4220 Macromolecules 1992, 25, 4220–4223

Communications to the Editor

Direct Imaging of Microstructures Formed in Aqueous Solutions of Polyamphiphiles

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Received February 28, 1992
Revised Manuscript Received May 26, 1992

Introduction. Polyamphiphiles are currently attracting considerable interest.[1–15] Indeed, in aqueous solution they give rise to hydrophobic microdomains which have some properties similar to those of micelles of conventional surfactants. In particular these microdomains can solubilize water-insoluble compounds.[5,9,15] One advantage of polyamphiphiles over conventional surfactants is that the critical micellar concentration of the former is negligibly small: microdomains are always present in solutions of polyamphiphiles, however small the stoichiometric concentration.[16–18] This is so because the repeat units are covalently linked and, therefore, successive hydrophobic groups are very close to each other. The local concentration is very high, much higher than, and independent of, the stoichiometric concentration. As a result the hydrophobic moieties self-associate, just as in solutions of conventional amphiphiles, forming hydrophobic microdomains at any concentration.

The existence of hydrophobic microdomains in aqueous solutions of polyamphiphiles has been well established,[1–14] mainly through the use of fluorescence probing techniques.[2,6,8–10,12–14] However, the size and shape of these microdomains remains a matter of controversy, which reflects the difficulties in determining the size of, or the number N_c of repeat units making up, a microdomain in solutions of polyamphiphiles. Indeed, these polymers combine the complexities of polyelectrolytes and of surfactant micelles. Hence, interpretation of experimental data obtained by means of classical methods in terms of microdomain size or molecular weight is extremely difficult, if not impossible. Most of the reported studies deal with copolymers of maleic acid or of either sodium or potassium maleate and of alkyl vinyl ether (referred to as PS*X*, *X* being the carbon number of the alkyl chain),[1,6–9,12,13] or 1-alkene.[2–5] N_c values were obtained by time-resolved fluorescence quenching (TRFQ)[2,6,9,10] or small-angle neutron scattering (SANS).[3,4] TRFQ studies were generally performed on polymers of a high degree of polymerization (DP). They concluded that several microdomains can form within a single polymer molecule, thus leading to a "string of beads" (microdomains) model, with bead size increasing and their distance decreasing with increasing alkyl side chain length.[6,9,10] For low DP (~25) it was reported[2] that molecules of poly(dipotassium maleate-*co*-octadecene) form a single microdomain each. However, SANS studies,[3,4] performed on the same low-DP polymer, indicated the formation of cylindrical microdomains from the assembly of several polymer molecules. It was also concluded[3,4] that low-DP homologous polymers with shorter side chains form ellipsoidal and even spherical micelles when the octadecene is replaced by 1-octene or 1-decene.

Recently cryo-transmission electron microscopy (cryo-TEM) has been successfully used to visualize and provide important information on the organized assemblies present in complex surfactant-containing systems such as viscoelastic micellar solutions or mixed lecithin/conventional surfactant systems.[19–24] The cryo-TEM method avoids the pitfalls usually encountered in studies of surfactant systems by electron microscopy.[25] This led us to apply cryo-TEM in an attempt to obtain new information on the structure adopted by polyamphiphiles in aqueous solution.

Materials. Two types of polyamphiphiles were investigated: (i) poly(disodium maleate-*co*-decyl and hexadecyl vinyl ether), i.e., PS10 and PS16, and (ii) poly[(vinylbenzyl)alkyldimethylammonium chloride] with the alkyl being octyl, dodecyl, and hexadecyl, referred to as P8STY, P12STY, and P16STY, respectively. The DPs of the PS10 and PS16 samples were 1000 ± 150 and 4000 ± 500, respectively. These polymers had been investigated by TRFQ, and the N_c values of the microdomains were evaluated.[9]

The P*X*STY polyamphiphiles were prepared by polymerization of the corresponding monomers in aqueous micellar solution under UV irradiation in the presence of an initiator, azobis(isobutyronitrile). A full account of the preparation of the monomers, of the characterization of their micellar properties in aqueous solution, of the kinetics of the micellar polymerization, and of the properties of the polymerized systems is given elsewhere.[26] For the time being it suffices to note that the DPs of the P*X*STY were all above 3000. The presence of microdomains was deduced from fluorescence probing in solutions of P16STY.[26] No microdomains were detected in solutions of P12STY and P8STY.[26]

It is noteworthy that the solutions of PS10 and PS16 were prepared by solubilization and neutralization of the corresponding poly(maleic acid-*co*-alkyl vinyl ether), whereas the solutions of P*X*STY were obtained by direct polymerization of micellar solutions of the corresponding monomers, followed by extensive dialysis to remove any unpolymerized material. Solid P16STY obtained by lyophilization did not redissolve in water.

Methods. Cryogenic temperature transmission electron microscopy (cryo-TEM) specimens were prepared as previously described.[19,20] Because small changes in concentration or temperature were not expected to alter the microstructure of the systems under investigation, no attempt was made to control temperature or humidity around the specimens during preparation. All specimens were prepared at a room temperature of 21–23 °C and a relative humidity of 50–60%.

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0024-9297/92/2225-4220$03.00/0 © 1992 American Chemical Society

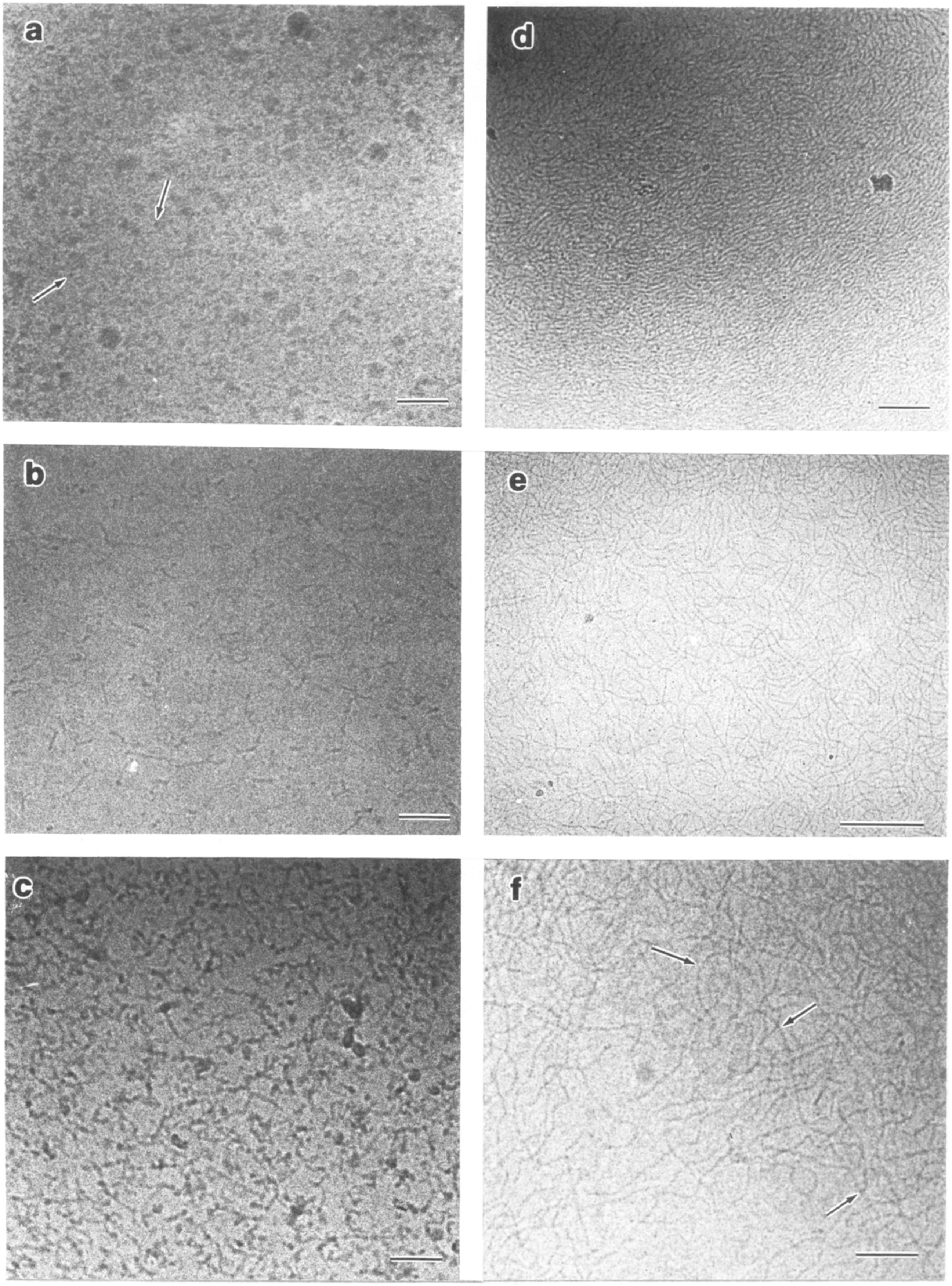

Figure 1. Cryo-transmission electron micrographs of vitrified aqueous solutions of polyamphiphiles: (a) PS10 solution showing globular micelles (indicated by arrows; bar = 100 nm). (b and c) P16STY solutions, 0.42 and 2.0%, respectively, with micrographs showing threadlike micelles (bars = 100 nm). (d–f) PS16 solution at concentration 0.40% with micelle entanglements seen in the three micrographs. Figure 1f shows an example of the band pattern (arrows). Bars = 200 (d and e) and 100 nm (f).

To prepare a thin vitrified specimen, a small droplet, about 5 μL in volume, was pipetted onto an electron microscope copper grid covered with a holey carbon film. The grid was held by tweezers mounted on a spring-loaded plunger of our vitrification system.[20] Most of the liquid was then blotted with filter paper wrapped around a flat metal strip, leaving thin fluid films spanning the holes (approximately 1–8 μm in diameter) in the carbon film. Upon triggering the plunger, the specimen was driven into liquid ethane at its freezing point (99 K) and vitrified. The vitrified specimen was then stored under liquid nitrogen. For microscopy the specimen was loaded into a Gatan 626 cooling-holder and transferred to the electron microscope, a JEOL JEM 2000FX, where it was maintained at about 100 K. Specimens were examined at an electron acceleration voltage of 100 kV. We used Kodak SO163 film developed for maximum electron speed.

Results and Discussion. Electron micrographs of P8STY and P12STY showed no organized structure in the vitreous ice. This is in agreement with the study of these systems by viscosity measurements, which showed a behavior of normal polyelectrolyte for these two polymers, and by fluorescence probing, which indicated the absence of microdomains.[26]

Cryo-TEM micrographs of PS10 solutions reveal the presence of globular micelles (Figure 1a). This is to be expected in view of our previous results, which had shown that small hydrophobic microdomains of aggregation number $N_c \approx 15$ are present in solutions of PS10 and that a PS10 molecule contains one microdomain per every 100 repeat units.[9]

Micrographs of vitrified P16STY and PS16 solutions (Figure 1b–f) show more interesting features, namely, cylindrical micelles: flexible for the former and very long and entangled for the latter. In fact, the micrographs for PS16 solutions are very similar to those obtained for systems of giant micelles such as the solutions of cetyltrimethylammonium bromide (CTAB) in the presence of sodium salicylate[27] or potassium bromide.[28]

The micrograph of Figure 1b is that of a fairly dilute solution (0.42%) of P16STY of DP 3100. It clearly shows individual threadlike micelles. Figure 1c corresponds to a more concentrated solution (2%) of P16STY of DP 9000. The density of the threadlike micelles is larger than that in Figure 1b, as expected. The micelles are still mostly discrete, with a few entanglements. The micelles in Figure 1b have a diameter of 6 ± 1.5 nm and a minimum length of 100–200 nm.[29] These values are consistent with the DP of the sample and with the string of beads (microdomains) model for polyamphiphiles inferred from time-resolved fluorescence quenching investigations.[9,26] It must be first emphasized that from the point of view of cryo-TEM a polyamphiphilic molecule which is organized as a string of beads, with the beads very close to each other, as postulated for PS16 and P16STY,[9,26] will appear in the micrographs as a flexible threadlike micelle, as observed. But if the polymer molecule contains microdomains which are far apart (as in the case of PS10), the electron micrographs will only show globular micelles, as the connecting segments would be invisible. For P16STY the microdomains have been found to be made of about 50 repeat units.[26] Using a value of 0.46 nm^3 for the volume of a hexadecyl chain,[30] the radius of the microdomain hydrophobic core is found to be 1.8 nm. As the head group contributes about 0.7 nm to the microdomain radius, the overall diameter of the microdomain is 5 nm, in agreement with the above value. Moreover, a DP of 3100 corresponds to some 60 microdomains, and thus the maximum micelle length would be 300 nm.

The micrographs of PS16 in Figure 1d–f show much longer micelles than those in parts b or c of Figure 1, even though the DP of the PS16 sample (about 4000) is close to, or smaller than, those of the P16STY samples and the concentration is comparable to that of the dilute P16STY solution. This suggests that the PS16 threadlike micelles are formed through a linear, end-to-end linking of several PS16 molecules. In fact similar micrographs were obtained for a PS16 sample of even lower DP. We leave it for the future, after more results become available. to discuss in full the reasons why PS16 threadlike micelles are formed by the assembly of several polymer molecules, while P16STY are not. At this stage we shall only point out that the density of hexadecyl chains, expressed as the number of chains per number of carbon atoms of the polymer backbone, is 1/4 for PS16 but 1/2 for P16STY. Besides, the charged groups are located closer to the polymer backbone in PS16 than in P16STY. These facts may contribute to the differences in behavior noted above between the PS*X* and P*X*STY polyamphiphiles.

An essential difference between the micrographs in parts b and d of Figure 1 is in the very large number of entanglements present in the PS16 system and their near absence in P16STY. This explains the difference of behavior between the solutions of these two polymers. Dilute P16STY solutions were viscous but not viscoelastic (no recoil occurred after rotating the solution), whereas the PS16 solution was not as viscous but showed viscoelasticity. This actually created some difficulty when going through the blotting process for thinning the film of the PS16 solution on the microscope grid before vitrification.

A last point to be discussed in this short report concerns the conformation of the polymer backbone in the two series of polyamphiphiles and particularly in the microdomains formed by PS16 and P16STY. The P*X*STY molecules have a higher density of alkyl side chains than the PS*X* molecules. This should result in a more rigid conformation which hinders the formation of microdomains, thereby providing a possible explanation to the fact that, whereas PS10 forms microdomains, P8STY and P12STY do not. In the microdomains of PS16 or P16STY the polymer backbone probably makes loops. The seam on a tennis ball may provide a schematic representation of the local backbone conformation. Either more disordered or more ordered conformations, as for instance, short helices (several turns), can be proposed for the microdomains. In the latter, these helices would be connected by short segments, comprising a few repeat units, where the alkyl chains would be in contact with water.

Figure 1f is a higher magnification image of the PS16 system showing a band pattern in threadlike micelles. This may be a result of the postulated helixlike conformation of their micelles.

The PS*X* series and similar polyamphiphiles are now systematically studied by cryo-TEM. The results will be reported in due time.

Acknowledgment. The research of Y.T. has been supported by grants from the United States–Israel Binational Science Foundation (BSF), Jerusalem, and from the Technion V.P.R. Fund–Steiner Research Fund. D.C., F.C., and R.Z. gratefully acknowledge financial support of Rhone-Poulenc, France.

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