Self-Organization of Poly(allylamine)s Having Hydrophobic Groups and Its Effect on the Interaction with Small Molecules. 2. Fluorescence Lifetime and ESR Techniques[1]

Toshihiro Seo,* Seiji Take,† Takayuki Akimoto, Kunihiro Hamada,‡ and Toshiro Iijima§

Department of Polymer Science, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152, Japan

Received December 31, 1990; Revised Manuscript Received April 9, 1991

ABSTRACT: Nanosecond fluorescence lifetime and ESR techniques were used to investigate the interaction of poly(allylamine)s (PAA) containing long alkyl or benzyl groups with fluorescent probes in an aqueous solution. The fluorescence decay curve was deconvoluted into two components: lifetimes τ_1 = 14–22 ns and τ_2 = 3–5 ns are assigned to the probes in the hydrophobic sphere of the amphiphilic polycation and in bulk water, respectively. The rotational correlation times (τ_R) for the spin probe were also obtained as greater than 10 ns and approximately 0.1 ns, respectively. The compact polymer domain is formed in a low concentration range (approximately 10^{-4} M) and keeps its structure up to the higher concentration range. The values of τ_1 increase with an increase in the chain length and the content of substituent groups. This result suggests that the lower the polarity of the domain, the greater the restriction of the probe located in the domain. The dodecylated PAA has a hard core in the interior of the domain, which loosens with a decrease in pH. On the other hand, the domain structure of the benzylated PAA has better sterical adaptability for probe molecules. The mobility of the probe was estimated to be lower in such a polymer assembly than in micelles formed from small surfactants.

Introduction

It is well-known that the various functions of the biosystem are based on the highly organized molecular assembly, which provides the fields of inclusion of the substrate and the subsequent reaction. Recently, such a molecular assembly was designed to provide a reaction field of a photoinduced electron-transfer reaction in a photoenergy transformation process. Examples of such systems are simple micelle, bimolecular membranes, liposome and LB (Langmuir–Blodgett) membranes, and so on.[2-6] In the amphiphilic polyelectrolytes a compact organized structure is formed when hydrophobic interaction of the side chains exceeds the electrostatic repulsion of the segment charge. In such polycations, dodecylated poly(4-vinylpyridine) (DQPVPy), the so-called "polysoap", which forms a compact intramolecular micelle structure, is known as a model for a globular protein in an aqueous solution.[7-9] In our previous work, the mechanism of the interaction between the polysoap and dye molecules and the mobility of probe molecules were examined by visible adsorption spectroscopy[10] and by the ESR technique.[11] As a result, it is estimated that the microenvironment of the polysoap is rigid and highly hydrophobic, and the motion of small molecules in it is quite restricted. It has also been reported that hydrolysis and decarboxylation reactions are accelerated by the addition of the polysoap.[12,13]

Klotz et al. found that the introduction of long alkyl hydrophobic groups to the branched poly(ethyleneimine) (b-PEI) with primary, secondary, and tertiary amino groups gives a large binding ability, comparable to that of bovine serum albumin (BSA) and a high catalytic activity in ester hydrolysis, similar to enzyme activity.[14-19] Sisido et al. investigated the dynamics of such polymers by means of the spin-labeled ESR method and fluorescence spectroscopy. It was concluded that those polymers have two kinds of domains: the hydrophobic cluster of low molecular mobility and the surrounding region of higher mobility, and the modified b-PEI binds the hydrophobic molecules more effectively than low molecular micelles.[20] Pshezhetskii et al. observed the high hydrolysis activity of the linear poly(ethyleneimine) with only the secondary amino group (l-PEI) when the long alkyl chain and benzyl groups are incorporated within it.[21,22]

In this context, poly(allylamine) (PAA)[23-25] is thought to be a promising material in which various substituent groups can be introduced through chemical modification, yielding a variety of functions. We have already reported that PAA has a greater ability for ester hydrolysis than b-PEI, and the introduction of β-cyclodextrin into PAA produces both selectivity and catalytic activity of the specific substrates.[26-28] As alkylated PAA produced considerable acceleration, it was thought that the inclusion of small substrate molecules was enhanced by the domain formed from alkyl side groups.[27-29]

In this connection, we prepared PAA derivatives containing various alkyl chains and benzyl groups with different degrees of substitution and investigated the interaction between such amphiphilic polymers and fluorescent probes using static fluorescence spectroscopy.[30] The results revealed that the polymer had high surface activity and formed a compact organized structure in an aqueous solution. Further, information was obtained not only in regards to the structure of the hydrophobic microdomain thus formed but also about the effects of the side chain and its contents on the polarity and the inclusion ability of small molecules.

In this work, the nanosecond fluorescence lifetime was determined and ESR measurements were carried out to obtain the more detailed information about the hydrophobic domain and, especially, the molecular mobility of small molecules in it. An examination of the results will shed more light on the whole picture of the molecular assembly consisting of PAA containing hydrophobic groups.

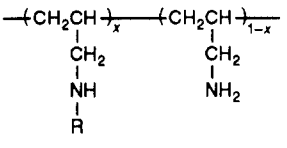
† Present address: Central Research Laboratories, Dai Nippon Printing Co., Ichigaya, Shijuku-ku, Tokyo 162, Japan.
‡ Present address: Department of Material Creation Chemistry, Shinshu University, Tokida, Ueda, Nagano 386, Japan.
§ Present address: Department of Textiles, Jissen Women's University, Oosakaue, Hino 191, Japan.

0024-9297/91/2224-4801$02.50/0

Experimental Section

Materials. Poly(allylamine) hydrochloride (PAA·HCl: $\bar{M}_w$ = 10 000), a scaly solid, donated by Nitto Boseki Co., was used after repeated precipitation from water–methanol.[26] Purification of dodecyltrimethylammonium bromide (DTABr), cetyltrimethylammonium (CTABr), and dodecylamine hydrochloride (DACl), has been described previously.[30] The buffer solution was prepared from 2-amino(hydroxymethyl)-1,3-propanediol (Nakarai Chemicals Co., analytical grade).[26,30] Methanol, ethanol, dioxane, and ethylene glycol were used after distillation.

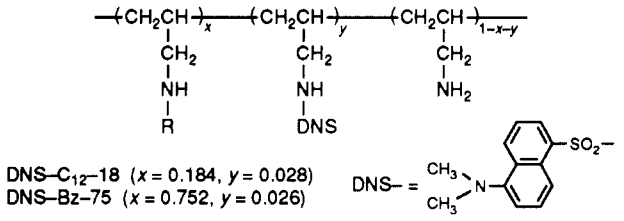
Poly(allylamine) Derivatives. PAA derivatives were prepared by the method described in our previous report.[27,30] Their chemical structure is shown in structure I. The polymer is



PAA (R = H)
R-X (R = C_4, C_8, C_{12}, Bz)

I

abbreviated as R-X, where "R" = *n*-butyl (C_4), *n*-octyl (C_8), *n*-dodecyl (C_{12}), or benzyl (Bz) groups and "X" denotes the mole percent of the substituted group (R) in a monomeric unit. The fluorescent labeled PAA (flPAA), DNS-C_{12}-18 and DNS-Bz-75 (structure II), has the same meaning as in the preceding paper.[30]

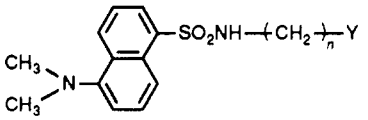


DNS-C_{12}-18 (x = 0.184, y = 0.028)
DNS-Bz-75 (x = 0.752, y = 0.026)

II

The content of the dansyl residue (DNS) is ca. 2 mol %.

Fluorescent Probes. The four dansylamino acid fluorescent probes used have the structural formulas shown in structure III. They are the same as described in our previous work.[30] DNS-



DNS-Gly (Y = COONa, n = 1)
DNS-Pip (Y = COONa, n = 3)
DNS-Tau (Y = SO_3Na, n = 2)
DNS-Eth (Y = OH, n = 2)

III

Gly, DNS-Pip, and DNS-Tau are different in their ionizing group and spacer length. DNS-Eth has no ionizing group.

Fluorescence Lifetime Measurement. The nanosecond fluorescence lifetime was determined at 25 °C with a Lifetime FS901 apparatus (Union Giken Co.), and a single-photon-counting method was used.[31,32] Prior to measurement, pure nitrogen gas was bubbled through the sample solution for 15 min to remove dissolved oxygen. The probe concentration [(4.88–6.05) × 10^{-6} M] was determined spectrophotometrically using ϵ_{328} = 4.60 × 10^2 $m^2 \cdot mol^{-1}$. An Ortec 9352 nanosecond light pulser was used as the light source. The samples were excited at 337 nm, and the emission was monitored at greater than 380 nm.

The results were analyzed by using eq 1, assuming two components.

$$F(t) = \alpha \exp(-t/\tau_1) + (1 - \alpha) \exp(-t/\tau_2) \quad (1)$$

$F(t)$ is the fluorescence decay, τ_1 and τ_2 are the lifetimes of species 1 and 2, respectively. α is the fraction of species 1. Curve fitting of the data with eq 1 by the nonlinear least-squares method gave the lifetime parameters. In the one-component system, the second term of eq 1 disappears.

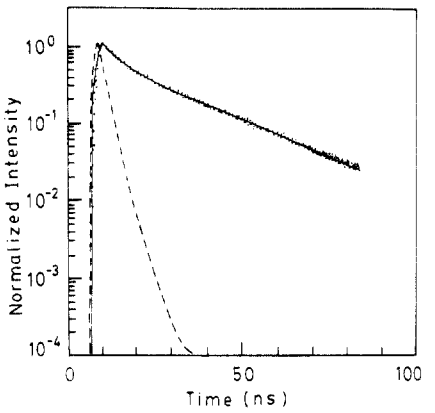
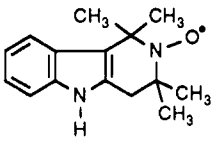


Figure 1. Typical fluorescence decay for a DNS-Eth/Bz-75 system in Tris buffer solution at 25 °C. C_p = 1.0 × 10^{-2} M; pH 9.2; μ = 0.04. The dashed line shows the excitation pulse profile. The solid line represents the optimal biexponential fit defined by τ_1 = 23.2 ns, τ_2 = 4.5 ns, α = 0.43 (see text).

An example of deconvolution into two components is illustrated in Figure 1. The ordinate and the abscissa indicate the fluorescence intensity and the time, respectively. The calculated line with the parameters given in the caption is in close agreement with the observed data for a DNS-Eth/Bz-75 system. In the case of C_p = 0, the decay curves for the four probes in the buffer solution were found to be best fitted with a single-exponential function with $\tau \doteq 3$ ns.

The static fluorescence spectra were measured with a Shimadzu RF-502 spectrofluorophotometer as reported previously.[30] The UV-visible absorption spectra were recorded on a Hitachi 556 double-beam spectrometer.

ESR Spectra. The spin probe SPII, kindly supplied by Professor McGregor, of type IV, a stable *N*-oxide radical, was used.[11,33] ESR spectra were obtained with a JEOL-FE3X ESR



IV

spectrometer as an X-band. The data analysis was carried out in the usual way.[11,33-35] To avoid spin–spin interaction, the concentration of the spin probe was kept at 1.0 × 10^{-4} M. The concentration of the polymer (C_p) and surfactants were fixed at 1.0 × 10^{-2} M.

Results and Discussion

(1) Fluorescence Lifetime. Effect of Polymer Concentration. Figure 2 shows the fluorescence lifetimes of various probes in a buffer solution of alkylated PAA. For the probes with an ionizable group, in both C_{12}-18 and Bz-75 systems, two lifetime components, τ_1 (16–22 ns) and τ_2 (3–7 ns) were obtained in the concentration range 1.0 × 10^{-4}–2 × 10^{-2} M. On the other hand, the uncharged probe, DNS-Eth, gave the two components at a higher polymer concentration than 10^{-3} M.

As the values of τ_2 are approximately the same as the lifetime in the absence of the polymer in a buffer solution, the τ_2 component corresponds to the probe molecules in bulk water, while the τ_1 component corresponds to that in the hydrophobic domain formed by the amphiphilic polyelectrolyte. $\tau_1 > \tau_2$ reflects the mobility of the probe molecules in the two phases. Furthermore, the constancy of τ_1 values with respect to polymer concentration indicates that the molecular assembly formed from the side chain

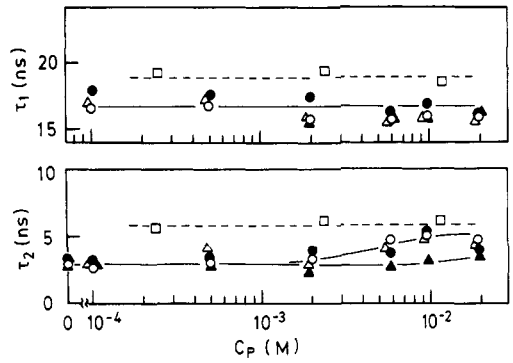


Figure 2. τ at various concentrations of C_{12}-18 in Tris buffer solution; pH 9.1; μ = 0.04. (○) DNS-Gly; (●) DNS-Pip; (△) DNS-Tau; (▲) DNS-Eth; (□) DNS-C_{12}-18.

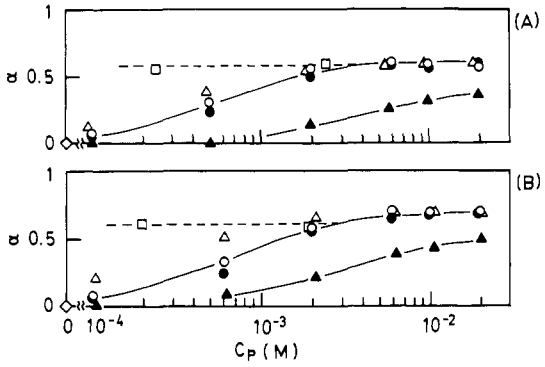


Figure 3. α at various concentrations of C_{12}-18 (A) and Bz-75 (B) in Tris buffer solution; pH 9.1; μ = 0.04. (○) DNS-Gly; (●) DNS-Pip; (△) DNS-Tau; (▲) DNS-Eth; (□) DNS-C_{12}-18 or DNS-Bz-75.

of the polymer is essentially unchanged over the polymer concentration range in this experiment.

A comparison of lifetimes of the four probes shows no clear differences, indicating that the degree of restriction practically does not depend on the ionizable group and the spacer methylene length in the probes. For every probe, τ_1 was found to be larger in Bz-75 than in C_{12}-18. This is consistently explained by the domain of the former having a better sterical adaptability for the inclusion of small molecules. This view is supported by the result obtained from the static fluorescence measurement on the same polymer systems.[30]

The component α, the fraction of τ_1, increases with an increase in the polymer concentration, for both C_{12}-18 and Bz-75 (Figure 3). This result indicates a shift of the partition of the fluorescent probe molecules from bulk water to the domain. The concentration of approximately 10^{-3} M, at which α increases in the case of DNS-Eth, accords with the results of the λ_{max} shift and the increase in the fluorescence intensity.[30] The smaller value of K_1 (the primary binding constant) of DNS-Eth, than of the other three probes, gives additional proof.[30] Thus, it is concluded that the electrostatic interaction between the primary amino cation of the polymer and the anionic probe plays an important role, in addition to hydrophobic interaction, in the inclusion of probe molecules in the domain.

It is interesting to note that α tends to saturate at the high polymer concentration with three charged probes. This may be connected with the fact that τ_2 increases by 2–4 ns in the high concentration range of the polymer. Here, in addition to the two phases, the hydrophobic domain giving τ_1 and the bulk water giving $\tau_2 \doteqdot 3$ ns, the existence of a "submicrodomain" is proposed. In the low

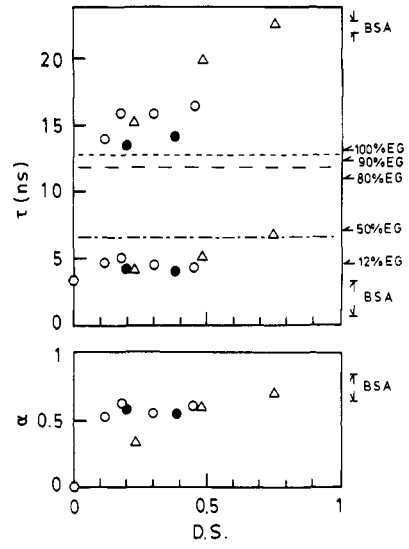


Figure 4. Effect of DS on τ and α for DNS-Gly in Tris buffer solution. $C_p = 1.0 \times 10^{-2}$ M; pH 9.1; μ = 0.04. (●) C_8; (○) C_{12}; (△) Bz; (– –) DTABr micelle; (- - -) CTABr micelle; (– · –) DACl micelle. Data for BSA are cited from ref 31.

concentration range the probes should be included by this submicrodomain, but the amount of inclusion has no effect on τ_2 as an averaged value. The increase of the amount of inclusion with an increase in the polymer concentration is believed to affect τ_2.

Fluorescent Labeled Polymer. The lifetimes and α values of flPAA, DNS-C_{12}-18, and DNS-Bz-75 systems were given in Figures 2 and 3. In each case the decay curves were deconvoluted into two components, τ_1 of 19–22 ns and τ_2 of 5–7 ns, just like in the fluorescent probe systems. This suggests the presence of a pendant fluorophore group in the polymer that is exposed to the bulk water.

The values of τ_1, τ_2, and α are practically independent of the polymer concentration. This fact means that the covalently bound fluorophore in flPAA is included in the hydrophobic domain of the polymer at low concentration. Comparing flPAA with the fluorescent probe system for C_{12}-18, we found that τ_1 of flPAA is 2 ns larger than that of the probe system. The argument that the labeled fluorophore is restricted much more and located mainly in the inner domain agrees with our previous results.[30] In regards to the Bz-75 system, τ_1 is the same in both cases. This can again be explained by the steric adaptability of benzylated PAA, as proposed in our previous paper.[30]

Effect of Chain Length and Degree of Substitution. The dependence of τ_1 and α on the degree of substitution (DS) in DNS-Gly/PAA derivative systems is shown in Figure 4. An increase in DS leads to an increase in τ_1, irrespective of the substituent group, C_8, C_{12}, and Bz. These results are the same as those obtained previously by static fluorescence probing[30] and are explained in the same context. In Figure 4 τ_1 values for various polymers are compared with the values in both an ethylene glycol (EG)–water mixture and a low molecular surfactant micelle. In every polymer system, the values are greater than that of pure EG ($\eta \doteqdot 20$ cP), the viscosity of which is at the same level as the surface viscosity of the surfactants,[36] suggesting that the probes exist in an environment of high motional restriction. The values of τ_1 are greater in the modified PAA than in the micelles of the surfactants, DTABr, CTABr, and DACl. The local environment of the domain formed by PAA derivatives is supposed to show a higher restriction of the probe molecules, as compared with the hydrophobic sphere of the surfactant micelle. These

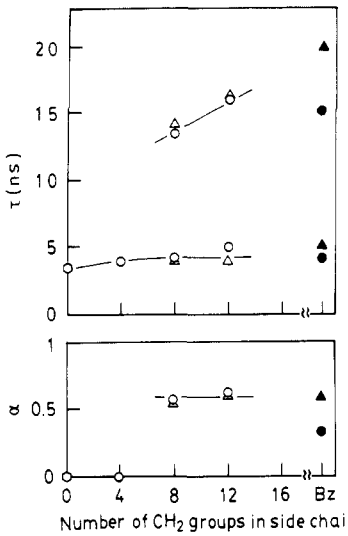


Figure 5. Effect of chain length on τ and α for DNS–Gly in Tris buffer solution. $C_p = 1.0 \times 10^{-2}$ M; pH 9.1; $\mu = 0.04$. Open symbols: alkyl–PAA. Filled symbols: Bz–PAA. (○, ●) DS ≒ 0.2; (▲, △) DS ≒ 0.4.

observations are consistent with our previous argument based on the static fluorometry. We found that in the polysoap (DQPVPy) system the restriction of the spin probe molecule in its assembled domain is greater than that in the micelle formed by dodecylpyridinium bromide (DPBr).[11] DPBr is the low molecular compound corresponding to DQPVPy.

τ_2 concerning the submicrodomain is about 5 ns for the polymer with a DS of less than 0.5, which corresponds to 12% of the EG system (η ≒ 2 cP). The higher τ_2, for Bz-75 (approximately 7 ns), which corresponds to 50% of the EG system (η ≒ 7 cP), may be ascribed to the formation of a thick layer of the submicrodomain that is due to the high DS. In the case of the C_{12}–PAA system, the values of α are approximately constant, irrespective of the DS, indicating the formation of the hydrophobic domain, which has a high ability of inclusion with a restricted mobility of included molecules. On the other hand, in the case of the Bz–PAA system, the molecular assembly is supposed to exhibit low polarity and high inclusion ability at higher DS. These arguments agree with the results of our previous work.[30]

Figure 5 gives the effect of chain length on lifetime. The C_4–PAA derivative can be analyzed by a single component, as unsubstituted PAA, and τ is a little larger than that in a buffer solution, suggesting the contribution of the submicrodomain. The longer chain length leads to two components, τ_1 and τ_2, and the increasing chain length gives the increase in τ_1. Comparing alkylated and benzylated PAA, we found that τ_1 has the same value at DS = 0.2, indicating that the degree of restriction of the probe is at the same level. The larger value of τ_1 in Bz–PAA of DS = 0.4 is attributed to the sterical adaptability between the polymer substituents, as discussed before.

Effect of pH. The effects of pH on the fluorescence lifetime of probe/PAA and flPAA systems are discussed when considering the results of static fluorescence spectroscopy.[30] Figure 6 shows the result for DNS–Gly/C_{12}-18 in pH 9.1 and 7.2 buffer solutions and an aqueous solution (pH 5.6). τ_1 and τ_2 for alkyl- and benzyl–PAA give approximately constant values with an increase in the polymer concentration and pH has practically no effect. On the other hand, on flPAA (Figure 7), τ_1 decreases with a decrease in pH for DNS–C_{12}-18, but τ_2 of DNS–C_{12}-18 and τ_1 and τ_2 of DNS–Bz-75 show no pH dependence. In

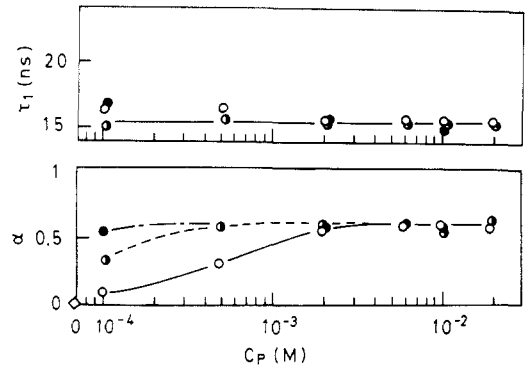


Figure 6. pH dependence of τ and α for DNS–Gly at various concentrations of C_{12}-18. (○) pH 9.1 Tris buffer solution (μ = 0.04); (◑) pH 7.2 Tris buffer solution (μ = 0.04); (●) aqueous solution (pH 5.6).

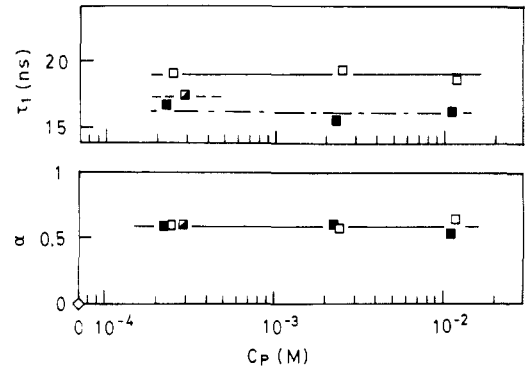


Figure 7. pH dependence of τ and α for DNS–C_{12}-18 at various concentrations. (□) pH 9.1 Tris buffer solution (μ = 0.04); (◪) pH 7.2 Tris buffer solution (μ = 0.04); (■) aqueous solution (pH 5.6).

Table I
τ_1 and λ_{max} of DNS–Gly/PAA Derivative and DNS-Labeled PAA Derivative Systems[a]

	τ_1, ns (λ_{max}, nm)		
	Tris buffer		
solvent system	pH 9.1, μ = 0.04	pH 7.2, μ = 0.04	water: pH 5.6
DNS–Gly/C_{12}-18	15.9 (515)	15.7 (520)	15.1 (520)
DNS–C_{12}-18	18.3 (508)	17.4[b] (517)[b]	16.2 (520)
DNS–Gly/Bz-75	22.7 (490)	23.0 (498)	22.0 (504)
DNS/Bz-75	21.7[c] (487)[c]	21.7 (498)	20.6 (505)

[a] $C_p = 1.0 \times 10^{-2}$ M, at 25 °C. [b] $C_p = 3.0 \times 10^{-4}$ M. [c] $C_p = 2.0 \times 10^{-3}$ M.

Table I, τ_1 of DNS–Gly/PAA and flPAA systems at the polymer concentration 1×10^{-2} M and λ_{max} are given.

λ_{max} shifts to the longer wavelength with a decrease in pH, indicating an increase of the polarity in the case of alkylated PAA. Lowering the pH may expand the structure of the polymer chain and therefore decrease the restriction of the fluorophore moiety of flPAA, which results in a decrease of τ_1. On the other hand, in the case of the probe systems, probe molecules reside near the outer side of the compact hydrophobic domain with a loose restriction; hence, τ_1 has a relatively small value. A decrease in pH loosens the hard core of the domain, enabling the inclusion of the probe into the domain, and, as a result, the total effects, the location, the polarity, and the degree of the restriction of the probe and the labeled fluorophore attain the same level.

In every system the parameters of Bz–PAA have almost the same values. At a low pH, λ_{max} indicates an expanded structure of the polymer, but there is little difference in

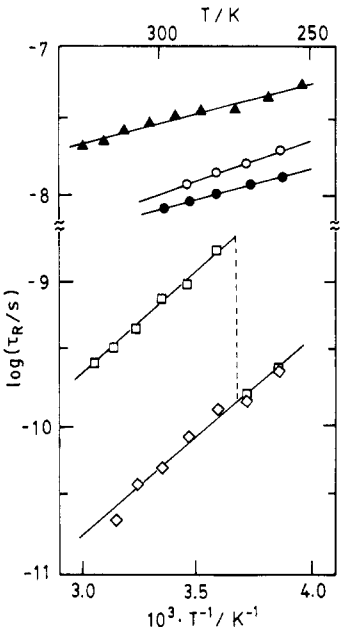


Figure 8. Arrhenius plots of τ_R for SPII in various systems. (◇) Tris buffer solution; (□) Tris buffer solution of CTABr; (○) Tris buffer solution of C_{12}-18; (●) aqueous solution of DQPVPy; (▲) C_{10}P4VP membrane. Data for DQPVPy and C_{10}P4VP are taken from ref 11.

τ_1. These results suggest that, in the domain formed by the benzyl side chain, the naphthalene moiety is included in the interior of the domain with a high sterical adaptability and is restricted by the hydrophobic and electrostatic interactions.

The change of α values of DNS–Gly/PAA systems with a change in pH is shown in Figure 6. The lower the pH, the greater the change in α at lower concentrations. This means that the contribution of the electrostatic interaction of the anionic probe with the primary amino cations is large at the lower concentrations, leading to an increase in inclusion. This finding is consistent with the drastic increase in the K_1 value, indicative of the binding ability of the probe, in the sequence pH 9.1 < pH 7.2 ≪ water.[30]

(2) ESR Spectra. ESR spectra of the spin probe, SPII, were measured in a pH 9.1 buffer solution, and the correlation time of the rotation τ_R was calculated by the usual method.[11,33-35]

The presence of C_{12}-18 was found to give a spectral component of a lower mobility of the spin probe, which is superimposed by the faster component. The former component is ascribable to the probe molecules included in the hydrophobic domain,[11] while the latter component was confirmed in the bulk solution, with coinciding τ_R values in both phases.

The Arrhenius plot of the component with the slower τ_R in the C_{12}-18 system is given in Figure 8. The figure shows that the mobility of the probe in the hydrophobic domain formed by alkylated PAA is restricted as compared with the bulk solution, in which τ_R is 2 orders higher. For comparison, τ_R of the same probe in dodecylated poly(4-vinylpyridine) (DQPVPy) is given in the same figure.[11]

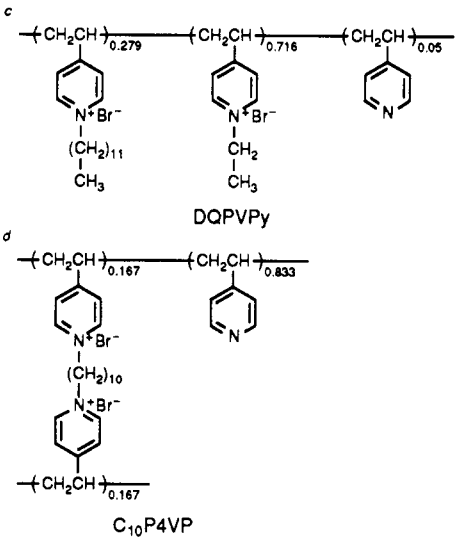
τ_R values of the C_{12}-18 system are slightly larger (but of the same order) than that of polysoap, suggesting a slightly lower mobility of the spin probe in the former system. The results of ESR investigations of spin-labeled quaternized (partially dodecylated) b-PEI[20] suggested that there are two domain components consisting of two layers, one, a hydrophobic cluster in the core with $\tau_R \doteq 1$ ns, and, the other, a region of the same segmental mobility of the main polymer chain, with $\tau_R \doteq 0.1$ ns. In comparison with the former value, τ_R of C_{12}-18 (greater than 10 ns) is 1 order higher, which corresponds to the value of the total rotational motion of the globular protein molecule.[20] This result indicates that the hydrophobic domain structure of C_{12}–PAA has a much higher degree of restriction of the probe molecules than that of C_{12}–PEI. In comparison of DQPVPy, which has the same level of restriction as C_{12}-18, with C_{12}–PEI, the former shows a higher value of τ_R. As both these polymers contain the same charged group, a loose structure due to branching of the latter is suggested.

τ_R in the micelle of the surfactant CTABr, as shown in Figure 8, is 1 order larger than that in bulk water, but 1 order smaller than that in the C_{12}-18 system. This result indicates a compact domain structure of the latter system in which the small molecules are restricted in their motion. This argument accords with the results of the fluorescence lifetime discussed above.

Table II
Activation Energies for Rotation in Various Systems

system	E_a^R,[a] kJ·mol^{-1}
buffer solution	26.0 ± 2.0
buffer solution of CTABr	27.0 ● 1.2
buffer solution of C_{12}-18	11.5 ● 0.6
aqueous solution of DQPVPy[c]	8.7 ± 1.6[b]
C_{10}P4VP membrane[d]	8.2 ● 1.6[b]

[a] For SPII used as the spin probe. [b] From ref 11.



The rotational activation energies, E_a^R, calculated from the Arrhenius plot, are given in Table II. The values in the buffer solution and surfactant micelle are similar, indicating the same mode of motion in both systems. On the other hand, in the C_{12}-18 system, the E_a^R value is close to that in the poly(4-vinylpyridine) quaternized membrane (C_{10}P4VP) in our previous report, suggesting a large restriction of the motion of SPII as in the polysoap (DQPVPy) system[11] and nylon 6 film at a low temperature.[34] The mode of the motion is believed to be vibrational.[11] Thus, it is concluded that the hydrophobic domain formed from C_{12}-18 is highly compact and rigid, similar to the solid membranelike nylon 6 film.

Now let us estimate the inclusion ability of the probe small molecules by means of eq 2; the spectral intensity is expressed as[37]

$$I = C2Y_{max}'(\Delta H_{pp})^2 \quad (2)$$

where I is the approximate relative intensity, $2Y_{max}'$ the peak-to-peak derivative amplitude, and ΔH_{pp} the peak-to-peak width. When we use the ratio of the values for

I for the two components, fast and slow, for the SPII/C_{12}-18 system, the fraction of the latter component is estimated to be 10/11; that is, the hydrophobic spin probe, SPII, is almost completely included in the hydrophobic domain. This result coincides with that obtained by the fluorescent probe technique.[30]

In both photophysical and ESR investigations the characteristics of the amphiphilic poly(allylamine) derivatives in aqueous solution were thus revealed. The polymer assembly is expected to have an enzymelike function in certain substrate inclusions and a resulting catalytic action. Further work on this line is in progress.

Acknowledgment. We are deeply indebted to Prof. Dr. Hiroshi Tomiyasu and Dr. Masayuki Harada of the Research Laboratory for Nuclear Reactors, Tokyo Institute of Technology, for the ESR measurements.

References and Notes

(1) Part 1 of this series is ref 30.
(2) Miyashita, T.; Murakata, T.; Yamaguchi, Y.; Matsuda, M. *J. Phys. Chem.* **1985**, *89*, 497.
(3) Meisel, D.; Rabani, J.; Meyerstein, D.; Matheson, M. S. *J. Phys. Chem.* **1978**, *82*, 985.
(4) Thomas, J. K. *Chem. Rev.* **1980**, *80*, 283.
(5) Delaire, J. A.; Rodgers, M. A.; Webber, S. E. *J. Phys. Chem.* **1984**, *88*, 6219.
(6) Morishima, Y.; Kobayashi, T.; Furui, T.; Nozakura, S. *Macromolecules* **1987**, *20*, 707.
(7) Strauss, U. P.; Gershfeld, N. L. *J. Phys. Chem.* **1954**, *58*, 747.
(8) Inoue, H. *Kolloid Z. Z. Polym.* **1964**, *195*, 102.
(9) Jorgensen, H. E.; Strauss, U. P. *J. Phys. Chem.* **1961**, *65*, 1873.
(10) Ando, Y.; Otonari, Y.; Koshii, T.; Komiyama, J.; Iijima, T. *Nippon Kagaku Kaishi* **1980**, 263; *Chem. Abstr.* **1980**, *92*, 147459q.
(11) Makino, Y.; Hamada, K.; Iijima, T. *Polym. J.* **1987**, *19*, 737.
(12) Rodulfo, T.; Hamilton, J. A.; Cordes, E. H. *J. Org. Chem.* **1974**, *39*, 2281.
(13) Kunitake, T.; Shinkai, S.; Hirotsu, S. *J. Org. Chem.* **1977**, *42*, 306.
(14) Klotz, I. M.; Stryker, V. H. *J. Am. Chem. Soc.* **1968**, *90*, 2717.
(15) Royer, G. P.; Klotz, I. M. *J. Am. Chem. Soc.* **1969**, *91*, 5885.
(16) Klotz, I. M.; Royer, G. P.; Sloniewsky, A. R. *Biochemistry* **1969**, *8*, 4752.
(17) Klotz, I. M.; Royer, G. P.; Scarpa, I. S. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 263.
(18) Johnson, T. W.; Klotz, I. M. *Macromolecules* **1973**, *6*, 788.
(19) Kiefer, H. C.; Congdon, W. I.; Scarpa, I. S.; Klotz, I. M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 2155.
(20) Sisido, M.; Akiyama, K.; Imanishi, Y.; Klotz, I. M. *Macromolecules* **1984**, *17*, 198.
(21) Pshezhetskii, V. S.; Lukyanova, A. P.; Kabanov, V. A. *J. Mol. Catal.* **1977**, *2*, 49.
(22) Pshezhetskii, V. S.; Nikolaev, G. M.; Lukyanova, A. P. *Eur. Polym. J.* **1977**, *13*, 423.
(23) Harada, S.; Hasegawa, S. *Makromol. Chem., Rapid Commun.* **1984**, *5*, 27.
(24) Kobayashi, S.; Tokunoh, M.; Saegusa, T.; Mashio, F. *Macromolecules* **1985**, *18*, 2357.
(25) Yonetake, K.; Seo, T.; Iijima, T. *J. Polym. Sci., Polym. Phys. Ed.* **1990**, *28*, 303.
(26) Seo, T.; Kajihara, T.; Iijima, T. *Makromol. Chem.* **1987**, *188*, 2071.
(27) Seo, T.; Kajihara, T.; Iijima, T. *Makromol. Chem.* **1990**, *191*, 1665.
(28) Seo, T.; Kajihara, T.; Miwa, K.; Iijima, T., to be submitted for publication.
(29) Ikeda, I.; Suzuki, K.; Takeuchi, S. *Kobunshi Ronbunshu* **1986**, *43*, 59.
(30) Seo, T.; Take, S.; Miwa, K.; Hamada, K.; Iijima, T., submitted for publication in *Macromolecules*.
(31) Seki, T.; Komiyama, J.; Iijima, T.; Wild, U. P. *Colloid Polym. Sci.* **1984**, *262*, 311.
(32) Wild, U. P.; Holzwarth, A. R.; Good, H. R. *Rev. Sci. Instrum.* **1977**, *48*, 1621.
(33) McGregor, R.; Iijima, T.; Sakai, T.; Gilbert, R. D.; Hamada, K. *J. Membr. Sci.* **1984**, *18*, 129.
(34) Hamada, K.; Iijima, T.; Mcgregor, R. *Polym. J.* **1985**, *17*, 1245.
(35) Hamada, K.; Iijima, T.; Mcgregor, R. *Macromolecules* **1986**, *19*, 1443.
(36) Turro, N. J.; Okubo, T. *J. Am. Chem. Soc.* **1981**, *103*, 7224.
(37) Wertz, J. E.; Bolton, J. R. *Electron Spin Resonance: Elementary Theory and Practical Applications*; McGraw-Hill: New York, 1972; Chapter 2, p 34.