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pH-induced phase transition control of thermoresponsive nano-micelles possessing outermost surface sulfonamide moieties

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ABSTRACT

Diblock copolymer comprising thermoresponsive poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide) (PIPAAm-*co*-DMAAm) and hydrophobic poly(benzyl methacrylate) blocks was prepared by reversible addition–fragmentation chain transfer radical polymerization. Terminal functionalization of thermoresponsive blocks with either pH-responsive sulfadimethoxine (SD) or hydroxyl groups was performed through coupling reactions with thiol groups exposed by the aminolysis of dithiobenzoate groups located at P(IPAAm-*co*-DMAAm) termini. Outermost surface functionalized polymeric micelles were formed through the multi-assemblies of end-functional diblock copolymers with low critical micelle concentration (3.1–3.3 mg/L) regardless of their terminal groups. Variety of outermost surface functional groups had little influence on nano-scale diameters of approximately 19 nm at various pH values. Although the zeta-potentials of nonionic (phenyl and hydroxyl) surface micelles were independent of pH values ranged 8.1–5.4, those of SD-surface polymeric micelles changed from −12 to −4 mV with reducing pH value, which caused by the protonation of surface SD units (pK_a = 6.2). In addition, lower critical solution temperature (LCST) of SD-surface micelles significantly shifted from 38.6 to 22.6 °C with lowering pH from 5.4 to 8.1. These pH-induced lower LCST shifts were caused by extremely increasing surface hydrophobicity through the charge neutralization of SD moieties and the subsequent promoted dehydration of corona-forming polymer chains. These results indicated that the phase transition behavior of thermoresponsive nano-micelles was particularly controlled by modulating the properties of outermost surface chemistry via specific signals (e.g., pH, light, and biomolecular interaction)

1. Introduction

Amphiphilic block copolymers spontaneously form multi-molecular assemblies, "polymeric micelles", possessing hydrophilic outer coronas and aggregated interior hydrophobic cores in aqueous media [1,2]. Nanoscopic structures and physicochemical characteristics of polymeric micelles are dictated by the block lengths and properties of polymer components [3]. Densely packed and extended hydrophilic polymer chains in the corona region play critical roles of improving micellar water-stability and reducing interaction with proteins [4,5]. In addition, various bioactive molecules (e.g., anti-cancer drugs, nucleic acid related molecules, and proteins) can be encapsulated into micellar cores by using specific interactions as the driving forces of micellar formation [6–9]. Therefore, polymeric micelles receive great attention in biomedical applications. Especially, pharmaceutical applications for cancer-targeting drug carriers have been extensively studied since the early 1990s [10,11]. This is attributed to that specific nanostructures and properties allow polymeric micelles to achieve their long-circulation in the bloodstream with avoiding the body defense system [12], and spontaneously accumulate at solid tumor sites due to relatively leaky tumor vasculature and the poor lymphatic drainage of macromolecules, called "enhanced permeability and retention (EPR) effect" [13]. Nowadays, next-generation polymeric micelle systems, which can change their structures and properties in response to specific physicochemical signals (e.g., heat, light, and pH), have been created for application in effective cancer chemotherapeutic systems [14,15].

In our previous works, polymeric micelles possessing thermoresponsive coronas have been designed using poly(*N*-isopropylacrylamide) (PIPAAm)-based block copolymers, and the controlled release of anti-cancer drugs and intracellular micellar uptake were successfully demonstrated with applied temperature changes [16–18]. PIPAAm exhibits a reversible phase transition across its lower critical solution temperature (LCST) in aqueous

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media [19]. Below the LCST, PIPAAm chain is water-soluble and exists in an extended random coil conformation, while undergoes a phase transition to water-insoluble aggregate through the dehydration of polymer chain across 32 °C. For utilizing this unique feature in biomedical applications, several methods for LCST regulation have been studied by introducing hydrophobic or hydrophilic moieties into PIPAAm main chains and/or to their termini [20–22]. In particular, the introduction of hydrophobes to the polymer termini promotes the dehydration of polymer chains and lowers polymer LCST, which depends on the molecular weight of polymers [21,22]. In the past few years, our research group reported outermost surface-functionalized thermoresponsive nano-micelles and demonstrated that surface groups have a distinct influence on thermoresponsive micellar behavior [23,24]. In our system, dramatically lowered LCST shifts are shown in only micelles possessing hydrophobic-terminated corona-forming PIPAAm derivatives, because surface-concentrated hydrophobes via polymer multi-assemblies extremely promote the dehydration of corona-forming chains [23]. If the physicochemical properties of outermost surface chemistry are changed by specific signals (e.g., pH variation and light), the thermoresponsive behavior of polymeric micelles can be particularly regulated without varying polymer components. Creation of these dual signal-responsive micelle systems may be useful for applications in drug delivery and sensing technologies.

Sulfonamide derivatives known as antibacterial agents are one of promising candidates as signal-sensing moieties introduced to outermost micellar surfaces. These compounds show weak acidic properties due to the ionization of N^1-amide bond in aqueous media and demonstrate significant water-solubility changes via environmental pH variation [25]. Especially, sulfadimethoxine (SD) derivatives are utilized as pH-responsive biomedical materials (e.g., cancer-targeted drug carrier systems) [26,27] due to their pK_a around 6.2, which is near physiological pH, tumor extracellular region (pH 7.2–6.5), and intracellular endosomal environment (pH 5–6). The present study focused on pH-regulated thermoresponsive micellar behavior through the pH-induced property changes of SD moieties located on outermost surfaces. Outermost surface SD-introduced thermoresponsive polymeric micelles was fabricated through the multi-assemblies of block copolymers comprising SD-terminated poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide) (PIPAAm-*co*-DMAAm) blocks and poly(benzyl methacrylate) (PBzMA) blocks. In addition, the effects of pH values on micellar characteristics and thermoresponsive behavior were further investigated, compared with polymeric micelles possessing individual nonionic phenyl (hydrophobic) and hydroxyl (hydrophilic) groups.

2. Experimental

2.1. Materials

N-isopropylacrylamide (IPAAm) (kindly provided by Kojin, Tokyo, Japan) was recrystallized from *n*-hexane. Benzyl methacrylate (BzMA) and *N*,*N*-dimethylacrylamide (DMAAm) were purchased from Wako Pure Chemicals (Osaka, Japan) and distilled under reduced pressure. 2,2′-Azobisisobutyronitrile (AIBN) (Wako Pure Chemicals) was recrystallized from methanol. Benzene, *N*,*N*-dimethylformamide (DMF), *N*,*N*-dimethylacetamide (DMAc), diethyl ether, 2-ethanolamine, acryloyl chloride, sodium hydroxide, acetone, dimethyl sulfoxide (DMSO), and pyrene were purchased from Wako Pure Chemicals, and were used as received. Sulfadimethoxine (Sigma–Aldrich, St. Louis, MO) and 2-iodoethanol (Sigma–Aldrich) were used without further purification. 2-Cyanopropyl dithiobenzoate (CPDB) was prepared by previously reported methods with slight modification [28,29]. Water used in this study was purified by a Milli-Q Synthesis A10 system (Millipore, Billerica, MA)

2.2. Synthetic and characterization procedure of polymers

A detailed synthetic procedures for poly(benzyl methacrylate)-*b*-poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide) [PBzMA-*b*-P(IPAAm-*co*-DMAAm)] by reversible addition–fragmentation chain transfer radical (RAFT) polymerization has been previously reported [23]. Briefly, BzMA (0.5 mol/L) was polymerized in benzene using CPDB (60 mmol/L) and AIBN (12 mmol/L) at 70 °C for 5 h. In the next step of thermoresponsive block synthesis, random copolymerization of IPAAm (1.33 mol/L) and DMAAm (0.66 mol/L) was conducted using PBzMA (23 mmol/L) as the macro-RAFT agent and AIBN (6 mmol/L) in benzene at 70 °C for 17 h. Number-averaged molecular weight (M_n) and chemical composition of polymers were both determined by a ^{1}H NMR instrument (INOVA 400, Varian, Palo Alto, CA) using chloroform-*d* ($CDCl_3$) (Sigma–Aldrich) as a solvent. Polydispersity index (PDI) of polymers was determined by a gel permeation chromatographic system (GPC) (Tosoh, Tokyo) with two columns (TSKgel-G3000H_{HR} and TSKgel-G4000H_{HR}) (Tosoh) at 40 °C using DMF containing 100 mmol/L LiCl as eluent (flow rate, 1.0 mL/min) and calibrated with polystyrene standards for PBMA and polyethylene oxide standards for block copolymers.

2.3. Aminolysis and conversion of polymer terminal groups

Acryloyl sulfadimethoxine was prepared by previously reported method with slight modification [25]. The obtained polymers (500 mg) and acryloyl sulfadimethoxine (30 mol equivalent to the terminal dithiobenzoate groups of polymers) were dissolved in 9 mL DMSO deoxidized by N_2 gas bubbling, and then 2-ethanolamine (20 mol equivalent to the terminal dithiobenzoate groups) was added to polymer solution. After changing solution color from pink to pale yellow, 1 mL of 100 mmol/L $NaHCO_3$/Na_2CO_3 buffer (pH 10.0) deoxidized by N_2 gas bubbling was dropped into the reactive solution, followed by reaction at 70 °C for 18 h in a nitrogen atmosphere. Reaction solution was dialyzed against methanol for 48 h at 5 °C using dialysis membrane (MWCO 1000, Spectra/Por 6) (Spectrum Medical Industries, Los Angeles, CA), and the white precipitate of unreacted SD derivative was removed by filtration. The filtrate was dialyzed against water for 48 h at 5 °C (MWCO 1000), and polymers were then recovered by freeze-drying. Hydroxylated diblock copolymers were prepared through the reaction of exposed terminal thiol groups with 2-iodoethanol. Detailed synthetic and purification procedures of block copolymers were described in our previous report [23].

2.4. Preparation and characterization of polymeric micelles

The chemical structures of PBzMA-*b*-P(IPAAm-*co*-DMAAm) diblock copolymers with various terminal groups and core-corona micellar formation are shown in Fig. 1. Micellar formation was spontaneously performed using a dialysis method [16]. Amphiphilic diblock copolymers were dissolved in DMAc, and the solutions were then dialyzed against distilled water at 5 °C for 24 h using a dialyzer with Spectra/Por 6 membrane (MWCO: 1000), followed by freeze-drying. The obtained surface-derivatized PBzMA-*b*-P(IPAAm-*co*-DMAAm) micelles were coded by the individual terminal groups of P(IPAAm-*co*-DMAAm) blocks as shown in Table 1 [SD-surface micelle: M(SD); phenyl-surface micelle: M(Phe); hydroxyl-surface micelle: M(OH)].

Micellar formation of SD-functional block copolymers were investigated by a ^{1}H NMR instrument (INOVA 400) using M(SD)