Research Article

Synthesis and the Micellar Characteristics of Poly(ethylene oxide)–Deoxycholic Acid Conjugates

Chulhee Kim, Sang Cheon Lee, Suk Won Kang, Ick Chan Kwon, Yong-Hee Kim, and Seo Young Jeong

Langmuir, **2000**, 16 (11), 4792-4797 • DOI: 10.1021/la9907634

Synthesis and the Micellar Characteristics of Poly(ethylene oxide)−Deoxycholic Acid Conjugates[1]

Chulhee Kim,* Sang Cheon Lee, and Suk Won Kang

Department of Polymer Science and Engineering, Inha University, Inchon 402-751, Korea

Ick Chan Kwon, Yong-Hee Kim, and Seo Young Jeong

Biomedical Research Center, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul 130-650, Korea

Received June 14, 1999. In Final Form: February 29, 2000

Novel polymeric amphiphiles were synthesized on the basis of poly(ethylene oxide) (PEO) as a hydrophilic segment and deoxycholic acid (DC) as a hydrophobic segment. Their micellar formation and characteristics in an aqueous phase were investigated by using ^{1}H NMR analysis, fluorescence techniques, light scattering, and transmission electron microscopy (TEM). The PEO−DC conjugates formed micelles in the aqueous phase with critical micelle concentrations (cmc's) in the range of 36−43 mg/L at 25 °C. The cmc values became higher at higher temperatures. The cmc's of the conjugates were much lower than that of the deoxycholic acid sodium salts. The mean diameters of the micelles were in the range of 120−180 nm, with a narrow size distribution. The TEM image showed micelles with a spherical shape. The partition equilibrium constants, K_v, of pyrene in the micellar solutions of the PEO−DC conjugate were 1.8×10^4 to 7.4×10^4 at 25 °C. The K_v value decreased as the temperature increased. The steady-state fluorescence anisotropy values (r) of 1,6-diphenyl-1,3,5-hexatriene (DPH) were 0.196−0.214 in PEO−DC solutions. The anisotropy values were not significantly dependent on the length of the hydrophilic PEO block. The fluorescence lifetime values for DPH in the PEO−DC conjugate solutions were in the range of 6.05−7.03 ns, and indicated that the core region of the micelles consisted of two regions with different hydrophobicity.

Introduction

Polymeric amphiphiles consisting of hydrophilic and hydrophobic segments have become attractive building blocks in the growing field of molecular self-assembly in an aqueous phase due to their unique solution properties and technical applications in various fields.[2−16] In analogy with low molecular weight amphiphiles and lipids, they undergo intermolecular association by hydrophobic segment leading to the construction of micelles or nanoaggregates, which have various morphological characteristics.[11−13] In addition, their hydrophobic core is surrounded by a hydrophilic outer shell so that the inner core can serve as a microcontainer for various substances. Therefore, there have been growing interests on the design and characterization of novel amphiphilic copolymers and hydrophobically modified water-soluble polymers which can self-assemble to form micelles or micelle-like aggregates in an aqueous phase.[18−20] In particular, poly(ethylene oxide) (PEO) has been selected as a host of a hydrophilic segment for a variety of polymeric amphiphiles.[3−6,21−24] However, there are few studies on the self-assembled structure of the polymeric amphiphile which consists of hydrophilic PEO and hydrophobic low molecular weight natural components. In this study, we describe the synthesis and the micellar characterization of novel polymeric amphiphiles based on PEO as a hydrophilic segment and deoxycholic acid (DC), a main component of bile acid, as a hydrophobic segment. PEO is a well-known biocompatible polymer that expresses low toxicity and, when located at the surface and interface, suppresses the protein and cellular adsorption. Bile acid is the main product of cholesterol metabolism and the most abundant

* To whom correspondence should be addressed. E-mail: chk@inha.ac.kr. Telephone: 82-32-860-7487. Fax: 82-32-865-5178.

(1) A preliminary account of this work was presented at the 216th ACS national meeting in 1998. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1998**, *39* (2), 258.
(2) Gref, R.; Minamitake, Y.; Peracchia, M. T.; Trubetskoy, V.; Torchilin, V.; Langer, R. *Science* **1994**, *263*, 1600.
(3) Xu, R.; Winnik, M. A.; Hallett, F. R.; Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 87.
(4) Wilhelm, M.; Zhao, C.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J.; Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 1033.
(5) Caldérara, F.; Hruska, Z.; Hurtrez, G.; Lerch, J.; Nugay, T.; Riess, G. *Macromolecules* **1994**, *27*, 1210.
(6) Xu, R.; Winnik, M. A.; Riess, G.; Chu, B.; Croucher, M. D. *Macromolecules* **1992**, *25*, 644.
(7) Hurter, P. N.; Hatton, T. A. *Langmuir* **1992**, *8*, 1291.
(8) Kataoka, K.; Kwon, G. S.; Yokoyama, M.; Okano, T.; Sakurai, Y. *J. Controlled Release* **1993**, *24*, 119.
(9) Yokoyama, M.; Kwon, G. S.; Okano, T.; Sakurai, Y.; Seto, T.; Kataoka, K. *Bioconjugate Chem.* **1992**, *3*, 295.
(10) Kwon, G. S.; Suwa, S.; Yokoyama, M.; Okano, T.; Sakurai, Y.; Kataoka, K. *J. Controlled Release* **1994**, *29*, 17.
(11) Gao, Z.; Varshney, S. K.; Wong, S.; Eisenberg, A. *Macromolecules* **1994**, *27*, 7923.
(12) Yu, K.; Eisenberg, A. *Macromolecules* **1996**, *29*, 6359.
(13) Zhang, L.; Eisenberg, A. *J. Am. Chem. Soc.* **1996**, *118*, 3168.
(14) Ma, Y.; Cao, T.; Webber, S. E. *Macromolecules* **1998**, *31*, 1773.
(15) Martin, T. J.; Procházka, K.; Munk, P.; Webber, S. E. *Macromolecules* **1996**, *29*, 6071.
(16) Martin, T. J.; Webber, S. E. *Macromolecules* **1995**, *28*, 8845.
(17) Patrickios, C. S.; Forder, C.; Armes, S. P.; Billingham, N. C. *J. Polym. Sci., Part* A: Polym. Chem. **1996**, *34*, 1529.
(18) Lee, S. C.; Chang, Y.; Yoon, J.-S.; Kim, C.; Kwon, I. C.; Kim, Y.-H.; Jeong, S. Y. *Macromolecules* **1999**, *32*, 1847.
(19) Lee, K. Y.; Jo, W. H.; Kwon, I. C.; Kim, Y.-H.; Jeong, S. Y. *Macromolecules* **1998**, *31*, 378.
(20) Tsuchida, E.; Yamamoto, K.; Miyatake, K.; Endo, K. *Macromolecules* **1997**, *30*, 4235.
(21) Nagasaki, Y.; Okada, T.; Scholz, C.; Iijima, M.; Kato, M.; Kataoka, K. *Macromolecules* **1998**, *31*, 1473.
(22) Kwon, G. S.; Naito, M.; Yokoyama, M.; Okano, T.; Sakurai, Y.; Kataoka, K. *Langmuir* **1993**, *9*, 945.
(23) Kabanov, A. V.; Nazarova, I. R.; Astafieva, I. V.; Batrakova, E. V.; Alakhov, V. Y.; Yaroslavov, A. A.; Kabanov, V. A. *Macromolecules* **1995**, *28*, 2303.
(24) Poppe, A.; Willner, L.; Allgaier, J.; Stellbrink, J.; Richter, D.; *Macromolecules* **1997**, *30*, 7462.

10.1021/la9907634 CCC: $19.00
Published on Web 05/05/2000

detergent-like molecule in the body.[25] In an aqueous media, bile salts can self-associate to form micelles, which play an important role in achieving adequate digestion and absorption of fats and lipid-soluble vitamins in the body. Thus, its physicochemical and thermodynamic properties such as micellization and aggregation kinetics in an aqueous phase have been extensively studied.[26−28] In this study, it is expected that the introduction of deoxycholic acid as a terminal group of PEO can induce self-association of PEO−DC conjugates leading to the formation of micelles in an aqueous phase. The synthetic strategy for PEO−DC conjugates in this study may allow the preparation of a biomimetic surfactant, which can be applied in the area of drug delivery. The preparation of PEO−DC conjugates was carried out by the reaction of α-methoxy-ω-aminopoly(ethylene oxide)s with an activated succinimide ester of deoxycholic acid. Micellar characteristics of this novel polymeric amphiphile in an aqueous phase were investigated using fluorescence techniques, dynamic light scattering, and TEM.

Experimental Section

Materials and Equipment. α-Methoxy-ω-aminopoly(ethylene oxide)s with M_n of 1000 and 5000 (Shearwater Polymers, Inc.) were purified by precipitation from methylene chloride into diethyl ether and were used after drying. Deoxychoilc acid (DC) (Sigma), *N*-hydroxysuccinimide (NHS) (Aldrich), dicyclohexylcarbodiimide (DCC) (TCI), 4-(dimethylamino)pyridine (DMAP) (TCI), pyrene (Aldrich), 1,6-diphenyl-1,3,5-hexatriene (DPH) (Aldrich), and 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP) (Aldrich) were used as received. THF was dried over sodium benzophenone ketyl and was distilled under nitrogen before use. Diethyl ether and *n*-hexane were used without further purification. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AC 250 spectrometer at 250 and 63 MHz, respectively. FT-IR spectra were recorded on a Perkin-Elmer Spectrum 2000 Explorer FT-IR spectrometer. Elemental analysis was performed on a CE instruments EA 1110(CHNS/O). Mass spectrum was obtained on a Hewlett-Packard 5890-JMS AX505WA spectrometer. Molecular weights and molecular weight distributions were determined using a GPC equipped with a Waters Associates 410 RI detector, 510 HPLC pump, and μ-Styragel columns with pore sizes of 10^2, 500, 10^3, and 10^4 Å. The eluent was THF, and the molecular weights were calibrated with polystyrene standards. UV−vis spectra were obtained on a Hewlett-Packard 8452A spectrophotometer.

Preparation of DC−NHS. To prepare the activated succinimide ester of DC, DCC (2.62 g, 12.7 mmol), and DMAP (0.078 g, 0.64 mmol) were added to a stirred THF solution (110 mL) of DC (5 g, 12.7 mmol) and NHS (1.46 g, 12.7 mmol). The reaction mixture was stirred at room temperature for 24 h under nitrogen. After filtration of dicyclohexylurea, the DC−NHS was obtained by column chromatography and was dried at 40 °C in vacuo before use: yield 20%; ^{1}H NMR (DMSO-d_6) δ 0.60 (s, 3H), 0.83 (s, 3H), 0.94 (d, 3H), 1.00−1.76 (m, 25H), 2.55−2.73 (m, 2H), 2.79 (s, 4H), 3.78 (s, 1H), 4.21 (d, 1H), 4.45 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 12.4, 16.7, 23.1, 23.5, 25.4, 26.1, 27.0, 27.1, 27.6, 28.6, 30.2, 30.5, 32.9, 33.8, 34.7, 35.1, 35.6, 36.3, 41.6, 46.0, 46.1, 47.5, 70.0, 71.0, 169.3, 170.2; FAB-MS *m*/*z* 488. Anal. Calcd for $C_{28}H_{43}NO_6$: C, 68.68; H, 8.85; N, 2.86. Found: C, 68.90; H, 8.78; N, 2.64.

Preparation of PEO−DC Conjugates. The PEO−DC conjugates were prepared by the reaction of DC−NHS with α-methoxy-ω-aminopoly(ethylene oxide). The synthetic procedure of a conjugate, PEO5000−DC, which consisted of PEO with M_n of 5000 and DC, is as follows. A THF solution of α-methoxy-ω-aminopoly(ethylene oxide) (M_n: 5000) (3 g, 0.6 mmol) and DC−NHS (0.88 g, 1.8 mmol) was stirred at reflux for 12 h under nitrogen. The pure PEO5000−DC conjugate was isolated by column chromatograpy and was precipitated twice into diethyl ether (yield 40%). PEO1000−DC conjugate was synthesized in an identical manner except that α-methoxy-ω-aminopoly(ethylene oxide) with M_n of 1000 was employed (yield 41%).

Sample Preparation. To prepare micellar solutions, the PEO−DC conjugate was dispersed in a phosphate-buffered saline (PBS) solution (pH 7.4) under gentle stirring for 3 h, followed by sonication for 30 min. For the measurement of fluorescence spectra of pyrene in micellar solutions, samples were prepared following a literature procedure.[18] The concentrations of sample solutions were varied from 5×10^{-5} g/L to 10 g/L. The final concentration of pyrene in the sample solutions was 6.0×10^{-7} M. For the measurements of steady-state fluorescence anisotropy and fluorescence lifetime of DPH in micellar solutions, samples were prepared following a literature procedure.[18,29]

Fluorescence Measurements. All the fluorescence measurements were performed using an ISS K2 spectrofluorometer with a thermostat cell unit. Samples were excited using a 300 W xenon arc lamp (ILC Technology). The measurement of pyrene excitation spectra was performed using a reference method.[18] For the excitation spectra, $\lambda_{em} = 391$ nm. Steady-state fluorescence anisotropy values (r) of DPH were determined in the L-format geometry of detection.[29] The excitation wavelength was 360 nm and the emission was measured at 430 nm. Fluorescence lifetimes of DPH were measured using an ISS K2 fluorometer equipped with a frequency synthesizer (Marconi Instruments) and an ISS-ADC interface for data collection and analysis.[18] The phase shift and demodulation ratios, using POPOP in ethanol as the reference ($\tau = 1.35$ ns), were recorded at 10 different modulation frequencies, logarithmically spaced (2.0, 3.3, 5.6, 9.3, 15.5, 25.8, 43.1, 71.9, 119.9, and 200.0 MHz). The excitation was operated at 360 nm. The emission was collected through a 408 nm cut-on filter. Fluorescence lifetimes were determined using a nonlinear least-squares program (ISS187) which minimized the reduced χ^2 for an exponentially good fit.

Light Scattering Measurements. Dynamic light scattering measurements were performed using a Brookhaven BI-200SM goniometer, BI-9000AT autocorrelator, and He−Ne laser (632.8 nm) (Research Electro-optics 35 mW). The sample solutions were purified by passing through a Millipore 0.45 μm filter. The hydrodynamic diameters (d) of micelles and the polydispersity factor of micelles, represented as μ_2/Γ^2, were calculated by using the Stokes−Einstein equation and the cumulant method, respectively.[30,31] CONTIN algorithms were used in the Laplace inversion of the autocorrelation function to obtain micelle size distribution.[4]

Transmission Electron Microscopy. Transmission electron microscopy (TEM) was performed on a Philips CM 200, operating at an acceleration voltage of 80 kV. For the observation of size and distribution of micellar particles, a drop of sample solution (concentration = 1 g/L) was placed onto a 300 mesh copper grid coated with carbon. About 2 min after deposition, the grid was tapped with filter paper to remove surface water, followed by air-drying. Negative staining was performed by using a droplet of a 5 wt % uranyl acetate solution. The samples were air-dried before measurement.[13]

Results and Discussion

Synthesis and Characterization of PEO−DC Conjugates. The PEO−DC conjugates were synthesized, as illustrated in Scheme 1, by the reaction of α-methoxy-ω-aminopoly(ethylene oxide)s and DC−NHS, an activated ester of DC, which was prepared by the reaction of DC with *N*-hydroxysuccinimide in the presence of DCC in THF. The molecular weights of α-methoxy-ω-aminopoly(ethylene oxide)s were 1000 and 5000, which respectively produced PEO1000−DC and PEO5000−DC. The forma-

(25) Small, D. M. In *The Bile Acids, Chemistry, Physiology, and Metabolism*; Nair, P. P., Kritchevsky, D., Eds.; Plenum Press: New York, 1971; p 249.
(26) Coello, A.; Meijide, F.; Rodríguez Núñez, E.; Vázquez Tato, J. *J. Pharm. Sci.* **1996**, *85*, 9.
(27) Gouin, S.; Zhu, X. X. *Langmuir* **1998**, *14*, 4025.
(28) Jover, A.; Meijide, F.; Rodríguez Núñez, E.; Vázquez Tato, J. *Langmuir* **1998**, *14*, 4359.
(29) Ringsdorf, H.; Venzmer, J.; Winnik, F. M. *Macromolecules* **1991**, *24*, 1678.
(30) Harada, A.; Kataoka, K. *Macromolecules* **1995**, *28*, 5294.
(31) Harada, A.; Kataoka, K. *Macromolecules* **1998**, *31*, 288.

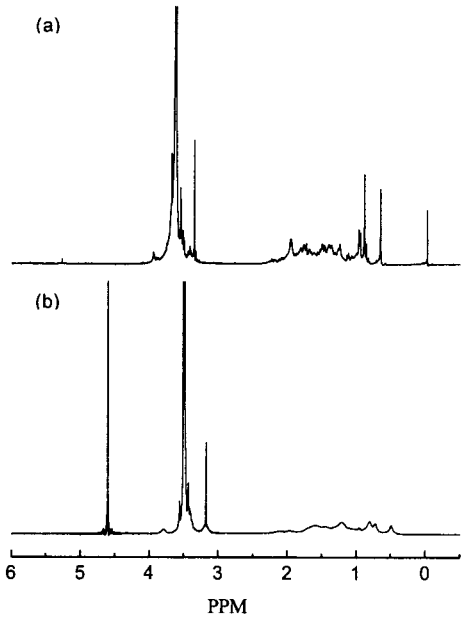
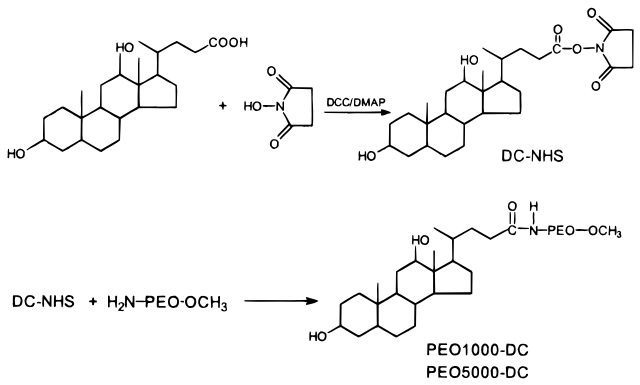


Figure 1. ^{1}H NMR spectra of PEO1000−DC in $CDCl_3$ (a) and D_2O (b).

Scheme 1



tion of amide linkage between PEO and DC was confirmed by the amide I band at 1653 cm^{-1} in FT-IR spectrum and amide carbon signal at 173.6 ppm in ^{13}C NMR. The molecular weights and compositions of PEO−DC conjugates were determined by the analysis of ^{1}H NMR spectrum. The ^{1}H NMR spectrum of PEO1000−DC in Figure 1a shows characteristic resonance peaks.

The number-average molecular weights of PEO−DC conjugates were determined by comparing the integration ratio of the protons in DC part at 0.65−2.30 ppm and the methylene protons in PEO at 3.60 ppm. The M_n's of PEO1000−DC and PEO5000−DC were 1400 and 5100, respectively. The gel permeation chromatograms, as shown in Figure 2, were narrow and did not show a trace of free PEO, reflecting the absence of free PEO and DC. The molecular weights and compositions of PEO−DC conjugates are summarized in Table 1.

Micelle Formation of PEO−DC Conjugates. The amphiphilic PEO−DC conjugates consisting of hydrophilic PEO and hydrophobic DC can self-associate to form micellar structure in an aqueous phase. The formation and characteristics of PEO−DC conjugate micelles were studied by using a NMR spectroscopy, fluorescence technique, dynamic light scattering, and TEM. The formation of micelles was demonstrated by examining the conjugate solutions using NMR spectroscopy.[22,32] Figure 1 shows the ^{1}H NMR spectra of PEO1000−DC in $CDCl_3$ (a) and D_2O (b), respectively. As clearly shown in spectrum b, small and broad signals assigned to DC moiety at 0.50−2.20 ppm were observed, which indicated limited molecular motion of DC moiety surrounded by the hydrophilic PEO. This result confirms the core−shell structure of PEO−DC conjugates in an aqueous phase. The critical micelle concentratons (cmc's) of PEO−DC conjugates were determined by a fluorescence technique using pyrene as a probe.[4,21−23,33] In Figure 3, the pyrene excitation spectra at various concentrations of PEO5000−DC are shown.

Above the critical concentration region, the gradual shift of pyrene spectra from 332 to 335 nm was observed, reflecting the change in vibrational structure of pyrene emission. The spectral shift in pyrene excitation spectra was utilized to determine the cmc's of PEO−DC conjugates. Figure 4 shows the intensity ratio (I_{335}/I_{332}) of pyrene excitation spectra depending on the concentration of PEO5000−DC and PEO1000−DC.

At low concentration ranges, the change in intensity ratios is negligible. As concentration increases, intensity ratios increase markedly, reflecting the partitioning of

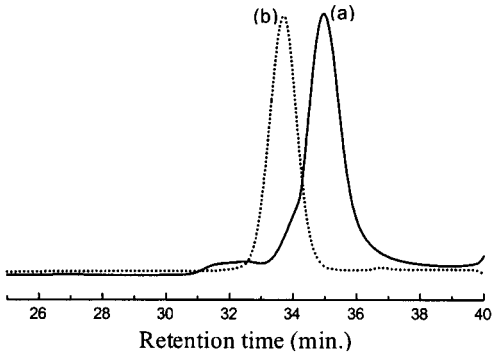


Figure 2. Gel permeation chromatograms of α-methoxy-ω-aminopoly(ethylene oxide) (M_n: 1000) (a) and PEO1000−DC (b).

Table 1. Molecular Weights and Compositions of PEO−DC Conjugates

conjugates	M_n[a]	wt % of deoxycholic acid[a]	M_w/M_n[b]
PEO1000−DC	1400	28	1.04
PEO5000−DC	5100	8	1.07

[a] Calculated by ^{1}H NMR. [b] Estimated by GPC.

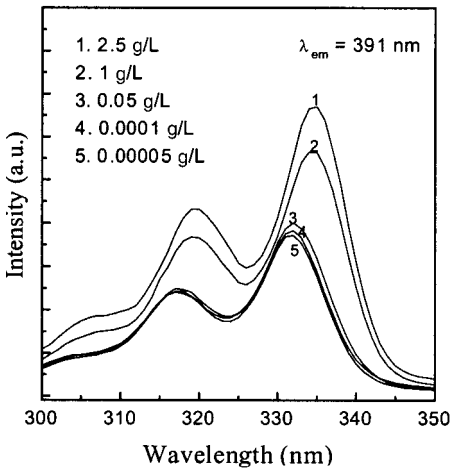


Figure 3. Excitation spectra of pyrene as a function of PEO5000−DC concentration in PBS solution.

(32) Weimer, M. W.; Scherman, O. A.; Sogah, D. Y. *Macromolecules* **1998**, *31*, 8425.

Table 2. Micellar Characteristics of PEO−DC Conjugates in PBS Solution

	cmc (mg/L)						K_v ($\times 10^{-4}$)				
conjugates	25 °C	30 °C	37 °C	45 °C	d^a (nm)	$\mu_2/\Gamma^{2\ b}$	25 °C	30 °C	37°C	45 °C	r[c]
PEO1000−DC	43	68	87	100	120	0.12	1.8	1.4	1.3	1.1	0.214
PEO5000−DC	36	61	63	73	180	0.07	7.4	6.5	5.8	5.5	0.196

[a] Mean diameters by dynamic light scattering at 25 °C. [b] Polydispersity factor. [c] Steady-state fluorescence anisotropy of DPH

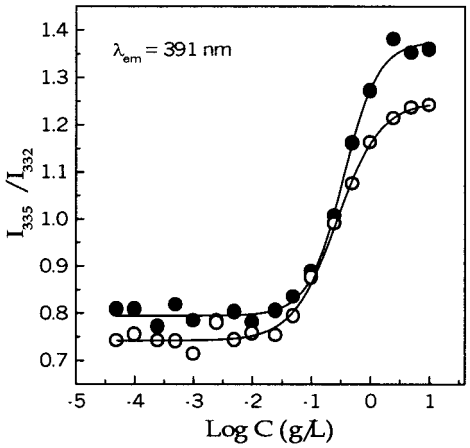


Figure 4. Plot of I_{335}/I_{332} (from pyrene excitation spectra) vs log C for PEO1000−DC (●) and PEO5000−DC (○) at 25 °C.

pyrene into the hydrophobic core region of micelles. Therefore, the cmc was determined from the crossover point at the low concentration range in Figure 4. The cmc's of PEO1000−DC and PEO5000−DC at 25 °C were 43 and 36 mg/L respectively, as listed in Table 2. The cmc's of the conjugates were much higher than those of other amphiphilic block copolymers.[4,18,21−23,33] However, they were much lower than 1 g/L for deoxycholic acid in water, indicating the formation of micelles at lower concentration.[25] The cmc values increased as the temperature of aqueous phase increased, as listed in Table 2.

This tendency could be ascribed to the enhanced solubility of bile acid in water upon increasing temperatures, which reduced the hydrophobicity of deoxycholic acid moiety in PEO−DC conjugates.[25] The mean hydrodynamic diameters (d) of PEO1000−DC and PEO5000−DC micelles, measured by dynamic light scattering, were 120 and 180 nm, respectively. Considering the block length of PEO−DC conjugates and the micellar sizes, it could be suggested that the micelles of PEO1000−DC would be multicore structure formed by the association of individual micelles rather than a simple core−shell type structure. In previous reports, micelles with a PEO outer shell are frequently found to form large aggregates in an aqueous phase.[34,35] In particular, Eisenberg et al. recently reported that the formation of large aggregates of individual micelles was more pronounced in the case of micelles with a shell from a relatively short PEO block (M_n = ∼2000), which could not evade hydrophobic interactions or van der Waals interactions between the exposed hydrophobic cores of micelles.[35] The polydispersity factors (μ_2/Γ^2) of the micelles, estimated by the cumulant method, were fairly low (0.07−0.12), suggesting narrow size distribution.[18,30,31] The micelles of PEO−DC conjugates were observed by TEM as shown in Figure 5.

The micelles of PEO1000−DC and PEO5000−DC are generally spherical. The size of micelles from TEM

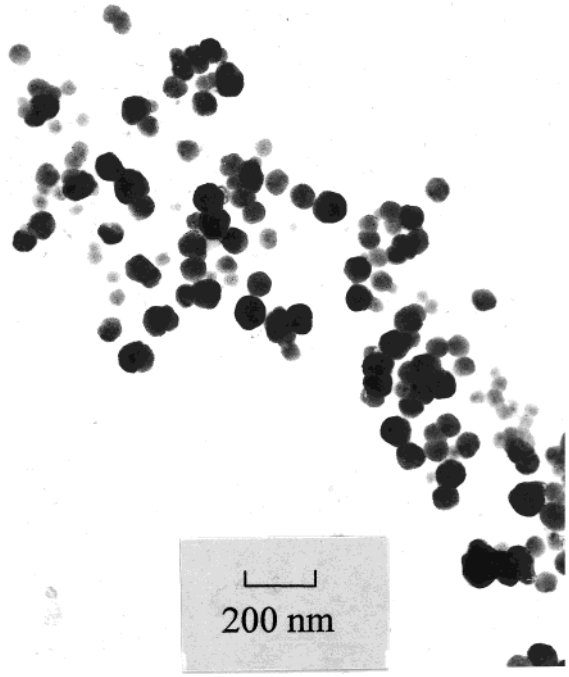


Figure 5. Transmission electron micrographs of PEO1000−DC micelles.

experiment might be smaller than that of micelles in an aqueous phase because of the collapse of micellar outer shell during the drying process in TEM experiment.

Binding Equilibrium of Pyrene. The hydrophobicity of the micellar core was estimated by measuring the equilibrium constant K_v for partitioning of pyrene between micellar phase and water. In this work, the equilibrium constant K_v is calculated following the method suggested by Wilhelm et al.[4] Assuming the simple equilibrium of pyrene binding to the micelles, the ratio of pyrene concentration in the micellar phase to water phase ($[Py]_m/[Py]_w$) can be correlated to the ratio of volume of each phase as expressed in

$$[Py]_m/[Py]_w = K_v V_m/V_w \quad (1)$$

Equation 1 can be rewritten as

$$[Py]_m/[Py]_w = K_v xc/1000\rho \quad (2)$$

where x is the weight fraction of deoxycholic acid, c is the concentration of the amphiphile, and ρ is the density of the deoxycholic acid core of micelles, which is assumed as that of deoxycholic acid (1.31 g/mL).[25] In the intermediate range of polymer concentration with substantial increases of intensity ratios (I_{335}/I_{332}), $[Py]_m/[Py]_w$ can be written as

$$[Py]_m/[Py]_w = (F - F_{min})/(F_{max} - F) \quad (3)$$

where F_{max} and F_{min} correspond to the average magnitude of I_{335}/I_{332} in the flat region of high and low concentration ranges in Figure 4, and F is the intensity ratio (I_{335}/I_{332}) in the intermediate concentration range of the conjugates. Combining eqs 2 and 3, K_v values of pyrene are determined

(33) Astafieva, I.; Zhong, X. F.; Eisenberg, A. *Macromolecules* **1993**, *26*, 7339.
(34) La, S. B.; Okano, T.; Kataoka, K. *J. Pharm. Sci.* **1996**, *85*, 85.
(35) Allen, C.; Yu, Y.; Maysinger, D.; Eisenberg, A. *Bioconjugate Chem.* **1998**, *9*, 564.

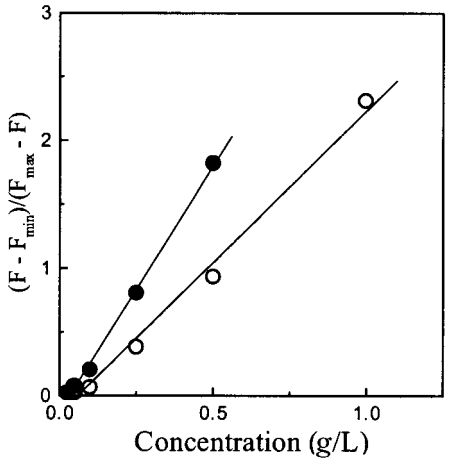


Figure 6. Plots of $(F - F_{min})/(F_{max} - F)$ vs concentration of PEO1000−DC at 25 °C (●) and at 45 °C (○).

by using a plot $(F - F_{min})/(F_{max} - F)$ versus PEO−DC concentration as shown in Figure 6-.

The K_v values at various temperatures are summarized in Table 2. The K_v values were 1.1×10^4 to 1.8×10^4 for PEO1000−DC, and 5.5×10^4 to 7.4×10^4 for PEO5000−DC, which suggests that the hydrophobicity of PEO−DC micellar core is lower than those of other amphiphiles such as sodium dodecyl sulfate ($K_v = 1.2 \times 10^5$) and PEO−polystyrene block copolymer micelles ($K_v = 3.0 \times 10^5$).[4,36] In the overall temperature range, the K_v values of PEO5000−DC were higher than those of PEO1000−DC, which reflected higher hydrophobicity of PEO5000−DC micellar core. The K_v values decreased as temperature increased, which resulted from the decrease in the hydrophobicity of DC core as discussed previously.

Microviscosity of Micellar Core. The microviscosity of the micellar core region was estimated by the measurement of the steady-state fluorescence anisotropy originated from the depolarization of DPH fluorescence due to the rotational diffusion of DPH. The anisotropy value increases upon increasing the microviscosity of the micellar core because the rotational diffusion of DPH is increasingly hindered. The anisotropy values, *r*, measured for PEO−DC conjugate micelles, are listed in Table 2. The anisotropy values were not significantly dependent on the length of the hydrophilic PEO block. It is worth comparing *r* values in Table 2 with those of SDS (0.070), poly(ethylene-*co*-maleic acid) (0.187), poly(1-decene-*co*-maleic acid) (0.225), poly(1-octadecene-*co*-maleic acid) (0.273), poly(2-ethyl-2-oxazoline)-*b*-poly(L-lactide) (∼0.270), and poly(2-ethyl-2-oxazoline)-*b*-poly(ϵ-caprolactone) (∼0.190).[18,37]

Fluorescence Lifetime. The site-specific information on the polarity was obtained from the fluorescence lifetime measurement of DPH using the phase and modulation method. The phase and modulation data at logarithmically spaced frequencies for DPH in the presence of PEO5000−DC are plotted in Figure 7.

The increase in phase angle and the decrease in modulation ratio were observed as the frequency increased. By analysis in biexponential functions, the goodness of fit (χ^2) was obtained more satisfactorily than with any other exponential functions, as judged by the χ^2 values (Table 3).

The single decay function and the three discrete exponential functions were not adequate for the reduction

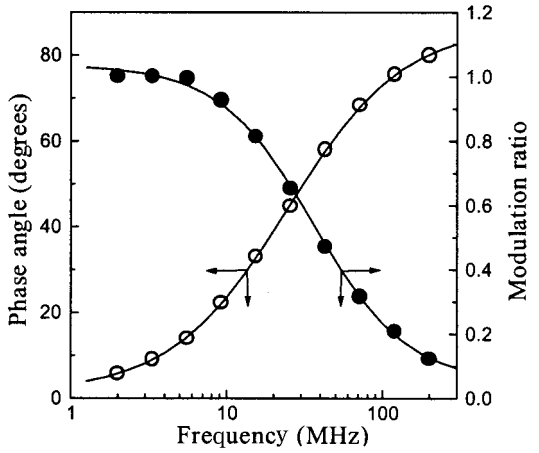


Figure 7. Frequency-dependent phase (○) and modulation (●) data for DPH in PEO5000−DC solution (polymer concentration = 2.5 g/L).

Table 3. Lifetime Data for DPH in PEO−DC Conjugate Solutions

conjugates	τ_1 (ns)	f_1[a]	τ_2 (ns)	f_2[a]	χ^2	$\langle\tau\rangle$[b](ns)
PEO1000−DC	6.93	0.80	2.25	0.20	5.29	6.05
PEO5000−DC	7.86	0.83	2.93	0.17	2.42	7.03

[a] Fractional intensity, $f_i = \alpha_i\tau_i/\sum\alpha_i\tau_i$, where α_i is preexponential factor representing the fractional contribution to the time-resolved decay of the component with a lifetime τ_i. [b] Average lifetime, $\langle\tau\rangle = \tau_1 f_1 + \tau_2 f_2$.

of χ^2 values. Therefore, it is suggested that the micelles may have two different hydrophobic microdomains. The microdomains with longer lifetime are the less polar regions.[18] Although the PEO−DC conjugates are suggested as muticore-structured micelles, each hydrophobic domain consists of less polar region (inner part) and more polar region. Therefore, the polarity of several hydrophobic domains constructing multicore could be averaged for two assumed domains, more polar region and less polar region. The fluorescence lifetimes of DPH in PEO5000−DC were larger than that in PEO1000−DC suggesting that the hydrophobic microdomain of PEO5000−DC was less polar than that of PEO1000−DC. This result is consistent with the K_v values in Table 2. The fraction values (f_1 and f_2) in Table 3 suggest that the more hydrophobic microdomain out of two different hydrophobic domains occupies the majority of the micellar inner core. For block copolymers such as poly(1-decene-*co*-maleic acid) and poly(1-octadecene-*co*-maleic acid), the DPH lifetime values of the main domain were reported as 10.62 and 11.58 ns, respectively.[37]

Conclusions

The novel polymeric amphiphiles, PEO−DC conjugates, were prepared and their micellar behavior in an aqueous phase was investigated. The critical micelle concentrations of PEO1000−DC and PEO5000−DC, depending on the temperature, were in the range of 43−100 mg/L and 36−73 mg/L, respectively. The cmc values of conjugates were much lower than that of deoxycholic acid sodium salts. The mean diameters of the micelles were in the range of 120−180 nm, with a narrow size distribution. The TEM image showed micelles with a spherical shape. The partition equilibrium constants, K_v, of pyrene were 1.1×10^4 to 1.8×10^4 for PEO1000−DC and 5.5×10^4 to 7.4×10^4 for PEO5000−DC micellar solutions, suggesting that the micellar core of PEO5000−DC is more hydrophobic than that of PEO1000−DC. K_v values decreased at higher

(36) Almgren, M.; Grieser, F.; Thomas, J. K. *J. Am. Chem. Soc.* **1979**, *101*, 279.
(37) McGlade, M. J.; Randall, F. J.; Tcheurekdjian, N. *Macromolecules* **1987**, *20*, 1782.

temperatures. The steady-state fluorescence anisotropy (r) values of DPH in PEO−DC conjugate solutions were in the range of 0.196−0.214. The analysis of the fluorescence lifetime of DPH in PEO−DC solutions indicated that the micellar core region consisted of two domains with different polarity. In addition, the hydrophobic core of PEO5000−DC was less polar than that of PEO1000−DC. Since the micelles constructed from PEO−DC conjugates are expected to express low toxicity in the body, they might be suitable as a carrier vehicle for delivering bioactive agents.

Acknowledgment. This work was supported by Korea Institute of Science and Technology. C.K. also thanks HOMRC for the support.

LA9907634