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Synthesis of Poly(*N*-isopropylacrylamide)−Poly(ethylene glycol) Miktoarm Star Copolymers via RAFT Polymerization and Aldehyde−Aminooxy Click Reaction and Their Thermoinduced Micellization

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ABSTRACT: A facile synthetic pathway to poly(*N*-isopropylacrylamide) (PNIPAM)−poly(ethylene glycol) (PEG) miktoarm star copolymers with multiple arms has been developed by combining reversible addition−fragmentation chain transfer (RAFT) polymerization and aldehyde−aminooxy "click" coupling reaction. Star PNIPAM with aldehyde functionalized core was initially prepared by the RAFT arm-first technique via cross-linking of the preformed linear macro-RAFT agents using a newly designed aldehyde-containing divinyl compound 6,6′-(ethane-1,2-diylbis(oxy))bis(3-vinylbenzaldehyde) (EVBA). It was then used as a multifunctional coupling agent for the subsequent formation of the second-generation PEG arms via the click coupling reaction between its aldehyde groups and aminooxy-terminated PEGs. The thermoresponsive micellization behavior of PNIPAM−PEG miktoarm star copolymer with different PEG arm numbers in water was also investigated. Opportunities are open for thermoinduced intermolecular or intramolecular micellization of PNIPAM−PEG miktoarm star copolymers via controlling the content ratio of PNIPAM and PEG, forming multimolecular micelles and unimolecular micelles, respectively.

Introduction

Double-hydrophilic copolymers, comprised of two different hydrophilic segments, can self-assemble into micelle-like aggregates in water if one segment becomes hydrophobic upon external stimulus such as a change in solution pH, temperature, and ionic strength.[1−4] This kind of copolymer has emerged as an important class of polymers due to their potential applications in drug delivery and as intelligent materials.[5] Poly(*N*-isopropylacrylamide) (PNIPAM) is one of the most widely studied thermoresponsive polymers and undergoes a reversible phase transition at low critical solution temperature (LCST) around 32 °C in water.[6] At temperature below the LCST, the intermolecular hydrogen bonding between PNIPAM chains and water molecules is dominant, and therefore PNIPAM is hydrophilic and soluble in water. At temperatures above the LCST, intramolecular hydrogen bonding between the amide groups in PNIPAM results in transition into compact and collapsed conformations of PNIPAM chains, and the polymeric chain becomes hydrophobic and insoluble in water. Poly(ethylene glycol) (PEG), as a typical hydrophilic segment, has several biomedical and pharmaceutical applications due to its specific properties such as nontoxicity, biodegradability, biocompatibility, and resistance to recognition by the immune system.[7] Therefore, the double-hydrophilic copolymers combining PNIPAM and PEG should be of popular interest. Compared to the numerous studies on block[8] and graft[9] copolymers, little work has focused on the double-hydrophilic star-shaped copolymers composed of PNIPAM and PEG, and the related investigations have been limited to the miktoarm star copolymers with few arms, mainly containing 3 and 4 arms.[10−12]

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Miktoarm star (also referred to as heteroarm star) copolymers contain two or more chemically different arms emanated from the same core. Compared to the synthesis of homoarm star polymers, the synthesis of miktoarm star copolymers is more difficult, and consequently their preparation is described in fewer reports.[13,14] Previously, the synthesis of miktoarm star copolymers was mainly accomplished by living anionic polymerization.[15−17] However, recent developments in living radical polymerization techniques, such as atom transfer radical polymerization (ATRP),[18] nitroxide-mediated radical polymerization (NMP),[19] and reversible addition−fragmentation chain transfer (RAFT) polymerization,[20] have expanded the ability to generate miktoarm star copolymers because of the wide range of applicable monomers and more facile operation conditions in comparison with living anionic polymerization. Among the methodologies developed for the synthesis of miktoarm star copolymers using living radical polymerization techniques, the "in−out" method[13−16,21,22] represents an important strategy for the synthesis of miktoarm star copolymers with a large number of arms (>20). In this method, a star polymer having the preserved dormant initiating sites in the cross-linked core, synthesized by the arm-first method, can be used as multifunctional initiators for the subsequent growth of the second generation of arms. As a result of sterically congested core, not all of the preserved initiating sites participate in the generation of the second arms, and therefore the number of the second generation of arms is always lower than the number of the first generation of arms.[23]

Continuing efforts have been made over the years to develop facile and efficient routes to synthesize miktoarm star copolymers with tunable arm number. Matyjaszewski and co-workers[23] have prepared a series of miktoarm star copolymers containing two or more types of arms with various arm number via one-pot cross-linking the mixture of different linear macroinitiators with a

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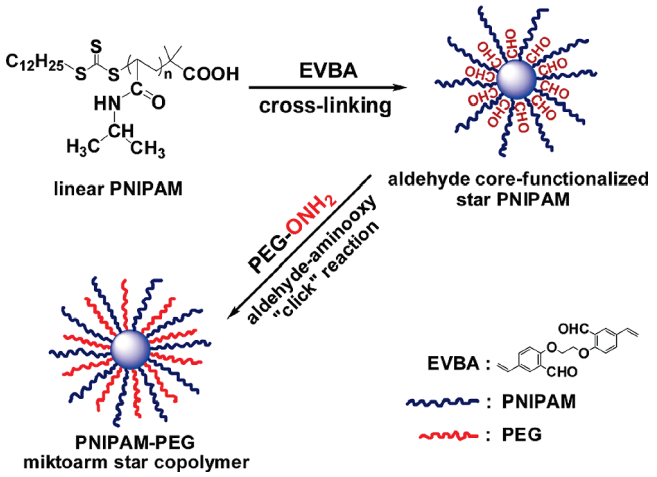
Scheme 1. Schematic Representation of the Synthesis of PNIPAM−PEG Miktoarm Star Copolymers via Combination of RAFT Polymerization and Aldehyde−Aminooxy Click Reaction



divinyl compound. This approach is simple and general; however, the synthesis of miktoarm star copolymers is challenging when macroinitiators with different activity are used, owing to the different incorporation rates of the macroinitiators. Highly efficient "click" reactions[24] were also used for the synthesis of miktoarm star copolymers.[14,22] The most popular "click" reaction is the copper-catalyzed alkyne−azide cycloaddition reaction, and various miktoarm star copolymers containing few arms with different chemical compositions have been synthesized by combining this click reaction with living radical polymerizations.[25,26] Reaction between an aminooxy and an aldehyde or ketone via the formation of an oxime linkage is another highly efficient "click" reaction.[27] This "click" reaction has the advantage that, in comparison to the most popular copper-catalyzed alkyne−azide click reaction, other than the reacting reagents no other auxiliaries including toxic metallic catalyst are required. Nevertheless, the potential of oxime click chemistry in the synthesis of polymers with complex topologies has yet to be fully explored although it has proven to be a versatile approach for synthesis of polymer−protein or drug conjugations.[28] So far nothing has been done on the application of this technique to prepare star polymers.

Herein, we report a facile synthetic pathway to double-hydrophilic miktoarm star copolymers composed of PNIPAM and PEG with multiple arms by combining RAFT arm-first technique and aldehyde−aminooxy click reaction (Scheme 1). Star PNIPAM with aldehyde-functionalized core was initially prepared by RAFT arm-first technique via cross-linking of the preformed linear PNIPAM macro-RAFT agent using an aldehyde-containing divinyl compound. It was then used as a multifunctional coupling agent for the subsequent formation of the second-generation PEG arms via the click reaction between its aldehyde groups and the aminooxy-terminated PEGs. The so far reported routes to synthesize miktoarm star copolymers using click chemistry usually involve a synthesis of multifunctional miktoinitiator and/or coupling agent, and thus only miktoarm star copolymers with a low number of arms have been synthesized.[26,29] Like the previous "in−out" method, our method also enables the facile preparation of miktoarm star copolymers having multiple arm numbers without the cumbersome synthesis of multifunctional miktoinitiator and/or coupling agents. However, in comparison to "in−out" method, the number of the second generation arms is more independent of the number of the first generation arms and can be tuned easily by varying the feed ratio of the aldehyde−aminooxy click reaction. Because aminooxy end groups can be easily incorporated into the polymers by living radical polymerizations using aminooxy-containing initiator[28] or by postmodification, this simple methodology can be extended to other miktoarm star structures. Except for the synthesis, the unique thermoresponsive micellization behaviors of the resulting new double-hydrophilic miktoarm star copolymer were also investigated.

Experimental Section

Materials. *N*-Isopropylacrylamide (NIPAM) (TCI, 98%) was recrystallized twice from *n*-hexane before use. 2,2′-Azobis(isobutyronitrile) (AIBN) (Shanghai Chemical Reagent Co., 99%) was purified by recrystallization from ethanol. Tetrahydrofuran (THF) (Shanghai Chemical Reagent Co., 99%) was refluxed with sodium chips under N_2 until dry and freshly distilled before use. *N*,*N*-Dimethylformamide (DMF) (Shanghai Chemical Reagent Co., 99%) was distilled under reduced pressure over CaH_2 before use. K_2CO_3 (Shanghai Chemical Reagent Co., 99%) and 1,2-dibromoethane (Aldrich, 99%) were used as received.

2-(Dodecylsulfanylthiocarbonylsulfanyl)-2-methylpropionic acid (DMPA),[30] 2-hydroxy-5-vinylbenzaldehyde (HVB),[31] and monoaminooxy end-functionalized poly(ethylene glycol) (PEG-ONH_2, molecular weight 2000)[32] were synthesized according to literature procedures.

Measurements. The number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) were measured by gel permeation chromatography (GPC) against PS standard in THF at a flow rate of 1.0 mL/min at 35 °C on three Waters Styragel columns (measurable molecular weight range: 100−5000, 500−30 000, and 5000−600 000) connected to a Waters 1515 pump and a Waters 2414 refractive index detector. The absolute weight-average molecular weight (M_w) of the polymers was determined by multiangle laser light scattering (MALLS) in THF at 40 °C on the chromatograph system equipped with a Waters 1515 pump, two PLgel 10 μm MIXED-B columns, a Waters 2414 refractive index detector, and a Viscotek 270 dual detector (dual laser light scattering, λ = 670 nm).

^{1}H NMR (300 MHz) spectra were recorded in $CDCl_3$ at room temperature on a Varian Unity Inova 300 spectrometer. Polymer samples for ^{1}H NMR and MALLS analysis were fractionated by preparative GPC (column: Ultrastyragel 10^4 Å).

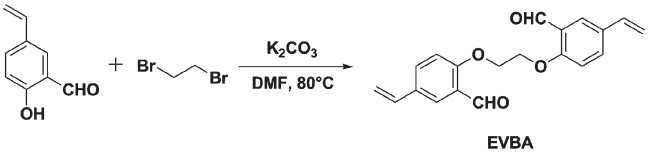
Elemental analysis was determined on a CHNS-Vario elemental analyzer. FAB mass measurement was carried out on a VG ZAB-HS spectrometer.

Dynamic light scattering (DLS) measurements were conducted at 25 and 60 °C on a Brookhaven BI-200SM apparatus with a BI-9000AT digital correlator and a He−Ne laser at 532 nm. Prior to the measurement, the sample solutions were filtered through hydrophilic poly(ether sulfone) syringe filters (SCAA-201, Anpel, 0.45 μm pore size). The data were analyzed by the CONTIN algorithm.

The light transmittance of the aqueous solution of the polymers was monitored at a wavelength of 500 nm on a PGENERAL TU-1901 UV/vis spectrophotometer. The operation was conducted from 25 to 60 °C at a heating rate of 0.2 °C/min.

Synthesis of Aldehyde Functionalized Divinyl Cross-Linker EVBA. The aldehyde functioned cross-linker EVBA was prepared by the reaction of 1,2-dibromoethane with HVB. A solution of 1,2-dibromoethane (7.52 g, 40 mmol) in DMF (5 mL) was added dropwise to a mixture of HVB (11.86 g, 80 mmol) and K_2CO_3 (11.06 g, 80 mmol) in DMF (20 mL). The mixture was stirred at 80 °C under N_2 for 12 h. The reaction mixture was poured into excess ice water and then extracted with ethyl acetate. The organic extract was washed with water and then brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was purified by silica gel chromatography eluted with acetate/*n*-hexane (20:80 v/v) to give EVBA as a light yellow solid (8.38 g, 65%); mp = 131−132 °C. ^{1}H NMR ($CDCl_3$), δ (TMS, ppm): 4.54 (m, 4H, $-CH_2-CH_2-$), 5.24 (d, J = 10.8 Hz, 2H, *trans*-CH_2), 5.69 (d, J = 17.4 Hz, 2H, *cis*-CH_2), 6.63 (dd, J_1 = 10.8 Hz, J_2 = 17.4 Hz, 2H, =CH−), 7.01 (d, J = 8.7 Hz, 2H, 6-aromtic proton), 7.6 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 2H, 4-aromtic proton), 7.88

Scheme 2. Synthesis of Aldehyde Functionalized Divinyl Cross-Linker



EVBA

(d, J = 2.4 Hz, 2H, 3-aromtic proton), 10.43 (s, 2H, aldehyde proton). Anal. Calcd for $C_{20}H_{18}O_4$: C 74.52; H 5.63. Found: C 74.29; H 5.66. FAB MS: m/z calcd for $C_{20}H_{18}O_4$ 322.34; found 322 (M^+).

Synthesis of PNIPAM Macro-RAFT Agent. For a typical example is given below. A mixture of NIPAM (4.972 g, 44 mmol), DMPA (160 mg, 0.44 mmol), AIBN (7.2 mg, 0.044 mmol), and DMF (11 mL) was degassed with three freeze−pump−thaw cycles, sealed under N_2, and heated at 60 °C. After 7 h at 55% conversion, the sealed ampule was cooled in an ice bath. The reaction mixtures were diluted with THF and then poured into a large amount of *n*-hexane. The precipitated polymer was washed with *n*-hexane and dried in vacuo to give the PNIPAM macro-RAFT agent with $M_{n,GPC}$ = 5000 and M_w/M_n = 1.07.

Synthesis of (PNIPAM)$_n$-polyEVBA Star Polymer. PNIPAM macro-RAFT agent (0.5 g, 100 μmol), EVBA (80.5 mg, 250 μmol), AIBN (1.6 mg, 10 μmol), and THF (5 mL) was degassed with three freeze−pump−thaw cycles, sealed under N_2, and heated at 60 °C. After the predetermined time, the sealed ampule was cooled in an ice bath. The reaction mixtures were diluted with THF and immediately analyzed by GPC. After 48 h, the star polymer yield was ca. 80%, estimated by comparing peak area of star polymer and unlinked linear polymer in GPC. Then a large amount of *n*-hexane was added to precipitate the star polymer. The star polymer was purified by fractional precipitation in THF/*n*-hexane.

Synthesis of (PNIPAM)$_n$-polyEVBA-(PEG)$_m$ Miktoarm Star Copolymers via Aldehyde−Aminooxy Click Reaction. The purified (PNIPAM)$_n$-polyEVBA ($M_{n,GPC}$ = 53 400 and M_w/M_n = 1.36; 100 mg, 0.086 mmol of aldehyde group, assuming 100% conversion of EVBA) and PEG-ONH_2 (molecular weight 2000, 260 mg, 0.130 mmol of aminooxy group) were dissolved in 10 mL of THF and stirred at room temperature for 24 h. The resulting miktoarm copolymer was purified by dialysis against THF for 24 h using a semipermeable membrane (cutoff molecular weight 5000) to remove the unreacted PEG-ONH_2.

Results and Discussion

Synthesis of (PNIPAM)$_n$-polyEVBA Star Polymer with Aldehyde Functionalized Core. For the synthesis of multiarm star polymers with aldehyde functionalized core, we employed the arm-first technique, in which preformed linear polymers with living dormant chain ends are joined together using a newly designed difunctional vinyl linking agent EVBA (Scheme 2) to form a microgel core carrying the linear arm on its surface. The trithiocarbonate terminated linear PNIPAM macro-RAFT agent was first prepared through RAFT polymerization of NIPAM using DMPA as RAFT agent and AIBN as initiator in DMF at 60 °C. After 7 h at 55% conversion, a well-defined PNIPAM macro-RAFT agent with $M_{n,GPC}$ = 5000 and M_w/M_n = 1.07 was obtained.

The obtained linear PNIPAM macro-RAFT agent was then allowed to react with the divinyl monomer EVBA in the presence of AIBN to induce polymer linking for the formation of star PNIPAM with aldehyde functionalized core. The RAFT approach was selected for preparation of star polymer because of its great tolerance to a wide range of functional groups including aldehyde groups.[33−37]

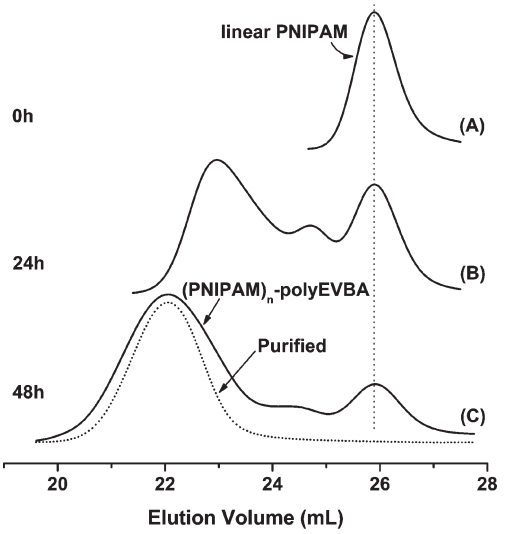


Figure 1. GPC traces for the formation of (PNIPAM)$_n$-polyEVBA star polymer at various reaction times. Experimental conditions: M_n of linear PNIPAM chains = 5000; [EVBA]/[PNIPAM-RAFT]/[AIBN] = 25/10/1, [EVBA] = 0.05 M, in THF at 60 °C. GPC conditions: RI detector, linear polystyrene as standard. (A) 0 h; (B) 24 h; (C) 48 h.

Since the amount of cross-linking agent is a crucial factor in the star polymer formation,[18] several trials were conducted to determine the suitable feed ratio of cross-linker EVBA to PNIPAM macro-RAFT agent (PNIPAM-RAFT). A higher ratio of [EVBA]/[PNIPAM-RAFT] > 6 led to insoluble gel, while a large amount of linear precursor remained un-cross-linked at a lower ratio of [EVBA]/[PNIPAM-RAFT] < 1.5. Thus, a feed ratio of [EVBA]/[PNIPAM-RAFT]/[AIBN] = 25/10/1 ([EVBA] = 0.05 M) was employed for the star polymer synthesis in THF at 60 °C. At a timed interval, the reaction mixtures were diluted with THF and immediately monitored by GPC.

The GPC curves in Figure 1 indicated that the amount of the linear precursor incorporated into the star polymers increased with the reaction time, which was confirmed by the decreasing GPC signal corresponding to the linear polymer and an increasing new GPC elution peaks shifting to higher molecular weight. After 48 h, the star polymer became the dominant population with a rather small amount of the unreacted linear polymer. The star polymer yield was ca. 80%, calculated from the GPC area ratio of the star polymer and the linear precursor.[18] The cross-linker EVBA was almost completely consumed, as evidenced by the disappearance of EVBA peak in GPC (retention time of EVBA at 30 min, not shown here).

After fractional precipitation of the star polymer in THF/*n*-hexane to remove the unreacted linear polymer, a monomodal GPC trace for (PNIPAM)$_n$-polyEVBA star polymer with narrow molecular weight distribution (M_w/M_n = 1.36) was detected (Figure 1C, dashed line). The purified star polymer was analyzed by ^{1}H NMR spectroscopy (Figure 2A). In addition to the characteristic signals of the methine proton (*b*, 3.8−4.2 ppm) from PNIPAM arms, the peak of aldehyde protons (*c*, 9.8−10.8 ppm) from polyEVBA core was distinct but with signal broadening and weakening due to the restricted rotation of the highly cross-linked polyEVBA core.[23] The ^{1}H NMR along with GPC results indicate the successful synthesis of the star PNIPAM with aldehyde functionalized core.

The absolute weight-average molecular weights (M_w) and the average number of arms per star polymer molecule (N_{arm}) were characterized by GPC with MALLS detector.

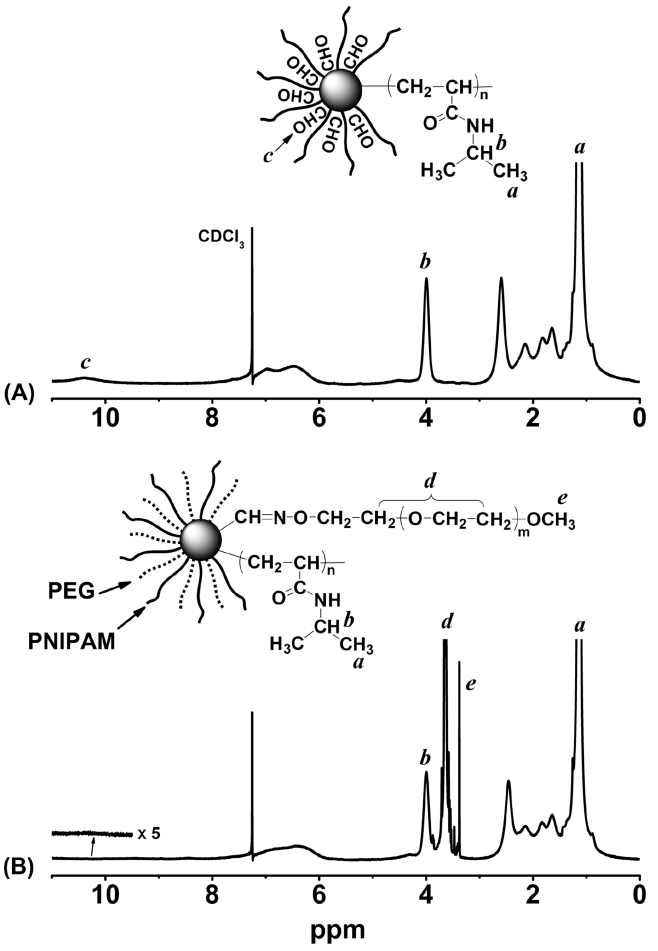


Figure 2. ^{1}H NMR spectra in $CDCl_3$ of $(PNIPAM)_n$-polyEVBA star polymer (A) and $(PNIPAM)_n$-polyEVBA-$(PEG)_m$ miktoarm star copolymer (B).

N_{arm} was calculated using the equation $N_{arm} = M_{w,star}\text{-Arm}_{wt\%}/M_{w,PNIPAM}$,[38] where $M_{w,star}$ and $M_{w,PNIPAM}$ are the absolute weight-average molecular weights of star polymer and linear PNIPAM precursor, respectively, measured from MALLS; $Arm_{wt\%}$ is the weight fraction of PNIPAM arm in the star polymer, determined on the basis of the feed ratio for the preparation of the star polymer. Consequently, the star polymer has 13 arms per molecule.

Synthesis of $(PNIPAM)_n$-polyEVBA-$(PEG)_m$ Miktoarm Star Copolymers via Aldehyde−Aminooxy Click Reaction. Aldehydes can react readily with aminooxy groups via the formation of a highly stable oxime linkage under quite mild conditions in a near-quantitative yield.[28] Thus, the obtained $(PNIPAM)_n$-polyEVBA star polymer bearing aldehyde groups within the core was allowed to simply click with PEG-ONH_2 (M_n = 2000) affording the synthesis of PNIPAM-PEG miktoarm star copolymer (Scheme 1). The click reaction was carried out in THF at room temperature for 24 h with a feed ratio of [PEG-ONH_2]/[−CHO group] = 1.5. GPC profile of the reaction mixtures showed two peaks for the expected $(PNIPAM)_n$-polyEVBA-$(PEG)_m$ miktoarm star polymer and PEG-ONH_2 used in excess (Figure 3B). After purification of the star polymer by dialysis against THF to remove the free PEG-ONH_2, the miktoarm star polymer displayed a narrow and monomodal GPC distribution (M_w/M_n = 1.12) shifting to higher molecular weight region compared with that of the precursor $(PNIPAM)_n$-polyEVBA (Figure 3C).

In order to confirm the formation of the PNIPAM-PEG miktoarm star copolymers, the miktoarm star copolymer

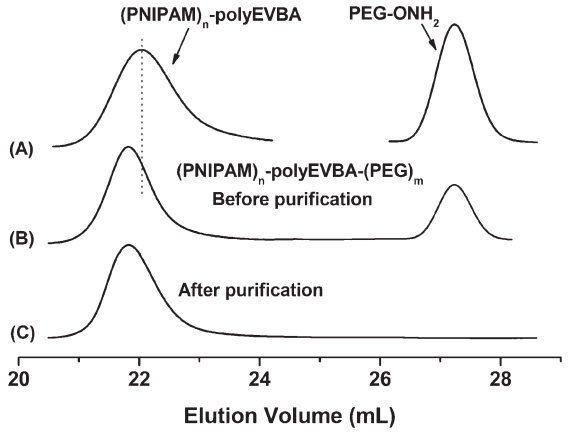


Figure 3. GPC traces of PNIPAM-PEG miktoarm star copolymer before (B) and after purification (C) prepared via click reaction between $(PNIPAM)_n$-polyEVBA star polymer and PEG-ONH_2 precursors (A).

was fractionated by preparative GPC and then analyzed by the ^{1}H NMR spectrum (Figure 2B). Apart from the peaks of the methine proton for PNIPAM, the characteristic resonances of methylene protons (*d*, 3.5−3.8 ppm) for PEG appeared, indicating the introducing of PEG segment into the star. Notably, the signal of aldehyde protons for its precursor (Figure 2A) completely disappeared, suggesting all the aldehyde groups located near the core surface had been almost reacted. On the basis of the integration areas of methine proton for PNIPAM and methylene protons for PEG and the degree polymerization of these two arms (44 for PNIPAM and 45 for PEG), the ratio of PEG and PNIPAM arm number N_{PEG}/N_{PNIPAM} was derived to be 2.92. Having value of N_{PNIPAM} = 13 determined previously by MALLS, N_{PEG} was thus calculated to be 38.

As expected, not all of aldehyde groups within the star core can be clicked by PEG-ONH_2 to form second-generation PEG arms due to the cross-linked nature of the star core. Comparing the observed N_{PEG} value (∼38) with the total amount of aldehyde groups in the star core (∼65 aldehyde groups per star, the calculated value based on the EVBA addition amount for the star PNIPAM formation) reveals that around 58% fraction of aldehyde groups, the "reactive" aldehyde groups located near the core surface, are accessible to PEG-ONH_2 and participate in the click reaction. It can be expected that the added PEG-ONH_2 should be clicked quantitatively when the initial PEG-ONH_2 amount added to the reaction was lower than that of the "reactive" aldehyde groups in the star core. In this case, the number of the second PEG arm can be controlled by varying the aminooxy/aldehyde feed ratio. In a ratio of [aminooxy]/[aldehyde] = 0.25:1, for example, PEG-ONH_2 was almost consumed, and the observed N_{PEG} was 14, which was in good agreement with the expected value (∼16) assuming 100% of click efficiency. The characterization data on PNIPAM-PEG miktoarm star polymers are given in Table 1. The molecular weights determined by GPC were lower than that by MALLS, which indicates that the star polymers were more compact than the linear counterparts with the same molecular weight.[39]

Thermoinduced Micellization of PNIPAM-PEG Miktoarm Star Copolymers in Aqueous Solution. The obtained new double-hydrophilic miktoarm star copolymer contains a permanently hydrophilic PEG block and a thermoresponsive PNIPAM block which exhibits a lower critical solution temperature (LCST) around 32 °C. At temperature below the LCST, the intermolecular hydrogen bonding between PNIPAM chains and water molecules is dominant, and

Table 1. Characterization of $(PNIPAM)_n$-polyEVBA Star Polymer and $(PNIPAM)_n$-polyEVBA-$(PEG)_m$ Miktoarm Star Copolymer

sample	M_n (GPC)	M_w/M_n (GPC)	M_w[c]	N_{PNIPAM}[f]	N_{PEG}[g]
$(PNIPAM)_{13}$-polyEVBA[a]	53 400	1.36	90 600[d]	13	
$(PNIPAM)_{13}$-polyEVBA-$(PEG)_{38}$[b]	65 300	1.12	166 600[e]	13	38
$(PNIPAM)_{13}$-polyEVBA-$(PEG)_{14}$[b]	61 100	1.17	118 600[e]	13	14

[a] Prepared by arm-first technique via cross-linking of the linear PNIPAM RAFT agent ($M_{n,GPC}$ = 5000, M_w/M_n = 1.07, M_w by MALLS = 5800) using aldehyde-containing divinyl compound EVBA. [b] Prepared by click reaction of aldehyde core-functionalized star PNIPAM and PEG-ONH_2, the feed ratio of [aminooxy]/[aldehyde] was 1.5:1 for $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{38}$ and 0.25:1 for $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{14}$. [c] Absolute weight-average molecular weight. [d] By GPC with MALLS detector. [e] Determined by combination of MALLS and ^1H NMR. [f] The average number of PNIPAM arms per star molecule: $N_{PNIPAM} = M_{w,star}Arm_{wt\,\%}/M_{w,PNIPAM}$. [g] Average number of PEG arms per star molecule: $N_{PEG} = (DP_{PNIPAM}I_{PEG,3.5-3.8}N_{PNIPAM})/(4DP_{PEG}I_{PNIPAM,3.8-4.2})$, $I_{PNIPAM,3.8-4.2}$ and $I_{PEG,3.5-3.8}$ are the ^1H NMR in $CDCl_3$ integration areas of methine protons for PNIPAM and methylene protons for PEG, respectively; DP is the degree of polymerization.

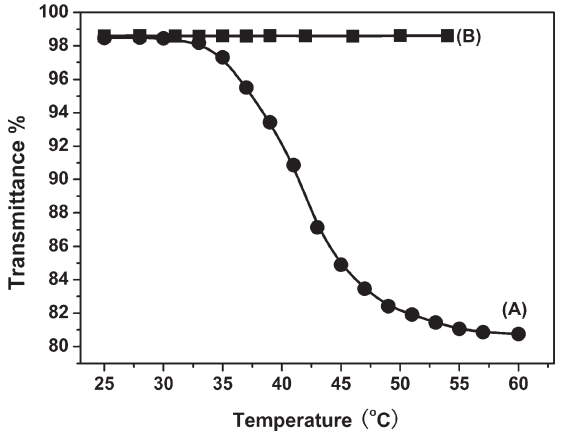
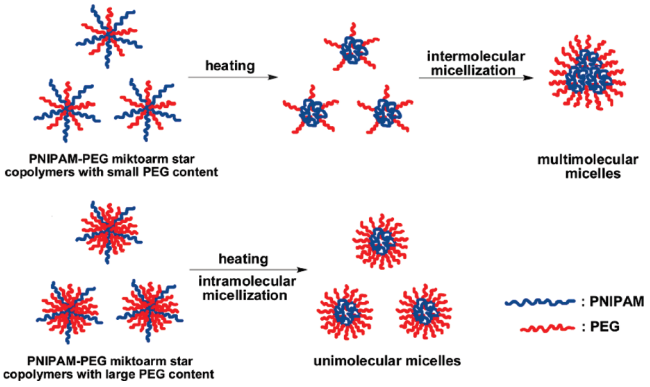


Figure 4. Temperature dependences of optical transmittance at 500 nm obtained for 10 mg/mL aqueous solutions of $(PNIPAM)_{13}$-poly-EVBA-$(PEG)_m$ miktoarm star copolymers with different PEG arms. (A) m = 14; (B) m = 38.

Scheme 3. Illustration of the Thermoinduced Micellization Behavior of the $(PNIPAM)_{13}$-polyEVBA-$(PEG)_m$ Miktoarm Star Copolymers with Different PEG Content



therefore PNIPAM is hydrophilic and soluble in water. At temperature above the LCST, intramolecular hydrogen bonding between the amide groups in PNIPAM results in transition into compact and collapsed conformations of PNIPAM chains, and the polymeric chain becomes hydrophobic and insoluble in water. Thus, PNIPAM-PEG miktoarm star copolymer might exhibit thermoresponsive micellization behavior in aqueous solution. Compared to linear counterparts (block copolymers), miktoarm star copolymers exhibit more complex phase-separation behavior in solution due to that two different kinds of arms are linked to a single junction point.[40] Thus, it may be interesting to investigate the micellization behavior of the synthesized PNIPAM-PEG miktoarm star copolymers. Miktoarm star copolymers $(PNIPAM)_n$-polyEVBA-$(PEG)_m$ with equal PNIPAM arm number (n = 13; M_n of PNIPAM ~5000) but different PEG arm number (m = 38 and 14, respectively; M_n of PEG ~ 2000) were used in order to check the effect of the miktoarm star copolymer composition on the thermo-induced micellization mechanism. The temperature sensitivity of the miktoarm star copolymers which should be strongly correlated with their micellization behavior in water was examined by turbidity measurements.

Figure 4 shows the temperature-dependent transmittance at 500 nm of the miktoarm star copolymer aqueous solutions (10 mg/mL). For the miktoarm star copolymer $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{14}$ having a small number of PEG arm and therefore a small PEG fraction (molar ratio of PEG to PNIPAM units ~1.1), the optical transmittance began to decrease around 31 °C (curve A), accompanied by a color change of the solution upon a temperature increase, from completely colorless and transparent to a blue tinge, indicating the formation of micelles. These results demonstrated that PNIPAM arms in the miktoarm star copolymer collapsed into hydrophobic segments upon a temperature increase, resulting in the formation of amphiphilic miktoarm star copolymer which subsequently assembled via intermolecular hydrophobic interactions (intermolecular micellization) to multimolecular micelles with collapsed PNIPAM core and stretched PEG corona (Scheme 3). To further confirm the formation of micelles, the size distributions of the miktoarm star copolymer aqueous solutions at 25 and 60 °C were measured by dynamic light scattering (Figure 5A). At 25 °C (below the phase transition temperature), the miktoarm star copolymer molecularly dissolves in water with a hydrodynamic radius of ~9.8 nm. At 60 °C (above the phase transition temperature), intermolecular micellization occurs, accompanied by an increase of hydrodynamic radius (~33.6 nm). Because the micelles formed here are small (size in nanoscale) and thus are not able to scatter light efficiently, the remaining transmittance at elevated temperature for an aqueous solution of $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{14}$ is still high (~80%, Figure 4, curve A). It is also necessary to point out that the thermal transition process of the miktoarm star copolymer is very broad and covers a temperature range from 31 to 55 °C, which is quite different from the sharp phase transition of PNIPAM homopolymers.

When more PEG arms were incorporated as in the case of $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{38}$ (molar ratio of PEG to PNIPAM units ~3), the transmittance did not change with temperature and was higher than 98% even at the highest temperature investigated (60 °C, Figure 4, curve B); thus, the solution was transparent over the experimental temperature range. Dynamic light scattering results (Figure 5B) showed that the hydrodynamic radius of the miktoarm star copolymer in aqueous solution at 60 °C (~8.1 nm) was almost consistent with that at 25 °C (~9.2 nm). These results suggested that upon heating of the aqueous solution of $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{38}$ no intermolecular aggregation occurred

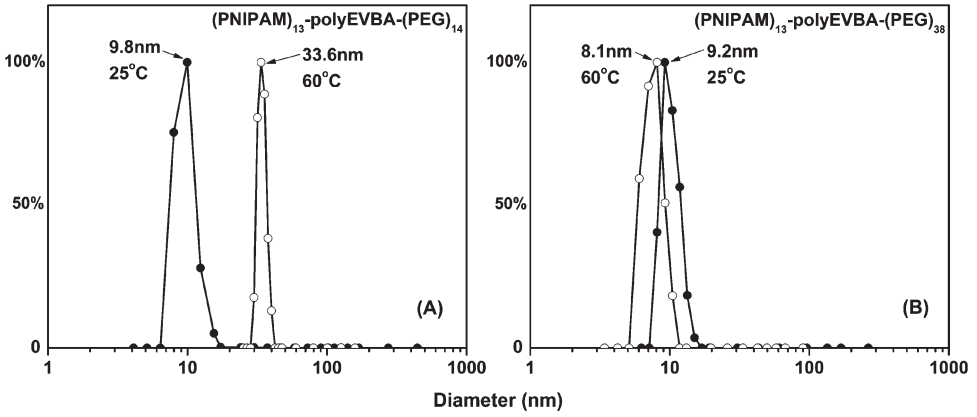


Figure 5. Size distributions (measure by dynamic light scattering at scattering angle 90°) of the $(PNIPAM)_{13}$-polyEVBA-$(PEG)_m$ miktoarm star copolymers at 25 and 60 °C. (A) $m = 14$; (B) $m = 38$.

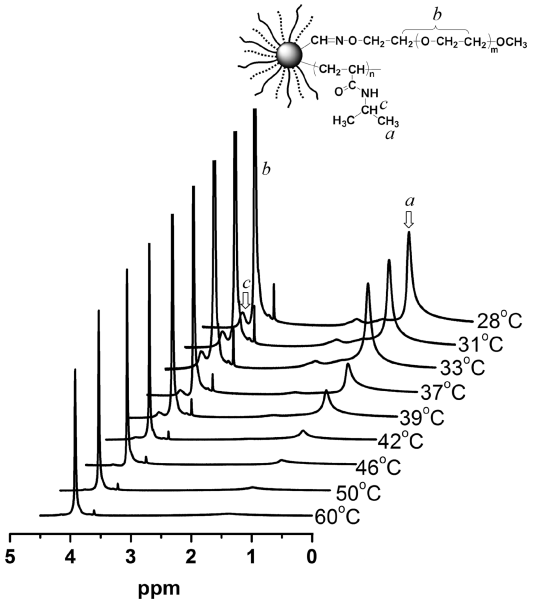


Figure 6. Temperature-dependent ^{1}H NMR spectra of $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{38}$ miktoarm star copolymers solution in D_2O.

although PNIPAM arms may collapse in the miktoarm star copolymer.

In order to find out whether the PNIPAM arms collapse at elevated temperature, ^{1}H NMR was employed to check the thermoresponsive behavior of the miktoarm star copolymer. ^{1}H NMR is a powerful and qualitative technique of reflecting the conformational change of polymer chain because the proton signals of collapsed polymer chain may become strongly attenuated or even vanish. Figure 6 shows representative temperature-dependent ^{1}H NMR spectra of $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{38}$ in D_2O solution (10 mg/mL) in the temperature range from 28 to 60 °C. The signal intensity due to PEG methylene protons (*b*) remained over the experimental temperature range except for slight chemical shift changes, whereas the signal intensities due to PNIPAM methine (*c*) and methyl (*a*) protons became attenuated at elevated temperatures, indicating thermoinduced collapse of PNIPAM chains did occur. By plotting the peak intensity ratio of *a*/*b* vs temperature, it was observed that the signal intensity from PNIPAM arms started to decrease around 33 °C and almost disappeared at 60 °C (Figure 7), which should correspond to the PNIPAM chain collapse process. Since the turbidity measurement and dynamic light

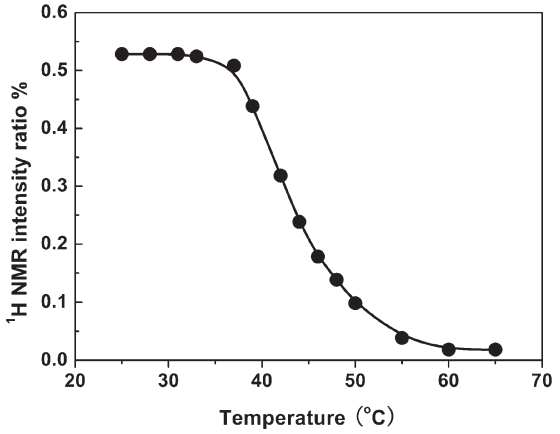


Figure 7. Plots of temperature vs the ratio of proton signal intensity of methyl (*a*) from PNIPAM arms to that of methylene (*b*) from PEG arms. Detailed temperature-dependent ^{1}H NMR spectra of $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{38}$ miktoarm star copolymers are shown in Figure 6.

scattering results have demonstrated that no intermolecular aggregation occurred for $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{38}$ in aqueous solution upon heating, it must undergo intramolecular micellization at elevated temperature to form unimolecular micelles consisting of collapsed and compact PNIPAM core and soluble PEG corona (Scheme 3). In contrast to the case for $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{14}$ having smaller number of PEG arms, the more PEG arms in $(PNIPAM)_{13}$-polyEVBA-$(PEG)_{38}$ may have a shielding effect to protect the collapsed PNIPAM segments against the intermolecular aggregation. Thus, for the PNIPAM-PEG miktoarm star copolymers, opportunities are open for thermoinduced intermolecular or intramolecular micellizations to form multimolecular micelles and unimolecular micelles, respectively, via controlling the content ratio of PNIPAM and PEG.

Conclusion

In summary, we have successfully demonstrated a facile synthetic pathway to PNIPAM-PEG miktoarm star copolymers with multiple arms using a combination of RAFT arm-first technique and aldehyde−aminooxy "click" coupling reaction. Star PNIPAM bearing multiple aldehyde functionalities in the core was initially prepared by RAFT arm-first technique via cross-linking of the preformed linear macro-RAFT agents using a newly designed aldehyde-containing divinyl compound EVBA. The aldehyde groups preserved in the star core was then allowed

to simply click with aminooxy-terminated PEGs to form PNIPAM-PEG miktoarm star copolymers. By controlling the content ratio of PNIPAM and PEG, the obtained double-hydrophilic miktoarm star copolymer may form multimolecular micelles and unimolecular micelles via micellization of intermolecular and intramolecular, respectively.

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