Unique Thermoresponsive Polymeric Micelle Behavior via Cooperative Polymer Corona Phase Transitions

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Densely packed polymer chains on solid substrates, polymer brushes, have been actively studied because of their unique physical properties (e.g., highly stretched and extended architectures, unique interfacial properties such as liquid wetting and inhibition of protein adsorption).[1,2] Amphiphilic block copolymers spontaneously form close-packed polymer assemblies (micelles) with an outer hydrophilic polymer corona and aggregated interior nanosized hydrophobic core in aqueous media. Relative micelle layer thicknesses and properties are dictated by molecular lengths and properties of polymer components.[3] These assembled polymeric micelles are utilized as unique tools for design of mixed polymer brushes using heterogeneous block copolymers, where specific interactions between each of the micellar polymers determines interfacial behavior. Polymeric micelles have also received extensive attention in drug delivery applications.[4] Densely packed corona-forming hydrophilic polymer chains exhibit reduced interactions with serum proteins,[5] and hydrophobic inner cores can incorporate hydrophobic drugs while maintaining their water-solubility due to the presence of the hydrated hydrophilic outer corona.[6] The specific nanoscopic structures and properties allow long circulation of polymeric micelles in the bloodstream while avoiding reticuloendothelial uptake. We previously reported polymeric micelles with thermoresponsive outer corona of poly(*N*-isopropylacrylamide) (PIPAAm) or its copolymers as next-generation drug carriers, and successful controlled release of anticancer drugs with applied temperature changes.[7] PIPAAm is water-soluble below its lower critical solution temperature (LCST), but undergoes a phase transition to water-insoluble aggregates above 32 °C.[8] In order to apply thermoresponsive systems for biomedical use, reliable LCST control is important. Several methods for LCST regulations use introduction of hydrophobic or hydrophilic moieties into PIPAAm main chains or at their termini.[9−11] Introduction of terminal hydrophobic groups lowers LCSTs compared with that of PIPAAm or PIPAAm possessing hydrophilic termini, and is strongly dependent on the molecular weight of polymer chains.[9,10]

We have recently designed surface-functionalized thermoresponsive polymeric micelles comprising well-defined end-functional amphiphilic diblock copolymers, and demonstrated that micelle surface chemistry has significant influence on micellar thermoresponse.[11] In our micelle system, dramatically lowered LCST shifts are observed only for polymeric micelles with hydrophobic phenyl groups at chain ends because surface-bound hydrophobes promote dehydration of thermoresponsive corona-forming polymer chains.[11] Thus, polymeric micelles can exhibit unexpected properties when using different block copolymers. Here we introduce a novel system to regulate micelle thermoresponse via a cooperative phase transition between heterogeneous end-functional (phenyl and hydroxyl) thermoresponsive polymer segments with identical molecular weights and compositions through multiassociation of diblock copolymer blends. This cooperative behavior should occur only under a close-packed polymer assembly such as a micelle outer corona.

We synthesized well-defined diblock copolymers comprising poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide) (PID) and poly(benzyl methacrylate) (PBzMA) by reversible addition−fragmentation chain transfer radical polymerization.[12−14] As a detailed synthetic procedure for these diblock copolymer has been previously reported,[11] only a brief description is included below: PBzMA was prepared using 2-cyanopropyl dithiobenzoate and 2,2′-azobis(isobutyronitrile) as a chain transfer agent (CTA) and an initiator, respectively (number-averaged molecular weight (M_n) = 2600, polydispersity index (PDI) = 1.06). In the next step, thermoresponsive block was synthesized by random copolymerization of IPAAm and *N*,*N*-dimethylacrylamide (DMAAm) using PBzMA as the macro-CTA. The obtained diblock copolymers have a narrow PDI of 1.14 as determined by gel permeation chromatography. Molecular weight and copolymer composition were both determined by ^{1}H NMR (M_n = 9400, IPAAm/DMAAm/BzMA = 39/23/14). Hydroxylation of thermoresponsive PID end groups was achieved by reaction of iodoethanol with exposed thiol groups after aminolysis of terminal dithioester bonds with 2-ethanolamine. Efficiency of terminal conversion, estimated from the methine IPAAm proton peaks (3.9 ppm) and methylene terminal moiety ($-S-CH_2\mathit{CH}_2OH$, 3.7 ppm), was 92% (data not shown). Formation of the outermost surface-functionalized polymeric micelles was obtained through self-association of diblock copolymers by aqueous dialysis (Scheme 1b). Series of end-functionalized diblock copolymers or their mixtures at various ratios (wt %) were dissolved in *N*,*N*-dimethylacetamide, followed by dialysis against deionized water at 10 °C.

The obtained polymeric micelles dispersed completely in water to become transparent solutions, independent of end-functional groups. This is due to incorporation of hydrophilic comonomer, DMAAm, into PIPAAm main chains to improve water solubility of the micellar corona. ^{1}H NMR spectra of PID/PBzMA micelles in D_2O at 20 °C showed mainly hydrophilic PID signals and complete loss of PBzMA resonance signals due to suppressed molecular motion of the aggregated hydrophobic chains. This is indicative of a stable core−corona formation with a highly viscous inner core (see Figure S1 in Supporting Information). Proton signals for hydrophobic phenyl groups consistent with a polar, hydrated environment were also observed in aqueous micelle solution (Figure S1 in Supporting Information). This suggests that terminal hydrophobic phenyl groups did not interact with or partition into the micelle inner cores due to highly hydrated PID chains existing as extended conformations.

We investigated influence of surface functional groups on hydrodynamic diameters of PID/PBzMA micelles by dynamic light scattering (DLS) measurements. A series of surface-functionalized (Phe, OH, and Phe/OH mixtures at equal wt %)

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Scheme 1. (a) Chemical Structure of Diblock Copolymers with Functional Terminal Groups and (b) Formation of Surface-Functionalized Thermoresponsive Polymeric Micelles Differing Only in Corona Terminal Chemistry

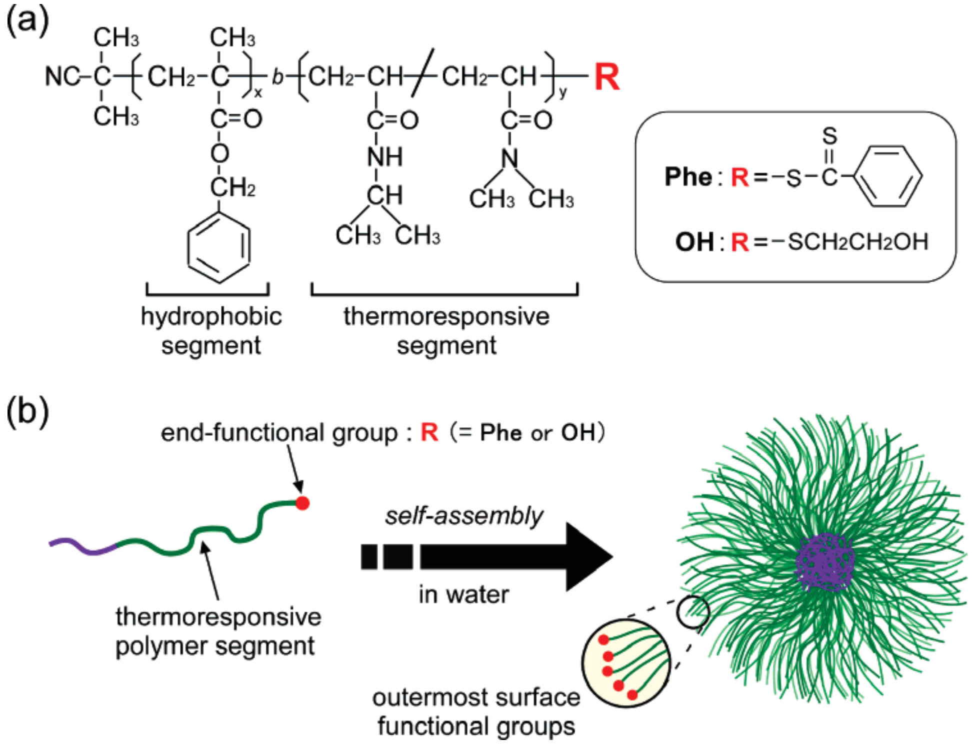

Table 1. Characterization of Surface-Functionalized Polymeric Micelles

code[a]	av diameter (nm) [b]	size polydispersity [b]	CMC (mg/L)[c]	LCST (°C)[d]
M(Phe)	21.4	0.029	2.1	22.7
M(OH)	20.8	0.016	2.2	41.3
M(Phe/OH)	21.3	0.010	2.2	34.4

[a] Coded by surface-functional groups. **M(Phe)** and **M(OH)** indicate micelles with respective phenyl and hydroxyl groups in their polymer termini. **M(Phe/OH)** means micelles comprising mixture of block copolymers with terminal phenyl and hydroxyl groups at equal wt%. [b] Determined by DLS at 20 °C ($n = 3$). [c] Determined from pyrene spectra. [d] Determined by optical transmittance changes in DPBS(−).

micelles all exhibit sizes of ca. 21 nm, independent of the outermost surface functional groups (Table 1). Polydispersity indexes of the micelle sizes estimated by cumulant analysis were considerably small (0.010−0.029, determined by DLS measurements); the values are sufficiently small to consider the prepared surface-functionalized micelles as essentially monodisperse by using well-defined polymers. Critical micelle concentration (CMC) values for the PID/PBzMA diblock copolymers were also evaluated from fluorescent excitation spectra of pyrene,[15] estimated to be approximately 2 mg/L (Table 1). These low CMC values are probably due to relatively low molecular weight of diblock copolymers and highly hydrophobic interaction between each core-forming PBzMA chain. These results indicate that the outermost surface groups (phenyl, hydroxyl, and their mixture) have little effect on hydrodynamic diameters and the CMC values of the prepared PID/PBzMA micelles.

Aqueous thermoresponsive behaviors of surface-functionalized polymeric micelles were investigated by measuring optical transmittance of micelle solutions in Dulbecco's phosphate buffered saline without calcium chloride and magnesium chloride (DPBS(−), pH 7.4) at various temperatures. Hydroxylated PID/PBzMA micelles (**M(OH)**) exhibit a thermoresponsive phase transition at 41.3 °C within a narrow temperature range. In contrast, micelles with surface phenyl groups (**M(Phe)**) demonstrated a dramatic LCST reduction to 22.7 °C even though molecular weights and compositions of PID chains are equivalent except for end-functional groups. We previously reported that a drastic decrease in LCST occurs only with surface hydrophobic functionalized micelles.[11] The lower LCST shift was thus attributed to the outermost surface phenyl groups, promoting dehydration of thermoresponsive corona-forming polymers. This results in alteration of micellar water-solubility to produce aggregation states. In addition, we suggested that this critical influence of surface chemistry on thermoresponsive behavior was based on a unique feature of micelle corona architecture: close-packed polymer-grafted interfaces facilitate a cluster effect for locally concentrated hydrophobic groups on the micelle surfaces, significantly enhancing dehydration of corona-forming polymers rather than that of independent thermoresponsive polymer chains in water.

Micelle solutions containing both **M(Phe)** and **M(OH)** micelles in equal weight showed two separated phase transitions corresponding to their respective phase transitions for both **M(Phe)** and **M(OH)** (Figure 1a). Of significant interest, **M(Phe/OH)** micelles comprising mixtures of phenyl- and hydroxyl-block copolymers demonstrated only one sharp transition (LCST = 34.4 °C). This temperature lies between the LCSTs of pure **M(Phe)** and **M(OH)** micelles. Furthermore, such thermoresponsive behavior was maintained even after stirring for more than a month (each polymer concentration was 0.25 mg/mL), indicating that the prepared PID/PBzMA micelles possess very stable core−corona structures, and no intermicellar exchange of diblock copolymers occur above their CMC (data not shown). Aqueous solutions of phenyl- and hydroxyl-terminated linear polymer PIPAAms showed individual phase transitions corresponding to each respective thermoresponsive bulk polymer solutions (Figure 1b). However, polymers within

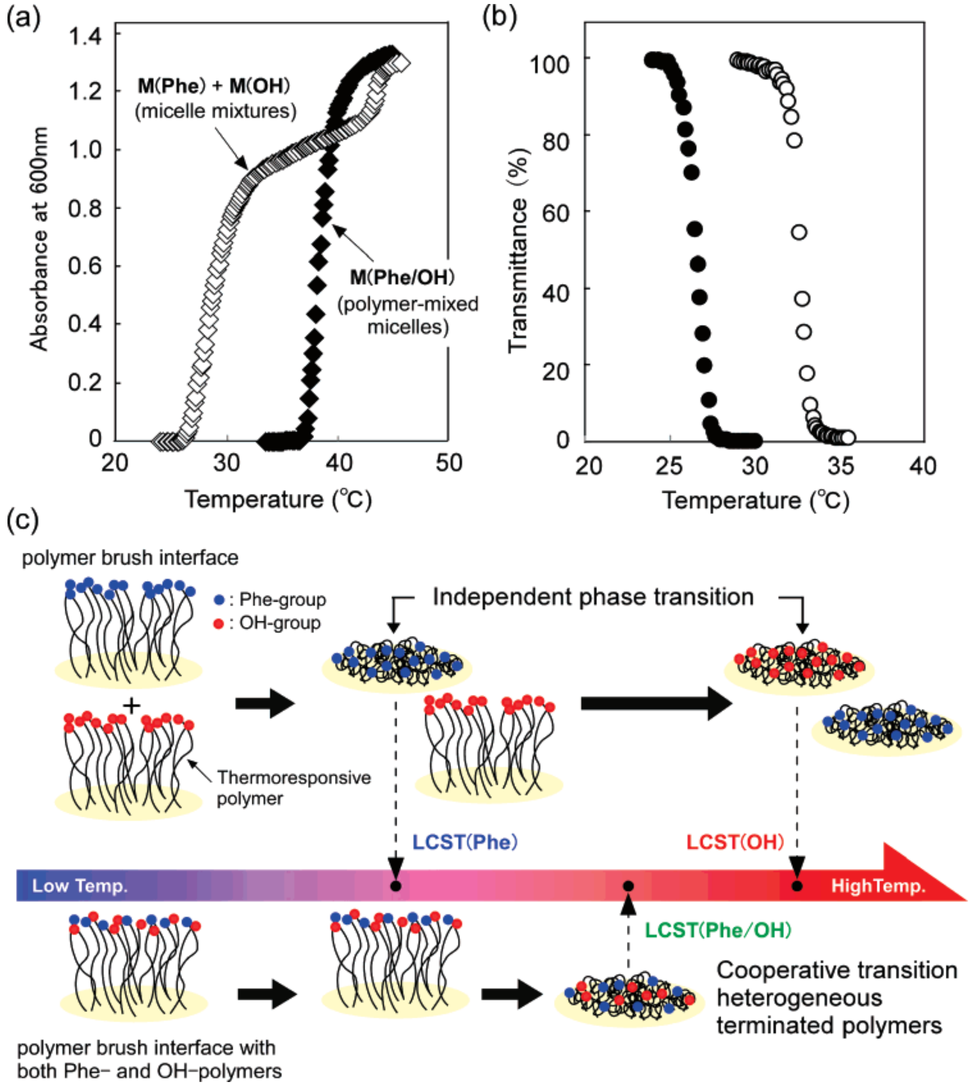

Figure 1. (a) Thermoresponsive behavior of polymeric micelle solutions (0.5 mg/mL in PBS); open diamond: mixed solution of **M(Phe)** and **M(OH)** pure micelles in equal wt %, and closed diamond: **M(Phe/OH)** mixtures of phenyl- and hydroxyl-block copolymers in equal wt % as uniform micelles. (b) LCST profiles of semitelechelic PIPAAm ($M_n = 6{,}400$, PDI = 1.15), closed circle: phenyl-PIPAAm and open circle: hydroxyl-PIPAAm (10 mg/mL in water, heating rate: 0.2 °C/min). (c) Schematic illustration of thermoresponsive polymer phase transition at a polymer brush interfaces such as micelle corona.

a densely packed polymer brush interface may easily be affected by surrounding microenvironment compared with solvated and free thermoresponsive polymer chains in aqueous milieu. Consequently, respective thermoresponsive polymers under close-packed conditions produced in micelle corona, are likely influenced considerably by surrounding polymer chains and/or end-functional groups, producing phase transitions not independently but cooperatively.

Furthermore, influence of Phe/OH end group ratios on micellar LCSTs was also investigated by using micelles comprising phenyl and hydroxyl block copolymer mixtures at various weight ratios. A series of micelles with various Phe/OH end group ratios demonstrated very sharp thermoresponsive phase transitions in between the LCSTs for **M(Phe)** and **M(OH)**, dependent on their compositions. LCSTs shifted to higher temperatures with increasing wt % of hydroxylated diblock copolymers (Figure 2). In traditional methods, LCSTs of micelles with PIPAAm-based corona are adjusted by introduction of hydrophobic or hydrophilic comonomers into PIPAAm chains at appropriate molar ratios. By contrast, in our current system, micelle thermoresponse can simply be regulated by blending ratios of diblock copolymers with identical molecular weights and compositions but differing in end-functional groups of the thermoresponsive corona-forming blocks.

In summary, this work indicates that thermoresponsive polymers differing only in end-functionalities induce phase transitions cooperatively only under dense-packed polymer brush conditions. This unique cooperative chain behavior in the hydrated micellar corona allows us to regulate monodispersed micelle thermoresponse by blending well-defined diblock copolymers with thermoresponsive segments having hydrophobic and/or hydrophilic termini without any variations in CMC value or micelle size. In future work, this concept of surface-functionalized thermoresponsive polymer systems should prove promising for creation of novel intelligent materials including targetable drug carriers and/or supramolecular sensors through surface chemistry modulations with applied signals such as pH, light, and biomolecular interactions.

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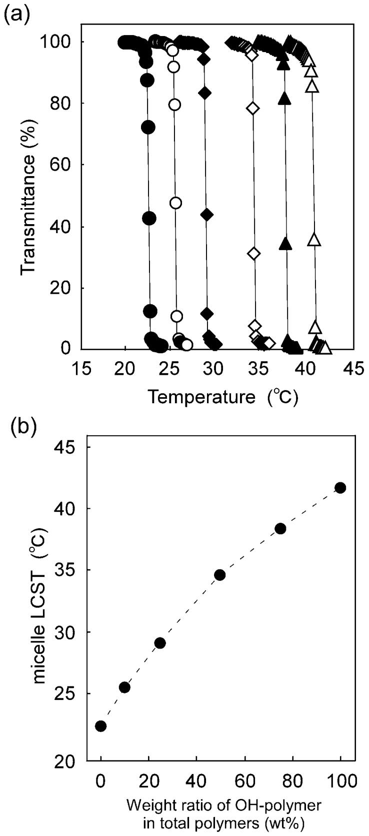

Figure 2. (a) LCST profiles for polymeric micelles comprising blends of block copolymers with phenyl and hydroxyl termini at various ratios in wt % (10 mg/mL in DPBS(-)); closed circle: Phe/OH = 100/0, open circle: 90/10, closed diamond: 75/25, open diamond: 50/50, closed triangle: 25/75, open triangle: 0/100. (b) LCST dependence of PID/PBzMA micelles on surface Phe/OH composition (10 mg/mL in DPBS(−), heating rate: 0.1 °C/min).

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Supporting Information Available: Text giving details of the synthesis of the block copolymers and of the experimental method and a figure showing ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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