Clustering of Poly(methacrylic acid) around Appended Binaphthyl Labels As Reflected by the Disruption of γ-Cyclodextrin Complexation and Racemization Kinetics

Sung Yun Yang, Gerald Schultz, Mark M. Green,* and Herbert Morawetz*

Herman F. Mark Polymer Research Institute, Polytechnic University, Six Metrotech Center, Brooklyn, New York 11201

Received November 30, 1998; Revised Manuscript Received February 5, 1999

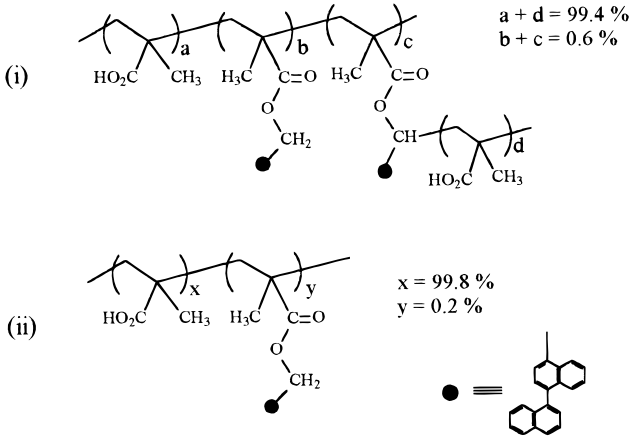
ABSTRACT: A derivative of 1,1′-binaphthyl (BN) was appended to poly(methacrylic acid) (PMA), and its reversible complexation with γ-cyclodextrin (CyD), which leads to an induced circular dichroism signal (ICD), was studied as a function of pH. The ICD was observed at pH above 6 but disappeared sharply at lower pH's where clustering of the chain led to encapsulation of the BN by the polyelectrolyte and prevented the approach of the CyD. Kinetic work based on the atropisomeric character of the BN showed the capsule to exert a strong and pH dependent restriction on the racemization of the BN. In syndiotactic PMA the racemization followed first-order kinetics at all pH values while, with atactic PMA as the carrier, the racemization at low pH was biphasic. Remarkably, the most restrictive relationship between the polyelectrolyte and the racemization of the probe is found at a degree of ionization just slightly below the point where the chain charge density disrupts the capsules. The behavior of the BN label appended to PMA was compared with its behavior when attached to poly(acrylic acid), poly(2-ethylacrylic acid), and an alternating maleic acid−butyl vinyl ether copolymer.

Introduction

The unusual characteristics of poly(methacrylic acid) (PMA) have been studied for more than 40 years. Whereas the ionization of a flexible polycarboxylic acid is expected to lead to a smooth increase of the chain extension (as is the case with poly(acrylic acid) (PAA),[1] PMA resists chain extension before a critical density of the ionic charges is attained.[2] Also, the p*K* of the acid groups, which is expected to increase smoothly with the polymer charge density, because of the increasing free energy required to remove a hydrogen ion from the polymer domain, exhibits a plateau between degrees of ionization of about 0.2−0.3.[3] This behavior has been shown by Leyte and Mandel[4] to be due to an equilibrium between two local conformations of the chain. Although this is not stated explicitly in their paper, it is implied that the more extended conformation has a higher degree of neutralization, so that the two forms have the same charge density, leading to the same p*K*. Calorimetric titration exhibits an endothermic peak in the plateau region[5] similar to a phase transition. However, the frequent description of the more contracted form as "globular" or "hypercoiled" is grossly misleading, since its unperturbed dimension is similar to that of poly(methyl methacrylate), a "well behaved" polymer.[6]

In 1968 Anufrieva et al.[7] showed that the fluorescence of the dye Auramine O added to a PMA solution decreases sharply with an increasing degree of ionization in the range where the p*K* plateau is observed. Later it was found that when an acid solution of PMA is mixed with an Auramine O solution in a stopped-flow apparatus, the fluorescence intensity increases at a rate independent of the PMA concentration, suggesting that the phenomenon is due to a unimolecular conformational transition following the association of the dye with the polymer.[8] There was little change in the solution viscosity, so that the transition had to be localized, presumably leading to a clustering of a section

Scheme 1. General Structure of the Binaphthyl Labeled Poly(methacrylic acid): (i) PMA-*co*-BN; (ii) PMA-a1-BN and PMA-a2-BN



of the chain around the dye. This would lead to increasing fluorescence intensity either because of the shielding of the dye from contact with water, a known quencher of the fluorescence of various dyes[9] or because of a rigidity of the local environment, reducing internal quenching.[10]

Other aromatic species, adsorbed or covalently bound to PMA, exhibit high fluorescence lifetimes and rotational relaxation times at low pH, where PMA clustering around the labels would be expected, and these drop sharply in the pH region where chain expansion disrupts the clusters.[11,12]

We have taken a stereochemical approach to the study of the PMA clusters around aromatic labels.[13] In using a 1,1′-binaphthyl (BN) label covalently attached to the chain via the carboxylic acid function (Scheme 1) in combination with dissolved γ-CyD in the aqueous solution, we could study the interference of the PMA chain with the complexation of the BN with the γ-CyD as

10.1021/ma9818533 CCC: $18.00
Published on Web 04/03/1999

Table 1. Characterization of Polymers

	PMA-a1-BN	PMA-a2-BN	PMA-s-BN	PAA−BN	PEA−BN	PBVEMA−BN
M_w	798 000	460 000[a]	286 000	350 000	40 000	10 000
M_w/M_n	1.67	1.04	1.81	2.0	1.8	2.01
f[c]	0.0013	0.002 (0.006)[b]	0.0019	0.002	0.0019	0.0019
$F_{meso\ dyad}$	0.294	0.258	0.02			
$F_{racemic\ dyad}$	0.706	0.742	0.98			

[a] Narrow disperse PMAs with various molecular weight were used also; M_w = 24 000−860 000 (dispersities are within 1 to 1.05). [b] For the deracemization kinetics of BN, a larger fraction (0.006) of the BN label was used. [c] Fraction of the label.

monitored by the induced circular dichroism (ICD) which occurs on incorporation of one of the naphthalene rings of the probe into the γ-CyD cavity. Also, BN is atropisomeric; i.e., the rotation of the naphthalene rings about the connecting 1,1′ bond interconverting its mirror forms is slow on a laboratory time scale. We could, therefore, use the BN racemization rate as a measure of the resistance to molecular motion within the cluster cavity. This allowed us[13] to obtain much more detailed information about the pH dependence of cluster formation than could be deduced by methods previously used.

We have also studied the behavior of the BN labels appended to other polycarboxylic acids, i.e., poly(acrylic acid) (PAA), poly(2-ethylacrylic acid) (PEA), and the alternating copolymer of butyl vinyl ether with maleic acid (BVEMA). The latter two polymers resemble PMA in exhibiting a p*K* plateau[14,15] due to a conformational transition in which a compact form is disrupted when the charge density reaches a critical value. In BVEMA this transition leads also to a sharp decrease in the emission intensity of fluorescent labels as they are transferred from an apolar to an aqueous environment analogous to the behavior of PMA labels when the polymer clusters are disrupted.[16]

Experimental Section

Preparation of Polymers with Appended Racemic Binaphthyl Residues. Three samples of poly(methacrylic acid) (PMA) were used. Atactic PMA-a1 was prepared by hydrogen peroxide initiated free radical polymerization. Methacrylic acid monomer (4.5 g) in distilled water (23 mL) was initiated by 30% H_2O_2 (160 μL) under argon at 90 °C. After 5 h, the reaction mixture was diluted with cold methanol. The polymer was precipitated in diethyl ether, purified by reprecipitation from methanol into acetone, and dried in a vacuum oven at 40 °C overnight. Atactic PMA-a2 with a low polydispersity (M_w = 460K, M_w/M_n = 1.04) was obtained by hydrolysis of the corresponding poly(*tert*-butyl methacrylate),[17] by dissolving 1 g in 100 mL of methanol and adding 2 mL of concentrated hydrochloric acid and refluxing for 12 h followed by precipitation in diethyl ether. Syndiotactic PMA-s [18] was obtained by the hydrolysis in concentrated H_2SO_4 of the syndiotactic poly(methyl methacrylate) (PMMA) The reaction time was 9 h.

Poly(acrylic acid) was purchased from Polysciences; poly(2-ethylacrylic acid) (PEA)[19] was a gift from Professor J. L. Thomas of Columbia University, used after purification by precipitation from methanol to diethyl ether. Poly(butyl vinyl ether-*co*-maleic acid) (BVEMA) was the hydrolysis product of the corresponding maleic anhydride copolymer obtained by free radical polymerization in toluene at 68 °C.[20] The concentrations of butyl vinyl ether, maleic anhydride, and azobisisobutyronitrile (AIBN) were 1.36, 1.36, and 0.00136 M, respectively. After 4 h, the reaction mixture became very viscous. The reaction was terminated by adding cold methanol. Benzene (3 × 5 mL into 20 mL of mixture) was added several times into the gelled solution to wash out low molecular weight oligomers and the remaining monomer. The product was dissolved in dried DMF and precipitated into diethyl ether. This alternating copolymer[20] was hydrolyzed by heating in water at 80 °C for 4 h followed by tumbling at room temperature for 24 h. A small fraction of the carboxyl groups of these polymers was reacted with either the racemic 4-bromomethyl-1,1′-binaphthyl or its enantiomer obtained by chiral chromatography (see below) to append the binaphthyl (BN) label to the polymer as follows:[21] To the solution of PMA (20 mg, 0.233 mmol) in DMF (2.5 mL) were added 1,8-diazabicyclo[5,4,0]-undec-7-ene (DBU) (35 μL, 0.233 mmol) at 0 °C and the resolved (*R*)- or (*S*)-4-bromo-1,1′-binaphthalene (0.95 mg, 0.0027 mmol). For the racemic system, the temperature was 50 °C and the reaction was conducted in DMSO. The mixture was stirred for 3 h in an ice−salt bath. The reaction was terminated by adding acetic acid. The polymer was obtained by precipitation into diethyl ether and dried with nitrogen gas. The labeled polymer (designated by the suffix BN) was purified by dialysis (Slide-A-Lyzer 10K dialysis cassettes, 3−15 mL, purchased from Pierce Co.) in distilled water below 5 °C.

4-Bromomethyl-1,1′-binaphthyl was prepared by bromination of 4-methyl-1,1′-binaphthyl (see below) with *N*-bromosuccinimide (NBS, 0.14 g) and AIBN (catalytic amount) in refluxing CCl_4 overnight. 4-Methyl-1,1′-binaphthyl was synthesized through cross coupling of 1-iodonaphthalene and the Grignard reagent of 1-bromo-4-methylnaphthalene catalyzed by bis(triphenylphosphine)palladium(II) dichloride following the literature.[22] 1,1′-Binaphthyl-4-acetic acid (BNA) was synthesized from 4-bromomethyl-1,1′-binaphthyl (300 mg, 0.867 mmol) by the bubbling of CO_2 (5−6 g) through the formed Grignard reagent (25.3 mg, 1.04 mmol of Mg in distilled THF).[23] After 8 h, 2 N HCl (3 mL) was added, and the mixture was stirred overnight. The above binaphthyl intermediates were analyzed by both carbon and hydrogen high field NMR with spectra in agreement with their structures.[24]

A copolymer of methacrylic acid with 1,1′-binaphthyl-4-methyl methacrylate (PMA-*co*-BN) was also prepared by free radical polymerization with AIBN in 1,4-dioxane at 65 °C.[25] As discussed below it was found to be branched, presumably because of chain transfer during the polymerization as evidenced by the sharp decreases of its molecular weight after acid hydrolysis. In acid solution it contained a gel fraction which had to be filtered off.[25]

Polymer Characterization. The weight average molecular weights and polydispersities (M_w/M_n) of the various polyelectrolytes studied were determined after exhaustive methylation[26] of the carboxylic acid groups followed by GPC analysis using a Waters 510 pump equipped with TSK Gel analytical columns, G4000H_{XL}, G5000H_{XL}, and GMH_{XL} (TosoHaas), calibrated with PMMA standards (Polyscience) in chloroform in line with a dual detector system consisting of Waters R410 differential reflectometer and 440 absorbance detector. The tacticities of the methylated PMA samples were determined using a Varian 200 MHz NMR.[24] The BN label content of the polymers was determined with a JASCO J-710 CD spectrometer in its UV mode with the extinction coefficient for 1,1′-binaphthyl-4-methanol (ϵ_{max} = 13,000 at λ_{max} = 285 nm in methanol) as an estimate of the extinction coefficient of the polymer bound binaphthyl group. These characteristics are listed in Table 1.

Preparation of Polymers with Optically Active BN Residues. The polymers carrying racemic BN label (0.01 M of polymer containing 0.1 M KCl) and racemic BNA were exposed to γ-CyD (1.85×10^{-3} M) at high pH and the deracemization of the BN (6.16×10^{-5} M) in the asymmetric complex was followed by the increase in the circular dichroism (CD) until a constant value was attained. When the pH was lowered below pH 6, the complexes between the cyclodextrin

and the BN label are fully dissociated.[13] For the racemization kinetic studies using the small enantiomeric excess obtained by the deracemization, cyclodextrin was removed by the following procedure: concentrated HCl solution was added to the polymer/cyclodextrin mixture and centrifuged to separate the precipitated polymer. The polymer was dissolved in distilled water, precipitated by HCl, and centrifuged again. The procedure was repeated several times, and the product was dissolved and dialyzed against a large amount of distilled water at 5 °C. A much more effective procedure to obtain polymers with BN residues of high enantiomeric purity used the reaction[21] of the PMA with pure (*R*)- or (*S*)-4-bromomethyl-1,1′-binaphthyl as described above. The enantiomers were separated using Waters HPLC equipment with a chiral column (Chiralcel OJ) with hexane/isopropyl alcohol (9/1) eluent. Each time 200 μL of a 0.05% solution was injected, and this was repeated a sufficient number of times to obtain a few milligrams of the optically pure 4-bromomethyl-1,1′-binaphthyl.

Fluorescence Spectroscopy. A Perkin-Elmer LS50B luminescence spectrophotometer was used. A series of fluorescence measurements at varying pH was carried out at a constant 0.01 base molar polymer concentration (since the fluorescent label was attached to polymer, the concentrations of the fluorophore were also constant). Absorbance at the excitation wavelength (290 nm) was 0.76 (path length = 1 cm; concentration = 6.0×10^{-5} M). Oxygen in the polymer solution was removed by bubbling N_2 through the polymer solution.

Racemization Kinetics. Polymers carrying the optically active BN labels (from either the γ-CyD deracemization or the procedure derived from the chiral chromatography) with a total concentration of PMA corresponding to 0.01 M carboxylic acid groups were dissolved in aqueous buffer solutions (pH 4.2, 4.75, 5.4, and 5.7 potassium acetate buffer; pH 6.2, 6.88, 7.45, 8.0, and 9.1 potassium phosphate buffer; and pH 10.18 borate buffer were used). KCl was added to obtain a total 0.072 M salt concentration. The pH was measured using an accumet model 15 pH meter within ± 0.01 error range (Fisher Scientific). The decay of optical activity at 20 °C was followed using a JASCO J-710 circular dichroism spectrometer. Circular dichroism spectra of the labeled polymer aqueous solutions were measured at wavelengths from 250 to 210 nm. Spectra were subtracted by the circular dichroism signal for the same buffer solution. ((*S*)-BN has a positive exciton: (+) and (−) signs at 229 and 218 nm.)

Results and Discussion

Spectroscopy of BN-Labeled PMA. When BN-labeled PMA was exposed at high pH to γ-CyD, an inclusion compound was formed in which one of the naphthalene residues was inserted into the cyclodextrin cavity, as evidenced by the nature of the induced circular dichroism (ICD) of the BN moiety[27] The CD increased with time due to the deracemization of the atropisomer, but this process is so slow (with a half-time of many hours) that the instantaneous ICD could be studied as a function of pH without interference from the deracemization. Table 2 lists the values obtained for PMA-a2-BN. It is seen that the ICD decreases slowly as the pH is reduced from 10.4 to 6.46 and disappears completely for pH less than 6. This change is perfectly reversible. This indicates that at low pH, where previous work has shown that sections of PMA form clusters around aromatic labels,[7,8,11,12] the clustering makes the CyD inaccessible to the BN. Above pH 6, the expansion of the polyion with increasing charge density leads to gradual disruption of the clusters and an increasing BN−CyD complexation, reflected in the increasing ICD.

Table 2 lists also the pH dependence of the emission intensity of BN attached to PMA-a2-BN and PMA-*co*-BN relative to the value observed at the highest pH where the electrostatic forces between the ionized carboxyls lead to a highly expanded PMA chain and eliminate cluster formation. In the case of PMA-a2-BN the emission intensity decreases very slowly between pH 4.43 and pH 7.40, but much more rapidly in more basic solutions. For PMA-*co*-BN the drop of the emission intensity as the pH is raised is much more pronounced. The decreasing emission intensity is accompanied by a shift of the emission maximum to longer wavelength, as would be expected from the more polar microenvironment of the label[28] as the clusters are disrupted at an increasing ionization of the PMA.

Table 2. pH Dependence of the Fluorescence (Excitation Wavelength, 290 nm) of PMA-a2-BN and PMA-*co*-BN and of the Induced Circular Dichroism Observed with PMA-a2-BN Immediately after Exposure to γ-Cyclodextrin in Water

	PMA-a2-BN			PMA-*co*-BN	
pH	intensity	emission max (nm)	ICD[a]	intensity	emission max (nm)
4.15			0	3.78	365
4.43	1.65	363	0		
5.27	1.59	364	0		
5.35			0		
5.86	1.56	367		3.15	365
5.96			0		
6.04			0		
6.21				1.72	380
6.46			42 200		
6.56	1.54	368	76 000		
7.02	1.50	374			
7.31				1.00	392
7.40	1.47	375	97 000		
7.80	1.13	375			
8.35				1.00	392
8.58	1.03	379	111 000		
8.76	1.00	382			
9.50			115 000		
10.40			116 000		

[a] Molar ellipticity (deg cm^2 dmol^{-1}).

The difference between the fluorescence behavior of PMA-a2-BN and PMA-*co*-BN requires some comment. During the free radical copolymerization of methacrylic acid with 1,1-binaphthyl-4-methacrylate leading to PMA-*co*-BN, chain branching occurs as documented by the observation that the intrinsic viscosity of PMA-*co*-BN dropped from 0.34 to 0.10 dL/g after acid hydrolysis. The free radicals are expected to abstract a hydrogen from the binaphthalene-bound methylene group leading to a resonance-stabilized radical and chain branching so that the BN at the branch point is attached directly to the chain backbone (Scheme 1), rather than being separated from it by three atoms in the linear chain of PMA-a1-BN. This may well account for the more pronounced effect of the clustering around the BN label on its fluorescence.

As pointed out in the Introduction, the increased emission intensity of aromatic labels enveloped by the PMA chain could be interpreted either as due to a shielding of the label from the aqueous environment[12] or as due to the rigidity of the local environment.[11] The study of Wang and Morawetz,[8] in which the behavior of the adsorbed dye Auramine O clustered within the PMA was compared with its behavior in solutions of varying polarity, concluded that the rigidity effect is probably dominant. It may be noted that the ICD increases slightly with increasing pH even beyond pH 8.76, where the constant fluorescence intensity suggests that the clusters are fully disrupted. It must then be

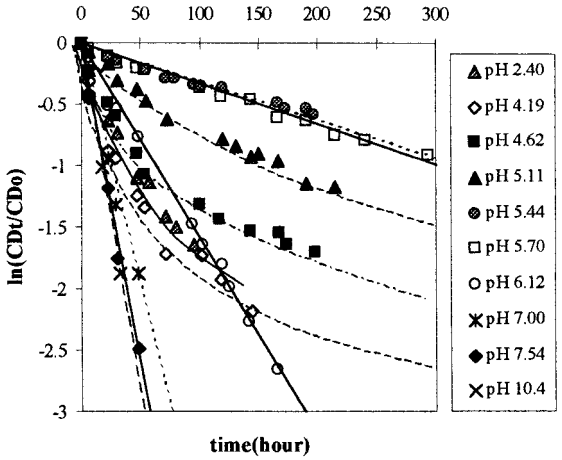


Figure 1. First-order kinetic polot of the racemization of PMA-a1-BN.

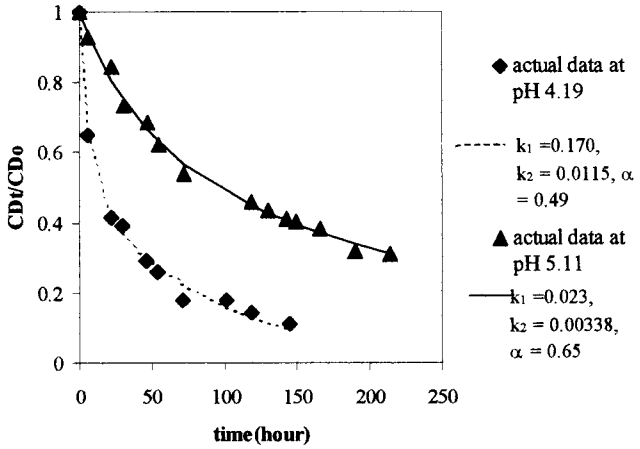


Figure 2. Curve fitting for the racemization data of PMA-a1-BN at low pH.

assumed that the CyD complexation is somewhat sensitive to the increasing charge of the chain.

The Racemization Kinetics of BN Attached to PMA. The availability of various molecular weights of PMA-a2-BN with narrow molecular weight distributions allowed us to verify that the racemization of the BN label, carried by a very small fraction of the monomer residues, is independent of the chain length of the polymer. In the early stages of our study it was shown that the racemization for PMA-*co*-BN follows first-order kinetics above pH 6 but is a biphasic process at lower pH values. The pH dependence of the racemization rate of the BN appended to the polydisperse PMA-a1 was then investigated in detail. Selected results are represented in Figure 1, which contains semilogarithmic plots of CD against time. Whereas the plots for the reaction above pH 5.43 are linear, indicating a simple exponential decay of optical activity, those at pH 5.11 and below exhibit a pronounced curvature. It was found that these curves could be closely fitted (see Figure 2) by

$$\mathrm{CD/CD_0} = \alpha \exp(k_1 t) + (1 - \alpha) \exp(k_2 t) \qquad (1)$$

where k_1 and k_2 are rate constants and α is the fraction of the BN residues racemizing by the faster rate. Figure 2 shows how closely the biphasic kinetics fit the experimental data obtained at various pH values.

The strong deviation from first-order kinetics at low pH was also observed for the racemization of PMA-*co*-BN. However, when the aqueous medium was replaced by water containing 30 vol % methanol, the racemization became much faster and almost monoexponential with a rate approaching that observed for BNA.[25] This suggests that the clustering of the PMA around the BN label, which restrains the racemization, is driven by hydrophobic forces.[29]

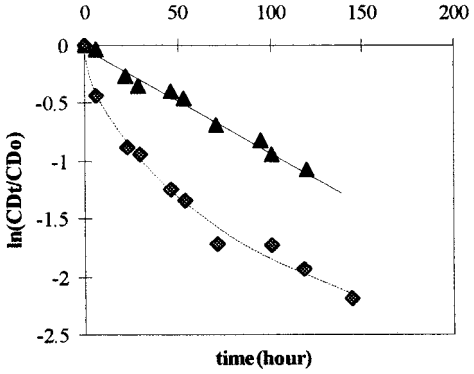


Figure 3. Comparison of the racemization kinetics of atactic (PMA-a1-BN), (◆) and syndiotactic PMA (PMA-s-BN) (▲) at pH 4.2.

It may be noted that the rate constant observed at pH 10.4, where the PMA is highly extended and would not be expected to interfere with the racemization of the label, is within the range of rate constants 0.040−0.076 h^{-1} reported for 1,1′-binaphthyl in various solvent media.[30] Surprisingly, the racemization rate constant at pH 11 of BNA, the low molecular analogue of the polymer side chain carrying the BN label, was 0.030 h^{-1} at pH 8.5,[25] only about half of the 0.052 h^{-1} at pH 7.54 and 0.057 h^{-1} at pH 10.4 found for PMA-a1-BN, where the BN label would be expected to be situated essentially in an aqueous microenvironment. We have no explanation for this property of BNA.

One of the surprises in this study was the dependence of the behavior of the BN label on the tacticity of the PMA to which it was attached. As seen in Figure 3, whereas a semilogarithmic plot of the optical activity against time is strongly curved for the atactic PMA-a1-Bn at pH 4.19, it is linear in the case of the syndiotactic PMA-s-BN at pH 4.20. The rate constant of 0.0083 h^{-1} found for the syndiotactic polymer is less than a tenth of 0.088 h^{-1}, the weighted average of the two rate constants and close to 0.0115 h^{-1}, and is the slow rate constant characterizing the racemization of the atactic polymer. The kinetic constants obtained for the racemization of PMA-a1-Bn and PMA-s-BN are listed in Table 3. Note the following features: (a) For the atactic polymer, the fast component of the kinetics decreases from 0.065 h^{-1} at pH 2.40 to 0.023 h^{-1} at pH 5.11 (the value of 0.17 h^{-1} at pH 4.19 was obtained for the best fit to the data but cannot be significant, since it is much higher than the rate constant observed for BN fully exposed to water). The slow component also decreases in this pH range from 0.0125 to 0.0034 h^{-1}. (b) Above pH 5.43, the racemization is monoexponential with k_2 slowly increasing with rising pH. (c) The data for PMA-s-BN fit well into the pH dependence of k_2 for PMA-a1-BN.

Since complexation of BN with cyclodextrin is fully inhibited in the pH range where the ICD goes to zero, there can be no doubt that the clusters enclosing the BN are stable under these conditions and the question then arises what is the reason for the reduction in the

Table 3. Kinetic Parameters for the Racemization of BN Appended to PMA-a1 and PMA-s[a]

pH	k_1 (h^{-1})[b]	k_2 (h^{-1})[c]	α[e]
2.40	0.065	0.0125	0.40
4.19	0.170	0.0115	0.49
(4.20)		(0.0083)[d]	
4.62	0.039	0.0032	0.65
5.11	0.023	0.0034	0.36
(5.43)		(0.0035)[d]	
5.43		0.0031[d]	
5.70		0.0033[d]	
6.12		0.016[d]	
7.00		0.040[d]	
(7.24)		(0.041)[d]	
7.54		0.052[d]	
10.40		0.057[d]	

[a] Data for BN appended to PMA-s are given in brackets. [b] The rate constant for the more rapidly racemizing fraction. [c] The rate constant for the more slowly racemizing fraction. [d] The rate constant for monoexponential decay. [e] Fraction of the more rapidly racemizing BN.

racemization rate on increasing the pH from 4.2 to 5.4 seen in the data in Table 3 and Figure 1. We may assume that the local conformation of the section of the PMA chain engaged in cluster formation undergoes with an increasing pH a local conformational change which leads to a tighter fit around the label and a more severe interference with its racemization motion. Such a conformational transition might be analogous to the one well documented for the unlabeled PMA chain, which is responsible for the p*K* plateau although one is still left with the question of what drives the change. The increased restriction to racemization could suggest that this conformational change intensified the hydrophobic interaction between the BN and the section of the PMA forming the cluster. On the other hand, Davenport and Wright[6] have argued that the transition responsible for the plateau region in unlabeled PMA is due to the difference between the strength of the hydrogen bond between two un-ionized carboxyls and that between an un-ionized and an ionized carboxyl and a similar principle may account for a local conformational transition of the PMA section involved in cluster formation.

The difference between the behavior of the atactic PMA-a1-BN and the syndiotactic PMA-s-BN is most significant. It suggests that at low pH the microenvironment of the label which permits it to racemize at the faster rate is due to a looser structure of the cluster wherever the PMA deviates from the syndiotactic configuration. Above pH 5.43, the loss of optical activity becomes faster with increasing pH, reflecting the destruction of the clusters with a rapid expansion of the polymer chain. Now the CD decays by first-order kinetics no matter what the stereoregularity of the polymer, indicating that the clustered and free BN labels are in kinetic equilibrium.

It is instructive to compare the results of the present study with previous investigations involving cluster formation of aromatic labels attached or adsorbed to PMA. Two fundamental differences should be pointed out: First, the nature of the motion involved in the racemization of BN is precisely defined, whereas the significance of the rotational diffusion data derived from fluorescence anisotropy is highly ambiguous. Ghiggino and Tan[11] assumed that the fluorescent label is rigidly attached to the surrounding PMA cluster, so that the rotational diffusion involves the entire cluster and can be used to estimate its size. In this study, they used the rather improbable model in which the cluster behaves as a freely rotating sphere, neglecting any restriction to its motion due to the attachment to the rest of the polymer chain. However, there is also no justification for neglecting any motion of the label within the cluster, and since we have found that the extensive motion involved in BN racemization can take place within the cluster, rotational diffusion of the much smaller labels used by Ghiggino and Tan may also have been possible. (A reliable approach to an estimate of the cluster size, based on the efficiency of the photoionization of embedded pyrenes, was reported by Chen and Thomas.[44]) Second, whereas studies of the fluorescence anisotropy involve excited molecules with a lifetime of the order of nanoseconds, the racemization of BN extends over many hours. Thus, if Ghiggino and Tan[11] and Bednář et al.[12] concluded from the biexponential decay of fluorescence intensity or anisotropy that the label was in two different environments, they merely showed that it cannot exchange between these environments within the excited lifetime of the label. By contrast, when we found that the racemization of PMA-a1-BN is biphasic below pH 5.43, we could conclude that the labels cannot interchange between these environments during the many hours of the experiment, i.e., a time longer by at least a factor of 10^{14}.

The interpretation of the changes in the fluorescence intensity of the clustered labels is also ambiguous. Ghiggino and Tan found that the decay in the emission intensity was monoexponential for the 9,10-dimethylanthracene label but biexponential for 9-methylanthracene. Since only the second label has a fluorescence intensity which increases with the viscosity of the medium, they concluded that the long lifetime fluorescence in the clustered labels is due to a local rigidity. On the other hand, Bednář et al. assigned the long lifetime component of the emission to a low polarity of the local environment, although it had been shown[31] that the dansyl label, which they used, changes its fluorescence in rigid media due to the formation of a twisted intramolecular-charge-transfer state on excitation.

Finally, it should be noted that Ghiggino and Tan found that the rotational diffusion of both their labels could be fitted by two correlation times even when they were adsorbed to un-ionized syndiotactic PMA. This is in striking contrast with our finding that the racemization of BN attached to syndiotactic PMA is monoexponential and suggests that attachment to the syndiotactic carrier may favor interchange of the label between two microenvironments over time scales longer than the fluorescence lifetime.

Deracemization Kinetics. When the complex of the PMA-bound BN with cyclodextrin was allowed to age at high pH, the CD approached exponentially its equilibrium value. This is associated with a deracemization of the BN label in the asymmetric CyD cavity. Within the complex the optical activity A would be expected to change with time proportionately to $A^* - A$, where A^* is the optical activity of BN in equilibrium with the asymmetric environment of the complex. Specifically

$$dA/dt = f^*k^*(A^* - A) - fkA \qquad (2)$$

where f^* and f are the fractions of BN inside and outside the complex, with k^* and k being the corresponding rate

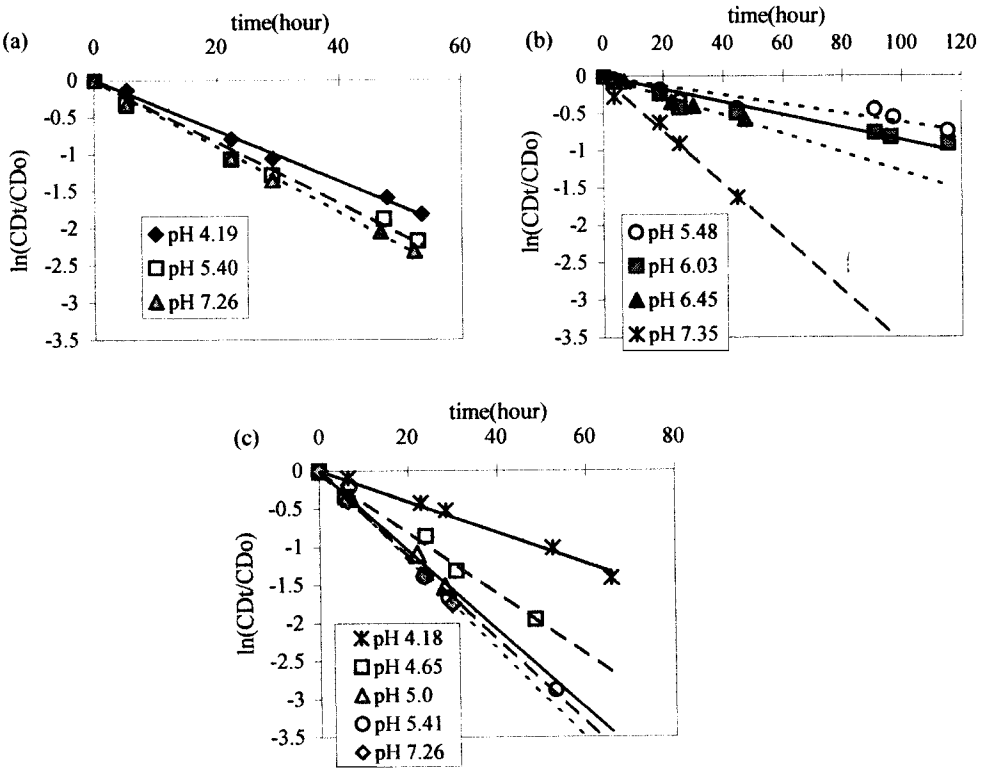


Figure 4. First-order kinetic plots of the racemization of 1,1′-binaphthyl appended to (a) poly(acrylic acid) (PAA−BN), (b) poly(2-ethylacrylic acid) (PEA−BN), and (c) poly(butyl vinyl ether-*co*-maleic acid) (PBVEMA−BN).

constants for the optical activity change. The observed rate constant for the deracemization of BN is then

$$k_{osb} = f^*k^* + fk \tag{3}$$

The equilibrium constant for the complexation of the PMA-a1-BN with cyclodextrin was found from the dependence of the ICD on the cyclodextrin concentration following the method of Ueno et al.[13,32] to be 1250 L/M at pH 10. With a cyclodextrin concentration of 0.0018 M, this leads to $f^* = 0.69$ and $f = 0.31$. Assuming that the complexation constant at 10.4 is identical to that at pH 10, using $k = 0.0575$ h^{-1} found for PMA-a1-BN at pH 10.4 and the observed deracemization rate constant $k_{osb} = 0.030$ h^{-1}, we obtain from eq 3 for k^*, describing the rate constant for the optical activity change within the complex

$$k^* = (k_{obs} - fk)/f^* = 0.018 \text{ h}^{-1} \tag{4}$$

so that passage over the energy barrier of the atropisomer is about three times slower in the complex than in the racemization of the free BN. This seems reasonable in view of the assumption that one naphthalene ring is in the cavity[33] so that the rotation of the attached naphthalene may be impeded by the rim structure of the CyD.

Racemization Kinetics and γ-CyD Complex Formation of BN Appended to Other Polycarboxylic Acids. With BN appended to poly(acrylic acid) (PAA), poly(2-ethacrylic acid) (PEA), and poly(butyl vinyl ether-*co*-maleic acid) (PBVEMA), the racemization followed first-order kinetics at all pH values (Figure 4). Figure 5 compares the pH dependence of the ICD for four BN-labeled polymers. At high pH, the values are similar for PAA−BN and for PMA-a2-BN, but much lower for PEA−BN and for PBVEMA−BN. This may be due to a competition of the ethyl and butyl group with the BN for inclusion into the CyD cavity. At low pH no ICD is observed with any of these polymers.

In PAA−BN the racemization rate is not significantly reduced at low pH, so that it appears that although the BN becomes inaccessible to cyclodextrin when the polymer is contracted at low ionization, the label remains in an aqueous environment where its motion encounters no hindrance. PEA−BN is insoluble below pH 5.43 and the increasing racemization rate in more basic solutions reflects, as with PMA-a1-BN, the gradual disruption of the clusters inhibiting molecular motion. Of particular interest is the behavior of PBVEMA. Whereas the ICD decreases smoothly with decreasing pH to a very small value at pH 5.43 (Figure 5), the fluorescence intensity remains constant, but increases by factors of 2.0 and 3.3 as the pH is further decreased to 5.0 and 4.2. The racemization rate decreases slightly with decreasing pH to 0.058, 0.055, and 0.052 h^{-1} at pH 7.3, 5.4, and 5.0, but then much more rapidly to 0.040 and 0.020 h^{-1} at pH 4.6 and 4.2. These changes are clearly associated with the well-known collapse of maleic acid copolymers with nonpolar comonomers at a low degree of ionization, where the apolar residues are aggregated in the interior of the coil. The transfer of much of the polymer during this transition has been shown to lead to a characteristic break in the pH dependence of the heat capacity and partial specific volume.[34]

It is striking how the different characteristics of the BN behavior respond in a different manner to this transition. Whereas the approach of the cyclodextrin to the BN label seems to be hindered even by a moderate contraction of the chain, the sharp increase in the emission intensity and the equally rapid decrease in the racemization rate appear to be associated with the drastic conformational transition in which the label is

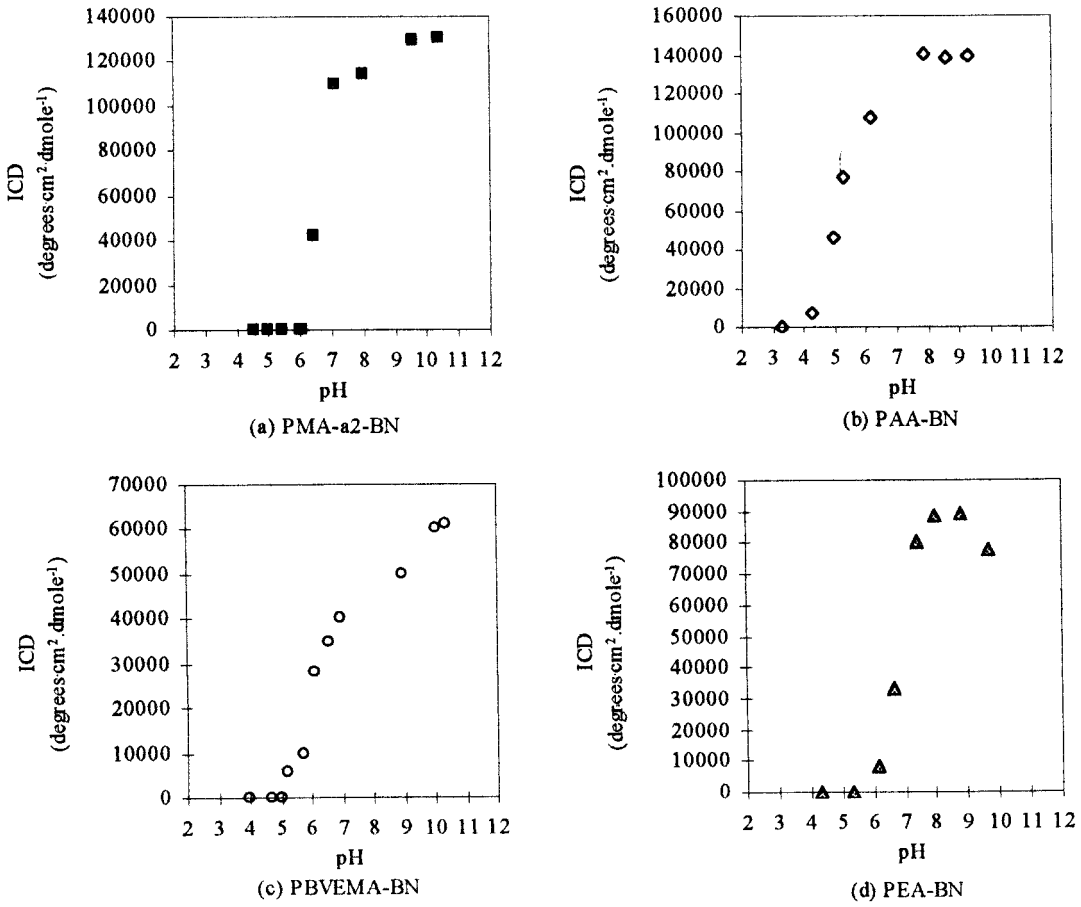


Figure 5. Comparison of induced circular dichroism vs pH of 1,1′-binaphthyl appended to (a) poly(methacrylic acid) (PMA-a2-BN), (b) poly(acrylic acid) (PAA−BN), (c) poly(butyl vinyl ether-*co*-maleic acid) (PBVEMA−BN) and (d) poly(2-ethylacrylic acid) (PEA−BN).

transferred from an aqueous to an apolar medium. It is also of interest that the change in the fluorescent intensity is much larger than observed with the BN-labeled PMA, suggesting that the interior of the PMA cluster is more polar than the interior of the collapsed PBVEMA.

Concluding Remarks

This investigation showed that the complexation with γ-cyclodextrin and the racemization kinetics of an atropisomeric aromatic label may be used as a measure of both the isolation of the label from water and the restriction of molecular motion within the cluster formed by PMA around BN.[35] Moreover, it showed that this restriction depends on pH within the low pH region where the clusters are stable, presumably because of a pH dependence of the local conformation of the portion of the PMA forming the cluster. It is probable that other aromatic labels which induce clustering of PMA at low pH can similarly move within the clusters. Thus, past interpretations of the rotational motion of the label as reflecting the motion of the whole cluster cannot be justified.

We have no clear understanding of why PMA should form clusters around aromatic labels at low pH. This is probably related to the solubilization of hydrophobic aliphatic or aromatic compounds by PMA.[36] However, the structural characteristics of these clusters are not understood.

We have found (data not reported) that when optically active 1,1′-binaphthyl is solubilized by PMA at low pH by adding a small amount of its solution in methanol to an aqueous PMA solution, the high fluorescence intensity indicates cluster formation, but the racemization rate is rapid, indicating no interference of the cluster with the molecular motion. It might have been expected that the hindrance to the racemization would be more severe with the BN attached to the molecular chain rather than merely adsorbed to it. Even so, the dramatic difference in the behavior of the covalently bound and adsorbed BN is interesting and reflects our poor understanding of the structural characteristics of the clusters.

As far as we know, poly(*N*-vinylpyrrolidone) is the only polymer other than PMA (and possibly PEA) that has been demonstrated to form clusters around aromatic labels [37] although other water soluble polymers with hydrophobic properties may be candidates for this kind of behavior.[38] It would be desirable to carry out on poly(*N*-vinylpyrrolidone) an investigation similar to the one in the present report.[39]

Finally, the sharp change of the ICD signal with small changes of pH (Table 2) in combination with the possibility of changing pH by irradiation[40] may allow applications for the labeled PMA in optical switching. A switch based on this system, which could be enclosed in a sol−gel matrix,[41] might be used in planar waveguides in which grating structures could be turned on and off.[42,43]

Acknowledgment. We are grateful to the Polymers and Chemistry Program of the National Science Foun-

dation and to the Office of Naval Research for support for this work.

References and Notes

(1) Kern, W. *Z Phys. Chem.* **1938**, *A181*, 283.
(2) Katchalsky, A.; Eisenberg, H. *J. Polym. Sci.* **1951**, *6*, 145.
(3) Arnold, R. *J. Colloid Sci.* **1964**, *12*, 549.
(4) Leyte, J. C.; Mandel, M. *J. Polym. Sci., Part A* **1964**, *2*, 1879. A recent X-ray scattering study by François and her collaborators has characterized this transition in terms of the change in the local conformation of the chain. See: Heiz, C.; Rawiso, M.; François, J. *Polymer* **1999**, *40*, 1637.
(5) Crescenzi, V.; Quadrifoglio, F. *J. Polym. Sci., A-2* **1972**, *10*, 367.
(6) Davenport, J. N.; Wright, P. V. *Polymer* **1980**, *21*, 293.
(7) Anufrieva, E. V.; Birshtein, T. M.; Nekrasova, T. N.; Ptitsyn, O. B.; Sheveleva, T. V. *J. Polym. Sci.* **1968**, *C16*, 3519.
(8) Wang, Y.; Morawetz, H. *Macromolecules* **1986**, *19*, 1925.
(9) McClare, W. O.; Edelman, G. M. *Biochemistry* **1966**, *5*. 1908 (b) Kenner, R. A. and Aboderin, A. A. *Biochemistry* **1971**, *10*, 4433.
(10) Oster, G.; Nishijima, Y. *J. Am. Chem. Soc.* **1956**, *78*, 1581.
(11) Ghiggino, K. P.; Tan, K. L. In *Polymer Photophysics*; Phillips, D., Ed.; Chapman and Hall: London, 1985; pp 341−376.
(12) Bednář, B.; Trněná, J.; Svoboda, P.; Vajda, S.; Fidler, V.; Procházka, K. *Macromolecules* **1991**, *24*, 2054.
(13) For a preliminary report of this work, see: Yang, S. Y.; Green, M. M.; Schultz, G.; Jha, S. K.; Müller, A. H. E. *J. Am. Chem. Soc.* **1997**, *119*, 12404.
(14) Sugai, S.; Nitta, K.; Ohno, N.; Nakano, H. *Colloid Polym. Sci.* **1983**, *261*, 159.
(15) Dubin, P. L.; Strauss, U. P. *J. Phy. Chem.* **1970**, *74*, 2842.
(16) Strauss, U. P.; Vesnaver, G. *J. Phys. Chem.* **1975**, *79*, 1558. (b) Strauss, U. P.; Schlesinger, M. S. *J. Phys. Chem.* **1978**, *82*, 1627.
(17) This polymer was a gift of Professor A. H. E. Müller of the University of Mainz. The preparation follows: Müller, A. H. E. *Makromol. Chem.* **1981**, *182*, 2863.
(18) This polymer was a gift of Professor F. Mikeš of the Institute of Macromolecular Chemistry of the Czech Academy of Sciences. See: Klesper, E.; et al. *Makromol. Chem.* **1974**, *175*, 523 for the details of its preparation.
(19) Ferritto, M. S.; Tirrell, D. A. *Polymer Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1990**, *31* (1), 242. Kim, J.; Tirrell, D. A. *Macromolecules* **1999**, *32*, 945; Linhardt, J. G.; Thomas, J. L.; Tirrell, D. A. *Macromolecules*, in press.
(20) Xi, F.; Bassett, W. Jr.; Vogl, O. *J. Polym. Sci., Polym. Chem. Ed.* **1983**, *21*, 891.
(21) Shimokawa, T.; Suzuki, T.; Nishikubo, T. *Polym. J.* **1994**, *26* (8), 967.
(22) Widdowson, D. A.; Zhang, Y. Z. *Tetrahedron* **1986**, *42*, 2111.
(23) *Organic Synthesis*; Horning, E. C., Ed.; John Wiley & Sons: 1955; Vol. III, p 553. (b) For further detail see: Jha, S. K. Doctoral thesis, Polytechnic University, 1998.
(24) We thank Dr. Shin Wang of the City University of New York for the use of the NIH supported NMR facility at the Staten Island campus. For details see ref 23b above.
(25) For details see: Schultz, G. Doctoral thesis, Polytechnic University, 1997.
(26) For references and procedures see: Yang, S. Y.; Green M. M. *Polymer Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1998**, *39* (2), 705.
(27) For induced circular dichroism of naphthalene derivatives see: (a) Kobayashi, N.; Minato, S.; Osa, T. *Macromol. Chem.* **1983**, *184*, 1983. (b) Shimizu, H.; Kaito, A.; Hatano, M. *J. Am. Chem. Soc.* **1982**, *104*, 7059.
(28) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1993; pp 190−200.; Weber, G.; Faris, F. J. *Biochemistry* **1979**, *18*, 3075.
(29) See: Tanford, S. *The Hydrophobic Effect*; Wiley: New York, 1973.
(30) Colter, A.; Clements, L. M. *J. Phys. Chem.* **1964,** *68*, 651.
(31) Kosower, E. M.; Dodiuk, H.; Tanizawa, K.; Ottolenghi, M.; Orbach, N. *J. Am. Chem. Soc.* **1975**, *97*, 2167.
(32) Ueno, A.; Moriwaki, F.; Osa, T.; Hamada, F.; Murai, K. *J. Am. Chem. Soc.* **1988**, *110*, 4323.
(33) Kano, K.; Tatasumi, M.; Hashimoto, S. *J. Org. Chem.* **1991**, *56*, 6579.
(34) Yamashita, F.; Kwak, J. C. T. *J. Polym. Sci., Part B.* **1987**, *25*, 1395.
(35) Binaphthyl racemization has been found to be of use as a probe of other environments. Naciri, J.; Spada, G. P.; Gottarelli, G.; Weiss, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 4352.
(36) Barone, G., Crescenzi, V., Liquori, A. M., Quadrifoglio, F. *J. Phys. Chem.* **1967**, *71*, 2341. (b) Barone, G., Crescenzi, V., Pispisa, B., Quadrifoglio, F. *J. Macromol. Chem.* **1966**, *1*, 761. (c) Morcellet-Sauvage, J., Morcellet, M., Loucheuz, C. *Makromol. Chem.* **1982**, *183*, 839.
(37) Molyneux, B. P.; Frank, H. P., *J. Am. Chem. Soc.* **1961**, *83*, 3169. (b) Tanaka, N.; et al. *Macromolecules*, in press.
(38) See ref 14 in ref 13 above.
(39) It should be noted in general that others have used chiral optical measurements to probe the properties of water-soluble polymers although not related to the present observations. For leading references see: (a) Huguet, J.; Vert, M.; Reix, M.; Sepulchre, M.; Spassky, N. *Polymer* **1979**, *20*, 961. (b) Villiers, C.; Braud, C.; Vert, M.; Chiellini, E. *Macromolecules* **1979**, *12*, 103. (c) Braud, C.; Vert, M. *Macromolecules* **1985**, *18*, 856. (d) Michailov, M.; Baldjieva, R. *Makromol. Chem.* **1969**, *123*, 135. (e) Maihailov, M.; Baldjieva, R. T., *C. R. Acad. Bulg. Sci.* **1974**, *27*, 1687.
(40) Wan, P., Shukla, D. *Macromolecules* **1993**, *93*, 571.
(41) Dave, B. C.; Dunn, B.; Valentine, J. S.; Zink, J. I. *Anal. Chem.* **1994**, *66*, 1120A−1127A. (b) Gill, I.; Ballesteras, A. *J. Am. Chem. Soc.* **1998**, *120*, 8587. (c) Wei, Y.; Jin, D.; Ding, T. *J. Phys. Chem. B* **1997**, *101*, 3318.
(42) Private communication from R. Schwerzel of Georgia Tech Research Institute.
(43) We have now seen the same sharp pH dependence for complexation with cyclodextrins for azo dyes bound to the PMA allowing wide variation in wavelength: Cheon, K. S.; van Delden, R.; Green, M. M. *Polymer Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1998**, *39* (2), 711.
(44) Chen, T. S.; Thomas, J. K. *J. Polym. Sci., Polym. Chem.* **1979**, *17*, 1103.

MA9818533