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Fluorescence Properties of Coumarin Laser Dyes in Aqueous Polymer Media. Chromophore Isolation in Poly(methacrylic acid) Hypercoils

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The photophysical properties of a series of coumarin laser dyes in an aqueous polyelectrolyte medium have been determined. The binding of the dyes to poly(methacrylic acid) (PMAA) in water has been demonstrated through the observation of a dramatic blue shift of fluorescence bands and increases in emission quantum yield, lifetime, and polarization. The systematic changes in fluorescence properties reveal that the hydrophobic dyes reside in relatively nonpolar (dry) and highly viscous microdomains for aqueous PMAA solutions at lower pH in which a compact conformation of the uncharged polyelectrolyte is important. The transition from a hypercoiled conformation (pH 4−8) can be followed readily by reference to coumarin fluorescence wavelength, quantum yield, or polarization data. Fluorescence parameters also serve as reporters of the low microscopic polarity and high viscosity of PMAA binding domains. Photophysical measurements are also reported for a coumarin moiety that has been covalently attached to the PMAA backbone. For the covalently bound dye, more subtle alterations of fluorescence properties are found over the entire range of pH examined, reflecting the close association with both compact and elongated (charged) forms of PMAA. Flash photolysis data have been obtained for polymer-bound coumarin 1, for which a low yield of dye triplet can be ascertained.

Introduction

In a series of earlier reports from this laboratory, the effects of medium on the emission properties of coumarin dyes that are important for lasing in the blue-green have been reported.[1−6] Changes in solvent polarity bring about alteration in the fluorescence wavelength, quantum yield, and lifetime for the coumarin laser dyes.[1,2,6] Self-quenching and product analysis and other photodegradation characteristics have been reported,[3,5] as well as coumarin dye quenching by electron transfer quenchers.[4] Aqueous media have also been of interest due to the anticipated benefits of reduced thermal gradients for water vs typical organic solvents under lasing conditions (less problematic influences of transient refractive index effects).[7,8] To this end the solubilization of normally insoluble coumarins in water with the aid of surfactant[9] or cyclodextrin[10] reagents has also been studied. The generally important result of these studies is that the rapid nonradiative decay that mitigates fluorescence for many coumarin dyes (especially for a medium of pure water) is suppressed when dye is sequestered in the less polar domain of an amphiphilic reagent.

In our study of solubilizing agents for the coumarins, we discovered the effectiveness of polyelectrolytes that are based on acrylate monomers, poly(acrylic acid) (PAA) and poly(methacrylic acid) (PMAA).[11] Especially effective was the latter polymer (PMAA), a polyelectrolyte that has been the object of numerous investigations. The intriguing feature of this class of water-soluble polymers is the dramatic change in microscopic and bulk properties that occurs on charging of the polymer chain. This change in polymer structure accompanies a rise in bulk medium pH in the range 5−7 as carboxylic acid moieties are deprotonated.[12] The conformational transition has been investigated using a wide array of physical techniques including potentiometric titration, NMR, and other spectroscopic methods.[13] Particularly pertinent to the present work are recent reports[14] of the behavior of fluorescence probes of the microenvironment of PMAA coils. In these studies organic fluorophores have been incorporated into polyelectrolyte domains through electrostatic or covalent attachment. The common result is the observation of some change in emission properties on alteration of the polymer shape and charge as PMAA is opened from a compact or globular form into an elongated rod-like conformation at higher pH's at which charged carboxylate units engage in electrostatic repulsion (transition region at pH 5−6). In other investigations of PMAA, the feature of hypercoiling at low pH has been exploited to alter the rates of bimolecular reaction, either through the partitioning of reagents between polymer and aqueous domains or through the co-binding of potential reactants.[15] An interesting early observation of relevance is the significant enhancement of the water solubility of aromatic polynuclear hydrocarbons by PMAA in its compact form.[16]

We report here the results of a study in which coumarin dyes have been solubilized in water through binding to PMAA. Dye fluorescence is remarkably enhanced in many instances, in certain cases to a degree not observed for other amphiphilic sequestering media. The coumarin dyes in turn provide sensitive probes of micropolarity and microviscosity, parameters that provide valuable insight to polyelectrolyte structure and the conformational transition in water.

Experimental Section

Materials. Coumarin dyes used in this study are shown in Scheme 1. Coumarins 102 (**3**), 153 (**4**), 120 (**5**), and 339 (**6**) were Kodak laser grade dyes and were used as received after checking for purity by chromatographic (TLC, silica gel, MeOH−CH_2Cl_2 = 1:3) and spectroscopic techniques. Coumarin 1 (**1**) (Aldrich) was purified by recrystallization from methanol−water. Coumarin 35 (**2**) was synthesized and purified according to a published procedure.[17] Atactic poly(methacrylic acid) (PMAA) was prepared by AIBN (2,2′-azobisisobutyronitrile) initiated polymerization of methacrylic acid[18] (freshly distilled under reduced pressure) in DMF with continuous nitrogen purging (60 °C, 12 h) and purified by multiple precipitation from methanol on addition of ethyl ether. The

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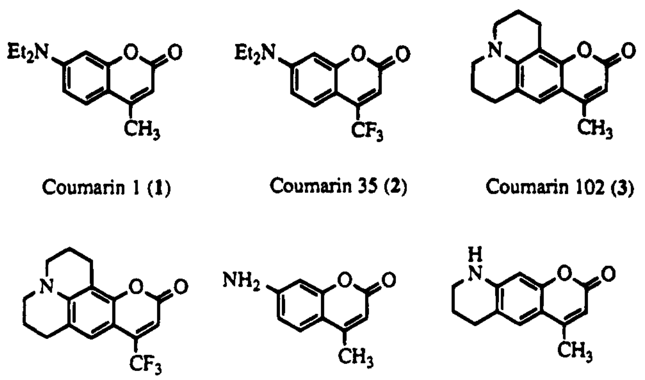
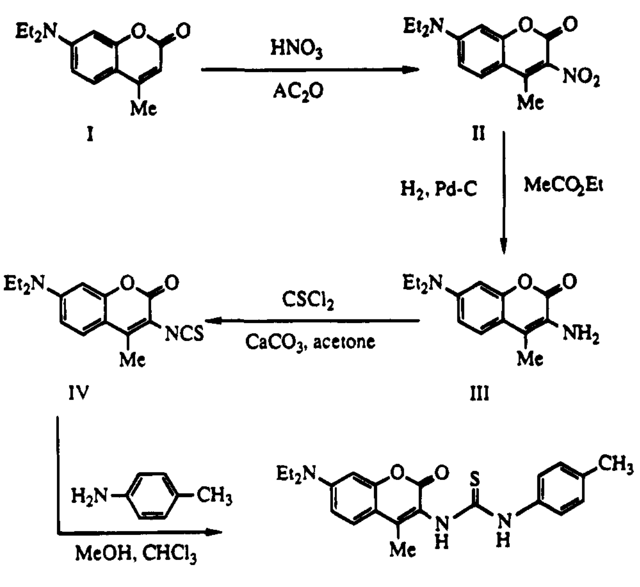
SCHEME 1



Coumarin 1 (**1**) Coumarin 35 (**2**) Coumarin 102 (**3**)

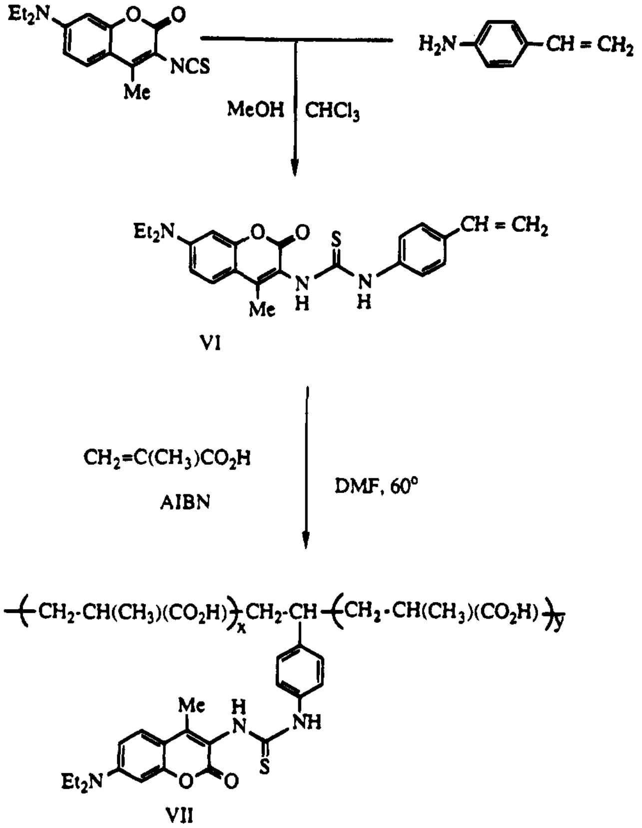
Coumarin 153 (**4**) Coumarin 120 (**5**) Coumarin 339 (**6**)

SCHEME 2



I II III IV V

SCHEME 3



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polymer was fractionated by the procedure described by Flory[19] except that each polymer solution was homogenized by cooling (i.e. the precipitated polymer was allowed to be redistributed between the gelled polymer phase and the solution phase by cooling on standing in a refrigerator). The second fraction obtained was used in photophysical measurements, having a number average molecular weight of 25 000 (determined by glass viscometry). Viscosity measurements were carried out for 1−2 g/dL of polymer solution in water (0.002 N in HCl) with an Ubbelohde viscometer having an outflow time for water of 338.5 at 25 °C. The molecular weight of the polymer was determined from the intrinsic viscosity using the equation[20]

$$[\eta] = (6.6 \times 10^{-4})M^{0.5}$$

where $[\eta]$ is the intrinsic viscosity of the polymer in the solvent used and M is the molecular weight of the polymer. The purity of the polymers was checked spectroscopically; no detectable absorption and emission were observed above 250 nm.

Acetic anhydride, acetone, chloroform, ethyl acetate, and methanol were Baker Analysed and nitric acid was Mallinckrodt AR grade. *N*,*N*-Dimethylformamide (DMF) was distilled over P_2O_5 and kept over molecular sieves. *p*-Toluidine, thiophosgene, and Pd (10%) on activated charcoal were obtained from Aldrich. *p*-Aminostyrene was purchased from Polysciences and 2,2′-azobisisobutyronitrile (AIBN) was obtained from Kodak.

Synthesis of Coumarin Derivatives and a Coumarin−Polymer (PMAA) Conjugate. The synthesis of different coumarin derivatives and the PMAA−coumarin conjugate are outlined in Schemes 2 and 3. The individual steps are described as follows:

*3-Nitro-7-(diethyl)-4-methylcoumarin (**II**).* 3-Nitro-7-(diethylamino)-4-methylcoumarin was prepared by nitration of 7-(diethylamino)-4-methylcoumarin (**1**) according to the method of Machida et al.[21] and reported in part by Chuang[22] with slight modifications. A solution of nitric acid in acetic anhydride (12.5 mL of HNO_3 and 100 mL of freshly distilled Ac_2O) was added gradually over a period of about 1 h to an ice-cooled solution of **1** (20 g, 0.090 mol) in 175 mL of freshly distilled acetic anhydride. To avoid overnitration, the temperature of the reaction bath was kept at −6 to −3 °C by adding sodium chloride. After addition of nitric acid and acetic anhydride, a yellow precipitate formed, which was identified as compound **1**. After about 5 h the precipitate redissolved, after which the reaction was stopped by adding excess ice−water to hydrolyze the excess acetic anhydride. After about 10 min of stirring, a yellow solid precipitated, and after filtration 14.5 g of nitrocoumarin product was obtained. The crude material consisted mainly of the 3-nitrocoumarin with smaller amounts of probably 6-nitro, 8-nitro, and dinitro derivatives.[21,22] From this mixture, 7.5 g (30% yield) of pure compound **II** was obtained by recrystallization from ethyl acetate (mp 170 °C). NMR ($CDCl_3$): δ 7.5, d, 1H, C(5)−H; 6.7, dd, 1H, C(6)−H; 6.5, d, 1H, C(8)−H; 3.5, q, 4H, N−CH_2−; 2.3, s, 3H, C(4)−CH_3; 1.6, t, 6H, N−C−CH_3. The absence of the proton resonance at C-3 in **1** (5.9 δ) confirmed the substitution pattern.

*3-Amino-7-(diethylamino)-4-methylcoumarin (**III**).* The nitrocoumarin (**II**) (3.0 g, 0.010 mol) was dissolved in 200 mL of ethyl acetate in a 500 mL hydrogenation vessel; 500 mg of palladium (10%, on activated charcoal) was added, and the reaction mixture was subjected to hydrogenation at 50 psi.

Aliquots were withdrawn at intervals of 2 h and tested by TLC (silica gel, methylene chloride) for progress of reaction. When all the nitro compound had disappeared ($\sim$12 h), the reaction mixture was filtered and the filtrate evaporated under vacuum to yield 2.5 g of a pale yellow solid material, which was recrystallized from ethanol. The pale yellow crystals (2.2 g, 80% yield) with mp 1−3 °C were identified as compound **III** from NMR data ($CDCl_3$): δ 7.4, d, 1H, C(5)−H; 6.7, dd, 1H, C(6)−H; 6.5, d, 1H, C(8)−H; 3.8, s, 2H, C(3)−NH_2; 3.4, q, 4H, N−CH_2−; 2.2, s, 3H, C(4)−CH_3; 1.2, t, 6H, N−C−CH_3.

3−(7−Diethylamino)−4−methylcoumarinyl)isothiocyanate (IV). Compound **III** (2.0 g, 0.008 mol) was dissolved in 200 mL of acetone, and 2.5 g of calcium carbonate was added. The suspension was heated to boiling, and 1.0 mL of thiophosgene was added. The reaction mixture was heated to reflux, and the progress of the reaction (about 10 min) was followed by TLC (silica gel, CH_2Cl_2). The reaction mixture was refluxed for an additional 20 min and filtered to remove calcium carbonate; excess thiophosgene was distilled off (oil bath, atmospheric pressure). In order to remove the thiophosgene completely, several portions of a small amount of acetone were added, and the evaporation was repeated. The reaction mixture was dried under vacuum to yield 1.5 g of a yellow solid, which was recrystallized from hot acetonitrile, needle-shaped crystals (1.2 g, 50% yield) (mp 157 °C) were obtained. The solid was identified as compound **IV** by NMR ($CDCl_3$): δ 7.6, d, 1H, C(5)−H; 6.8, dd, 1H, C(6)−H; 6.5, d, 1H, C(8)−H; 3.6, q, 4H, N−CH_2−; 2.4, s, 3H, C(4)−CH_3; 1.2, t, 6H, N−C−CH_3.

1-(4−Methylphenyl)-3-(7-(diethylamino)-4-methylcoumarinyl)-thiourea (V). Compound **IV** (57.6 mg, 0.20 mmol) was dissolved in 20 mL of methanol with the aid of 5 mL of chloroform, and *p*-toluidine (2.14 g, 0.02 mol) was added to this solution. The reaction mixture was stirred at room temperature, and after about 12 h, complete conversion of the isothiocyanate was observed (TLC, silica gel, $CHCl_3$). The reaction mixture was dried under vacuum, and the residual solid was dissolved in 20 mL of $CHCl_3$. The unreacted *p*-toluidine was removed by extraction with 200 mL of 1 N aqueous HCl. After evaporation *in vacuo*, the dried $CHCl_3$ extract gave a solid that was recrystallized from 1:1 benzene−cyclohexane. The solid (47 mg, 60% yield) (mp 183 °C) was identified as compound **V** from the high-resolution mass spectrum (m/e = 395.1668; calculated for $C_{22}N_{25}N_3O_2S$ = 395.1667) and from NMR data ($CDCl_3$): δ 7.7, 5H, C(5)−H and 4H of tolyl ring; 6.55, dd, 1H, C(6)−H; 6.4, d, 1H, C(8)−H; 3.3, q, 4H, N−CH_2−; 2.3, s, 3H, C(4)−CH_3; 2.1, s, 3H, C(4′)−CH_3; 1.1, t, 6H, N−C−CH_3.

1−(4−Vinylphenyl)−3−(7−(diethylamino)−4−methylcoumarinyl)thiourea (VI). Compound **IV** (57.6 mg, 0.20 mmol) was dissolved in 10 mL of MeOH with the aid of 5 mL of chloroform, and *p*-aminostyrene (238 mg, 2.0 mmol) was added to this solution. The reaction mixture was stirred at room temperature, and the progress of reaction was followed by TLC (silica gel, $CHCl_3$). After reaction for 24 h and removal of the solvent under vacuum, the residual material was dissolved in 2 mL of CH_2Cl_2, applied to a silica gel column (15 × 2 cm, 230−400 mesh), and eluted with chloroform. In this manner compound **VI** was separated ($\sim$20 mL of eluant) from excess *p*-aminostyrene ($\sim$50 mL of eluant) and any byproduct. A slightly sticky crystalline solid was obtained by evaporation of the column eluant. After recrystallization from benzene−cyclohexane (1:1, v/v), 27 mg (35% yield) of a yellow solid (mp 197 °C) was obtained. The solid was identified as compound **VI** from the high-resolution mass spectrum (m/e = 407.1668, calculated for $C_{23}H_{25}N_3O_2S$ = 407.1667) and the NMR spectral data ($CDCl_3$): δ 7.7, 5H, C(5)−H and 4H of tolyl ring; 6.6, dd, C(4′)−C(H); 6.55, dd, 1H, C(6)−H; 6.4, d, 1H, C(8)−H; 5.65, d, 1H, and 5.2, d, 1H, C(4′)−C−CH_2; 3.3, q, 4H, N−CH_2−; 2.3, s, 3H, C(4)−CH_3; 1.1, t, 6H, N−C−CH_3.

Synthesis and Characterization of the Conjugate of PMAA and coumarin Derivative (VII). The PMAA−coumarin conjugate was synthesized by AIBN (2,2′-azobisisobutyronitrile) initiated copolymerization of methacrylic acid and coumarin derivative **VI** in DMF.[18] Freshly distilled methacrylic acid (2.0 mL, 25 mmol) was dissolved in DMF (20 mL), and compound **VI** (0.02 g, 0.05 mmol) was added to the solution. The polymerization and subsequent purification of the polymer were carried out by a method similar to that used for preparation of PMAA.[18,19] The purified polymer (1.2 g) was dried under vacuum, and its molecular weight (45 000) was determined by glass viscometry. From a comparison of the measured absorbance of the conjugate in methanol (λ_{max} = 373 nm, as observed for **V**) with the molar extinction coefficient of the model compound **V** ($\epsilon_{max} = 2.5 \times 10^4\ M^{-1}\ cm^{-1}$, MeOH), the concentration of the coumarin moiety in the conjugate was calculated. In this way, an R/D ratio ([total polymer residues]/[dye-tagged residues]) of 1600 was obtained for the conjugate. The number of residues for a PMAA chain of average molecular weight 45 000 is 523, so that the probability that polymer chains carried only one coumarin moiety is quite high.

General Techniques. Absorption spectra were recorded on a Perkin-Elmer Model 552 spectrophotometer. Emission spectra were measured on Perkin-Elmer MPF-44A and SLM 48000 fluorimeters. Stock solutions (2.0 mM) of different coumarin dyes were prepared in ethanol by dissolving measured amounts of solid materials. A decimolar stock solution of PMAA was prepared in deionized water. Dye−polymer solutions of desired R/D (ratio of concentration of polymer residue and dye) were prepared by mixing specific amounts of dye and polymer stock solutions in deionized water. The pH of the aqueous solutions was recorded on a Fisher-Accumet pH meter. Dilute NaOH and HCl aqueous solutions were used to control pH. All experiments were performed at room temperature with air-saturated solutions unless otherwise specified. For measurement of absorption spectra, 1−10 μM dye solutions were employed in 1 cm path length quartz cells. For obtaining corrected fluorescence spectra, and fluorescence quantum yield and lifetime data with the SLM 48000 fluorimeter, dye concentrations were kept low so that the absorbance at excitation wavelengths was less than 0.2 (quantum yields for dyes in ethanol[6] were used as standards). Corrections for refractive indices of solvents[24] were included in calculations of Φ_F.

Fluorescence polarization was measured in the L-format on both Perkin-Elmer MPF-44A and SLM 48000 fluorimeters.[23] Excitation at the maximum of the long-wavelength absorption band led to fluorescence monitored at the maximum of the emission band. Concentrations of dye solutions were 10 μM (absorbance values $\sim$0.2 at λ_{ex}). Reproducibility of measured values of P was within ±5%. Measured polarization values were converted to anisotropy values (r) using the relation $r = (2P)/(3 - P)$.

Fluorescence lifetimes were determined by the phase and modulation method.[25] Excitation wavelengths were set toward the blue edge of the visible absorption band, and the total emission was detected without an emission monochromator. Cutoff filters (420, 475, and 515 nm) were used to prevent Raman-scattered light from entering the detection chamber. An aqueous solution of glycogen (scatterer) was used as standard. Modulation frequencies in the range 10−250 MHz were used.

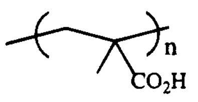
Laser flash photolysis was performed using a Quantel YG-581-10 Nd:YAG laser and associated electronics. The frequency-tripled 355 nm beam (~60-80 mJ/pulse) was employed for excitation following procedures previously reported.[14a,26] Dye solutions of 10–100 μM concentration were used in 2 cm × 1 cm rectangular Pyrex cells. Irradiation was carried out at ambient temperature, and the solutions were deaerated prior to photolysis after purging with argon (ca. 15 min).

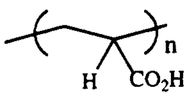
Solubility of Coumarin Dyes in Aqueous Polymer Solution. Excess dye was stirred overnight in PMAA solution at pH 3.0. The resulting filtered solution was measured for optical density at the absorption maximum observed for a solution of the dye in ethanol–water (1:4). The concentration of the polymer-substituted dye was calculated from the absorbance of the solution using the molar extinction coefficient of the dye in ethanol–water (1:4, v/v). The effect of polymer conformation on its dye solubilizing power was examined by ionizing the polymer by addition of dilute aqueous NaOH solution.

Results and Discussion

Enhancement of Solubility of Coumarin Dyes in Water by Addition of Poly(methacrylic acid) (PMAA). Some coumarin dyes, especially the fluorinated ones, are almost insoluble in water. Enhancement of water solubility for coumarins has been previously observed in aqueous micellar[9] and cyclodextrin[10] solutions. Poly(methacrylic acid) in its hypercoiled conformation (pH < 4.0) was examined for similar solubilizing potential;[11,16] significant enhancement of solubility of the dyes in water was observed in the presence of PMAA (Table 1) for moderately concentrated polymer solutions. About a 100-200-fold increase in solubility was observed for the most water-insoluble dyes, **2** and **4**, values generally better than those obtained for surfactant and cyclodextrin media.[9–11]

The dye solubilizing power of the polymer was found to be strongly dependent on its state of ionization. When a saturated solution of **4** in 0.1% aqueous PMAA was titrated with NaOH, a gradual decrease of absorbance was observed at pH values above 4.5. At pH's above 7.0, absorbances were identical to those in water in the absence of the polymer. This experiment demonstrated that the dye solubilizing influence of the compact hypercoiled form of the polymer is lost upon expansion and charging of the polymer chain. In contrast, the homologous polymer, poly(acrylic acid) (PAA), failed to demonstrate any enhancement of coumarin solubility in water. Hence, the solubilizing power of PMAA results from the added hydrophobic interactions originating from the pendant methyl groups,[12,15] which provide lipid-like microdomains within the compact polymer coil.


PMAA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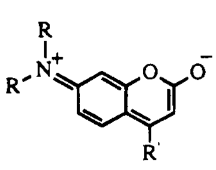
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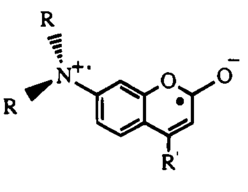
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TABLE 1: Solubility of Coumarin Dyes in Aqueous Poly(methacrylic acid)[a]

medium	1 (10^{-5} M)	2 (10^{-5} M)	3 (10^{-5} M)	4 (10^{-5} M)
water	10.0	0.58	13	0.20
0.1% PMAA (pH 3.0)	110	10.0	35	2.2
1.0% PMAA (pH 3.0)	200	110	52	21

[a] 0.1% and 1.0% w/v PMAA solutions correspond to concentrations of 0.012 and 0.12 M residues of the polymer, respectively.

TABLE 2: Photophysical Parameters for Coumarin 1 in Solvents and in Aqueous PMAA at pH 3 and at Different R/D[a]

medium	λ_a (nm)	λ_f (nm)	Φ_F[b]	$P(r)$[b]
ethanol	373	450	0.73	0.02 (0.01)
water	380	468	0.09	0.02 (0.01)
R/D = 50	380	438	0.18	0.10 (0.09)
R/D = 100	380	435	0.23	0.14 (0.11)
R/D = 500	380	433	0.48	0.25 (0.17)
R/D = 1000	380	432	0.61	0.31 (0.23)
R/D = 2000	380	432	0.65	0.32 (0.24)

[a] [dye] = 10 μM; pH was maintained by adding dilute aqueous HCl and NaOH solutions; R/D = [PMAA residues]/[dye] for aqueous polymer solutions. [b] Fluorescence quantum yield and polarization (anisotropy) values on excitation at the maximum of the long-wave absorption band.

Alteration of Photophysical Parameters of Coumarin 1 on Solubilization in Aqueous Poly(methacrylic acid). In ethanol solution coumarin **1** absorbs at 373 nm ($\epsilon = 2.2 \times 10^4$ M^{-1} cm^{-1}) and emits strongly at 450 nm.[6,7,27] A 7 nm red shift in the absorption spectrum, and a 18 nm red shift in the emission spectrum of the dye, was observed for aqueous solution. Photophysical parameters for the dye in aqueous PMAA at moderately acidic pH (~3.0) are collected in Table 2. The incorporation of dye molecules in the compact polymer domain is indicated by the gradual increase of the fluorescence polarization (P), or anisotropy (r), upon addition of the polymer. The phenomenon is demonstrated in the plot for polarization data as a function of R/D in Figure 1. Above R/D 500 the sharp increase in the anisotropy parameter is diminished, and a plateau is reached above R/D 1000, where dye molecules are completely sequestered in the polymer domain. The increase in fluorescence polarization on solubilization of the coumarins is the result of association of dye molecules with the hypercoiled polymer microdomain, a host–guest interaction that introduces a hindrance to dye mobility (limited rotational diffusion of chromophores within the fluorescence lifetime).

Binding with the polymer does not lead to any significant change in the absorption spectrum of **1**, but a large (~36 nm) blue shift of the emission spectrum is observed (Table 2). For solvatochromic coumarin dyes, a hypsochromic shift of emission wavelength for less polar solvents is a well-documented phenomenon.[1,2,6,7] For **1**, the blue shift of emission in passing from water to aqueous PMAA indicates less solvent stabilization of the fluorescence polar excited state of the dye in polymeric solution compared to pure water (i.e., the PMAA hypercoil is substantially less polar than water).[12–14] The model of a relatively polar intramolecular charge-transfer (ICT) state that is responsible for emission for the 7-aminocoumarins has been previously proposed.[1,2,6,7] The solvatochromism of emission bands is readily understood in terms of partial charge separation for the ICT state (normally depicted by the canonical form shown below). Calculations using Lippert's equations[28,29] involving absorption and emission energies in different solvents for **4** showed a difference in dipole moment of about 5 D between the ground state and the excited (S_1, ICT) state.

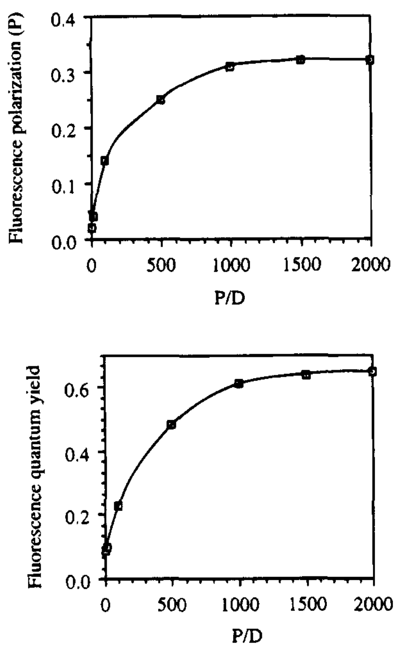

Figure 1. Dependence of fluorescence polarization (upper panel) and fluorescence quantum yield (lower panel) for coumarin **1** on addition of polymer; P/D = [PMAA residue]/[dye]; for aqueous solution of **1**, [dye] = 10 μM and pH = 3.0.

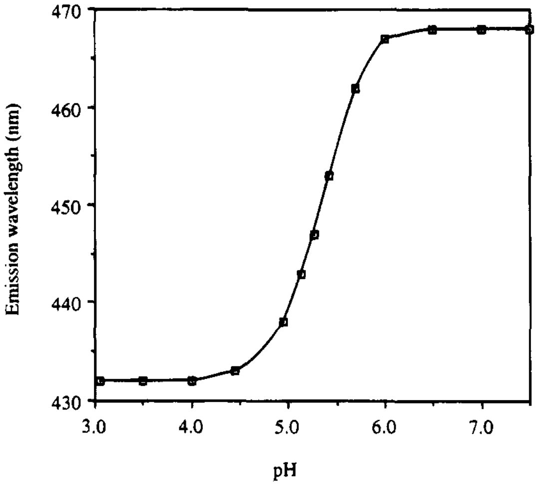

Figure 2. Emission wavelength maximum for **1** (10 μM) in aqueous PMAA (R/D 1000) at different pH values (λ_{ex} = 428 nm).

The variation of emission wavelength for **1** in an aqueous solution of PMAA at R/D 1000 as a function of pH is shown in Figure 2. The blue-shifted emission observed for the dye in the PMAA hypercoil prevails up to pH 4.0, above which a gradual red shift of the emission is observed. At high pH (>6.0) the emission spectrum of the dye resembles that in water; the shift in emission maximum is accompanied by a sharp decrease in the fluorescence polarization (~0.02). The change of emission wavelength of the dye with pH takes place in the pH range 4−6, the region of conformational transition of the polymer from the hypercoiled to the elongated form.[12,13] Thus, it is clear that with the unfolding of the polymer chain, the dye is gradually exposed to the aqueous environment, and little or no association of the dye exists with the charged rod-like polymer.

Photophysical Parameters for Other Coumarin Dyes in Aqueous Poly(methacrylic acid). Since for **1** the effects of PMAA solubilization on photophysical properties were optimized at R/D 1000, and the hypercoiled conformation of the polymer is most suitable in dissolving hydrophobic solutes in the polymer domain, further study with other coumarin dyes was limited to the conditions of R/D 1000 and pH 3.0. Photophysical properties of other dyes selected for study (Scheme 1) in aqueous polymer solution along with the values for water and ethanol solutions are collected in Table 3. Only coumarin dyes carrying tertiary nitrogen at the 7-amino position were solubilized in the PMAA hypercoil, as reflected by high values of emission polarization (*P*). Dyes containing primary (**5**) and secondary (**6**) nitrogen at the 7-amino position are not solubilized in the polymer microenvironment, but rather remain in the bulk aqueous medium (consistently low polarization). Thus, no significant effect of added polymer is observed on the photophysical properties of **5** and **6**, structures in which the amino nitrogen provides an additional site for H-bonding with water[30] (in addition to interaction with the carbonyl oxygen) and favors residence in the water microphase.

TABLE 3: Photophysical Parameters for Coumarin Dyes in Different Media[a]

dye	medium	λ_a (nm)	λ_f (nm)[b]	$P(r)$[b]
1	ethanol	373	450	0.01 (0.01)
	water	380	468	0.06 (0.04)
	aq PMAA	380	432	0.32 (0.24)
2	ethanol	402	508	0.03 (0.02)
	water	408	530	0.01 (0.01)
	aq PMAA	403	468	0.31 (0.23)
3	ethanol	388	472	0.01 (0.01)
	water	397	488	0.01 (0.01)
	aq PMAA	397	447	0.29 (0.21)
4	ethanol	422	530	0.01 (0.01)
	water	432	548	0.01 (0.01)
	aq PMAA	422	488	0.31 (0.23)
5	ethanol	353	427	0.02 (0.01)
	water	343	435	0.02 (0.01)
	aq PMAA	341	437	0.02 (0.01)
6	ethanol	378	442	0.01 (0.01)
	water	374	457	0.01 (0.01)
	aq PMAA	372	456	0.04 (0.03)

[a] [dye] = 10 μM; for aqueous PMAA, [residues] = 10 mM (R/D = 1000) and pH = 3.0. [b] Excitation at the long-wavelength absorption band maximum.

Among the four PMAA-solubilized coumarin dyes, some interesting differences are observed that are related to dye structure. The fluorinated dyes **2** and **4** show larger solvatochromic polar red shifts of the emission maximum (~60 nm alteration in wavelength for cyclohexane → water) compared to the nonfluorinated dyes **1** and **3** (~40 nm) and measurable (although smaller) shifts in absorption maxima.[2,6,7] The nonpolar nature of the polymer environment provides less solvent stabilization of the coumarin emissive ICT state and is the origin of the blue shift of emission for the PMAA hypercoil-bound dyes. For the fluorinated dyes (**2** and **4**) the presence of the trifluoromethyl group at the 4-position introduces additional stabilization of the excited state (a more polar ICT) through an inductive (electron withdrawing) effect. Thus, in polar environments the emission of these dyes displays more Stokes shift than the dyes containing a methyl group at the 4-position. In the nonpolar environment of the polymer microdomain, solvent stabilization of the excited state dipole is limited and the Stokes shift of fluorescence dramatically reduced. The slight red shift of the absorption of the fluorinated dyes in the pure water medium is consistent with a solvent effect on the relative energy of the (unrelaxed) Frank-Codon excited state; that is, the more polar fluorinated dyes have a relatively larger ground state dipole moment that "fixes" through H-bonding water molecules for stabilization of the FC ICT state, a feature that is less pronounced or absent for ethanol and aqueous PMAA media. On increasing pH a gradual release of dye molecules from the

TABLE 4: Fluorescence Quantum Yield and Lifetime Data and Computed Radiative and Nonradiative Decay Constants for Coumarin Dyes in Different Media[a]

dye	medium	Φ_F[b]	τ_F(ns)[b]	$k_f \times 10^{-7}$ (s^{-1})	$k_{nd} \times 10^{-7}$ (s^{-1})
1	ethanol	0.73	3.3	22	8.2
	water	0.09	<0.5	18	>182
	aq PMAA	0.62	4.1[c]	14	7.7
2	ethanol	0.09	<0.5	18	>182
	water	0.03	<0.5	6.0	>194
	aq PMAA	0.49	5.5[d]	8.4	8.8
3	ethanol	0.95	4.9	19	1.0
	water	0.95	5.8	16	0.86
	aq PMAA	0.98	5.4	18	0.38
4	ethanol	0.38	4.8	7.9	13.0
	water	0.10	<0.5	20	>180
	aq PMAA	0.62	6.6	9.4	5.8

[a] [dye] = 5.0 μM; for aqueous PMAA, [residues] = 5.0 mM (R/D = 1000) and pH = 3.0. [b] Excitation at the maximum of the long-wavelength absorption band. [c] Global value; somewhat better fit of decay data with two-component model with τ_1 = 0.70 ns (11%) and τ_2 = 4.7 ns (89%). [d] Global value; somewhat better fit of decay data with two-component model with τ_1 = 1.8 ns (4%) and τ_2 = 5.8 ns (96%).

polymer domain to the bulk aqueous medium is indicated, as with **1**, by a gradual red shift in fluorescence emission. At a pH higher than 7.0, when the polymer attains an elongated rod-like conformation, dye molecules become totally free from the polymer environment since very low values of fluorescence polarization ($P \sim 0.02$) are shown.

Fluorescence Quantum Yield and Lifetime Data for PMAA-Bound Coumarin Dyes. Coumarins are generally highly fluorescent when dissolved in solvents such as ethanol or methanol, but for some dyes the fluorescence efficiency is significantly diminished in water.[1,2,6,9,10] The fluorescence quantum yields of the PMAA-bound dyes are presented in Table 4 along with the values for ethanol and water solvents. For the dyes **1**, **2**, and **4**, whose emission is quenched in water, solubilization in the PMAA hypercoil rejuvenates fluorescence intensity. In two cases the fluorescence quantum yields of polymer-bound dye are significantly higher than the values in ethanol. Exceptional is the case of coumarin **3**, which has a very high value of Φ_F in water, a value which is maintained for the aqueous PMAA medium.

Fluorescence lifetime values for the dyes in different media are also presented in Table 4. Values for **1**, **2**, and **4** in water are too low to be measured reliably with present fluorimeter capabilities (τ_f < 0.5 ns represents a conservative limit). Measurements were carried out using 10–20 modulation frequencies in the range 10–150 MHz, and the fitted result was an average of all measurements. In every case attempts were made to fit the experimental parameters with single-, double-, or even triple-exponential decays, and the results with the best values of χ_r^2 were taken. For PMAA solutions generally, relatively high average values of τ_F were obtained. For **1** and **2** the phase and modulation data were better fit with a biexponential decay, but for the more structurally compact hydrophobic dyes, **3** and **4**, a single-component lifetime was observed.

The principal model proposed previously[6,7] to understand the effects of medium on fluorescence lifetimes (and quantum yields) focuses on the relative energy of the coumarin ICT excited state and its propensity to evolve into the fully charge-spearated, nonemissive TICT state. Polar solvents stabilize the TICT state to a greater extent than ICT, leading to an accelerated rate of ICT → TICT conversion; subsequent depletion of the TICT form returns molecules to the ground state, completing a channel for nonradiative deactivation for dyes that are structurally capable of twisting about the 7-amino function (**1** and **2**, but not **3** and **4**).

Although often cited, the nonradiative decay mechanism that proceeds via the TICT species is in fact clearly indicated only for those dye structures in which the amine function is dialkyl substituted (providing a terminal moiety with a low ionization potential) and capable of sustaining a full positive charge (in the twisted form), and, ideally, further supported by having electron-withdrawing groups in the pyran ring (e.g., CF_3 as in **2**).[6] An alternative mechanism of radiationless decay has been proposed for the coumarins involving a solvent-assisted "umbrella" motion (out-of-plane deformation at the amine function).[30a] This mechanism, reflected in an observed *increase* in nonradiative decay with longer chain alcohols (e.g., for **5** in less polar ROH) and associated with specific interactions of solvent (including H-bonds) with the emissive state,[30a] appears to be most appropriately applied to coumarin derivatives less substituted at the 7-amino position.

Recent attempts to directly observe the TICT species for **1** in several polar solvents have not been successful, using transient absorption techniques restricted to the >35 ps time domain or through examination of spectra for a coumarin (153) in a supersonic jet.[31] On the other hand, evidence from experiments involving low-temperature glasses for a 6-aminocoumarin[32] from high-pressure studies of fluorescence frequency shifts for **1** and **3**[33,34] has been viewed as consistent with the importance of an intervening twisted state. In a recent time-resolved fluorescence study of **1** and **3** in various solvents, an assignment was made of a short-lived decay component (<0.5 ps) to the ICT → TICT process for **1** in water.[35] For alternate coumarin structures with carboxylate substituents that show similar solvent-dependent (enhanced) nonradiative decay, a twist mechanism of deactivation involving an ester group in the 3-position has also been proposed.[36]

In comparison to a pure water medium, the relatively nonpolar and microviscous interior (vide infra) of the PMAA hypercoil resists conversion of ICT to TICT forms, thus extending the quantum yields of ICT emission. The rise in fluorescence lifetimes generally observed for polymer-bound species parallels this trend, also reflecting the distribution of coumarin fluors in a dense restrictive microdomain. The influences of medium on the fluorescence lifetime and decay kinetics for the coumarins (particularly **4**) have in fact been studied extensively and in general show complicated multicomponent patterns of decay for a variety of polar solvents.[28,34,37] Typically, the emission decay data have been satisfactorily fit to biexponential functions, the principal rationale for which has focused on solvation dynamics that results in a time-dependent Stokes shift of the fluorescence.[36] According to this theory, multicomponent decays result from a natural distribution of fluorophore solvate structures that evolve with time, differ slightly in energy in polar media, and are therefore associated with a distribution of fluorescence frequencies, ν_f (and the associated radiative decay constants). An alternative rationale[38] deemphasizes the role of solvent relaxation and focuses on the inhomogeneous distribution of static structures (related to a "two-state" model also proposed[35]) that contribute to different portions of the fluorescence decay (for example, higher energy fluors, emitting to the blue, contribute a shorter lifetime to the global decay function).

The main point to be made about the present fluorescence data is that all parameters (enhanced emission yield, lifetime, polarization, and a reduced Stokes shift) agree in requiring a location of dye molecules in the nonpolar polymer microphase (away from bulk water, vide infra). The observation of singular long-lifetime components for the fluorescence decay of **3** and

TABLE 5: Comparison of Photophysical Parameters for Coumarin Dyes Dissolved in Aqueous PMAA and in Aqueous Glycerol Solution[a]

dye	medium	λ_a (nm)	λ_f (nm)[b]	Φ_F[b]	τ_F[b]
1	aq PMAA	385	432	0.62	4.7
	aq glycerol (75%)	386	454	0.31	2.0
	aq glycerol (95%)	385	454	0.52	3.8
2	aq PMAA	403	468	0.49	5.8
	aq glycerol (75%)	415	524	0.07	1.0
	aq glycerol (95%)	414	524	0.18	2.5
3	aq PMAA	400	447	0.98	5.4
	aq glycerol (75%)	400	482	0.92	5.1
	aq glycerol (95%)	400	482	0.93	5.1
4	aq PMAA	422	488	0.62	6.6
	aq glycerol (75%)	438	545	0.19	2.5
	aq glycerol (95%)	436	545	0.22	3.2

[a] [dye] = 5.0 μM; for aqueous PMAA, [residue] = 5.0 mM (R/D = 1000) and pH = 3.0. [b] Excitation at the long-wavelength absorption band maximum.

4 in PMAA is consistent with the monoexponential decay behavior observed for coumarins in nonpolar media[34,36] (the polymer microphase is remarkably free of polar water molecules, vide infra, that would reorient around nascent charge in emissive ICT species). Nonetheless, a warning against oversimplification regarding a uniqueness for binding sites should be registered, since the precise location of sequestered dye molecules in the compact form of PMAA cannot be determined with our measurements. Indeed, one must anticipate some distribution of binding sites within the PMAA microenvironment.[12,14] Although some uniformity in the binding domain for dyes **3** and **4** is indicated, the anticipated greater complexity in binding is perhaps at least partially revealed by the two-component decays for **1** and **2**, dyes with flexible chains associated with the 7-position and less capable of achieving highly compact arrangements within polymer microdomains.

With the caveats in mind regarding inhomogeneous distributions of dye–polymer structures, the dissection of microscopic rate constants for radiative and nonradiative decay of the singlet excited states remains worthwhile for comparison purposes (Table 4). Values were computed from the common relations $k_f = \Phi_f/\tau_f$ and $k_{nd} = (1 - \Phi_f)/\tau_f$. In the calculation of parameters for water solutions, the lifetimes for **1**, **2**, and **4** have been taken as 0.5 ns for computation of k_f values, and k_{nd} constants then were determined as lower limits. The data show that the dyes have comparable rates for radiative decays in all the media measured and for the nonradiative decay (k_{nd}) for the dyes dissolved in ethanol and aqueous PMAA. For aqueous solutions the values for the nonradiative rate constants are very high, consistent with the intervention of the facile twisting deactivation mechanism (ICT → TICT) for **1** and **2**. The fast decay for **4** results from the dramatic reduction in the energy gap separating the emissive ICT and ground states.[6]

Photophysical Parameters for Coumarin Dyes in Aqueous Glycerol Solution: Microviscosity of the PMAA Hypercoil. The high values of fluorescence quantum yield and lifetime for PMAA-bound coumarin dyes originate from residence of dye molecules in a highly viscous environment within the polymer microdomain. To examine this hypothesis, photophysical parameters for coumarin dyes were measured in 75% and 95% aqueous glycerol solution (Table 5). For all the coumarin dyes studied, the solvatochromic red shift of fluorescence is far more prominent than that for PMAA, consistent with the high polarity of the alcohol. For **1**, a 95% (v/v) glycerol solution in water can only partially attain the enhancement of fluorescence quantum yield that results from dissolution of the dye in hypercoiled PMAA. For **2** and **4**, even data for 95% glycerol solvent fall short of the effect produced by PMAA on emission intensity and lifetime. As reporters, the coumarins reveal therefore very high values of microviscosity (>500 cP, 20 °C, the value for 95% glycerol solution), consistent with other data that suggest highly restrictive rigid microdomains for PMAA hypercoils.[11,12,14] High values of fluorescence polarization are also observed for the coumarins in 95% glycerol (P = ca. 0.30), implicating a restriction of molecular rotational diffusion in this solvent, again in parallel with findings for dye in PMAA and consistent with a reduced free volume experienced by dye in these media.[39]

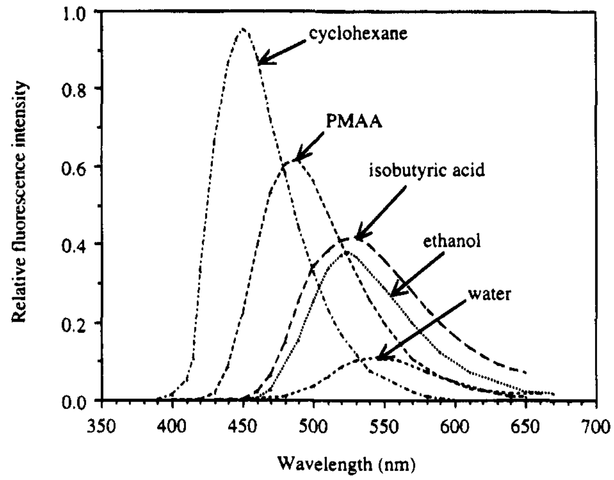

Figure 3. Emission spectra for **4** (5.0 μM) in different media; for PMAA solution, pH = 3.0 and R/D = 1000.

Micropolarity of the PMAA Hypercoil. The emission frequency maximum of the solvatochromic coumarin dyes is known to be a useful quantitative reporter of polarity of the medium.[1,2,6,9] In order to have an appreciation of the micropolarity of the PMAA hypercoil, a comparison was conducted of the emission of **4** in a variety of solvents (Figure 3). The regular blue shift of the emission peak and enhancement of emission intensity on deployment of less polar solvents reveal that residence in the PMAA hypercoil provides an environment that is much less polar than *tert*-butyl alcohol or ethyl acetate. Systematic correlation of coumarin absorption and emission frequencies with solvent parameters, π^* and α, has been observed by Jones et al.[2] The π^* scale of solvent polarity was developed by Kamlet and Taft[40] and reflects solvent polarity–polarizability in the absence of specific interactions such as hydrogen bonding. The Kamlet/Taft parameter, α, is an empirically derived constant reflecting the ability of the solvent to donate a hydrogen bond.[41]

Plots of emission frequencies for **4** in different solvents vs π^* and α parameters are presented in Figure 4 (data in part from ref 2). The PMAA hypercoil shows a π^* value equivalent to approximately 0.40 and an α value of approximately 0.45. To have an appreciation of these values, it should be noted that cyclohexane has the reference π^* value 0.00, ethyl acetate, 0.55, and acetonitrile, 0.75. On the α scale, water has a value of 1.17, ethanol, 0.83, and *t*-butyl alcohol, 0.62. Notably, reporter **4** provides a larger dynamic range for assessment of micropolarity for the π^* (non H-bonding) solvents and a much diminished response to changes in H-bonding capability within the α series of alcohols (that is, a "low polarity" ROH solvent with small ϵ will yield a substantial fluorescence red shift), again a probable reflection of the effectiveness of specific H-bonding in stabilization of the emissive state.[30,37d,38] The most striking feature of the data for the PMAA microdomain is the extent to which hydrogen bonding (and even moderate dipolar medium

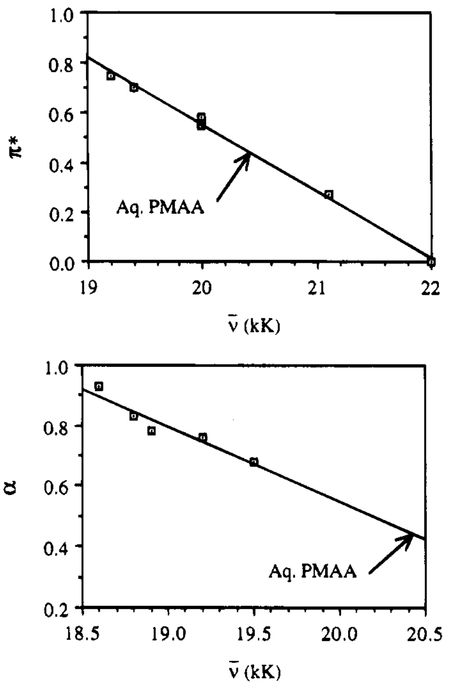

Figure 4. Emission energy (units of kK) for **4** in different solvents vs the solvent polarity/polarizability parameter, π^* (top panel), and the hydrogen bonding parameter, α (bottom panel). The solvents in order of decreasing polarity (parameter value) are, for π^*, acetone, acetonitrile, tetrahydrofuran, ethyl acetate, ethyl ether, and cyclohexane, and, for α, methanol, ethanol, *n*-butanol, 2-propanol, and *tert*-butyl alcohol.

interactions) is essentially absent, according to the micropolarity values reported by **4** (nominally, a dielectric constant, ϵ = ca. 5, and H-bond capabilities far inferior to *t*-BuOH are indicated). A revealing reference point is the finding that the solubilization site for coumarin **4** in a sodium dodecylsulfate micelle corresponded to α = 1.05 in a similar comparison, consistent with the notion that micelle binding sites are relatively "wet".[9] The specific interaction of bound coumarin with a "buried" carboxyl moiety is also not well indicated, given the difference in spectral shift for PMAA vs a model "residue", isobutyric acid (Figure 3).

Photophysical Properties of a Coumarin–PMAA Conjugate. Dye loading of the PMAA–coumarin covalent conjugate (Scheme 3) was calculated to be equivalent to a polymer residue/dye ratio, R/D, = 1600. Photophysical parameters for the conjugate were examined in aqueous solution at two different pH's (values of 3 and 8, for which, respectively, the hypercoil and elongated rod structures were assumed for the polymer). The photophysical parameters of the model compound **V** (Table 6) are significant. Compared to other coumarin dyes in this study, fluorescence is diminished even in ethanol ($\Phi_F = 0.10$). The fluorescence lifetime is also below the measurement limit ($\tau_F < 0.5$ ns). The presence of the thiourea linkage at the 3-position offers an additional mode of nonradiative deactivation of the excited singlet state.[35] Incorporation in the PMAA hypercoil enhances the fluorescence quantum yield (0.32) by about 3-fold, and unfolding of the polymer to the elongated rod-like structure eliminates the polymer effect as expected.

The effect of covalent conjugation with PMAA on the photophysical properties of the analogous coumarin moiety is quite remarkable. A high value of the fluorescence quantum yield (0.65), about 7 times higher than the value for the model compound in ethanol, is observed for the polymer-conjugated coumarin dye at pH 3.0. The blue-shifted fluorescence with a high value for quantum yield and polarization (0.32) indicates residence of the dye chromophore inside the polymer hypercoil.

TABLE 6: Photophysical Parameters for PMAA-Coumarin Covalent Conjugate (VII) in Aqueous Solution Compared to Model Compound (V) in Aqueous PMAA[a]

system	medium	λ_a (nm)	λ_f (nm)[b]	Φ_F	P	τ_F (ns)
V	ethanol	373	464	0.10	0.02	<0.5
	R/D 1600 (pH 3.0)	395	448	0.32	0.29	2.1[c] 3.5 (79%) 0.9 (21%)
	R/D 1600 (pH 8.0)	395	480	0.07	0.03	<0.5
conjugate **VII**	pH 3.0	390	458	0.65	0.32	4.2[c] 3.8 (83%) 7.1 (17%)
	pH 8.0	388	480	0.23	0.22	1.9[c] 2.9 (71%) 0.5 (29%)

[a] For the model compound and conjugate, [dye (chromophore)] = 5.0 μM. [b] Excitation at the maximum of long-wavelength absorption band. [c] Values from analysis of single-exponent decay (poor fit); following values from analysis of two-component decays.

Moreover, the fluorescence yield of 0.23 at pH 8.0 (for the rod-like charged polymer in water) is a significantly higher value than those normally observed for aminocoumarins with mobile diethylamino groups or for model structure **V**. An unusually high value of fluorescence polarization (0.22) for the conjugate at pH 8.0, as compared to the low values of about 0.02 for the parameter for unbound coumarin dyes indicates considerable restriction to the rotation of the chromophore, even when the dye is attached to the *open* polymer coil for which considerable exposure to water is expected. Emission titration of the PMAA–coumarin conjugate in aqueous solution indicated the gradual unfolding of the polymer upon ionization, as revealed by the red shift of emission with increasing pH. The conformational transition of the polymer is found to take place in the pH range 4.5–6.5. A molecular model for a segment of PMAA residues that provides some insight for the degree of interaction of chromophore and the open form of the polyelectrolyte is shown in Figure 5. Although the model is only partially illustrative, it may be the case that residual interactions between the pendant chromophore and polymer chain segments serve to minimize water contacts for the hydrophobic moieties, even in the high-pH regime.

Fluorescence lifetime measurements reveal a heterogeneity of binding sites for the conjugated dye. For pH 3.0 polymer solutions (hypercoiled PMAA) the phase and modulation data could not be fit to a clean single-exponential decay; a better fit ($\chi_r^2 = 0.74$) was obtained for a biexponential decay with lifetimes of 7.1 ns (17%) and 3.8 ns (83%). Both of the components of lifetime indicate the presence of the dye chromophore in the polymer domain, the longer component indicating a situation where some chromophore units are more deeply buried in the polymer matrix. A biexponential decay is also observed for the polymer–dye conjugate system at higher pH (8.0) when the polymer attains the elongated rod-like conformation exposing dye chromophore to the bulk aqueous medium. One of the components is very short (0.5 ns), indicating a contact of (some) chromophores with water (vide supra); somewhat surprisingly, the much larger fraction of the fluorescence decay is associated with chromophores exhibiting slow decay times (2.9 ns), indicating limited interaction with free water. Apparently, there is some remaining limitation on the effectiveness of solvation by water of the open (charged) polymer that may involve polarization of solvent and the side-chain dye moiety in the strong local electrostatic field of the charged polyelectrolyte. A distinction cannot be presently made as to whether the emission frequency shift to the red associated

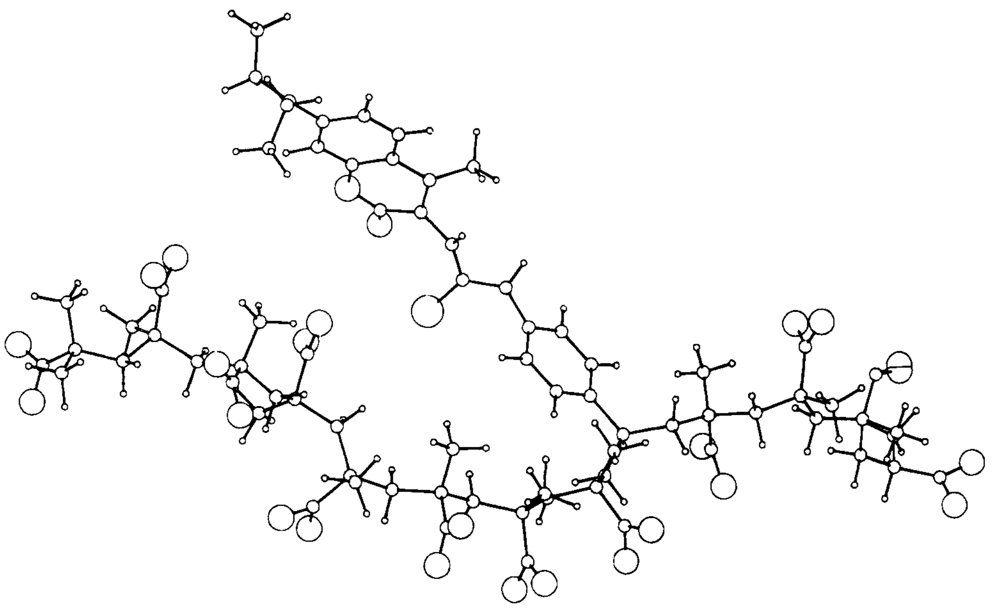

Figure 5. CHARMm model of PMAA–coumarin conjugate (**VII**), consisting of a dye moiety attached to a polymer segment of 12 methacrylic acid residues assembled in an elongated form (high pH).

with charging (opening) the polymer on elevation of bulk pH is due to exposure to the ionic field (a kind of "salt effect" which is precendented[42]) or to interaction with neighboring water.

Laser Flash Photolysis of PMAA-Bound Coumarin Dyes. Laser flash photolyis was performed on coumarin dyes solubilized in aqueous PMAA in order to determine the fate of any phototransients, including especially dye triplet excited states.[6] Excitation of the polymer solutions of **2**, **3**, and **4** (355 nm) (pH = 3.0) failed to produce any detectable transients, and the ground state bleach of the dyes was found to recover completely within a few tens of nanoseconds. For **1** in aqueous polymer solution (R/D = 1000, pH = 3.0) the situation was quite different. Although the major transient again grew and decayed within the laser pulse length (ca. 7 ns), several hundred microseconds was required for the recovery of about 10% of the ground state bleach at 380 nm. The transient expected in the known triplet absorption region for **1**, although weak, could be detected for aqueous PMAA solutions (~600 nm).[3,43] The relatively higher yield of phototransients and the longer time required for the assumed decay of dye triplets in the polymer environment are consistent with triplet counting experiments that show for **1** a higher triplet yield for lower polarity media[6] and a relative isolation of dye triplets upon incorporation into the microdomain of PMAA.[14]

Conclusions

Water solubility of coumarin laser dyes is significantly enhanced in the presence of poly(methacrylic acid) (PMAA), when the polymer exists in the hypercoiled conformation. Emission polarization data indicate that dye molecules are sequestered in the hydrophobic microdomain of the compact conformation of the (largely uncharged) polymer that predominates for aqueous solutions at moderately acidic pH (3.0). Such enhancement of coumarin solubility in water is not affected by poly(acrylic acid) (PAA), indicating that for PMAA hydrophobic interaction plays the critical role, either directly by van der Waals contact with pendant methyl groups or within a cluster of these hydrophobic groups that provide a pocket for dye incorporation. On ionization of the polymer at higher pH (8.0), the dye-binding capacity is diminished; the binding phenomenon is absent for the charged rod-like conformation of the polyelectrolyte.

Under conditions of binding with PMAA, the emission spectra of solvatochromic[2] coumarin dyes display dramatic blue shifts (compared to the fluorescence in polar solvents), indicating the residence of dye molecules in a nonpolar environment, a medium not unlike a hydrocarbon solvent in micropolarity. A comparison with aqueous glycerol media suggests that microviscosity for the PMAA interior corresponds to that of nearly pure glycerol (>500 cP at 20 °C). The dramatic quenching of fluorescence generally observed for most coumarins in water is reversed upon incorporation of dyes in the PMAA polymer domain. For the fluorinated coumarins, the fluorescence quantum yield values are even higher than the values found for ethanol solution. The effect of the nonpolar, highly viscous environment of the polymer domain is to raise the energy of the emissive ICT state and to slow the nonradiative decay, ICT → TICT. The PMAA-bound coumarin dyes are highly fluorescent (generally, $\Phi_f > 0.5$); in fact PMAA is superior to surfactant or cyclodextrin amphiphiles[9–11] in providing a microenvironment that inhibits nonradiative deactivation.

For the covalently bound coumarin **VII**, more subtle alterations of fluorescence properties were found for the entire range of pH examined, reflecting the close association of dye chromophores with both compact and elongated (charged) forms of PMAA. Especially noticeable for the covalent conjugate is the moderate fluorescence quantum yield (0.23) of the dye when the polymer is in the elongated form, indicative of some remaining restriction of the distorting motions associated with nonradiative decay. The extended PMAA conformation appears to expose dye to water as expected, but also appears to retain a remnant of the sequestered local environment associated with the polymer in its globular form.

The enhancement of water solubility and fluorescence quantum yield of coumarin dyes upon binding with PMAA could be of practical importance in the use of these dyes as the active medium in dye lasers. The high emission yields and the absence of the induction of triplet formation upon incorporation in the polymer domain (except for **1**) make such deployment of the coumarin dyes of the present study especially attractive.

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References and Notes

(1) Jones, G., II; Jackson, W. R.; Halpern, A. H. *Chem. Phys. Lett.* **1980**, *72*, 391.

(2) Jones, G., II; Jackson, W. R.; Kanoktanaporn, S. *Optics Commun.* **1980**, *33*, 315.

(3) Jones, G., II; Bergmark, W. R.; Jackson, W. R. *Optics Commun.* **1984**, *50*, 320.

(4) Jones, G., II; Griffin, S. S.; Choi, C.; Bergmark, W. R. *J. Org. Chem.* **1984**, *49*, 2705.

(5) Jones, G., II; Bergmark, W. R. *J. Photochem.* **1984**, *26*, 179.

(6) Jones, G., II; Jackson, W. R.; Choi, C.; Bergmark, W. R. *J. Phys. Chem.* **1985**, *89*, 294.

(7) Jones, G., II Photochemistry of Laser Dyes In *Dye Laser Principles with Applications*; Duarte, F. J., Hillman, L. W., Eds.; Academic Press: New York, 1990.

(8) Drexhage, K. H.; Erickson, G. R.; Reynolds, G. A. *Optics Commun.* **1975**, *15*, 399.

(9) Jones, G., II; Jackson, W. R.; Kanoktanaporn, S.; Bergmark, W. R. *Photochem. Photobiol.* **1985**, *42*, 477.

(10) (a) Nag, A.; Chakrabarty, T.; Bhattacharyya, K. *J. Phys. Chem.* **1990**, *94*, 4203. (b) Scypinski, S.; Drake, J. M. *J. Phys. Chem.* **1985**, *89*, 2432. (c) Bergmark, W. R.; Davis, A.; York, C.; Macintosh, A.; Jones, G., II. *J. Phys. Chem.* **1990**, *94*, 5020.

(11) Jones, G., II; Rahman, M. A. *Optics Commun.* **1993**, *97*, 140.

(12) (a) Chu, D. Y.; Thomas, J. K. In *Photochemistry and Photophysics*; Rabek, J. F., Ed.; CRC Press: Boca Raton, FL, 1991; Vol. III, Chapter 2. (b) Ghiggino, K. P.; Tan, K. L. In *Polymer Photophysics*; Phillips, D., Ed.; Chapman and Hall: New York, 1985. (c) Dubin, P. L.; Strauss, U. P. In *Polyelectrolytes*; Selegny, E., Ed.; D. Reidel Publishing Co.: Dordrecht, Holland, 1975; p 3. (d) Leyte, J. C.; Mandel, M. J. *Polym. Sci., Part A−1* **1964**, *2*, 1879.

(13) (a) Morishima, Y. *Adv. Polym. Sci.* **1992**, *104*, 51. (b) Katchalsky, A.; Spitnik, P. *J. Polym. Sci.* **1947**, *2*, 433. (c) Mandel, M.; Leyte, J. C.; Stadhonder, M. G. *J. Phys. Chem.* **1967**, *71*, 603. (d) Mandel, M. *Eur. Polym. J.* **1970**, *6*, 807. (e) Mandel, M.; Stork, W. H. *J. Biophys. Chem.* **1974**, *2*, 127.

(14) (a) Jones, G., II; Oh, C. *J. Phys. Chem.* **1994**, *98*, 2367. (b) Stramel, R. D.; Webber, S. E.; Rodgers, M. A. J. *J. Phys. Chem.* **1991**, *95*, 960. (c) Chu, D. Y.; Thomas, J. K. *Macromolecules* **1987**, *20*, 2133. (d) Wang, Y.; Morawetz, H. *Macromolecules* **1986**, *19*, 1925. (e) Guillet, J. E.; Rendall, W. A. *Macromolecules* **1986**, *19*, 224. (f) Park, J. W.; Park, Y. H. *Bull. Korean Chem. Soc.* **1986**, *7*, 137. (g) Tan, K. L.; Trealor, F. E. *Chem. Phys. Lett.* **1980**, *73*, 234.

(15) Jager, J.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **1984**, *106*, 3331; *J. Org. Chem.* **1985**, *50*, 1474.

(16) Barone, G.; Crescenzi, V.; Liquori, A. M.; Quadrifoglio, F. *J. Phys. Chem.* **1967**, *71*, 2341.

(17) Schimitschek, E. J.; Trias, J. A.; Taylor, M.; Celto, J. E. *IEEE J. Quantum Electron.* **1973**, *QE9*, 781.

(18) Chu, D. Y.; Thomas, J. K. *Macromolecules* **1989**, *22*, 1165.

(19) Flory, P. J. *J. Am. Chem. Soc.* **1943**, *65*, 372.

(20) Katchalsky, A.; Eisenberg, H. *J. Polym. Sci.* **1951**, *6*, 145.

(21) Machida, M.; Ushijima, N.; Takahashi, T.; Kanaoka, Y. *Chem. Pharm. Bull.* **1977**, *25*, 1289.

(22) Chuang, H. Ph.D. Thesis, Boston University, 1987.

(23) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983; Chapter 5.

(24) Busselle, F. J.; Haig, N. D.; Lewis, C. *Chem. Phys. Lett.* **1980**, *72*, 533.

(25) (a) Reference 23, Chapter 3. (b) Lakowicz, J. R.; Laczko, G.; Cherek, H. *Biophys. J.* **1984**, *46*, 463. (c) Gratton, E.; Limkerman, M.; Lakowicz, J. R.; Maliwal, B. P.; Cherek, H.; Liczko, G. *Biophys. J.* **1984**, *46*, 479.

(26) (a) Jones, G., II; Malba, V.; Poliakoff, E. *Photochem. Photobiol.* **1985**, *42*, 451. (b) Jones, G., II; Haney, W. A. *J. Phys. Chem.* **1986**, *90*, 5410.

(27) Drexhage, K. H. In *Topics in Applied Physics*, Volume 1: Dye Lasers, 2nd ed., Schäfer, F. P., Ed.; Springer-Verlag: Berlin, 1977; p 163.

(28) Maroncelli, M.; Fleming, G. R. *J. Chem. Phys.* **1987**, *86*, 6221.

(29) (a) Reference 23, Chapter 7. (b) Lippert, V. E. *Z. Electrochem.* **1957**, *61*, 962. (c) Mataga, N.; Kaifu, Y.; Koizumi, M. *Bull. Chem. Soc. Jpn.* **1956**, *29*, 465.

(30) (a) Arbeloa, T. L.; Arbeloa, F. L.; Tapia, M. J.; Arbeloa, I. L. *J. Phys. Chem.* **1993**, *97*, 4704. (b) Masilamani, V.; Chandrasekar, V.; Sivaram, B. M.; Sivasankar, b.; Natarajan, S. *Opt. Commun.* **1986**, *59*, 203.

(31) (a) Yip, R. W.; Wen, Y. X. *J. Photochem. Photobiol., A: Chem.* **1990**, *54*, 263. (b) Taylor, A. G.; Bowman, W. G.; Jones, A. C.; Guo, C.; Phillips, D. *Chem. Phys. Lett.* **1988**, *145*, 71.

(32) Rettig, W.; Klock, A. *Can. J. Chem.* **1985**, *63*, 1649.

(33) Kutty, A. P. G.; Venkateswaran, S.; Vaidya, S. N.; Kartha, V. B. *J. Phys. Chem.* **1993**, *97*, 7132.

(34) Ramalingam, A.; Palanisamy, P. K.; Masilamani, V.; Sivaram, B. M. J. *J. Photochem. Photobiol., A: Chem.* **1989**, *49*, 89.

(35) Yip, R. W.; Wen, Y.-X.; Szabo, A. G. *J. Phys. Chem.* **1993**, *97*, 10458.

(36) Van Gompel, J. A.; Schuster, G. B. *J. Phys. Chem.* **1989**, *93*, 1292.

(37) (a) Maroncelli, M.; Fee, R. S.; Chapman, C. F.; Fleming, G. R. *J. Phys. Chem.* **1991**, *95*, 1012. (b) Maroncelli, M.; MacInnis, J.; Fleming, G. R. *Science* **1989**, *243*, 1674. (c) Maroncelli, M.; Kumar, V. P.; Papazyan, A. *J. Phys. Chem.* **1993**, *97*, 13. (d) Moog, R. S.; Bankert, D. L.; Maroncelli, M. *J. Phys. Chem.* **1993**, *97*, 1496.

(38) Agmon, N. *J. Phys. Chem.* **1990**, *94*, 2959.

(39) Abdel-Mottaleb, M. S. A.; Antonious, M. S.; Abo-Aly, M. M.; Ismaiel, L. F. M.; El-Sayed, B. A.; Sherief, A. M. K. *J. Photochem. Photobiol., A: Chem.* **1989**, *50*, 259.

(40) Kamlet, M. J.; Taft, R. W. *J. Am. Chem. Soc.* **1977**, *99*, 6027, 8325.

(41) Taft, R. W.; Kamlet, M. J. *J. Am. Chem. Soc.* **1976**, *98*, 2886.

(42) Chapman, C. F.; Maroncelli, M. *J. Phys. Chem.* **1991**, *95*, 9095.

(43) The broad triplet transient for **1**[44] can be more readily detected in modest yield on flash lamp excitation using a 22 cm absorption cell.[6]

(44) Dempster, D. N.; Morrow, T.; Quinn, M. F. *J. Photochem.* **1973**, *2*, 329.