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Assembly behavior of double thermo-responsive block copolymers with controlled response temperature in aqueous solution

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ABSTRACT

We reported the synthesis of double thermo-responsive block copolymers comprised of a poly(*N*-isopropylacrylamide (NIPAAm)) block and a poly(NIPAAm-*co*-*N*-(hydroxymethyl)acrylamide (HMAAm)) block, which was synthesized using an atom transfer radical polymerization (ATRP) technique. The cloud point of the poly(NIPAAm-*co*-HMAAm) (NH) block could be easily controlled by altering the HMAAm content. Below the cloud point of polyNIPAAm (N) block, the block copolymers were completely dissolved. Between the cloud points of each block, the block copolymer formed the aggregate structure that could be constructed with the hydrated NH shell and the dehydrated N core. Moreover with increases in temperature, the diameter decreased due to the dehydration of the NH block shell. The response was completely reversible by changes in temperature, as confirmed by ^{1}H NMR, turbidity, and FE-SEM measurements.

1. Introduction

Recently, the self-assembly behavior of amphiphilic block copolymers in solution has been the focus of much attention due to their wide range of potential applications [1–8]. Assembly is generally triggered by hydrophobic or electrostatic interactions and coordinate bonding. The size and number of polymers in the structure, as well as their physicochemical characteristics (e.g., critical association concentration (CAC) and critical micelle concentration (CMC)) are affected by molecular weight, hydrophilicity, hydrophobicity, charge (or lack thereof), sequence of block copolymers, and other related factor. In recent years, many block copolymers possessing various characteristics have been prepared by controlled living radical polymerizations (CRPs), such as, nitroxide-mediated polymerization (NMP) [9], reversible addition fragmentation chain transfer (RAFT) methods [10,11], and atom transfer radical polymerization (ATRP) [12,13]. These methods have enabled the reliable synthesis of well-defined chain structures with a narrow molecular weight distribution. For example, polyNIPAAm-*b*-poly(L-glutamic acid-*co*-L-lysine) was prepared with stimuli-responsiveness to temperature and pH variation in aqueous solutions, and therefore this assembly was able to change in terms of surface properties in a manner dependent on the environment [14]. Moreover, biotin-poly(ethylene glycol)-*b*-poly(NIPAAm-*co*-HMAAm) was also designed and prepared. At higher temperatures, the block copolymer formed self-assembled micelles, which were composed of biotin at the outermost layer. The micelles were mixed with FITC-avidin to form FITC-avidin-block copolymer complex via biotin–avidin interactions. And, to use the biotin–transferrin, the complex could bond to the certain type of cancer cell [15]. These results were suggested that multifunctional micelles possess were expected great potentials for tumor-targeting chemotherapies.

The behavior of multiple stimuli-responsive polymers has been intensively studied within various frameworks. Multi thermo-responsive polymers are of especial interest due to the triggering of various intelligent systems via changes in temperature. PolyNIPAAm is among the most extensively studied thermo-responsive polymers that exhibit a lower critical solution temperature (LCST) in water at approximately 32 °C. Due to this characteristic behavior in solution, polyNIPAAm has been utilized for biomedical applications, such as thermo-responsive hydrogels [16,17], drug delivery [18–20] and tissue engineering [21,22]. The LCST can be controlled by copolymerization of NIPAAm with a hydrophilic monomer, which raises the LCST, or hydrophobic monomer, which lowers the LCST. In addition, compelling studies have been conducted to examine the effects of changing the LCST, using block- or graft-copolymerization [23,24], immobilization [25], and terminal

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modification [26,27], and altering other factors. Recently, multiple thermo-responsive block polymers have been synthesized that include a polyNIPAAm block [28]. For example, polyNIPAAm-*b*-poly(3-[*N*-(3-methacrylamidopropyl)-*N*,*N*-dimethyl]ammoniopropane sulfonate) was synthesized with a double thermo-response using two blocks that possessed both LCST and UCST. The prepared block copolymers could dissolve in aqueous solution at specific temperature [29]. PolyNIPAAm-*b*-poly(*N*-acryloylpyrrolidine)-*b*-poly(*N*,*N*-dimethylacrylamide) was synthesized to consist of two thermo-responsive blocks and one soluble block. Such tri-block copolymers can be made to exhibit various conformational changes via alteration of the sequence of the three blocks [30]. Moreover, polyNIPAAm-*b*-poly(2-(dimethylamino)ethyl methacrylate), which consists of a dendritic core and double thermo-responsive corona, was synthesized [25]. These block copolymers have exhibited a constant LCST under aqueous conditions. Thus, it remains useful to control the LCST by simple methods when designing multiple thermo-responsive block copolymers.

In our previous studies, we attempted to develop easier methods that would facilitate the preparation of double thermo-responsive block copolymers [31]. As a result, polyNIPAAm-*b*-poly(NIPAAm-*co*-HMAAm) was synthesized using a one-pot ATRP technique. First, the preparation protocol required investigation of the time-conversion profile on NIPAAm. Then, at the determined time point, the secondary monomers (HMAAm and other types of NIPAAm) were added to the solution. We successfully carried out block copolymer preparation to easily obtain the desired structures, as expected, the resulting copolymers exhibited the double thermo-response, as based on investigation of the N and NH blocks.

In order to observe the thermo-responsive characteristics of polyNIPAAm-*b*-poly(NIPAAm-*co*-HMAAm)s in more detail, in the present study we designed block copolymers with a controlled cloud point for the NH block, expecting that the resulting block copolymers would form reversible aggregate structures due to dehydration of the NH block. We focused the thermo-responsive assembly behaviors (e.g., size change, reversibility) in the study, which were investigated by means of ^{1}H NMR analysis, turbidity measurements, dynamic light scattering (DLS), and field-emission scanning electron microscopy (FE-SEM).

2. Materials and methods

2.1. Materials

NIPAAm was kindly provided by Kohjin (Tokyo, Japan) and was recrystallized from a mixture of benzene and hexane. HMAAm was obtained from Wako Pure Chemical Industries (Osaka, Japan) and purified by recrystallization from ethanol and dried *in vacuo*. Tris (2-(dimethylamino)ethyl)amine (Me_6TREN) was synthesized and purified according to the protocol given in previous reports [32,33]. Ethyl 2-bromoisobutyrate (EBB) and copper(I) bromide (CuBr) were obtained from Sigma–Aldrich (USA) and were used as received. Other chemicals and solvents were commercially available. Water used in this study was purified with a Millipore Milli-Q system.

2.2. Synthesis of polyNIPAAm-b-poly(NIPAAm-co-HMAAm)s

PolyNIPAAm-*b*-poly(NIPAAm-*co*-HMAAm)s were synthesized by ATRP. The typical preparative procedure (e.g., 10 mol% of HMAAm content in the NH block) was as follows. First, EBB (1.95 mg, 0.0125 mmol) and NIPAAm (1131 mg, 10 mmol) were dissolved in 4 mL of a water/DMF mixture (3:5 v/v%). Then, water (1 mL) containing Me_6TREN (5.5 µl, 0.025 mmol) and CuBr (3.63 mg, 0.025 mmol) were separately prepared. After three freeze–pump–thaw cycles, these two solutions were mixed to prepare the N block at 20 °C for 15 min. Next, a 1:1 v/v% water/DMF mixed solution (2.5 mL) containing NIPAAm (205 mg, 1.8 mmol) and HMAAm (70 mg, 0.69 mmol) was added to the reaction mixture in order to prepare the NH block. This polymerization reaction was carried out at 20 °C for 24 h. The resulting copolymers were purified at the ambient temperature by dialysis against ethanol for 3 days and using pure water for the next 4 days. After 3 days lyophilization, the block copolymers were yielded as a white powder. The conversion and HMAAm contents were estimated by ^{1}H NMR analysis. The molecular weight (M_n) and the polydispersity index (M_w/M_n) of the block copolymers were measured by gel permeation chromatography (GPC) at 15 °C using a TOSOH TSK-GEL GMPW$_{xl}$ and a G3000PW$_{xl}$ column (Tosoh, Tokyo, Japan) connected to a Shodex RI-101 refractive index detector (Shoden Inc., Tokyo, Japan). Water containing 0.1 M NaCl was used as an eluent, and a poly(ethylene oxide) standards were used for molecular weight calibration.

2.3. ^{1}H NMR spectra measurements

To determine the extent of monomer conversion in the polymerization process and to trace polymer chain mobility in response to temperature change, the ^{1}H NMR spectra were recorded with a 400 MHz JNM-GSX400 spectrometer (JEOL, Tokyo, Japan). As solvents, dimethyl sulfoxide-d_6 (DMSO-d_6) was used for the monomer conversions and comonomer contents, and D_2O was used to determine the thermo-responsive properties.

2.4. Transmittance measurements

The thermo-responsive behavior of the block copolymers was estimated by turbidity measurements using a UV–vis spectrometer (Jasco V-550, Tokyo, Japan). The block copolymers were dissolved in aqueous solution with the given concentration. Light transmittance was continuously recorded at a wavelength of 500 nm, and a heating rate of 1.0 °C/min. The cloud points of the polymer solutions were defined as the temperatures at which transmittance was 50%.

2.5. Dynamic light scattering (DLS) measurements

DLS measurements were conducted with an Otsuka Electronics Photal FPAR-1000HL spectrometer (Otsuka Electronics Co., Ltd., Osaka, Japan). The light source was a 10 mW He–Ne laser. All samples were prepared by filtration with a 0.45 µm Millipore filter and the samples were kept in a given temperatures to reach the equilibrium prior to the measurements. We obtained the data using the marquadt method.

2.6. Field-emission scanning electron microscopy (FE-SEM) measurements

The morphology of the assembly was observed using field-emission scanning electron microscopy (Hitachi High-Technologies S-4100H, Tokyo, Japan). The block copolymer solutions were first heated at a given temperature in the thermostat bath, and then a small drop of the solution was deposited onto a preheated gold surface, and the sample was subsequently dried at the same temperature under atmospheric pressure. The sample was then coated with Pt using a Magnetron Sputtering System. Observations were performed at 5.0 kV.

3. Results and discussion

3.1. Syntheses of polyNIPAAm-b-poly(NIPAAm-co-HMAAm)s

HMAAm is a non-ionic acrylamide-type hydrophilic monomer and is readily available for the preparation of the functional copolymers of a desirable chain length and controlled content by