Synthesis of Amphiphilic Three-Armed Star Random Copolymers via Living Radical Polymerization and their Unimolecular Folding Properties in Water

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Summary: Amphiphilic three-armed star random copolymers were synthesized by ruthenium-catalyzed living radical copolymerization of hydrophilic poly(ethylene glycol) methyl ether methacrylate (PEGMA) and hydrophobic dodecyl methacrylate (DMA). Their amphiphilic star random copolymers with 10-50 mol% DMA efficiently self-folded in water with intramolecular hydrophobic interaction to form compact unimolecular micelles. Owing to PEG segments, star copolymers were thermoresponsive to induce lower critical solution temperature-type phase separation in water.

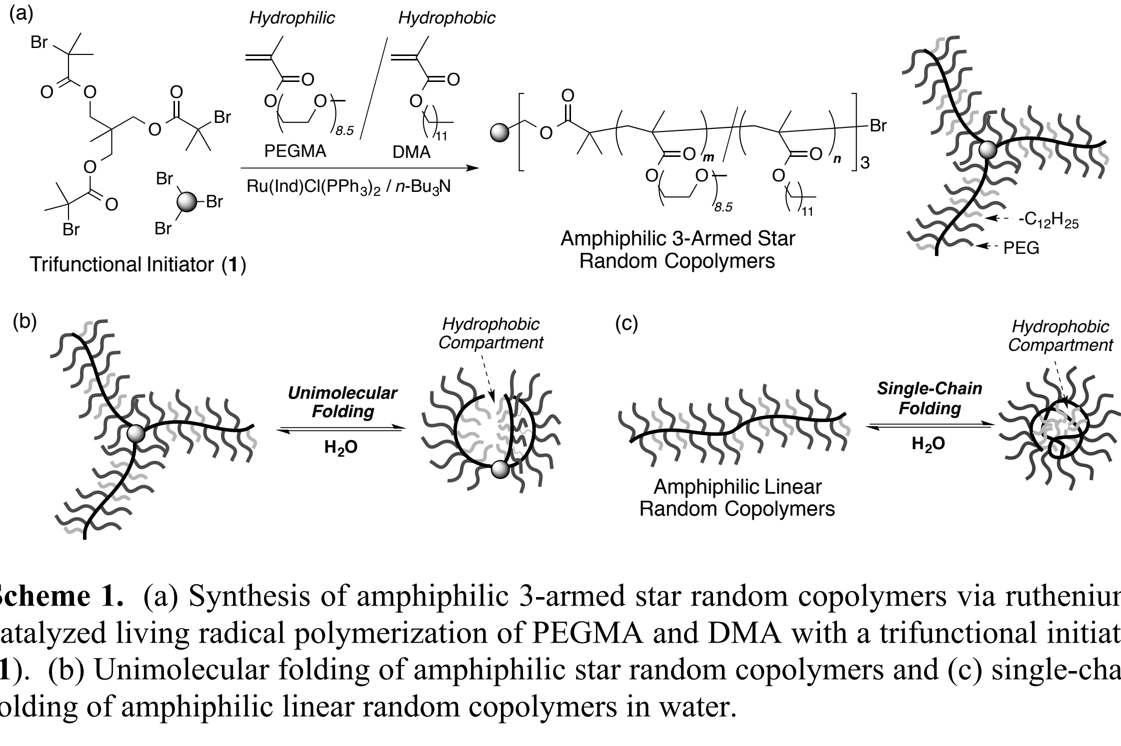
Keywords: star polymer; amphiphilic copolymer; folding; unimer micelle; living radical polymerization; hydrophobic interaction

Introduction

Protein and enzyme form globular tertiary structures carrying specific inner spaces and cavities that drive unique functions such as selective catalysis and recognition in water.[1] The key is no doubt the self-folding of polymer chains of precision primary structure via site-specific association of the pendant functional groups. In particular, amphiphilicity of polymers bearing both hydrophilic and hydrophobic units plays an important role for the self-folding conformation in water, where the hydrophobic segments come inside to effectively induce intramolecular hydrophobic interaction for stabilization of the resulting globular structure.

Chemical crosslinking or physical association of synthetic linear polymers also gives various globular polymeric materials applicable as functional spaces: 1) microgel-core star polymers;[2-4] 2) micelles, vesicles, and polymersomes;[5-7] and 3) unimer micelles and single-chain polymeric nanoparticles (SCPNs).[8-10] The third unimer micelles and SCPNs are generally obtained from the intramolecular folding of functional and/or amphiphilic linear polymers with non-covalent (hydrophobic, hydrogen-bonding etc.) interaction and/or the intramolecular crosslinking of their linear (or folding) polymers with covalent bond.[8-24] They are thus structurally regarded as synthetic alternatives of proteins and enzymes. More importantly, primary structure for unimer micelles and SCPNs can be controlled more precisely and designed more straightforwardly than that for the former two globular materials obtained from the intermolecular crosslinking and/or association of multi polymer chains. Namely, molecular weight, composition and sequence (random, gradient, block, alternating) of monomers (functional groups), and terminal functional groups for linear polymers can be first designed on demand with appropriate living polymerization systems and then directly reflected to those for objective single-chain compartments.

Focusing on these features, we and other groups have recently developed unimer micelles and SCPNs via the single-chain folding of amphiphilic random copolymers of a hydrophilic poly(ethylene glycol) methyl ether methacrylate (PEGMA) and a hydrophobic methacrylate with intramolecular hydrophobic and/or hydrogen-bonding interaction in water (Scheme 1c).[17-21] Typically, random copolymers of PEGMA and dodecyl methacrylate (DMA), synthesized by living radical polymerization,[25-33] undergo single-chain folding in water to be compact unimer micelles up to 40 mol% DMA content and in turn multi-chain aggregation over 50 mol% DMA.[21] The self-folding structure is reversible, i.e., unfolded by the addition of alcohol, and more mobile upon temperature, but is still maintained at high temperature (<80 °C) and concentration (< ~6 wt%). Owing to PEG pendants, such folding polymers further showed thermosensitive solubility [lower critical solution temperature (LCST)] in water.[21,34,35]



Scheme 1. (a) Synthesis of amphiphilic 3-armed star random copolymers via ruthenium-catalyzed living radical polymerization of PEGMA and DMA with a trifunctional initiator (**1**). (b) Unimolecular folding of amphiphilic star random copolymers and (c) single-chain folding of amphiphilic linear random copolymers in water.

Herein, we report on the self-folding of amphiphilic three-armed star random copolymers in water as a new class of unimer micelles comprising a single star polymer (Scheme 1). Owing to the branched structure, amphiphilic star random copolymers not only might possess self-folding properties distinct from previous linear counterparts[21] but also could extend the design scope of unimolecular compartments in terms of molecular weight, local density, size, folding structures, among others. Living radical polymerization with multi-functional initiators is a promising strategy to produce well-controlled, functional star polymers with precision arm numbers.[26] Thus, in this work, amphiphilic 3-armed star random copolymers were prepared by ruthenium-catalyzed living radical polymerization of PEGDMA and DMA with a trifunctional initiator (**1**) (Scheme 1a). PEGMA/DMA star random copolymers up to 50 mol% DMA efficiently self-folded with intramolecular hydrophobic interaction in water to be unimer micelles. The folding properties were investigated in detail, compared with those for corresponding linear PEGMA/DMA random copolymers. The star polymers further showed thermoresponsive solubility in water.

Experimental Section

Materials. For the synthesis of a trifunctional initiator (**1**), 2-bromo-2-methylpropanoyl bromide (Aldrich, purity >98%) and 1,1,1-tris(hydroxymethyl)ethane (Aldrich, purity >99%) were degassed by triple vacuum-argon purge cycles before use. Triethylamine (TCI, purity >99%) was purified by distillation before use. Dry THF (Wako, dehydrated) was used as received. For polymerization and characterization, poly(ethylene glycol) methyl ether methacrylate [PEGMA; $CH_2=CMeCO_2(CH_2CH_2O)_nMe$: $M_n = 475$; $n = 8.5$ on average; Aldrich] and dodecyl methacrylate (DMA: Wako; purity >95%) were purified by an inhibitor removal column (Aldrich) and degassed by triple vacuum-argon purge cycles before use. $Ru(Ind)Cl(PPh_3)_2$ (Aldrich) was used as received and handled in a glove box under moisture- and oxygen-free argon ($H_2O < 1$ ppm, $O_2 < 1$ ppm). n-Bu_3N (TCI, purity >99%) was degassed before use. Tetralin (1,2,3,4-tetrahydronaphthalene: Kishida Chemical; purity >98%; an internal standard for ^1H NMR analysis of monomer conversion) was dried overnight over calcium chloride and distilled from calcium hydride under reduced pressure before use. Toluene was purified before use; passing it through a purification column (Glass Contour Solvent Systems: SG Water USA). Reichardt's dye (Aldrich, purity >90%) was used as received.

Characterization. Molecular weight distribution (MWD) curves, number-average molecular weight (M_n), peak top molecular weight (M_p), and M_w/M_n ratio of polymers were measured by size-exclusion chromatography (SEC) in DMF containing 10 mM LiBr at 40 °C (flow rate: 1 mL/min) on three linear-type polystyrene gel columns (Shodex KF-805L: exclusion limit = 4×10^6; particle size = 10 μm; pore size = 5000 Å; 0.8 cm i.d. × 30 cm) that were connected to a Jasco PU-2080 precision pump, a Jasco RI-2031 refractive index detector, and a Jasco UV-2075 UV/vis detector set at 270 nm. The columns were calibrated against 10 standard poly(MMA) samples (Polymer Laboratories: M_n = 1000–1200000; M_w/M_n = 1.06–1.22) or 10 standard poly(ethylene oxide) samples (Polymer Laboratories: M_n = 1460–737000; M_w/M_n = 1.03–1.07). MWD curves and M_p of polymers were measured by SEC in H_2O at 30 °C (flow rate: 1 mL/min) on a silica gel column (TOSOH TSKgel G4000SW$_{XL}$: exclusion limit = 7×10^6; particle size = 8 μm; 0.8 cm i.d. × 30 cm) that was connected to the same pump and detectors as those in DMF. The column was calibrated against 10 standard poly(ethylene oxide) samples (Polymer Laboratories: M_n = 1460–737000; M_w/M_n = 1.03–1.07). Proton or carbon nuclear magnetic resonance (^{1}H or ^{13}C NMR) spectra were recorded in $CDCl_3$ on a JEOL JNM-ECA500 spectrometer operating at 500 (^{1}H) or 125 (^{13}C) MHz. Absolute weight-average molecular weight (M_w) of polymers in DMF or H_2O was determined by multi-angle laser light scattering (MALLS) equipped with SEC on a Dawn E instrument (Wyatt Technology: Ga-As laser; λ = 690 nm). The SEC was performed in DMF containing 10 mM LiBr at 40 °C (flow rate: 1 mL/min) on three linear-type polystyrene gel columns (Shodex KF-805L) or in H_2O at 30 °C (flow rate: 1 mL/min) on a silica gel column (TOSOH TSKgel G4000SW$_{XL}$), that were connected to a Jasco PU-2080 precision pump, a Jasco RI-1530 refractive index detector, and a Jasco UV-1570 UV/vis detector set at 270 nm. Refractive index increment (dn/dc) was measured in DMF at 40 °C on an Optilab DSP refractometer (Wyatt Technology: λ = 690 nm; c < 2.5 mg/mL). Dynamic light scattering (DLS) was measured on Otsuka Photal ELSZ-0 equipped with a semi-conductor laser (wavelength: 658 nm) at 25 °C. The measuring angle was 165° and the data was analyzed by CONTIN method. UV/Vis spectra were recorded on Shimadzu UV-1800 in H_2O, H_2O/acetone (19/1), and acetone at 25 °C (optical path length = 1.0 cm).

Synthesis of a trifunctional initiator (1). In 100 mL round-bottomed flask filled with argon, 2-bromo-2-methylpropanoyl bromide (16 mmol, 1.96 mL) was added to a solution of 1,1,1-tris(hydroxymethyl)ethane (3.5 mmol, 0.42 g) and triethylamine (24 mmol, 3.3 mL) in dry THF (60 mL) at 0 ^{o}C. The reaction mixture was stirred at 25 ^{o}C for 18 h. After the evaporation of the reaction solution, diethyl ether (25 mL) and distilled water (50 mL) were poured into the flask. The aqueous phase was separated and extracted by diethyl ether (25 mL), and the ether extracts were combined with the organic layer. The combined organic phase was washed with water three times, ammonia water, and brine, and was dried over anhydrous Na_2SO_4 overnight. After the ether was removed in vacuo, a pure solid product [1,1,1-tris(2-bromoisobutyryloxymethyl)ethane: **1**] was obtained (0.99 g, 50% yield). ^{1}H NMR [500 MHz, $CDCl_3$, 25 ^{o}C, $\delta = 7.26$ ($CHCl_3$)] $\delta = 4.12$ (s, 6H), 1.94 (s, 18H), 1.17 (s, 3H). ^{13}C NMR [125 MHz, $CDCl_3$, 25 ^{o}C, $\delta = 77.0$ ($CDCl_3$)] $\delta =$ 171.2, 66.6, 55.4, 39.7, 30.7, 16.9.

Synthesis of Amphiphilic 3-Armed Star Polymers (S1-S6). The synthesis of amphiphilic 3-armed star polymers (**S1**-**S6**) was carried out by syringe technique under argon in baked glass tubes equipped with a three-way stopcock via ruthenium-catalyzed living radical polymerization. A typical procedure for **S4** was given: In a 30 mL glass tube, $Ru(Ind)Cl(PPh_3)_2$ (0.008 mmol, 6.3 mg) was placed. Then, toluene (5.8 mL), tetralin (0.1 mL), a 400 mM toluene solution of n-Bu_3N (n-Bu_3N = 0.16 mmol, 0.4 mL), PEGMA (2.4 mmol, 1.1 mL), DMA (1.6 mmol, 0.47 mL), and a 120 mM toluene solution of **1** (**1** = 0.016 mmol, 0.13 mL) were added sequentially in that order into the tube at 25 ^{o}C under argon (The total volume of the reaction mixture: 8 mL). The glass tube was placed in an oil bath kept at 80 ^{o}C. At predetermined intervals, the mixture was sampled with a syringe under dry argon, and the reaction was terminated by cooling the solution to –78 °C. The monomer conversion was determined by ^{1}H NMR in $CDCl_3$ with tetralin as an internal standard. The quenched reaction solutions were evaporated to dryness. The crude product was purified by silica gel column chromatography with toluene as an eluent, and precipitated into hexane, to give **S4**. SEC (DMF, PMMA std.): M_n = 52300 g/mol; M_w/M_n = 1.26. dn/dc (DMF) = 0.045. SEC-MALLS (DMF, 0.01 M LiBr): M_w = 122300 g/mol. ^{1}H NMR [500 MHz, $CDCl_3$, 25 ^{o}C, $\delta = 7.26$ ($CHCl_3$)] δ 4.2–4.0 ($-COOC\underline{H}_2CH_2O-$), 4.0–3.8 ($-COOC\underline{H}_2CH_2CH_2-$), 3.7–3.5 ($-OCH_2CH_2O-$), 3.4–3.3 ($-OCH_3$), 2.1–1.7 ($-C\underline{H}_2C(CH_3)-$), 1.7–1.5 ($-COOCH_2C\underline{H}_2(CH_2)_9CH_3$), 1.5–1.2 ($-COOCH_2CH_2(C\underline{H}_2)_9CH_3$), 1.2–0.7 ($-COO(CH_2)_{11}C\underline{H}_3$, $-CH_2C(C\underline{H}_3)-$).

Results and Discussion

Synthesis of Amphiphilic Three-Armed Star Random Copolymers

Amphiphilic three-armed star random copolymers (**S1-S6**) were synthesized by living radical polymerization of PEGMA and DMA with a trifunctional initiator [**1**: 1,1,1-tris(2-bromoisobutyryloxymethyl)ethane] and a ruthenium catalytic system [Ru(Ind)Cl(PPh_3)$_2$/*n*-Bu_3N][17,21] in toluene at 80 °C (Table 1, Figure 1). To investigate the effects of hydrophobic DMA content on the self-folding properties of star polymers in water, the feed ratio of PEGMA and DMA against an initiator (**1**) was systematically varied in the constant total monomer concentration (500 mM): [PEGMA]$_0$/[DMA]$_0$/[**1**]$_0$ = 500/0/2 (**S1**), 450/50/2 (**S2**), 400/100/2 (**S3**), 300/200/2 (**S4**), 250/250/2 (**S5**), 200/300/2 (**S6**) mM. Degree of polymerization (DP) of PEGMA and DMA for a single arm chain [***m*** = ([PEGMA]$_0$ x conv.)/(300 x [**1**]$_0$), ***n*** = ([DMA]$_0$ x conv.)/(300 x [**1**]$_0$)] at 90% conversion is given: ***m***/***n*** (90% conv.) = 75/0 (**S1**), 67.5/7.5 (**S2**), 60/15 (**S3**), 45/30 (**S4**), 37.5/37.5 (**S5**), 30/45 (**S6**).

Table 1. Characterization of Amphiphilic 3-Armed Star Random Copolymers (**S1-S6**)[a]

Code	PEGMA/ DMA[b]	Time (h)	Conv. (%)[c] PEGMA/DMA	M_n[d]	M_w/M_n[d]	***m***/***n***$_{calcd}$[e]	$M_{n,calcd}$[f]	$M_{w,DMF}$[g]	A_{DMF}[h]	$M_{w,H2O}$[g]	A_{H2O}[i]
S1	10/0	9	92/-	58900	1.30	77/0	109800	158600	1.11	135300	0.85
S2	9/1	9	90/85	66400	1.26	68/7	102100	146600	1.14	128000	0.87
S3	8/2	9	87/84	62000	1.26	58/14	93800	143200	1.21	115100	0.80
S4	6/4	9	88/87	52300	1.26	44/29	85300	122300	1.14	123000	1.01
S5	5/5	9	89/89	43400	1.25	37/37	81600	119600	1.17	147700	1.23
S6	4/6	11	83/84	26300	1.33	28/42	72000	138000	1.44	-	-

[a] [PEGMA]$_0$+[DMA]$_0$/[**1**]$_0$/[Ru(Ind)Cl(PPh_3)$_2$]$_0$/[*n*-Bu_3N]$_0$ = 500/2.0/1.0/20 mM in toluene at 80 °C.

[b] Feed ratio of PEGMA and DMA: [PEGMA]$_0$/[DMA]$_0$ = 500/0 (**S1**), 450/50 (**S2**), 400/100 (**S3**), 300/200 (**S4**), 250/250 (**S5**), 200/300 (**S6**) mM.

[c] Monomer conversion: determined by ^{1}H NMR.

[d] Number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n): determined by SEC in DMF (10 mM LiBr) with PMMA standard calibration.

[e] DP of PEGMA (***m***) and DMA (***n***) for a single arm chain, calculated from the monomer feed ratio and conversion: ***m*** = ([PEGMA]$_0$ x conv.)/(300 x [**1**]$_0$); ***n*** = ([DMA]$_0$ x conv.)/(300 x [**1**]$_0$).

[f] Molecular weight of star polymers calculated from DP (***m*** and ***n***) and formula weight of monomers.

[g] Absolute weight-average molecular weight (M_w) in DMF (10 mM LiBr) or H_2O: determined by SEC-MALLS.

[h] Association number in DMF: $A_{DMF} = M_{w,\,DMF}/(M_{n,calcd} \times M_w/M_n)$.

[i] Association number in H_2O: $A_{H2O} = M_{w,\,H2O}/M_{w,\,DMF}$.

In all cases, PEGMA and DMA were efficiently and smoothly polymerized up to 83-92% conversion to give well-controlled star polymers with narrow molecular weight distribution (**S1-S6**: M_n = 26300 – 66400; M_w/M_n = 1.25 – 1.33, by SEC in DMF with PMMA standard calibration, Table 1, Figure 1e). Independently of the monomer feed ratio, PEGMA and DMA were simultaneously consumed at the same reaction rate [Figure 1a (**S2**), 1b (**S4**), 1c (**S5**)]. These results support that PEGMA and DMA are randomly introduced into arm chains.

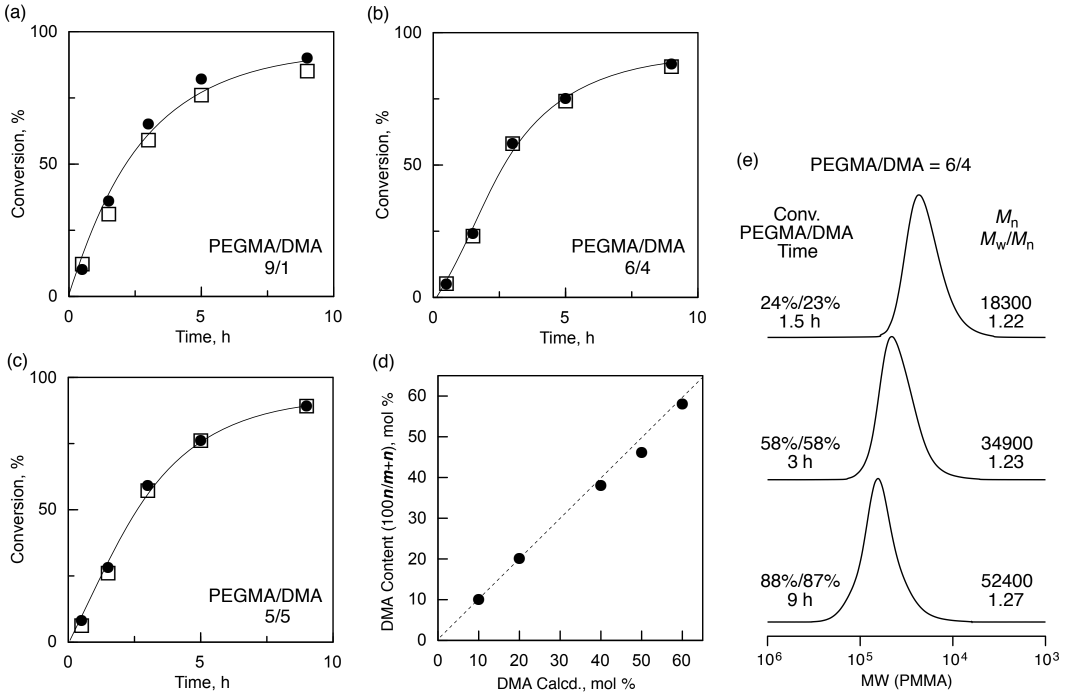


Figure 1. Ruthenium-catalyzed living radical polymerization of PEGMA and DMA with **1**: $[PEGMA]_0+[DMA]_0/[\mathbf{1}]_0/[Ru(Ind)Cl(PPh_3)_2]_0/[n\text{-}Bu_3N]_0$ = (a) 450+50, (b, e) 300+200, (c) 250+250/2.0/1.0/20 mM in toluene at 80 °C; (a-c) time conversion curves (filled circle: PEGMA, open square: DMA); (d) DMA content in polymers (**S2-S6**) determined by ^{1}H NMR as a function of DMA content calculated from monomer feed ratio and conversion; (e) SEC curves of products with 40 mol% DMA.

Products were analyzed by ^{1}H NMR in $CDCl_3$ at 25 °C. Typically, a star polymer with 40 mol% DMA (**S4**) showed characteristic methylene and methyl protons of PEG chains (***c***: 4.2–4.0 ppm; ***d***: 3.7–3.5 ppm; ***e***: 3.4–3.3 ppm) and those of dodecyl groups (***f***: 4.0–3.8 ppm; ***g***: 1.7–1.5 ppm; ***h***: 1.5–1.2 ppm; ***i***: 0.9–0.7 ppm) in addition to those of a methacrylate backbone (***b***: 2.1–1.7 ppm; ***a***: 1.2–0.7 ppm) (Figure 2). In all cases, the composition of PEGMA and DMA in products, calculated from the area ratio of methylene protons of PEGMA and DMA (***c*** and ***f***), was in good agreement with the monomer feed ratio (Figure 1d).

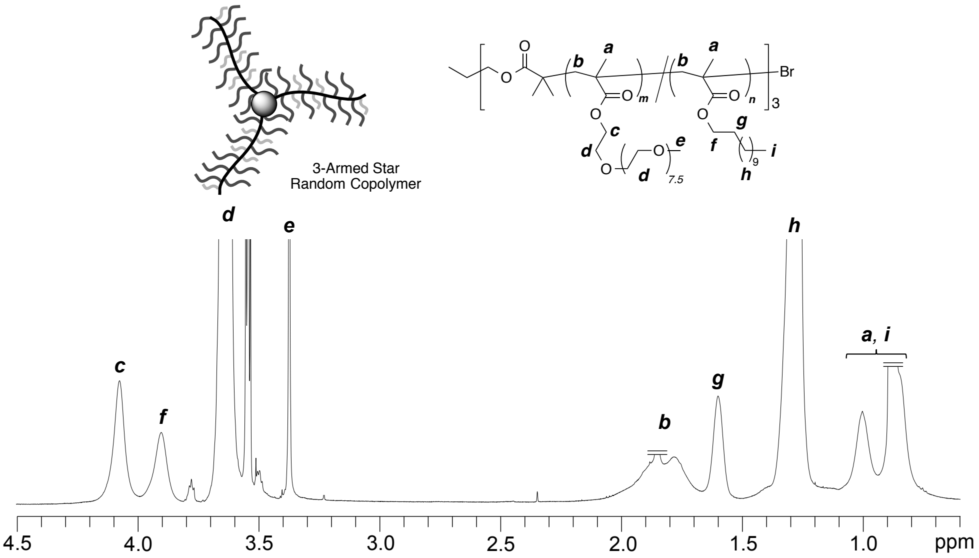


Figure 2. ^{1}H NMR spectrum of **S4** (***m***/***n*** = 44/29) in $CHCl_3$ at 25 °C.

Unimolecular Folding of Star Polymers in Water

Folding properties of **S1**-**S6** in water were investigated with multi-angle lasar light scattering coupled with SEC (SEC-MALLS) and dynamic light scattering (DLS), in comparison with those of linear PEGMA/DMA random copolymers [PEGMA/DMA (***m***/***n***) = 200/0, 180/20, 160/40, 120/80, 100/100, 80/120, $M_{\mathrm{w,DMF}}$ (MALLS) = 89000 – 143000, $M_{\mathrm{w}}/M_{\mathrm{n}}$ (SEC) = 1.2 – 1.4].[21] Here, the total DP (***m*** + ***n*** = 200), monomer composition, and $M_{\mathrm{w}}/M_{\mathrm{n}}$ for linear copolymers are almost consistent with those for **S1**-**S6** (total DP = 210 – 230), to conclude that different properties between **S1**-**S6** and linear counterparts are attributed to the branched structure. Assuming that amphiphilic star polymers unimolecularly fold with intramolecular hydrophobic interaction in water to be compact unimer micelles, they keep their original molecular weight but may become smaller in water than those in good organic solvents such as DMF and CH_2Cl_2.

Based on these criteria, absolute weight-average molecular weight of **S1**-**S6** ($M_{\mathrm{w,DMF}}$) was first determined by SEC-MALLS in DMF (Table 1). $M_{\mathrm{w,DMF}}$ for **S1**-**S6** was ranging from 119600 to 158600, which was almost consistent with the weight-average molecular weight ($M_{\mathrm{w,calcd}}$), respectively calculated from the molar feed ratio of monomers to initiator ($[\mathrm{PEGMA}]_0/[\mathbf{1}]_0$, $[\mathrm{DMA}]_0/[\mathbf{1}]_0$), conversion and molecular weight ($M_{\mathrm{n,PEGMA}}$, $F\mathrm{w}_{\mathrm{,DMA}}$) of monomers, and molecular weight distribution ($M_{\mathrm{w}}/M_{\mathrm{n}}$): $M_{\mathrm{w,calcd}} = M_{\mathrm{n,calcd}}$ x ($M_{\mathrm{w}}/M_{\mathrm{n}}$); $M_{\mathrm{n,calcd}}$ = ($[\mathrm{PEGMA}]_0$ x conv. x $M_{\mathrm{n,PEGMA}}$)/(100 x $[\mathbf{1}]_0$) + ($[\mathrm{DMA}]_0$ x conv. x $F\mathrm{w}_{\mathrm{,DMA}}$)/(100 x $[\mathbf{1}]_0$). Association number of their samples in DMF, defined as $A_{\mathrm{DMF}} = M_{\mathrm{w,DMF}}/M_{\mathrm{w,calcd}}$, was thus almost 1; **S1**-**S6** are unimolecularly dissolved in DMF without intermolecular aggregation.

Solubility of star polymers (**S1**-**S6**) in water was then examined. **S1**-**S5** were homogeneously soluble in water but **S6** with high DMA content (60 mol%) became emulsion. Analyzed by SEC-MALLS in H_2O, **S1**-**S5** showed almost single modal SEC curves [Figure 3a,b: dash lines from refractive index (RI) detector] and had absolute weight-average molecular weight ($M_{w,H2O}$) of 115100 – 147700 (Table 1). Since $M_{w,H2O}$ is close to $M_{w,DMF}$, respectively, **S1**-**S5** still exist as unimer in water [association number in water: $A_{H2O} = M_{w,H2O}/M_{w,DMF} = \sim 1$].

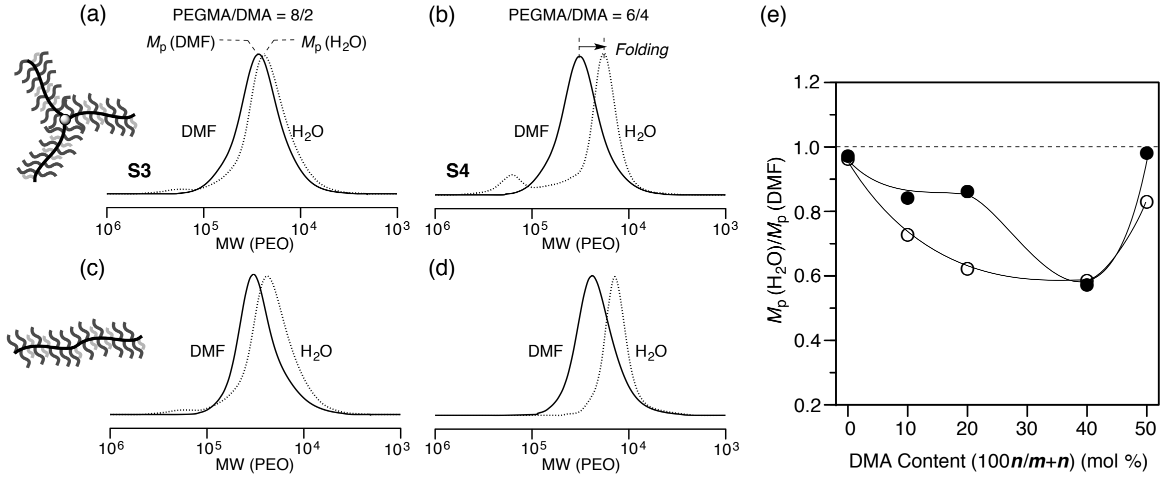


Figure 3. SEC curves in DMF or H_2O for (a, b) 3-armed star PEGMA/DMA random copolymers (a: **S3**, b: **S4**) and (c, d) linear PEGMA/DMA random copolymers (c: 160/40, d: 120/80). (e) SEC peak top molecular weight ratio [$M_p(H_2O)/M_p(DMF)$] for 3-armed star PEGMA/DMA random copolymers (**S1**-**S5**: filled circle) and linear PEGMA/DMA (***m***/***n***) random copolymers (***m***/***n*** = 200/0 – 100/100: open circle).

Importantly, the SEC peak-top molecular weight [M_p (H_2O)] for **S2**-**S4** in water, based on poly(ethylene oxide) standard calibration, was smaller than that in DMF [M_p (DMF)] [Figure 3a,b: dash lines (H_2O) vs. solid lines (DMF)], which indicate that **S2**-**S4** are more compact in water than in DMF. The folding efficiency in water was evaluated with the ratio of M_p (H_2O) to M_p (DMF) [M_p (H_2O)/M_p (DMF)], where M_p (H_2O) was normalized by M_p (DMF) reflecting their original structures in good solvent (Figure 3e). M_p (H_2O)/M_p (DMF) slightly decreased from 0.97 (**S1**: DMA 0 mol%) to ~0.85 with increasing DMA content up to 20 mol% and sharply decreased to a minimum of 0.57 at 40 mol% DMA, and in turn increased to 0.98 at 50 mol% DMA. Thus, it revealed that a star polymer with 40 mol% DMA (**S4**) efficiently collapses to be most compact in water against that in DMF, as well as a corresponding linear PEGMA/DMA (120/80) copolymer (Figure 3d,e).[21] **S4** has similar compactness ratio in water to the linear counterpart.

Hydrodynamic radius (R_h) of **S1**-**S5** was further determined by DLS in H_2O and CH_2Cl_2 (Figure 4a). R_h in H_2O was constant around 12 – 13 nm up to 20 mol% DMA content (**S1**-**S3**) and sharply decreased to 8 – 9 nm over 40 mol% DMA (**S4**, **S5**), whereas R_h in CH_2Cl_2 was almost constant (~13 nm), independent of DMA content (**S2**-**S5**). These results support that star polymers with 40 - 50 mol% DMA (**S4**, **S5**) are more compact in water than in CH_2Cl_2. R_h for **S1**-**S5** was larger than that for corresponding linear counterparts in both H_2O and CH_2Cl_2 (Figure 4b), which is attributed to the 3-armed, branched structures of **S1**-**S5**. It should be noted that **S5** with 50 mol% DMA unimolecularly folds in water, whereas a corresponding linear PEGMA/DMA (100/100) random copolymer induces bimolecular aggregation in water (A_{H2O} = 2).[21] The different folding/aggregation properties also most likely arise from the branched structure of main chains; i.e., 3-armed star polymers can fill the local space with their dodecyl pendants more effectively than linear counterparts to preferentially induce unimolecular folding with intramolecular hydrophobic interaction even at high DMA content (50 mol%).

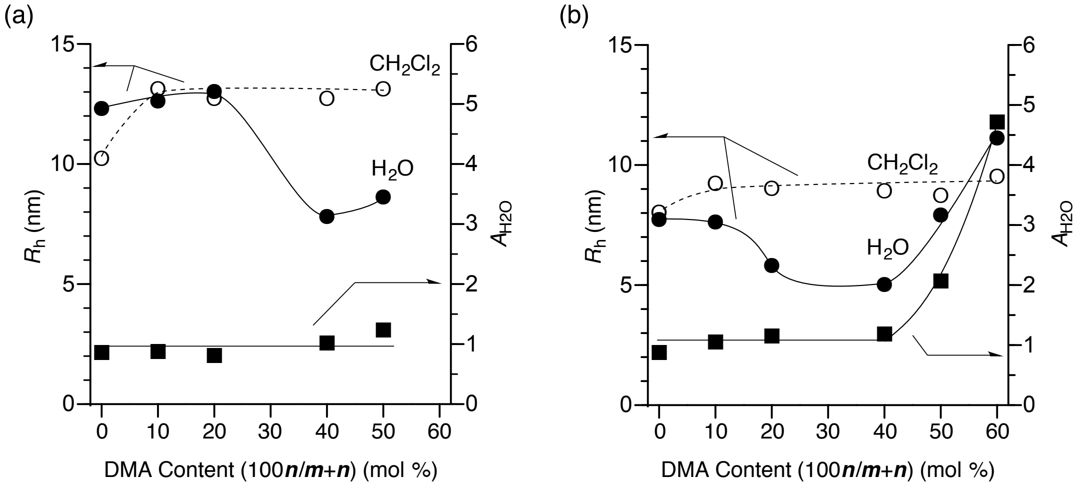


Figure 4. Hydrodynamic radius (R_h) in CH_2Cl_2 (open circle) or H_2O (filled circle) and association number in H_2O (A_{H2O}, filled square) for (a) 3-armed star PEGMA/DMA random copolymers (**S1**-**S5**) and (b) PEGMA/DMA (***m***/***n***) random copolymers (***m***/***n*** = 200/0 – 80/120) as a function of DMA content in polymers.

Hydrophobicity of folded star polymers in water was examined with UV/Vis measurements of the aqueous solutions with a solvatochromic Reichardt's dye (**RD**).[21,36] The pyridinium *N*-phenolate betaine dye induces blue shift of the UV-vis absorption originating from intramolecular charge transfer by increasing solvent polarity, typically as the following: λ_{max} = 675 nm in acetone (dash line); 455 nm in H_2O/acetone (19/1, v/v, long dash line) (Figure 5). In the presence of a 20 mol% DMA star copolymer (**S3**), λ_{max}

of **RD** was observed at 547 nm in H_2O/acetone (19/1, v/v), indicating that **RD** was encapsulated into a folded **S3** in water to reflect the inner hydrophobic environment. Such negative solvatochromic shift with **S3** was larger than that with a PEGMA star homopolymer (**S1**: λ_{max} = 509 nm). Thus, dodecyl pendants (n-$C_{12}H_{25}$) of **S3** in fact effectively associate to give unimolecular hydrophobic nanospaces in water.

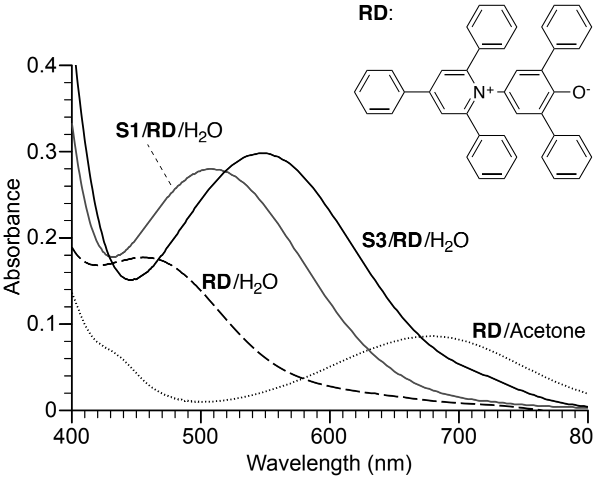


Figure 5. UV-vis spectra of Reichardt's dye (**RD**) (long dash line), **RD** with **S1** (gray solid line), and **RD** with **S3** (black solid line) in H_2O/acetone (19/1, v/v) and **RD** in acetone (dash line) at 25 °C: $[\text{polymer}]_0/[\textbf{RD}]_0$ = 0.0045 or 0/0.45 mM.

Thermosensitive Solubility

Owing to their pendant PEG units, **S1**-**S5** are also expected to show thermosensitive solubility in water. Thus, cloud point (Cp) of the aqueous solutions was measured by temperature-dependent UV-vis spectroscopy monitored at λ = 660 nm. Here, Cp was defined as 50% transmittance of their solutions that were heated at 1 °C/min. **S1-S5** sharply exhibited lower critical solution temperature (LCST)-type phase separation in water (Figure 6a). The resulting Cp gradually decreased from 92 °C to 65 °C with increasing DMA content from 0 to 50 mol% [Figure 6b, Cp = 92 (**S1**), 87 (**S2**), 85 (**S3**), 75 (**S4**), and 65 (**S5**) °C]. The Cp temperatures were identical to those for linear PEGMA/DMA (***m***/***n***) random copolymers with corresponding DMA content (***m***/***n*** = 200/0 – 100/100),[21] demonstrating that the branched, three-armed structure is independent of PEG-based thermosensitive solubility in water.

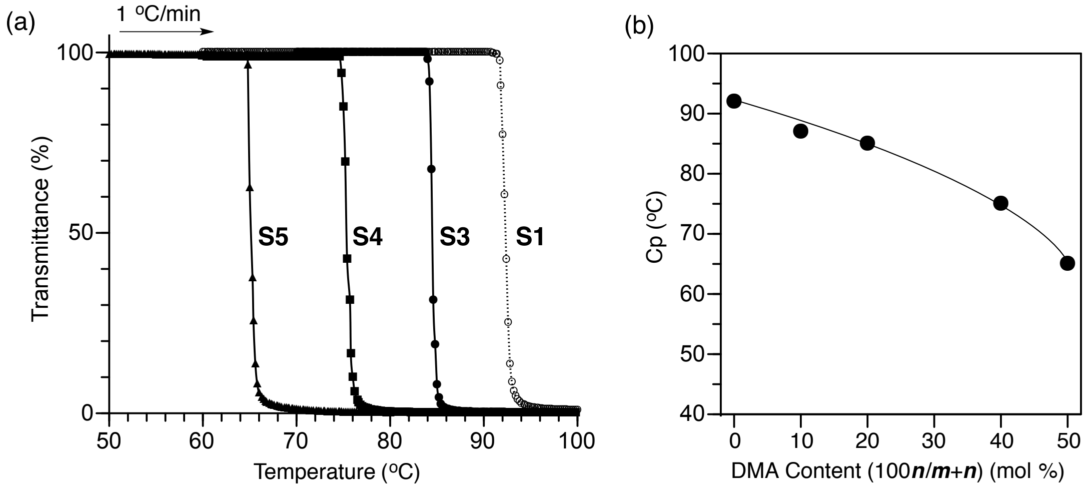


Figure 6. (a) Transmittance of aqueous solutions of **S1**, **S3**-**S5** as a function of temperature (heating rate: 1 °C/min) and (b) Cp of the aqueous solutions of **S1**-**S5**: [polymer] = 4 mg/mL.

Conclusion

In conclusion, amphiphilic three-armed star random copolymers were successfully synthesized by ruthenium-catalyzed living radical polymerization of hydrophilic PEGMA and hydrophobic DMA with a trifunctional initiator. The star copolymers with 10 – 50 mol% DMA were homogeneously soluble and unimolecularly folded with intramolecular hydrophobic interaction in water to be compact unimer micelles carrying hydrophobic domains. Owing to the branched structure, three-armed star copolymers exhibit folding properties distinct from linear counterparts in water: 1) folding star copolymers have hydrodynamic radius larger than folding linear copolymers; 2) a 40 mol% DMA star copolymer leads to the most compact structure as well as a corresponding linear copolymer; 3) a 50 mol% DMA star copolymer undergoes unimolecular folding, whereas a linear counterpart induces bimolecular association. Additionally, the star polymers showed LCST-type phase separation in water; the cloud points decreased from 92 to 65 °C with increasing DMA content (0 - 50 mol%). Thus, amphiphilic star random copolymers are a new class of thermosensitive, self-folding polymers in water to be applicable as unimolecular functional compartments.

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