Fluorescent Probe Investigation of a Water-Soluble Polyphosphazene

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ABSTRACT: Three fluorescent probes were utilized to investigate the microenvironment created by a water-soluble polyphosphazene copolymer in aqueous solution. The combination of intensity, wavelength shift, and vibrational analysis measurements of the three small-molecule probes is used to postulate an environment at high pH (with no added electrolyte) that is similar in polarity to methanol. In the absence of excess small-molecule electrolyte, negatively charged probes are repelled by the charge on the polymer. Addition of small-molecule electrolyte results in solubilization of the negatively charged probes. However, pyrene, even in the absence of added electrolyte (NaCl), is solubilized. Investigation of pyrene fluorescence quenching demonstrates the ability of the polyphosphazene copolymer to bind positively charged ions (Tl⁺) and repel negatively charged ions (I⁻). Neutral quenchers such as nitromethane readily penetrate the microenvironment of the polymer.

Introduction

Due to their potential utilization in a variety of applications where water solubility is essential, there is interest in the synthesis and characterization of water-soluble polyphosphazenes. A water-soluble (at high pH) copolymer derivative (shown as I) of poly(methylphen-

I, $n = m = 1$
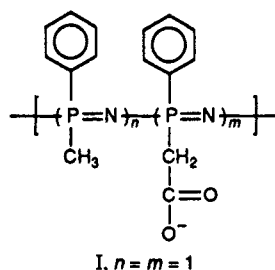
ylphosphazene) containing 50% carboxylated functionalities of $CH_2COO^-M^+$ was recently synthesized and characterized by Wisian-Neilson et al.[1] In addition, it was determined that the salt derivatives of I in aqueous solutions could be precipitated by addition of a dication salt ($CuCl_2$), presumably via an ionic cross-linking mechanism.

In view of the novelty of these water-soluble polyphosphazenes and in anticipation that other equally interesting and potentially useful water-soluble polyphosphazenes might be developed, it is deemed essential to characterize the solution properties of the substituted polymer I. In view of the success in utilizing fluorescent probes to characterize the conformational structure of a number of typical polyelectrolytes [poly(acrylic acid), poly(methacrylic acid), copolymers of maleic anhydride and long-chain alkenes, etc.], we have chosen to evaluate the structure of I in aqueous solutions using several luminescent molecules, which have been well-characterized with respect to their photophysical properties in different environments. Thus three probes, 1-anilino-8-naphthalene (ANS), 2-p-toluidinylnaphthalene-6-sulfonic acid (TNS), and pyrene, all

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of which operate by different methods to report on the environment in which they are placed,[2-11] are used to gain a realistic description of the carboxylated polyphosphazene polymer I in aqueous solution at high pH.

Experimental Section

The polyphosphazene polymer I was prepared as described previously.[1] Pyrene (Aldrich) was purified by column chromatography. ANS (Aldrich) was recrystallized several times from deionized water, and TNS (Aldrich) was used as received. KI, CH_3NO_2, $TlNO_3$, and (dimethylamino)pyridine (DMAP) were used as obtained from Aldrich. KOH was used to adjust all aqueous solutions (prepared from deionized water) to a pH of 12. The polymer concentration was kept constant at 0.19 mg/mL. NaCl was obtained from Aldrich. Each sample was degassed with N_2 (10 min before measuring the fluorescence). When performing the pyrene quenching studies, samples were degassed for 3 min in between each incremental addition of the quencher before taking the next measurement. Fluorescence spectra (arbitrary units) were recorded on a Spex Fluorolog-2 fluorescence spectrometer. Fluorescence decay curves were measured with a PRA single-photon-counting instrument. The nonlinear iterative deconvolution technique was used to fit the decay profiles.

Results and Discussion

The ANS fluorescent probe can exist in the excited single state in two forms, a nonplanar state with peak emission maximum (λ_{max}) at 520 nm and a planar intermolecular charge-transfer (CT) state with λ_{max} at 460 nm.[3] The medium viscosity and polarity dictate the relative population of each state and thus the fluorescence spectrum in a given environment. In a polar medium, the emission at 520 nm is enhanced, while in a nonpolar medium the 460-nm emission predominates. Additionally, the emission at 520 nm in a polar environment is lower than the 460-nm emission in a nonpolar environment. These properties of ANS have promoted its use as a reporter of microenvironment for polyelectrolytes such as poly(methacrylic acid)[3] and copolymers of 2-isopropenylnaphthalene (2IPN) and maleic acid (MAA) in aqueous media.[2]

Figure 1 shows the fluorescence of ANS ($\lambda_{ex} = 377$ nm) in pure water and in the presence of polymer I (pH = 12.0) at several NaCl salt concentrations (the spectra in Figure 1 are corrected for low-level anomalous fluorescence from pure polymer/salt solutions). There is little difference
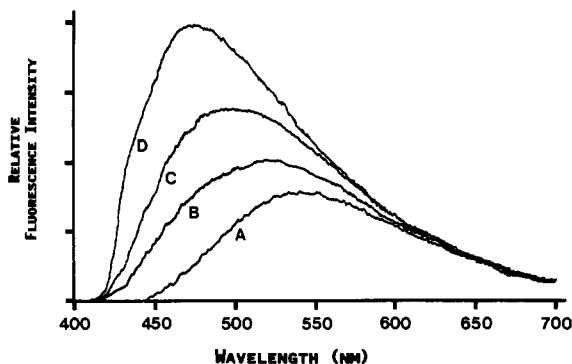
Figure 1. Plot of relative fluorescence intensity versus emission wavelength for ANS. λ_{ex} = 377 nm; polyphosphazene at 0.19 mg/mL; ANS at 1 × 10⁻⁵ M; for NaCl concentrations of (A) 0.00 M, (B) 0.17 M, (C) 0.34 M, and (D) 0.50 M, in water at pH = 12.

between the fluorescence maximum of ANS in pure water (not shown here) and in an aqueous solution of polymer I with no NaCl added (curve A). Apparently, if there is a hydrophobic microenvironment created by the phosphazene polyelectrolyte, it does not effectively solubilize ANS, at least in the absence of additional indifferent electrolyte such as NaCl. These results are reasonable since, as pointed out by Sturtevant and Webber[2] for the 2-IPN/MAN copolymer system, ANS is negatively charged and in the absence of indifferent electrolyte should experience repulsion by the deprotonated form of I at high pH in pure water. The addition of electrolyte to the solution could well alter the ability of I to provide a stable hydrophobic environment for ANS, and in fact, as the concentration of NaCl increases to 0.5 M, the spectra intensify and shift to lower wavelengths. The increase in fluorescence intensity in the solution with the highest NaCl concentration is well over 200% with a peak maximum at about 470 nm. This is a comparable, if not larger, change to that experienced by ANS in the work reported by Sturtevant and Webber[2] for the 2-IPN/MAA copolymer. The environment, as reflected by the fluorescence wavelength maximum, surrounding ANS is similar to that experienced by the probe in methanol, which has a dielectric constant of 32.7 and a dipole moment of 1.7 D.[4] The enhanced intensity and blue shift in the fluorescence maximum of ANS could result either from a change in the coil with addition of NaCl to form a hydrophobic environment *or* from lowering of the repulsion between ANS and the deprotonated form of I to allow transport into the hydrophobic environment. At this juncture, although we can define the polymer solution of I with high NaCl as providing a somewhat hydrophobic environment, we are still left with the dilemma of characterizing the microenvironment created by I in aqueous media in the absence of electrolyte. Before attempting to solve this problem and thus to describe the microenvironment of an aqueous solution of I with added electrolyte, we first provide supporting evidence for the hydrophobic environment at high NaCl concentrations.

Additional evidence for the hydrophobic environment created by I in water (pH = 12) is provided by the fluorescence results in Figure 2 for TNS (λ_{ex} = 345 nm) in aqueous solutions of I with added NaCl (the spectra of Figure 2 were corrected for low-level anomalous fluorescence from pure polymer/salt solutions). The wavelength of the fluorescence maximum (435 nm) of TNS is relatively independent of the salt concentration, but the fluorescence does exhibit the usual increase in intensity[5] typical of TNS in a hydrophobic environment. The absence of fluorescence in an aqueous solution of I with no NaCl added is in excellent agreement with the results for ANS: the same
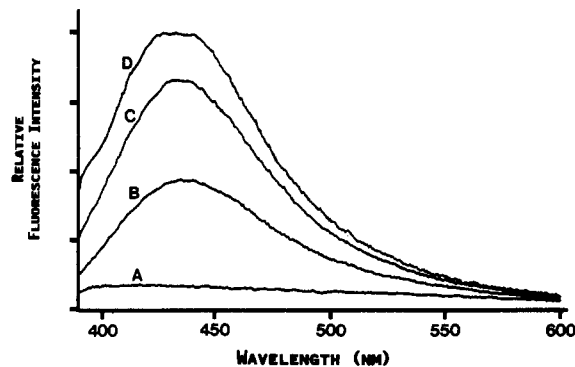

Figure 2. Plot of relative fluorescence intensity versus emission wavelength for TNS. λ_{ex} = 345 nm; polyphosphazene at 0.19 mg/mL; TNS at 1 × 10⁻⁵ M; for NaCl concentrations of (A) 0.00 M, (B) 0.17 M, (C) 0.34 M, and (D) 0.50 M, in water at pH = 12.
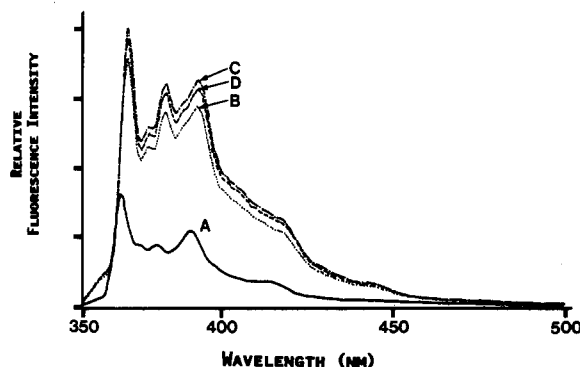

Figure 3. Plot of relative fluorescence intensity versus emission wavelength for pyrene. λ_{ex} = 336 nm; polyphosphazene at 0.19 mg/mL; pyrene at 5 × 10⁻⁷ M; for NaCl concentrations of (A) 0.10 M (no polyphosphazene), (B) 0.00 M (with polyphosphazene), (C) 0.10 M (with polyphosphazene), and (D) 0.34 M (with polyphosphazene), in water at pH = 12.

arguments applied to account for the ANS results can be extended to TNS since TNS is also a negatively charged species and would be expected to be repelled by the deprotonated form of I (at pH = 12) upon attempting to enter any hydrophobic environment created by I (in the absence of added NaCl electrolyte).

Having established the hydrophobic nature of the polyelectrolyte I in a high-pH medium in the presence of added NaCl using two hydrophobic probes TNS and ANS, the task of providing additional information about the hydrophobic microenvironment, particularly in an aqueous solution with no added electrolyte, and its ability to solubilize hydrophobic small molecules remains. Figure 3 shows the fluorescence of pyrene (λ_{ex} = 336 nm) in pure water plus 0.1 M NaCl at pH = 12 (curve A) as well as in an aqueous solution (pH = 12) of polymer I (curve B). Two observations can be made: first, the overall fluorescence of pyrene intensity increases dramatically in the presence of I, and, second, the relative intensity of the third peak (λ_{em} = 384 nm) in the vibrational fine structure of pyrene increases with respect to the intensity of the first peak (λ_{em} = 373 nm); i.e., the 3:1 ratio increases from 0.55 to 0.79. This increase is indicative of a shift in environment from a purely aqueous medium (dielectric constant of water is 78.54) to a medium with a polarity similar to that of methanol, which has a 3:1 ratio of 0.75[7] and as noted before has a dipole moment of 1.70 D and a dielectric constant of 32.7.[4] Thus, the results for pyrene provide additional insight into the nature of polyphosphazene I in purely aqueous media, suggesting that indeed I, in aqueous solution at pH = 12, provides a microenvironment that is somewhat hydrophobic (i.e., much like
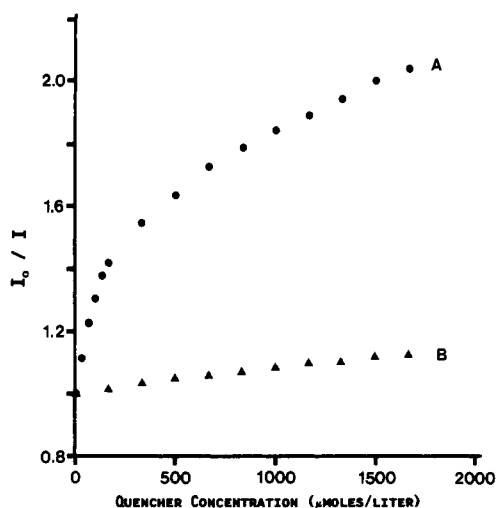
Figure 4. Stern–Volmer plot of pyrene (5×10^{-7} M) and polyphosphazene (0.19 mg/mL) for (A) TlNO$_3$ and (b) KI, in water at pH = 12.

Figure 5. Stern–Volmer plot of pyrene (5×10^{-7} M) and polyphosphazene (0.19 mg/mL) for (A) CH$_3$NO$_2$ and (B) DMAP, in water at pH = 12.

that of methanol) and capable of solubilizing pyrene.

The lifetime of pyrene in water with no polyelectrolyte present is 174.9 ns. The fluorescence decay of the pyrene probe in aqueous solution of polyelectrolyte at pH = 12 (no added NaCl electrolyte) readily fits to a double-exponential decay function, with lifetimes of 174.2 and 360.2 ns reflecting a dual environment, one very polar (τ = 174.2 ns) and one relatively nonpolar (τ = 360.2 ns). From the preexponential terms used to obtain the double-exponential fit to the decay curve, it can be inferred that approximately 83% of the pyrene molecules are in a more hydrophobic environment while only about 17% are in an aqueous environment. A similar analysis has been applied by Olea and Thomas[10] to account for the multienvironment experienced by pyrene in an aqueous solution of poly(methacrylic acid).

Before further progression in the discussion of the pyrene results for the simple solution of I in water, it should be noted that addition of excess NaCl (up to 0.5 M) did not alter significantly either the 3:1 ratio or the intensity of the pyrene fluorescence (Figure 3, curves C and D). Apparently, even with no added NaCl electrolyte, the pyrene probe in the presence of I is in a moderately hydrophobic environment (like that of methanol), which does not change appreciably in its ability to further "solubilize" pyrene. The 3:1 ratio of 0.79 in pH = 12 medium in the presence of I is significantly greater than the 3:1 ratio of about 0.55 reported by Olea and Thomas[10] for poly(methacrylic acid) in aqueous solution at a pH above 7. The phosphazene backbone, even in a high-pH medium with the acid functionalities existing as carboxylate salts, is capable of providing a limited hydrophobic microenvironment without added electrolyte (NaCl). The fact that the 3:1 ratio remains constant in the presence of 0.5 M NaCl predicts that the environment experienced by pyrene is not greatly altered by the addition of electrolyte.

In summarizing the results for the pyrene probe, we conclude that pyrene is capable of detecting the partial hydrophobic nature of the solution of I in the absence of added NaCl. The repulsion argument previously extended to account for the inability of TNS and ANS to interrogate effectively the microenvironment created by I in aqueous media (no added NaCl) seems to be the best explanation of the results in view of the data generated from the pyrene probe analysis. Furthermore, the absence of an appreciable change in pyrene fluorescence intensity or alteration of the 3:1 ratio with added NaCl reflects the static nature
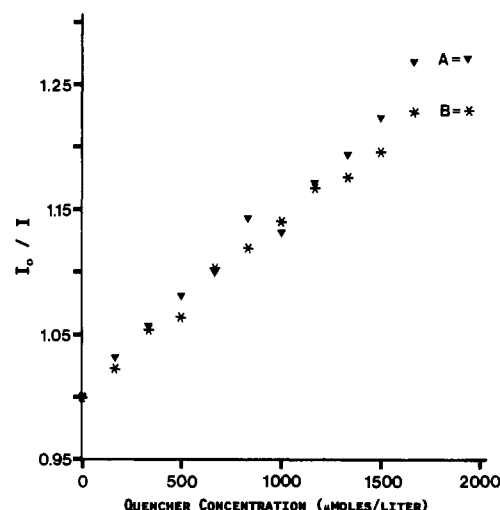
of the microenvironment and its lack of dependence on an added electrolyte in further enhancing the hydrophobic nature of the microenvironment created by I.

Additional information about the microenvironment experienced by pyrene in the solution of I in aqueous media can be obtained by quenching the fluorescence of pyrene with a variety of small-molecule quenchers, i.e., TlNO$_3$, CH$_3$NO$_2$, KI, and DMAP [4-(dimethylamino)pyridine], each of which is capable of producing a different degree of quenching, depending upon environmental considerations. Figures 4 and 5 show Stern–Volmer (see eq 1) plots of pyrene fluorescence intensity (384 nm) as a

$$I_0/I = 1 + K_{sv}[Q] \qquad (1)$$

I_0 = fluorescence (384 nm)

intensity of unquenched pyrene

I = fluoresence (384 nm)

intensity of pyrene in the presence of quencher

K_{sv} = Stern–Volmer quenching constant

[Q] = quencher concentration

function of quencher concentration (Figure 4, KI and TlNO$_3$; Figure 5, CH$_3$NO$_2$ and DMAP). We should note that all results for quenching were essentially independent of the wavelength (384 or 373 nm) monitored. In Figure 4, the ionic quenchers yield quite different results depending upon the charge of the quenching ion (I$^-$ or Tl$^+$). In the case of I$^-$, the plot is linear but with a low slope (K_{sv} = 71.9), indicating very inefficient quenching. This is quite reasonable, and in accord with other results for quenching of pyrene fluorescence by I$^-$ in polyelectrolyte solutions[11] it is a direct result of charge repulsion between I$^-$ and the negative charge of the carboxylate groups on the polyphosphazene I. In contrast, quenching by Tl$^+$ is much more efficient but nonlinear. Before we provide an explanation for the results for Tl$^+$ quenching and its relatively high quenching efficiency, it is instructive to consider the reported[10,11] attraction between Tl$^+$ ion and negatively charge carboxylate groups of similar polyelectrolytes. The binding of Tl$^+$ to a polyelectrolyte has been postulated to result in its enhanced access to the pyrene molecules located in the hydrophobic core of the

polyelectrolyte and thus a larger quenching rate. (Of course, the small percentage of pyrene molecules that are in a more polar environment would naturally be accessible to quenching by free Tl^+ ions in solution.) How is this brief analysis of Tl^+ ions and their reported binding to polyelectrolytes useful in interpreting the results in Figure 4? The large quenching efficiency predicted for bound Tl^+ is obvious, at least as reflected in the initial slope of the Stern–Volmer plot at lower Tl^+ concentrations. But what about the reduced quenching efficiency at higher Tl^+ concentrations? This could well result from an inability of the polyelectrolyte to accommodate additional Tl^+ ions at higher concentrations, thus resulting in free Tl^+ ions, which quench pyrene molecules associated with the polyphosphazene polyelectrolyte by a diffusion process rather than by static quenching. If the plot for Tl^+ in Figure 4 is divided into an initial rapid static/dynamic combination quenching process (concentration of $TlNO_3$ ranging from 0.0 to 1.0×10^{-4} M) and a slower primarily diffusion-controlled quenching process at higher concentrations (concentration of $TlNO_3$ ranging from 8.33×10^{-4} to 1.67×10^{-3} M), quenching constants of 3067 and 310 are obtained, respectively. The very large initial quenching constant (3067) for bound Tl^+ ions is similar to the value (3250) obtained by Auroa and Turro[11] for intensity quenching of the fluorescence of bound pyrene in a pyrene-substituted poly(acrylic acid) polymer. Interestingly, the Stern–Volmer constant obtained for quenching of pyrene in the present case at higher Tl^+ concentrations (310) is rather close (relatively) to the value obtained[11] for lifetime quenching of the bound pyrene in the poly(acrylic acid) case (642). If we assume that the 310 value for dynamic Stern–Volmer quenching of pyrene fluorescence (polyelectrolyte I solution) at higher Tl^+ ion concentrations corresponds to the lifetime quenching value for K_{sv} obtained by Aurora and Turro (642), then the difference (3067 – 310 = 2756; defined as the static quenching component K_s) is almost the same as the 2608 value for K_s from ref 11 for bound pyrene. Although we are comparing two quite different methods for estimating K_s for two completely different systems, agreement between the two values obtained is noteworthy. Finally, we should mention that we do not include lifetime quenching data, which in our case, due to the two component decay, is difficult to interpret.

Figure 5 shows Stern–Volmer plots for the two nonionic quenchers CH_3NO_2 and DMAP. Both quenchers have Stern–Volmer K_{sv} quenching constants (K_{sv} = 148.7 for CH_3NO_2 and K_{sv} = 133.1 for DMAP) intermediate between the K_{sv} constants for I^- and Tl^+ (at higher concentrations). This seems quite reasonable in view of the nonionic nature of both CH_3NO_2 and DMAP. The rather low value of K_{sv} for DMAP deserves comment. In the fluorescence quenching work reported by Aurora and Turro for bound pyrene in a pyrene-substituted poly(acrylic acid) system,[11] DMAP was found to give an unusually large K_{sv} value, approximately 2 orders of magnitude greater than that of CH_3NO_2. In the present case for polyphosphazene I and nonbound pyrene, the K_{sv} value for DMAP is almost the same as that for CH_3NO_2. The large value reported for the DMAP–pyrene-substituted poly(acrylic acid) system was attributed to preassociation of DMAP and pyrene induced by hydrophobic interactions. Apparently, in the microenvironment created by polyphosphazene I at high pH (pH = 12), the hydrophobic core in which pyrene exists is not

conducive to ground-state association; hence, the K_{sv} constant is essentially identical with that obtained for CH_3NO_2.

Conclusions

The results in this paper provide insight into the nature of the hydrophobic environment created by a polyphosphazene polyelectrolyte in aqueous media. With use of ANS, TNS, and pyrene fluorescent probes, the polyphosphazene polymer is depicted as providing a microenvironment at high pH (pH = 12) that is similar in polarity to that of methanol. Interestingly, even in the absence of added NaCl electrolyte, a hydrophobic environment capable of solubilizing pyrene is generated. The hydrophobic environment is no doubt created by the methyl and phenyl groups attached to the parent phosphazene backbone. Steady-state fluorescence quenching for pyrene by a negatively charged ionic species suggests that the microenvironment is "protected" by the negatively charged carboxylate salt species on the polyphosphazene, which are present on 50% of the phosphazene repeat units. Conversely, the negatively charge carboxylate groups can accommodate and bind a limited concentration of positively charged ions (Tl^+) and enhance quenching of pyrene (at least for low Tl^+ concentrations). Uncharged quencher species [CH_3NO_2 and (dimethylamino)pyridine] are unaffected by the negative charge on the phosphazene in high-pH solutions. Unfortunately, due to the limited pH range in which the water-soluble polyphosphazene is soluble, it is impossible to conduct a comprehensive investigation of the effect of pH on the polymer microenvironment in solution using fluorescent probes. The composite results in this paper, however, clearly illustrate the potential of water-soluble polyphosphazenes and their ability to form a moderately hydrophobic environment in aqueous media, even at high pH and in the absence of added electrolyte. By selective substitution of side groups, it should be possible to generate polyphosphazene polyelectrolytes that display a variety of properties in aqueous media.

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