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ORIGINAL CONTRIBUTION

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Direct preparation of core cross-linked micelles in a nonselective solvent

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Abstract This is the first light scattering study demonstrating that the size of micelles, the aggregation number, and the mobility of the core blocks of the micelles could be controlled by the length of the cross-linker in the micellar cores. The core cross-linked micelles were prepared using a poly[(4-pyridinemethoxymethyl)styrene]-*block*-polystyrene (PPySt-*b*-PSt) diblock copolymer and perfluoroalkyl dicarboxylic acid. The PPySt-*b*-PSt copolymer formed the micelles in THF, a nonselective solvent, in the presence of the perfluoroalkyl dicarboxylic acid. The light scattering studies demonstrated that the micellar size and aggregation number were dependent on the chain length of the perfluoroalkyl dicarboxylic acid. Perfluoroazelaic acid produced micelles with a larger hydrodynamic radius and higher aggregation number than tetrafluorosuccinic acid. The micellization proceeded through the formation of the pyridinium carboxylate and the cross-linkage between the PPySt blocks via the dicarboxylic acid. The core cross-linked micelles were thermally stable and maintained its structure with changes in the temperature. A ^{1}H NMR analysis revealed that the micelles prepared by perfluoroazelaic acid had more mobility of the core blocks than those by tetrafluorosuccinic acid.

Keywords Poly[(4-pyridinemethoxymethyl)styrene]-*block*-polystyrene (PPySt-*b*-PSt) · Perfluoroalkyl dicarboxylic acid · Micelles · Hydrodynamic radius · Aggregation number

Introduction

Micelles with structures in which the individual constituents are cross-linked, have attracted considerable attention in recent years because of the stability of the micelles. The steric and thermodynamic stability of the micelles is indispensable in a drug delivery system for the well-controlled release of drugs. The cross-linking prevents the micelles in blood from collapsing under the high dilution condition below the critical micelle concentration [1]. As a result, the cross-linking increases the blood circulation times and allows drugs to be administered over prolonged periods of time. For the preparation of the cross-linked micelles, two methods of cross-linking have been found: one is cross-linking of the micellar shells, and the other is core cross-linking. These two different methods have their respective advantages. The shell cross-linked micelles have the potential to produce nanosized hollow particles by removal of the cores after cross-linking the shells and then the particles can encapsulate hydrophilic drugs inside them [2–5]. The core

cross-linking can increase the stability of the micellar structures without affecting the drug loading capacity, leading to the temporal control of the hydrophobic drug release. While a number of publications on the shell cross-linked micelles have been released [6–11], a few micelles with the core cross-linked were prepared. Bates et al. [12] obtained the core cross-linked micelles of poly(ethylene oxide)-*b*-poly(butadiene) by cross-linking the poly(butadiene) blocks using a $K_2S_2O_8/Na_2S_2O_5$–$FeSO_4$ redox initiation system. Henselwood and Liu [13] prepared poly(2-cinnamoylethyl methacrylate)-*b*-poly(acrylic acid) micelles with the cinnamoyl moieties cross-linked by UV irradiation. Kataoka and coworkers prepared micelles using block copolymers with polymerizable groups at the hydrophobic chain end. They obtained poly(D,L-lactide)-*b*-poly(ethylene glycol) with methacryloyl groups at the chain end of the poly(D,L-lactide) blocks, and polymerized the groups by a radical initiator after the micellization [14, 15]. Similarly, Kissel et al. [16] prepared the micelles of poly(ε-caprolactone)-*b*-poly(ethylene glycol) through the radical polymerization of the double bonds introduced into the poly(ε-caprolactone) blocks. Kataoka et al. [17] also demonstrated that the introduction of thiol groups into the core blocks was effective for preparing the core cross-linked micelles using poly(ethylene glycol)-*b*-poly(L-lysine).

We have reported the micelle formation of nonamphiphilic block copolymers consisting entirely of hydrophilic blocks [18–22]. The micellization was performed for poly(vinylphenol)-*block*-polystyrene using α,ω-diamine. The micellization proceeded through the hydrogen bond cross-linking between the poly(vinylphenol) blocks via the α,ω-diamine. We also released publications on the micellization of a nonamphiphilic poly[(4-pyridinemethoxymethyl)styrene]-*block*-polystyrene diblock copolymer (PPySt-*b*-PSt), using monofunctional perfluoroalkyl carboxylic acid [23, 24]. This micellization was induced by the salt formation of the pyridinium carboxylate. During the micellization, the size of the micelles, the aggregation number, the critical micelle concentration, and the thermostability of the micelles were dependent on the length of the perfluoroalkyl chain of the acid.

We found thermally stable micelles with cross-linked cores for the nonamphiphilic PPySt-*b*-PSt copolymer using bifunctional perfluoroalkyl carboxylic acid. The core cross-linked micelles formed from the nonamphiphilic copolymer have the potential to include solvophilic substances in the micellar cores. This paper describes the direct preparation of the core cross-linked micelles through the micellization induced by salt formation and by cross-linking of the micellar cores.

Experimental

Instrumentation

^{1}H NMR spectra were obtained with Bruker ARX-500 and JEOL GSX-500 FT NMR spectrometers. Light scattering experiments were performed with a Photal Otsuka Electronics DLS-7000 super dynamic light scattering spectrometer equipped with an LS-71 control unit, an LS-72 pump controller, and an argon ion laser operating at $\lambda = 488$ nm.

Materials

The PPySt-*b*-PSt diblock copolymer was prepared as previously reported [24]. The copolymer had the molecular weight of M_n(PPySt-*b*-PSt) = 12000-*b*-33000 determined by ^{1}H NMR. THF was purified by refluxing over sodium for several hours and then distilled. Tetrafluorosuccinic acid (Cf-2b) and perfluoroazelaic acid (Cf-7b) were purchased from the Aldrich Chemical Co. and Daikin Chemicals Sales, Ltd., respectively, and were used without further purification. THF-d_8 was also purchased from the Aldrich Chemical Co. and was used without further purification.

Light scattering measurements: general procedure

PPySt-*b*-PSt (10 mg) was dissolved in THF (3 ml), and using a syringe, the resulting solution was injected through a microporous filter into a cell. The solution was subjected to light scattering measurement at 20 °C. After the measurement, 2 μl of a solution of Cf-7b (91 mg, 0.207 mmol) in THF (0.5 ml) was added to the copolymer solution in the cell, and the mixture was shaken vigorously. The solution was allowed to stand at 20 °C for 10 min, then subjected to light scattering again. This procedure was repeated until distribution of the unimers disappeared completely in nonnegatively constrained least-squares (NNLS) analysis [25].

Results and discussion

The PPySt-*b*-PSt diblock copolymer shows no self-assembly into micelles in THF, because this solvent is nonselective for the copolymer. Light scattering study demonstrated that the copolymer formed micelles in this solvent in the presence of Cf-7b. Figure 1 shows variation in the intensity distribution of the hydrodynamic radius of the copolymer through the micellization by Cf-7b. The distribution was obtained by the NNLS analysis. In the absence of Cf-7b, the copolymers showed the distribution

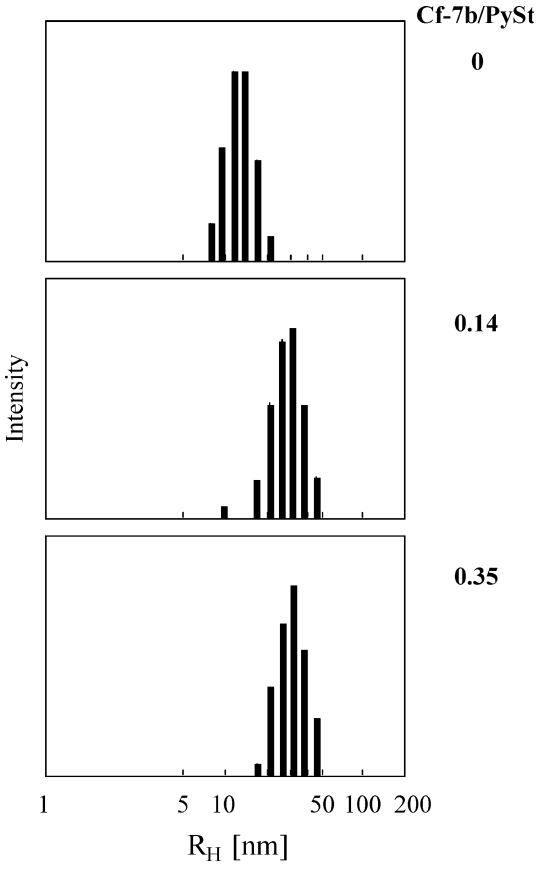

Fig. 1 Variation in the intensity distribution of the hydrodynamic radius of the PPySt-*b*-PSt diblock copolymer through the micellization at 20 °C in THF using Cf-7b. [copolymer] = 3.33×10^{-3} g/ml

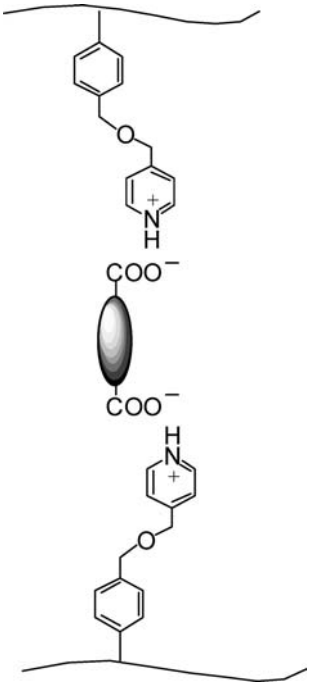

Fig. 2 Micelle formation of PPySt-*b*-PSt by the perfluoroalkyl dicarboxylic acid

at 9 nm of the hydrodynamic radius. This distribution was attributed to unimers, the isolated copolymers. When Cf-7b was added to the copolymer solution at 0.14 as the molar ratio of Cf-7b to the PySt unit (Cf-7b/PySt), another distribution appeared at a 24-nm hydrodynamic radius. The distribution at 9 nm was still observed at this molar ratio, although the intensity was small. At Cf-7b/PySt = 0.35, the distribution of the unimers disappeared completely, indicating that all the copolymers were engaged in forming the micelles. The micellization should proceed by the salt formation of the pyridinium carboxylate and by the cross-linking of the PPySt blocks via Cf-7b (Fig. 2). On the basis of our previous results on the micellization of the copolymer using monofunctional perfluoroalkyl carboxylic acid [23], it was found that the cross-linking was indispensable to provide the micelles. The perfluoroalkyl monocarboxylic acid was required to have the number of carbons in the perfluoroalkyl chains over 8 to produce the micelles of the copolymer.

We explored the effect of the chain length of the perfluoroalkyl dicarboxylic acid on the micellization. Figure 3 shows variation in the hydrodynamic radius

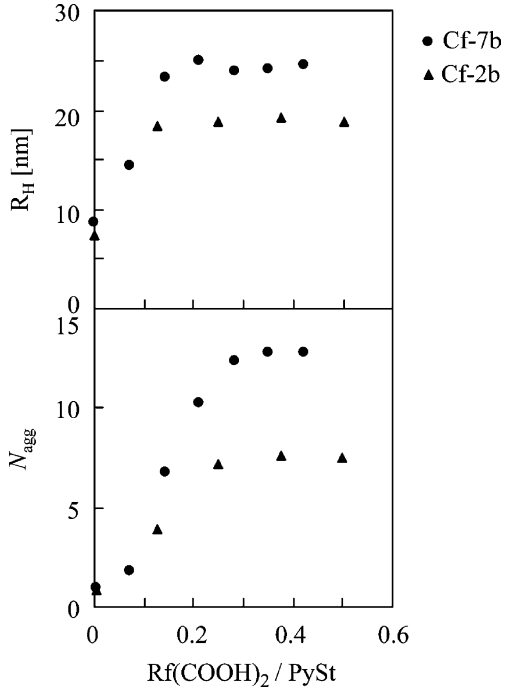

Fig. 3 Variation in the hydrodynamic radius and the aggregation number of the PPySt-*b*-PSt copolymer through the micellization by Cf-2b and Cf-7b. [copolymer] = 3.33×10^{-3} g/ml. Solvent: THF. Temperature: 20 °C

and aggregation number of the copolymer during the micellization by Cf-7b and Cf-2b. The aggregation numbers were represented as relative aggregation numbers estimated with the relative scattering intensity. This estimation is based on the fact that the copolymer concentration is unchanged during the micellization and that the interaction of the PySt moieties and the perfluoroalkyl carboxylic acid has no influence on the scattering intensity. In fact, the addition of trifluoroacetic acid to the copolymer solution made no changes in the scattering intensity and the hydrodynamic radius of the copolymer. Consequently, the relative scattering intensity may be regarded as an apparent aggregation number estimated approximately. The hydrodynamic radius and aggregation number increased with an increase in the molar ratio of Cf-7b or Cf-2b to the PySt unit and became constant over a certain ratio. Cf-7b and Cf-2b made a small difference in the ratio at the cmc, although the ratios were below 0.5 for both the acids. Cf-2b provided smaller micelles than Cf-7b. The hydrodynamic radii of the micelles were estimated as 24.5 nm for Cf-7b and 19.1 nm for Cf-2b. The micellar size was dependent on the length of the perfluoroalkyl chain. Additionally, the aggregation numbers of the micelles were 13 for Cf-7b and 8 for Cf-2b, respectively.

^{1}H NMR revealed that the micelle formation proceeded through the cross-linking by the salt formation between the PySt unit and the perfluoroalkyl dicarboxylic acid. Figure 4 shows the ^{1}H NMR spectra of the PySt monomer in the absence and presence of Cf-2b. Signals of the vinyl protons were observed at 5.13, 5.71, and 6.68 ppm. Signals originating from the phenyl were discerned at 7.36 and 7.29 ppm. The former was assigned to the protons located at the 2 positions neighboring to the carbons attached to the vinyl, and the latter to those at the 3 positions. Signals of the two different methylenes attached to the phenyl and pyridine rings were observed at 4.57 and 4.50 ppm, respectively. The aromatic protons of the pyridine ring were discerned at 8.41 and 7.20 ppm. The signals at 8.41 ppm were assigned to the protons at the 2 positions adjacent to the N, while those at 7.20 ppm to the 3 positions. When Cf-2b was added to the monomer solution, the signals at 8.41 ppm were shifted to 8.50 ppm. The shift was also made for the signals from 7.20 ppm to 7.36 ppm, with the result that those were overlapped with the signals for the 2 positions of the phenyl. The signal of the methylene attached to the pyridine ring was overlapped with the signal of the benzyl protons as a result of the addition of Cf-2b. Even after the addition, the signal intensities were in good agreement with the chemical structure of the PySt monomer. This identification of the intensity ratios with the chemical structure suggests that none of the protons composing the monomer were shielded from the magnetic resonance in the presence of Cf-2b.

In contrast with the PySt monomer, the PPySt-*b*-PSt diblock copolymer aggregates into the micelles in the presence of Cf-2b. Figure 5 shows the ^{1}H NMR spectra of the copolymer in the absence and presence of Cf-2b. In the absence of Cf-2b, proton signals for the 2 and 3 positions on the pyridine rings were observed at 8.40 and 7.15 ppm, respectively. Signals for the two different

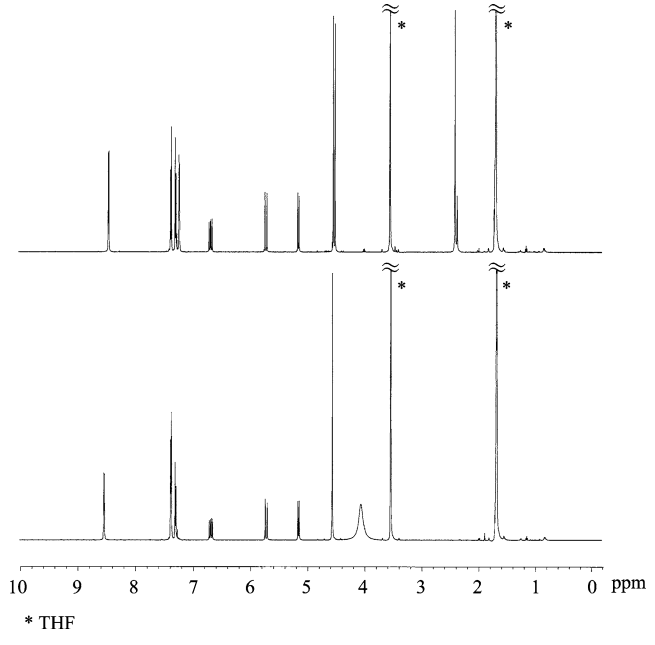

Fig. 4 ^{1}H NMR spectra of the PySt monomer in the absence (*upper*) and presence of Cf-2b (*lower*). Cf-2b/PySt = 0.5. Solvent: THF-d_8. [PySt] = 3.33×10^{-3} g/ml

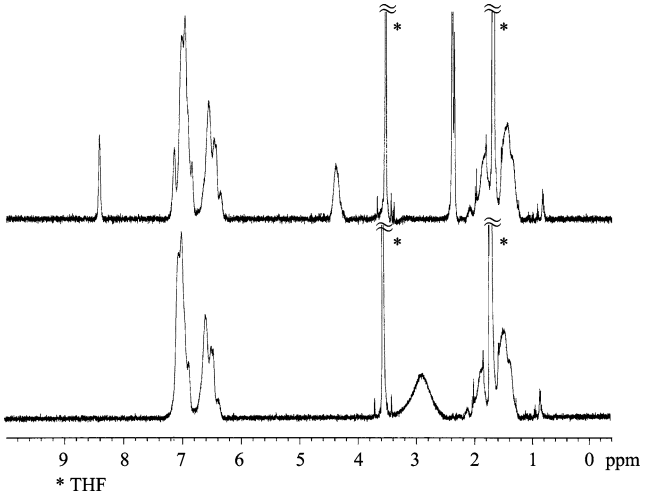

Fig. 5 ^{1}H NMR spectra of the PPySt-*b*-PSt diblock copolymer in the absence (upper) and presence of Cf-2b (lower). Cf-2b/the PySt unit = 0.5. Solvent: THF-d_8. [PPySt-*b*-PSt] = 3.33×10^{-3} g/ml

methylene protons attached to the pyridine and phenyl rings were observed at 4.2–4.5 ppm as a somewhat broad signal. In the presence of Cf-2b, no observation of the signals was made for the 2 and 3 positions on the pyridine rings and for the methylene. The copolymer formed the micelles having the PPySt cores cross-linked by Cf-2b, resulting in that the protons of the PySt moieties were shielded from the magnetic resonance by the PSt coronas.

The micellization showed slight temperature-dependence. Figure 6 shows variation in the hydrodynamic radius and the aggregation number of the copolymer through the micellization by Cf-7b at each temperature. There was a slight difference in the critical micelle concentration for the temperatures, suggesting that the temperature had a negligible effect on the micellization. This is a remarkable difference in the temperature-dependence on the micellization between by the perfluoroalkyl monocarboxylic acid and by the dicarboxylic acid. The micellization by the monocarboxylic acid showed large temperature-dependence and required more monocarboxylic acid to produce the micelles at higher temperature [23].

The micelles prepared by the perfluoroalkyl dicarboxylic acids also made a marked difference in the thermostability from those by the monocarboxylic acid. The micelles formed by Cf-7b were stable for the change in the temperature, whereas the micelles by the monocarboxylic acid were dissociated into the isolated unimers by increasing the temperature [23]. Figure 7 shows variability in the hydrodynamic radius and aggregation number of the micelles when the temperature of the micellar solution was increased from 10 °C to 40 °C and then was decreased from 40 °C to 10 °C. The micellar solution was prepared at 10 °C at Cf-7b/PySt = 0.35. The micelles showed good hysteresis in the hydrodynamic radius. The micellar size slightly increased as a result of increasing the temperature; however, it reverted to the original value by decreasing the temperature. The hydrodynamic radius of the micelles reversibly changed for the variation of the temperature. Consequently, the increase in the micellar size by increasing the temperature should be based on the swelling of the micelles. On the other hand, there was a small difference in the aggregation number at 10 °C before and after the hysteresis. The NNLS analysis revealed that this difference was based on the reorganization of the micelles by the changes in the temperature. The intensities of the micelles around a 50-nm hydrodynamic radius in the distribution decreased after the hysteresis (Fig. 8). Accordingly, the change in the aggregation number of the micelles after the hysteresis can be accounted for the increase in the aggregation number by the reorganization of the micelles. The micelles prepared by Cf-2b showed the similar tendency in the thermostability to those by Cf-7b.

The ^{1}H NMR analysis also revealed that Cf-2b and Cf-7b made a difference in the mobility of the micellar cores. Figures 9 and 10 show the variability in the signal intensity of the micelles prepared with Cf-7b and Cf-2b

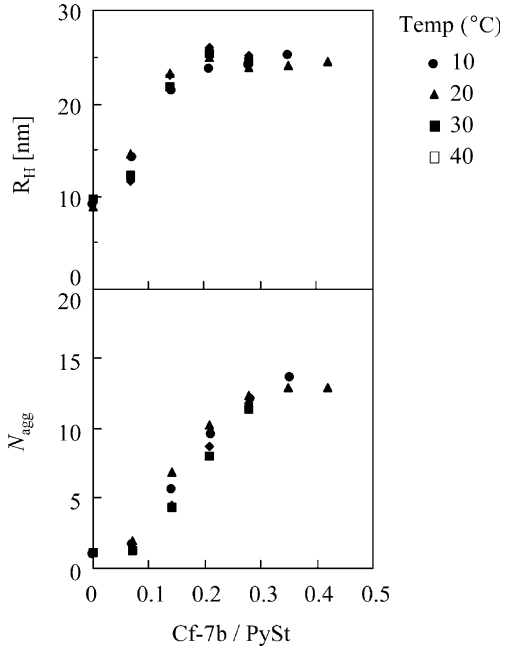

Fig. 6 Variation in the hydrodynamic radius and aggregation number of the PPySt-*b*-PSt copolymer through the micellization by Cf-7b at each temperature. [copolymer] = 3.33×10^{-3} g/ml

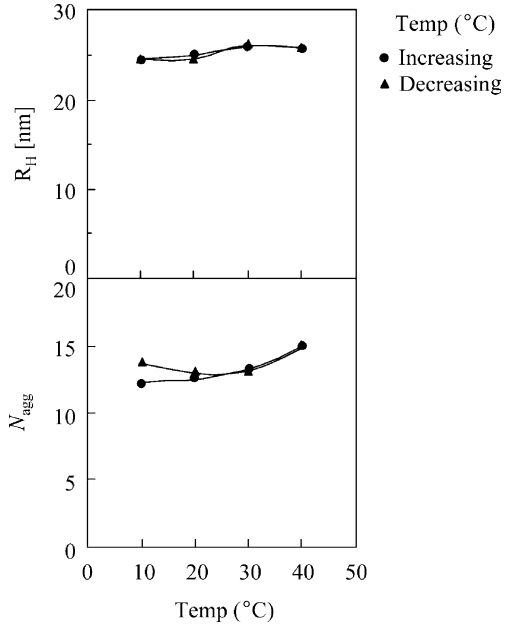

Fig. 7 Hysteresis curves of the PPySt-*b*-PSt micelles with Cf-7b for the temperature. Cf-7b/PySt = 0.35. [copolymer] = 3.33×10^{-3} g/ml

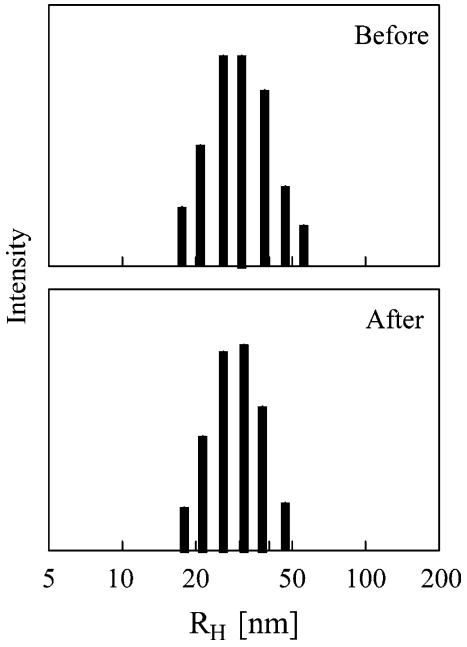

Fig. 8 Intensity distribution of the hydrodynamic radius of the micelles at 10°C before and after the hysteresis. Cf-7b/PySt = 0.35. [copolymer] = 3.33×10^{-3} g/ml

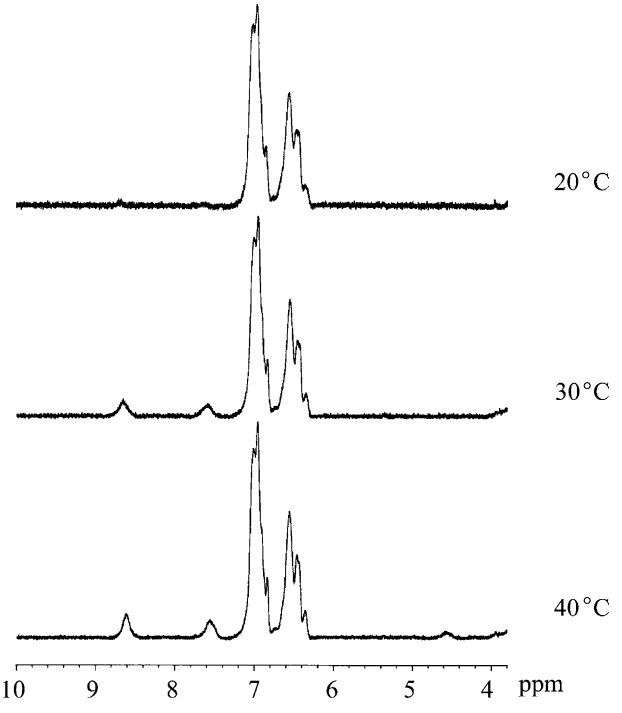

Fig. 9 Variability in the ^{1}H NMR signals of the PPySt-*b*-PSt micelles with Cf-7b for the temperature. Cf-7b/the PySt unit = 0.5. Solvent: THF-d_8. [copolymer] = 3.33×10^{-3} g/ml

for the change in the temperature. For the micelles with Cf-7b, the signals of the pyridine rings and the methylene attached to the aromatic rings were hardly observed

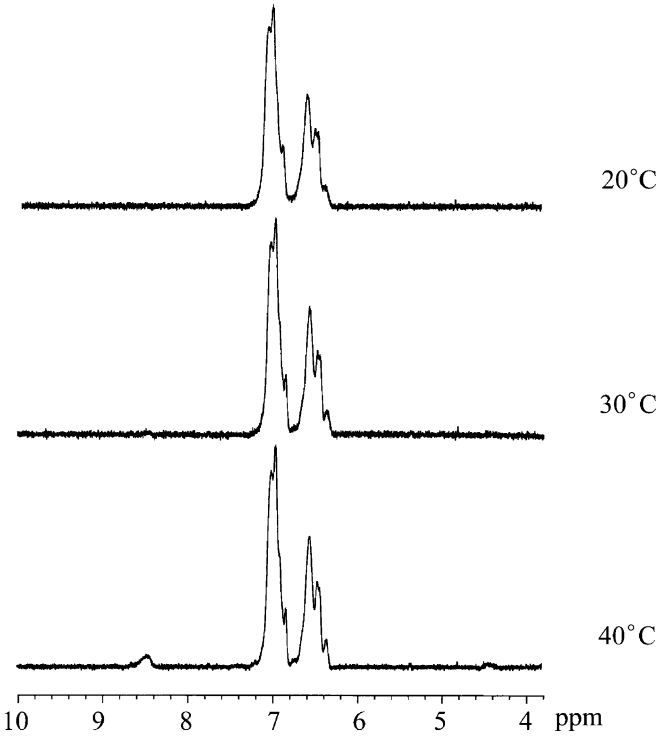

Fig. 10 Variability in the ^{1}H NMR signals of the PPySt-*b*-PSt micelles with Cf-2b for the temperature. Cf-2b/the PySt unit = 0.5. Solvent: THF-d_8. [copolymer] = 3.33×10^{-3} g/ml

at 20 °C. When the temperature was raised to 30 °C, the signals of the pyridine rings were discerned at 8.67 ppm for the 2 positions and at 7.60 ppm for the 3 positions. These signals were observed more sharply at 40 °C, with the small shifts to the higher magnetic field side. The methylene signals were also discerned at this temperature. On the other hand, for the micelles with Cf-2b, the signals of the pyridine rings for the 2 positions and the methylene were barely discerned even at 40 °C. Cf-2b produced the micelles having the cores cross-linked more tightly because of the shorter perfluoroalkyl chain than Cf-7b, resulting in that the PPySt cores were more shielded from the magnetic resonance. Consequently, the micelles prepared with Cf-2b are regarded to be more structurally stable for the temperature.

Conclusions

We attained the direct preparation of the core cross-linked micelles using the nonamphiphilic PPySt-*b*-PSt diblock copolymer. The copolymer showed micellization in the presence of the perfluoroalkyl dicarboxylic acid in the nonselective solvent. The micellization proceeded through the salt formation and the cross-linking of the PPySt blocks via the dicarboxylic acid. The light scat-

tering studies demonstrated that the micellar size and aggregation number were dependent on the chain length of the perfluoroalkyl dicarboxylic acid. The acid with longer perfluoroalkyl chain produced the larger micelles with higher aggregation number. The micelles were thermally stable because of the cross-linked cores. The micelles almost maintained the structure for the changes in the temperature, although those were slightly swelled as a result of increasing the temperature. The dicarboxylic acid with the shorter perfluoroalkyl chain produced structurally stable micelles with less mobility of the cores.

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