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Time-Resolved Fluorescence Study of Chain Dynamics. 1. Poly(methacrylic acid) in Dilute Water Solutions

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ABSTRACT: Fluorescence decay and polarization of dansyl-labeled poly(methacrylic acid) (PMA) and poly(acrylic acid) (PAA) have been studied as a function of pH. The decay measurements have shown that, at low pH, PMA chains form highly compact hydrophobic clusters, which are joined by short extended polymer chains. During the transition from the compact to the expanded form, the size of the clusters decreases up to a limit, beyond which the clusters disintegrate completely to an expanded polymer chain. The effect of PMA molar mass and ionic strength on this process was investigated.

Introduction

The solution as well as some other properties of poly(methacrylic acid) (PMA) as a function of the degree of ionization, α, has been the subject of a number of studies. Viscometric measurements[1–3] of PMA and PAA along with the calorimetric ones[4,5] have led to the prevalent view that PMA, in contrast with PAA, exists at low α (at low pH) in a highly compact, hypercoiled conformation. The main cause of the compact form of PMA is seen in the hydrophobic interactions of the methyl groups, although a certain effect of intramolecular hydrogen bonds between the ionized and un-ionized carboxyl groups should not be excluded. In most of the studies the conformational transition of PMA from the compact to the expanded form, brought about by repulsive Coulombic interactions of the ionized carboxyl groups, is assumed to be highly cooperative.[3] Some of the studies, however, suggest a progressive rather than a cooperative change.

The conformational transition of PMA has been recently intensively monitored by means of fluorescence measurements.[7–10] The results of some of these measurements are interpreted by using a model of the PMA chain, which is composed of compact clusters mutually interlinked with flexible extended parts of a polymer chain.[8] To study the equilibrium and the kinetics of the conformational transitions of PMA, dansyl (5-(dimethylamino)-1-naphthalenesulfonate) covalently attached to a polymer chain[10] has been recently employed as a fluorescence label. In these measurements the sensitivity of dansyl fluorophore to the polarity of the microenvironment has been utilized. Similarly, as in the case of copolymers of maleic acid with vinyl ethers,[11,12] the change in the environment of the dansyl label from the hydrophobic interior of the PMA coil to the aqueous medium, due to the conformational changes in the PMA, is accompanied by a decrease in the fluorescence intensity and a red shift in the emission maximum. The sensitivity of the dansyl label to the polarity of the microenvironment has been also successfully employed in studying the association equilibrium and kinetics of formation of polymer complexes[13,14] and in describing the properties of lipid bilayer systems.[15]

In order to find the explanation for some phenomena observed in static fluorescence measurements, we used of time-resolved fluorescence to study dansyl labels covalently bonded to PMA and PAA as a function of pH. Fluorescence depolarization provided information on the dynamics of the polymer chains of PMA and PAA as a function of their degree of ionization as well as some ideas of the structure of the compact and the expanded forms of PMA.

Experimental Section

Materials. The dansyl-labeled PMA (Dan-PMA), dansyl-labeled PAA (Dan-PAA), and low molecular weight analogues 1-(dimethylamino)-5-[(aminoethyl)sulfonamido]naphthalene (Dan-A) and 1-(dimethylamino)-5[((methacryloylamino)ethyl)sulfonamido]naphthalene used in this study were prepared as previously described.[11,13,14] Table I lists the characteristics of all the polymers employed.

Fluorescence Measurements. The time-dependent fluorescence polarization anisotropy, $r(t)$, is defined by[16]

$$r(t) = \frac{I_{\parallel}(t) + I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)} \tag{1}$$

where $I_{\parallel}(t)$ and $I_{\perp}(t)$ are the time-dependent intensities of fluorescence parallel and perpendicular to the polarization plane of the exciting radiation.

To measure fluorescence with subnanosecond resolution, the method of pulse spectrofluorometry with time-correlated photon counting was employed. The measurements were carried out on an Edinburgh Instruments Model 288 T spectrofluorometer in a multiplex regime.[17]

The fluorescence decay curves were fitted by single- or double-exponential functions. The parameters describing the experimental curves were found in the particular cases using the nonlinear least-squares method so that the statistical test yielded the best correlation parameters (standard χ^2). Fluorescence lifetimes were calculated partly from the fluorescence decay measured at the magic angle ($F_m(t)$) and partly from the sum ($S(t) = F(t)_{\parallel} + 2F(t)_{\perp}$). In calculations from $F_m(t)$ and $S(t)$ deconvolution was performed. A comparison of the results thus obtained provided an estimate of accuracy of these independent measurements.

The fluorescence lifetimes were calculated from the experimental curves having typically 10^4 counts at the maximum. In a few cases, the data with a relatively lower number of counts at the maximum had to be used due to very low quantum yield. The reliability of data is in all cases characterized by the standard deviation listed with the data in the tables. For rotational correlation times data were used with a number of counts at a maximum higher than 10^5 (especially for distribution calculations and double-exponential anisotropy fits).

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Table I
Characterization of Polymers Used in This Study

polym designatn	$[\eta]$, mL g^{-1}	$M_v \times 10^{-4}$, g mol^{-1}	dansyl content, mol %
Dan-PMA1	44	2.7	0.9
Dan-PMA2	149	29.5	1.0
Dan-PMA3	151	30.4	0.2
Dan-PPA	192	28.6	1.8

Computation of the "Distribution" of Fluorescence Lifetimes. Due to the fact that the fluorescence labels are attached at random to the different polymer chains, we attempted to evaluate the fluorescence data considering a distribution of fluorescence lifetimes resulting from the fluorophore interactions with different microenvironments. In the case of two sufficiently different fluorescence lifetimes, the procedure is as follows.[17] First, fluorescence lifetime estimates are derived from the double-exponential fit. Small, but systematical, departures of the experimental data from the regression curve in the short time region are largely due to the distribution of the shorter lifetimes τ_1, whereas in the longer time region, the distribution of the longer lifetimes τ_2 is the most important source of systematical departures. Second, the fit is refined iteratively by varying the parameters of the Gaussian distribution of the short-lifetime component (i.e., the value in the maximum, τ_{1m}, and the distribution width, $\Delta\tau_1$) while keeping the τ_2 value constant; the initial τ_1 value is used as the starting approximation for τ_{1m}. After this refinement, the Gaussian distribution of τ_{2m}, $\Delta\tau_2$, is sought. If τ_{2m} differs from τ_2 by more than $\Delta\tau_2$, the whole cycle must be repeated using τ_{1m} and τ_{2m} as the input values. Though this method does not permit simultaneous and independent determination of parameters of a two-peak distribution, it the τ_{1m} and τ_{2m} values differ significantly, the parts of the fluorescence decay curves in the region of short or long times are markedly influenced by only one peak of the distribution curve, and the method offers reliable data within the assumed analytical form of the peak. The method could be used only for high-quality data with about 10^5 counts at the maximum.

In calculating the rotational correlation times, we have employed eq 1, the individual components of fluorescence decay being calculated by deconvolution.

Results

Fluorescence Decay. Equilibrium measurements of the dependence of the fluorescence intensity of the dansyl label covalently bonded to PMA and PAA on the pH of the water solution reported in ref 10 showed that maximum fluorescence occurs at pH 4.

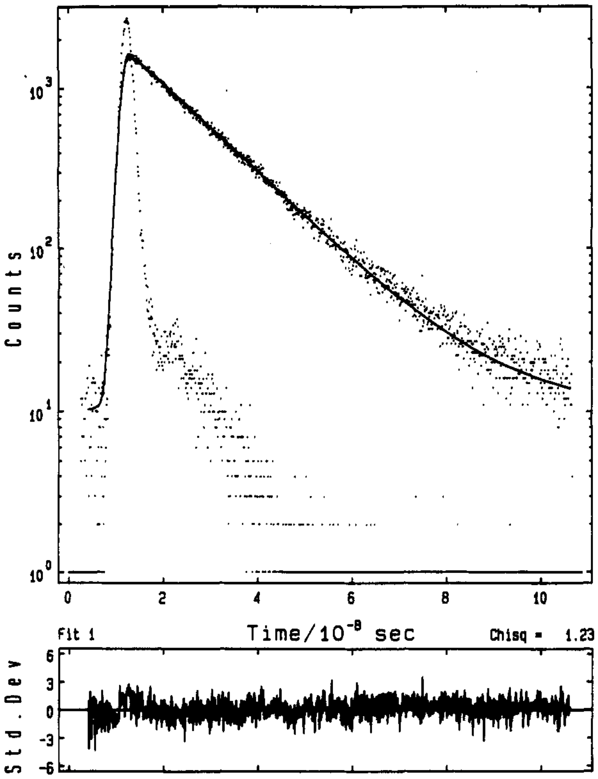

Figure 1. Fluorescence decay of the dansyl label attached to Dan-PMA1 at pH 3.92 (for lifetimes, see Table II): points, experimental data; solid line, double-exponential fit.

Table II
Lifetimes τ_1^F and τ_2^F and a Relative Amplitude A_1^{rel} for a Fast Process Calculated from Decay Fluorescence Measurements

sample	solvent	τ_1^F, ns	SD	τ_2^F, ns	SD	A_1^{rel}, %
Dan-PMA1	water (pH 3.92)	7.85	0.41	21.94	0.04	8
Dan-PAA	water (pH 3.88)	3.35	0.14	10.15	0.10	34
Dan-MA[a]	water (pH 3.88)	3.67	0.35			100
Dan-A[b]	methanol			11.75	0.05	
Dan-A[b]	paraffin oil			14.77	0.04	
Dan-PMA1	1,4-dioxane/50 vol % water	5.57	0.39	14.13	0.05	11
Dan-PMA1	1,4-dioxane/50 vol % methanol			18.13	0.02	

[a] Dan-MA = 1-(dimethylamino)-5-(β-methacryloylaminoethyl)sulfonamidonaphthalene. [b] Dan-A = 1-(dimethylamino)-5-(β-aminoethyl)sulfonamidonaphthalene.

The measurement of the time dependence of the fluorescence intensity of the dansyl label attached to PMA and PAA at pH 3.88 could be reliably fitted with a double-exponential function (see, e.g., the dependence for Dan-PMA1 in Figure 1). The parameters of this function provide two lifetimes τ_1^F and τ_2^F, which, together with a relative amplitude of the faster component, A_1^{rel}, are listed in Table II. The values of τ_1^F and τ_2^F for Dan-PMA1 are more than twice as high as those for Dan-PAA. The measurements of a low molecular analogue Dan-MA in water at pH 3.88, as well as that of Dan-A in methanol and paraffin oil (see Figure 2), were reliably fitted with a single-exponential function. The values of the lifetime obtained from the single-exponential fit differ, however, significantly as seen from Table II. The τ^F value at pH 3.88 is the lowest and within experimental error is identical with the value τ_1^F for Dan-PAA in water at pH 3.88, while the values τ^F obtained by measurements in methanol and paraffin oil are between the values τ_2^F for Dan-PAA and Dan-PMA1. A similar dependence of τ^F on solvent polarity has been observed by Li et al.[18] for 1-(dimethylamino)-5-naphthalenesulfonamide (Dan-AM) and ascribed to different emitting states in polar and nonpolar solvents. The high sensitivity of τ^F values of the dansyl label to polarity of the microenvironment of PMA (water environment) has been confirmed by measurements of Dan-PMA1 in mixtures of 1,4-dioxane/50 vol % water and 1,4-dioxane/50 vol % methanol. In the 1,4-dioxane/water mixture two lifetimes were found, whereas in 1,4-dioxane/methanol only a longer lifetime indicating a smaller polarity of the microenvironment of the polymer chain (see Table II) was observed.

The values of a shorter, τ_1^F, and a longer, τ_2^F, lifetime together with values A_1^{rel} as a function of pH are plotted for Dan-PMA1 and Dan-PAA in Figure 3 and those for Dan-PMA2, Dan-PMA3, and Dan-PAA in Figure 4. In the acid region up to pH 5–6 (depending on the ionic strength of the solution) the values τ_2^F for all the Dan-PMA studied are in the range of 18–22 ns, and their fraction

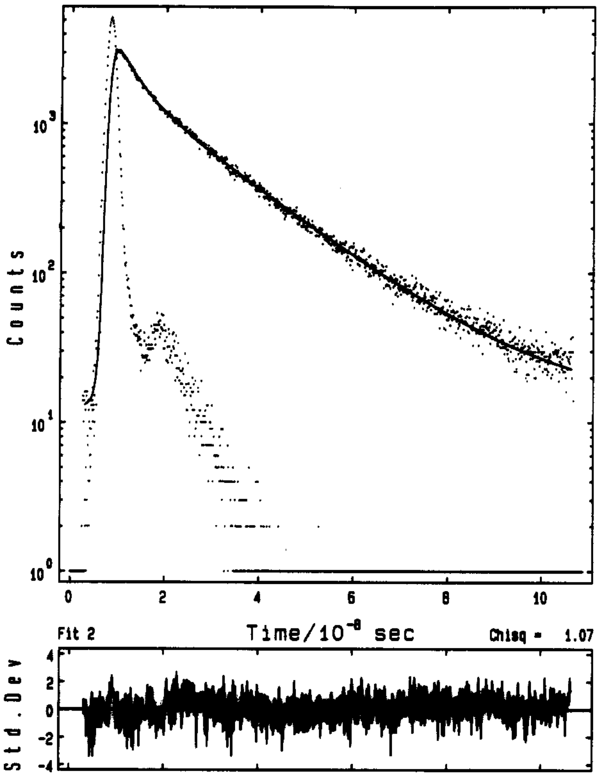

Figure 2. Fluorescence decay of the Dan-A in paraffin oil (for lifetimes, see Table II): points, experimental data; solid line, single-exponential fit.

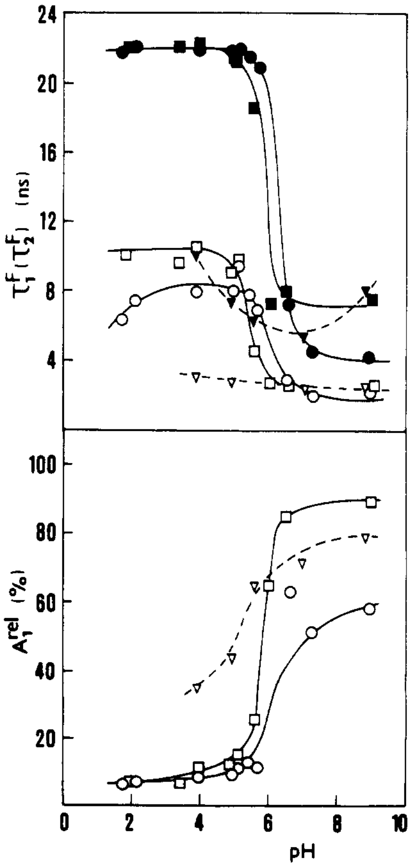

Figure 3. Dependence of the fluorescence lifetimes (τ_1^F, τ_2^F) and relative amplitude of the fast process (A_1^{rel}) upon pH: empty points, fast process; filled points, slow process; Dan-PMA1, $I = 0.5$ (○); Dan-PMA1, $I = 0.5$ (□); Dan-PAA, $I = 0.05$ (▽).

is close to 90%. These high values of τ_2^F indicate that most of fluorescence labels are located in the highly non-

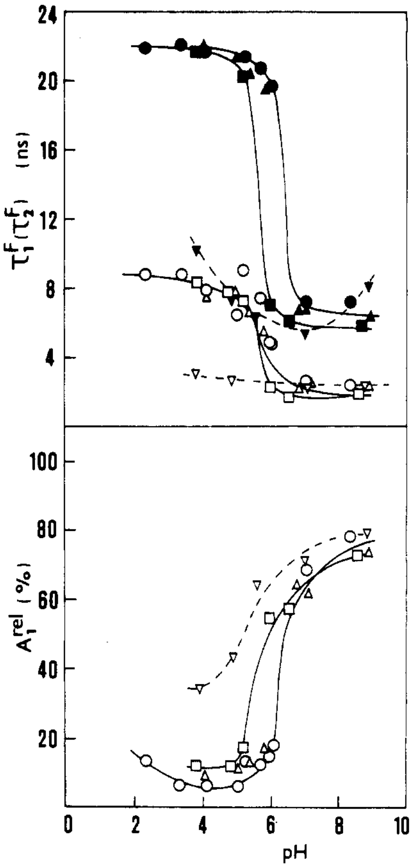

Figure 4. Dependence of the fluorescence lifetimes (τ_1^F, τ_2^F) and relative amplitude of the fast process (A_1^{rel}) upon pH: empty points, fast process; filled points, slow process; Dan-PMA2, $I = 0.05$ (○); Dan-PMA3, $I = 0.05$ (△); Dan-PMA3, $I = 0.5$ (□); Dan-PAA, $I = 0.05$ (▽).

polar microenvironment. Between pH 5.5 and 6.5 the values of τ_2^F drop precipitously for Dan-PMA1 at $I = 0.5$ and for Dan-PMA2 and Dan-PMA3 to the region of 6–8 ns, for Dan-PMA1 at $I = 0.05$ the τ_2^F value drops to 4 ns. Simultaneously the fraction of the shorter lifetimes rises steeply. These observed changes reflect a principal increase in the polarity of the microenvironment of the fluorescence label with increasing pH. In the case of Dan-PMA1 at $I = 0.5$ the value of τ_2^F at pH 9 approaches the value obtained for Dan-MA in water (see Table II). The shorter lifetime τ_1^F lies in the acid region in the range of 7–10 ns, and again at pH 5.5 or 6.5 (depending on the ionic strength of the solution) it drops to the region of 2–3 ns. Of quite a different character is the dependence of τ_1^F and τ_2^F on pH for Dan-PAA (see Figures 3 and 4, respectively). While τ_2^F passes in the pH range from 4 to 9 through a minimum at pH 7, its values varying between 6 and 10 ns, the value of the shorter lifetime τ_1^F changes very little (in the range 2.68–3.35 ns) in this pH interval. In the same way the fraction of the τ_1^F for Dan-PAA varies in a far narrower range (see, e.g., Figure 3).

Figure 5 shows the distribution of lifetimes for Dan-PMA2 at three pHs describing the respective dependence regions in Figure 4. The distribution of τ_2^F values with a maximum in the region of 22 ns, which indicates a highly nonpolar microenvironment of the label, is, like the distributions of lifetimes with maxima in the region of 3–10 ns depending on the pH, relatively broad. The distribution of τ_1^F values in the region of 2–3 ns, indicating a highly polar aqueous microenvironment of the label, is, on the contrary, very narrow. The width of distribution

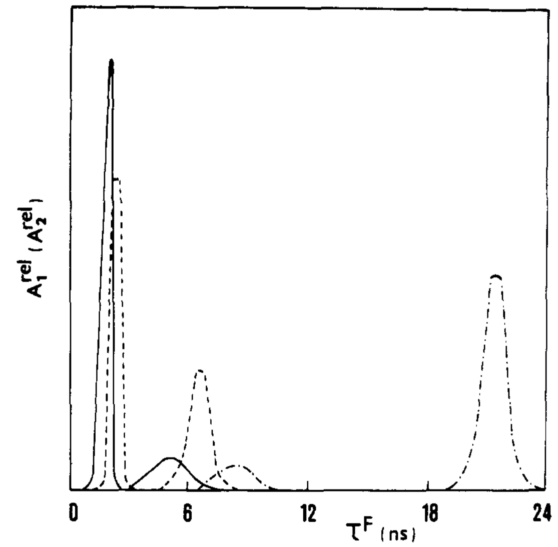

Figure 5. Distribution of fluorescence lifetimes τ^F for Dan-PMA2 at different pH: pH 8.7 (—); pH 6.0 (- - -); pH 3.88 (– · –).

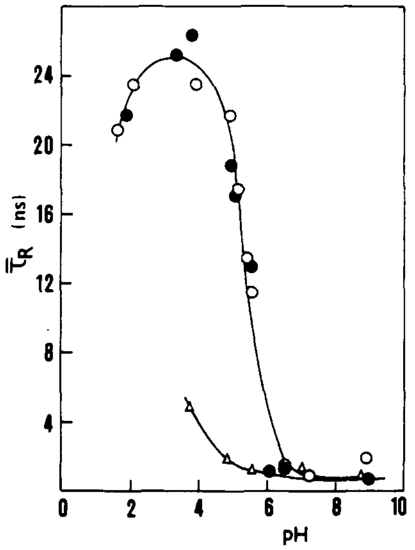

Figure 6. Dependence of the rotational correlation times ($\bar{\tau}_R$) upon pH: Dan-PMA1, $I = 0.5$ (○); Dan-PMA1, $I = 0.5$ (●); Dan-PAA, $I = 0.05$ (△).

of the τ_1^F and τ_2^F values at different pH values reflects the uniformity in the polarity of the microenvironment of the label.

Fluorescence Depolarization. From the measurements of fluorescence depolarization the values of rotational correlation times $\bar{\tau}_R$ were calculated by using the method described above. The measurement of the lifetime showed that in all the cases studied two "kinds" of labels, differing in the lifetimes, can be found in the system. Nevertheless, owing to the prevalent fraction of one component and the low accuracy of data at high and low pH values, we employed a single-exponential function to fit the decay of the fluorescence depolarization. The $\bar{\tau}_R$ values are thus the mean values of rotational correlation times corresponding to labels with different lifetimes.

The dependence of the $\bar{\tau}_R$ values for Dan-PMA1 and Dan-PAA on pH are plotted in Figure 6, and those for Dan-PMA2, Dan-PMA3, and, for comparison, also those for Dan-PAA, in Figure 7. Figure 6 shows that the $\bar{\tau}_R$ for Dan-PMA1 (independent of the ionic strength of solution) has a maximum in the pH range of 3.5–4.0. The value dropped precipitously by a factor 20 on passing to pH 6.5. Over the some range of pH, $\bar{\tau}_R$ for Dan-PAA decreases smoothly by a factor of 5. At pH > 6 the $\bar{\tau}_R$ values for Dan-PMA1 and Dan-PAA are practically identical, approaching that for Dan-MA ($\bar{\tau}_R = 0.8$). Of similar character are the dependencies of $\bar{\tau}_R$ for Dan-PMA2 and Dan-PMA3 in Figure 7. In this case, however, the $\bar{\tau}_R$ values depend slightly on the ionic strength of the solution. The difference between the maximum and the minimum value of $\bar{\tau}_R$ for solutions with $I = 0.5$ is still more pronounced than that in Figure 6. The observed changes of $\bar{\tau}_R$ for Dan-PMA as a function of pH obviously reflect the conformational transition of the polymer chain affecting the mobility of the fluorescence label. These average values of $\bar{\tau}_R$ do not allow a direct estimate of size of the individual intramolecular clusters. Consequently, we fitted the dependence of fluorescence anisotropy for Dan-PMA1 at $I = 0.5$ (see Figure 8) and several pH values with a double-exponential function reflecting two values of rotational correlation times that can be assigned to fluorescence labels situated in two microenvironments of different polarity (see Table III). The values for the shorter rotational correlation time τ_1^R are, within experimental error, practically pH independent, being very close to the value $\bar{\tau}_R$ for the low molar mass Dan-MA ($\bar{\tau}_R = 0.8$). The values for the longer correlation time τ_2^R depend on pH, similar to the $\bar{\tau}_R$ (see Figure 6), indicating that the dansyl labels are in this case firmly entrapped in a cluster of a highly hydrophobic character with a maximum at pH 3.84. The calculations for pH 8.9 suggest (from the point of view of the mobility studied) the presence of only one type of fluorescence labels, which corresponds quite well with the measurements of fluorescence decay (see Figure 3).

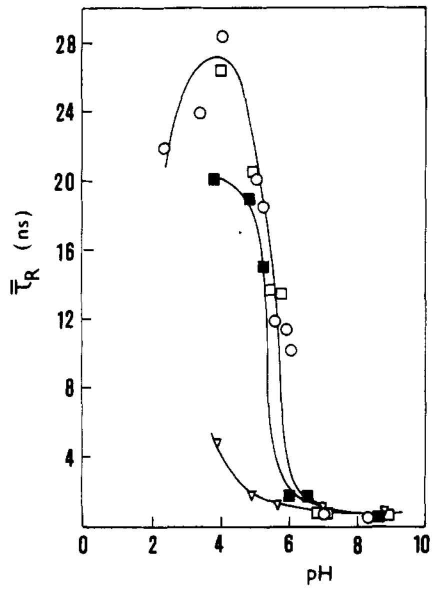

Figure 7. Dependence of the rotational correlation times ($\bar{\tau}_R$) upon pH: Dan-PMA2, $I = 0.05$ (○); Dan-PMA3, $I = 0.05$ (□); Dan-PMA3, $I = 0.5$ (■); Dan-PAA, $I = 0.05$ (▽).

The pH dependence of the ratio r_∞/r_0 expressing the fraction of relaxation processes with a relaxation time significantly longer than the time of fluorescence decay of the dansyl label is plotted in Figure 9. For Dan-PMA1 r_∞/r_0 (like $\bar{\tau}_R$) does not depend on the ionic strength of the solution; between pH 1.6 and 4.0 it lies in the range of 0.3–0.4 independent of pH. For solutions with pH > 4 r_∞/r_0 decreases almost linearly with increasing pH, being very close at pH 9 to the value for Dan-A (in methanol and/or paraffin oil, $r_\infty/r_0 = 0.01$–0.02). The pH dependence of r_∞/r_0 for Dan-PMA2 and Dan-PMA3 has quite a different character. In the pH range of 2.3–4.0 r_∞/r_0 decreases gradually (for solutions with $I = 0.05$) with

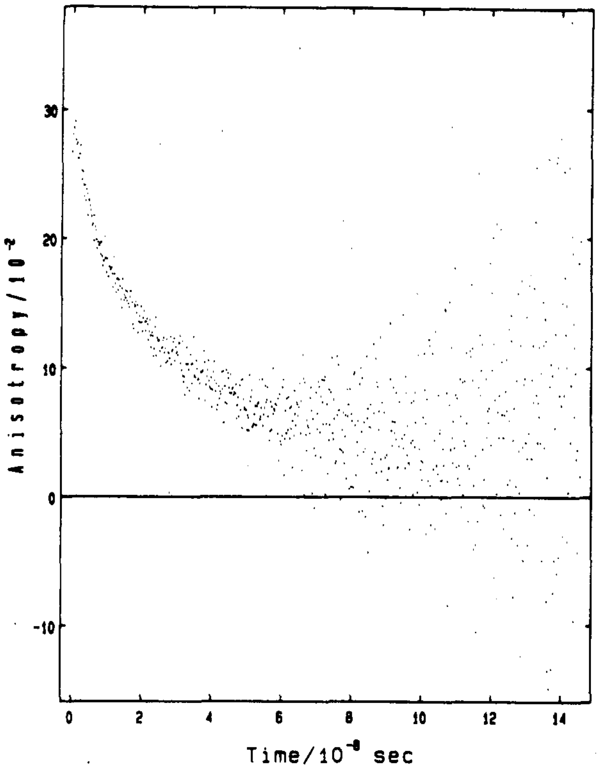

Figure 8. Fluorescence anisotropy decay for Dan-PMA1 at pH 3.84 and $I = 0.5$.

Table III
Rotational Correlation Times of Dan-PMA1, $I = 0.5$

pH	τ_1^R, ns	SD	τ_2^R, ns	SD	χ^2
1.89	1.57	0.70	25.03	1.93	1.15
3.39	2.48	1.18	28.21	2.19	1.17
3.84	2.04	0.56	36.50	2.11	1.10
5.57	0.62	0.22	19.73	2.32	0.99
8.90	0.75	1.06	0.73	0.40	1.16

increasing pH; in the pH range of 4.0–6.0, this value, like $\bar{\tau}_R$, drops from 0.4–0.45 to near 0.1, which remains constant, within experimental error, on any further increase of pH.

The dependence of r_∞/r_0 for Dan-PAA for pH > 4.0 is similar to the dependencies for Dan-PMA2 and Dan-PMA3, but the values r_∞/r_0 decrease from the value 0.23 to values ranging from 0.05 to 0.08, being higher than those for a low molecular weight analogue Dan-A. Experiments have also shown that for Dan-PMA3, in contrast to Dan-PMA1, the r_∞/r_0 values at pH < 5 are higher than those in a solution with a higher value of ionic strength, which corresponds well with the influence of the ionic strength on $\bar{\tau}_R$ (see Figure 7).

Discussion

The high lifetime value τ_2^F for the dansyl label attached to PMA in the range of low pH values cannot be attributed only to the low polarity of the microenvironment. Analogously, high values have been found for Dan-MA[15] in polar 1-butanol at a temperature of 190 K and attributed to low fluidity of the microenvironment. In our case the dansyl label thus seems to be very tightly entrapped in the coiled clusters of the polymer chain, formed as a result of hydrophobic interactions of methyl groups. The high rigidity of the clusters is thus, along with the hydrophobic character, the reason for very high lifetime values. The rigidity of the microenvironment of the compact form of PMA also accounted for the high fluorescence intensity in noncovalently bonded dyes.[8,9,19-22] The $\bar{\tau}_R$ values in Figures 6 and 7 as well as the τ_2^F values in Table II indicate that the size of these clusters reaches a maximum around pH 4.0, within experimental error independent of the molar mass of Dan-PMA. The decrease in the $\bar{\tau}_R$ values, similar to the τ_2^R (see Table III) in the region of pH < 4, indicating a decrease in the size of clusters is obviously the result of hydrogen bonding between the ionized and un-ionized carboxyls. In the region of pH 4.5–6.0, where a transition of the polymer chain from the contracted to the expanded form is expected on the basis of titration[23] and fluorescence[10,19-22,24-26] measurements, there is both a sudden decrease in τ_2^F (as well as of τ_1^F) and a rapid decrease in the $\bar{\tau}_R$ and τ_2^R values. These changes can be accounted for by the disintegration of the clusters as a result of repulsive Coulombic interactions of ionized carboxyls. The pH value at which a change in the microenvironment polarity of the dansyl label is indicated decreases with an increasing ionic strength of the solution, as also indicated by titration[23] as well as fluorescence[22,24] measurements. The $\bar{\tau}_R$ values for Dan-PMA1 of lower molar mass do not depend on the ionic strength of the solution, but for Dan-PMA3 with a molar mass higher by almost 1 order of magnitude (see Table I) a similar shift may be observed as in the case of lifetimes. The τ_1^F values for Dan-PMA in the pH range where the polymer chain is in a "hypercoiled" form indicate that a small part of the polymer chain has a microenvironment polarity close to that of the prevalent part of the PAA chain (see the τ_2^F values). It is somewhat surprising that even at a pH where the PMA is found in the expanded form a smaller part (15–25%) of the microenvironment of the polymer chain has a relatively low polarity and that PAA behaves in a similar way. The largest part of the polar (aqueous) microenvironment in the expanded form was found for Dan-PMA1 at $I = 0.5$, which is probably a

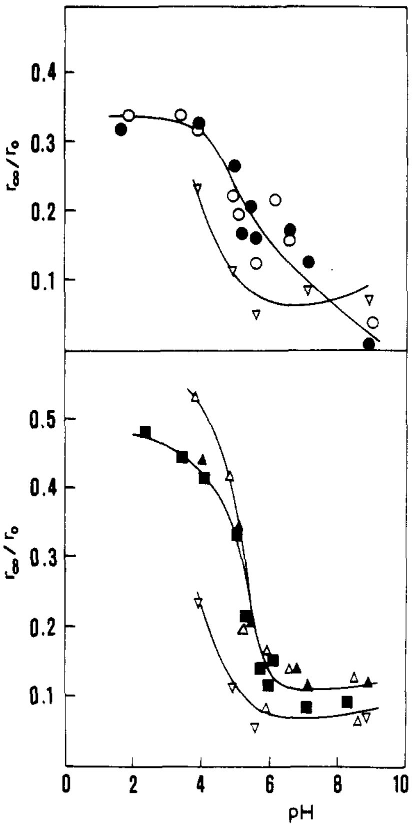

Figure 9. Dependence of relative fluorescence anisotropy (r_∞/r_0) on pH: Dan-PMA1, $I = 0.05$ (●); Dan-PMA1, $I = 0.5$ (○); Dan-PMA2, $I = 0.05$ (■); Dan-PMA3, $I = 0.05$ (▲); Dan-PMA3, $I = 0.5$ (△); Dan-PAA, $I = 0.05$ (▽).

result of the low molar mass of this sample in comparison with Dan-PMA2 or Dan-PMA3, as well as Dan-PAA. The polymer chain of Dan-PMA1 is likely to possess in basic solution an almost perfect rodlike shape. In the pH range where PMA is in the highly expanded form most of the microenvironment of the polymer chain (60–90%) is waterlike. Moreover, the distribution in Figure 5 shows unambiguously the almost homogeneous character of the microenvironment, as indicated by the dansyl label, which exists only in the highly polar microenvironment.

Titration measurements in the studies of Horsky and Morawetz[24] indicated some influence of the hydrophobic fluorescent labels on the conformational transition of PMA. Our measurements show, however, that both the values τ_1^F and τ_2^F as well as $\bar{\tau}_R$ for Dan-PMA2 and Dan-PMA3, which differ in the amount of the covalently attached dansyl labels, are practically identical. The amount of the attached labels thus does not influence, as indicated by our measurements, the conformational transitions of PMA.

On the basis of all these data, one can surmise the arrangement of the polymer chains of PMA and PAA as a function of the pH of the water solution (degree of ionization). At low pH the PMA chain forms highly compact clusters, which are joined by short extended parts of a polymer chain whose microenvironment polarity is identical with that of the "hydrophobic" areas of PAA. During the transition from the compact to the expanded form, the clusters are likely to shrink without changing their internal structure (the polarity of the microenvironment of the dansyl labels entrapped in them). A relatively broad distribution of τ_2^F values seems to reflect the dynamic phenomena of the polymer chain, resulting also in clusters with different sizes. The size of the clusters, however, as follows from the values $\bar{\tau}_R$ (Figures 6 and 7) and τ_2^R in Table III, decreases only to a certain limit, beyond which, on a further increase of carboxyl ionization, the clusters disintegrate completely to expand to a regular polymer chain, similar in character to the PAA chain. Our conclusions are in good agreement with the measurements obtained by the fluorescence study of noncovalently bound fluorescence probes[8,9,19–22] and with the results of a covalently bound label in the polymer chain.[8a,27]

A rather surprising result of our measurement is the lack of homogeneity in the microenvironment polarity even in PAA chains, although, as evident from the $\bar{\tau}_R$ value (e.g., Figure 6), they do not contain any larger organized structures such as those that exist in PMA. This need not necessarily imply a tendency of the chain to isolate the hydrophobic label from the aqueous medium but may be due to a different structure of water, which would affect the microenvironment polarity[28] of the polymer chain and would not affect significantly the mobility of the label. This idea is supported by the practically pH-independent τ_1^R values in Table III, corresponding to the dansyl labels in microenvironments of different polarity. In the basic pH region, where the polymer chains of PMA and PAA are expanded and only an aqueous microenvironment could be expected, 10–20% of microenvironment of the polymer chain has a "nonpolar" character, as discussed above. The differences found among the individual samples obviously reflect a more random chain character of the polymer with a higher molar mass, which could result in a different type of solvation and consequently also in hydrophobic interactions that would cause changes in the structure of the aqueous solvation. The interpretation of the differences observed between high and low molar mass PMA is confirmed by the r_∞/r_0 values in Figure 9. The r_∞/r_0 ratio also indicates that the polymer chain of PMA contains, besides the above discussed clusters and extended parts of the polymer chain, structures that markedly exceed the size of clusters and thus contribute to fluorescence anisotropy. Due to the short lifetime of the dansyl label, however, their movement could not be covered by our measurements. Since, as stated above, the size of clusters is limited, at low pH values a mutual association of clusters is likely to occur in some polymer chains, with the consequent reduction of interactions of the hydrophobic parts of the polymer chain with water molecules.

Time-resolved fluorescence measurements of fluorescence decay and depolarization of the PMA and PAA with attached dansyl labels show the conformational transition of PMA from the compact to the expanded form to be a progressive continuous process over several pH units.

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