Self-Organization of Poly(allylamine)s Containing Hydrophobic Groups and Its Effect on the Interaction with Small Molecules. 1. Static Fluorometry

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Received November 9, 1990; Revised Manuscript Received March 5, 1991

ABSTRACT: Amphiphilic cationic polyelectrolytes, poly(allylamine)s (PAA), with long alkyl groups or a benzyl group show high surface activity in aqueous solutions. Viscosity measurements indicated that the polymers were in a compact conformation. The present work examines the interaction of the amphiphilic polymers with various fluorescent probes and compares the behavior of the fluorescent-labeled polymers. Dodecylated PAA with a degree of substitution (DS) of more than 0.12 and benzylated PAA (DS > 0.5) were found to form a hydrophobic domain similar to a micelle formed by the usual surfactants. It is suggested that at pH 9 the former has a compact core in the interior of the domain and that the latter is sterically capable of accepting probe molecules. The hydrophobic domain is less polar and more dense than the surfactant micelle. The longer the alkyl groups in PAA and the higher the DS, the lower the polarity of the domain is and the higher the inclusion of the substrate is. At pH 9, the inclusion of small molecules by the domain is mainly due to hydrophobic interactions; electrostatic interactions play a minor role. On the other hand, it is suggested that in a neutral aqueous solution conformational change of the polymer exerts an influence on the inclusion and electrostatic interaction makes a contribution.

Introduction

In many of the functions in biopolymer system as, for example, enzymatic reactions, immunology, transport phenomena, and so on, the interaction of macromolecules, proteins, and low molecular compounds is of great importance. Much work on interaction has been carried out in this connection, using various molecules as probes, such as dyes and fluorescent and spin probes. Of these, the very sensitive fluorescent method gives information about local polarity and mobility of the environment, as well as the binding behaviour of the probes.[1-3] In our previous work, the hydrophobic environment and the depth of cleft in bovin serum albumin were investigated by dansyl-amino acid probes.[4]

Recently, the application of the fluorescence method has been frequently reported in connection with complex biopolymer and on biomimetic model systems, including micellar systems. Work on conformational changes of poly(methacrylic acid) as a function of pH[5,6] and the hydrophobic environment of sodium poly(styrenesulfonate),[7,8] poly(vinylpyrrolidone),[9,10] and amphiphilic alternate copolymers[11,12] and block copolymers[13,14] are further examples. Work on an organized molecular assembly as a reaction field of photoinduced electron transfer has also been reported.[15-18]

Quaternized poly(vinylpyridine) with long alkyl chains is a well-known cationic polyelectrolyte that has a hydrophobic domain in aqueous solution and is called a polysoap. Compact intramolecular micelle formation has been proved by viscosity,[19] light scattering,[20] and surface tension.[21] In our previous work on the mechanism of interaction between dyes and polysoaps[22] and the mobility of spin probes in a polysoap solution,[23] it was found that the microenvironment of the polysoap is much more rigid and hydrophobic than that of low molecular surfactant micelles. The strongly hydrophobic microdomain formed by polysoaps is known to serve as a reaction field to accelerate hydrolysis and decarboxylation reactions.[24,25] On the other hand, Klotz et al. reported that flexible branched poly(ethyleneimine) (b-PEI) with a hydrophobic side group has a high binding ability with low molecular weight compounds, which is as good as that of bovine serum albumin, and exhibits a high catalytic ability in ester hydrolysis similar to that of an enzyme.[26-31] They investigated the interaction between side groups of the hydrophobic b-PEI labeled with a pyrene residue.[32] Sisido et al. also investigated the same polymer labeled with stable free radicals. They concluded that the domain structure consists of two parts, the hydrophobic cluster, which has low mobility, and the surrounding region, which has high mobility.[33] Pshezhetskii et al. found a high hydrolysis activity for linear poly(ethyleneimine) (l-PEI) containing benzyl and long alkyl groups. They explained the activity as caused by a compact structure similar to that of globular proteins.[34,35] It is worthwhile mentioning that b-PEI has primary, secondary, and tertiary amino groups whereas l-PEI has only a secondary amino group in the main chain.

Recently, Harada et al. prepared poly(allylamine) of high molecular weight,[36] with a side chain containing primary amino group of high reactivity. The polymer was convertible to various functional polymers by chemical modification.[37,38] We have reported that PAA has a higher hydrolysis reactivity than b-PEI and the introduction of β-cyclodextrin as a side chain in the polymer produces both a specific selectivity of the substrate and a high catalytic activity.[39-41] On the other hand, alkylated PAA was found to show a significantly accelerate hydrolysis, and it was suggested that the domain formed by the substituted side chain enhances the inclusion of the substrates.[40-42] In this connection, in view of the structure, function, and the formation of a reaction field, PAA derivatives with hydrophobic groups are interesting polymers. However, this topic has not been systematically

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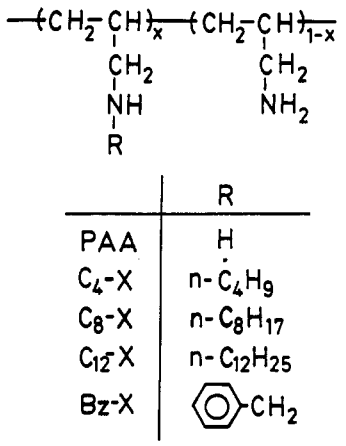

Figure 1. Poly(allylamine) derivatives.

investigated.

In the present work, the amphiphilic properties of the PAA derivatives by the introduction of long alkyl chains and benzyl groups were investigated. The interaction between those polymers and some fluorescent probes varying in polarity was studied by static fluorometry. A comparison of the results obtained both from the fluorescent probe and fluorescent-labeled polymer systems can give systematic information about both the polarity of the local environment and the inclusion ability of small molecules.

Experimental Section

Materials. Poly(allylamine) hydrochloride (PAA·HCl), M_w = 10 000, supplied by Nitto Boseki Co. was used after repeated precipitation from water/methanol. *n*-Butyl bromide, *n*-octyl bromide, and *n*-dodecyl bromide (all G.R. Grade, from Wako Pure Chemical Industries), benzyl chloride (G.R. Grade, Kanto Chemical Co.), dansyl chloride (DNS-Cl) (G.R. Grade, Tokyo Kasei Co.), and 2-aminoethanol (1st Grade, Tokyo Kasei Co.) were used without further purification.

Dodecyltrimethylammonium bromide (DTABr) and dodecylamine hydrochloride (DACl) (G.R. Grade, Tokyo Kasei Co.) were recyrstallized from acetone and ethanol, respectively. Cetyltrimethylammonium bromide (CTABr) (G.R. Grade, Nakarai Chemicals) was used without purification.

pH and ionic strength (μ) were adjusted with Tris buffer, 2-amino-2-(hydroxymethyl)-1,3-propanediol (Analytical Grade, Nakarai Chemicals), dissolved in hydrochloric acid aqueous solution. Distilled methanol, ethanol, 1-propanol, and dioxane were used.

***N*-Alkylated and Benzylated Poly(allylamine)s.** Various alkyl bromides or benzyl chloride were added to a PAA solution in methanol and kept 48 h at 50 °C, as described in our previous reports.[40,41] The products were precipitated in an ether/hexane mixture, filtrated, and dried in vacuo, yielding white powders. Their structures were confirmed by IR, NMR, elemental analysis, and DTA. Figure 1 shows the structural formulas of PAA derivatives, and in the same figure is given the notation of the polymers studied, where the symbol X means the content of alkyl and benzyl groups (mol %).

Fluorescent Probes. The chemical structures and the abbreviations of the probes used are shown in Figure 2. 5-(Dimethylamino)-1-naphthalenesulfonic acid (DNSA) (G.R. Grade, Tokyo Kasei Co.) was purified by recrystallization from ethanol. Potassium 2-(*p*-toluidinyl)naphthalene-6-sulfonate (TNS) (Fluorometry Reagent, Aldrich Co.) was used without further purification. 5-(Dipropylamino)-1-naphthalenesulfonic acid (PNSA), dansylglycine (DNS-Gly), dansylpiperidic acid (DNS-Pip), and dansyltaurine (DNS-Tau) were kindly supplied by Dr. T. Seki.[4] Dansyl ethanol amine (DNS-Eth) was prepared from DNS-Cl and 2-aminoethanol in ethanol at room temperature for 24 h, by the method of Onodera.[43] The product was extracted with chloroform and recrystallized from benzene, giving yellowish green needles (mp 104–105 °C). In this work, the probes used

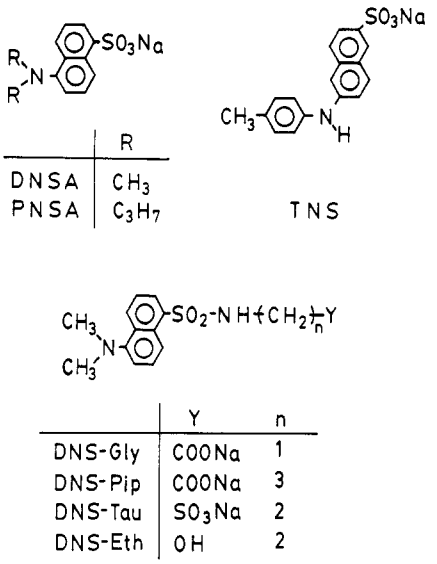

Figure 2. Fluorescent probes used.

Table I
λ_{max} of Fluorescent Probes in Various Solvents

probe	λ_{max}, nm			
	H_2O (63.1)[a]	methanol (55.5)[a]	ethanol (51.9)[a]	1-propanol (50.7)[a]
DNSA	508	476	462	458
PNSA	508	477	465	460
TNS	500	456	437	433
DNS-Gly	570	526	518	509
DNS-Pip	570	523	516	510
DNS-Tau	570	524	515	512
DNS-Eth	570	525	518	513

[a] Dimroth's solvent polarity parameter, E_T(25 °C), by: Dimroth, K.; Reichardt, C.; Siepmann, T.; Bohlman, F. *Liebigs Ann. Chem.* **1963**, *661*, 1; **1963**, *669*, 95.

was classified into two groups, the Laurent and the dansyl amino acid groups. DNSA, PNSA, and TNS, which contain the same naphthalenesulfonic acid moiety with different hydrophobic groups, are in the former group. DNS-Gly, DNS-Pip, and DNS-Tau, which contain the same fluorophor but different ionic groups and spacer moieties, belong to the latter group, as does the nonionic DNS-Eth.

The maximum emission wavelengths (λ_{max}) of these probes are given in Table I. In each case λ_{max} shifts to a shorter wavelength when polarity decreases (decrease in the E_T value); thus λ_{max} is a function of the polarity of the environment. With the exception of TNS, the λ_{max} of the six 1,5-substituted probes, i.e., two Laurent's acids and four DNS-amino acids, is approximately the same in the same solvent, suggesting the same fluorescent characteristics for different substituent groups.

Fluorescent-Labeled PAA Derivatives. PAA derivatives covalently bound with the dansyl group, DNS-C_{12}-18 and DNS-Bz-75 (Figure 3) were synthesized by mixing the aqueous solution of the polymer with a solution of dansyl chloride in dioxane for 40 h at room temperature. The solvent was then removed by vacuum distillation and the residue dissolved in water and then dialyzed with an ethanol/water mixture in a Visking tube for 4 days. The dialyzate was condensed, methanol was then added and the dialyzate was precipitated in ether. After the mixture was dried a white powder was obtained. The degree of substitution was then determined spectrophotometrically, using ϵ_{326} = 4.6×10^3 $M^{-1}\cdot cm^{-1}$.

Viscosity and Surface Tension. The reduced viscosity of a 0.1 g/100 mL polymer solution was measured at 25 °C with a Ostwald viscometer. Pure water and Tris buffer (pH 8.74, μ = 0.05) was used as solvent.

The surface tension at room temperature was determined with an electrosurface tensiometer (ESB-Vl, Kyowa Kagaku Ltd.). The same solvent as above was used and a concentration of 1.0×10^{-2} M.

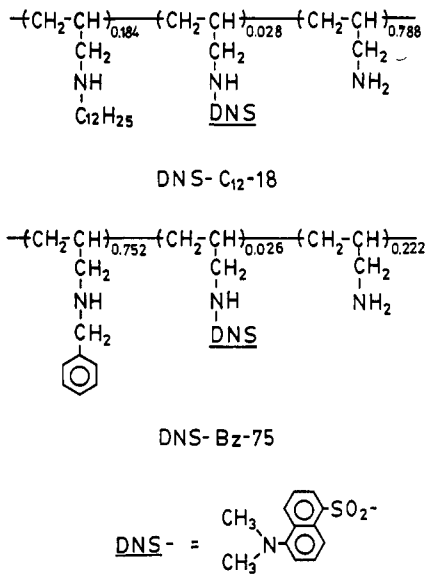

Figure 3. Fluorescent-labeled PAA derivatives.

Static Fluorescence Spectra. The fluorescence spectra were determined at 25 °C with a Shimadzu RF 502 spectrophotometer, described in a previous paper.[4] Nitrogen was bubbled through the sample for 15 min before measurement. The concentration of fluorescence probes was adjusted to (4.88–6.38) $\times 10^{-6}$ M. For the determination of concentration, the following values were used:

probe	λ, nm	ϵ_λ, M·cm^{-1}
DNSA	312 nm	4.55×10^3 [44]
PNSA	312 nm	4.55×10^3 [44]
TNS	366 nm	4.08×10^3 [45]
DNS-amino acid	326 nm	4.06×10^3 [46]

Extinction wavelengths were set at 366 nm for TNS and 330 nm for the other probes. Emission spectra were automatically corrected by a compensator. The fluorescence intensity was standardized with a 0.1 M sulfuric acid solution of quinine bisulfate.

UV and visible absorbance were determined with a double-beam spectrophotometer, Hitachi 556.

Calculation of the Binding Constant.[41,47] The primary binding constant, K_1 (M^{-1}), was easily evaluated by keeping the initial concentration of a fluorescent probe, $C_0(M)$, and changing the polymer concentration, C_p (M repeating units), when the system consists of a single equilibrium expressed by

$$P + F \rightleftarrows P{\cdot}F$$

where P, F, and P·F represent the polymer, the probe, and the polymer–probe complex, respectively.

If the concentration of the free probe is C_f and its concentration entrapped in the polymer chain is $C_b(M)$, eq 1 is obtained when $C_p \gg C_0$ and $C_0 = C_f + C_b$:

$$K_1 = \frac{[P{\cdot}F]}{[P][F]} = C_b/\{(C_p - C_b)C_f\} \approx C_b/(C_pC_f) \quad (1)$$

Bearing in mind that the mole fraction of the probe entrapped in the polymer domain is $X_b = (I - I_0)/(I_{max} - I_0)$ and $C_b/C_f = X_b/(1 - X_b)$, eq 1 gives

$$I = \{(I_0 - I)/(K_1 \times C_p)\} + I_{max} \quad (2)$$

where I is the observed fluorescence intensity at given C_p and I_0 and I_{max} are respectively the intensities at $C_p = 0$ and at the complete complexation of the probes.

With use of the least-squares method, the K_1 value was determined from the slope of the straight line in a plot of of I against $(I_0 - I)/C_p$ and the intercept on the I axis gave I_{max} (see Figure 7 in the following section). As in the case of TNS, $I \gg I_0 \approx 0$, only I was used. For the other probes giving the weak intensity in a aqueous solution, I/I_0 was used for the analysis in place of I.

Table II
Aqueous Properties of Alkylated and Benzylated Poly(allylamine)s

polymer	DS[b]	HLB[c]	γ,[d] dyn·dm^{-1}	η_{sp}/C, dL·g^{-1} in buffer[e]	η_{sp}/C, dL·g^{-1} in water[f]
PAA[a]	0	11.7	60.8	0.548	3.11
C_4-23	0.230	8.9	55.5	0.817	1.40
C_8-20	0.200	7.6	45.0	0.133	0.161
C_8-38	0.377	5.8	43.5	0.129	0.379
C_{12}-12	0.120	7.9	53.2	0.434	0.210
C_{12}-18	0.184	6.8	31.4	0.090	0.412
C_{12}-30	0.303	5.3	29.8	0.150	1.05
C_{12}-45[a]	0.454			0.821	0.445
Bz-23	0.233	8.0	51.9	0.603	0.865
Bz-48	0.476	6.1	48.8	0.067	0.671
Bz-75	0.750	4.9	42.5	0.044	0.459

[a] Hydrochloride salt of polymer. [b] Degree of substitution of alkyl or benzyl group. [c] Calculation based on polymer composition using the empirical relation, given by HLB = Σ(hydrophilic group numbers)/Σ(oleophilic group numbers) × 10 where the hydrophilic group numbers of amino group and benzene ring are 70 and 15, respectively, and the oleophilic group number per carbon atom is 20. [d] $C_p = 1.0 \times 10^{-2}$ M. Tris buffer (pH 8.74, $\mu = 0.05$), at room temperature. [e] $C_p = 0.1$ g·dL^{-1}. Tris buffer (pH 8.74, $\mu = 0.05$), at 25 °C. [f] $C_p = 0.1$ g·dL^{-1}. Pure water (pH 5.6, $\mu = 0$), at 25 °C.

In general, the absolute value of the intensity of fluorescence depends on the polarity of the medium and the amounts included in the polymer domain. On the other hand, in eq 2, K_1 and I_{max} separately reflect the amount of inclusion and the polarity of the medium, respectively. Accordingly, the use of both the measurements gives clear insight into the fluorescence behavior.

Results and Discussion

(1) Amphiphilic Polyelectrolytes. Surface Activity. In Table II are given the data of 10 poly(allylamine) derivatives of different degrees of alkyl and benzyl substitution.

The surface activity, γ, is a measure of amphiphilicity. The amphiphilic character is revealed clearly in the table by the introduction of the hydrophobic group in poly(allylamine) when the surface tension is decreased. The larger alkyl groups, such as C_8 and C_{12}, and the benzyl group lead to a larger decrease in surface tension. The values of the hydrophile–lipophile balance (HLB) are expected to correlate convincingly with the surface tension. Similar behavior has been reported for "polysoap", poly(*N*-decyl-4-vinylpyridinium bromide) (DQPVpy),[21] and amphiphilic polyanion systems.[17]

Viscosity. To obtain information about the molecular conformation of these amphiphilic polymers, the reduced viscosity of the polymer solutions was determined. The highest viscosity in water is observed for unsubstituted PAA as is the decrease in viscosity with the introduction of the hydrophobic group (Table II). This indicates that unsubstituted PAA has an extended chain conformation based on the electrical repulsion of the charged groups. On the other hand, PAA derivatives have a compact micellelike structure because of the hydrophobic interaction of alkyl and benzyl groups. The decrease in viscosity in buffer solutions, as compared with water, is explained by screening of the charge of the polymer by the buffer salts. As for the effect of the substituent groups, in the cases of C_8 and C_{12}, the decrease is shown clearly even at the low degree of substitution, DS = 0.2 and 0.12, respectively. However, a higher degree of substitution, DS > 0.5, is required in the case of the benzyl derivative to show a clear decrease in viscosity. This behavior was observed in

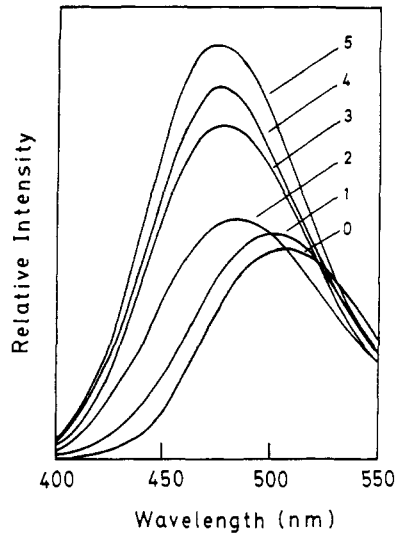

Figure 4. Fluorescence emission spectra of DNSA at different concentrations of C_{12}-18 in pH 9.1 Tris buffer (μ = 0.04). C_P: (1) 0 M; (2) 5.7×10^{-4} M; (3) 2.3×10^{-3} M; (4) 1.1×10^{-2} M; (5) 2.3×10^{-2} M.

linear poly(ethyleneimine)s (l-PEI), substituted with alkyl and benzyl groups.[35] It is of interest that a viscosity minimum is observed in C_{12} at DS = 0.18, as compared with the monotonous decrease in viscosity with DS in the C_8 and Bz systems. For steric reasons,[25] the high DS of the longer alkyl group may cause a difficulty in forming a compact structure, as in the case of DQPVPy.[19]

It is concluded from these results that PAA derivatives with hydrophobic groups have an amphiphilic character and form a compact hydrophobic domain in aqueous solution. A study of the interaction of these polymers with fluorescent probes, described in the next section, was carried out mainly with the polymers C_{12}-18 and Bz-75, which have a high surface activity and low viscosity.

(2) Static Fluorescence Spectra. Laurent's Acid Probes. In this section the polarity of the hydrophobic domain and the inclusioin ability of small molecules by the domain were investigated by means of fluorescent probe technique. The fluorescence emission spectra of the C_{12}-18/DNSA system in Tris buffer at pH 9.1 are shown in Figure 4. Figure 5 gives a plot of maximum emission wavelength (λ_{max}) and the fluorescent intensity ratio (I/I_0) as a function of polymer concentration. An increase in polymer concentration causes a shift of λ_{max} to a shorter wavelength and an increase in intensity. The unsubstituted PAA/DNSA system, on the other hand, shows no such spectral change, indicating that a hydrophobic domain is formed in the buffer solution of C_{12}-18 and it includes the probe molecules. This is consistent with the surface activity and viscosity behavior of the same polymer solutions, just as discussed above. Similar fluorescent behavior was observed in the Bz-75 system (Figure 6).

As can be seen in Figures 5 and 6, polymer concentrations at which λ_{max} and I/I_0 (or I) change shift to lower values when the hydrophobicity of the probes increases in the sequence of DNSA < PNSA < TNS. This suggests that the larger the nonpolar character of the probes, the stronger the hydrophobic interaction is. The limiting values of λ_{max} at the high concentration of the polymer are given in Table III. The primary binding constant K_1 and I_{max}/I_0 (or I_{max}), obtained from the linear relation of eq 2 as shown in Figure 7, are also listed in the same table.

The values of K_1 for both C_{12}-18 and Bz-75 increase in the sequence of DNSA < PNSA < TNS, which clearly indicates the greater inclusion ability of the larger hydrophobic interaction systems. Of the probes DNSA and PNSA, which have similar fluorescent characteristics, PNSA emits at a shorter wavelength for C_{12}-18, suggesting that PNSA, which has greater hydrophobicity, is included more firmly by the hydrophobic domain. On the other hand, λ_{max} is approximately the same for both probes in the case of Bz-75, indicating a similar location of the probes in the hydrophobic domain. Another measure of the polarity of the medium, I_{max}/I_0, supports the evidence for this argument.

K_1 of C_{12}-18 and Bz-75 is always greater for the former, for every probe. In the side chain of PAA, the secondary amino group, substituted by a hydrophobic group, for steric reasons, interacts less electrostatically, which is in contrast

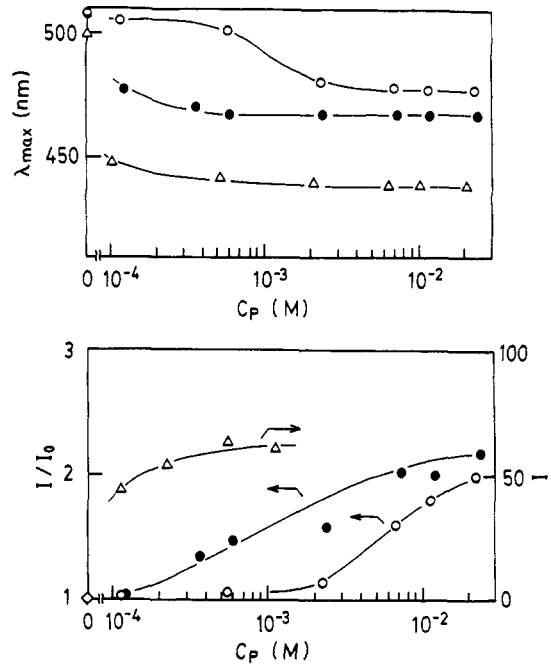

Figure 5. λ_{max} and I/I_0 or I as a function of the concentration of C_{12}-18 in pH 9.1 Tris buffer (μ = 0.04): (○) DNSA; (●) PNSA; (△) TNS.

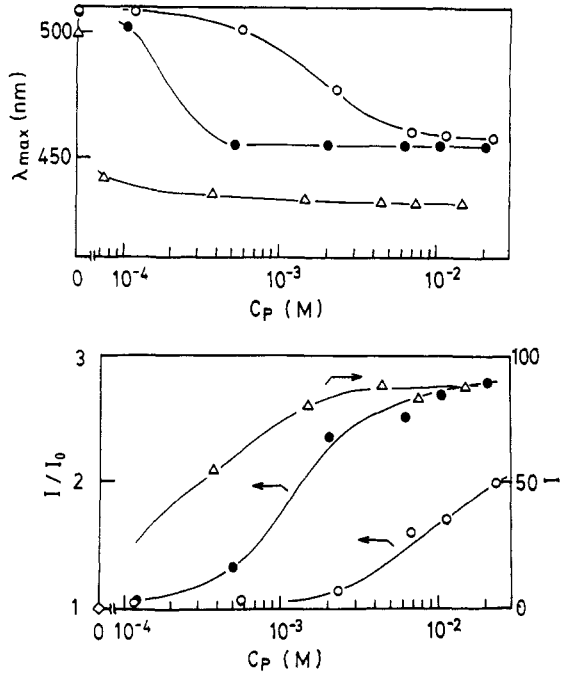

Figure 6. λ_{max} and I/I_0 or I as a function of the concentration of Bz-75 in pH 9.1 Tris buffer (μ = 0.04): (○) DNSA; (●) PNSA; (△) TNS.

Table III
Fluorescence Parameters of Laurent's Acid Probes in Buffer Solutions of PAA Derivatives

polymer	probe	λ_{max},[a] nm	K_1,[b] M^{-1}	I_{max}/I_0
C_{12}-18	DNSA	477	340 ± 70	2.03 ± 0.14
	PNSA	467	2200 ± 200	2.19 ± 0.06
	TNS	439	72000 ± 25000	59[c]
Bz-75	DNSA	459	220 ± 70	2.80 ● 0.50
	PNSA	455	1300 ● 200	2.87 ± 0.12
	TNS	432	32000 ± 110	108[c]

[a] $C_p = 1.0 \times 10^{-2}$ M. Tris buffer (pH 9.1, $\mu = 0.04$), at 25 °C. [b] Binding constant per unit monomolarity of polymer. [c] I_{max}.

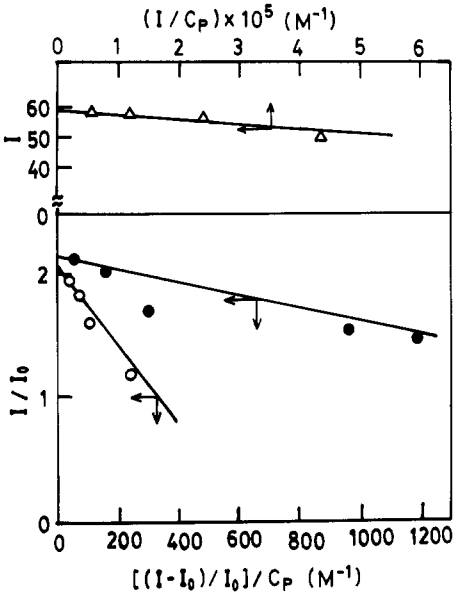

Figure 7. Plots of I versus $(I - I_0)/C_p$ to determine the binding constant for DNSA- (○), PNSA- (●), and TNS (△)/C_{12}-18 systems.

with the positively charged amino group in the side chain of PAA. Thus Bz-75 may have a smaller inclusion ability than C_{12}-18.

Dansyl-Amino Acid Probes. In this section are described the results of an investigation into the effects of the distance between the ionic and fluorophoric groups and the effect of the charge. The dependence of λ_{max} and I/I_0 of DNS-amino acid probes on the polymer concentration is shown in Figures 8 and 9. Table IV gives λ_{max} (at $C_p = 1 \times 10^{-2}$ M), K_1, and I_{max}/I_0. In both systems, C_{12}-18 and Bz-75, the values of λ_{max}, K_1, and I_{max}/I_0 are the same for DNS-Gly and DNS-Pip, that is the methylene spacer length has no effect. When comparing sulfonic- and carboxylic-type probes, the above parameters have practically the same values in DNS-Tau and DNS-Gly, suggesting the location of the probes in the domain is similar and that there is no difference in inclusion abilities. As the nonionic DNS-Eth has a considerably smaller K_1, compared with three anionic probes, in the inclusion of small molecules at pH 9.1, electrostatic attraction should play a role, in addition to hydrophobic interaction. The same values of λ_{max} and I_{max}/I_0 of four probes for C_{12}-18 and Bz-75 systems indicate a small effect of the probe charge on the location of the probes in the polymer domain.

Of the three probes, with the exception of DNS-Eth, K_1 for C_{12}-18 is larger than that for Bz-75, and nonionic DNS-Eth has approximately the same value for both polymers. These are the same tendencies as found in Laurent's acid probes. The electrostatic contribution of the primary amino cation on the inclusion is again verified.

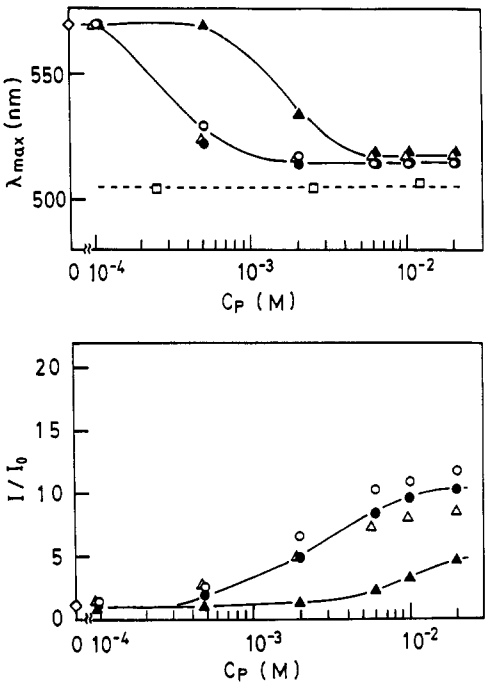

Figure 8. λ_{max} and I/I_0 as a function of the concentration of C_{12}-18 in pH 9.1 Tris buffer ($\mu = 0.04$): (○) DNS-Gly; (●) DNS-Pip; (△) DNS-Tau; (▲) DNS-Eth; (□) DNS-C_{12}-18.

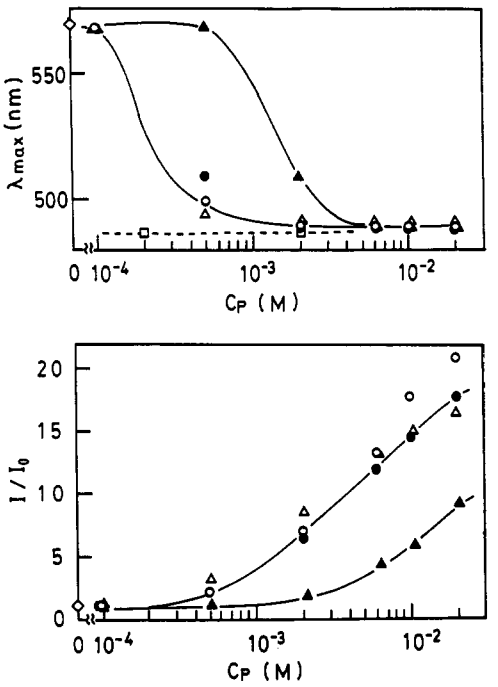

Figure 9. λ_{max} and I/I_0 as a function of the concentration of Bz-75 in pH 9.1 Tris buffer ($\mu = 0.04$): (○) DNS-Gly; (●) DNS-Pip; (△) DNS-Tau; (▲) DNS-Eth; (□) DNS-Bz-75.

Effects of Chain Length and DS. The influence of the chain length of PAA derivatives on the fluorescence behavior of DNSA, DNS-Gly, and TNS is shown in Figures 10–12.

PAA derivatives, except C_4-0.2, have shorter λ_{max} than unsubstituted PAA, which proved again the domain formation of the less polar local environment. The minimum length for the formation of the domain is estimated to be C_8. At DS = 0.2, the alkylated PAA forms a less polar domain than Bz-PAA, which suggests a more compact packing of alkyl groups than of benzyl rings and leads to the less polar domain structure.

Table IV
Fluorescence Parameters of Dansyl-Amino Acid Probes in Buffer Solutions of PAA Derivatives

polymer	probe	λ_{max},[a] nm	K_1,[b] M^{-1}	I_{max}/I_0
C_{12}-18	DNS-Gly	515	410 ± 30	13.3 ± 0.7
	DNS-Pip	515	440 ± 110	12.5 ± 0.5
	DNS-Tau	518	440 ± 19	10.4 ± 0.2
	DNS-Eth	520	43 ± 5	9.6 ± 0.7
Bz-75	DNS-Gly	490	200 ± 30	24 ■ 3
	DNS-Pip	489	190 ± 3	22.2 ■ 0.2
	DNS-Tau	492	365 ± 16	20.1 ± 0.4
	DNS-Eth	490	40 ± 4	20 ± 2

[a] $C_p = 1.0 \times 10^{-2}$ M. Tris buffer (pH 9.1, $\mu = 0.04$), at 25 °C. [b] Binding constant.

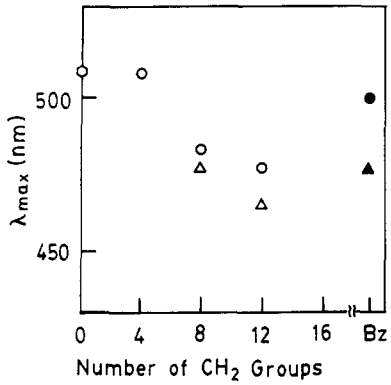

Figure 10. Dependence of λ_{max} of DNSA on side-chain length in pH 9.1 Tris buffer ($\mu = 0.04$). $C_p = 1.0 \times 10^{-2}$ M. Open symbols, alkyl-PAA; filled symbols, Bz-PAA. (○) DS = 0.2; (△) DS = 0.4.

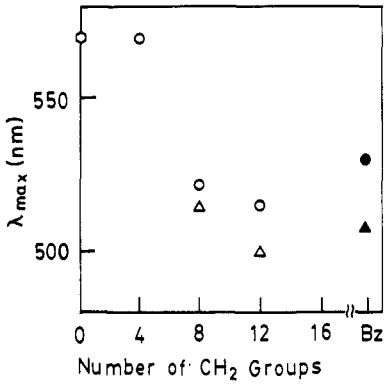

Figure 11. Dependence of λ_{max} of DNS-Gly on side-chain length in pH 9.1 Tris buffer ($\mu = 0.04$). $C_p = 1.0 \times 10^{-2}$ M. Open symbols, alkyl-PAA; filled symbols, Bz-PAA. (○) DS = 0.2; (△) DS = 0.4.

λ_{max} of DNSA, PNSA, and TNS plotted against DS of the polymer is shown in Figures 13–15. For every probe, PAA derivatives give a shift in λ_{max} to a shorter wavelength, the shift increasing with the DS. The more hydrophobic the side group, the more compact and less polar the local domain is.

From the inspection of λ_{max} values, the domain formed by PAA derivatives should have a similar polarity to that of methanol or ethanol. The polarity of TNS is less than that of DNSA in the system of alkylated PAA, suggesting that it is located more in the interior of the domain. Surprisingly, as the steric adaptability of probe molecules with benzylated PAA is better than with alkylated PAA, even DNSA is located in the interior of the domain. The fact that λ_{max} of DNS-Gly is at a shorter wavelength than that of DNSA suggests that the fluorophor group, which is linked through a long spacer group in DNS-Gly, may reflect the more inner location in the hydrophobic domain.

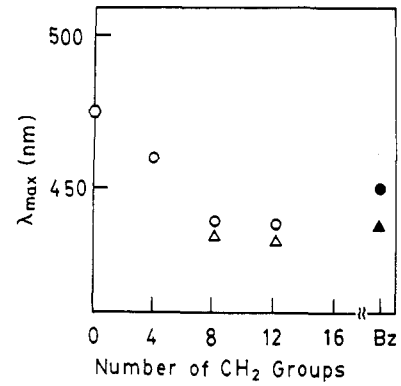

Figure 12. Dependence of λ_{max} of TNS on side-chain length in pH 9.1 Tris buffer ($\mu = 0.04$). $C_p = 2.0 \times 10^{-3}$ M. Open symbols, alkyl-PAA; filled symbols, Bz-PAA. (○) DS = 0.2; (△) DS = 0.4.

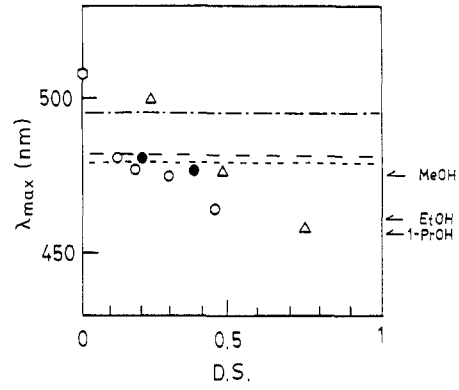

Figure 13. Dependence of λ_{max} of DNSA on DS of PAA derivatives in pH 9.1 Tris buffer ($\mu = 0.04$). $C_p = 1.0 \times 10^{-2}$ M. (●) C_8; (○) C_{12}; (△) Bz; (– –) DTABr micelle; (- - -) CTABr micelle; (– - –) DACl micelle.

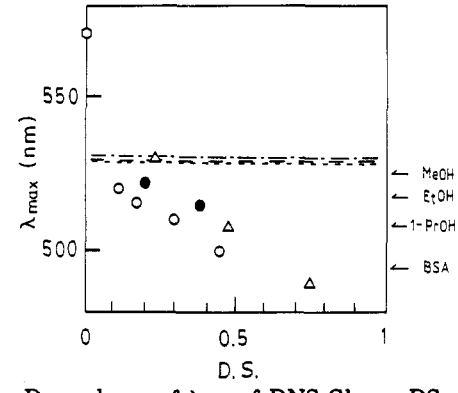

Figure 14. Dependence of λ_{max} of DNS-Gly on DS of PAA derivatives in pH 9.1 Tris buffer ($\mu = 0.04$). $C_p = 1.0 \times 10^{-2}$ M. (●) C_8; (○) C_{12}; (△) Bz; (– –) DTABr micelle; (- - -) CTABr micelle; (– - –) DACl micelle.

On the other hand, comparison with the micelle systems of surfactants, DTABr (cmc = 1.5×10^{-2} M at 25 °C),[48] CTABr (cmc = 9.2×10^{-4} M at 25 °C),[48] and DACl (cmc = 1.5×10^{-2} M at 25 °C),[48] shows that the local domains of the PAA derivatives used here are less polar. The side chains of the polymer may form a more compact, subsequently less polar, structure than that of surfactants. Furthermore, a λ_{max} = 430–440 nm in the TNS system (Figure 15) indicates a lower polarity of the domain, compared with that formed by PAA covalently bound β-cyclodextrins (λ_{max} = 456 nm).[41] Using the results for DNS-Gly (Figure 14), the domain structure in C_{12}-45 and Bz-75 is estimated to have a similar characteristics to the hydrophobic cleft of bovine serum albumin (BSA).[4]

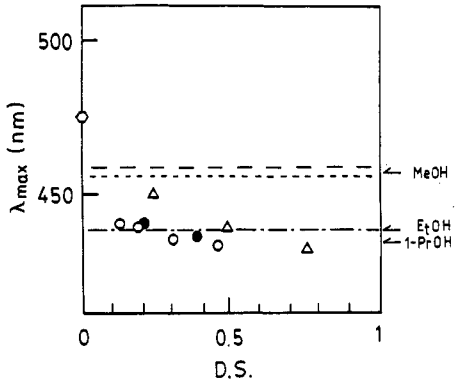

Figure 15. Dependence of λ_{max} of TNS on DS of PAA derivatives in pH 9.1 Tris buffer (μ = 0.04). $C_p = 2.0 \times 10^{-3}$ M. (●) C_8; (○) C_{12}; (△) Bz; (– –) DTABr micelle; (- - -) CTABr micelle; (– – –) DACl micelle.

Table V
Fluorescence Parameters of DNSA in Buffer Solutions of PAA Derivatives

polymer	λ_{max},[a] nm	K_1,[b] M^{-1}	I_{max}/I_0
C_4-23	508		
C_8-20	481	190 ± 20	1.68 ± 0.05
C_{12}-12	481	250 ● 40	2.16 ± 0.14
C_{12}-18	477	340 ± 70	2.03 ± 0.14
C_{12}-30	475	410 ± 40	2.04 ± 0.06
C_{12}-45	465	250 ± 30	2.58 ± 0.14
Bz-23	500	120 ● 30	1.35 ● 0.05
Bz-48	477	180 ± 30	2.11 ± 0.14
Bz-75	459	220 ± 60	2.80 ± 0.50

[a] $C_p = 1.0 \times 10^{-2}$ M. Tris buffer (pH 9.1, μ = 0.04), at 25 °C. [b] Binding constant.

Inclusion Ability of PAA Derivatives. In Table V, I_{max}/I_0, λ_{max}, and K_1 in the systems using DNSA as fluorescent probe are summarized to estimate the relation between the structure of PAA derivatives and the inclusion ability of small molecules.

With respect to the same sequences, λ_{max} and I_{max}/I_0, it is noticed that both parameters reflect the polarity of the domain atmosphere. A low λ_{max} shown by the long alkyl chain and a large DS in the polymer correspond to a large K_1. The lower the polarity of the domain, the greater the hydrophobic interaction in the inclusion is. In the case of C_{12}-45, the low value of K_1—despite the low polarity—can be explained by the hindrance effect on the inclusion because of the crowded long alkyl chain. This argument is in accord with the explanation of the viscosity behavior of dodecylated PAA, as discussed in the preceding section on viscosity.[19,25] From the low result of K_1 for Bz-23, it follows that the DS of Bz-PAA should be at least 0.5 to form an effective domain to include small molecules. For alkylated PAA, K_1 is generally large, indicating the formation of the effective structure of the domain.

Fluorescent-Labeled PAA. As a model of a polymer domain system which completely incorporates the probe molecules, fluorescent-labeled PAA (flPAA) was investigated.

As shown in Figure 16, λ_{max} of DNS-C_{12}-18 and DNS-Bz-75 is constant irrespective of polymer concentration. The results suggest the polymer has a compact structure even at low concentration (10^{-4} M). With changes in pH 9.1 buffer, pH 7.2 (buffer), and water, the values of λ_{max} shift to longer wavelengths. This is attributed to the increase in the charge of the polymer flPAA, in which the pK_a of the amino group is ca. 9.[37] However, even in aqueous solution (pH = 5.6) the hydrophobic domain is supposed to be maintained as evidenced by λ_{max}.

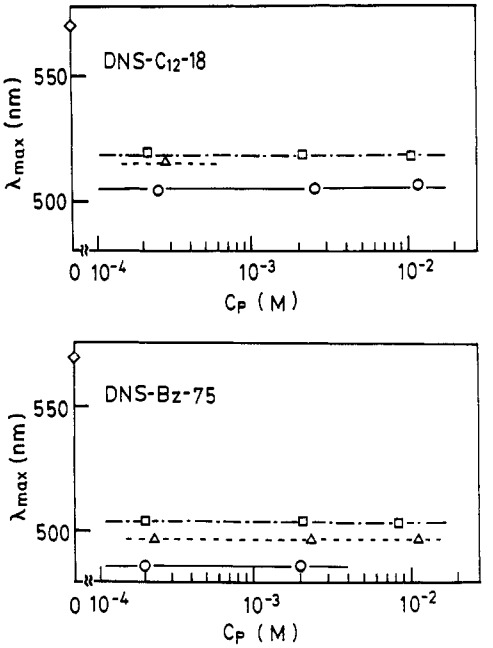

Figure 16. λ_{max} as a function of the concentration of DNS-C_{12}-18 and DNS-Bz-75 at different pHs. pH: (○) 9.1 (Tris buffer, μ = 0.04); (△) 7.2 (Tris buffer, μ = 0.04); (□) 5.6 (pure water, μ = 0).

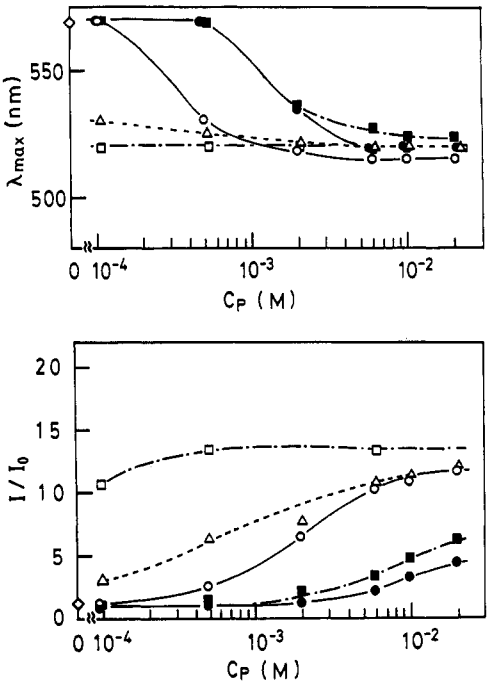

Figure 17. λ_{max} and I/I_0 as a function of the concentration of C_{12}-18 at different pHs. Open symbols, DNS-Gly; filled symbols, DNS-Eth. pH: (○) 9.1 (Tris buffer, μ = 0.04); (△) 7.2 (Tris buffer, μ = 0.04); (□) 5.6 (pure water, μ = 0).

Effect of pH. The fluorescent behavior of the probe/PAA derivative and flPAA systems have been compared, varying the pH. For the comparison DNS-Gly was chosen as the probe molecule. The results at pH 9.1 (buffer) and 7.2 (buffer) and in water are given in Figures 17 and 18 and in Table VI.

The only difference in λ_{max} is observed for C_{12}-18 at pH 9.1 (compare Figures 16 and 17). It is estimated that in alkylated PAA at pH 9.1 the interior of the domain core is compact and does not incorporate the probe molecules. On the other hand, in flPAA the covalently bound fluorophor group exists inherently in such a core. At pH 7.2 or in aqueous solution (pH = 5.6) the core structure may

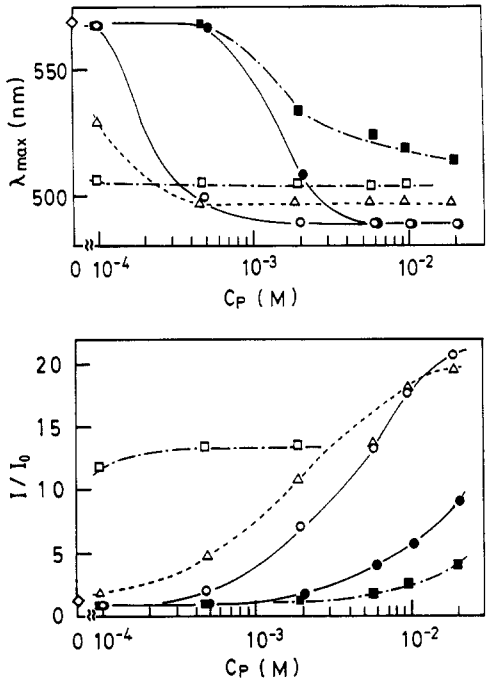

Figure 18. λ_{max} and I/I_0 as a function of the concentration of Bz-75 at different pHs. Open symbols, DNS-Gly; filled symbols, DNS-Eth. pH: (○) 9.1 (Tris buffer, $\mu = 0.04$); (△) 7.2 (Tris buffer, $\mu = 0.04$); (□) 5.6 (pure water, $\mu = 0.04$).

Table VI
pH Dependence of λ_{max} for DNS-Gly/PAA Derivative and DNS-Labeled PAA Derivative Systems

system	λ_{max},[a] nm at pH 9.1[b]	7.2[b]	5.6[c]
DNS-Gly/C_{12}-18	515	520	520
DNS-C_{12}-18	508	517[d]	520
DNS-Gly/Bz-75	490	498	504
DNS-Bz-75	487[e]	498	505

[a] $C_p = 1.0 \times 10^{-2}$ M, at 25 °C. [b] Tris buffer ($\mu = 0.04$). [c] Pure water ($\mu = 0$). [d] $C_p = 3.0 \times 10^{-4}$ M. [e] $C_p = 2.0 \times 10^{-3}$ M.

Table VII
pH Dependence of Fluorescence Parameters for DNS-Gly or DNS-Eth/PAA Derivative System

polymer	probe	pH	λ_{max},[a] nm	K_1,[b] M^{-1}	I_{max}/I_0
C_{12}-18	DNS-Gly	9.1[c]	515	410 ± 30	13.3 ± 0.7
		7.2[c]	520	640 ± 40	13.3 ± 0.7
		5.6[d]	520	61000 ± 8000	13.7 ± 0.7
	DNS-Eth	9.1[c]	520	43 ± 5	9.6 ± 0.7
		5.6[d]	523	72 ± 25	10.1 ± 1.1
Bz-75	DNS-Gly	9.1[c]	490	200 ± 30	24 ± 3
		7.2[c]	498	640 ± 40	20.4 ± 0.7
		5.6[d]	504	67000 ± 3000	13.3 ± 0.2
	DNS-Eth	9.1[c]	490	40 ± 4	20 ± 2
		5.6[d]	520	25 ± 12	10 ± 4

[a] $C_p = 1.0 \times 10^{-2}$ M, at 25 °C. [b] Binding constant. [c] Tris buffer ($\mu = 0.04$). [d] Pure water ($\mu = 0$).

loosen because of the electrostatic repulsion, enabling the inclusion of the probe molecules. In contrast, for benzylated PAA the adaptability of the side group and the probe molecule is expected to be sterically good, even at pH 9.1.

The effect of the charged group of the probe molecules on the polarity and the inclusion ability of the domain can be derived from Figures 17 and 18. It can be seen in Table VII that for DNS-Gly the polarity increases with decrease in pH, while the measure of inclusion ability, K_1, increases. This is attributable to the additional contribution of electrostatic attraction to the inclusion of small molecules.

In the case of uncharged DNS-Eth, K_1 for C_{12}-18 increases with lowering pH but it decreases for Bz-75. On the other hand, λ_{max} and I_{max}/I_0 change in a similar manner to DNS-Gly, indicating the same type of location of the probes in the domain, irrespective of their charged group. These observations are explained as follows: the domain core of C_{12}-18 loosens with decrease in pH, which makes it easy to include DNS-Eth, while that of Bz-75, which is inherently less compact than alkylated PAA, loosens too much, which results in decreasing the inclusion ability. Thus in the inclusion of small molecules, in which hydrophobic interactions play the sole role, the domain structure formed by the alkyl and benzyl groups in PAA is perturbed differently by the change in pH.

From the fluorimetric results together with the data obtained by surface tension and viscosity measurements, it can be concluded that long-chain alkyl- and benzyl-substituted polycations, poly(allylamine) derivatives, are amphiphilic and form a compact micellar conformation in aqueous solution. Both the pendant groups covalently linked to PAA and pH-dependent conformational change of the polymer are found to affect the inclusion ability of the hydrophobic domain. Further work on these polymer micelles by means of pulse fluorometry and ESR technique will be described in a subsequent paper.

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Registry No. PAA (homopolymer), 30551-89-4; C_4-Br, 134288-31-6; C_8-Br, 101321-16-8; C_{12}-Br, 101321-17-9; Bz-Cl, 134288-32-7; DNSA, 21263-87-6; PNSA, 134288-27-0; TNS, 53313-85-2; DNS-Gly, 134288-28-1; DNS-Pip, 134288-29-2; DNS-Tau, 134288-30-5; DNS-Eth, 5282-89-3; DNS-C_{12}-18, 134288-33-8; DNS-Bz-75, 134288-34-9.