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Article

Thermo-Responsive and Biocompatible Diblock Copolymers Prepared via Reversible Addition-Fragmentation Chain Transfer (RAFT) Radical Polymerization

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Abstract: Poly(2-(methacryloyloxy)ethyl phosphorylcholine)-*b*-poly(*N*,*N*-diethyl acrylamide) (PMPC$_m$-PDEA$_n$) was synthesized via reversible addition-fragmentation chain transfer (RAFT) controlled radical polymerization. Below, the critical aggregation temperature (CAT) the diblock copolymer dissolved in water as a unimer with a hydrodynamic radius (R_h) of *ca.* 5 nm. Above the CAT the diblock copolymers formed polymer micelles composed of a PDEA core and biocompatible PMPC shells, due to hydrophobic self-aggregation of the thermo-responsive PDEA block. A fluorescence probe study showed that small hydrophobic small guest molecules could be incorporated into the core of the polymer micelle above the CAT. The incorporated guest molecules were released from the core into the bulk aqueous phase when the temperature decreased to values below the CAT because of micelle dissociation.

Keywords: block copolymers; micelles; RAFT polymerization; water-soluble polymers; critical aggregation temperature

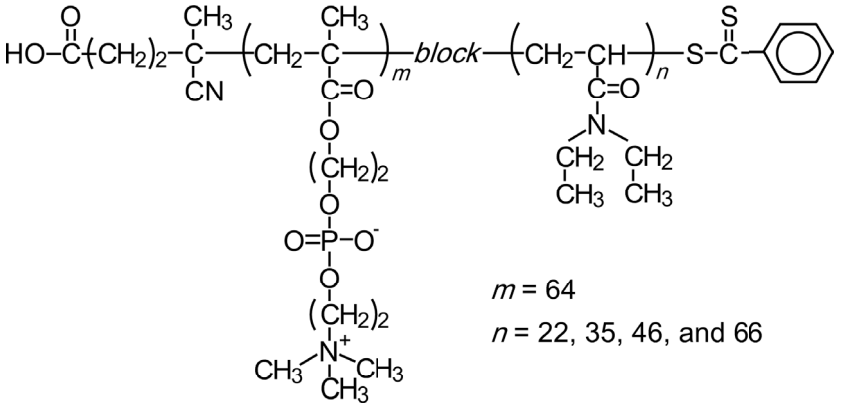
1. Introduction

Phospholipids are a component of lipid bilayers in cell membranes; thus, they are of interest in the biochemical and biomedical fields as unique substrates. The phosphorylcholine group of 2-(methacryloyloxy)ethyl phosphorylcholine (MPC) is a component of cell membranes [1,2]. MPC can be polymerized with other vinyl monomers, and the properties and functions of MPC polymers can be controlled by changing comonomers. Polymers with water-soluble MPC units show excellent biocompatibility and antithrombogenicity.

In water, many *N*-substituted polyacrylamides show phase separation upon heating [3], and the temperature at which phase transition occurs is called the lower critical solution temperature (LCST). The most extensively studied polymer is poly(*N*-isopropylacrylamide) (PNIPAM) [4]. Below the LCST, the PNIPAM chains are dissolved as extended coils, while above the LCST they collapse into compact globules in a coil-to-globule transition [5]. Poly(*N*,*N*-diethylacrylamide) (PDEA) is also a thermo-responsive polymer similar to PNIPAM. The LCST of PDEA is reported to be between 25 and 35 °C, depending on molecular weight, concentration, and external additives [6]. The LCST is mainly governed by the balance between hydrogen bonds and hydrophobic interactions among alkyl side chains [7]. Therefore, the LCST is accompanied by sharp changes in the degree of hydration, chain mobility, hydrophobicity, and so on.

Lowe *et al.* [8] reported the synthesis of diblock copolymers ($PMPC_m$-$PDEA_n$) composed of hydrophilic PMPC and thermo-responsive PDEA blocks and investigated their thermo-responsive association behavior in water using NMR and dynamic light scattering (DLS) measurements. We were interested in the detailed thermo-responsive association behavior of $PMPC_m$-$PDEA_n$ and the potential for controlled release of guest molecules, such as drugs, in the cores of polymer micelles. We focused on diblock copolymers based on a hydrophilic shell-forming PMPC block and a thermo-responsive core-forming PDEA block (Figure 1). $PMPC_m$-$PDEA_n$ can be molecularly dissolved in water below the LCST for the PDEA block, as the PDEA block is hydrophilic under these conditions. However, when the temperature is elevated above LCST, the PDEA block becomes hydrophobic because of the breaking of hydrogen bonding interactions between the pendant amide bond in the PDEA block and water molecules, leading to the formation of micelles with a dehydrated PDEA core and hydrated PMPC shells. These polymer micelles can incorporate in their hydrophobic core hydrophobic guest molecules, such as pyrene and adriamycin hydrochloride (ADR).

Figure 1. Polymer structure of diblock copolymers ($PMPC_m$-$PDEA_n$).



2. Experimental Section

2.1. Materials

2-(Methacryloyloxy)ethyl phosphorylcholine (MPC) was prepared as previously reported and recrystallized from acetonitrile [9]. *N*,*N*-Diethyl acrylamide (DEA) was dried over 4 Å molecular sieves and purified by distillation under reduced pressure. 4-Cyanopentanoic acid dithiobenzoate (CPD) was synthesized according to the method reported by McCormick and co-workers [10]. Pyrene was purified by recrystallization from methanol. 4,4′-Azobis(4-cyanopentanoic acid) (V-501, >98%) and adriamycin hydrochloride (ADR), from Wako Pure Chemical, were used as received without further purification. Water was purified with a Millipore Milli-Q system. Other reagents were used as received.

2.2. Preparation of Poly(2-(methacryloyloxy)ethyl phosphorylcholine)-Based Chain Transfer Agent ($PMPC_{64}$)

A poly(2-(methacryloyloxy)ethyl phosphorylcholine) (PMPC)-based chain transfer agent was prepared using a modified method reported previously [11]. MPC (50.2 g, 170 mmol) was dissolved in 290 mL of water, and then CPD (696 mg, 2.31 mmol) and V-501 (80.7 mg, 0.288 mmol) were added to the solution. The mixture was deoxygenated by purging with Ar gas for 30 min. Polymerization was carried out at 70 °C for 2 h. After cooling the reaction mixture with an ice bath, the solution was dialyzed against pure water for a week. $PMPC_{64}$ was recovered by freeze-drying (19.1 g, 38.0% conversion). The number-average molecular weight (M_n(NMR)) and degree of polymerization (DP) for $PMPC_{64}$ were 1.89×10^4 and 64, respectively, as estimated from ^{1}H nuclear magnetic resonance (NMR) terminal group analysis. The molecular weight distribution (M_w/M_n), estimated from gel-permeation chromatography (GPC), was 1.19.

2.3. Preparation of Diblock Copolymers

A typical procedure for block copolymerization is as follows. $PMPC_{64}$ (2.00 g, M_n(NMR) = 1.89×10^4, M_w/M_n = 1.19), DEA (2.58 g, 20.3 mmol), and V-501 (5.92 mg, 0.0210 mmol) were dissolved in 32.0 mL of methanol. The solution was placed in a glass ampoule and outgassed on a vacuum line by six freeze-pump-thaw cycles, and then the ampoule was vacuum-sealed. Polymerization was carried out at 70 °C for 24 h. The reaction mixture was poured into a large excess of diethyl ether to precipitate the resulting polymer. The polymer ($PMPC_{64}$-$PDEA_{66}$) was purified by reprecipitation from methanol into a large excess of diethyl ether (2.64 g, 57.6% conversion). The M_n(NMR) and M_w/M_n values for $PMPC_{64}$-$PDEA_{66}$ were 2.82×10^4, M_w/M_n = 1.21, respectively.

To investigate the relationship between polymerization time and conversion, the monomer conversion was determined by ^{1}H NMR spectroscopy. Predetermined amounts of DEA, $PMPC_{64}$, and V-501 were dissolved in methanol. The solution was transferred to an NMR tube containing an NMR lock tube of D_2O and degassed by purging with Ar gas for 30 min. The cap was sealed and the solution was heated at 70 °C in a preheated oil bath for varying lengths of reaction time. Polymerization was terminated by rapid cooling with an ice bath. The monomer conversion estimated from ^{1}H NMR was monitored as a function of reaction time.

2.4. Measurements

GPC measurements were performed with a GPC system composed of a Tosoh DP-8020 pump, a Tosoh RI-8021 refractive index detector, and Shodex 7.0 μm bead size GF-7M HQ column (molecular weight range 10^7–10^2) using a phosphate buffer (pH 9) containing 20 vol% acetonitrile as an eluent at a flow rate of 0.6 mL/min at 25 °C. Sample solutions were filtered with a 0.2-μm pore size membrane filter before measurements. The M_n(GPC) and M_w/M_n for the polymers were calibrated with standard sodium poly(styrenesulfonate) samples.

^{1}H NMR spectra were obtained with a Bruker DRX-500 spectrometer. The sample solutions of the block copolymers at a polymer concentration (C_p) of 5.0 g/L for ^{1}H NMR measurements were prepared in D_2O containing 0.1 M NaCl. The temperature was changed from 24 °C to 60 °C with a heating or cooling rate of 1 °C/min.

Percent transmittance (T%) for 0.1 M NaCl aqueous solutions of diblock copolymer was measured with a U-3000 spectrophotometer (Hitachi, Tokyo, Japan) with a 1.0 cm path length quartz cell at various temperatures. The temperature was changed from 20 °C to 60 °C with a heating or cooling rate of 0.5 °C/min.

Static light scattering (SLS) and dynamic light scattering (DLS) measurements were performed at 25 °C with an Otsuka Electronics Photal DLS-7000HL light scattering spectrometer equipped with a 5000E multi-τ digital time correlator (ALV, Langen, Germany). Sample solutions for SLS and DLS measurements were filtered with a 0.2-μm pore size membrane filter. For SLS measurements, a He-Ne laser (10 mW at 632.8 nm) was used as a light source. The weight-average molecular weight (M_w), z-average radius of gyration (R_g), and second virial coefficient (A_2) values were estimated from the relation:

$$\frac{KC_p}{R_\theta} = \frac{1}{M_w}\left(1 + \frac{1}{3}\left\langle R_g^2 \right\rangle q^2\right) + 2A_2C_p \quad (1)$$

where R_θ is the Rayleigh ratio, $K = 4\pi^2 n^2 (dn/dC_p)^2/N_A\lambda^4$, with n being the refractive index of the solvent, dn/dC_p being the refractive index increment against C_p, N_A being Avogadro's number, and λ being the wavelength (= 632.8 nm), while $q = (4\pi n/\lambda)\sin(\theta/2)$ with θ being the scattering angle. By measuring R_θ for a set of C_p and θ, values of M_w, R_g, and A_2 were estimated from Zimm plots. Toluene was used for calibration of the instrument. Values of dn/dC_p were determined with an Otsuka Electronics Photal DRM-1020 differential refractometer at a wavelength of 632.8 nm. For DLS measurements, an He-Ne laser (10 mW at 632.8 nm) was used as a light source. The hydrodynamic radius (R_h) was calculated using the Einstein-Stokes relation $R_h = k_BT/6\pi\eta D$, where k_B is Boltzmann's constant, T is the absolute temperature, and η is the solvent viscosity.

Excitation emission spectra for pyrene were recorded on a Hitachi F-2500 fluorescence spectrophotometer. A pyrene-saturated aqueous stock solution was prepared as previously [12]. Sample solutions were prepared by mixing aliquots of stock solutions of the polymer and pyrene (2.0×10^{-7} M) in 0.1 M NaCl aqueous solutions. Excitation spectra were monitored at 390 nm. Excitation and emission slit widths were maintained at 2.5 and 20 nm, respectively.

2.5. Release Experiments

The release of ADR from the polymer micelles and blank experiments were performed according to a modified literature procedure [13]. The release was monitored by a V-530 UV/VIS spectrophotometer (Jasco, Tokyo, Japan) equipped with a magnetic stirrer using a 1.0 cm path length quartz cell as a diffusion cell contained in a small glass cell separated from the surrounding solution by a dialysis membrane with a molecular weight cutoff of 15,000. First, 0.3 mL of a 0.1 M NaCl aqueous solution of ADR (0.06 mM) with and without $PMPC_{64}$-$PDEA_{66}$ (C_p = 50 g/L) was put inside a small glass cell. The temperature of the solution was maintained at 25 and 50 °C. The small glass cell, capped with a dialysis membrane with an o-ring, was immersed into 3.0 mL of 0.1 M NaCl with same the temperature as that of the blank and polymer solutions in the quartz cell. The cumulative amount of ADR released from the small glass cell was monitored by absorption at 480 nm.

3. Results and Discussion

We prepared $PMPC_{64}$ via RAFT using CPD as a water-soluble CTA. The M_n(NMR) value, calculated from ^{1}H NMR peak area intensities of pendant methylene protons and terminal phenyl protons (Table 1), was 1.89×10^4. The M_n(GPC) and M_w/M_n values were 8.62×10^3 and 1.19, estimated from GPC. A notable observation was the marked deviation of M_n(GPC) from M_n(NMR). To verify the true molecular weight of $PMPC_{64}$, SLS measurements were performed. The M_w value of 2.04×10^4 determined by SLS was in fair agreement with M_n(NMR), using M_w/M_n = 1.19. It should be mentioned that M_n(GPC) values estimated by GPC are only apparent values, probably because sodium poly(styrenesulfonate) was used as a standard for molecular weight calibration, compared to $PMPC_{64}$, which has a bulky phosphorylcholine side chain.

Table 1. Characteristics of poly(2-(methacryloyloxy)ethyl phosphorylcholine)-*b*-poly(*N*,*N*-diethyl acrylamide) ($PMPC_m$-$PDEA_n$).

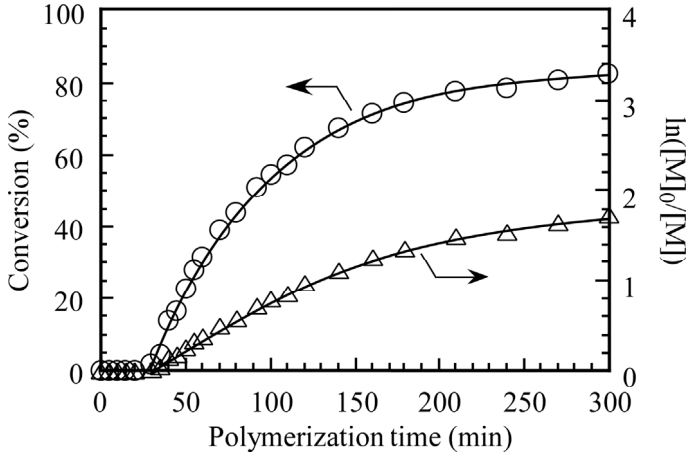
Sample code	M_n(NMR) [a] $\times 10^{-4}$	M_n(GPC) [b] $\times 10^{-3}$	M_w(SLS) [c] $\times 10^{-4}$	M_w/M_n [b]	$A_2 \times 10^4$ (mol·mL·g^{-2}) [c]	R_g (nm) [d]	dn/dC_p mL/g
$PMPC_{64}$	1.89	8.62	2.04	1.19	7.71	9.6	0.139
$PMPC_{64}$-$PDEA_{22}$	2.26	8.10	2.95	1.26	7.34	13.7	0.134
$PMPC_{64}$-$PDEA_{35}$	2.43	8.35	3.06	1.26	4.42	16.2	0.139
$PMPC_{64}$-$PDEA_{46}$	2.57	8.30	3.46	1.22	4.09	18.3	0.138
$PMPC_{64}$-$PDEA_{66}$	2.82	8.30	3.89	1.21	7.81	22.6	0.146

[a] estimated by ^{1}H NMR in methanol-d_4; [b] estimated by GPC; [c] estimated by SLS in 0.1 M NaCl aqueous solution; [d] estimated by DLS at C_p = 5.0 g/L in 0.1 M NaCl aqueous solution at 25 °C.

To prepare the thermo-responsive diblock copolymer, DEA was polymerized via RAFT radical polymerization in methanol using the $PMPC_{64}$ macro-chain transfer agent. Figure 2 shows the pseudo-first-order kinetics plot for the polymerization of DEA in the presence of $PMPC_{64}$ at 70 °C under Ar in methanol containing a sealed tube of D_2O to lock the NMR frequency. Monomer consumption was monitored by ^{1}H NMR spectroscopy as a function of polymerization time. There was an induction period of *ca.* 30 min, which may have been due to a slow rate of formation of the radical

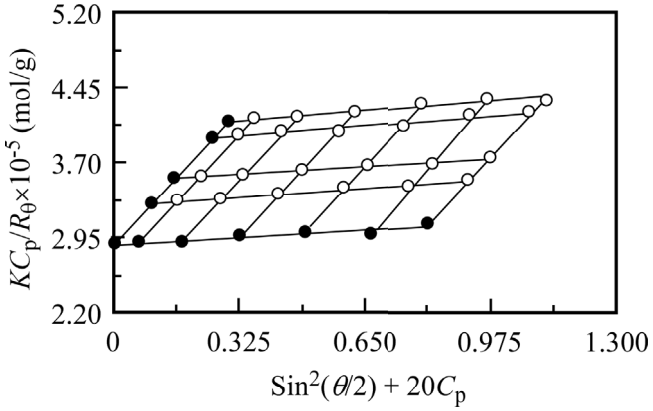
fragment [14]. A monomer conversion of 83% was reached within 5 h. The downward curvature was observed, which indicates a decrease in the concentration of propagating radicals.

Figure 2. Time–conversion (○) and the first-order kinetic plots (△) for polymerization of DEA in the presence of $PMPC_{64}$ (M_n(NMR) = 1.89 × 10^4, M_w/M_n = 1.19) in methanol at 70 °C. $[M]_0$ and [M] are the concentrations of the monomer at polymerization time = 0 and the corresponding time, respectively.



The M_n(GPC) values estimated from GPC were only apparent values because sodium poly(styrenesulfonate) was used as a standard for molecular weight calibration. To verify the true molecular weight of $PMPC_m$-$PDEA_n$, SLS measurements were performed (Figure 3). The M_n(NMR) for $PMPC_m$-$PDEA_n$ was calculated from ^{1}H NMR in D_2O at 25 °C (Figure 4a). The M_w(SLS) values of $PMPC_m$-$PDEA_n$, determined by SLS, were in fair agreement with M_n(NMR), using M_w/M_n. Apparent M_w, R_g, and A_2 for the diblock copolymers at 25 °C, determined by SLS measurement, are listed in Table 1. The dn/dC_p values at 633 nm for the polymers at 25 °C are listed in Table 1. The same $PMPC_{64}$ macro-chain transfer agent of M_n(NMR) = 1.89 × 10^4 was used to prepare a series of $PMPC_{64}$-$PDEA_n$ with different PDEA block lengths. The DP values for PMPC and PDEA blocks were calculated based on ^{1}H NMR. Table 1 lists the molecular parameters of the polymers.

Figure 3. Typical example of a Zimm plot for $PMPC_{64}$-$PDEA_{46}$ in 0.1 M NaCl at 25 °C. The polymer concentration was varied from 5 to 10 g/L. The Rayleigh ratio (R_θ) is determined by subtracting the solvent scattering from the total scattering for the solutions.



To study heat-induced association of the diblock copolymers, ^{1}H NMR spectra for the block copolymers were measured at different temperatures in D_2O containing 0.1 M NaCl. Figure 4 compares typical ^{1}H NMR spectra for $PMPC_{64}$-$PDEA_{66}$ measured at 25 and 60 °C. At 25 °C the diblock copolymer chains are fully solvated and molecularly dissolved in water—*i.e.*, a "unimer" state—and all signals expected for each block were observed. The resonance bands in the 0.8–1.4 ppm region were attributed to the sum of the α-methyl protons in the main chain and the methyl protons in the pendant *N*,*N*-diethylamino group. The methylene protons in the main chain of the PMPC and PDEA blocks were observed at 1.6 and 2.0 ppm, respectively. The resonance peak at 2.6 ppm was attributed to the methine proton in the main chain of the PDEA block. The resonance bands at 3.1–3.5 ppm were assigned to the methyl protons of the pendant trimethyl ammonium group of the PMPC block and the methylene protons in the pendant *N*,*N*-diethylamino group of the PDEA block, respectively. The methylene protons in the pendant phosphorylcholine group of the PMPC block were observed at 3.6–4.5 ppm. The composition of the block copolymer was determined from the area intensity ratio of the resonance bands due to the methine proton in the main chain of the PDEA block at 2.6 ppm and the methylene protons of the pendant phosphorylcholine of the PMPC block at 3.6 ppm at 25 °C, because these peaks were relatively isolated from the other peaks. The intensity of resonance peaks corresponding to the PDEA block decreased at an elevated temperature of 60 °C, which implies poor solvation and reduced mobility of the PDEA block. Considering its chemical structure, the block copolymer should form a core-shell-type polymer micelle with dehydrated PDEA blocks forming a core and hydrophilic PMPC blocks forming a shell.

Figure 4. Comparison of ^{1}H NMR spectra for $PMPC_{64}$-$PDEA_{66}$ at 25 °C (**a**) and 60 °C (**b**) in D_2O containing 0.1 M NaCl at C_p = 5.0 g/L. Asterisks represent peaks due to solvent.

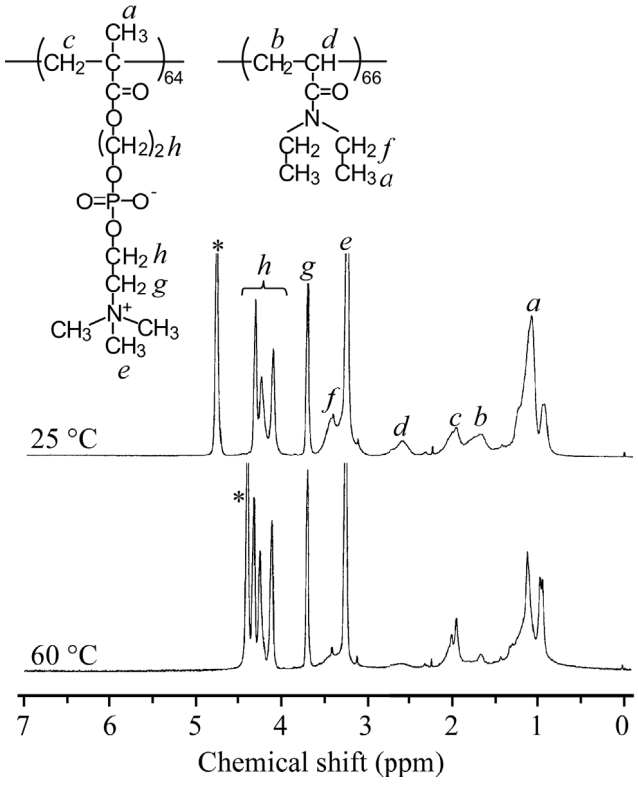
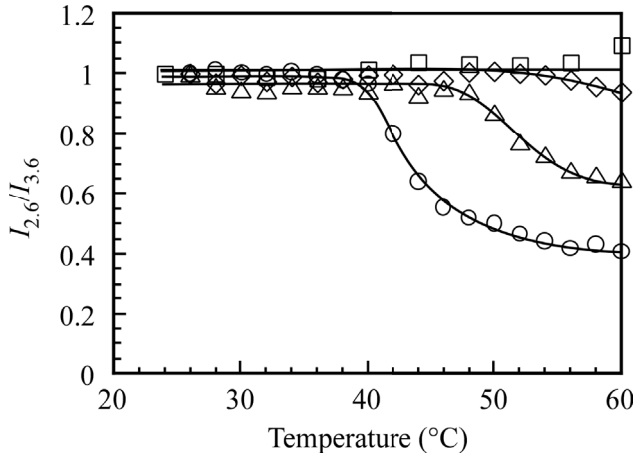


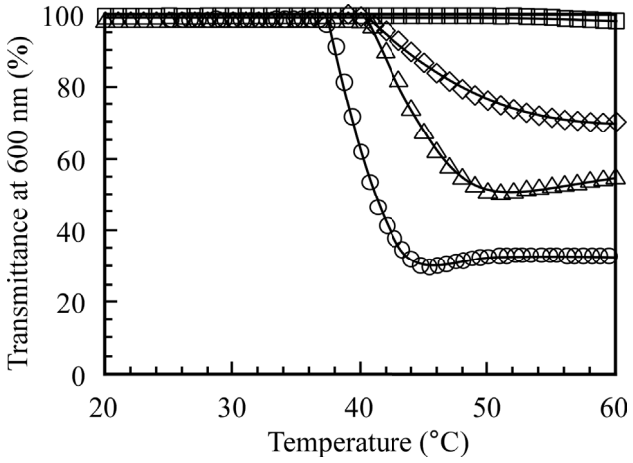
Figure 5 shows the peak intensity ratio for the methine proton at 2.6 ppm in the main chain of the PDEA block and the methylene protons of the pendant phosphorylcholine group of the PMPC block at 3.6 ppm as a function of temperature. The peak intensity ratio is normalized with the ratio at 25 °C. When the temperature is increased from 24 °C, the normalized intensity ratios for $PMPC_{64}$-$PDEA_{35}$ and $PMPC_{64}$-$PDEA_{22}$ were almost constant, independent of the temperature. The normalized peak intensity ratios for $PMPC_{64}$-$PDEA_{66}$ and $PMPC_{64}$-$PDEA_{46}$ were practically constant below 37 and 47 °C, respectively. The intensity ratios for $PMPC_{64}$-$PDEA_{66}$ and $PMPC_{64}$-$PDEA_{46}$ decreased with increasing temperature, reaching minimum values of 0.4 and 0.6, respectively. These findings indicate that the motion of the methine protons in the PDEA blocks for $PMPC_{64}$-$PDEA_{66}$ and $PMPC_{64}$-$PDEA_{46}$ was restricted above a certain temperature. These results suggest that the onset of micellization occurs at lower temperatures for polymers with longer DEA block lengths.

Figure 5. Peak intensity ratio of $I_{2.6}/I_{3.6}$ normalized to the ratio at 25 °C for $PMPC_{64}$-$PDEA_{66}$ (○), $PMPC_{64}$-$PDEA_{46}$ (△), $PMPC_{64}$-$PDEA_{35}$ (◇), and $PMPC_{64}$-$PDEA_{22}$ (□) in D_2O containing 0.1 M NaCl at C_p = 5.0 g/L as a function of temperature.



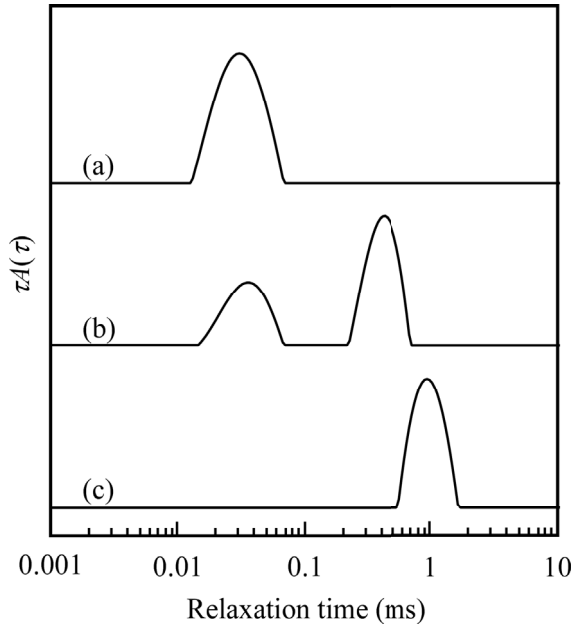
In 0.1 M NaCl aqueous solution, the diblock copolymer undergoes a transition from a molecularly dissolved unimer state at low temperatures to a micellar state above the critical association temperature (CAT). Figure 6 shows values of *T*% monitored at 600 nm for an aqueous solution of the diblock copolymers at C_p = 5.0 g/L. The *T*% value for $PMPC_{64}$-$PDEA_{66}$ is 100% below 38 °C and the solution shows no Tyndall scattering. *T*% decreases with increasing temperature, reaching 30% above 50 °C. Tyndall scattering at 50 °C, which is characteristic of micellar solutions, was visually confirmed. The solution, which was turbid above the CAT, became clear again when the solution was cooled below the CAT. The CAT values for the diblock copolymers were estimated from a break in the *T*% *versus* temperature plot. The CAT values for $PMPC_{64}$-$PDEA_{66}$, $PMPC_{64}$-$PDEA_{46}$, $PMPC_{64}$-$PDEA_{35}$, and $PMPC_{64}$-$PDEA_{22}$ were estimated at 38, 43, 44, and 54 °C, respectively. The CAT values for $PMPC_m$-$PDEA_n$ increased as the thermo-responsive PDEA block length decreased. These observations are consistent with the aforementioned ^{1}H NMR data (Figure 5).

Figure 6. Percent transmittance (T%) at 600 nm for 0.1 M NaCl aqueous solutions of $PMPC_{64}$-$PDEA_{66}$ (○), $PMPC_{64}$-$PDEA_{46}$ (△), $PMPC_{64}$-$PDEA_{35}$ (◇), and $PMPC_{64}$-$PDEA_{22}$ (□) at C_p = 5.0 g/L as a function of temperature.



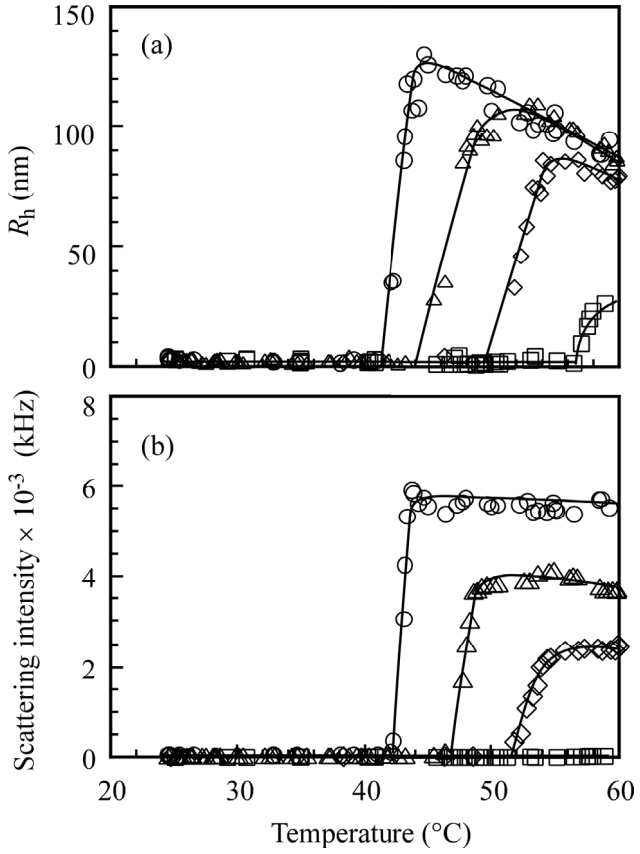
Heat-induced association behavior for the diblock copolymers was confirmed by DLS measurements. The hydrodynamic radius (R_h) for the diblock copolymer was measured in 0.1 M NaCl aqueous solution. Figure 7 shows DLS relaxation time distributions for $PMPC_{64}$-$PDEA_{66}$ at C_p = 5.0 g/L at 25, 42, and 60 °C. The distributions were unimodal at 25 and 60 °C with different relaxation times. A faster relaxation mode at 25 °C was attributed to a unimer state with R_h = 3.2 nm, whereas the slower relaxation time at 60 °C was attributed to polymer aggregates with R_h = 95 nm. At 42 °C, the relaxation time distribution was found to be bimodal. The fast and slow modes were attributed to unimers and polymer aggregates.

Figure 7. Typical examples of DLS relaxation time distributions for $PMPC_{64}$-$PDEA_{66}$ at C_p = 5.0 g/L in 0.1 M NaCl at 25 °C (**a**), 42 °C (**b**), and 60 °C (**c**).



The R_h values for the diblock copolymers, determined by DLS measurement, are plotted as a function of temperature in Figure 8a. The R_h values for the diblock copolymers below a certain temperature were in the order of *ca.* 5 nm, suggesting that all of the polymers existed in a unimer state. Upon an increase in temperature, the R_h values for $PMPC_{64}$-$PDEA_{66}$, $PMPC_{64}$-$PDEA_{46}$, $PMPC_{64}$-$PDEA_{35}$, and $PMPC_{64}$-$PDEA_{22}$ began to increase at 42, 44, 50, and 57 °C. These observations indicate the formation of polymer micelles above certain temperatures. As temperature was further increased, the R_h values for $PMPC_{64}$-$PDEA_{66}$, $PMPC_{64}$-$PDEA_{46}$, and $PMPC_{64}$-$PDEA_{35}$ started to decrease. These observations suggest that aggregates of the PDEA blocks became more compact due to further dehydration of the PDEA blocks as the temperature was increased beyond the CAT, or the aggregation number (N_{agg}) of the multipolymer aggregate decreased with increasing temperature. The scattering intensity is proportional to M_w, because the intensity is linearly related to R_θ/C_p (*i.e.*, $R_\theta/C_p \propto M_w$). Therefore, the observation that the scattering intensities are nearly constant above CAT (Figure 8b) suggests that N_{agg} is practically constant. The absolute scattering intensity is not important to obtain DLS data, however for SLS measurements the absolute scattering intensity is important to determine correct R_θ. The accurate SLS data for the polymer micelle solutions at high temperature cannot be obtained, because there is multiple scattering from the turbid solution at 60 °C (Figure 6).

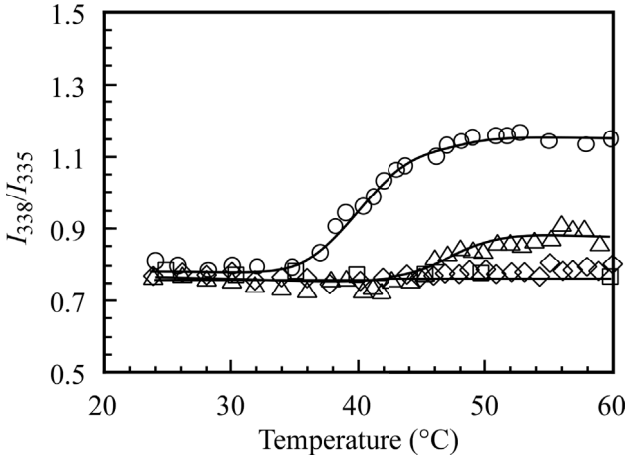
Figure 8. (**a**) Hydrodynamic radius (R_h) and (**b**) scattering intensity for $PMPC_{64}$-$PDEA_{66}$ (○), $PMPC_{64}$-$PDEA_{46}$ (△), $PMPC_{64}$-$PDEA_{35}$ (◇), and $PMPC_{64}$-$PDEA_{22}$ (□) at C_p = 5.0 g/L in 0.1 M NaCl as a function of temperature.



Excitation spectra of pyrene in 0.1 M NaCl aqueous solution in the presence of the block copolymers at varying temperatures were obtained. The excitation spectrum in the case of $PMPC_{64}$-$PDEA_{66}$ showed peaks associated with the (0-0) band of pyrene at 335 nm below the CAT, and the peak shifted to 338 nm at 60 °C. It is known that the (0-0) band in pyrene excitation spectra in water shifts to longer wavelengths when pyrene is solubilized in hydrophobic domains [15]. Thus, we estimated the ratio of the intensity at 338 nm relative to that at 335 nm (I_{338}/I_{335}), and this is plotted in Figure 9 as a function of temperature. It is known that the dithiobenzoate group at the polymer chain end prepared via RAFT is responsible for pyrene fluorescence quenching [16]. We measured only the relative intensity ratio of the excitation emission spectra. Therefore, the quenching effect can be ignored to some degree. The I_{338}/I_{335} values for $PMPC_{64}$-$PDEA_{35}$ and $PMPC_{64}$-$PDEA_{22}$ were almost constant independent of the temperature. This observation indicates that the hydrophobic domain formed from the PDEA blocks could not incorporate the pyrene probes, presumably because of the low hydrophobicity of the core. As temperature was increased, the I_{338}/I_{335} value for $PMPC_{64}$-$PDEA_{66}$ and $PMPC_{64}$-$PDEA_{46}$ began to increase at 34 and 44 °C. When the temperature was increased from 25 to 60 °C and subsequently decreased to 25 °C, the thermo-responsive emission spectral changes were found to be completely reversible without hysteresis.

The CAT values for the block copolymers were measured using various methods, including ^{1}H NMR, *T*%, R_h, scattering intensity, and pyrene fluorescence. These CAT values did not always coincide, depending on the measurement method. For example, the CATs for $PMPC_{64}$-$PDEA_{66}$ estimated from ^{1}H NMR, R_h, and fluorescence methods were 37, 42, and 36 °C, respectively, presumably due to the difference in the sensitivity of the measurement methods.

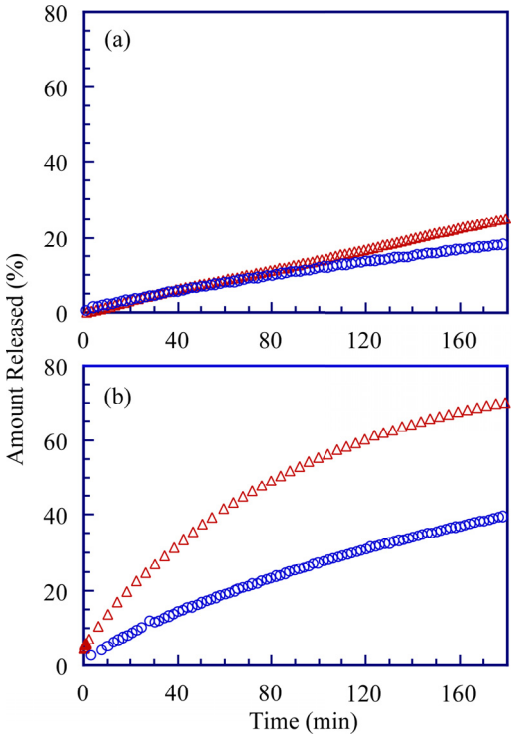
Figure 9. I_{338}/I_{335} in pyrene excitation spectra in the presence of $PMPC_{64}$-$PDEA_{66}$ (○), $PMPC_{64}$-$PDEA_{46}$ (△), $PMPC_{64}$-$PDEA_{35}$ (◇), and $PMPC_{64}$-$PDEA_{22}$ (□) at C_p = 5.0 g/L as a function of temperature in 0.1 M NaCl aqueous solution.



The capture and release of small hydrophobic guest drugs by $PMPC_m$-$PDEA_n$ in 0.1 M NaCl aqueous solution may be easily controlled by changing the temperature. We studied the thermo-responsive release behavior of $PMPC_{64}$-$PDEA_{66}$, applying a dialysis method, with ADR as a guest drug [17]. Above the CAT, $PMPC_{64}$-$PDEA_{66}$ formed polymer micelles with a hydrophobic core, sufficient to keep ADR captured in the interior. Below the CAT, however, the polymer micelles dissociated, and ADR was released from the core into the aqueous bulk phase. Figure 10 represents the

time course of the cumulative permeation of ADR through the dialysis membrane at 25 and 50 °C. For comparison, data without $PMPC_{64}$-$PDEA_{66}$ at 25 and 50 °C are presented along with control data. At 25 °C, the rate of permeation of ADR through the membrane was almost same in the presence and absence of $PMPC_{64}$-$PDEA_{66}$; however, the permeation rate of ADR without $PMPC_{64}$-$PDEA_{66}$ at 50 °C was much faster than that at 25 °C. At 50 °C, the permeation rate of ADR from the polymer micelle was slow compared to the control experiment without the polymer. From these observations, it was concluded that the $PMPC_{64}$-$PDEA_{66}$ polymer micelle can retain captured ADR in its core at 50 °C. It is noteworthy that the release rate from the $PMPC_{64}$-$PDEA_{66}$ polymer micelle at 50 °C was slightly higher than that at 25 °C. This observation suggests that the polymer micelle cannot completely incorporate ADR into its core at 50 °C, because the diffusion coefficient of ADR at 50 °C is much higher than at 25 °C. To create a temperature-responsive micelle containing such a molecule without leaking the hydrophobic medication at temperatures higher than the CAT, it is necessary to improve the molecular design of the diblock copolymer—for example, by extending the hydrophobic block length.

Figure 10. Cumulative ADR release to environment at 25 °C (**a**) and 50 °C (**b**). In the blank release (△), ADR (0.06 mM) solution without polymer was released into 0.1 M NaCl aqueous solution. ADR solution with $PMPC_{64}$-$PDEA_{66}$ (○) was released into 0.1 M NaCl aqueous solution.



4. Conclusions

Thermo-responsive diblock copolymers were prepared via RAFT controlled/living radical polymerization. Diblock copolymers, composed of hydrophilic biocompatible PMPC blocks of the same DP (= 64) and thermo-responsive PDEA blocks with different DPs (= 22, 35, 46, and 66), were synthesized. These diblock copolymers formed polymer micelles composed of a hydrophobic PDEA core and hydrophilic PMPC shells in water above the CAT. The CAT values decreased as the DP of

the PDEA block increased. $PMPC_{64}$-$PDEA_{66}$ and $PMPC_{64}$-$PDEA_{46}$ can incorporate hydrophobic guest molecules such as pyrene into the hydrophobic PDEA core above the CAT. Guest molecules such as ADR can also be incorporated into the hydrophobic PDEA core above the CAT, which allows controlled release from the core to the bulk aqueous phase below the CAT. However, leakage of ADR from the hydrophobic core above the CTA cannot be entirely prevented. Therefore, to avoid the leakage of guest molecules from the core above the CAT, it is necessary to improve the system by increasing the chain length of PDEA block.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Inoue, Y.; Nakanishi, T.; Ishihara, K. Adhesion force of proteins against hydrophilic polymer brush surfaces. *React. Funct. Polym.* **2011**, *71*, 350–355.
2. Ueda, T.; Oshida, H.; Kurita, K.; Ishihara, K.; Nakabayashi, N. Preparation of 2-methacryloyloxyethyl phosphorylcholine copolymers with alkyl methacrylates and their blood compatibility. *Polym. J.* **1992**, *24*, 1259–1269.
3. Plate, N.A.; Lebedeva, T.L.; Valuev, L.I. Lower critical solution temperature in aqueous solutions of *N*-alkyl-substituted polyacrylamides. *Polym. J.* **1999**, *31*, 21–27.
4. Heskins, M.; Guillet, J.E. Solution properties of poly(*N*-isopropylacrylamide). *J. Macromol. Sci. Chem.* **1968**, *2*, 1441–1455.
5. Wu, C.; Zhou, S. Laser light scattering study of the phase transition of poly(*N*-isopropylacrylamide) in water. 1. Single chain. *Macromolecules* **1995**, *28*, 8381–8387.
6. Idziak, I.; Avoce, D.; Lessard, D.; Gravel, D.; Zhu, X.X. Thermosensitivity of aqueous solutions of poly(*N*,*N*-diethylacrylamide). *Macromolecules* **1999**, *32*, 1260–1263.
7. Schild, H.G. Poly(*N*-isopropylacrylamide): Experiment, theory and application. *Prog. Polym. Sci.* **1992**, *17*, 163–249.
8. Yu, B.; Lowe, A.B.; Ishihara, K. RAFT synthesis and stimulus-induced self-assembly in water of copolymers based on the biocompatible monomer 2-(methacryloyloxy) ethyl phosphorylcholine. *Biomacromolecules* **2009**, *10*, 950–958.
9. Ishihara, K.; Ueda, T.; Nakabayashi, N. Preparation of phospholipid polylners and their properties as polymer hydrogel membranes. *Polym. J.* **1990**, *22*, 355–360.
10. Mitsukami, Y.; Donovan, M.S.; Lowe, A.B.; McCormick, C.L. Water-soluble polymers. 81. Direct synthesis of hydrophilic styrenic-based homopolymers and block copolymers in aqueous solution via RAFT. *Macromolecules* **2001**, *34*, 2248–2256.

11. Yusa, S.; Fukuda, K.; Yamamoto, T.; Ishihara, K.; Morishima, Y. Synthesis of well-defined amphiphilic block copolymers having phospholipid polymer sequences as a novel biocompatible polymer micelle reagent. *Biomacromolecules* **2005**, *6*, 663–670.
12. Yusa, S.; Kamachi, M.; Morishima, Y. Hydrophobic self-association of cholesterol moieties covalently linked to polyelectrolytes: Effect of spacer bond. *Langmuir* **1998**, *14*, 6059–6067.
13. Tang, Y.; Liu, S.Y.; Armes, S.P.; Billingham, N.C. Solubilization and controlled release of a hydrophobic drug using novel micelle-forming ABC triblock copolymers. *Biomacromolecules* **2003**, *4*, 1636–1645.
14. Donovan, M.S.; Lowe, A.B.; Sumerlin, B.S.; McCormick, C.L. RAFT polymerization of *N*,*N*-dimethylacrylamide utilizing novel chain transfer agents tailored for high reinitiation efficiency and structural control. *Macromolecules* **2002**, *35*, 4123–4132.
15. Yusa, S.; Sakakibara, A.; Yamamoto, T.; Morishima, Y. Reversible pH-induced formation and disruption of unimolecular micelles of an amphiphilic polyelectrolyte. *Macromolecules* **2002**, *35*, 5243–5249.
16. Yusa, S.; Konishi, Y.; Mitsukami, Y.; Yamamoto, T.; Morishima, Y. pH-responsive micellization of amine-containing cationic diblock copolymers prepared by reversible addition-Fragmentation chain transfer (RAFT) radical polymerization. *Polym. J.* **2005**, *37*, 480–488.
17. Yokoyama, M.; Kwon, G.S.; Okano, T.; Sakurai, Y.; Seto, T.; Kataoka, K. Preparation of micelle-forming polymer-drug conjugates. *Bioconjugate Chem.* **1992**, *3*, 295–301.