Free-Radical Synthesis of Poly(2-ethylacrylic acid) Fractions of Low Polydispersity: Effects of Molecular Weight and Polydispersity on the pH-Dependent Conformational Transition in Aqueous Solutions

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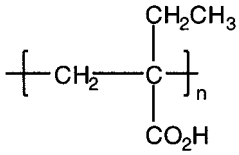
Introduction

The interaction of the hydrophobic polyelectrolyte, poly(2-ethylacrylic acid) [PEAA, **1**], with phospholipid bilayer membranes has been studied extensively in this laboratory over the past fifteen years.[1] This system has been tailored to create lipid vesicles that respond—via release of contents—to changes in pH,[2] temperature,[3] light intensity,[4,5] or concentration of a solute such as glucose.[6]



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PEAA undergoes a conformational transition from an expanded coil to a more compact structure upon acidification in aqueous solutions.[7] Acidification of PEAA/lipid mixtures causes increased membrane permeation at low concentrations (<3% w/w PEAA/lipid). At higher concentrations (~50% w/w PEAA/lipid), the polymer triggers structural reorganization from membrane vesicles at high pH to mixed polymer−lipid micelles at low pH.[1]

These properties make PEAA a candidate for use in the development of functionalized drug carriers for pharmaceutical applications. To extend the versatility of this system, one would like to be able to control the pH at which release of contents takes place. Tuning of the "critical pH" is necessary for targeting of different intracellular destinations characterized by different degrees of acidity.[8,9] Control of the critical pH can be attained either by altering the properties of the bilayer membrane, or by variation of the polymer chain structure. Membrane properties have been altered through choice of constituent lipids (e.g., by selection of the most appropriate lengths of the acyl chain substituents or through the use of saturated vs unsaturated chains),[10] through the inclusion of cholesterol in the bilayer,[11] or through the use of polymerizable lipids.[12,13] Modulation of polymer chain structure has been achieved through variations in tacticity,[2] molecular weight,[14] and chemical composition (by copolymerization with methacrylic acid).[15] All of the aforementioned studies have demonstrated useful control over the transition pH.

In the present paper, the effect of molecular weight on the conformational transition of PEAA in aqueous solution is described with special attention paid to the role of polydispersity on the shape of the transition. We also demonstrate that solvent fractionation is effective in reducing the polydispersity of a PEAA sample obtained from bulk free-radical polymerization and in providing PEAA fractions of various molecular weights.

Experimental Section

Materials. Diethyl ethylmalonate, diethylamine, and pyrene were used as received from Aldrich Chemical Co. Azobis(isobutyronitrile) (AIBN) was purchased from Aldrich and recrystallized from methanol. Formaldehyde solution (37% w/w) and cellulose dialysis tubing (Spectra/Por 6, MWCO 1000) were purchased from Fisher Scientific Co.

Synthesis of Poly(2-ethylacrylic acid). 2-Ethylacrylic acid was prepared from diethyl ethylmalonate by a procedure published earlier.[16] The monomer was vacuum distilled (bp 50 °C/1 mmHg) and placed in ampules. AIBN (5 mol %) was added and the ampules were subjected to four freeze−degas−thaw cycles and sealed under vacuum. Polymerizations were carried out in bulk and run at 60 °C for 24 h. The resulting slurry was dissolved in methanol and precipitated into diethyl ether. The precipitated polymer was collected by filtration, dissolved in pH 9 phosphate buffer, and dialyzed against water for 4 days in cellulose dialysis tubing (MWCO = 1000).

Solvent Fractionation. Solvent fractionation was carried out using methanol and diethyl ether as solvent and nonsolvent, respectively. PEAA was dissolved in methanol at a concentration of 50 mg/mL and precipitated by slow dropwise addition of ether while stirring vigorously. Successive fractions of the polymer were precipitated by taking the filtrate (or the supernatant) and adding more ether. Fractions that were insoluble in mixtures of diethyl ether and methanol with ether-to-methanol ratios of 1:1, 6:1, 10:1, and a final fraction that was soluble in the 10:1 mixture were isolated.

Molecular Weight Determination. Molecular weights of fractionated samples were determined by aqueous gel permeation chromatography (GPC) using two TSK columns (TSK 3000PW, TSK 5000PW) and a differential refractometer. Calibration was performed using five poly(ethylene oxide) (PEO) standards of narrow molecular weight distribution and molecular weights ranging from 10^3 to 10^5 (Waters). PEAA was dissolved in pH 9 phosphate buffer (0.034 M) at a concentration of 2 mg/mL. The buffer contained 0.3 M NaCl in order to suppress coil expansion.[17]

Fluorescence Measurement. The conformational transition of PEAA in solution was monitored by observing the steady-state fluorescence of codissolved pyrene.[18] A polymer stock solution of low buffer capacity was prepared by dissolving PEAA (4 mg/mL) and pyrene (200 μM) in 5 mM phosphate buffer of pH 8. Phosphate buffers (100 mM) ranging in pH from 8 to 5.3 were prepared by mixing varying ratios of monobasic and dibasic phosphates. Samples for fluorescence measurements were prepared by mixing 0.5 mL of the polymer solution with 1.5 mL of phosphate buffer at various solution pH, to give final concentrations of 1 mg/mL PEAA and 50 μM pyrene and constant ionic strength ($I = 0.229$). Pyrene was excited at 337 nm, and the conformational transition was followed by measuring the intensity of the fluorescence emitted at 373 nm (peak 1) and at 384 nm (peak 3) using a Perkin-Elmer MPF-66 fluorescence spectrophotometer.

Data Analysis. The data presented in Table 2 were derived from numerical analysis of the first-derivative plots obtained

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Table 1. Molecular Weight Data[a] for PEAA Samples Obtained from Solvent Fractionation

sample[b]	M_w	M_n	PDI
Unfractionated	32 000	14 000	2.2
1:1	23 000	16 000	1.4
6:1	8000	6200	1.3
10:1	5000	4400	1.2
Soluble	4800	4100	1.2

[a] Determined by GPC with PEO calibration. [b] Ratios in the sample column refer to the ether:methanol ratio used to precipitate the individual fractions.

Table 2. Transition Midpoints and Widths Obtained from Fluorescence Measurements

	peak 1 (373 nm)		peak 3 (384 nm)	
sample	FWHM	transition pH	FWHM	transition pH
unfractionated	0.33	5.93	0.41	5.93
1:1	0.22	6.01	0.21	6.02
6:1	0.34	5.83	0.33	5.82
10:1	0.28[a]	5.42	0.29[a]	5.42
soluble fraction	0.22[a]	5.34	0.23[a]	5.34

[a] FWHM value was obtained by doubling the pH difference between the midpoint (peak value) and the higher pH half-maximum value of the first-derivative plot.

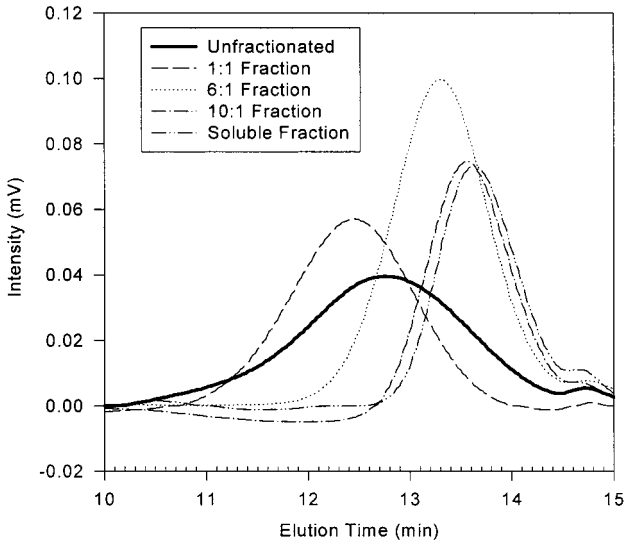

Figure 1. Gel permeation elution chromatograms for PEAA samples obtained from solvent fractionation. Ratios refer to the ether:methanol ratios used to precipitate the individual fractions.

for each of the fractions. The transition midpoint is taken as the position of the maximum in the first derivative, and the transition width is the width of the first-derivative peak at half its maximum value.

Results and Discussion

Solvent fractionation was used to reduce the polydispersity of a PEAA sample obtained from a bulk free-radical polymerization. Fractionation was carried out by inducing precipitation of PEAA from methanolic solutions through addition of diethyl ether, a nonsolvent for the polymer. The highest molar mass species precipitate first so that fractions of decreasing molar mass are obtained as the proportion of nonsolvent is increased. Gel permeation chromatography (Figure 1) shows that each of the PEAA fractions obtained by ether precipitation is of lower polydispersity (1.2−1.4) than the initial sample obtained via radical polymerization (polydispersity 2.2). Molecular weight data are presented in Table 1. Weight-average molecular weights (estimated on the basis of poly(ethylene oxide) GPC standards) range from 4800 to 32 000.

The effects of molecular weight and polydispersity on the conformational transition of PEAA in aqueous solution were monitored by fluorescence spectroscopy with pyrene as a probe. Pyrene has been used previously to study the conformational states of PEAA[14] and poly(methacrylic acid) [PMA][18,19] in water. The utility of pyrene as a probe of polyelectrolyte conformation arises from the fact that the emission intensities of peak 1 (373 nm) and peak 3 (384 nm) increase in nonpolar environments. The peak 3/peak 1 ratio is also dependent on the polarity of the environment, and varies from 1.65 in hexane to 0.64 in water.[18]

The conformational transition was monitored for four different fractions of PEAA, and the behavior of each fraction was compared to that of the unfractionated sample. The results are presented in Figure 2a and b, which show the peak 1 and peak 3 intensities vs pH for all samples. The transition midpoint ranges from pH 6.0 for the highest molecular weight sample, to pH 5.3 for the lowest molecular weight sample. Furthermore, for the 10:1 and soluble fractions, the transition is not complete at the lowest pH obtainable with the phosphate buffer system. The peak 3/peak 1 ratios measured for all five of the PEAA samples also signal the conformational transition: Ratios range from 0.93 ± 0.02 for the polymer chain in the compact state to 0.63 ± 0.02 in the expanded state, which is consistent with prior results.[14]

The molecular weight effect on the conformational transition can be explained, at least in part, by the molecular weight dependence of the ionization behavior of PEAA. Although ionization of poly(acrylic acid) shows no molecular weight dependence, titration curves of PMA[21] and PEAA[22] exhibit significant molecular weight effects in the transition region, where shorter chains behave as stronger acids than their higher molecular weight counterparts. Thus, even the simplest assumption (i.e., that the conformational transition occurs at a fixed value of the degree of ionization) would require that the solution pH must be reduced further to induce collapse of short PEAA chains.

The role of polydispersity in determining the breadth of the conformational transition was examined by fitting the data in Figure 2a and b to a logistic function.[22] The first derivative of the resulting function was calculated, and the full width at half-maximum (fwhm) of the derivative curve was taken as a measure of the transition width. In Figure 2c and d, the first derivative plots for the 1:1 and unfractionated samples (which have similar number-average molecular weights) are shown. The fractionated sample exhibits reduced transition width, which we attribute to a decrease in polydispersity (PDI of 1.4 vs 2.2). The transition widths and midpoints for each of the fractions are presented in Table 2. Note that for the 10:1 and soluble fractions, the width at half-maximum was obtained by doubling the pH difference between the midpoint (peak value) and the higher pH half-maximum value of the first derivative plot, because these fractions did not fully complete the transition at the minimum pH value investigated.

Conclusions

The pH-dependent conformational transition of PEAA and the capacity of the polymer to cause reorganization

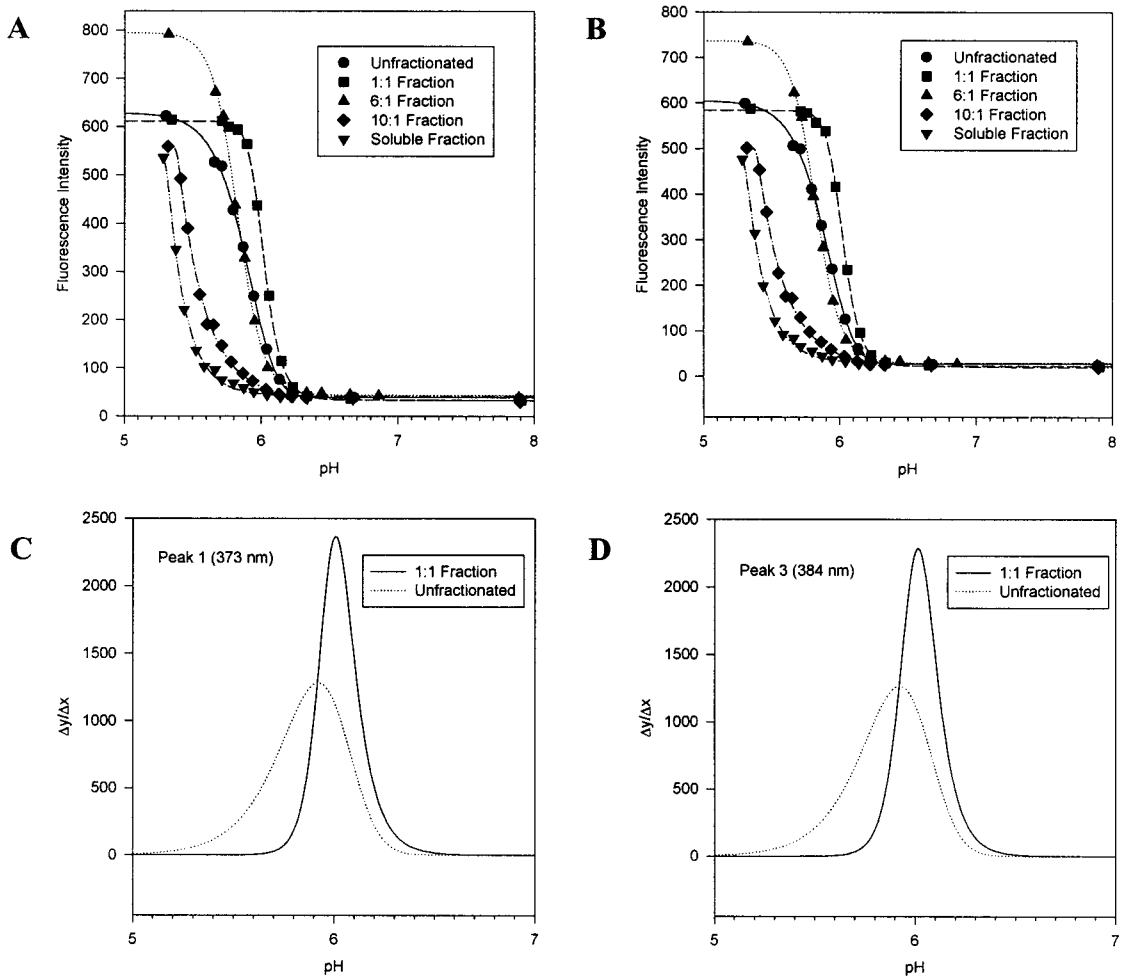

Figure 2. Fluorescence intensity at 373 nm (Peak 1, A) and 384 nm (Peak 3, B) for pyrene dissolved in phosphate-buffered solutions of PEAA of different molecular weights. First derivative plots for the 1:1 and the unfractionated samples demonstrate the effect of polydispersity on the transition width (Peak 1, C and Peak 2, D).

of bilayer membranes and release of encapsulated material, have been well documented. Precise control of these events at a predetermined "critical pH" is necessary in applications such as liposomal drug delivery. We have demonstrated that (i) the conformational transition of PEAA shifts to lower pH as the chain length of the polymer is reduced and (ii) the breadth of the conformational transition can be reduced by using samples of lower polydispersity. PEAA samples of low polydispersity were obtained readily by solvent fractionation of a sample prepared by bulk free-radical polymerization.

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