Complex Formation between Poly(acrylic acid) and Pyrene-Labeled Poly(ethylene glycol) in Aqueous Solution

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ABSTRACT: Pyrene groups were attached to poly(ethylene glycol) (PEG) at both chain ends to allow pyrene excimer fluorescence to be used as a molecular probe of the complexation between PEG and poly(acrylic acid) (PAA). The excimer-to-monomer intensity ratio, I_D/I_M, was measured under two conditions to distinguish intramolecular and intermolecular excimer formation. The first is a system with PAA and a mixture of 1% of labeled PEG and 99% of unlabeled PEG; the second is one with PAA and 100% of end-tagged PEG. The decrease in I_D/I_M for the intramolecular excimer showed that the addition of PAA to PEG induces a decrease of intramolecular mobility of the PEG chain. On the other hand, the fluorescence behavior of the intermolecular excimer showed that the local concentration of PEG is increased in the vicinity of PAA as a result of hydrogen bond interaction. Both phenomena were more pronounced in the PAA–PEG complex formed from the PAA of higher molecular weight.

Introduction

The objective of this study is to utilize fluorescence spectroscopy to examine complex formation between proton-donor poly(acrylic acid) (PAA) and proton-acceptor poly(ethylene glycol) (PEG). The hydrogen-bonded complex observed for this system has been intensively investigated by many researchers.[1,2] Many kinds of analytical methods have been employed, including conductivity, potentiometry, viscometry, sedimentation, turbidity, and IR, NMR, and Raman spectroscopies. However, the application of fluorescence techniques is still quite limited.

Anufrieva et al. applied the polarized luminescence method to the stoichiometric hydrogen-bonding complexes between PEG and poly(methacrylic acid) (PMAA) or PAA in aqueous solution. It was found that the relaxation time τ_w of anthracene-labeled PMAA and PAA became much longer upon complexation with PEG: from 77 to 290 ns for PMAA and from 23 to 50 ns for PAA.[1,3] The same phenomenon was also observed in anthracene-labeled PEG, for which τ_w changed from <1 to 350 ns in the PMAA–PEG complex. The τ_w was independent of polymer concentration within the interval studied (0.03–1%). They concluded from the similarity of τ_w values between those of PMAA and PEG polymers as well as the increase in τ_w that the mobility of component polymers in the complex is restricted by a fairly long continuous linear succession of bonds between monomer units of the complementary polymer chains, very much like a ladder structure.

Morawetz et al. employed PAA labeled with the fluorescent dansyl group (Dan-PAA) to study hydrogen-bonding polymer complexes.[4-7] The characteristic feature of the dansyl group that was employed involved the fluorescence intensity, which is about an order of magnitude greater in organic solvents than in water. Complex formation between aqueous solutions of the Dan-PAA and PEG or poly(vinylpyrrolidone) (PVP) led to a sharp increase in the fluorescence intensity, since water is being displaced from the vicinity of the dansyl groups.[5]

In addition, the kinetics of complexation was also investigated. Anufrieva et al. demonstrated that if one of the component polymers labeled with a chromophore is added to the system that has already formed the complex, the substitution from the original polymer chain in the complex (unlabeled) to the added chain (labeled) can be monitored by the change in the fluorescence depolarization with time.[8] More recently, Morawetz and co-workers measured the kinetics of complexation as well as that of dissociation using a stopped-flow apparatus with the dansyl group, where the change in fluorescence intensity was used as a probe.[7] Both processes had low activation energies (3–4 kcal/mol) but very large negative entropies of activation (−36 and −43 eu, respectively). They concluded that complex formation involved an initial diffusion-controlled hydrogen bonding, which leads to the small activation energy. This was followed by extensive conformational transitions of the two polymer chains necessary to achieve the additional hydrogen bonding to stabilize the complex. This leads to the large negative entropy for complexation.

In this study excimer formation between pyrene groups attached to PEG chain ends was utilized as a molecular probe. Both intramolecular and intermolecular excimer formation are employed to analyze the complex formed between PAA and PEG. Various molecular weights are used for PEG and PAA samples to determine the effects of relative chain length of these component polymers, i.e., PAA < PEG, PAA = PEG, PAA ≫ PEG. The influence of stoichiometry and a modest concentration variation on the fluorescence data are discussed.

Table I
Molecular Weights of Samples

sample name	mol wt	polydispersity
PAA(1850)	M_n = 1000	2.1
	M_v = 1850	
	M_w = 2100	
	M_z = 4200	
PAA(4600)	M_n = 2100	2.4
	M_v = 4600	
	M_w = 5100	
	M_z = 9300	
PAA(890K)	M_v = 890 000	
PEG(4800)	M_w = 4800	1.05
PEG(9200)	M_w = 9200	<1.10
PEG*(4800)	*a*	*a*
PEG*(9200)	*b*	*b*

[a] Synthesized from the PEG(4800). [b] Synthesized from the PEG(9200).

Experimental Section

Materials. Three different molecular weights of PAA were used for the experiments as shown in Table I. Samples denoted PAA(1850) and PAA(4600) were purchased from Polysciences Inc. The company reports number-average, viscosity-average, weight-average, and *z*-average molecular weights on these two materials, denoted by M_n, M_v, M_w, and M_z, respectively, in the table. The polydispersities of PAA(1850) and PAA(4600) were 2.1 and 2.4, respectively. The number inside the parentheses of the sample name shows the viscosity-average molecular weight in the case of PAA samples. The materials obtained commercially were in the form of aqueous solutions which were freeze-dried.

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They did not contain any fluorescent impurity and so were used without further purification. The third PAA sample was synthesized by a conventional radical polymerization.[9] Acrylic acid was purified by distillation at low pressure: 325 K at 2.6 kPa. Polymerization was carried out under a nitrogen atmosphere at 318 K using ammonium persulfate as an initiator. After purification of the product by multiple precipitation in dilute HCl, chlorine ions were removed by dialysis against water. The molecular weight was determined by viscosity measurement in 1,4-dioxane solution at 303 K. Under this Θ condition, the Mark–Houwink constants in the expression $[\eta] = KM^a$ are known to be $K = 8.5 \times 10^{-4}$ [mL/g] and $a = 0.50$.[10] The viscosity-average molecular weight was found to be 8.9×10^5 and the sample is denoted PAA(890K).

Pyrene end-labeled poly(ethylene glycol) (PEG*) was synthesized by direct esterification between poly(ethylene glycol) and 1-pyrenebutyric acid (PBA).[11] Monodisperse (polydispersity <1.10) samples of PEG of molecular weights 4800 and 9200 were purchased from Polysciences Inc. PBA was obtained from Eastman Kodak and purified by recrystallization in dry toluene. The reaction between PEG and PBA was carried out in toluene for 48 h at 383 K under a nitrogen atmosphere using *p*-toluenesulfonic acid monohydrate as catalyst. Water was removed during the reaction via azeotropic distillation using a Dean–Stark water trap to drive the esterification to completion. The product was purified by repeated precipitation in anhydrous ether from THF solution. To verify that the high reaction temperature had not degraded the PEG, the untagged and tagged PEG samples were analyzed by gel permeation chromatography in THF using a series of Waters Styragel columns. The polydispersities of the products were unchanged and the peak positions of molecular weight shifted to slightly higher values as expected from the effect of newly attached pyrene groups. Tagging percentages were calculated by UV absorption with methyl 1-pyrenebutyrate as the model compound, which was obtained from Molecular Probes, Inc. The products were determined to be fully labeled at both ends with ±5% accuracy and were denoted PEG*(4800) and PEG*(9200), where the asterisk designates a pyrene fluorescence label.

Spectroscopy. The UV–visible absorption spectra were measured with a Cary 210 spectrophotometer manufactured by Varian. The excitation spectra were measured by a Spex Fluorolog 212 spectrofluorometer. The monomer excitation spectrum was monitored at 376 nm and the excimer excitation spectrum was monitored at 500 nm in the scanning excitation range between 300 and 370 nm.

The fluorescence spectra were taken with a spectrofluorometer that has been described previously.[12] Excitation was at 343 nm, corresponding to the ^1L_a band of the pyrene ring. The output from the picoammeter was sent through an analog-to-digital converter to a PDP 11/23 computer, and the raw data were corrected by the spectral response function. The excimer-to-monomer intensity ratio, I_D/I_M, was calculated from the areas for an excimer and a monomer, respectively. In order to get the vibrational structure of the monomer entity, 1-pyrenebutyric acid was used as a model compound. The excimer area was then calculated by subtraction of the monomer area from the total area.

The concentration of the PEG* aqueous solution was selected to be at a high level, as long as there was no self-absorption effect. The PEG* sample was dissolved in glass-distilled deionized water and adjusted to 1.0×10^{-3} or 2.0×10^{-3} M per repeating unit. PAA was also dissolved in distilled deionized water and adjusted to 1.0×10^{-1} or 2.0×10^{-1} M per repeating unit. The PAA concentration was selected to be 100 times greater than that of PEG* in order to neglect the effect of dilution upon its addition to PEG*. The composition of the complex was described by the molar ratio of the two repeating units, [PAA]/[PEG], because the complex is known to be formed between a carboxylic acid of PAA and an ether oxygen of PEG.[1,2] To prepare a sample, 4 mL of the PEG* solution was placed in a quartz tube and the PAA solution was added to the PEG* with a microsyringe. The sample was newly prepared for each data acquisition. A water bath was used to keep the temperature of the sample solution at 303 K.

Results

1. Fluorescence Spectrum of PEG* Aqueous Solution.

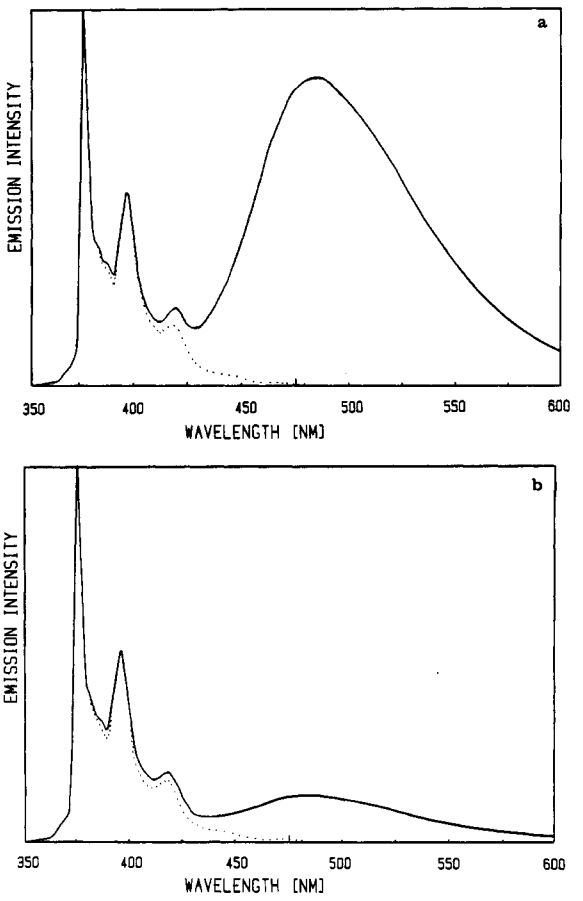

Figure 1. (a) Emission spectrum of 1×10^{-3} M PEG*(4800) aqueous solution at 303 K. (b) Emission spectrum of 1×10^{-3} M PEG*(9200) aqueous solution at 303 K. The spectrum shown by a dotted line is for 1-pyrenebutyric acid.

The fluorescence spectra of 1×10^{-3} M PEG* aqueous solution at 303 K exhibited emission both from the locally excited pyrene chromophore (monomer) and also from the excimer, as shown in Figure 1. The emission from a monomer entity observed between 370 and 430 nm was assumed to have the same envelope as 1-pyrenebutyric acid, which is shown as a dotted line in Figure 1. The vibrational structure consists of three distinct peaks with two shoulders after the first. The broad structureless band centered at 480 nm is due to the excimer. The excimer band usually can be fit to a Gaussian shape unless there are multiple excimer states. However, the excimer band observed here showed tailing at longer wavelengths so that force-fitting of a Gaussian shape to the excimer emission was considered improper. Therefore, the bandwidth and position derived from imposed Gaussian fitting are not reported here. The excimer area was calculated by subtraction of the monomer area from the total envelope area. The excimer-to-monomer intensity ratio, I_D/I_M, was then calculated.

We note at the outset that there are two types of excimer formation in the PEG* aqueous solution. The first type of excimer-forming site (EFS) results intermolecularly by association between chromophores from two different polymer chains. The number of these sites is directly dependent on the local concentration of labeled chains. The second type of excimer site arises from association between aromatic rings on the same polymer chain. In the present case, it means that cyclization of the labeled PEG chain must occur. Since this type of excimer is intramolecular, it is independent of the chain concentration.

Although pyrene end-tagged poly(ethylene oxide) has been studied extensively in organic solution by Cuniberti[11]

and Winnik et al.,[13] few results have been reported on PEG* in aqueous solution. Water is a particularly interesting solvent for PEG not only because it is a good solvent but also because the strong-hydrogen-bonding affinity of the ether oxygen of PEG chain plays an important role in the polar medium. The latter effect results in the association of the proton-acceptor PEG polymer chain with proton-donor polymeric acids. In order to set the stage for the subsequent studies on polymer association, we will first focus on the peculiar characteristic of aqueous solutions by comparison of the fluorescence behavior of PEG in aqueous solution with that in organic solutions.

In Winnik's paper on the effects of solvent on end-to-end cyclization of PEG,[13] the excimer-to-monomer intensity ratio, I_D/I_M, was observed to be inversely proportional to solvent viscosity, η_0, in nonprotonic solvents, as expected for a diffusion-controlled process. However, in water and methanol the extent of excimer emission and the rate of intramolecular excimer formation were substantially greater than one would infer on the basis of solvent viscosity alone. They explained this phenomenon by assuming that the pyrene chromophores are not uniformly distributed outside and inside the polymer coil. They suggested that the hydrophobic chromophore is undoubtedly better solvated by the environment within the polymer coil than if the pyrene were surrounded completely by water or methanol. This situation would be expected to lead to an increased I_D/I_M due to a reduced distance of separation between pyrenes.

Although this proposal seems plausible, it must be remembered that on the order of 95% or more of the volume enclosed by a polymer coil in solution consists of solvent molecules.[14] Thus, there might not be an appreciable difference between the environment afforded to a pyrene group whether it is on the surface or the interior of a coil. An alternative explanation that includes the spirit of the Winnik proposal, if not the same physical foundation, is that the interactions between the pyrene groups and the water environment are sufficiently unfavorable that some form of aggregation of pyrene groups occurs. Since the molecular weight of the PEG* is expected to influence the compatibility of PEG* in aqueous solution, it was of interest to repeat the Winnik experiment with both our PEG*(4800) and PEG*(9200) samples. To do so, I_D/I_M values were determined in several solvents at the same concentration as for the Winnik study. The results are shown in Figure 2, which shows plots of I_D/I_M vs. $1/\eta_0$, where η_0 is solvent viscosity at 25 °C.

The PEG* in the aqueous solution showed the same qualitative behavior as observed by Winnik. However, the deviation of I_D/I_M of the aqueous solution from the value expected from the diffusion-controlled cyclization process was considerably larger in the shorter PEG chains. In the PEG*(4800) aqueous solution, the observed I_D/I_M value was 8.4 times greater than the value expected for the diffusion-controlled mechanism, i.e., the value given by the straight line in Figure 2a at the appropriate viscosity of water. In the PEG*(9200) aqueous solution, the actual value of I_D/I_M was 3 times greater. These results tend to support the aggregation proposal in that the effect is reduced for the higher molecular weight PEG* in which the more extensive hydrogen-bonding interaction between the backbone units and water molecules would enhance the thermodynamic compatibility. A more detailed study of the effect of concentration and of PEG* molecular weight on the aggregation of PEG* in aqueous solution is being pursued separately.[15]

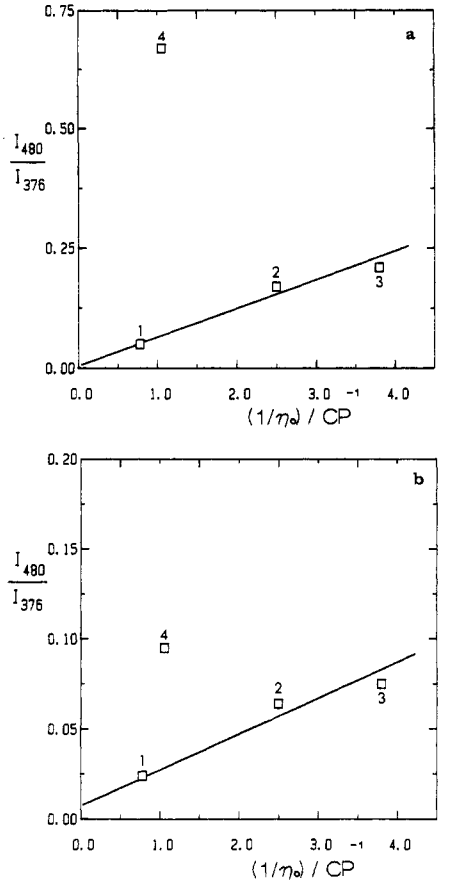

Figure 2. Excimer-to-monomer intensity ratio of (a) PEG*(4800) and (b) PEG*(9200) in various solvents. The ratio was calculated from the peak heights at 480 and 376 nm for excimer and monomer intensities, respectively. (1) Dioxane, (2) ethyl acetate, (3) acetone, and (4) water.

It is sufficient for the present paper merely to note that some PEG* inhomogeneity is clearly indicated in the aqueous solution prior to addition of the proton donor. Although there may be an equilibrium between initially associated PEG* chains and free PEG* chains, the primary thrust of the present work is based on the assumption that initial aggregation does not appreciably influence subsequent interaction with PAA. This essentially means that the initial aggregate is considered to result from weak interactions.

2. Intramolecular Excimer Formation in the PEG*–PAA Complex. Intramolecular excimer formation has been reported extensively in past studies[11,13] because of its rather direct applicability to treatment of experimental data. However, the objective of the present work was to elucidate intermolecular excimer formation as well as intramolecular excimer formation in the PAA–PEG* complex system. Therefore, under such a complicated situation, it is necessary to distinguish the two types of excimers clearly. For this purpose, a solution of 1% of PEG* (both chain ends tagged by chromophores) and 99% of PEG (untagged) was prepared. We assumed that the mixture of 1% PEG*–99% PEG solution allowed the behavior of an individual PEG chain to be examined in terms of intramolecular excimer formation. As a result, we assumed that the contribution of intermolecular excimer formation could be elucidated by the difference in data between the fully tagged PEG system and the 1% PEG*–99% PEG system. The intermolecular excimer formation will be discussed in the third section.

The fluorescence spectra of the 1% PEG*–99% PEG mixture were measured upon addition of PAA aqueous

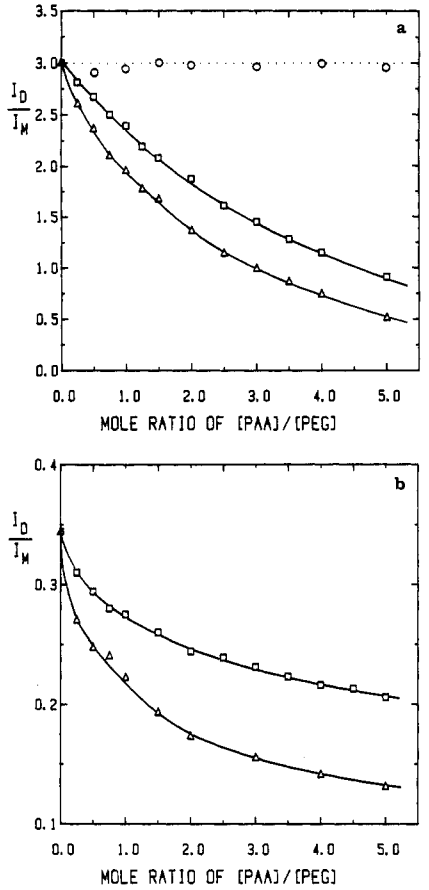

Figure 3. Change in the intramolecular excimer-to-monomer intensity ratio with the addition of proton donors at 303 K. (a) Measured for the mixture of 1% PEG*(4800) and 99% PEG(4800) (total PEG concentration, [PEG* + PEG] = 1×10^{-3} M; concentration of PAA, [PAA] = 1×10^{-3} M; concentration of PAA, [PAA] = 1×10^{-1} M): (△) PAA(890K); (□) PAA(1850); (○) acetic acid. (b) Measured for the mixture of 1% PEG*(9200) and 99% PEG(9200) (total PEG concentration, [PEG* + PEG] = 2×10^{-3} M; concentration of PAA, [PAA] = 2×10^{-1} M): (△) PAA(890K); (□) PAA(1850).

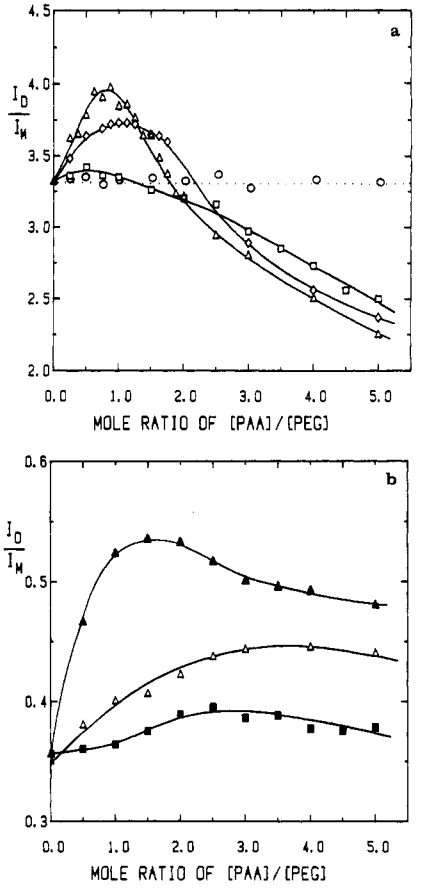

Figure 4. (a) Change in the ratio of excimer-to-monomer intensity of PEG*(4800) with the addition of PAA at 303 K ([PEG*] = 1×10^{-3} M; [PAA] = 1×10^{-1} M): (△) PAA (890K); (◇) PAA(4600); (□) PAA(1850); (○) acetic acid. (b) Change in the ratio of excimer-to-monomer intensity of PEG*(9200) with the addition of PAA at 303 K: (▲) 2×10^{-3} M PEG*(9200) + 2×10^{-1} M PAA-(890K); (△) 1×10^{-3} M PEG*(9200) + 1×10^{-1} M PAA(890K); (■) 2×10^{-3} M PEG*(9200) + 2×10^{-1} M PAA(1850).

solution. The total concentration of labeled and unlabeled PEG was fixed to be 1×10^{-3} M per repeating unit. The PAA concentration was selected to be 100 times higher than that of the total PEG in order to neglect the effect of dilution by its addition to PEG. Figure 3a shows the results for (1% PEG*(4800) + 99% PEG(4800)) and Figure 3b presents those for (1% PEG*(9200) + 99% PEG-(9200)). The higher initial value of I_D/I_M in Figure 3a compared to that in Figure 3b is due to the larger aggregation of terminal pyrene groups of the shorter PEG chain in water as well as due to the larger diffusivity of the shorter PEG chain facilitating intramolecular excimer formation, as described in the previous section. It was found from Figure 3 that the addition of PAA induced a tremendous decrease in I_D/I_M of PEG* where PAA(890K) caused a greater decrease than did PAA(1850). It is interesting to note in comparing parts a and b of Figure 3 that at the [PAA]/[PEG] composition ratio equal to 5, PEG(4800) showed a larger decrease in I_D/I_M than PEG-(9200) relative to each original value for association with PAA(1850) and PAA(890K). However, at the stoichiometric [PAA]/[PEG] ratio equal to unity, the extent of decrease was 20% and 35% in PEG–PAA(1850) and PEG–PAA(890K), respectively, where there was no difference between PEG(4800) and PEG(9200). Note that even though the PAA–PEG complex is reported to form a stoichiometric complex,[1,2] there was no discontinuity at the stoichiometric ratio. The I_D/I_M ratio continued to drop even in the presence of excess PAA. However, acetic acid did not cause any change over the whole composition range.

3. Intermolecular Excimer Formation in the PEG*–PAA Complex. Next, the same experiments were repeated with the aqueous solution of fully tagged PEG materials (PEG*) at the same polymer concentration. Figure 4 shows the change in I_D/I_M for PEG* (fully tagged) by addition of the PAA proton donor. The drastic difference compared to Figure 3 is apparent. Figure 4a contains data on 1×10^{-3} M PEG*(4800) with various PAA's at 303 K. The initial I_D/I_M value was higher than that in Figure 3a due to the contribution of intermolecular excimer formation. The difference also means that 90.4% of the I_D/I_M value in the 1×10^{-3} M PEG*(4800) is ascribed to the intramolecular excimer and 9.6% to the intermolecular excimer. In addition, this figure shows that PAA(890K) and PAA(4600) cause an initial increase in I_D/I_M followed by a decrease up to ca. two-thirds of the initial I_D/I_M value at [PAA]/[PEG] = 5. However, PAA-(1850) did not increase substantially at the beginning and this was followed by a gradual decrease ending up a higher I_D/I_M value than other PAA systems at [PAA]/[PEG] = 5. Finally, although acetic acid is supposed to be another type of proton donor, it had absolutely no effect over the whole region, in the same fashion as that in Figure 3a. Furthermore, in the case of PEG*(9200), given in Figure 4b, the change in I_D/I_M was smaller compared to Figure

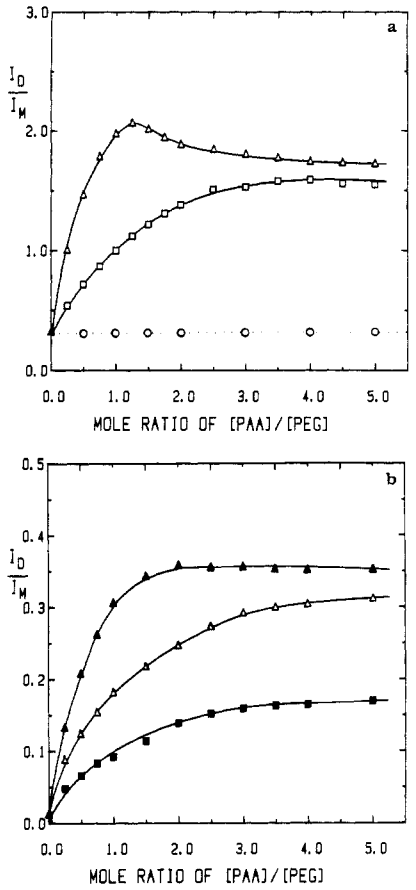

Figure 5. Change in the intermolecular excimer-to-monomer intensity ratio with the addition of PAA at 303 K. It was calculated by the subtraction of data in Figure 3 from those in Figure 4. (a) PEG*(4800): (△) 1×10^{-3} M PEG*(4800) + 1×10^{-1} M PAA(890K); (□) 1×10^{-3} M PEG*(4800) + 1×10^{-1} M PAA(1850); (○) 1×10^{-3} M PEG*(4800) + 1×10^{-1} M acetic acid. (b) PEG*(9200): (▲) 2×10^{-3} M PEG*(9200) + 2×10^{-1} M PAA(890K); (△) 1×10^{-3} M PEG*(9200) + 1×10^{-1} M PAA(890K); (■) 2×10^{-3} M PEG*(9200) + 2×10^{-1} M PAA(1850).

4a at the same condition. When the concentration was doubled, the increase in I_D/I_M was observed more clearly. It appears that the complex formation is facilitated upon increase of the PAA molecular weight and/or the polymer concentration. For the 1.0×10^{-3} M PEG*(9200) system, the intermolecular excimer contribution amounted to only 2.9% of the initial I_D/I_M; for 2.0×10^{-3} M PEG*(9200) it amounted to 4.2%.

As the next step, in order to obtain the net contribution of the intermolecular excimer, the smoothed I_D/I_M data shown in Figure 3 were subtracted from the corresponding smoothed data in Figure 4. The results are given in Figure 5. In Figure 5a on the intermolecular excimer formation of PEG*(4800), the data for 1×10^{-3} M PEG* with PAA(890K) reach a maximum at about the stoichiometric ratio. On the other hand, PAA(1850) increased more slowly and reached a plateau region at [PAA]/[PEG] = 3. This shows that the higher molecular weight of PAA causes stronger intermolecular aggregation at low [PAA]/[PEG] ratios. However, at higher [PAA]/[PEG] ratios, the molecular weight dependence of PAA was very slight. In Figure 5b, the I_D/I_M increased more gradually than that in Figure 5a. The PEG(9200)–PAA system with higher molecular weight of PAA showed the larger value of I_D/I_M after reaching the plateau, where that of PAA(890K) system showed a value over twice that of the PAA(1850) system. Intermolecular excimer formation was found to be influenced by the concentration again. At the higher concentration, more

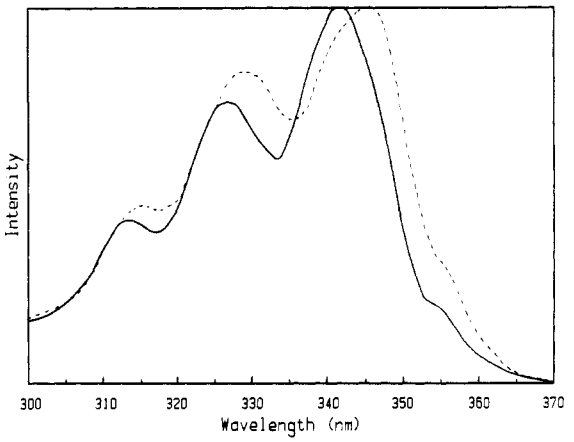

Figure 6. Excitation spectra of PEG*(4800): (a) monitored at 376 nm (solid line); (b) monitored at 500 nm (dotted line).

excimers were formed intermolecularly.

4. Excitation Spectra for PEG* Aqueous Solution. From the preceding results it should be apparent that excimer formation between pyrene groups in aqueous solution is very different from that in organic solvent. This was manifested in the higher I_D/I_M value compared to that expected from a diffusion-controlled process and in the excitation spectra as shown in Figure 6. The spectrum monitored at the monomer emission maximum at 376 nm was well resolved and differed from the more poorly resolved and red-shifted spectrum monitored in the range of the excimer emission at 500 nm. This second spectrum corresponds to the aggregated pyrene moieties. This difference in excitation spectra is due to a ground-state interaction between pyrene groups, which could also lead to the excited-state interaction of excimer fluorescence. Thus the excimer formed in this system is essentially different from that in organic solvents where the pyrene excimer can be formed independent of any ground-state interactions.[13] This type of excimer fluorescence used as a probe of intermolecular complexation leads to several important points, as will be described in the following discussion section.

Discussion

From these observations on the intermolecular and intramolecular excimers formed between PEG chain ends, we infer several important effects of complexation on the component polymers.

First, it is worth noting that the fluorescence technique was more sensitive to intermacromolecular complex formation than conventional methods, such as potentiometry, viscometry, and turbidimetry. From these latter methods, Antipina et al. concluded that in order for a complex to be formed between poly(methacrylic acid) (PMAA) and PEG, the PEG molecular weight should not be less than 2000, while any significant interaction between PAA and PEG started only when the molecular weight of the PEG was around 6000.[16] In addition, Ikawa et al. also reported that they did not observe any change in viscometric data when the PEG molecular weight was less than 1760 for the PMAA–PEG complex or 8800 for the PAA–PEG complex, when their polymer concentration was 3×10^{-3} M at 298 K.[17] However, in the present study on complexation, the fluorescence technique was shown to be sensitive enough to observe the interaction between PEG*(4800) and PAA with a wide range of molecular weight between 1850 and 890 000.

Second, the longer chain of PAA showed a much stronger interaction with PEG. This is particularly evident in the larger decrease in the plots of Figure 3, which will

be discussed later. In general, the effect of molecular weight of the proton-donating polymers on complexation has received little attention, although the effects of PEG molecular weight dependence have appeared in several papers. For example, Antipina et al. observed that for PEG of molecular weight greater than 6000, the reduced viscosity at the stoichiometric ratio started to drop drastically with complexation and that the degree of decrease in viscosity depended on the PEG molecular weight. Longer chains of PEG reduced the viscosity a greater amount for molecular weights up to 40000 at 298 K.[16] The present observation of the molecular weight dependence of PAA on complexation is similar to the results of the molecular weight dependence of PEG. Only the proton-donor analogue of low molecular weight, acetic acid, did not interact with PEG. This means that the driving force toward the formation of a hydrogen bond between an ether and a monocarboxylic acid in aqueous solution is very small and that a stable complex can be formed only by the cooperative interaction of many such groups. Moreover, the observation that the I_D/I_M value was constant in the acetic acid–PEG system implies that the ester linkage to label pyrene groups was stable in this study. This is because the I_D/I_M would be expected to decrease if ester group hydrolysis occurred.

Third, upon complexation with PAA, PEG chains start to aggregate along the added PAA due to hydrogen bond interactions. This was observed as an initial increase in I_D/I_M in Figure 5. An increase in PAA molecular weight and in polymer solution concentration facilitates intermolecular excimer formation. The interesting point here is that the excimer is still able to be formed between pyrenes at the PEG chain ends intermolecularly even though the system has a larger molecular weight of PEG than that of PAA as observed in both the PEG*–PAA(1850) systems.

Fourth, the prescence of excess PAA does not cause distribution of PEG chains to unreacted PAA in the time range studied here because the intermolecular excimer showed a constant value at higher [PAA]/[PEG] ratios. In a previous study employing a sedimentation diagram, Papisov et al. reported that the PEG oligomers distributed in a random manner into the PAA matrix up to a stoichiometric ratio.[18] By contrast, in the PMAA–PEG system in water where the unassociated PMAA matrix possesses a specific globular conformation in an aqueous solution, the distribution of oligomers occurs according to the "all-or-none" principle; i.e., one part of the matrix polymer is completely bound and the other is practically free. Polarized luminescence has also been employed to elucidate the distribution of PEG oligomers in the proton-donating matrix.[1] The relaxation times for labeled PMAA* and PAA* chains in the presence of PEG were quite different, depending on the degree of filling of the matrix with the oligomer. Thus, in our PAA–PEG system, it seems that the aggregation of PEG along PAA is occurring in a homogeneous way, not in the fashion of the "all-or-none" principle until [PAA]/[PEG] = 2–3. However, above that composition ratio there are two kinds of PAA chains in the system; one participates in the complexation with PEG and the other remains unreacted.

Fifth, the addition of PAA induced a decrease in I_D/I_M of intramolecular excimer formation of PEG, as given in Figure 3. Even though the PAA–PEG complex was observed to exhibit 1/1 stoichiometry in past studies,[1,2] I_D/I_M for the isolated chain decreases continuously with an increase in the [PAA]/[PEG] molar composition ratio. It seems due to a decrease in intramolecular mobility of PEG chain because it is supposed to affect the cyclization rate of the PEG chain directly. Anufrieva et al. also demonstrated that the relaxation time of the component polymer chains becomes longer with complexation.[1,3] The smaller decrease in I_D/I_M for PAA(1850) than for PAA(890K) seems due to the fact that the shorter chain of PAA has the weaker interaction with PEG, and thus a smaller loss of intramolecular mobility of PEG chain is promoted.

Bednář et al. investigated the PAA–PEG system by randomly labeling PAA with a dansyl group.[4] They proposed that the dansyl-labeled PAA chain is in contact with PEG only at widely separated regions along its contour and that the PAA chain is stretched out between these contact points. They concluded that some of the dansyl labels are removed from the aqueous environment while others are in even more intimate contact with water than in the unassociated dansyl-labeled PAA chains. They used several kinds of PEG with molecular weights between 1.6×10^4 and 2.43×10^6, while the molecular weight of their PAA was 2.86×10^5. Thus, even their shortest PEG chain is several times longer than our samples. In the PAA–PEG complex between the shortest chain of PEG and the PAA, they observed that the dansyl label had efficient access to the PEG, thereby creating a more hydrophobic environment. By contrast, the PAA with the longer chains of PEG was in even more intimate contact with water than in the unassociated PAA solution.

Finally, as Antipina proposed, there may exist two types of equilibrium in cooperative reactions occurring on the macromolecular level and within the associated pair of carboxylic acid and ether oxygen.[16] The former is dependent on the molecular weight and concentration of the component polymers, whereas the latter is simply governed by the reaction between proton donor and proton acceptor. They observed that in the PMAA–PEG system with a low molecular weight of PEG such as 2000 or 3000, the dilution of solution containing an equimolar ratio of monomer units of the complex components resulted in the dissociation of the complex. However, it did not occur in the system with high molecular weight PEG. Since higher molecular weight of component polymer is required to stabilize the complex in the PAA–PEG system than in the PMAA–PEG system,[16,17] the first type of equilibrium might affect substantially the complex composition of PAA–PEG in our experiment. The data in Figure 5 showed that the composition seemed to locate between 2 and 3.5 of [PAA]/[PEG], depending on molecular weight and concentration. In this case, the complex system does not have a perfect match of the two complementary polymers, presumably involving a considerable number of unbound functional groups.

Summary

Pyrene excimer fluorescence was used as a sensitive proximity probe in the intermacromolecular complex system. Although numerous papers have been published on the PAA–PEG complex, the present work provides new information on interactions between component polymers. At 1 (or 2) $\times 10^{-3}$ M the PEG* solution shows excimers mostly formed intramolecularly, although some excimer formation results from pyrene chromophore aggregation in water. Upon addition of PAA solution, the intramolecular mobility of the PEG chain was suppressed, resulting in decreased intramolecular excimer formation. Simultaneously the local concentration of PEG is increased in the vicinity of PAA. Excimer formation seems to result from an arrangement of pyrene groups which is already preformed in the ground state. The effect of complexation is observed to be more pronounced in the PEG–PAA with

a higher molecular weight of PAA.

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Topological Constraints and Their Influence on the Properties of Synthetic Macromolecular Systems. 1. Cyclic Macromolecules

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ABSTRACT: Monte Carlo simulations of closed random walks on a body-centered cubic lattice are used to investigate the influence of the average knot structure on the root mean square radius of gyration R_g and scattering function $P(q)$ of flexible ring polymers obtained by cyclization under Θ-conditions. Calculations are carried out for walks of 10 to 160 steps. Considered as an equivalent chain model for ring polystyrene, this corresponds to molecular weights $M_n \leq 120000$. Up to 37% of the ring molecules are shown to be knotted. The influence of the number and kind of knots on R_g is presented in some detail. Removing part or all of the unknotted rings produces a more pronounced maximum in the Kratky plot of the scattering function. Comparisons are made with experimental small-angle neutron scattering data for cyclic polystyrene in deuteriated cyclohexane.

1. Introduction

The recent synthesis of ring-shaped polystyrenes via anionic polymerization has led to a number of interesting experimental studies involving these polymers.[1-6] It is believed that a detailed investigation of the properties of ring polymers, in dilute solution and in the melt, may ultimately result in a better understanding of the first principles governing the behavior of macromolecules.

Experiments carried out in dilute solution at the Θ-temperature for linear polystyrene (cyclohexane, 35 °C) show a positive second virial coefficient A_2 for ring polystyrene.[1] Theoretically, this was already predicted by Vologodskii et al.,[7,8] who showed that the condition of conservation of the topological state of the system (absence of linkage, or linkage of a definite type such as catenates) leads to entropy-type interactions between the chains. These interactions were shown to be quite appreciable and effectively forbid any mutual penetration of unlinked polymer rings. Hence, intermolecular excluded volume effects will be present in dilute solutions of ring polymers in Θ-solvents of the corresponding linear polymers. For linear polymers, the Θ-temperature (normal Θ-temperature) is generally considered to be the temperature of unperturbed dimensions, vanishing second virial coefficient, and onset of phase separation.[9] Candau et al.[10] showed that for star polymers, these three properties do not necessarily occur at the same temperature. Combining the strongly increased density inside the coil of a highly branched polymer with a segment density dependent χ-parameter, they were able to show that the temperature of unperturbed dimensions, Θ_α, is in general lower than the temperature, Θ_{A_2}, of vanishing second virial coefficient. Both are below the Θ-temperature of the corresponding linear polymer. The density inside the coil of a ring polymer is also higher than for a linear polymer, but the difference is too small[11] to give rise to similar effects.

The topological interaction between two unlinked ring polymers is always present and gives rise to a positive second virial coefficient at the normal Θ-temperature. However, if rings of all types of knot structures are present in proportions dictated by ring closure under Θ-conditions, the Θ-temperature of linear polymers should be the temperature of unperturbed dimensions of ring polymers, too. If, on the other hand, ring closure takes place under conditions giving rise to a nearly complete absence of knots, as is the case for cyclization in good solvents, the situation is somewhat different. It is generally believed that knot-free rings (trivial rings) should also show intramolecular excluded volume behavior due to the topological constraints, even at the normal Θ-temperature. So far, numerical simulations have not been able to confirm this. des Cloizeaux et al.[12] went as far as investigating unknotted

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