Polymerization and Conformational Transition of Poly(methacrylic Acid) Probed by Electronic Spectroscopy of Aminoacridines

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ABSTRACT: 9-Aminoacridinium vinyl monomers with intramolecular charge transfer (ICT) state were studied as molecular probes for copolymerization with methacrylic acid. The fading of the dye color after its addition to the poly(methacrylic acid) (PMA) chain leads to sweeping spectral changes from a broad absorption band in the red region before the polymerization to structured absorption and emission bands in the blue region after labeling. Also, the dyes bound lost their multiexponential decay behavior due to the ICT process and have only a single lifetime of the locally excited state of the aminoacridinium fluorophore. The conformational transition of PMA is sharply determined at pH = 5.2 by time-resolved fluorescence anisotropy. This probe technique also indicates strong polymer segments interactions prior to the critical transition of the chain opening with increases of the pH of the aqueous PMA solution.

Introduction

Spectroscopic methods based on molecular photophysical properties have been used to monitor polymerization processes[1−5] and to study the conformational transition of polymer in solution such as poly(methacrylic acid) (PMA).[6−10] However, a significant change in fluorescence during the polymerization process is required for a proper application of molecular probes. Pyrene acrylic and its methyl ester[2] and compounds with intramolecular charge transfer (ICT) dynamics, such as [*p*-(*N*,*N*-diakylamino)-benzylidene] malononitriles,[1] have been used as fluorescent probes for the cited purposes. When a fluorescent probe is hosted or labeled in PMA, the change of its emission with pH can indicate the conformational transition of PMA with pH between 4 and 6, depending on the probe used and the property measured. At low pH, the conformation of PMA is a compact coil of uncharged carboxylate units and hydrophobic cluster of the methyl groups. When the pH increases, the carboxylates units become charged, and the electrostatic repulsion and solvation of the clusters drives the polymer chain to an elongated ionic form.[6−7] Pseudoisocyanine (PIC^+) attached to PMA in its compact conformation changes its quantum yield by a 600-fold and fluorescence lifetime varies from a few picoseconds to 2.7 ns when the pH goes across the entire region of the conformational transition of the polymer in aqueous solution.[9]

Time-resolved fluorescence anisotropy (TRFA) is an important spectroscopic method to measure the rotational diffusion of molecular probes in several media.[11−23] For instance, polyamines labeled with fluorescein were target systems for TRFA measurements, and the study of the dynamics of the polyamine chain have indicated a higher conformational flexibility in polylysine when compared to poly(allylamine).[19] Because of its high selectiveness, TRFA has been used to estimate the hydrodynamic properties of polymers and dendrimers[22,23] as well as the polymer chain dynamics and segment mobility in solution.[11−13,16] TRFA is capable of indicating the change in hydrophobic/hydrophilic balance of acrylamide copolymer with temperature of the solution,[24] and also it is a proper technique to search for interpolymer complexation of binary polymer solution where one of the chains is labeling with a fluorescent probe.[25]

In the study of the conformational transition of PMA, there are two classical reports using TRFA method.[11,12] However, with dansyl[11] or with 1-vinylnaphthalene and acenaphthylene[12] labeled to PMA, the presence of multiexponential fluorescence decay or a monoexponential behavior restricted to a limited pH range were observed. These facts can give additional complexity in the fluorescence anisotropy analysis.

In this work, 9-aminoacridinium derivatives having ICT character[26,27] are used as new molecular probes for studing copolymerization and conformational transition of PMA. However, for the later purpose, after dye labeling, the lack of any significant change in its band shape and intensity of the absorption and emission spectra, or even, in its lifetime and emission quantum yield with solution pH in the range of 2−9, lead us to study this process using TRFA.

Experimental Section

Synthesis and Characterization of Acridine Derivatives Attached in PMA. The 9-aminoacridine derivatives were prepared and characterized as described in a recent publication.[26,27] The copolymers were synthesized using benzyl peroxide (BP, 5 mg/mL) thermal-initiated copolymerization of methacrylic acid (MA, 1.0 g/mL, previously distilled under reduced pressure) with 9-aminoacridinium derivatives (1% in weight), all reactants in ethanol solution (under nitrogen atmosphere) at 70 °C, resulting in the copolymers as shown in Figure 1. These materials were purified by multiple precipitations from methanol on addition of ethyl acetate. The viscosity-averaged molecular weights were determined by capillary viscosimetry from the intrinsic viscosity in 0.002 M of HCl solution (T = 298 K), and using the Mark−Houwink equation with known parameters for PMA.[28]

Instrumentation. Absorption measurements were performed on a Cary 5G-Varian spectrophotometer, and the corrected steady-state fluorescence spectra were recorded on a CD-900 Edinburgh spectrofluorimeter at 298 K. The pH of the aqueous solutions was measurement on a Micronal pH meter. Dilute HCl and NaOH aqueous solutions were used to control pH. Fluorescence decays in solution were measured by time-correlated single photon counting technique using a CD-900 Edinburgh spectrometer equipped with Glan-Thompson polarizers and a Peltier-cooled PMT (Hamamatsu R955) or a Hamamatsu R3809U-50 MCP as a photon detector. The

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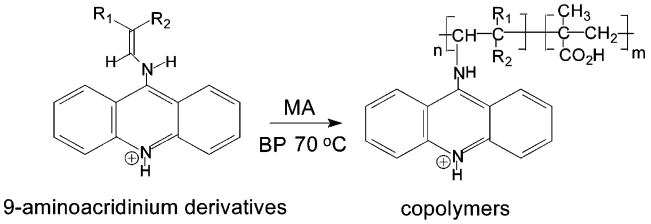

Figure 1. Schemes of the 9-aminoacridinium derivatives and of the copolymerization route with methacrylic acid (MA) using benzyl peroxide (PB). ($m \gg n$). Compound I, $R_1 = R_2 =$ COOEt; compound II, $R_1 =$ CN, $R_2 =$ COOEt; compound III, $R_1 = R_2 =$ CN.

light pulse was provided by frequency doubling the 200-fs laser pulse of Mira 900 Ti−Sapphire laser pumped by a Verdi 5 W Coherent. The laser-pulsed frequency was reduced by using a Conoptics pulse-picker system. The fluorescence decays were analyzed by a reconvolution procedure and using global analysis methods.[29] The time-dependent fluorescence anisotropy, $r(t)$, is calculated by

$$r(t) = \frac{I_{VV}(t) - I_{VH}(t)}{I_{VV}(t) + 2I_{VH}(t)} \quad (1)$$

where the subscripts VV and VH are the polarization intensity data in parallel and perpendicular measurements, respectively. By consideration of an isotropic molecular rotor with a single rotational relaxation time τ_r, the fluorescence intensities are given by

$$I_{VV}(t) = \exp(-t/\tau_0)[1 + 2r_0 \exp(-t/\tau_r)] \quad (2)$$

$$I_{VH}(t) = \exp(-t/\tau_0)[1 - r_0 \exp(-t/\tau_r)] \quad (3)$$

In the equations above, r_0 is the initial value of anisotropy and τ_0 the fluorescence lifetime of the probe. The value of τ_0 is determined from the decay taken at magic angle configuration (54.7° from vertical polarizer) and analyzed by single-exponential fit. Equations 2 and 3 were used in global fitting with linked parameters r_0 and τ_r to determine the best global values.

Results and Discussion

9-Aminoacridine Probes for Copolymerization. The target dyes used to monitor the copolymerization are very interesting probes because both of their electronic absorption and emission vary with reaction time. The change in the absorption of the ICT band of 9-aminoacridinium monomers with maxima at 456, 470, and 490 nm for the compounds I, II, and III, respectively, can be used to report the copolymerization process in solution. The bleaching of this strong ICT band in the red together with the rising of a structured UV band, as illustrated in Figure 2 with UV maxima at $\lambda = 381$ and 401 nm for probe II, is ascribed to a quantitative addition of the vinyl dye to the poly(methacrylic acid) chain during the copolymerization process. This assumption is also supported by the close similarity of the UV band shape observed with the standard absorption spectrum of 9-aminoacridinium and, moreover, from the comparison of fluorescence lifetime (see next section). It is worthwhile to mention that these changes are visible to the eye, and the solution color goes from a dark orange to a pale yellow after copolymerization. In addition to the changes in absorption upon incorporation of the dye in the polymer, a concurrent intensity increase of the fluorescence is observed upon excitation at 400 nm. The increase of the structured emission band in the region of 425 nm, due to the loss of conjugation of the original dye with copolymerization, is illustrated in Figure 2B for case of dye II.

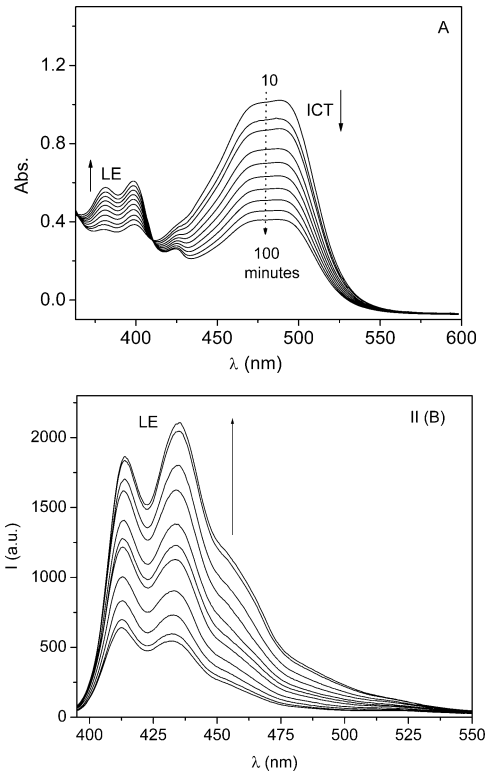

Figure 2. (A) Changes in the absorption spectrum of compound II with polymerization time. (B) Increase of the fluorescence emission band intensity with reaction time of polymerization from 10 to 100 minutes. $\lambda_{exc} = 390$ nm.

When the vinyl double bond of the dye-monomer is lost in the chain addition process, the charge conjugation from the extended π-system is precluded, and thus the optical ICT transition is no longer possible for the dye bound in PMA. The presence of a clear isosbestic point at 409 nm (see Figure 2A) gives additional support to the proposed mechanism in which the dye stay in two forms, the free and bound species. The UV−visible absorption changes of the aminoacridines are very similar to those observed with auramine vinyl probes upon polymerization with acrylic monomers,[30] although the emission properties of free and bound dyes are completely distinct.

The kinetics of the dye addition to the polymer chain can be followed by absorption measurements. By use of a classical Gugenheim method,[31] the first-order rate constant of free dye fading or its incorporation in the polymer chain can be determined. A typical plot is given in Figure 3, and from it the average first-order rate constant calculated is $1.4 \pm 0.2 \times 10^{-2}$ min^{-1}. By consideration that the initial concentration of the dye is 7×10^{-5} M, this rate constant is converted into a second-order value of 3.3 M^{-1} s^{-1}. Such a value is much lower than the propagation rate constant of methacrylic acid polymerization reported in the literature[32] (670 M^{-1} s^{-1} at 296 K at pH = 8.0), which indicates that the PMA polymer chain is labeled with a low percent of the dye probes used. The viscosity-averaged molecular weights of the copolymers I, II, and III were determined as 146, 155, and 148×10^3 g/mol, respectively. The average number of bound dyes per polymer chain was evaluated in the range of 4−5, considering the dye incorporation of 1% in weight. This would give 350 up to 440 methacrylic acid monomers for each dye molecule in the polymer material.

Photophysics of the Aminoacridines Bound to PMA. When the new acridinic dyes are chemically bound to PMA, they present similar photophysical behavior as that of the precursor 9-aminoacridinium with structured absorption and emission

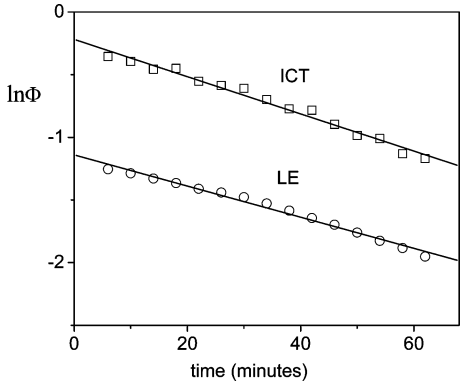

Figure 3. Data treatment of the change in absorbance of dye II measured at the ICT and LE bands according to the classical Gugenheim method.[31] The change in absorbance between time t and $t + \Delta t$, where Δt is a constant increment larger than the half-life of apparent first-order reaction, is represented by Φ.

Table 1. Photophysical Parameters of 9-Aminoacridinium Derivatives Bound to PMA

system	solvent	λ_{abs} (nm)	λ_{em} (nm)	τ (ns)	χ^2
I	methanol	381, 401, 423	430, 454, 483	14.1	1.048
	pH = 2	383, 402, 424	435, 456, 483	16.5	1.060
	pH = 9	381, 402, 425	435, 455, 484	15.3	1.030
II	methanol	381, 401, 423	429, 454, 481	13.8	1.026
	pH = 2	381, 401, 424	430, 455, 484	15.5	1.071
	pH = 9	382, 401, 422	430, 454, 382	15.4	1.035
III	methanol	381, 401, 423	429, 454, 481	13.8	1.026
	pH = 2	382, 402, 424	432, 455, 484	15.1	1.130
	pH = 9	383, 402, 423	434, 454, 483	14.8	1.029

bands. The maxima of these bands in different media are listed in Table 1. Typical absorption and emission spectra of the dye-PMA systems are given in Figure 4 for the copolymer I in aqueous solution at pH = 2 and in methanolic solution as well. In addition, time-resolved fluorescence decays of all dye−PMA samples were of monoexponential character, and the measured fluorescence lifetimes (see values in Table 1) are close to the lifetime of the precursor cation dye 9-aminoacridinium that is about 15 ns.[33]

However, contrasting with some fluorescent dyes that when labeled or hosted in PMA show great changes in the photophysical parameters along a pH titration curve of PMA, 9-aminoacridine copolymers do not have such behavior in the range of pH from 2 to 9, which includes the region of the conformational transition of PMA. The profile and intensity of the absorption and emission bands of the dye−PMA systems are practically constant with pH. Also, the fluorescence lifetime of the bound dye changes less than 8% and the decay is monoexponential (see Table 1 for details). It should be mentioned that the dye stays in a monoprotonated form in the pH range of 2−9, because the free base is only formed at higher pH of about 10, and the diprotonated form appears only at pH lower than 1.[34]

TRFA. The fluorescence decays of the 9-aminoacridine derivatives exhibit a monoexponential behavior with pH in the range of 2−9, which would simplify the application of fluorescence anisotropy as a tool to study the dye−PMA structure and dynamics in solution. Typical fluorescence decays observed with parallel (VV) and crossed (VH) excitation to emission polarization are illustrated in Figure 5 for copolymer III in aqueous solution at pH = 2.

By use of eqs 2 and 3 in global analysis, the rotational relaxation time (τ_r) of the probe in all three dye-PMA systems in an extended range of solution pH was determined. The plot of τ_r as a function of pH of the PMA solution is given in Figure 6. There is a common behavior of τ_r for the three dye−PMA systems, an initial increase from 9 ns at pH 2 to about 11−15 ns at pH 4, followed by a drop in a sigmoidal shape down to the value of 2 ns when in pH = 6, and then it remains practically constant up to pH = 9.

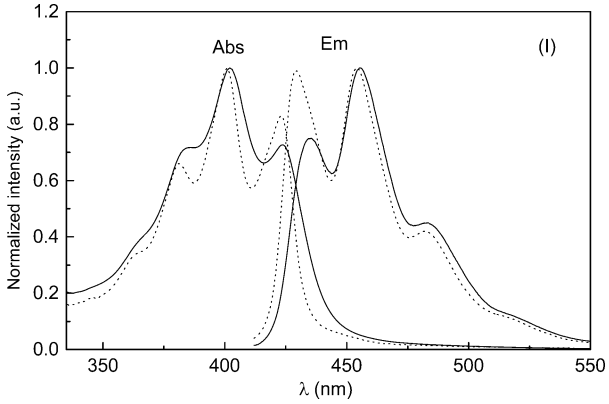

Figure 4. Normalized absorption and emission spectra (λ_{exc} = 390 nm) of the acridinic dye I bound to PMA in methanol (dotted line) and aqueous (pH = 2) solutions (solid line). A polymer concentration of 10 mg/mL was used.

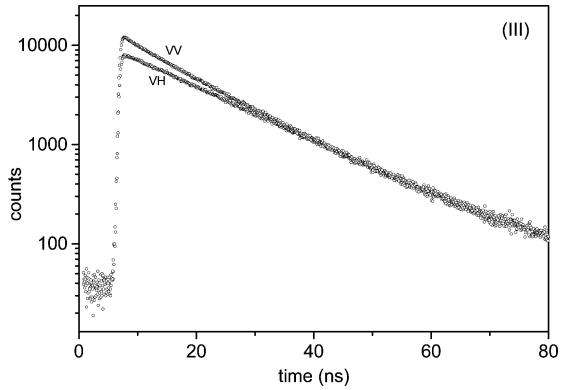

Figure 5. Fluorescence decays of 9-aminoacridinium in copolymer III in water solution (pH = 2) measured in two light polarization directions (VV and VH are vertical and horizontal polarization emissions with respect to the excitation in vertical polarization, respectively). λ_{exc} = 400 nm.

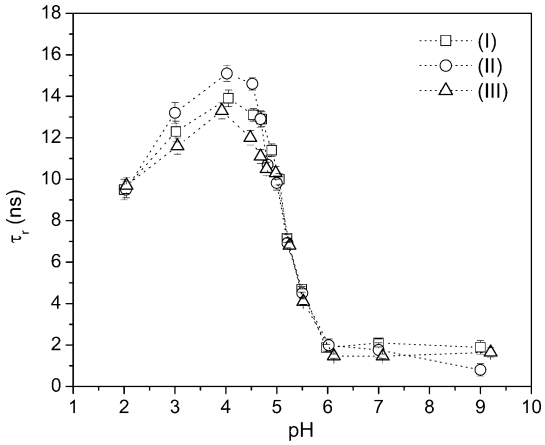

Figure 6. The rotational relaxation time of the 9-aminoacridinium probes bound to PMA as a function of the pH of the solution at 298 K. λ_{exc} = 400 nm and λ_{em} = 460 nm.

This change in the rotational relaxation time of the probe bound PMA is well illustrated by the time resolved anisotropy at different pHs in the case of copolymer III reported in Figure 7. Slow to fast depolarization is clearly recognized in the anisotropy behavior with increase of pH.

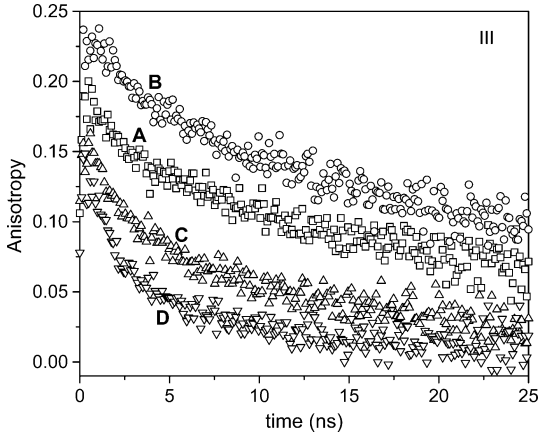

Figure 7. Anisotropy decays of 9-aminoacridinium (compound III) bound to PMA in different pH values. (A) pH = 2.0, (B) pH = 3.8, (C) pH = 5.5, (D) pH = 6.0. λ_{exc} = 400 nm and λ_{em} = 460 nm.

The dipole moments of ground and excited states of the first singlet transition of the 9-amino acridinium are nearly collinear with the short axis of the heterocycle,[35] and thus probe rotation around the amino bound will not give substantial fluorescence depolarization. Depolarization should occur mainly as a result of rotational diffusion of the probe and neighboring polymer segments in a cooperative mode producing bending and wobbling motions of the dye. This segment's interactions would explain the increase in the rotational relaxation time with the first change of pH as shown in Figure 6. At low pH, the PMA chain is a compact coil due to uncharged carboxylate groups and hydrophobic character of the methyl groups that may form clusters. An increase in pH produces charged carboxylates, which by interaction with polymer segments through hydrogen bonds in addition to the electrostatic pairing with the cationic dye can form a tiny and large cluster around the fluorescent probe, therefore explaining the initial increase in τ_r. However, further increase of the pH charges the polyelectrolyte even more, disrupts the hydrophobic cluster, and leads to a cooperative conformational transition of PMA. Such a critical point can be taken as the middle of the sigmoidal curve of τ_r as a function of pH. The value of critical pH from the data in Figure 6 is 5.2, which is in agreement with our previous results of PMA conformational transition.[30]

In the conformational transition region, all of these three dye−PMA copolymers have very similar behavior. Also, for pH larger than 6 where the polymer chain is completely open to solvent interaction, the rotational relaxation times have about the same value of τ_r = 1.7 ns in the three systems, and such a value approaches the average value found when the copolymers are dissolved in methanol to which τ_r = 1.25 ns (data not shown). The difference could be accounted by the lower viscosity of methanol (0.55 cP) when compared with the water viscosity (0.89 cP) at 298 K. Nevertheless, these values of τ_r in dye−PMA systems are much higher than the value observed for the free 9-aminoacridinium (precursor probe) in the same solvents (0.15 and 0.22 ns in methanol and water, respectively[18]). This comparison would indicate that, in dye−PMA systems studied, some kind of viscous drag effect of the neigboring dye polymer segments is slowing down the rotational diffusion of the bound dye even when the polymer conformation is an open solvated chain as occurs at high pH.

The model of exponential relaxation of the anisotropy of the fluorescent dye bound to the PMA chain is an approximation, and therefore τ_r should be seen as an average value of the rotational relaxation motions of the dye in the polymer−solvent environment. Nevetheless, any attempt to use the classical Stokes−Einstein equation, $\tau_r = \eta V_h/kT$, where η and V_h are the local viscosity and the hydrodynamic volume of the molecular rotor, is a problem because neither of these two parameters is well defined or even constant along the change in pH, and also they may fluctuate quite significantly along just a single polymer chain. However, in the high pH region where the polymer is an open chain well solvated by water (0.89 cP at 298 K), the value found of τ_r = 1.7 ns will predict, by simple calculation using a spherical rotor, a molecular radius of 12 Å. This value is about twice the molecular diameter of the bound dye, and, therefore, the depolarization motion should involve the additional viscous friction of an elongated chain segment with size not less than that of four methacrylic monomers.

Conclusions

Aminoacridine vinyl monomers with electron-withdrawing groups are outstanding spectroscopic probes for copolymerization process with acrylic monomers. The spectral changes in absorption and emission during copolymerization due to the loss of the ICT character are well defined, allowing an easy and practical tool to follow the polymerization process. On the other hand, fluorescence anisotropy of the copolymers is a suitable method to study polymer dynamics and structural changes in solution, as demostrated from the study of pH induced conformational transition in dye−PMA systems.

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