Preparation and Characterization of a pH-Responsive Core Cross-linked Polymer Micelle

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Abstract. Poly(ethylene glycol)-b-poly(2-(diethylamino) ethyl methacrylate-co-2-cinnamoyloxyethyl acrylate) (PEG-b-P(DEA/CEA)) was prepared by reversible addition–fragmentation chain transfer (RAFT) controlled radical polymerization. pH-responsive association behaviour of PEG-b-P(DEA/CEA) in 0.1 M NaCl was characterized by dynamic light scattering (DLS). As solution pH is increased from an acidic pH, the hydrodynamic radius \( R_h \) increases, indicative of the polymer micelle formation. The formation of a micelle was also supported by static light scattering (SLS) data. The cinnamoyl groups in the core of the polymer micelle undergo photodimerization, yielding cross-links between polymer chains. The core of the polymer micelle was fixed, which was confirmed by DLS, SLS, and small angle X-ray scattering (SAXS) techniques. When pH is decreased to 3, \( R_h \) of the core cross-linked (CCL) polymer micelle slightly increases due to the protonation of the DEA unit in the cross-linked core. The reversible pH-induced swelling and shrinking behaviour can be observed.

1. Introduction

Stimuli-responsive polymer micelles composed of amphiphilic diblock copolymers have received considerable attention as a candidate for controlled delivery applications, including drug delivery system (DDS) [1-3]. However, if polymer micelles are used for this purpose, they may experience dilution below the critical micelle concentration (cmc), leading to dissociation into unimers. In such a case, the encapsulated guest molecules cannot be delivered to the target. On the other hand, core cross-linked (CCL) polymer micelles are expected to have a much higher stability of the encapsulation of guest molecules at a diluted concentration than uncross-linked polymer micelles.

We prepared a stimuli-responsive CCL micelle in water by a photo-cross-linking technique using diblock copolymer micelles. We reported on the preparation of a pH-responsive copolymer (Scheme 1), poly(ethylene glycol)-b-poly(2-(diethylamino) ethyl methacrylate-co-2-cinnamoyloxyethyl acrylate) (PEG-b-P(DEA/CEA)). PEG-b-P(DEA/CEA) was synthesized from precursor polymer (PEG-b-P(DEA/HEA)) prepared via reversible addition-fragmentation chain transfer (RAFT) radical polymerization using PEG based chain transfer agent (PEG-CTA) [4-6]. The PEG block is soluble in water independent of pH. On the other hand, the solubility of DEA depends on pH. CEA is the photo-cross-linkable unit. A CCL micelle was prepared by UV-irradiation. The CCL micelle will be expected for the application of its solubilizing ability for hydrophobic molecules and controlled release by changing pH.

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2. Experimental

2.1. Materials. DEA was passed through basic alumina columns to remove inhibitor. 2-Hydroxyethyl-acrylate (HEA) was dried and purified by distillation under reduced pressure. PEG-CTA was synthesized according to a modified literature procedure [7].

2.2. Preparation of Precursor Diblock Copolymer. Precursor diblock copolymer (PEG-b-P(DEA/HEA)) was prepared by RAFT polymerization. DEA (3.08 g, 16.6 mmol) was dissolved in 30 mL of water, and the solution pH was adjusted to 7 using HCl (6.0 M). PEG-CTA (750 mg, 0.316 mmol), HEA (1.54 g, 13.3 mmol), and 4,4’-azobis (4-cyanopentanoic acid) (27.9 mg, 0.0995 mmol) were added to the solution. Polymerization was carried out at 70 °C for 3 h under Ar atmosphere. The block copolymer was purified by dialysis against pure water. PEG-b-P(DEA/HEA) was recovered by a freeze-drying technique (yield 3.71 g). Number-average molecular weight ($M_n$) and molecular weight distribution ($M_w/M_n$) determined by gel-permeation chromatography (GPC) are $1.00 \times 10^4$ and 1.09, respectively. The total number-average degree of polymerization (DP) for DEA and HEA is 55, and the molar contents of DEA and HEA units in the P(DEA/HEA) block are 67 and 33 mol %, respectively, estimated by $^1$H NMR. To investigate relationship between $M_n$ and conversion, predetermined amounts of neutralized DEA, HEA, PEG-CTA, and initiator were dissolved in D$_2$O. The solution was heated at 70 °C under Ar atmosphere for varying lengths of time. Conversion and $M_n$ were characterized by $^1$H NMR and GPC, respectively.

2.3. Introduction of Cinnamoyl Group to PEG-b-P(DEA/HEA). Cinnamoyl chloride (1.10 g, 6.60 mmol) and PEG-b-P(DEA/HEA) (3.20 g, 0.283 mmol) were dissolved in pyridine (28.0 g, 0.354 mol), and the mixture was stirred for 22 h at room temperature. The mixture was poured into a large excess of diethyl ether to precipitate the polymer. PEG-b-P(DEA/CEA) was recovered (yield 3.34 g). The conversion of HEA to CEA was almost completed confirmed by $^1$H NMR.

2.4. Micelle Cross-Linking. PEG-b-P(DEA/CEA) was dissolved in a 0.1 M NaCl aqueous solution, and the pH was adjusted to 3 or 10, where the polymer concentration ($C_p$) is 5.0 g/L. The photodimerization of cinnamoyl groups was carried out by using an Asahi Spectra MAX-300 equipped with 300-W Xe lamp and a 275-nm cut-off filter. The light intensity was 20 mW/cm$^2$ at 350 nm. The change in absorption spectra of PEG-b-P(DEA/CEA) during photoirradiation was monitored by UV-visible absorption spectra.

2.5. Measurements. GPC analysis was performed using with a SB-804 HQ column working at 40 °C under a flow rate of 0.6 mL/min. A 0.3 M Na$_2$SO$_4$ aqueous solution containing a 0.5 M acetic acid was used as eluent. $^1$H NMR spectra were obtained with a Bruker DRX-500 spectrometer. Light scattering data were obtained at 25 °C with an Otsuka Electronics Photol DLS-7000HL equipped with an ALV5000/EPP multi-$r$ digital time correlator. A He-Ne laser (10.0 mW at 632.8 nm) was used as a light source. Sample solutions for light scattering measurements were prepared with 0.1 M NaCl, which were filtered with a 0.2 μm pore size membrane filter. In dynamic light scattering (DLS) measurements, to obtain the relaxation time distribution, $\tau_A(\tau)$, the inverse Laplace transform (ILT) analysis was performed using the algorithm REPES [8]. The hydrodynamic radius ($R_h$) is calculated using the Einstein–Stokes relation. In static light scattering (SLS) measurements, the angular dependence of the excess absolute time-averaged scattered intensity, also known as the Rayleigh ratio $R_{\omega}(\theta)$, was measured [9]. SAXS measurements were carried out at the BL40B2 of SPring-8, Harima, Japan using an incident X-ray with the wavelength, $\lambda = 0.150$ nm. The measured sample was 5.0 g/L of the CCL micelle in a 2 mm glass capillary. The SAXS scattering intensity from isolated particle in

![Scheme 1. Chemical structure of PEG-b-P(DEA/CEA)](image)
solution was used to model fitting to ignore the contribution of interparticle interaction. The hard sphere and spherical core-shell models were used to analyze the scattering intensity from the CCL micelle [10, 11].

3. Results and Discussion

We studied dependence of $M_n$ and $M_w/M_n$ on the average conversion for the random copolymerization of DEA and HEA using PEG-CTA via RAFT (Figure 1). $M_n$ increased almost linearly with the conversion, while $M_w/M_n$ remained nearly constant independent of conversion. All the values of $M_n$ are close to the theoretical values predicted from the living mechanism.

Before UV-irradiation, the $R_h$ and $M_w$ values of PEG-$b$-P(DEA/CEA) at pH 3 were 4.0 nm and 3.5 \times 10^4, respectively, indicated that the polymer was dissolved as a unimer. On the other hand, at pH 10 the $R_h$ and $M_w$ values of the polymer were 12.5 nm and 3.2 \times 10^6, respectively, indicated that the polymer aggregated to form micelle. At pH 10, the photodimerization of the pendent cinnamoyl groups leads to the cross-linking of different polymer chains in the micelle core. The $R_h$ value for the CCL micelle at pH 10 was found to be the same as that of the un-cross-linked polymer micelle at pH 10. When the solution pH was decreased to 3, the $R_h$ value for the CCL micelle increased from 12.5 to 13.6 nm, which suggests that the CCL micelle remains. The CCL micelle core is swollen in acidic solutions due to the protonation of the DEA units in the cross-linked core. The pH-induced reversible swelling and shrinking of the CCL micelle was observed. The values of aggregation number ($N_{agg}$) can be calculated from the ratio of weight-average molecular weight ($M_w$) for the polymer micelle or the CCL micelle and the unimer. The

### Table 1. Light Scattering Data for Unimer, Polymer Micelle, and CCL Micelle

| Sample       | pH | $R_h$ (nm) | $M_w \times 10^4$ | $N_{agg}$ |
|--------------|----|------------|------------------|-----------|
| Unimer       | 3  | 4.0        | 3.5              | -         |
| Micelle      | 10 | 12.5       | 320              | 92        |
| CCL micelle  | 3  | 13.6       | 340              | 98        |

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![Figure 1. Dependence of $M_n$ and $M_w/M_n$ on the monomer conversion in the polymerization of DEA and HEA in the presence of PEG-CTA in D$_2$O at 70 °C under Ar atmosphere.](image1)

![Figure 2. SAXS data for a CCL micelle aqueous solution containing 0.1 M NaCl at pH 3. The data were fitted to the monodisperse hard sphere model (---) or to the monodisperse spherical core-shell model (—).](image2)
light scattering data for PEG-b-P(DEA/CEA) as a unimer at pH 3, the polymer micelle at pH 10, and the CCL micelle at pH 3 are summarized in Table 1.

Figure 2 shows SAXS data for the CCL micelle in 0.1 M NaCl at pH 3. The data were examined by fitting to a hard sphere model and a spherical core-shell model. The data are reasonably well fitted to the spherical core-shell model over the entire q range, whereas the hard sphere model does not yield a good fit. By fitting the SAXS data to the spherical core-shell model, radius of core and thickness of shell values were estimated to be 9 and 3 nm, respectively. This result indicates that the core of cross-linked P(DEA/CEA) is surrounded by PEG shell. The overall radius of the CCL micelle estimated from SAXS data (= 12 nm) is fairly close to that estimated from light scattering data.

4. Conclusion
PEG-b-P(DEA/HEA) was prepared by RAFT polymerization using PEG-CTA followed by chemical modification into PEG-b-P(DEA/CEA). PEG-b-P(DEA/CEA) exhibited reversible micellization and dissociation induced by changing solution pH. The polymer formed micelles at pH 10. The core of the polymer micelle can be cross-linked by UV-irradiation at pH 10. The $R_h$ values for the CCL micelle at pH 3 and 10 were 12.5 and 13.6 nm, respectively. The CCL micelle indicated reversible swelling and shrinking by changing solution pH. The SAXS data suggested that the CCL micelle composed of cross-linked pH-responsive core and PEG shell.

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