Fluorescence Anisotropy Studies of Polyelectrolyte Mobility and Interpolyelectrolyte Complexation in Aqueous Solution

Introduction. Much interest has been shown in recent years in the study of water-soluble polymers. In this respect, fluorescence techniques have been used (see for example refs 1–3) to complement the more conventional armory of methods in the study of the physical behavior of polyelectrolytes in aqueous media. In addition to studies of molecular mobility and conformational behavior, fluorescence techniques have been used to investigate interpolyelectrolyte complexation.[2-10] However, there have been relatively few reports of the use of luminescence anisotropy and in particular of time-resolved polarization data in this context. Recently, Heyward and Ghiggino[11] reported on fluorescence polarization determination of rotational correlation times for the complex formed between poly(acrylic acid), PAA, and poly(ethylene oxide), PEO, in an aqueous medium. In this paper we report on initial studies of the interpolymer complexation of PEO with poly(methacrylic acid), PMAA. PMAA represents a particularly interesting case for study since the additional hydrophobic interactions introduced by the methyl substitutents in PMAA relative to those operative in PAA result in *hyper*coiling of the PMAA in acid media wherein the facility for complexation via hydrogen-bonding interactions becomes apparent. It is information regarding the effects of complexing macromolecules upon the hypercoil and the dynamics and nature of the resultant complex that we seek in our current investigations.

Experimental Section. Poly(methacrylic acid) labeled with 1-vinylnaphthalene (1 mol %) was synthesized by free-radical polymerization at 60 °C in benzene by using AIBN as initiator.[12] The polymer was purified by multiple reprecipitation prior to spectroscopic characterization.[12] The molar mass characteristics of the resultant labeled PMAA are as follows: M_w = 105K; M_w/M_n = 1.9.

Steady-state fluorescence measurements were made on a Perkin-Elmer MPF-3 spectrometer. Time-resolved data were obtained by using the method of time-correlated single-photon counting using either excitation from a thyratron-gated coaxial flashlamp [Edinburgh Instruments 199] or radiation from the Synchrotron Radiation Source (SRS), Daresbury, U.K.[13,14]

The pH of aqueous polyelectrolyte solution (final concentration 10^{-3} wt % in polymer) was varied by addition of HCl or NaOH as required. Poly(ethylene oxide)s of nominal molar masses 100 000 (Aldrich), 6000, and 400 (Koch-Light) were used as supplied.

Results and Discussion. Steady-State Excitation. Estimates of segmental relaxation times, ρ, under conditions of steady-state excitation have been obtained by using the eq 1 developed by Perrin[15]

$$(p^{-1} - {}^1/_3) = (p_0^{-1} - {}^1/_3)(1 + 3\tau/\rho) \qquad (1)$$

where p is the degree of polarization and requires independent determination of τ, the excited-state lifetime of the fluorescent label, and p_0, its intrinsic polarization. In this instance p_0 values for the 1-VN label in the hypercoiled polyacid environment were obtained by extrapolation of p^{-1} values to zero τ,[16] quenching the excited-state dynamically by using CH_3NO_2.[12,17] The resultant relaxation time for PMAA at 298 K and pH 3 was 160 ± 10 ns.[12,17] Comparison of this value with that of 18 ± 2 ns obtained for the loosely coiled state, extant at pH 11, demonstrates the restrictions upon segmental mobility induced by hypercoiling.

Addition of PEO (of any molar mass) to PMAA solutions at pH 11 had no affect on the degree of polarization of emission obtained from the 1-VN label, indicative of the fact that complexation between the polymers does not occur when the PMAA is fully ionized.

Similarly, at pH 3, addition of low molar mass oligomers (e.g., PEO of molar mass ca. 400) did not effect any changes in p at any PMAA/PEO weight ratio between 0.01 and 100. However, as the molar mass of the PEO is increased above some critical value such that cumulative H-bonding interactions between carboxylic and ether functions results in interpolymer binding, the radiation emitted by the 1-VN label becomes progressively polarized up to a mole ratio of 1.[17] Similar behavior has been observed for the complexation of PAA and PEO.[9,11]

Such studies of fluorescence polarization not only clearly indicate that complexation occurs between the two macromolecules but also illustrate the severe restrictions on segment mobility that ensue from such interactions. It is obvious that the rotational relaxation time of the complex is very much longer than that of the hypercoiled PMAA value of 160 ns. More importantly, the rotational relaxation time of the complex would appear to be considerably greater than that (ca. 138 ns[11]) of the corresponding PAA/PEO species. Unfortunately, problems exist in attempting to quantify the relaxation time of the PMAA/PEO complex through steady-state data.

First, the p^{-1} value is extremely close to that of p_0^{-1} (as estimated in the hypercoiled state). This in turn introduces a high degree of error into the determination of ρ from eq 1. This problem could be overcome in principle through the adoption of a label of longer lived excited state (ideally one would wish to "match" the fluorescence lifetime with that expected of the relaxation). However, a further complication is the fact that there is evidence that the intrinsic polarization, p_0, may vary as the label's environment changes from that of the hypercoiled state to that of the complex. In the case of PEO6000 the p^{-1} values at relative PMAA/PEO ratios greater than unity continue to decrease to values inferior to that of p_0^{-1} in the hypercoil.[12] Unfortunately, it is not obvious how one might estimate p_0 for the labeled complex (e.g., by the addition of an organic quencher) without the risk of disrupting its structure. Finally, although nonfluorescent blanks were employed in order to guard against the contamination of the sampled emissions by stray excitation, such data distortion cannot be compensated for with complete confidence. Data contamination by scattered light *would* artificially depress the p^{-1} values, as observed.

In principle, all aberrant features of the experiment conducted in steady state can be obviated by the use of time-resolved measurements. Hence, we can check whether the rotational relaxation time of the PMAA/PEO complex really is as long as the steady-state experiment infers.

Time-Resolved Anisotropies. In the time-resolved approach, the time profiles of the components of fluorescence intensity employing an analyzer set parallel [$i_\parallel(t)$] and perpendicular [$i_\perp(t)$] to the vertically polarized excitation, used for photoselection of the labels, are recorded. These components are combined to furnish the time-resolved anisotropy, $r(t)$, as follows:

$$r(t) = \frac{i_\parallel(t) - i_\perp(t)}{i_\parallel(t) + 2i_\perp(t)} \qquad (2)$$

The anisotropy can then be analyzed by using whatever mathematical model seems appropriate to the situation under study. In our experiments we have analyzed the

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Table I
Rotational Correlation Times Derived from Time-Resolved Anisotropy Decays for 1-VN-Labeled PMAA at 298 K

pH	method of anal.[a]	τ_{c1}/ns	τ_{c2}/ns
11	IR	8.7	
	DA	8.9	0.6
3	IR	37.4	1.5
	DA	31.5	0.4

[a] IR = impulse reconvolution; DA = direct analysis of raw anisotropy data (without deconvolution).

anisotropy decay in terms of one or two exponential terms, i.e.

$$r(t) = A_1 \exp(-t/\tau_{c1}) + A_2 \exp(-t/\tau_{c2}) \qquad (3)$$

where, for a pseudo spherical rotor, $A_2 = 0$ and $3\tau_{c1} = \rho$. Details of the deconvolution procedures employed will be published elsewhere.[17] Data quoted in this note have been derived either through direct analysis of the anisotropy decay, without deconvolution, or through the impulse reconvolution approach[18] [cf. Table I].

At high pH, the relaxation of the expanded polymer coil is characterized by a relatively short relaxation time and the individual components $i_{\parallel}(t)$ and $i_{\perp}(t)$ of the overall anisotropy rapidly converge within the accessed time window (cf. Figure 1a). The anisotropy decay behavior for the 1-VN–MAA system can be reasonably well-described by a single-exponential decay function in analysis by the impulse reconvolution method. A rotational relaxation time, ρ, of the order of 26 ns results. Direct analysis of the anisotropy decay yields a comparable relaxation time. However, adequate statistical fitting requires introduction of a further short-lived *minor* component (corresponding to a ρ value of ca. 1.8 ns). Since deconvolution cannot be applied in direct analyses of anisotropy, caution is necessary. It is likely that this short component does not enjoy *physical* significance. In the event that such a component *were* to prove real, it might be ascribable to the influence of restricted (librational) motions of the polymer chain or to "wobbling" movements of the label independent of the macromolecule.

At low pH the effect of hypercoiling on the relaxation behavior is evident in the individual intensity components (cf. Figure 1b), which converge slowly over the time range sampled. Neither method is capable of achieving an adequate description of the fluorescence temporal profiles through the involvement of a single-exponential anisotropy model. However, the shorter-lived component is of minor significance. The longer component (corresponding to a relaxation time of ca. 112 ns) constitutes 99% of the total decay intensity as analyzed by impulse reconvolution. Direct anisotropy fitting to a double exponential yields a value of 95 ns for this major component to the molecular reorientation. Neither estimate agrees well with the steady-state value. The reason for this discrepancy is not apparent at present but may reflect both the intrinsic susceptibility of the steady-state approach to the distorting influences of scattered radiation and uncertainties in the estimated value of p_0 (especially in instances where the relaxation *is* not capable of approximation to a single relaxation time).

Addition of PEO in a quantity such as to form a 1:1 complex produces a dramatic change in the appearance of the parallel and perpendicular components of the anisotropy decay as shown by comparison of Figures 1 and 2. These data provide convincing evidence that the *indications* given by steady-state analyses of a long relaxation time associated with a very rigid complex are correct. The implications of these findings are that while

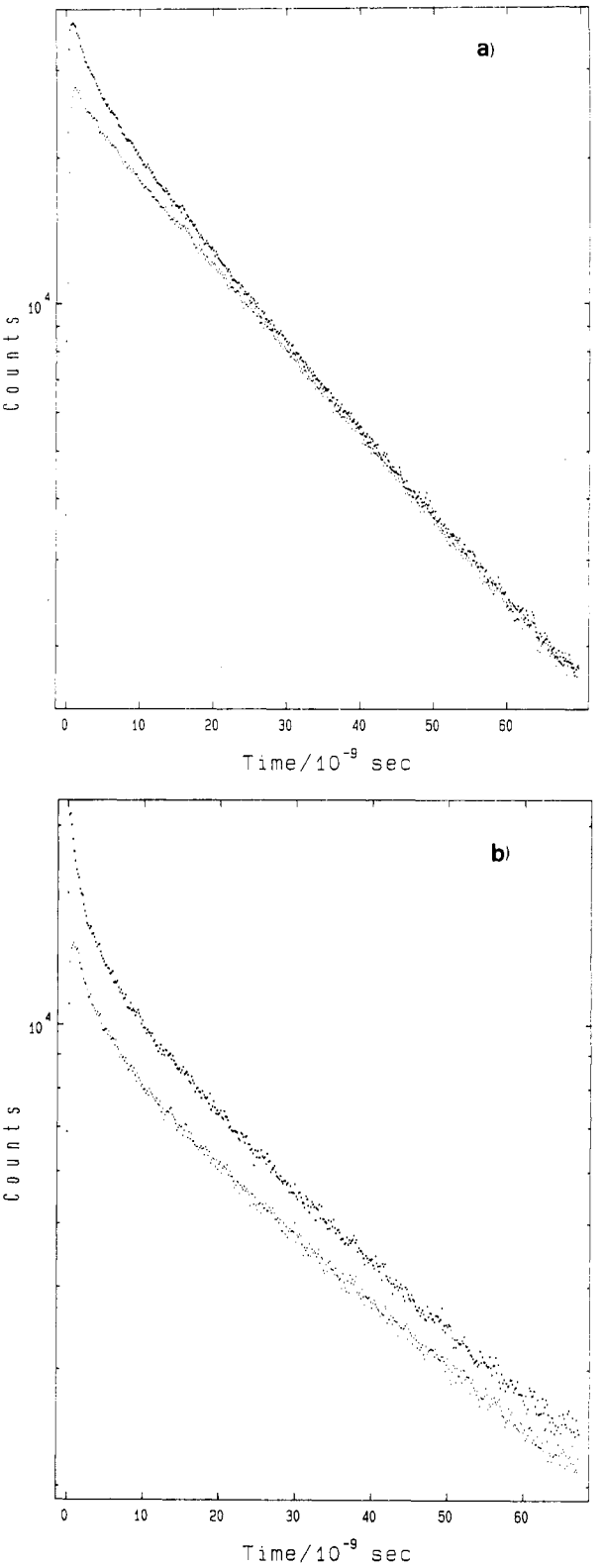

Figure 1. Parallel (upper) and perpendicular (lower) fluorescence intensity decay curves following excitation with vertically polarized light (λ_e = 290 nm) analyzed at 340 nm from 1-VN–MAA in aqueous medium at (a) pH = 11.0 and (b) pH = 3.0.

complexation requires "unwinding" of the hypercoiled state, this, in turn, is replaced by an extremely rigid (e.g., "ladder-type"?) structure for the PEO/1-VN–MAA species. It may be that the relaxation time for the complex characterizes the whole macromolecule ("end over end") motion rather than that of a segment whereas that of the PMAA hypercoil is segmental. (There *are* indications that the hypercoiled form of PMAA is not a discrete entity but rather consists of globular sections joined by loosely coiled segments of

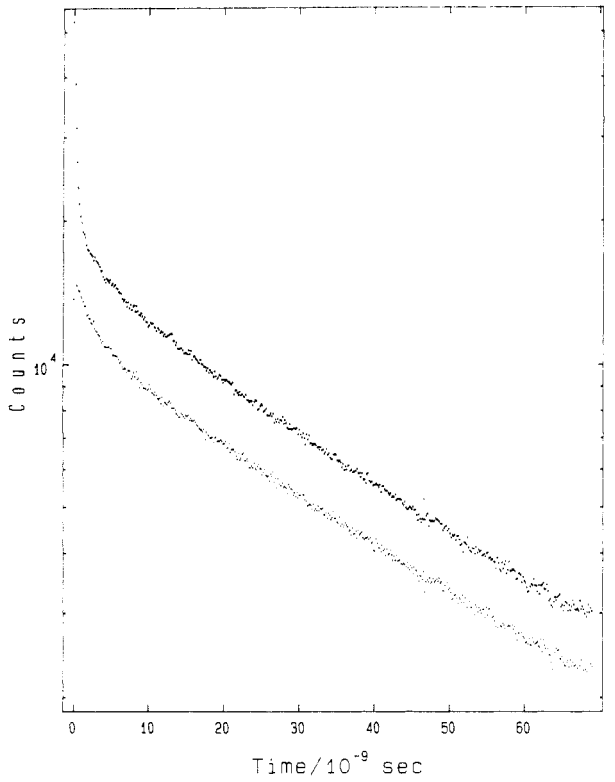

Figure 2. Parallel (upper) and perpendicular (lower) fluorescence intensity profiles following excitation with vertically polarized radiation (λ_e = 290 nm) analyzed at 340 nm from the 1-VN–MAA/PEO 1:1 complex at pH = 3.0.

greater relaxational freedom.[1] Unfortunately, the implications of the data are also that the 1-VN label chosen for these experiments may not be wholly appropriate for study of the complexation process and product. Early analyses of these data indicate that the relaxation time occupies the *microsecond* domain. Consequently, future studies will require labeling with a species whose luminescence characteristics more closely match those of the complex's reorientation. Such studies are currently in progress.

Conclusions. It has been shown that interpolyelectrolyte complexation between PMAA and PEO is both molar mass and pH dependent. Furthermore, these initial investigations have shown that while naphthyl labels are appropriate for the study of the relaxation behavior of PMAA itself at all pH values in dilute aqueous solution, longer lived excited states are likely to be necessary for accurate determination of relaxation parameters of the PMAA/PEO complex itself.

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