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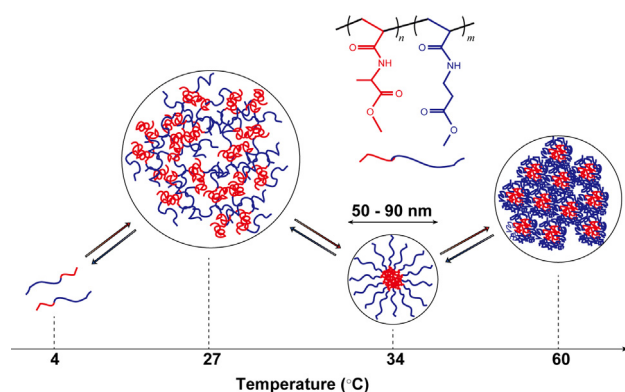
Temperature induced self-assembly of amino acid-derived vinyl block copolymers via dual phase transitions



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GRAPHICAL ABSTRACT



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ABSTRACT

The unique thermoresponsive phase behaviors of diblock copolymers from amino acid-derived vinyl monomers have been demonstrated in view of variation in the aggregation state in water. Amino acid-based block copolymers composed of *N*-acryloyl-Ala-methylester (NAAME) and *N*-acryloyl-βAla-methylester (NAβAME) are successfully synthesized by RAFT polymerization. The resultant block copolymers poly(NAAME₄₈-*b*-NAβAME_{*m*}) contain a constant degree of polymerization ($DP = 48$) of the poly(NAAME) block, but the DP of the poly(NAβAME) block varies ($m = 80-122$). The turbidimetry subjected to these copolymer aqueous solutions exhibits two LCST transitions upon heating. In the 1st LCST region, the block copolymer forms a relatively loose-molecular packing, while large aggregates due to partial dehydration of polymer molecules, which subsequently transform into a stable micelle structure in a region of 30–39 °C. Finally, a tight aggregate composed of the dehydrated micelles is formed. Temperature-dependent ¹H NMR spectroscopy of the diblock copolymers also supports such a postulation for the dual phase transitions and stable micelle structure formation. In addition, a typical salting-out effect is observed in the thermal behavior of the polymer, but a serious cytotoxic effect is not observed in NIH/3T3 cells, suggesting that the novel diblock copolymers are relevant for biomedical applications.

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1. Introduction

Research on thermoresponsive polymers has recently increased due to potential nanotechnology and biotechnology applications [1]. For instance, water-soluble polymers exhibiting a lower critical

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solution temperature (LCST) in water may be useful for several biomedical applications such as smart bioactive surfaces, phase separation immune-assays, and hyperthermia-induced drug delivery [1–4]. Poly(*N*-isopropylacrylamide) (PNIPAM), which shows an LCST in water around 32 °C [5], is so far the most studied thermo-sensitive polymer in bioapplications. Its transition temperature can be shifted to the human body temperature by copolymerization with hydrophilic comonomers [6].

Recently, we reported that the LCST/UCST (upper critical solution temperature) behaviors of amino acid-derived vinyl polymers depend on the kind of amino acid and the chemical modification of the side chain group. Additionally, we found that the copolymerization of two vinyl monomers of different amino acids forms thermo-sensitive copolymers with a widely tunable LCST [7]. For example, random copolymers of NAAME and *N*-acryloyl-Gly-methylester exhibit LCST values between 18 °C and 73 °C, which can be precisely adjusted by varying the copolymer composition. These thermo-sensitive polymers are very promising for biomedical applications since they principally contain bio-compatible amino acid pendants. Despite the potential of amino acid-based vinyl polymers, research has been limited to scattered publications [8–14]. Here we newly synthesize a block copolymer composed of poly(NAAME) block and poly(*N*-acryloyl- β Ala-methylester) (NA β AME) block, both of which exhibit LCST-type transitions at different temperatures (18 °C and 45 °C), respectively. Block copolymers exhibiting both of UCST and LCST transitions offer an interesting hydrophobic and hydrophilic block segment, which makes self-assembly into micelles in water possible. Moreover, by varying the surrounding conditions, they can self-assemble into two different micelle structures (schizophrenic block copolymer) [15]. Such unique thermoresponsive behaviors have been observed for various diblock vinyl copolymers [16–20]. The amino acid-based block copolymers have also exhibited unique phase behaviors. For example, the proline-based block copolymers have shown both of LCST and UCST offered a soluble–insoluble–soluble transition in aqueous media upon elevating the temperature [21]. The thermal behavior of triblock copolymers with triple LCST transitions have been examined. As a result, each block is responsible for one of the transitions, inducing the formation of a micelle-like aggregate with various aggregation morphologies that depend on the block sequence [22,23]. More recently, a triple thermo-responsive block copolymer composed of PNIPAM and poly(methacrylamide) bearing a zwitterionic side chain group exhibits two UCST-type and one LCST-type transitions [24].

In this study, we describe the preparation of well-controlled amino acid-based block copolymers poly(NAAME_{*n*}-*b*-NA β AME_{*m*}) and their unique thermal behaviors in water. The LCST value of each block segment is 18 °C (poly(NAAME)) and 45 °C (poly(NA β AME)). However, the block copolymers exhibit unexpected phase transitions; as the temperature increases, the transparent aqueous solution becomes turbid around 20 °C. Its turbid solution becomes almost transparent at 34 °C, but further increasing the temperature provides a completely turbid solution.

2. Materials and method

2.1. Materials

The monomers NAAME and NA β AME were prepared according to the literature [7]. The chain transfer agent (CTA) benzyl 1*H*-pyrrole-1-carbodithioate (BPCT) was purchased from Sigma-Aldrich and used as received. The initiator α,α' -azobisisobutyronitrile (AIBN), other solvents, and materials were purchased from Wako Pure Chemical Industries (Japan) and used as received,

except for dimethylformamide (DMF), which was purified by distillation under nitrogen atmosphere prior to use.

2.2. Synthesis of poly(NAAME₄₈) macro-CTA

A typical RAFT synthesis of poly(NAAME₄₈) macro-CTA employed the following procedure. A polymerization glass tube was charged with NAAME (2.0 g, 12.7 mmol), BPCT (59 mg, 0.25 mmol), AIBN (21 mg, 0.13 mmol), BPCT/AIBN molar ratio = 2.0), and DMF (2.1 mL). The glass tube was degassed by repeating a freeze–vacuum–nitrogen filling–thaw cycle. It was then sealed into an ampule. Polymerization was carried out at 60 °C for 2 h. The resulting poly(NAAME) (NAAME conversion = 67%) was purified by precipitation with diethylether and dried *in vacuo*. The average degree of polymerization (*DP*) of this macro-CTA was 48, determined by ¹H NMR spectroscopy and size exclusion chromatography (SEC) analysis (eluent: THF; PDI (*M_w*/*M_n*) = 1.15).

2.3. Synthesis of Poly(NAAME₄₈-*b*-NA β AME_{*m*})

A typical protocol for the synthesis of the diblock copolymer poly(NAAME₄₈-*b*-NA β AME₁₀₃) was as follows: poly(NAAME₄₈) macro-CTA (0.452 g, 0.064 mmol), NA β AME (1.00 g, 6.4 mmol; target *DP* = 100), and AIBN (0.005 g, 0.030 mmol; macro-CTA/AIBN molar ratio = 2.1) were dissolved in distilled DMF (2.1 mL). The sealed polymerization ampule was purged with nitrogen and placed in an oil bath at 60 °C for 24 h. The resulting copolymer, which was purified by reprecipitation with diethylether (NA β AME conversion = 67%) was analyzed by ¹H NMR spectroscopy and SEC (eluent: THF; *M_n* = 24,000, *DP* of NA β AME (*m*) = 103, PDI = 1.22)). Diblock copolymers with different value of *m* were prepared in the same manner as above by changing the monomer NA β AME conversion.

2.4. Methods and procedures

2.4.1. Spectroscopies

¹H NMR spectra for the monomers (NAAME and NA β AME) were recorded in *d*₆-DMSO and D₂O, respectively, while those for macro-CTA and the diblock copolymer were recorded in *d*₆-DMSO and *d*₄-methanol, respectively, using a 400 MHz JNM-AL spectrometer (JEOL Co., Japan). For the phase transition experiments, the ¹H NMR spectra were collected at different temperatures from 5 °C to 70 °C in D₂O at a copolymer concentration of 1.0 wt%. In turbidimetry, visible absorption spectra were recorded at 600 nm for 1.0 wt% aqueous solutions of various polymers between 4 °C and 70 °C at a rate of 1 °C min^{−1} using a V-650 spectrophotometer (JASCO Ltd., Japan) equipped with a Peltier type thermostated cell holder coupled with a controller JASCO PTC-423L. To determine the critical aggregation concentration (CAC) by using the dye-method with pyrene as a fluorescent probe, the fluorescence spectra were recorded on a FP-8300 fluorophotometer (JASCO Ltd., Japan).

2.4.2. Size exclusion chromatography (SEC)

The molecular weights and polydispersities of the homopolymers and the diblock copolymers were determined by SEC using an LC-netII/AD (JASCO Ltd., Japan) equipped with a refractive index (RI) detector (Sodex GF-710 F columns; eluent, THF; flow rate, 1 mL min^{−1}; temperature, 40 °C). Standard poly(methacrylate)s (PMMA) were purchased from GL Science Inc. (Japan) and used as the calibration standard.

2.4.3. Microscopic observations

The AFM images were collected at ambient temperature on a SPM9700 (Shimadzu Co., Japan) operated by tapping using a silicon

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