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Time-Resolved Fluorescence Anisotropy Measurements on Fluorescently Tagged Amphiphilic Micelles

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Block copolymers of polystyrene-*block*-poly(methacrylic acid) form multimolecular micelles in mixtures of 1,4-dioxane with a surplus of methanol at ambient temperatures. Micelles consist of compact polystyrene cores surrounded by outer shells formed by poly(methacrylic acid) and are in a reversible equilibrium with nonmicellized copolymer chains (unimers). A series of light scattering, ultracentrifugation, and fluorimetric measurements was performed on micellizing systems of end-tagged copolymers. Complex time-resolved fluorescence anisotropy decays may be explained by a distribution of fluorophores in two microenvironments, i.e., in compact polystyrene micellar cores and in unimer coils.

KEY WORDS: Time-resolved fluorescence anisotropy; light scattering; amphiphilic block copolymer micelles.

INTRODUCTION

Amphiphilic block copolymers of polystyrene-*block*-poly(methacrylic acid) form spherical multimolecular micelles at ambient temperatures in water and aqueous buffers [1–3] and also in organic selective solvents [i.e., solvent for poly(methacrylic acid) and a nonsolvent for polystyrene]. Micellization equilibrium is frozen in water-rich solvents [2,3] and properties of micelles are controlled to a great extent by dissociation of carboxylic groups [2,3].

In selective organic solvents (e.g., mixtures of 1,4-dioxane with a high content of methanol), dissociation of carboxylic groups is suppressed and micellization is similar to that of nonpolar block copolymers in organic solvents [4]. At concentrations higher than the critical micelle concentration (CMC), spherical micelles, M, coexist in a reversible equilibrium with a certain concentration (equal to CMC) of nonmicellized copolymer chains, called unimers, U. Micellization process obeys the scheme of a closed association [5],

$$nU \overset{K}{\rightleftharpoons} M \qquad (1)$$

where n is the association number and K is the micellization constant. Micelles are fairly monodisperse in mass and size. A typical multimolecular block copolymer micelle contains several tens to few hundreds of copolymer chains and consists of a small and dense spherical core formed by insoluble blocks and an outer shell (corona) formed by soluble blocks. The density of the core is fairly high (0.7–0.8 g cm^{-3}) and spatially constant. The density of the shell is considerably lower than that of the core and decreases from the core/shell interface to the micelle periphery (average density, 0.15–0.25 g cm^{-3}). Polymeric micelles are very small colloid objects (hydrodynamic radius, R_h, ca. 10–30 nm) for their high particle molar mass (10^6–10^7 g mol^{-1}). A reversible exchange of copolymer chains exists between unimers and micelles. The rate of that exchange depends strongly on the selectivity of the used solvent [6,7].

Micellization of block copolymers in selective solvents resembles, in many respects, that of soaps and de-

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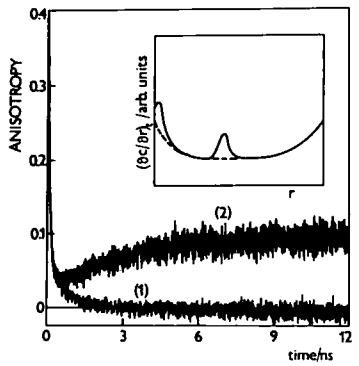

Fig. 1. Curve 1: Time-resolved fluorescence anisotropy decay for polystyrene-*block*-poly(methacrylic acid), N_1SMA, sample in a good solvent for both blocks (1,4-dioxane/10 vol% methanol); copolymer concentration, $c = 5.10^{-3}$ g·cm^{-3}; $T = 25$°C. Curve 2: Fluorescence anisotropy decay for mixed micelles (90 wt% SMA and 10 wt% N_1SMA) in a mixture 1,4-dioxane/73 vol% methanol for the same experimetnal conditions as before. Insert: Sedimentation velocity diagram for mixed micelles (90 wt% SMA and 10 wt% N_1SMA), $c = 5.10^{-3}$ g·cm^{-3}, in 1,4-dioxane/73 vol% methanol for the following experimental conditions: 50,000 rpm, time of sedimentation, $t = 15$ min, $T = 25$°C.

tergents in water. In contrast to most detergent systems, micellization of block copolymers is an enthalpy-driven process [4]. Properties of polymeric micelles in an equilibrium system are influenced by a complex interplay of enthalpic and entropic contributions to the Gibbs function of the system and depend on the strength of interactions of segments of both blocks with solvent molecules.

Fluorescence methods are suitable experimental techniques for studying fluorescently tagged micellizing copolymers. The aim of this paper is to describe and properly interpret typical time-resolved fluorescence anisotropy decays obtained for systems of micellizing copolymers in organic solvents. We would like to emphasize the complexity of the problem and the danger of misinterpretation of the data and to point out the absolute necessity of combining the fluorimetric measurements with other experimental techniques (such as light scattering, ultracentrifugation, etc.).

EXPERIMENTAL

Polymers. Diblock polystyrene-*block*-poly(methacrylic acid), **SMA**, with a narrow distribution of molar masses (weight-average molar mass, $M_w = 6.10^4$ g mol^{-1}; molar fraction of polystyrene, ca. 0.45), and an identical, but end-tagged (with 2-vinylnaphthalene at the end of the polystyrene block, slightly less than one fluorophore per chain), N_1**SMA**, were supplied by Prof. P. Munk and Dr. C. Ramireddy from the University of Texas at Austin. Details on the preparation are given in Ref. 1.

Experimental Techniques. Steady-state (SPEX Fluorolog 2 fluorimeter) and time-resolved fluorimetry (time-correlated single-photon counting technique with a synchronously pumped and cavity-dumped dye laser, Coherent 701-3D; circulating rhodamine 6G), static (SOFICA 42 000) and quasielastic (Brookhaven BI 2030), light scattering, ultracentrifugation (Spinco E), and NMR (General Electric QE 300) were used for studying polystyrene-*block*-poly(methacrylic acid) micelles. Details are given in Refs. 2 and 3.

RESULTS AND DISCUSSION

Both the polystyrene-*block*-poly(methacrylic acid) sample, SMA, and the fluorescence end-tagged sample, N_1SMA, dissolve well in dioxane-rich mixtures with methanol. Those mixtures are good solvents for both polystyrene and poly(methacrylic acid). Copolymer chains form isolated random coils with a low density of segments (0.03–0.05 g cm^{-3}). Pendant fluorophores are exposed to solvent molecules and are quite mobile.

Fluorescence decays for N_1SMA sample in good solvents are almost single-exponential. In the mixture 1,4-dioxane/10 vol% methanol at $T = 25$°C, the average fluorescence lifetime $\langle\tau_F\rangle$, is ca. 30 ns. The corresponding time-resolved fluorescence anisotropy, $r(t)$, is shown in Fig. 1 (curve 1). Anisotropy decays fast to zero. The decay is roughly double-exponential (τ_{r1}, ca. 90 ps; τ_{r2}, ca. 1.0 ns), indicating an efficient depolarization due in part to the fast one-dimensional rotational diffusion of the fluorophore around the C–C single bond and to a slower and complex diffusion motion of the end of a chain.

In solvents rich in methanol, which are selective solvents for poly(methacrylic acid), multimolecular micelles with compact polystyrene cores are formed. Those micelles coexist in equilibrium with unimers. Fluorophores in micellar form of the sample are trapped in rigid polystyrene cores. Their motion is strongly restricted. Fluorophores are relatively close to each other and anisotropy decay may be affected by possible excitation energy migration. To suppress this effect, we have prepared mixed micelles from nontagged sample, SMA, and a small fraction (10 wt%) of the tagged sample, N_1SMA. In that case, the average fluorophore-to-fluorophore distance is ca. 7.5 nm and energy migration may be neglected.

As a typical system, solution of multimolecular micelles in a mixed solvent 1,4-dioxane/73 vol% methanol at $T = 25°C$ was chosen and results for that particular system are presented. Micelles were characterized by a combination of light-scattering techniques and ultracentrifugation. The hydrodynamic radius of micelles measured by quasielastic light scattering, R_h, was 23.4 nm. Quasielastic light-scattering measurement yields a z-average of $(R_h)^{-1}$ and therefore only values for micelles were obtained [2,3]. Analysis of the data indicated a low polydispersity of micellar sizes. Sedimentation velocity measurements (Fig. 1, insert) indicate the simultaneous presence of micelles (the fast-moving peak) and unimers (the slow-moving peak).

Time-resolved anisotropy is shown in Fig. 1 (curve 2). The unusual shape of the curve is slightly surprising. Anisotropy decays fast to a minimum value of ca. 0.04 and then grows again to a constant value of ca. 0.10, which does not change until the complete decay of the fluorescence emission. The corresponding polarized and total fluorescence decays are strongly multiexponential.

An effective anistropy with minima at early times and rises (or even maxima) at later times may be explained on the basis of the distribution of fluorophores into two (or more) microenvironments: the first, where both the fluorescence lifetime, τ_{F1}, and the depolarization correlation time, τ_{r1}, are short, and the latter where both τ_{F2} and τ_{r2} are long. The effective fluorescence intensity, $I^{ef}(t)$, for such a system (containing, for simplicity, single-exponential fluorophores) is given by the following formula [8]:

$$I^{ef}(t) = x_1A_1 \exp(-t/\tau_{F1}) + x_2A_2 \exp(-t/\tau_{F2}) \quad (2)$$

where x_i are molar fractions of fluorophores in individual microenvironments and A_i reflect the nonequal quantum yields. The effective anisotropy, $r^{ef}(t)$, is described by the following equation:

$$r^{ef}(t) = [x_1F_1(t)r_1(t) + x_2F_2r_2(t)]/I^{ef}(t) \quad (3)$$

where $F_i(t)$ are the fractional intensities, $F_i(t) = A_i \exp(-t/\tau_{Fi})/\tau_{Fi}$.

Combination of light-scattering and ultracentrifugation measurements unambiguously shows the coexistence of multimolecular micelles with a significant fraction of unimers. Fluorophores in micellar cores are embedded in dense and nonpolar medium formed by segments of entangled polystyrene blocks and both the fluorescence lifetime and the depolarization correlation time are long. Nonmicellized copolymer chains are assumed to form a slightly perturbed random coils [9]. Blocks in one unimer chain are only little segregated and a collapse of the insoluble block is only partial and almost insignificant. The average copolymer conformation resembles the letter "C." Fluorophores are exposed to the polar solvent and quite mobile—the fluorescence lifetime is shorter than that in micellar cores and the fluorophore mobility is similar to that in good solvents (i.e., the rotational depolarization is fast). For pendant naphthalene fluorophores, there is no significant shift in emission wavelength with the polarity of the microenvironment and Eqs. (2) and (3) describe well the fluorescence properties of the system.

It should be noted, however, that even in micelles with a low fluorophore loading, fluorescence depolarization due to energy migration still may occur. Our extensive Monte Carlo simulations of insoluble block conformations in micellar cores (performed as simulations of tethered chain conformations in spherical volumes) [10] indicate a nonrandom distribution of the chain ends and a certain increase in their density toward the core center. A covalent attachment of fluorophores to the chain ends may alter interactions with the microenvironment and lead to a partial fluorophore clustration. Studies of those effects are in progress and will be presented together with detailed experimental data on various micellizing systems and results of Monte Carlo simulations in the near-future.

In this report we wanted to show a high complexity of the behaviour of fluorescently tagged polymeric micelles. Changes in polymer conformations and compactness are often deduced from changes in fluorescence anisotropy (sometimes only steady-state measurements are considered). We wanted to show that information provided by other experimetnal techniques are needed before conclusions concerning polymer structure are being made in complex systems on the basis of fluorescence measurements.

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