pH-Induced Fluorescence Quenching of Anthracene-Labeled Poly(2-vinylpyridine)

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ABSTRACT: The fluorescence intensity of mid- or end-tagged anthracene-labeled poly(2-vinylpyridine) in acidic aqueous solution is very dependent on the degree of protonation (α). In the absence of protonation (e.g., in methanol solution) there is no appreciable intrapolymer fluorescence quenching. We presume that the quenching is the result of electron transfer from the excited anthracene to neighboring pyridinium units. Even a qualitative fit to a simple Bernoullian statistical model based on the state of nearest-neighbor protonation was not possible unless we propose that the protonation of pyridine units that are adjacent to the chromophore is significantly favored compared to pyridines located elsewhere on the chain. There is a change in the slope of the relative fluorescence vs α curve in the vicinity of 0.42−0.45 that suggests some kind of conformational transition at this degree of protonation.

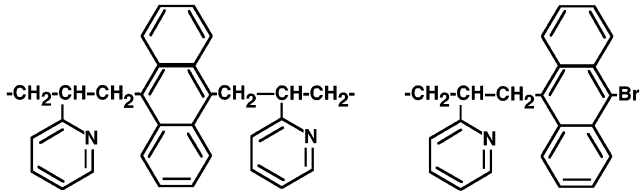
Introduction

There have been many studies of polyelectrolytes with chemically attached chromophores,[1] including our previous studies on poly(methacrylic acid) with mid- or end-tagged anthracene moieties.[2] In general, for weak polyacids the fluorescence quantum yield and the access of quenching species to the chromophore are a strong function of the solution pH, reflecting the expansion or contraction of the polyelectrolyte coil and the changes in the linear charge density. We undertook the present experiments with the same objective, but of course for a polypyridine chain the coil expansion will increase as the pH of the solution is lowered. It was found that the fluorescence quantum yield changed dramatically as a function of pH, becoming almost unmeasurable as the pH approached 2. (The minimum pH required to dissolve poly(2-vinylpyridine is approximately 4.) The fluorescence intensity of the two polymers, A-m-PVP and BA-e-PVP (see Scheme 1), was studied as a function of the extent of protonation, α. The fluorescence intensity is qualitatively proportional to $(1 - \alpha)^2$ and $(1 - \alpha)$ for A-m-PVP and BA-e-PVP, respectively, as would be expected for a quenching mechanism that is related to the probability that the adjacent pyridine moieties are *not* protonated. However, to achieve even a semiquantitative fit to the data, it is necessary to propose that the pyridines adjacent to the anthracene groups are preferentially protonated compared to those located in the main chain. Unfortunately, it is not possible to obtain a very precise value of the preferential protonation constant from these data fits, but we estimate an equilibrium constant on the order of 10 for this proton exchange.

Experimental Section

Monomers. 2-Vinylpyridine (2-VP) (Scientific Polymer Products) were passed through inactive alumina to remove inhibitor and predried by stirring over CaH_2 for at least 24 h and stored in the presence of CaH_2 at 0 °C until needed. To remove any traces of water, 2-VP was exposed to sodium metal. To slow the inevitable polymerization of monomer by exposure to the reactive metal, thin shavings of metal were used instead of the more usual sodium mirror. This allows ample time for purified monomer to be transferred to the receiving ampule before significant polymerization has occurred. Because of the high boiling point of 2-VP (20 °C at 1 Torr), a warm water bath (∼45 °C) was necessary to cryodistill the monomer. Also, it was often necessary to warm the manifold with a heat gun to prevent condensation of the monomer in the line. Once in the presence of sodium metal the monomer was allowed to stir for ∼45−60 min under vacuum until the appearance of a purple/gray color, the scavenging anion, indicated purity. At this point, the monomer was cooled to −78 °C and opened to vacuum to remove the small quantities of hydrogen gas formed by the reaction of sodium and water. The monomer was then warmed to ∼ 45 °C and cryodistilled into an ampule and stored at 0 °C.

Scheme 1



Initiator. Cumyl potassium was used as the initiating species although it is more difficult to prepare than reagents such as *n*-butyllithium. Because of the much larger size of the K^+ counterion relative to Li^+, the ion pair readily dissociates to yield the highly reactive free cumyl anion, resulting in extremely rapid initiation. This results in the production of fairly monodisperse substances (polydispersity ∼ 1.05−1.20).[3] Cumylpotassium also possesses a characteristic deep red color that can easily be titrated to determine precise initiator concentration.

9,10-Dibromomethylanthracene. 9,10-Dichloromethylanthracene (Aldrich) was used without further purification. 3.0 g (1 equiv) of 9,10-dichloromethylanthracene solid was extracted into a solution of 6.0 g (5.3 equiv) of sodium bromide in 220 mL of acetone using a Soxhlet apparatus and allowed to reflux under nitrogen for 18 h according to the procedure developed by Golden.[4] Because of the photoreactive nature of the product, the material was protected from room light exposure during the reaction and subsequent purification process. The reaction mixture was cooled to room temperature

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and filtered. The yellow residue, which was mostly monobrominated material, was collected and extracted a second time in the presence of sodium bromide using toluene solvent to yield the desired dibrominated species. The final residue was collected, recrystallized from toluene, and freeze-dried as an emulsion in benzene to yield the pure, dry product. Note that purification via chromatography using alumina or silica stationary phases results in decomposition of the product into a variety of species as detected by TLC. MS: *m*/*z* 367 (M^+), 366, 365, 364, 363, 285, 283, 205. H NMR: δ 5.50 (s, 2 X $-CH_2-$), 7.63−7.70 (m, 2,3,6,7-H), 8.32−8.40 (m, 1,4,5,8-H); mp >300 °C in agreement with Golden.[4]

9-Bromomethyl-10-bromoanthracene. 9-Methylanthracene (Aldrich) was reacted with 1.70 mL of bromine (6.2 equiv) in the presence of triphenylphosphine in acetonitrile. After 1 h of reaction at room temperature the mixture was cold filtered and allowed to air-dry in a fume hood to remove excess bromine vapors. The residue was dissolved in dioxane and rotary-evaporated to further remove excess bromine. This dark yellow residue was twice recrystallized from chloroform (40 mL of chloroform per 750 mg of material) to obtain pure product. The material was freeze-dried from dioxane to obtain the product in a dry powder form. MS: (M^+) *m*/*z* 191, 269, 270, 271, 272 (parent molecule signals 349, 350, 351 not present in significant quantities). H NMR: δ 5.46 (s, $-CH_2-$), 7.56−7.70 (m, 2,3,6,7-H), 8.22−8.32 (d, 4,5-H), 8.56−8.64 (d, 1,8-H) in agreement with Wang.[5] MP: 178 °C (char onset), 193−195 °C (melt) (Wang: 200−202 °C).

Preparation and Termination of Polymers. The polymerization reactor is described in detail elsewhere.[6] THF was cryodistilled into the vessel at −78 °C followed by pressurization with high-purity nitrogen. The cold THF was allowed to warm to near room temperature at which time existing impurities in the solvent were eliminated by titration with the initiator solution. The persistence of a light peach color was indicative of a dilute solution of unreacted cumylpotassium and signified the end point of the titration. The vessel was again cooled to −78 °C, and the appropriate amount of initiator solution was added and allowed to mix thoroughly. Since no known termination pathway exists for the polymerization of 2-VP, this monomer was added quickly so as to ensure that all chains initiate simultaneously. After 1 h of stirring at −78 °C a small aliquot of the polymer solution was isolated for subsequent study via a siphon and terminated with methanol. The desired anthracene terminating solution was then added to the vessel and allowed to react with the living chain ends for 1.5−2 h at −78 °C. The mixture was then slowly warmed to room temperature for an additional hour as per the procedure developed by Valeur and co-workers to form the labeled polymer products.[7]

Analysis and Purification of Labeled Polymers. GPC of all labeled polymers was performed before and after any purification method used. The use of fluorescence and absorption detection chromatograms allowed us to evaluate just the labeled population and were used to determine tagging efficiency (within experimental error, 98−100% efficient for both polymers). Note that for the doubly terminated polymer A-m-PVP a smaller than stoichiometric amount of anthracene terminating agent was added, so there remains in the final mixture some PVP homopolymer which makes no contribution to the photophysical results. The GPC number-average molecular weight and PDI for the A-m-PVP polymer were 28 600 (target 30 000) and 1.26 (determined from the UV detection signal), and for BA-e-PVP these quantities were 19 100 (target 20 000) and 1.23.

Dialysis was employed to remove traces of any unreacted chromophores. (Obviously, these could considerably compromise the photophysical studies.) A concentrated solution of the polymer in methanol (∼10 mg/mL) was placed into a Spectra/Por series 1 cellulose membrane tubing with a molecular weight cutoff of 6000−8000, which was sufficient to allow low molecular weight impurities to pass while retaining the high polymer. The solution was dialyzed against an outer solution of the same solvent that was changed every 3−4 h until the presence of impurities migrating into this solution could no longer be detected by UV−vis absorption spectroscopy. This procedure usually involved 8−10 outer solution changes. Note that, prior to use, the membrane tubing was immersed in 5% acetic acid solution for 10 min to remove trace metals. The tubing was then conditioned to methanol environment by a 30−45 min soaking in pure water followed by similar soakings in water/methanol mixtures of progressively higher methanol content. The polymer solution retained inside the membrane tubing was evaporated, dissolved in dioxane, filtered, and freeze-dried to obtain the pure polymer in dry form.

Instrumentation and Equipment. Steady-state and time-resolved measurements were performed on a SPEX DM3000 Fluorolog-τ2 spectrofluorometer that has been described in our earlier publications.[2] The quantum yields of A-m-PVP and BA-e-PVP were obtained in methanol and aqueous solution by referencing to a 9-methylanthracene standard in cyclohexane taking into account differences in solution refractive index ($\phi_{ref} = 0.29$).[8] Solutions were prepared with chromophore concentrations of $\sim 10^{-5}$ M. Self-absorption, which can result in erroneously low quantum yields, is minimal at these concentrations. Both the labeled polymers and the standard were excited at 365 nm.

Stock Solutions and Fluorescence Quenching by HCl Addition. A known amount of labeled polymer was placed into a clean, dry volumetric flask. To this was added a predetermined amount of standardized 0.1 M HCl to give a final solution with pH ∼4 assuming that, at these concentrations, ∼25% of 2-vinylpyridine repeating units are protonated. The polymer solution was then diluted with deionized water to yield a 2-vinylpyridine repeating unit concentration within the range 1.8−2.4 mM. The measured pH value was always within the range 3.90−4.10, which corresponds to the minimum amount of HCl required to dissolve the labeled polymers. Solutions of A-m-PVP and BA-e-PVP in mixed methanol (MeOH)/water solvents were prepared by dissolving a known amount of the labeled polymer in MeOH followed by slow addition of deionized water while stirring to give the final MeOH/H_2O volume ratio. The 2-vinylpyridine repeating unit concentrations for these solutions were similar to that prepared using dilute HCl.

Because of the observation of systematic changes in the fluorescence spectrum of the labeled materials as a consequence of prolonged room light exposure, the solutions were protected from light during storage, which was not allowed to exceed 1 week. The sensitivity of A-m-PVP fluorescence to atmospheric oxygen was found to be very similar to that for A-m-PMA.[2] For this reason, solutions of A-m-PVP were bubbled with nitrogen for at least 20 min immediately after solution preparation, and each time the solution stock was opened. Such precautions were not necessary for BA-e-PVP since, like BA-e-PMA, this material appears to be much more stable.

Steady-State Measurements. 2.600 mL of labeled PVP stock solution was placed into a quartz cuvette, equipped with a screw cap with a gas inlet/outlet, and the solution bubbled was with nitrogen for 15 min. The samples were excited at 365 nm, and the emission was recorded from 390 to 500 nm with the signal referenced to the excitation intensity (the S/R mode). Small amounts of a 35 mM HCl stock solution (∼3−25 μL) were added between each subsequent measurement and allowed to mix for 2−3 min with N_2 bubbling prior to the acquisition of each spectrum. The decrease of fluorescence by dilution from the addition of quencher was taken into account in later analysis. It has been reported that at high degrees of polymer protonation excitation of pyridinium units at 260 nm yields an emission centered at 390 nm.[9] However, with 365 nm excitation the fluorescence observed in our experiments is strictly from the anthracene label.

Results and Discussion

Protonation Equilibrium of Labeled Polymers. Measuring the pH of the labeled polymer solutions at every addition of HCl allows us to calculate the degree

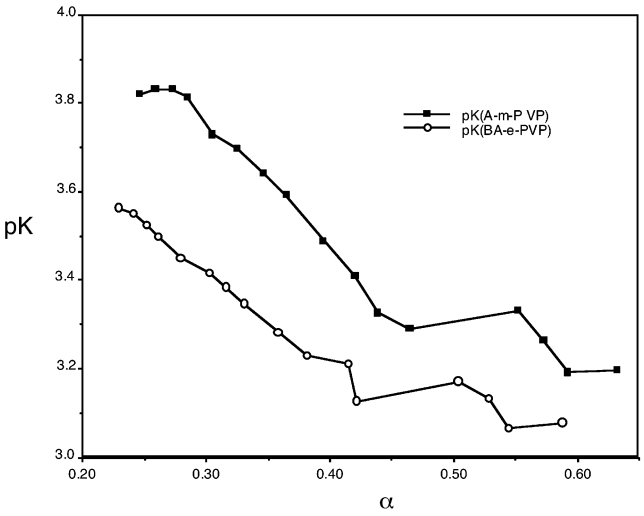

Figure 1. Effect of α on p$K_{d,app}$ for A-m-PVP and BA-e-PVP based on eq 1 of the text.

of protonation of the polymer, α, as follows:

$$\alpha = \frac{[PH^+]}{[P] + [PH^+]} = \frac{[HCl]_{init} - [H^+]}{[P]_{init}} \qquad (1)$$

where [P] and [PH^+] represent the concentration of unprotonated and protonated pyridine units, respectively, and $[P]_{init}$ is the total amount of pyridine added to the solution. [H^+] is given by 10^{-pH}, and $[HCl]_{init}$ is the HCl concentration assuming no consumption of ions by polymer protonation.

As is common with polyelectrolytes, the equilibrium constant for protonation of the pyridine repeating unit is a function of α and decreases significantly with increasing α as a result of increasing electrostatic repulsive forces between protonated pyridine units. The apparent dissociation constant, K_d, for the pyridinium ion can be expressed as

$$pK_{d,app} = pH + \log\left(\frac{\alpha}{1-\alpha}\right) \qquad (2)$$

Values of p$K_{d,app}$ vs α are displayed in Figure 1 for both A-m-PVP and BA-e-PVP in dilute HCl. p$K_{d,app}$ values of 3.56 and 3.82 were observed for the A-m-PVP and BA-e-PVP polymer solutions prepared at pH 4, respectively ($\alpha_{A-m-PVP} = 0.229$, $\alpha_{BA-e-PVP} = 0.243$). Note that these values are far removed from the pK_d value of 6.12 for the model compound 2-ethylpyridine in water, which is nearly constant over the full range of α. A similar result was reported by Kirsh et al.[10] The p$K_{d,app}$ curves for the two materials are surprisingly different, with p$K_{d,app}$ values for BA-e-PVP that are approximately 0.2 pK_d units lower than those for A-m-PVP over the entire α range investigated. One might think that this difference is the result of differing molecular weights for the two materials ($DP_{A-m-PVP} = 272$; $DP_{BA-e-PVP} = 182$). However, Kirsh and co-workers reported identical potentiometic titration results for poly(2-vinylpyridine) with molecular weights ranging from 3.0×10^4 (DP = 286) to 1.0×10^6 (DP = 9524) in 45/55 EtOH/H_2O.[10] It is possible that the pK_d difference is an effect of the chromophore and its location. This suggests that the introduction of fluorescence probes can significantly perturb the polymer from its unlabeled state.[11]

Table 1. Fluorescence Quantum Yield for A-m-PVP and BA-e-PVP in Different Solvents

polymer	solvent	pH	ϕ_{fl}
A-m-PVP	MeOH		0.63
	80/20 MeOH:H_2O		0.59
	60/40 MeOH:H_2O		0.44
	40/60 MeOH:H_2O		0.40
	20/80 MeOH:H_2O		0.42
	5/95 MeOH:H_2O		0.28
	H_2O (HCl)	4.08	0.054
	H_2O (HCl)	1.96	$<10^{-3}$
BA-e-PVP	MeOH		0.15
	H_2O (HCl)	4.27	0.078
	H_2O (HCl)	1.94	$<10^{-3}$

Photophysics of Labeled Polymers in Mixed MeOH/H_2O Solution. The fluorescence quantum yield, ϕ, for A-m-PVP and BA-e-PVP was found to be $<10^{-3}$ at the pH required for complete protonation (<2), whereas ϕ values of 0.63 and 0.15 were found for A-m-PVP and BA-e-PVP in pure MeOH (Table 1). The quantum yield of A-m-PVP in different MeOH/H_2O mixtures decreases steadily upon the addition of water. We presume that the decrease in the quantum yield of A-m-PVP with increasing water content is the result of fluorescence quenching due to partial protonation of the polymer by water (see the next subsection).

The solubility of BA-e-PVP is very different than A-m-PVP. In particular, it is insoluble in MeOH/H_2O mixtures for MeOH volume fractions below 40%. Thus, we did not determine the quantum yield values in the limit of nearly pure H_2O for this material.

Fluorescence Quenching of Labeled Polymers by Protonation in Aqueous Solution. We propose that electron transfer from excited-state anthracene to neighboring pyridinium units is the mechanism for fluorescence quenching:

$$A + h\nu \rightarrow {}^1A^*$$
$${}^1A^* + P_AH^+ \rightarrow A^{\bullet+} + P_AH^{\bullet}$$
$$A^{\bullet+} + P_AH^{\bullet} \rightarrow A + P_AH^+ \qquad (3)$$

In eq 3, A represents the anthracene label and P_AH^+ represents a pyridinium unit adjacent to the anthracene label. While it is conceivable that unprotonated pyridine units might also participate in quenching, the fluorescence quantum yield value obtained for A-m-PVP in MeOH (0.63, see previous section) is identical to that reported by Cherkasov et al. for 9,10-dimethylanthracene in the same solvent.[12] This suggests that in the absence of acid pyridine units do not participate in fluorescence quenching for pure MeOH solutions.

An oxidation potential of 0.665 V has been measured by Zhang et al. for 9,10-dimethylanthracene in DMSO, referenced to the Fc^+/Fc (ferrocene) electrode.[13] Although no reduction potential data were found for the pyridinium ion, Raghavan and Iwamoto have obtained a value of −1.32 V for the 1-methylpyridinium ion in acetonitrile, referenced to the standard calomel electrode (SC).[14] Conversion to the standard hydrogen electrode (SHE) yields a ΔG value of −2.12 V for the transfer of an electron from the anthracene label to a neighboring pyridinium unit. For most anthracene derivatives the 0−0 transition energy for S_0-S_1 absorption is within the range 3.10−3.35 eV. Although we do not have the corresponding redox value for PVP, it

seems plausible that the proposed electron-transfer quenching mechanism is thermodynamically allowed.

The fluorescence intensity of the 9-methylanthracene (9-MA) solution described above was then monitored as pyridine and HCl were added in succession. Addition of pyridine up to 1.8 mM to the 9-MA solution did not result in any fluorescence quenching. Addition of HCl to give a maximum concentration of 2.4 mM resulted in a 4.8% decrease in the fluorescence intensity of the 9-MA/pyridine solution. The quenching data can be fit to the linear Stern−Volmer quenching expression, and assuming a fluorescence lifetime of 5.2 ns for 9-MA (in pure ethanol),[8] the second-order rate constant for pyridinium ion quenching, k_q, is found to be 5.8×10^9 M^{-1} s^{-1}, e.g., approximately a diffusion-controlled reaction. Chu and Thomas have reported that cetylpyridinium chloride quenches the pyrene excited singlet state at the diffusion-limited rate (ca. 1.5×10^{10} M^{-1} s^{-1}).[15]

To test any physical model to describe the HCl quenching behavior observed for the labeled polymers, it would be convenient to determine the initial fluorescence intensity values in the absence of HCl (I_0). Unfortunately, a minimal amount of HCl is required to prevent polymer precipitation in pure water. As mentioned earlier, the quenching data in Figure 2 are referenced to the fluorescence intensity in the presence of 0.586 or 0.600 mM HCl for A-m-PVP and BA-e-PVP, respectively (I_{init}). The HCl quenching data for A-m-PVP and BA-e-PVP are plotted in Figure 2 as the ratio I_{init}/I (= $R(\alpha)$) vs α (α_{min} = 0.229 and 0.243 for A-m-PVP and BA-e-PVP, respectively).

While the ratio $R(\alpha)$ for both labeled polymers increases smoothly over the entire range of data, changes of the slope occur in the range α ca. 0.42−0.45. This may be the result of counterion condensation occurring as α approaches the critical charge density. According to Manning's counterion condensation theory, the critical degree of protonation, α_c, at which chlorine counterions will condense onto the polyelectrolyte is 0.357, assuming the average axial spacing between pyridine units to be 2.55 Å.[16] The slope changes for both polymers occur at values slightly higher than this. We speculate that the jump in a near α_c corresponds to a conformational transition associated with achieving a critical linear charge density along the PVP chain (perhaps analogous to the so-called "hypercoiling" in PMA). The only literature we find concerning the effect of charge density on PVP is the report of Topp et al. on quaternized PVP in aqueous solution.[17] They find that there is a substantial change in the intrinsic viscosity for a degree of quaternization between 0.09 and 0.25. Their highest value is at approximately the lowest degree of protonation we are able to study. There is no conformational change suggested by their data for α ∼ 0.42. Therefore, if there is a conformational transition that is responsible for the change of the $R(\alpha)$ slope, it must be localized in the immediate vicinity of the fluorescence probes.

A Bernoullian Model of Fluorescence Quenching. From the discussion in the previous section we propose that the mechanism of fluorescence quenching is via electron transfer to a protonated pyridine moiety, and because this is expected to be a short-range process, we focus on the state of protonation of the nearest neighbors of the anthracene chromophores.

First we consider A-m-PVP in which the anthracene has two nearest pyridine neighbors. If x is the probability that a pyridine is protonated, then we may write the following for the anthracene fluorescence intensity:

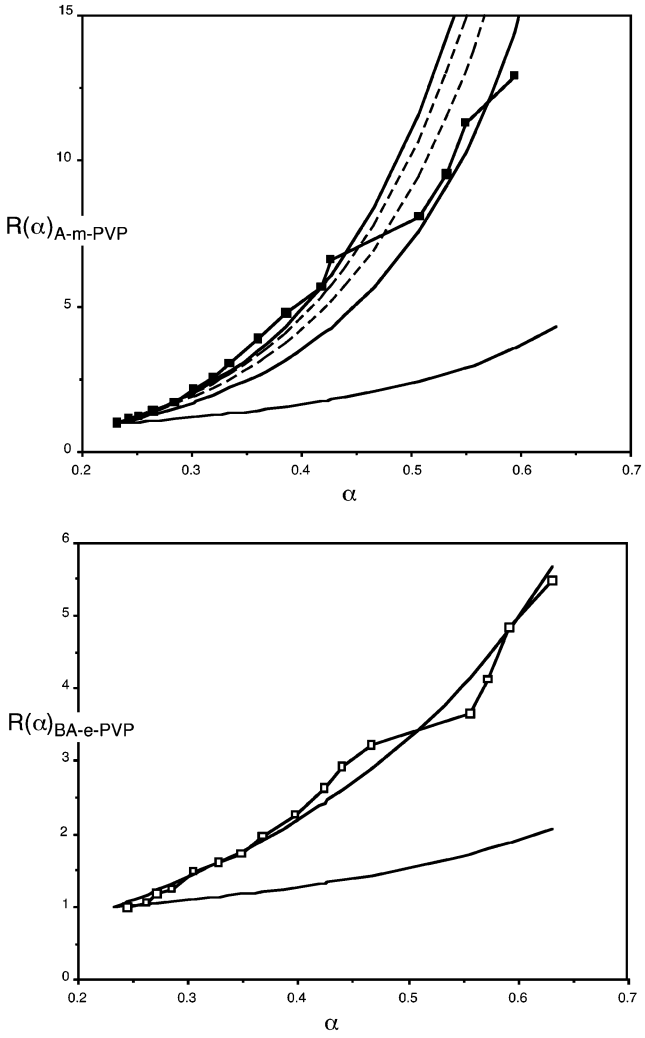

Figure 2. $R(\alpha)$ for A-m-PVP (upper) and BA-e-PVP (lower) as a function of α and different K values applied to the Bernoullian quenching model. For A-m-PVP the model curves are the smooth curves from the lowest curve up, K = 1, 8.9 (best least-squares fit), 20, 50, and infinity. For BA-e-PVP only K = 1 and K = infinity are shown.

$$I_{\mathrm{A-m-PVP}}(x) = \phi_{\mathrm{fl}}{}^{\mathrm{A-m-PVP}}[(1-x)^2 + 2xf_1 + f_2x^2] \quad (4)$$

The first term inside the brackets is the probability that neither pyridine is protonated, in which case the fluorescence of this population is unchanged from the unquenched reference state (with a fluorescence quantum yield of $\phi_{\mathrm{fl}}{}^{\mathrm{A-m-PVP}}$). The next two terms represent the probability of one or two protonated neighbors, and f_1 and f_2 represents the relative quantum yield in this case. Presuming that the pyridines quench efficiently and independently, it is reasonable to imagine that $f_1 = 2f_2$. If the quenching is very fast (as expected from our model compound quenching discussed previously), then both f_1 and f_2 would be expected to be very small. Because there is a minimum degree of pyridine protonation that is required for polymer solubility, the data presented in Figure 2 are for the ratio

$$R(\alpha)_{\mathrm{A-m-PVP}} = I_{\mathrm{A-m-PVP}}(\alpha_{\mathrm{min}})/I_{\mathrm{A-m-PVP}}(\alpha) \quad (5)$$

If we simply substitute α for x in eq 4, then the shape of the predicted $R(\alpha)$ curve is not a good match to the experimental findings. In particular, the experiment results resemble the prediction of eqs 4 and 5 only if the neighboring pyridines are assumed to have a higher degree of protonation than the average value (α).

If there is a preferential protonation of neighboring pyridines, we can write

$$PH^+ + P_A = P + P_AH^+ \tag{6}$$

with an equilibrium constant K. This yields the following relationship for x in terms of α:

$$x = \frac{\alpha K}{1 + \alpha(K - 1)} \tag{7}$$

According to this approach, the fluorescence quenching ratio, $R(\alpha)$, is a function of f_1, f_2, and K (and we expect f_1 and f_2 to be very small). Substituting for x in eq 4, one obtains the rather unwieldy expression

$$I_{A-m-PVP}(\alpha) = \phi_{fl}^{A-m-PVP} \frac{(1 - \alpha)^2 + 2f_1K\alpha + (2f_1K(K - 1) + f_2K^2)\alpha^2}{(1 + \alpha(K - 1))^2)} \tag{8}$$

If we ignore f_1 and f_2, then

$$R(\alpha)_{A-m-PVP} = \left\{\frac{(1 - \alpha_{min})}{1 + \alpha_{min}(K - 1)} \frac{1 + \alpha(K - 1)}{1 - \alpha}\right\}^2 \tag{9}$$

A similar approach predicts the following for BA-e-PVP:

$$I_{BA-e-PVP}(x) = \phi_{fl}^{BA-e-PVP}[(1 - x) + xf_1] \tag{10}$$

where the value of f_1 is not necessarily the same as for A-m-PVP because of different electronic factors, the differing lifetime of the excited state, etc. In any case, f_1 is expected to be small. Substituting for x using eq 10 and ignoring f_1, we obtain

$$R(\alpha)_{BA-e-PVP} = \frac{1 - \alpha_{min}}{1 + \alpha_{min}(K - 1)} \frac{1 + \alpha(K - 1)}{1 - \alpha} \tag{11}$$

From eqs 9 and 11 we see that if the preferential protonation constant K is equal for these two polymers that

$$R(\alpha)_{BA-e-PVP} = (R(\alpha)_{A-m-PVP})^{1/2} \tag{12}$$

Equation 12 suggests that a square-root relationship should exist between $R(\alpha)_{BA-e-PVP}$ and $R(\alpha)_{A-m-PVP}$. In Figure 3 we present this comparison and this expectation is realized, especially for α values between α_{min} and ca. 0.4.

Fitting the full Bernoullian model to the experimental data is not so straightforward because there are two or three independent parameters that affect the calculated $R(\alpha)$ curve in different ways. Roughly the values of f_1 and f_2 determine the dynamic range of the quenching while K shifts the x values to values larger than α (assuming $K > 1$). By far the preferential protonation constant K has the largest influence on the fit of the experimental data to the models, and we find that f_1 and f_2 can be taken to be zero within experimental error.[18] A nonlinear least-squares fit to the $R(\alpha)_{A-m-PVP}$ data for all α values using eq 8 (with f_1 and f_2 constrained to zero) yields $K_{A-m-PVP} = 8.9 \pm 1.1$, but fitting just the points between α_{min} (0.229) and 0.42 (e.g., before the "break" in the $R(\alpha)_{A-m-PVP}$ curve) yields very large and nonphysical values for $K_{A-m-PVP}$. The reason for this is that if K is much larger than unity, eq 9 becomes

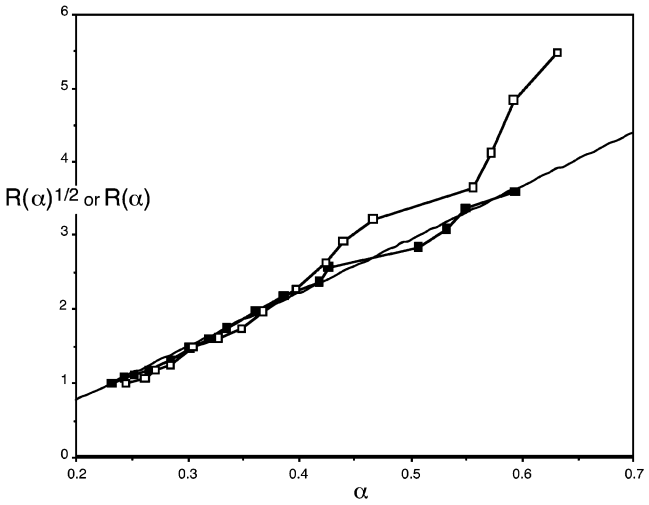

Figure 3. Plot of $(R(\alpha)_{A-m-PVP})^{1/2}$ (solid symbols) and $R(\alpha)_{BA-e-PVP}$ (open symbols) vs α.

$$R(\alpha)_{A-m-PVP} = \left\{\frac{1 - \alpha_{min}}{\alpha_{min}} \frac{\alpha}{1 - \alpha}\right\}^2 \tag{13}$$

e.g., independent of K. As can be seen from Figure 2, this limiting case fits the initial data reasonably well, while using $K = 8.9$ forces a fit through the experimental values for $\alpha > 0.42$. Also illustrated in Figure 2 is the predicted $R(\alpha)_{A-m-PVP}$ curve for $K = 1$, which clearly does not fit the data at all. The other K values presented (20 and 50) illustrate that obtaining a value of K from fitting the experimental data will have very poor precision, but we can certainly state that for the Bernoullian model to be applicable K must be equal to or larger than 10. For BA-e-PVP ($\alpha_{min} = 0.243$) the large K limiting form is a reasonably good fit to all the data (Figure 2), but once again there is a small "break" in the curve between $\alpha = 0.45$ and 0.55.

It is also possible to estimate the value of K from the absolute value of the fluorescence intensity using eqs 8 and 10. If the fluorescence quantum yield in pure methanol is taken to be equal to $\phi_{fl}^{A-m-PVP}$ or $\phi_{fl}^{BA-e-PVP}$, then we can write (ignoring the f_1 and f_2 terms)

$$I_{MeOH}/I(\alpha_{min}) = \left\{1 + \frac{\alpha_{min}K}{1 - \alpha_{min}}\right\}^n \tag{14}$$

where $n = 1$ and 2 for BA-e-PVP and A-m-PVP, respectively. Using the values in Table 1 and $\alpha_{min}^{A-m-PVP} = 0.229$ and $\alpha_{min}^{BA-e-PVP} = 0.243$, we obtain the estimate $K_{A-m-PVP} = 8.0$ and $K_{BA-e-PVP} = 2.9$. The former is reasonably close to the value obtained from the least-squares fit, but the latter is much smaller than expected from Figure 2.

We are not aware of any literature precedent that would explain why pyridines adjacent to anthracene moieties would be preferentially protonated. The pyri-

dinium ion could be stabilized by the relatively electron-rich anthracene moiety provided that there is sufficient mobility in the $-CH-CH_2-$ bridge to permit their juxtaposition.

Summary

The fluorescence intensity of mid- or end-tagged anthracene-labeled PVP in acidic aqueous solution is very dependent on the degree of protonation (α). This result was unexpected, and no precedent could be found in the literature. We presume that the quenching is the result of electron transfer from the excited anthracene to neighboring pyridinium units. (Unprotonated pyridine does not quench the anthracene singlet state.) Even a qualitative fit to a simple Bernoullian statistical model based on the state of nearest-neighbor protonation was not possible unless we propose that the protonation of pyridine units that are adjacent to the chromophore is significantly favored compared to pyridines located elsewhere on the chain. There is a change in the slope of the relative fluorescence vs α curve in the vicinity of 0.42−0.45 that suggests some kind of conformational transition at this degree of protonation.

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