Mixed Micelle Formation through Stereocomplexation between Enantiomeric Poly(lactide) Block Copolymers

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Block copolymer micelles have received tremendous interest due to current and potential applications in the fields of materials science, bioengineering, and the pharmaceutical industry.[1−4] Continued advances in these industrial fields depend on the ability to manufacture precisely controlled building blocks (i.e., nanostructured materials) tailor-made for specific applications in a simple manner.[5,6] The use of mixed micelles formed from a mixture of two block copolymers, A−B and A−C, with distinctly different properties can be a plausible candidate that is capable of tuning the resulting properties and meeting various requirements for specific applications.[7−15] In addition, self-assembled particles with discrete, attractive interaction sites, so-called "patches", on the surface can lead to an unprecedented diverse specific spectrum of particle anisotropy, including branched, colloidal molecules, faceted polyhedra, rods and ellipsoids, patterned assemblies, and many other exotic structures, as the molecular simulations predicted.[16−19] Recently, Srinivas and Pitera suggested from a series of coarse-grain (CG) molecular dynamics (MD) simulations that a binary mixture of two different diblock copolymers with a common hydrophobic block but sufficiently dissimilar hydrophilic blocks reliably self-assembles into a "patchy" spherical micelle in water, with phase separation of the two hydrophilic blocks on the surface of the micelle.[19] However, the overwhelming majority of polymers differing in chemical nature are usually incompatible, although a sufficient difference in the properties between shell-forming blocks is necessary for the mixed micelles to be effective in applications. The formation of stable mixed micelles requires their spontaneous formation due to strong interactions between two blocks constituting either core or shell of micelles. Traditionally, it has been achieved by (i) the cross-linking of either core or shell of premixed micelles[9] and (ii) the polymer−polymer complex formation mediated by noncovalent interactions between blocks.[10−13] Despite the improved stability of the chemical cross-linking, this approach may not be optimal in the encapsulation of a guest molecule or biodegradability. Here, we are interested in a simple and versatile approach to stabilized mixed micelles through the use of noncovalent interactions that are still dynamic. Well-studied examples for mixed micelles driven by noncovalent interactions include (i) a polyelectrolyte complex between oppositely charged block ionomers,[10,11] (ii) a hydrogen-bonding complex between a poly(methacrylic acid) and a polyether or polyol,[12] and (iii) a nucleic acid pair between block copolymers tagged with a H-bonding complementary nucleic acid.[13] Despite the attractiveness of previous reports, their micellization behavior has largely been limited to the strong affinity between shell-forming blocks (cases i and ii) or the micelle formation in organic medium (case iii), depending on the natures of noncovalent interactions. Thus, a strategy to yield stable mixed micelles in an aqueous environment from significantly different shell-forming block copolymers is highly desirable for application in biomedicine including drug-delivery systems (DDS).

Polylactides (PLA) have attracted a great deal of attention because they are biodegradable, producible from renewable resources, and nontoxic to the human body for potential use in medicine and bioengineering.[4,20−22] As lactide monomers have two stereoisomers, L- and D-compounds, there are three types of polylactides: optically active poly(L-(−)-*S*-lactide) (PLLA) and poly(D-(+)-*R*-lactide) (PDLA) and racemic poly(DL-lactide) (PDLLA). It has been reported that the PLLA/PDLA mixtures in either the melt or solution form stereocomplexes with distinctive physical and chemical stability due to the interaction between L-lactyl and D-lactyl unit sequences.[23] Sarasua et al. showed that the stable stereocomplex formation stems from H-bonding force from specific $CH_3\cdots O{=}C$ and $C_\alpha H\cdots O{=}C$ interactions between both PLA stereoisomers from a combined study with FT-IR spectroscopy and molecular modeling.[24] This interesting behavior of stereocomplexation has attracted an increasing interest in the design of stabilized polymeric micelles for the delivery of drugs.[25−27] For example, Leroux et al. showed that stereocomplex block copolymer micelles obtained from mixtures of poly(ethylene glycol)-*block*-poly(L-lactide) (PEG−PLLA) and poly(ethylene glycol)-*block*-poly(D-lactide) (PEG−PDLA) exhibited enhanced kinetic stability and redispersion properties superior to micelles prepared with isotactic or racemic polymer alone.[25] The objective of this study is to explore the possibility of a stable PLA stereocomplexation as another noncovalent driving force to improve mixing between dissimilar polymer chains and form stable mixed micelles. Here, we describe the preparation of mixed micelles from mixture of PEG−PDLA with poly(*N*-isopropylacrylamide)-*block*-poly(L-lactide) (PNIPAAM−PLLA). The PNIPAAM is a typical example of thermally responsive polymers, which show a temperature-induced collapse from an extended coil to a globular structure in water upon heating above 32 °C near to the human body temperature, called lower critical solution temperature (LCST).[28] These types of polymers that display a physicochemical response to stimuli have been widely explored as potential injectable drug-delivery systems.[29,30] The proposed strategy of this study relies on compensating the repulsion between the shell-forming PEG and PNIPAAM blocks by using the peculiarly strong stereocomplexation between PDLA and PLLA blocks constituting the hydrophobic core of mixed micelles.

Scheme 1 shows the synthesis of PNIPAAM−PLLA and PEG−PDLA block copolymers. PNIPAAM−PLLA (6K−2K, M_n: 8380 g/mol, PDI: 1.13) block copolymer was prepared from a dual-headed initiator containing an alkoxyamine and a primary hydroxyl group through nitroxide-mediated polymerization of *N*-isopropylacrylamide and subsequent ring-opening of lactide.[31,32] PEG−PDLA (5K−2K, M_n: 6970 g/mol, PDI: 1.04)

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Scheme 1. Synthesis of (a) PNIPAAM−PLLA and (b) PEG−PDLA Block Copolymers

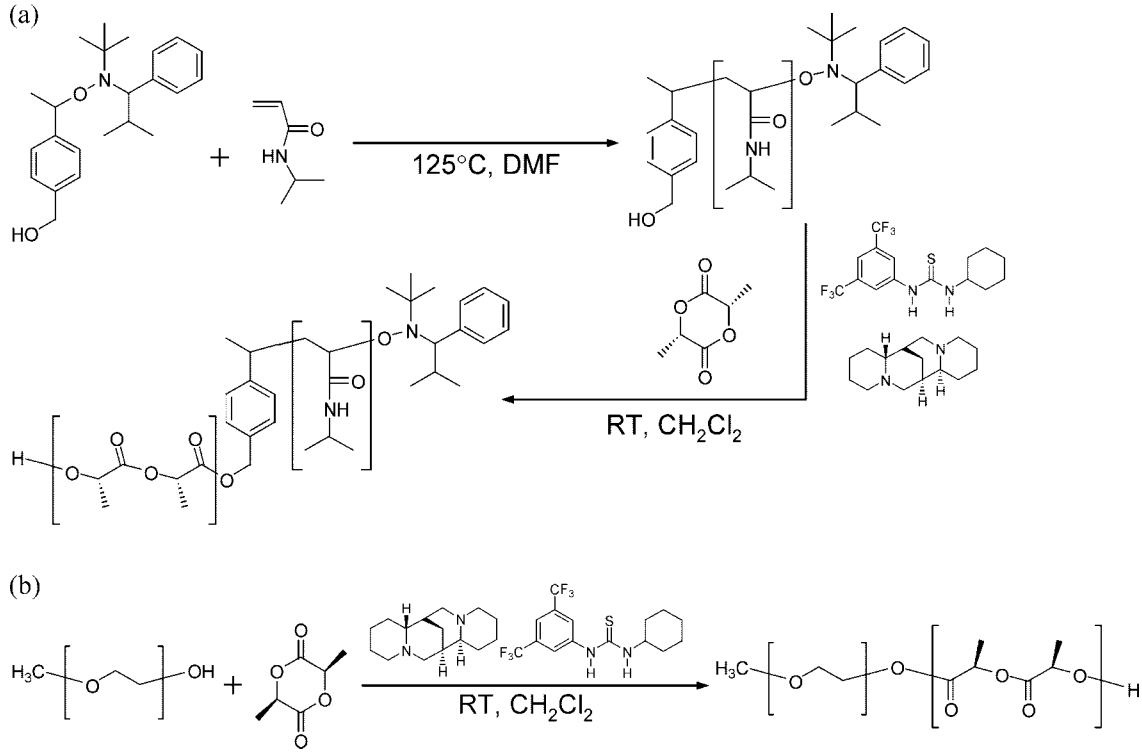

Table 1. Characteristics of Block Copolymers and Their Resulting Micelles

sample	M_n (g/mol)	PDI[c]	T_m[d] (°C)	LCST[e] (°C)	D_h[f] (nm)	PDI[f]	cmc[g] (mg/L)	D_g [h] (nm)	D_g/D_h[h]	N_{agg}[h]
PNIPAAM−PLLA + PEG−PDLA			196	21	37 (44)	0.12 (0.10)	5.0 (7.5)	30 (33)	0.81 (0.75)	27 (127)
PNIPAAM−PLLA	8380[a]	1.13		21	32 (39)	0.09 (0.10)	7.9 (11.2)	27 (31)	0.84 (0.79)	24 (91)
PEG−PDLA	6970[b]	1.04	110		28	0.11	25.1	23	0.82	38

[a] Molecular weight of PNIPAAM−PLLA was obtained from GPC. [b] Molecular weight of PEG−PDLA was obtained from NMR. [c] Polydispersity index by GPC in THF using PS standards. [d] Melting point from DSC. [e] Lower critical solution temperature in aqueous solution. [f] Hydrodynamic diameter and polydispersity index of aqueous solution by dynamic light scattering. [g] Critical micelle concentration obtained by fluorescence spectroscopy. [h] Diameter of gyration and aggregation numbers obtained from static light scattering. The values in parentheses are measured at 15 °C below the LCST.

was prepared by using methoxy PEG (M_n of 5000 g/mol) as a macroinitiator for ring-opening polymerization. The ring-opening of enantiomeric lactides was performed in a glovebox using thiourea and tertiary amine catalysts designed for bifunctional activation of both monomer and alcohol through hydrogen bonding.[33] The utility of this catalytic system was demonstrated through the synthesis of narrowly dispersed PLA blocks with predictable table molecular weights (Table 1). Full details on the synthesis and characterization of both block copolymers are described in the Supporting Information.

Mixed micelles of PNIPAAM−PLLA and PEG−PDLA were prepared by directly dissolving two block polymers in deionized water at a mass ratio of 1:1 (Figure 1a). Although our block copolymers of PEG−PDLA (5K−2K) and PNIPAAM−PLLA (6K−2K) with a relatively short PLA block could self-disperse in water to form pure or mixed micelles, some additional procedures including a cooling down of PNIPAAM solution and sonication techniques were applied to facilitate micellization and aqueous dispersion of block copolymers.[27] Typically, the aqueous solution of mixture was cooled down in a refrigerator for 1 h, sonicated for 1 h, and stirred for 24 h at an ambient condition to equilibrate the micelle formation before further analyses. Differential scanning calorimetry (DSC) on samples prepared by freeze-drying the block copolymer solutions (∼5 mg/mL) was used to confirm the formation of mixed micelles through the PLA stereocomplexation (Figure 1b). The PEG−PDLA copolymer clearly shows a T_m (∼110 °C) of stereoregular PDLA, while a T_g (∼125 °C) of PNIPAAM is overlapped with the T_m of PLLA in the PNIPAAM−PLLA. The DSC trace of block copolymer mixtures, PNIPAAM−PLLA + PEG−PDLA, shows a T_m (∼55 °C) of PEG, T_m (∼110 °C, weak) of PLA homocrystallites, T_g (∼125 °C) of PNIPAAM, and T_m (196 °C, strong) of PLA stereocomplex crystallites (Figure 1b and Figure S1 in the Supporting Information). The melting temperature of PLA stereocomplex crystallites was significantly higher than the respective polymers (∼110 °C), and predominant compared to that of PLA homocrystallites, which shows the successful formation of mixed micelles through stereocomplexation between stereoregular PLA cores. The block copolymer mixture also shows a T_m of PEG and T_g of PNIPAAM without a significant shift from that for the pure homopolymer and copolymer, showing a microphase-separated morphology between the two hydrophilic blocks of PEG and PNIPAAM. The TEM image of mixed micelles air-dried from the dilute solution confirms the formation of spherical nanoparticles with sizes of 20−40 nm although it was very difficult to discern the patched surface morphologies due to their small feature size.

The temperature-responsive behavior of the mixed micelles was monitored by the turbidity measurements and DSC method. Figure 2a shows the temperature dependence of the transmittance of the polymer aqueous solution at 500 nm. The cloud-point temperatures for both PNIPAAM−PLLA and PNIPAAM−PLLA + PEG−PDLA were similarly observed to

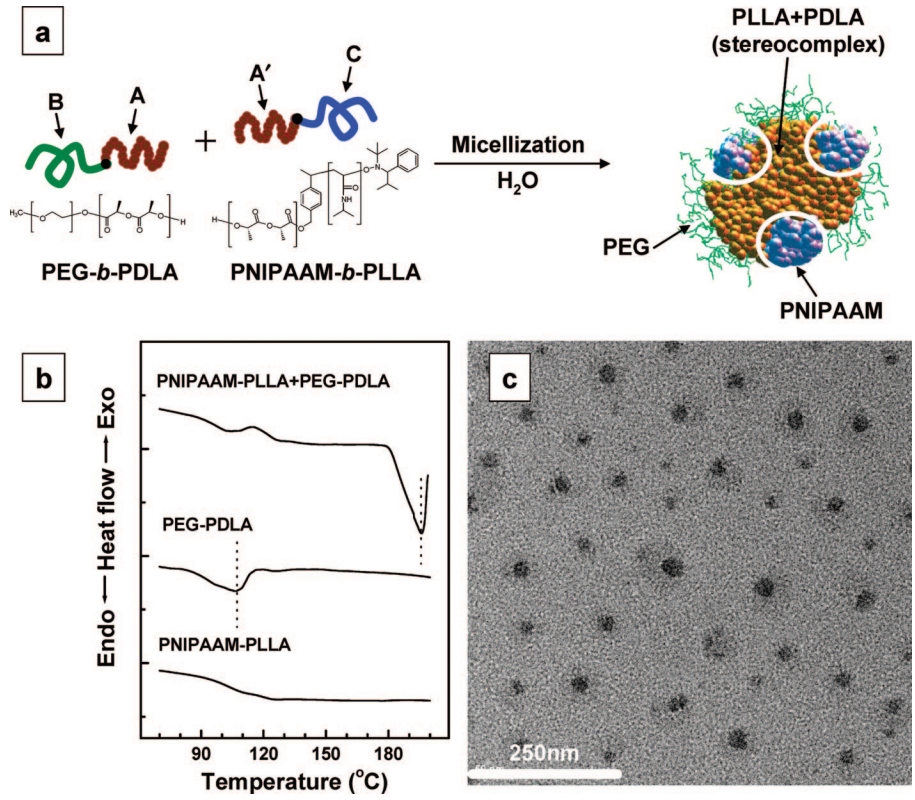

Figure 1. Mixed micelles formed from dissimilar stereocontrolled block copolymer mixture. (a) The PEG−PDLA + PNIPAAM−PLLA mixed micelles were prepared by dispersing both block copolymers in deionized water. (b) DSC thermograms. (c) TEM image of air-dried micelles shows the stereocomplex mixed micelles with diameters of 20−40 nm.

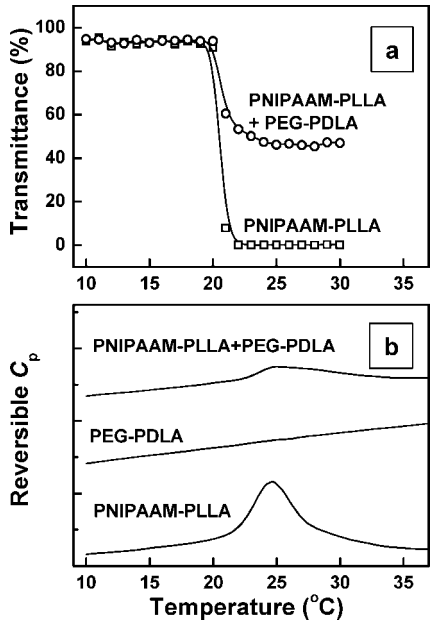

Figure 2. (a) Transmittance curves and (b) DSC thermograms of aqueous block copolymer solutions. The LCST values for pure PNIPAAM block copolymer and mixtures were determined from the cloud points from transmittance measurement and onset of the endothermic peak in DSC.

be ∼20 °C, a little lower than that (∼32 °C) of conventional PNIPAAM polymers. This may be attributed to the incorporation of hydrophobic PLA blocks and/or the hydrophobic group at the distal end of PNIPAAM, as similarly observed in other cases of PNIPAAM block copolymers with more hydrophobic or hydrophilic blocks.[30,34,35] The phase transition temperatures, T_{tr} (∼21 °C), determined from onset of endothermic peak in a DSC thermograms for the respective samples represent a good agreement with the value from the transmittance (Figure 2b). Both transmittance and DSC results for micelles solutions showed that the mixed micelles and PNIPAAM−PLLA micelles have the same LCST value. It indicates that the thermoresponsiveness of PNIPAAM was not affected by the presence of PEG blocks. These mixed biodegradable micelles having the LCST of about 20 °C (below our normal body temperature, i.e., 37 °C) have great potential for use as a local drug delivery carrier. For example, an anticancer drug can be loaded into the micelles, and the drug-loaded micelles can be injected into a surface tumor, where the micelles would precipitate due to the phase change and stay in the tumor tissues for the drug to perform its functions.

Finally, we investigated the effects of stereocomplex micellization on the micelle forming ability in aqueous solution. For the measurements at different temperatures of 15 or 25 °C, the aqueous solutions of block copolymer micelles formed before were kept for 10 min at the selected temperatures. The distinctive change accompanied by mixed micelles through stereocomplex formation was observed in the critical micelle concentrations (cmc), an important parameter to anticipate the in vivo performance under so-called sink conditions. The measurement of cmc values was done with steady-state fluorescence spectroscopy using pyrene as a probe.[36] Interestingly, the value for PNIPAAM−PLLA + PEG−PDLA mixture is much lower than those of single block copolymers at the same temperature of 25 °C, as shown in Figure 3a. This shows that the stabilized self-association driven by the strong PLA stereocomplexation lowers the cmc of mixed micelles, as observed in stereocomplex

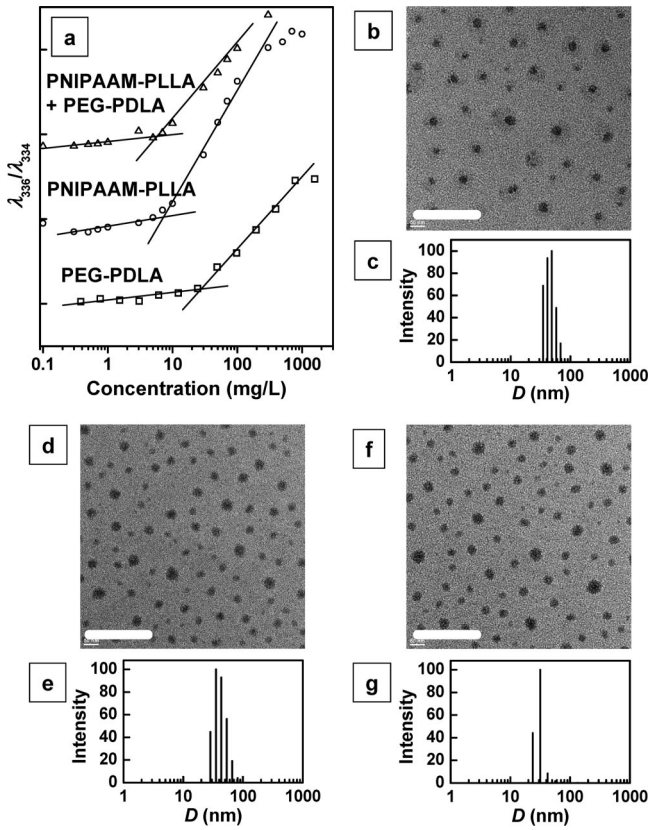

Figure 3. Critical micelle concentrations (cmc) and sizes of polymeric micelles in aqueous solutions. (a) The cmc values were determined from plot of intensity ratios, $\lambda_{336}/\lambda_{334}$, from fluorescence spectra of pyrene (at 25 °C). (b−g) Sizes of micelles were obtained from TEM images and DLS size distributions of PNIPAAM−PLLA + PEG−PDLA (b, c), PNIPAAM−PLLA (d, e), and PEG−PDLA (f, g) samples prepared and dried at 15 °C. Scale bars are 200 nm (b, d, f).

block copolymers.[26] The PNIPAAM−PLLA exhibits a lower cmc value than the PEG−PDLA, and the value of PNIPAAM block copolymer at 25 °C is a little lower than that at 15 °C (values in parentheses in Table 1), indicating that the PNIPAAM blocks are also acting as a supplemental hydrophobic core at a temperature above the LCST (~20 °C) to reduce cmc values. All the water-soluble aggregates form nanoscopic spheres with diameters (D_h) ranging from 20 to 40 nm, as shown in TEM and dynamic light scattering (DLS) in Figure 3b−g, which shows a good agreement with those of PEG−PLA reported by other group.[27] Micelles formed from polymers with high molecular weight distribution (PDI), for example, poly(*N*-isopropylacrylamide-*co*-*N,N*-dimethylacrylamide)-*b*-poly(DL-lactide-*co*-glycolide) with PDI of 1.5,[37] poly(*N*-isopropylacrylamide-*co*-*N,N*-dimethylacrylamide-*co*-2-aminoethyl ethacrylate)-*b*-poly(10-undecenoic acid) with PDI of 1.5,[38] and folate-conjugated poly(*N*-isopropylacrylamide-*co*-*N,N*-dimethylacrylamide-*co*-undecenoic acid) grafted with cholesterol having PDI of 1.78,[39] had a wide size distribution with polydispersity index of 0.23, 0.27, and 0.32, respectively. The micelles reported in this study have a relatively narrow size distribution due to narrow molecular weight distribution (Table 1). A ratio of R_g/R_h that is equal to 0.774 supports the formation of core−shell micelles, while any value equal to or bigger than 1 indicates the formation of vesicles or rodlike micelles, respectively.[40−42] The R_g/R_h values for our block copolymer micelles are close to 0.774, which indicates the formation of core−shell micelles. Additional properties of block copolymers and their resulting micelles are measured and summarized in Table 1, as similarly measured elsewhere.[43] Full details on the experimental procedures are described in the Supporting Information.

In summary, stereocomplexation of enantiomeric PLA blocks was successfully exploited to form mixed micelles from mixture of dissimilar PNIPAAM−PLLA and PEG−PDLA block copolymers. The proposed strategy of this study that relies on the strong noncovalent interaction between the core-forming hydrophobic blocks affords significant opportunities in the hydrophilic shell of micelles for tuning final properties and meeting various requirements for applications. The temperature-responsive stable micelle in an aqueous environment this study demonstrates would be potentially suitable for the biomedical polymeric assemblies to transport and deliver biologically active agents such as potential injectable drug-delivery systems. Exploiting the PLLA−PDLA stereocomplex bridges in conjunction with an appropriate molecular design can be a simple and versatile route to expand the scope of possible morphologies of supramolecular structures.

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Supporting Information Available: Details on the experimental procedure for the synthesis of block copolymers and their characteristics. This material is available free of charge via the Internet at http://pubs.acs.org.

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