric titration data. Therefore, it is important to clarify whether Tl^+ has an affinity toward polyanions different from those of alkali metal cations.

In the present study, we investigated the effects of some added alkali metal salts on the fluorescence quenching of the Phen-labeled NaAA−AAm copolymers by Tl^+ and attempted to clarify whether Tl^+ is condensed on the polyanions preferentially over such alkali metal cations as Li^+, Na^+, and K^+.

Experimental Section

Materials. The terpolymers of sodium acrylate (NaAA), acrylamide (AAm), and *N*-(9-phenanthrylmethyl)methacrylamide (PhenMAm) were prepared as reported previously.[3] The contents of the PhenMAm unit in the terpolymers were calculated from the absorbances at 347 nm (0−0 band of phenanthrene) in UV absorption spectra by assuming that the molar extinction coefficient (ϵ) of the PhenMAm unit is the same as that of 9-phenanthreneacetic acid ($\epsilon = 710$ cm^{-1} M^{-1} at 347 nm) in aqueous solution at pH 8.5. The contents of the NaAA unit in the terpolymers were determined by titration; a polymer solution of a known concentration was first acidified to pH 3 by adding aqueous HCl and then titrated with aqueous NaOH.

Analytical grade $TlNO_3$, $LiNO_3$, $NaNO_3$, and KNO_3 were used without further purification. Water was doubly distilled and deionized by passing through an ion-exchange column.

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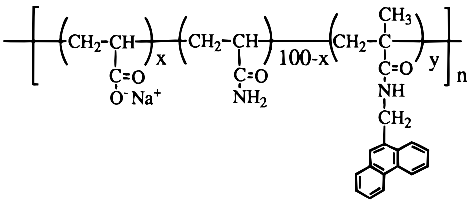
Chart 1



AP90 : x = 90 mol% , y = 2.6 mol%
AP34 : x = 34 mol% , y = 1.5 mol%

Measurements. Sample solutions for the steady-state and time-dependent fluorescence measurements were prepared by volumetric dilution of stock solutions of the polymers, $TlNO_3$, $LiNO_3$, $NaNO_3$, and KNO_3 in pure water. The diluted solutions of the polymer, $TlNO_3$, and one of the alkali 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 residue 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 residues at 297 nm. Prior to measurements, the sample solutions were deaerated by bubbling with Ar for 30 min.

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.

Results

The Phen-labeled copolymers of NaAA and AAm used in the present study are of two different charge densities, i.e., $\xi = 2.5$ and 0.9, ξ being 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 polyanion.[10] In the text, the polymers are abbreviated as AP90 and AP34, the numbers indicating the mole percent content of the NaAA unit on the basis of the total NaAA and AAm units in the polymers (Chart 1). Since the sequence distribution of the NaAA and AAm units in the copolymers is random, the "average" ξ value for each polymer can be calculated from the average b value based on the copolymer composition.[16,17] The amounts of the Phen label tagged on AP90 and AP34 are 2.6 and 1.5 mol %, respectively. In aqueous solution, the Phen labels are forced to experience microenvironments in the vicinity of the polymer backbone because of their covalent bonding.

Steady-State Fluorescence Studies. Manning's theory[10] predicts that if $\xi > 1$, monovalent countercations condense onto a polyanion until the fraction $1 - \xi^{-1}$ of polymer charge is neutralized. The remaining polymer charge is compensated for by atmospheric counterions through Debye−Hückel interactions. Previously, we reported on fluorescence quenching of the Phen-labeled NaAA−AAm copolymers by Tl^+ in aqueous solution.[3] Fluorescence quenching by Tl^+ occurs only via close contact with the Phen label due to a "heavy atom effect",[21] which may preclude long-range quenching due to electron transfer, if not completely. In Stern−Volmer plots, the copolymers with $\xi > 1$ showed an upward curvature, whereas the copolymers with $\xi < 1$ showed a straight line, and we attempted to explain the upward curvature in terms of a mixed static and dynamic quenching; the static quenching by condensed Tl^+ and the dynamic quenching by atmospheric Tl^+.[3]

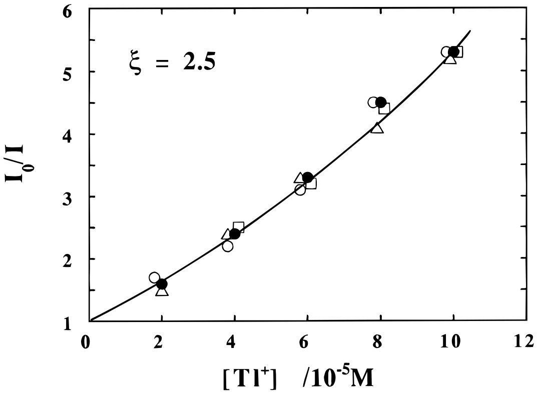


Figure 1. Stern−Volmer plots for fluorescence quenching of AP90 by Tl^+ in the absence (●) and presence of 8.0×10^{-5} M $LiNO_3$ (○), $NaNO_3$ (△), and KNO_3 (□).

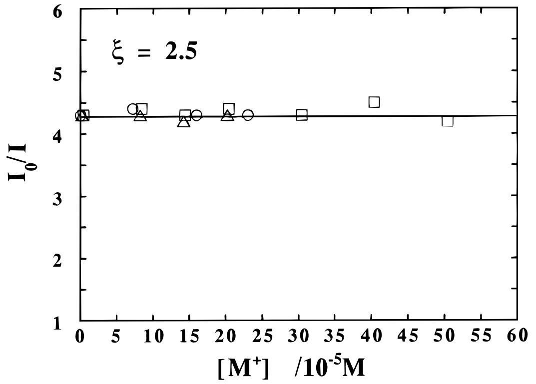


Figure 2. Plots of I_0/I at a constant concentration of 8.0×10^{-5} M Tl^+ for AP90 as a function of the concentrations of added Li^+ (○), Na^+ (△), and K^+ (□) in the nitrate form. I_0 and I represent the Phen fluorescence intensities in the absence and presence of Tl^+, respectively.

According to Manning's theory, 60% of the charge in AP90 ($\xi = 2.5$) is compensated for by condensed Na^+ ions in a quencher-free solution. When Tl^+ is added, some of the condensed Na^+ may be replaced by added Tl^+, 60% of the polymer charge remaining to be neutralized. Figure 1 compares the Stern−Volmer plots for AP90 in the absence and presence of various added alkali metal salts. We had expected that the contribution of quenching by the condensed Tl^+ would have been decreased by adding the salts because we had thought that some of the condensed Tl^+ ions should have been replaced by the added cations. However, we recognized that fluorescence quenching for AP90 was not affected by added alkali metal salts, as can be seen in Figure 1.

In Figure 2, I_0/I values at a constant concentration (8.0×10^{-5} M) of Tl^+ are plotted as a function of the concentration of added salts, where I_0 and I are the fluorescence intensities in the absence and presence of 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 remain intact even though the salts are added beyond the background Na^+ concentration which is far beyond the Tl^+ concentration. These results indicate that Tl^+ ions condensed on AP90 are not expelled by added Li^+, Na^+, and K^+ ions.

In the case of AP34 ($\xi = 0.94$), Manning's theory predicts that no Tl^+ condensation occurs because $\xi < 1$. The Stern−Volmer plot for AP34 shows a straight line

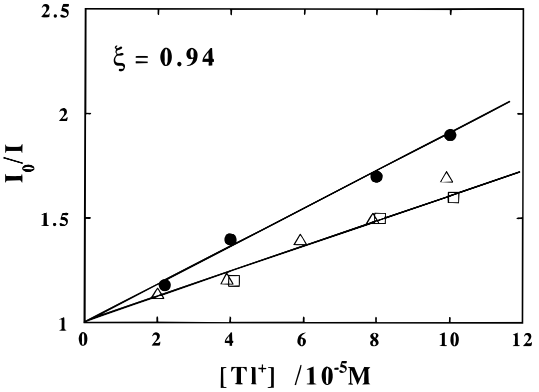


Figure 3. Stern−Volmer plots for fluorescence quenching of AP34 by Tl$^+$ in the absence (●) and presence of 8.0 × 10^{-5} M NaNO$_3$ (△) and KNO$_3$ (□).

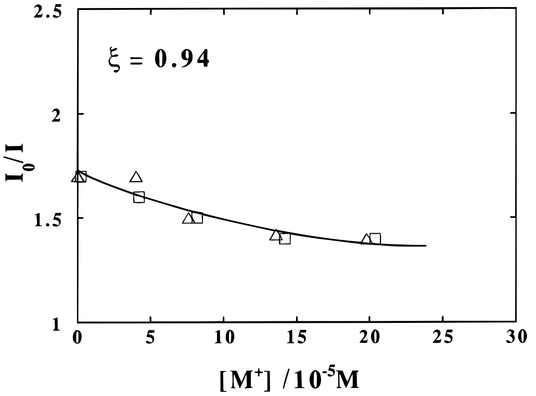


Figure 4. Plots of I_0/I at a constant concentration of 8.0 × 10^{-5} M Tl$^+$ for AP34 as a function of the concentrations of added Na$^+$ (△) and K$^+$ (□) in the nitrate form.

due to complete dynamic quenching by only atmospheric Tl$^+$ (Figure 3). In contrast to the AP90 case, fluorescence quenching of AP34 significantly decreases by adding alkali metal salts (Figure 3). As shown in Figure 4, I_0/I decreases with increasing concentration of the added salts even in a low concentration regime. The fluorescence quenching occurs via collision of atmospheric Tl$^+$ with the Phen label in AP34. When an alkali metal salt is added, the thickness of the ionic atmosphere decreases and some atmospheric Tl$^+$ ions are displaced with alkali metal cations, leading to a decreased chance for the Phen label to collide with Tl$^+$.

Fluorescence Decay Studies. Figure 5 compares fluorescence decay curves for AP90 in the absence and presence of Tl$^+$ in aqueous solution. A quencher-free solution of AP90 shows a single-exponential decay with a lifetime of 36 ns. The decay becomes increasingly fast with the increasing amount of added Tl$^+$. The decay curves, in the presence of Tl$^+$, are best-fitted with a double-exponential function

$$I(t)/I(t_0) = \alpha \exp(-t/\tau_1) + (1-\alpha)\exp(-t/\tau_2) \quad (1)$$

where τ_1 and τ_2 are the lifetimes of the fast and slow decay components, respectively, and α is the fraction of the fast decay component.

In Figure 6 are plotted the fluorescence lifetimes and the fraction of the short lifetime component as a function of the concentration of added Tl$^+$. The lifetime of the shorter-lived component is ca. 7 ns, independent of the concentration of Tl$^+$, while the lifetime of the longer-lived component decreases progressively with increasing Tl$^+$ concentration. An important observation to be noted is that the fraction of the shorter-lived component markedly increases as the Tl$^+$ concentration is increased, although its lifetime remains the same. In conjunction with the steady-state fluorescence quenching data, it may be reasonable to consider that the shorter-lived component arises from quenching by condensed Tl$^+$ and that the longer-lived component is a result of collisional quenching by atmospheric Tl$^+$.

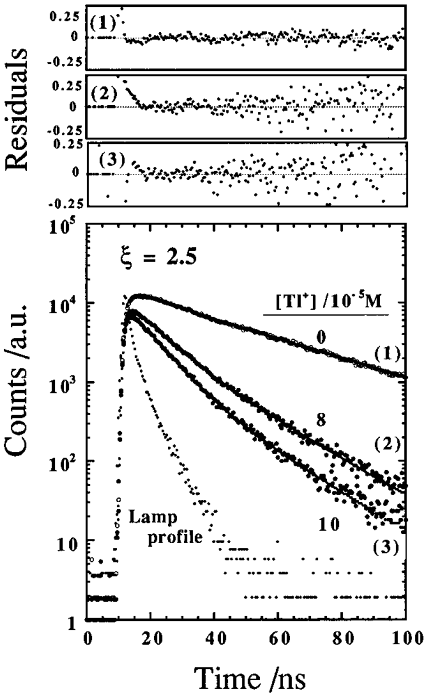


Figure 5. Fluorescence decay profiles for AP90 in the absence and presence of Tl$^+$. The concentrations of Tl$^+$ are indicated in the figure.

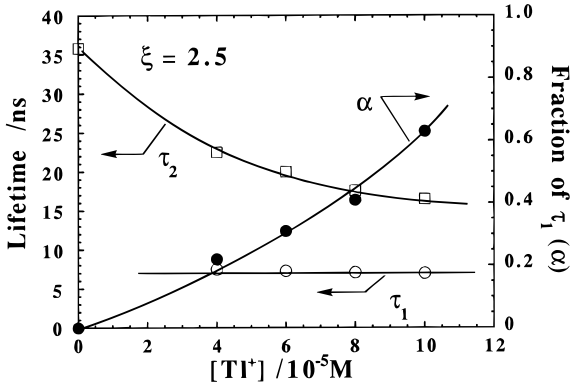


Figure 6. Lifetimes of the shorter- and longer-lived components and the fraction of the shorter-lived component for AP90 plotted as a function of the concentration of Tl$^+$.

The fluorescence decays are not affected by added alkali metal cations. In Figure 7, the lifetimes of both the shorter- and longer-lived components and the fraction of the shorter-lived component at a constant concentration (8.0 × 10^{-5} M) of Tl$^+$ are plotted against the concentration of added KNO$_3$. There is no effect of added K$^+$ (from KNO$_3$) on the lifetimes and their fractions in the concentration range of (0−6.0) × 10^{-4} M KNO$_3$, indicating that the condensed Tl$^+$ ions are not replaced by K$^+$ ions.

Fluorescence decay curves for AP34 in Tl$^+$-free solutions are essentially the same as those for AP90. In the presence of Tl$^+$, however, the decay nature of AP34 is considerably different from that of AP90; i.e., the

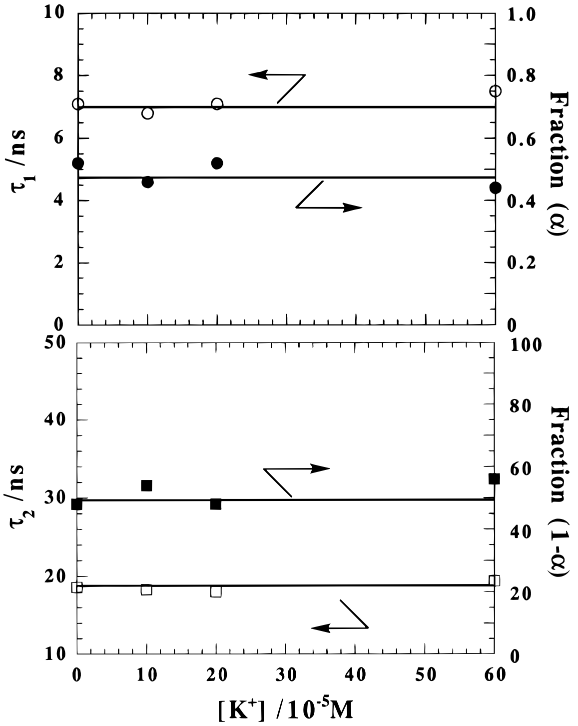


Figure 7. Lifetimes of the shorter- and longer-lived components and their fractions for AP90 at a constant concentration of 8.0×10^{-5} M Tl$^+$ plotted as a function of the concentration of added KNO$_3$.

decay curves for AP34 are practically single exponential with decreased lifetimes due to dynamic quenching by atmospheric Tl$^+$. The decreased lifetime of AP34 becomes longer when alkali metal salts are added, which is an indication that the atmospheric Tl$^+$ ions are displaced with the added alkali metal cations.

Discussion

In the fluorescence decays of AP90 in the presence of varying concentrations of Tl$^+$, the lifetime of the shorter-lived component remains the same while its fraction increases with increasing Tl$^+$ concentration (Figure 6). If the quenching occurs via noncollisional interactions between the Phen label and site-bound Tl$^+$, the lifetime of the short-lived component should decrease with an increase in the Tl$^+$ concentration due to a decrease in the average distance between the Phen and bound Tl$^+$ sites. In addition, there may be a concurrent decrease in the peak count (intensity) in the fluorescence decay curves due to a lifetime component shorter than the time resolution of the instrument employed. However, there was no significant difference in the peak counts when compared to the decay data in the absence and presence of Tl$^+$ under identical conditions. These observations indicate that the fluorescence quenching by condensed Tl$^+$ is not "static" (in the sense that quenching occurs between fixed sites without any dynamic contribution), but some dynamic process may be involved in the encounter between the Phen label and Tl$^+$. Namely, Tl$^+$ ions may be territorially bound to the polyanion, and they may be able to diffuse rapidly in the axial direction of the polymer backbone to encounter with the Phen labels.

As we discussed previously,[3] the exchange between the condensed and free Tl$^+$ ions is so slow compared with the fluorescence life of the Phen label that the contribution of the counterion exchange within the lifetime of the Phen fluorescence should be negligible; relaxation times of counterion redistribution in the radial direction around a rodlike polyion are on the order of 10^{-7} s, at the shortest, according to the literature.[22,23] However, the results of the present study suggest that a territorially-bound monovalent cation may diffuse along a polyanion backbone with a relaxation time comparable to the lifetime of the shorter-lived fluorescence component of the Phen label, i.e., on the order of 10^{-9} s.

In the kinetic model that we previously proposed,[3] we assumed a static quenching based on the Perrin model for the quenching by condensed Tl$^+$. However, the present study suggests that the previous assumption of the static quenching in the kinetic model is not appropriate.

On the basis of the steady-state and time-dependent fluorescence data we can conclude that Tl$^+$ ions condensed on the polyanions are not replaced by added Li$^+$, Na$^+$, and K$^+$ ions but atmospheric Tl$^+$ ions are displaced with the alkali metal cations. However, if there is a complete preference in the binding of Tl$^+$ over Na$^+$ (the original counterion for the NaAA unit in the polyanions), all Tl$^+$ ions should be condensed onto AP90 in the concentration range shown in Figure 6 and the quenching should totally be due to the condensed Tl$^+$; i.e., all Tl$^+$ ions may be condensed when [Tl$^+$] < 2.04×10^{-4} M, according to Manning's theory, given that the concentration of the COO$^-$ residue in AP90 is 3.45×10^{-4} M in this experiment. However, Figure 6 shows that there is a significant contribution of quenching by atmospheric Tl$^+$ even in a lower concentration regime. This suggests that the binding of Tl$^+$ is preferential over Na$^+$ but not completely preferential; i.e., there are some atmospheric Tl$^+$ ions before the Tl$^+$ condensation reaches its saturation. From the above considerations, the territorial binding seems to be more reasonable than the site binding to explain the experimental results. However, it is an open question why territorially-bound Tl$^+$ cannot be replaced by alkali metal cations.

Conclusions

Interactions of Tl$^+$ with Phen-labeled NaAA−AAm copolymers with different charge densities ($\xi = 2.5$ and 0.94) were studied by fluorescence techniques in aqueous solution with added alkali metal salts. The steady-state fluorescence of the polyanion with $\xi = 2.5$ is efficiently quenched by Tl$^+$. The fluorescence decays, in the presence of Tl$^+$, consist of two components, fast and slow decay components attributed to the quenching by condensed and free Tl$^+$ ions, respectively. Both the steady-state and time-dependent fluorescences indicate that the fluorescence quenching by Tl$^+$ (8.0×10^{-5} M) is not affected by the addition of Li$^+$, Na$^+$, and K$^+$ ions ($(0-5.0) \times 10^{-4}$ M), indicating that polymer-bound Tl$^+$ ions are not replaced by the added alkali metal cations. Namely, Tl$^+$ ions are preferentially bound to the polyanions over the Li$^+$, Na$^+$, and K$^+$ ions. On the other hand, the polyanion with $\xi = 0.94$ shows much less effective quenching. In the fluorescence decay, there is no such fast decay component due to the quenching by bound Tl$^+$; i.e., fluorescence quenching is only caused by atmospheric Tl$^+$. The fact that fluorescence quenching is significantly reduced by added alkali metal cations indicates that the atmospheric Tl$^+$ ions are displaced with the added cations.

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