(14) Matsuo, M.; Ozaki, F.; Sugawara, S.; Ogita, T. *Macromolecules* **1980**, *13*, 1187.
(15) Sawatari, C.; Muranaka, T.; Matsuo, M. *Polym. J.* **1983**, *15*, 33.
(16) Hashimoto, T.; Todo, A.; Kawai, H. *Polym. J.* **1978**, *10*, 521.
(17) Matsuo, M.; Tamada, M.; Terada, T.; Sawatari, C.; Niwa, M. *Macromolecules* **1982**, *15*, 985.
(18) Moritani, M.; Hayashi, N.; Utsuo, A.; Kawai, H. *Polym. J.* **1971**, *2*, 74.
(19) Blundell, D. J. *Acta Crystallogr., Sect. A* **1970**, *A26*, 472.
(20) Blundell, D. J. *Acta Crystallogr., Sect. A* **1970**, *A26*, 476.
(21) Clough, S.; van Aartsen, J. J.; Stein, R. S. *J. Appl. Phys.* **1965**, *36*, 3072.
(22) Hosemann, R.; Bagchi, S. N. "Direct Analysis of Diffraction by Matter"; North-Holland Publishing Co.: Amsterdam, 1962.
(23) Hashimoto, T.; Nagatoshi, K.; Todo, A.; Hasegawa, H.; Kawai, H. *Macromolecules* **1974**, *7*, 264.

Poly(α-amino acids) Carrying Amphiphilic Side Chains. Synthesis, Conformation, Hydrophobic Binding, and Induced Circular Dichroism

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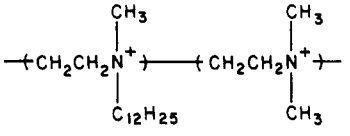
ABSTRACT: Poly(L- and D-glutamine) derivatives carrying hydrophobic groups and quaternized amino groups were prepared. Their circular dichroism indicated the charged-coil conformation in water. The formation of hydrophobic clusters in aqueous solution was evidenced by enhanced excimer formation of 4-(1-pyrenyl)butyric acid in the presence of the amphiphilic poly(α-amino acids). The efficiency of hydrophobic binding by the amphiphilic polymer was much higher than that by low molecular weight detergents and similar to that by quaternized laurylpoly(ethylenimine). Induced circular dichroism was observed when certain cyanine dyes were adsorbed onto the amphiphilic poly(α-amino acids). However, no circular dichroism or circularly polarized fluorescence was detected when chromophores or fluorophores less bulky than the cyanine dyes were mixed with the amphiphilic poly(α-amino acids). It was therefore concluded that the amphiphilic poly(α-amino acids) provide a hydrophobic environment that is achiral in a local region but chiral in a spatially extended region.

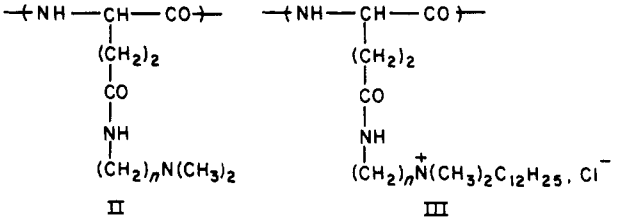
Structures and functions of molecular assemblies of amphiphilic substances in water are of current interest in membrane-mimetic chemistry.[1] Polymers may play an important role in this field, since the main chain may stabilize the assembly structures, which are otherwise unstable physically and chemically. Attempts have been reported recently to reinforce the bilayer structure by a polymeric framework.[2-5] Polymers carrying amphiphilic side chains have been reported previously.[6,7] For example, quaternized laurylpoly(ethylenimine) (I) was shown to bind



I

hydrophobic molecules effectively in aqueous solution.[8] This polymer detergent was found to have the following advantages over low molecular weight detergents. First, smaller amounts of amphiphilic groups are required to bind a given amount of hydrophobic substrate than in the case of low molecular weight detergents. This property of polymer detergents leads to a higher effective concentration of substrate taken up in the hydrophobic cluster. Second, the amphiphilic polymer forms micelle-like clusters even at the lowest concentration examined (10^{-6} M with respect to the lauryl group). This means that the polymer detergent has virtually no critical concentration at which the hydrophobic cluster begins to appear. Third, the amphiphilic polymer can be recovered by ultrafiltration, which is especially important from a practical point of view.

Since the main chain of poly(ethylenimine) is highly branched and has a randomly coiled conformation, the hydrophobic cluster should have no ordered structure. In order to develop novel amphiphilic assemblies that have a regular and chiral structure, we have undertaken a synthesis of poly(α-amino acids) carrying amphiphilic side chains. In this article, the syntheses of poly(L- and D-glutamine) derivatives having cationic side chains (II) and amphiphilic side chains (III) are described. The hydrophobic binding of a pyrenyl chromophore by these polymers was examined on the basis of the excimer formation, and the chirality of the polymeric cluster was studied by the induced circular dichroism of cyanine dyes adsorbed onto the cluster.



II

L-3 ($n = 3$, L isomer)
D-3 ($n = 3$, D isomer)
L-6 ($n = 6$, L isomer)

III

L-3-12 ($n = 3$, L isomer)
D-3-12 ($n = 3$, D isomer)
L-6-12 ($n = 6$, L isomer)

Besides the general advantages of polymeric detergents mentioned above, the amphiphilic poly(α-amino acids) are expected to show the following characteristics. First, the chiral and possibly helical polypeptide main chain may provide a novel amphiphilic structure with a high degree of ordering and chirality, which one may utilize as a medium for stereospecific reactions and interactions. Second,

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since the polypeptide chain is biodegradable, the amphiphilic polymer may be utilized as a biocompatible support for a drug delivery system. In the present investigation our interest has been focused on the first point.

Experimental Section

Synthesis of Poly[N^{ϵ}-(3-(dimethylamino)propyl)-L- and -D-glutamine] (L-3 and D-3). Poly(γ-benzyl L-glutamate) ($M_w \sim 50\,000$, $M_w/M_n \sim 1.8$) (0.5 g) prepared in our laboratory was swollen with 3 mL of chloroform, and 3-(dimethylamino)-propylamine (5 mL) was added dropwise. The mixture was stirred at 60 °C under a nitrogen atmosphere. After the IR absorption characteristic of the ester group (1720 cm^{-1}) disappeared (3 days), the polymer was precipitated with ether and reprecipitated repeatedly from methanol (solvent)/ether (nonsolvent). Elemental analysis showed complete (99%) conversion, and the yield was quantitative. The polymer was soluble in acidic water and alcohols. The D isomer was prepared by the same procedure.

Quaternization of L-3 and D-3 with Lauryl Bromide (Synthesis of L-3-12 and D-3-12). The polymer prepared above (0.35 g) was dissolved in dimethylformamide (12 mL), and lauryl bromide (1.0 mL) was added dropwise. The solution was stirred at 35 °C under a nitrogen atmosphere for 24 h. The solution was poured into ether and purified by repeated reprecipitation with methanol/ether. Elemental analysis indicated nearly quantitative quaternization (98%). No amino group was detected by titration with hydrochloric acid.

The polymer was subjected to ultrafiltration using a Diaflo PM30 membrane against 0.05 M NaCl to remove the bromide ion, which would disturb excimer formation of bound fluorophores. The polymer solution was further purified by ultrafiltration against distilled water and then lyophilized. The yield from L-3 was 80%. The polymer was soluble in water and alcohols. Its molecular weight was estimated to be 3.7×10^4 by gel chromatography (calibration with standard polystyrenes). The D polymer having an approximate molecular weight of 4.5×10^4 was also prepared.

Synthesis of L-6 and L-6-12. These polymers were prepared by a similar procedure using 6-(dimethylamino)hexylamine prepared from 6-aminohexanoic acid, instead of 3-(dimethylamino)propylamine. The reaction of 6-(dimethylamino)hexylamine with poly(γ-benzyl L-glutamate) was quantitative, as shown by elemental analysis. The quaternization of poly[N^{ϵ}-(6-(dimethylamino)hexyl)-L-glutamine] with lauryl bromide proceeded to only 70% conversion. The approximate molecular weight of the quaternized polymer was 3.5×10^4 by gel chromatography (polystyrene standard).

Preparation of poly[N^{ϵ}-(12-(dimethylamino)dodecyl)-L-glutamine] was also attempted. The reaction of poly(γ-benzyl L-glutamate) with 12-(dimethylamino)dodecylamine proceeded up to 70% conversion but the resulting polymer was insoluble in any organic solvents. Subsequent quaternization was therefore impossible.

Other Reagents. 4-(1-Pyrenyl)butyric acid (PBA) (Koch-Light) was recrystallized twice from benzene. Cyanine dyes were purchased from Nippon Kanko Shikiso Co. and used without further purification.[9] Water was distilled twice and used to prepare 0.02 M Tris buffer.

Measurements. Circular dichroism was measured on a Jasco J-20 spectropolarimeter. Fluorescence spectra were recorded on a Hitachi MPF-4 at room temperature. Decay curves of monomer fluorescence were measured with a Hitachi time-resolved photometer. A saturated aqueous solution of $NiSO_4$ was used as a band-pass filter for the excitation light, and a band-pass glass filter was inserted in the emission beam to remove monomer fluorescence. The decay curves were analyzed by a least-squares method to obtain the time constants, taking a finite width of exciting pulse into account. The static and dynamic fluorescence measurements were carried out after nitrogen gas was bubbled through the aqueous solution for 20 min. The fluorescence quantum yield was evaluated with quinine sulfate solution as a standard (q = 0.55). The circularly polarized fluorescence spectrum was recorded on a Jasco FCD-1F instrument.[10]

Results and Discussion

Conformations of the Cationic and the Amphiphilic Poly(α-amino acids). The circular dichroism (CD) spectra of the cationic and the amphiphilic poly(α-amino acids) in water at different pHs and in water/methanol mixture are shown in Figures 1–3. Despite the varying

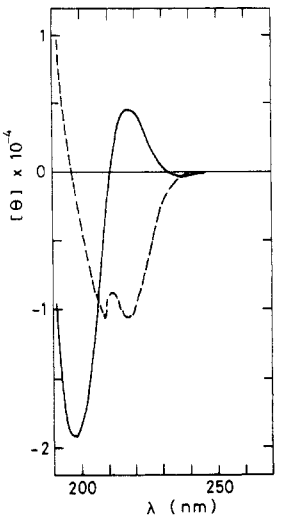


Figure 1. CD spectra of L-3 in aqueous solution at pH 7.2 (—) and pH 10.5 (– – –). [Gln unit] = 1.0×10^{-4} M.

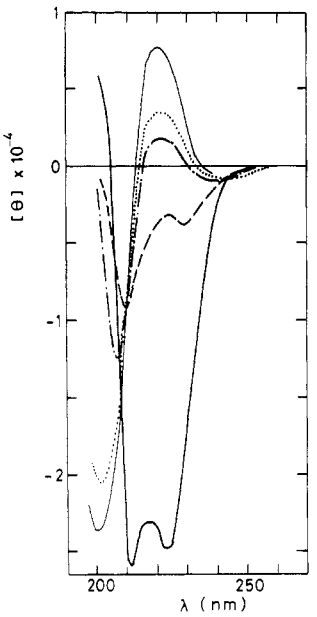


Figure 2. CD spectra of L-3-12 in methanol/water mixture (pH 7.2). Methanol volume fraction: 1.0 (—), 0.9 (– – –), 0.8 (– · –), 0.5 (· · ·), and 0 (—). [Gln unit] = 1.0×10^{-4} M.

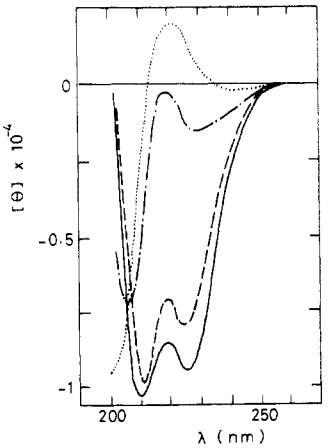


Figure 3. CD spectra of L-6-12 in aqueous solution at pH = 10.0 (—), 9.2 (– – –), 8.5 (– · –), and 7.8 (· · ·). [Gln unit] = 1.0×10^{-4} M.

distances from the polypeptide main chain to the positive charge, the cationic polymers L-3, D-3, and L-6 showed very similar pH-dependent conformational changes in aqueous solution, which resemble those of poly(L-lysine).[11,12] For example, the pH dependence of the CD spectrum of L-3 is shown in Figure 1. At pH 10.5, the polymer assumes an α-helical conformation, and at pH 7.2, it takes a "charged-coil" conformation.

There is some controversial discussion on the charged-coil conformation.[13] Woody stated that it should be classified as a kind of random coil conformation, in which some regions of conformational space are particularly favored.[13] However, it appears to us that the charged-coil should be distinguished from random coil conformations and may have some ordering along the chain, because it shows strong and specific CD peaks and, as will be shown in this paper, it induces strong CD of some chromophores that are bound to the polypeptide chain. Despite the fact that the "charge-coil" conformations have been also observed in some nonionic polypeptides,[14,15] the term will be used in the following discussion.

The amphiphilic polymer with short side chains (L-3-12) assumes an α-helical conformation in methanol but the charged-coil conformation appeared when more than 20% Tris buffer was added to the methanol solution (Figure 2). The quaternized polymer remained in the charged-coil conformation in alkaline pH regions or even in the presence of 0.01 M NaCl.

The amphiphilic polymer with long side chains (L-6-12) assumes an α-helix in pH regions higher than pH 9 (Figure 3). The presence of α-helical conformation in L-6-12 may be understood partially by the presence of about 30% tertiary amino groups which are not quaternized. Another reason should be that the L-6-12 polymer has long spacer chains between the main chain and the positive charges. The long spacer chain may reduce the repulsive interactions between positive charges and hence minimize destabilization of the α-helix. In methanol/water mixture L-6-12 polymer showed a transition from an α-helix to charged-coil conformation at a methanol content of 80–90%. The methanol content at the transition point was lower than that for L-3-12 polymer (90–100%, Figure 2). Thus a greater stability of the α-helical conformation of L-6-12 polymer as compared with that of L-3-12 polymer was again suggested.

The CD amplitudes in Figures 1–3 are lower than the usual values for α-helix or for charged-coil conformation. The lower value may suggest incomplete helix formation of low molecular weight polymer components which have been detected by the GPC analysis. However, the presence of the latter will not affect the following discussion.

Binding of 4-(1-Pyrenyl)butyric Acid by the Amphiphilic Polymers. The binding property of the hydrophobic cluster of amphiphilic polymers can be studied by the excimers of fluorophores.[8] As a hydrophobic fluorophore that is soluble in water in a monomeric form, 4-(1-pyrenyl)butyric acid (PBA) was employed. Figure 4 shows the intensity ratio (I_E/I_M) of the excimer (502 nm) to monomer (376 nm) fluorescence plotted against the concentration of lauryl groups of L-3-12 and D-3-12 polymers. PBA showed only monomer emission in the absence of the amphiphilic polymers, but excimer emission appeared when about the same amount of lauryl groups was present in the solution. Excimer formation is most evident when the number of lauryl groups is 3–6 times as large as that of the fluorophore. Above this optimum concentration of the polymer, the excimer-to-monomer fluorescence ratio decreased, which may be due to a decrease in the effective

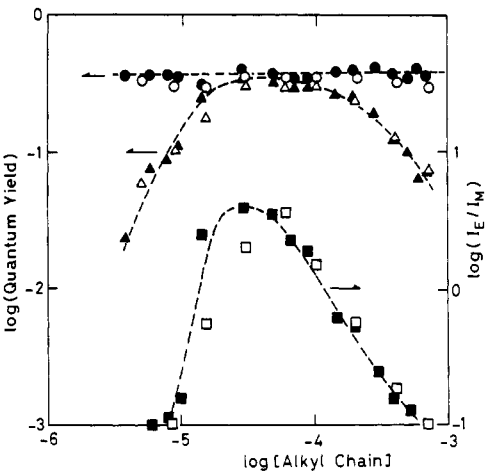


Figure 4. Excimer/monomer intensity ratios observed in the fluorescence spectra of 4-(1-pyrenyl)butyric acid (1.0×10^{-5} M) in the presence of L-3-12 (■) or D-3-12 (□), excimer quantum yield in the presence of L-3-12 (▲) or D-3-12 (△), and total (excimer + monomer) quantum yield in the presence of L-3-12 (●) or D-3-12 (○). pH 7.2, room temperature.

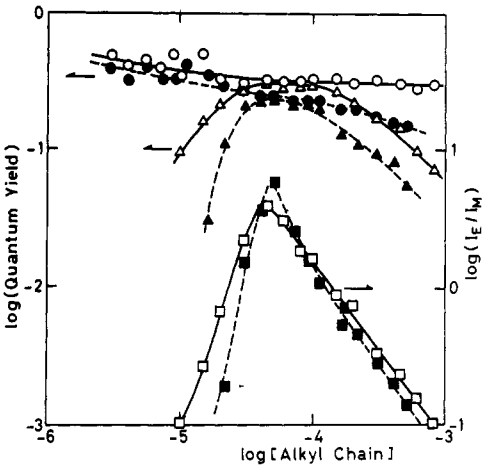


Figure 5. Excimer/monomer intensity ratios observed in the fluorescence spectra of 4-(1-pyrenyl)butyric acid (1.0×10^{-5} M) in the presence of L-6-12 at pH 7.2 (□) and 10.5 (■), excimer quantum yield at pH 7.2 (▲) and 10.5 (▲), and total quantum yield at pH 7.2 (○) and 10.5 (●).

concentration of bound PBA in the polymeric cluster. The behavior shown in Figure 4 is essentially the same as that observed with quaternized laurylpoly(ethylenimine)[8] but is quite different from that with low molecular weight detergents such as cetyltrimethylammonium chloride (CTAC) or lauryltrimethylammonium chloride (LTAC). For the latter cases the optimum concentrations were higher by more than an order of magnitude.[8] Thus the advantage of polymer detergent was again demonstrated.

In Figure 4 are plotted the fluorescence quantum yield of excimer (ϕ_E) and the total (excimer + monomer) fluorescence quantum yield (ϕ_T). Invariance of ϕ_T is especially noteworthy, although there is no reason to expect equality of quantum yields for the three species: free monomer in solution, bound monomer in the cluster, and excimer in the cluster. The invariance of ϕ_T may suggest the absence of any quenching processes in the polymeric cluster. The absence of any quenching process is further evidenced when Figure 4 is compared with the similar plot for quaternized laurylpoly(ethylenimine)[8] or for L-6-12 (Figure 5), which show a substantial decrease of ϕ_T at high polymer concentrations, due to quenching by tertiary amino groups remaining in these polymers.

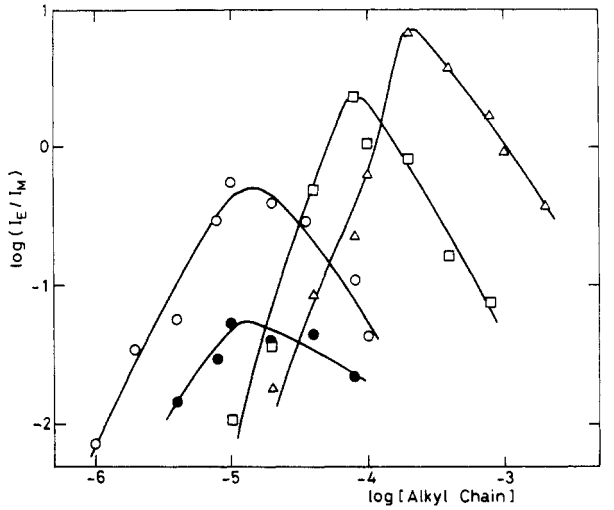


Figure 6. Excimer/monomer intensity ratios observed in the fluorescence spectra of 4-(1-pyrenyl)butyric acid at the concentration of 1×10^{-7} (●), 1×10^{-6} (○), 1×10^{-5} (□), and 1×10^{-4} M (△) in the presence of D-3-12. pH 7.2, room temperature.

Figure 5 shows the same kind of plot for the L-6-12 polymer at pH 7.2 and 10.5. Although the amphiphilic polymer assumes different conformations at the two pHs, the binding behavior was not affected by the conformational change. The significant decrease of ϕ_T at high concentrations in alkaline solution is explainable in terms of quenching by tertiary amino groups.

As stated above, quaternized laurylpoly(ethylenimine) binds hydrophobic substrates when the concentration of alkyl chain becomes comparable to the substrate concentration. It is somewhat strange that the same behavior is observed with the present amphiphilic poly(α-amino acids). Since the present polymers assume a charged-coil or α-helical conformation under the conditions of the binding study, it seems unlikely that the amphilic side chains, which are arranged along the extended chain, would form large aggregates. It is therefore concluded that some intermacromolecular aggregates contribute to the cluster formation, or even very small aggregates that are formed by the folding of the charged polymer can bind the fluorophore effectively.

Figure 6 shows the excimer/monomer fluorescence intensity ratios at various fluorophore concentrations plotted against the concentration of D-3-12. The optimum polymer concentration shifts to a lower value when the concentration of PBA decreases to 1×10^{-6} M in the system. The behavior observed in the PBA concentration range 10^{-6}–10^{-4} M is very similar to that observed with quaternized laurylpoly(ethylenimine) and has been interpreted as due to excimer formation in *intra*macromolecular clusters, in which the PBA molecules are highly concentrated.[8] However, the optimum polymer concentration remained unchanged when the PBA concentration was as low as 10^{-6}–10^{-7} M. The presence of the critical polymer concentration for eximer formation implies the contribution of *inter*macromolecular association for the hydrophobic cluster formation. Since the onset of the intermacromolecular association occurred at as low a concentration as 10^{-5} M, it would not have been detected unless the fluorescence probe had been used.

Usually intermacromolecular association of poly(α-amino acids) is accompanied by a conformational transition to a β-structure. However, this was not true in the present case, as shown in Figure 1–3. The amphiphilic polypeptides may associate by hydrophobic interactions between the amphiphilic side chains, and the contribution

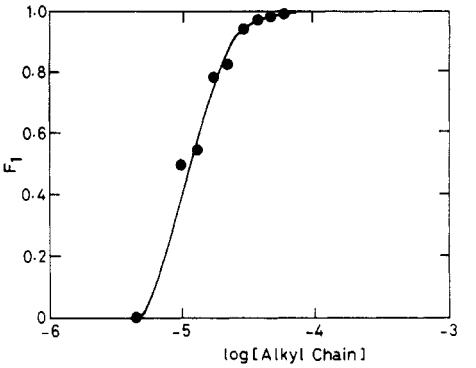


Figure 7. Fraction of 4-(1-pyrenyl)butyric acid (1.0×10^{-5} M) bound to D-3-12. pH 7.2, room temperature.

of hydrogen bonding between the main chains, which induces the β-structure, may not be important.

The fraction of PBA molecules bound to the amphiphilic poly(α-amino acids) can be quantitatively evaluated from the analysis of the biphasic decay curves of the monomer fluorescence of PBA.[8] The free PBA molecules in bulk solution showed a single-exponential decay with a lifetime of about 120 ns, whereas the bound PBA decayed with a lifetime of about 10 ns. The decay curves were analyzed by a least-squares method, and the fractions of the bound fluorophore were obtained. The results are plotted in Figure 7 against the polymer concentration.

The uptake of 1×10^{-5} M of PBA begins at a polymer concentration where the number of alkyl chains is equal to that of PBA molecules, as indicated above by the excimer/monomer intensity ratios in Figure 4. All PBA molecules are bound when the lauryl chain/PBA molar ratio reaches 5. It is therefore evident that only a few alkyl chains are enough to bind hydrophobic substrates effectively. The binding behavior of the amphiphilic poly(α-amino acid) (Figure 7) is much the same as that observed for quaternized laurylpoly(ethylenimine)[8] but quite different from the behavior of low molecular weight detergents such as CTAC and LTAC. In the latter cases, about 20 CTAC and even more LTAC molecules were necessary to bind 1 PBA molecule at a PBA concentration of 1.0×10^{-5} M.

To conclude this section, the amphiphilic poly(α-amino acids) were found to form a new class of polymeric cluster which binds hydrophobic substrates very efficiently.

Induced Circular Dichroism of Cyanine Dyes Bound to the Amphiphilic Poly(α-amino acids). The induced circular dichroism (ICD) of dyes bound to chiral assemblies is diagnostic for the dissymmetric structure of the assemblies. Studies have been made on the ICD of dyes bound to poly(L-lysine).[16] In this study, circular dichroism was measured on such chromophores as PBA, pyrene, anthracene, and 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) in the presence of the amphiphilic poly(α-amino acids). However, no ICD whatsoever was detected in these cases. The absence of ICD implies that the hydrophobic clusters of the poly(α-amino acids) are not dissymmetric, at least in the neighborhood of the bound dyes. Circularly polarized fluorescence (CPF) spectra were also measured for the above chromophores in the presence of the amphiphilic polymers. Again, no CPF signal was detected either in the monomer fluorescence or in the excimer region. The absence of fluorescence dissymmetry in the excimer region indicates that the orientation of two chromophores in the excimer is not subjected to the dissymmetrical perturbation.

However, the dissymmetry of the polymeric system was evident when spatially extended chromophores having two

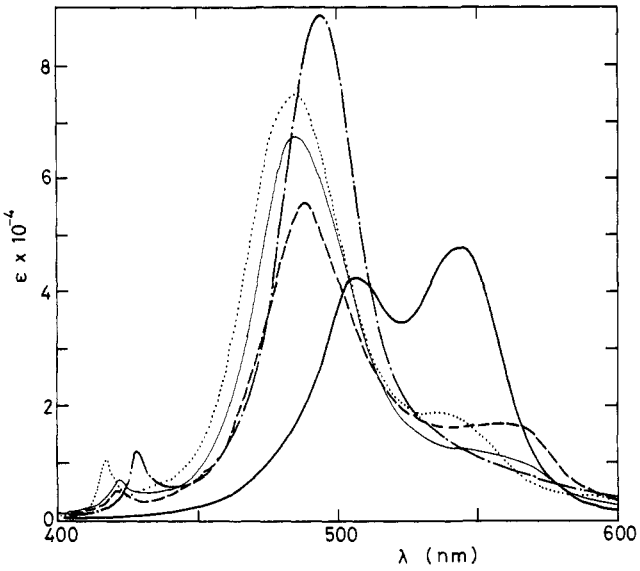


Figure 8. Absorption spectra of a cyanine dye III (2.0×10^{-5} M) in the presence of L-3 at pH 10.5 (—) and 7.2 (···), in the presence of L-3-12 at pH 7.2 (– – –), and in the presence of L-6-12 at pH 10.5 (–·–) and at 7.2 (—). The spectrum in the presence of L-3-12 at pH 10.5 is almost indistinguishable from that at pH 7.2. [Gln unit] = 1.0×10^{-4} M.

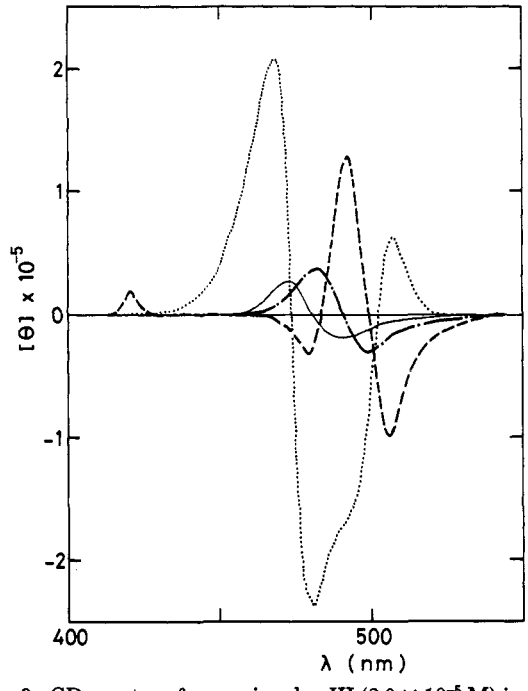
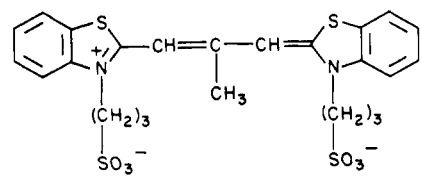


Figure 9. CD spectra of a cyanine dye III (2.0×10^{-5} M) induced by L-3 at pH 7.2 (···), by L-3-12 at pH 7.2 (– – –), and by L-6-12 at pH 10.5 (–·–) and 7.2 (—). The spectrum induced by L-3-12 at pH 10.5 is almost indistinguishable from that at pH 7.2. No CD was induced by L-3 at pH 10.5. [Gln unit] = 1.0×10^{-4} M.

negatively charged sites were bound to the poly(α-amino acids). Figures 8 and 9 show the absorption and CD spectra of the cyanine dye IV,[17] respectively, in the presence of the poly(α-amino acids). Intense ICD's were observed in this case. The origin of the ICD may be either a dissymmetric stacking of the dyes on the polymer or a skewed configuration of the dye molecule induced by binding to the chiral polymer. The first interpretation is more probable, since the ICD appears at the absorption band that is assignable to a dimer or an aggregate of the dye. Indeed, the absorption peak at 553 nm in Figure 8, which is seen in the presence of the L-3 polymer, is also observed in a dilute methanol solution of the dye and assigned to a monomer absorption. No ICD was detected in this region (Figure 9). On the other hand, the peaks at wavelengths shorter than 500 nm were seen only when the chromophore was bound to the polymer. These absorptions should be assigned to a dimer or higher aggregates, and strong ICD's were observed in these absorption regions.



IV

The ICD spectra depended on the nature, conformation, and ionization state of the polymers. The L-3-12 polymer showed an identical ICD at pH 7.2 and 10.5, where the amphiphilic poly(α-amino acid) takes the same (charged coil) conformation. The L-6-12 polymer changed its conformation from a charged coil at pH 7.2 to an α-helix at pH 10.5. The ICD spectra at the two pH values differed only slightly, implying that the chiral structure of the polymeric cluster was not much affected by the conformation change of the main chain. The poly(α-amino acid) L-3, which carries no hydrophobic side chains, also induced a large CD when the side chains were ionized at pH 7.2. But the ICD disappeared when the pH was raised to 10.5, at which pH the side chains are not ionized. Therefore, the ICD of L-3 is caused only by the electrostatic interactions between the positively charged side chains and the negatively charged dyes. The pattern of the ICD spectrum in the presence of L-3 polymer differed substantially from those in the presence of the amphiphilic polymers. Therefore, the configuration of the bound dyes was found to depend strongly on the nature of the dye–polymer interaction.

Similar ICD spectra were observed with other cyanine dyes having two negatively charged sites. A small ICD was also detected with diphenylhexatriene. Therefore, it was concluded that the amphiphilic poly(α-amino acids) afford hydrophobic clusters, which show no local dissymmetry but show some chirality in a spatially extended domain, which may have a size comparable to that of the dye aggregates.

Attempts were also made to detect a stereoselective excimer formation when chiral fluorophores such as *N*-acetyl-L-1-pyrenylalanine (Ac-L-1-PyrAla),[18] *N*-(benzyloxycarbonyl)-L-phenylalanine pyrenylamide, and *N*-(benzyloxycarbonyl)-L-proline pyrenylamide were solubilized in the amphiphilic polymer solution. However, no significant difference of the I_E/I_M ratios were detected between the L-3-12 and the D-3-12 polymers. The absence of the stereoselectivity in the excimer formation indicates again the absence of local dissymmetry in the polymeric cluster of the amphiphilic poly(α-amino acids).

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Registry No. Lauryl bromide, 143-15-7; 3-(dimethylamino)-propylamine, 109-55-7; 6-(dimethylamino)hexylamine, 1938-58-5; 12-(dimethylamino)dodecylamine, 91228-39-6; 4-(1-pyrenyl)butyric acid, 3443-45-6.

References and Notes

(1) Fendler, J. H. "Membrane Mimetic Chemistry"; Wiley-Interscience: New York, 1982.
(2) Kusumi, A.; Singh, M.; Tirrell, D. A.; Oehme, G.; Singh, A.;

Samue, N. K. P.; Hyde, J. S.; Regan, S. L. *J. Am. Chem. Soc.* **1983**, *105*, 2975.
(3) Akimoto, A.; Dorn, K.; Gross, L.; Ringsdorf, H.; Schupp, H. *Angew. Chem.* **1981**, *93*, 108.
(4) Kippenberger, D.; Rosenquist, K.; Odberg, L.; Tundo, P.; Fendler, J. H. *J. Am. Chem. Soc.* **1983**, *105*, 1129.
(5) Kunitake, T.; Nakashima, N.; Takarabe, K.; Nagai, M.; Tsuge, A.; Yanagi, H. *J. Am. Chem. Soc.* **1981**, *103*, 5945.
(6) Fendler, J. H.; Fendler, E. J. "Catalysis in Micellar and Macromolecular Systems"; Academic Press: New York, 1975.
(7) Klotz, I. M. *Adv. Chem. Phys.* **1978**, *39*, 109.
(8) Sisido, M.; Akiyama, K.; Imanishi, Y.; Klotz, I. M. *Macromolecules* **1984**, *17*, 198.
(9) We are indebted to Professor T. Kunitake and Dr. N. Nakashima of Kyushu University for suggesting the use of the cyanine dyes.
(10) Sisido, M.; Egusa, S.; Okamoto, A.; Imanishi, Y. *J. Am. Chem. Soc.* **1983**, *105*, 3351.
(11) Walton, A. G.; Blackwell, J. "Biopolymers"; Academic Press: New York, 1973.
(12) Tiffany, M. L.; Krimm, S. *Biopolymers* **1969**, *8*, 347.
(13) Woody, R. W. *J. Polym. Sci., Macromol. Rev.* **1977**, *12*, 181.
(14) Mattice, W. L.; Lo, J.-T.; Mandelkern, L. *Macromolecules* **1972**, *5*, 729.
(15) Mattice, W. L. *Biopolymers* **1974**, *13*, 169.
(16) (a) Hatano, M.; Yoneyama, M.; Sato, Y.; Kawamura, Y. *Biopolymers* **1973**, *12*, 2423. (b) Sato, Y.; Woody, R. W. *Biopolymers* **1980**, *19*, 2021. (c) Yamamoto, H.; Nakazawa, A.; Hayakawa, T. *J. Polym. Sci., Polym. Lett. Ed.* **1983**, *21*, 131. (d) Yamamoto, H.; Nakazawa, A. *Chem. Lett.* **1983**, 47. (e) Yamamoto, H. *Makromol. Chem.* **1983**, *184*, 1479.
(17) Nakashima, N.; Kunitake, T. *J. Am. Chem. Soc.* **1982**, *104*, 4261.
(18) Egusa, S.; Sisido, M.; Imanishi, Y. *Chem. Lett.* **1983**, 1307.

Small-Angle X-ray Scattering Study of Micelle Formation in Mixtures of Butadiene Homopolymer and Styrene–Butadiene Block Copolymer

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ABSTRACT: The small-angle X-ray scattering technique is utilized to study the formation of micelles in mixtures containing polybutadiene homopolymer (M_n = 2350) with much smaller amounts (0.5–8 wt %) of styrene–butadiene diblock copolymer (M_n = 25000, 52.2 wt % styrene). The following quantities, characterizing the structure of the micelle core consisting of styrene blocks swollen with polybutadiene, have been evaluated as a function of temperature and the copolymer concentration: the radius of gyration of the core, the degree of swelling of the core, the number of block copolymer molecules forming a micelle, and the volume of a core. In addition, the critical micelle concentration (i.e., the minimum copolymer concentration necessary for micelle formation) and the number density of micelles as a function of the concenration were also determined. The degree of swelling of micelle cores by polybutadiene increases steadily with increasing temperature. The micelle core size is fairly independent of the concentration and, as the temperature is raised, at first remains unchanged but then increases rapidly before it finally dissolves completely. The temperature of dissolution increases with concentration of the copolymer. The micelle core volumes, determined by two independent methods (one by the Guinier analysis and the other from the ratio $I(0)/Q$), agree well with each other.

I. Introduction

In a recent series of papers we reported on the study of thermodynamic behavior of polymer systems containing block copolymers. In particular, we investigated,[1] by means of the small-angle X-ray scattering technique, the thermal transition occurring in diblock and triblock copolymers from an ordered microdomain structure to a disordered homogeneous structure. We further investigated,[2,3] by means of small-angle X-ray scattering and turbidity measurements, mixtures of a diblock copolymer with a homopolymer with regard to the solubility of the homopolymer and the effect of the homopolymer concentration on the thermal transition of the block copolymer. In the present work we continue our effort to understand the phase transition and phase separation behaviors of block copolymer systems. In contrast to previous systems studied, we now take up mixtures in which the block copolymer is present as a minority component. Specifically, we investigate mixtures containing a small concentration (up to 8%) of a styrene–butadiene diblock copolymer (50%–50%) dispersed in a low molecular weight polybutadiene. At high temperature and at low concentration the block copolymer is molecularly dissolved. As the temperature is lowered below a certain temperature (which depends on the concentration) block copolymer molecules aggregate into micelles. In this work we utilize the small-angle X-ray scattering technique and determine the critical micelle concentration as a function of temperature and the size and degree of swelling of the micelles as a function of temperature and concentration.

The formation of block copolymer micelles in solutions of selective solvents, i.e., small-molecule solvents which are good for one of the blocks but poor for the other, has been studied by others by small-angle X-ray scattering[7] as well as by light scattering[8–12] and by sedimentation velocity measurements.[7,10,13] In comparison to these, the mixture of a block copolymer with a homopolymer offers advantages both experimentally and theoretically. Interpretation of small-angle scattering data is more straightforward in the system containing a homopolymer, since it has a two-phase structure (that is, has only two regions of differing electron density) whereas the system containing a solvent has a three-phase structure. Theoretical interpretation is simpler in the system containing a homopolymer because the homopolymer shares the same repeat unit with one of the blocks and thus only a single polymer–polymer interaction parameter is required to characterize the thermodynamic behavior of the mixture. The value of the interaction parameter between the styrene and butadiene units has previously been determined[14] from a

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