Compartmentalization Technologies via Self-Assembly and Cross-Linking of Amphiphilic Random Block Copolymers in Water

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Supporting Information

ABSTRACT: Orthogonal self-assembly and intramolecular cross-linking of amphiphilic random block copolymers in water afforded an approach to tailor-make well-defined compartments and domains in single polymer chains and nanoaggregates. For a double compartment single-chain polymer, an amphiphilic random block copolymer bearing hydrophilic poly(ethylene glycol) (PEG) and hydrophobic dodecyl, benzyl, and olefin pendants was synthesized by living radical polymerization (LRP) and postfunctionalization; the dodecyl and benzyl units were incorporated into the different block segments, whereas PEG pendants were statistically attached along a chain. The copolymer self-folded via the orthogonal self-assembly of hydrophobic dodecyl and benzyl pendants in water, followed by intramolecular cross-linking, to form a single-chain polymer carrying double yet distinct hydrophobic nano-compartments. A single-chain cross-linked polymer with a chlorine terminal served as a globular macroinitiator for LRP to provide an amphiphilic tadpole macromolecule comprising a hydrophilic nanoparticle and a hydrophobic polymer tail; the tadpole thus self-assembled into multi-compartment aggregates in water.

Self-assembly and/or cross-linking of amphiphilic copolymers in water is fundamental technologies to create compartmentalized materials (e.g., micelles, vesicles, and nanogels).1–7 Among them, constructing microdomains within nano-aggregates, i.e., multicompartimentalization, allows one to introduce different functional units into discrete but closely located nanospaces; this attracts attention to create selective, unique, and bioinspired functions.2–4 Such multicompartimentalized materials have been prepared by intermolecular self-assembly of multiple block copolymers whose block segments carry different properties, solubility, and miscibility (e.g., ABC triblock copolymers). In natural biopolymers, proteins and enzymes consist of amphiphilic polymers with inherent primary structure, where hydrophilic and hydrophobic monomer units are sequenced along a chain. As a result, they site-specifically self-fold in water via the orthogonal self-assembly of pendant functional groups, according to the programmed monomer sequences, to form single-chain compartmentalized structures that carry precision cavities and/or discrete domains.

For compartmentalized synthetic single-chain polymers, self-folding of random copolymers is recognized as a promising strategy.7–14 Typically, amphiphilic random copolymers of hydrophilic poly(ethylene glycol) methyl ether methacrylate (PEGMA; Mn = 475) and hydrophobic dodecyl methacrylate (DMA) autonomously self-fold into unimer micelles with dynamic, hydrophobic compartments in water (∼10 nm).12a Uniquely, the copolymers have threshold chain length (degree of polymerization: DPth) suitable for single-chain folding, dependent on the hydrophobic DMA composition (e.g., 40 mol % DMA; DPth = ~200). Thus, PEGMA-based amphiphilic random block copolymers, where hydrophobic units with different miscibility are respectively incorporated into the block segments of DPth, potentially induce double self-folding via selective self-assembly of the hydrophobic pendants in water to give single-chain double compartment polymers.

We herein report the self-assembly and cross-linking of amphiphilic random block copolymers in water to design precision multicompartments in single polymer chains or nanoaggregates (Scheme 1). Typically, distinct but connected domains are successfully built in single polymer chains via the orthogonal intramolecular self-assembly of the hydrophobic

Scheme 1. Nanocompartment Polymers Created via the Self-Folding and Cross-Linking of Amphiphilic Random/Block Copolymers in Water

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pendants (A, B) of a A/C−B/C random block copolymer (C: hydrophilic). This is a strategy to tailor-make small domains (<10 nm) in macromolecules, distinct from multicompartiment micelles via the intermolecular self-assembly of ABC triblock copolymers.

For this, we designed an amphiphilic random block copolymer (P1-O) bearing different hydrophobic pendants (A: dodecyl; −C12H25, B: benzyl: −CH2Ph) in respective block segments, whereas hydrophilic poly(ethylene glycol) methyl ether (PEG) pendants (C) were statistically distributed along a chain. P1-O induced double self-folding via the “orthogonal” self-assembly of the hydrophobic pendants (A, B) in water, followed by intramolecular cross-linking,12b to provide a single-chain cross-linked polymer (S1) with distinct hydrophobic dodecyl (A) and benzyl (B) cores both of which are covered by PEG. It should be noted A/B double domains are constructed via the phase separation between simple hydrophobic pendants. In contrast, an amphiphilic random copolymer statistically bearing both dodecyl and benzyl pendants without segmentation (P3-O) gave a single-chain cross-linked polymer with a dodecyl and benzyl-mixed core. Single-chain cross-linked polymers obtained with ruthenium-catalyzed living radical polymerization (LRP) carry a chlorine terminal originating from the precursors. Thus, the cross-linked polymers served as “globular” macroinitiators for LRP, giving an amphiphilic tadpole macromolecule consisting of a hydrophilic nanoparticle and a hydrophobic linear polymer (P4). The tadpole intermolecularly self-assembled into multi-compartment aggregates in water. Thus, amphiphilic random block copolymers opened ways to design compartmentalized materials structurally close to proteins.

Two synthetic approaches were investigated for single-chain A/B-double compartment polymers (S1, S2): (1) direct intramolecular cross-linking of double self-folded amphiphilic random block copolymers in water and (2) iterative synthesis via random copolymerization with a single-chain cross-linked macroinitiator, self-folding, and intramolecular cross-linking (Schemes 1 and 2). To achieve orthogonal self-folding of the respective block segments, the content of hydrophobic monomers including olefin pendants were set as 40 mol % (including 20 mol % hydrophobic olefin), whereas degree of polymerization on respective block segments was targeted to 200.

For the first block segment including dodecyl units, PEGMA, DMA, and 2-hydroxyethyl methacrylate (HEMA) were copolymerized with a ruthenium catalyst [RuCp2Cl2(PPh3)2]/4-(dimethylamino)-1-butanol and a chloride initiator (ethyl 2-chloro-2-phenylacetate) in ethanol at 40 °C to give a well-controlled PEGMA/DMA/HEMA random copolymer with narrow molecular weight distribution [Conv. >80%, 31 h, 52,000, Mn/Mn = 1.14, by size exclusion chromatography (SEC) in DMF with PMMA calibration, Figure S1, Table S1]. The degree of polymerization was determined by 1H nuclear magnetic resonance (NMR) spectroscopy: PEGMA/DMA/HEMA (I/m/p) = 99/33/33; total DP = 165 (Figure S1). Subsequently, block copolymerization of PEGMA, benzyl methacrylate (BzMA), and HEMA was conducted with the random copolymer as a macroinitiator to provide an amphiphilic random block copolymer (P1: Mn = 115,000, Mn/Mn = 1.13 by SEC, Table S1, Figure S2). Conformed by 1H NMR (Figure S3), P1 consisted of a random A/C segment including DMA (I/m/p = 99/33/33) and a random B/C segment including BzMA (a/n/q = 115/35/35). Then, P1 was treated with 2-isocyanatoethyl methacrylate (IEMA) and dibutyltin dilaurate in CH2Cl2 at 25 °C ([OH][IEMA] = 1/10) to quantitatively give an olefin-bearing random block copolymer (P1-O: Mn (NMR) = 137,000, Table S2, Figure S3).

The self-folding of P1-O by hydrophobic effect in water was confirmed by SEC and 1H NMR (Figure 1a and S4). Analyzed by SEC with poly(ethylene oxide) calibration, SEC peak-top molecular weight of P1-O in water was smaller than that in DMF (Mpk = 33,000 in water, 54,500 in DMF). 1H NMR measurement of P1-O in D2O further revealed that the proton signals of hydrophobic dodecyl, benzyl, and olefin pendants and methacrylate backbones were broad (Figure S4). The intramolecular cross-linking of P1-O was carried out with 2,2′-azidodisobutirnitrile (AIBN) in water under UV irradiation (λ = 375 nm) at 25 °C: [polymer]/[AIBN] = 10/0.6 mg/mL. The reaction proceeded without macroscopic gelation up to 94% olefin conversion in 82 h to give a cross-linked S1 (Figure S4). S1 had smaller peak-top molecular weight in DMF than P1-O [Mpk =
74 300 (S1), 122 000 (P1-O), $M_n/M_w$ (S1) = 1.11 by PMMA calibration, Figure 1b, whereas absolute weight-average molecular weight of S1 by SEC-MALLS almost agreed with that of P1-O [$M_{\text{w}} = 219 000$ (S1), 196 000 (P1-O)]. These results support that S1 has self-folded and compact structure even in organic solvents.

Another synthetic approach involves use of a single-chain cross-linked polymer as a macroinitiator (Scheme 2b). The chlorine-capped macroinitiator ($M_n = 38 000, M_n/M_w$ = 1.17) was prepared by intramolecular cross-linking of an olefin-bearing PEGMA/DMA/HEMA random copolymer in water (Figure S5). Then, ruthenium-catalyzed living radical copolymerization of PEGMA, BzMA, and HEMA was conducted with the macroinitiator, giving a block copolymer consisting of a globular nanoparticle and linear amphiphilic random copolymer bearing benzyl units (P2). An olefin-bearing P2-O, obtained via the postfunctionalization of P2 with IEMA, was cross-linked with AIBN in water to provide a single-chain cross-linked S2 ($M_n = 240 000, M_n/M_w$ = 1.20, Figure S6). The structure was similarly confirmed by SEC-MALLS (Tables S2 and S3).

A cross-linked polymer bearing both dodecyl and benzyl units in a single core (S3) was also designed, as a controlled sample against double compartment polymers (S1, S2). S3 was obtained from the intramolecular cross-linking of a self-folded P3-O in water [$S3: M_n = 98 900$ by SEC-MALLS, $l/m/n/p = 95/15/16/31$, Figure S6]; P3-O was similarly synthesized by LRP and postfunctionalization (S1).

The double compartment structures of S1 and S2 were evaluated by small-angle X-ray scattering (SAXS) and transmission electron microscopy (TEM) (Figure 2 and S7). S1 and S2 were analyzed by SAXS in DMF or water ([polymer] = 10 mg/mL), compared with a single-chain cross-linked polymer with a DMA core (macroinitiator for P2-O). All of the cross-linked polymers had globular structures in the solvents, whereas the pair-distance distribution functions $P(r)$ were dependent on the monomer sequences. S1 showed a peak with a shoulder at large size region ($r = 4.2$, 8.6 nm), whereas the single-chain cross-linked polymer only exhibited a unimodal peak without such a shoulder ($r = 3.6$ nm). S2 obtained via an iterative synthetic approach also had almost identical $P(r)$ to S1 in both DMF and water (Figure S7). These results strongly support that, independent of synthetic pathways, both S1 and S2 had double domains comprising dodecyl or benzyl units in single polymer chains. Thus, an amphiphilic random block copolymer bearing dodecyl and benzyl pendant actually self-folded in water via the orthogonal phase separation of dodecyl and benzyl pendant, resulting in a single-chain double compartment polymer. Additionally, the double compartment structure of S1 was observed by TEM (Figure 2c). Such double domains were not observed for S3 with a dodecyl and benzyl-mixed core (Figure 2d).

To evaluate the local distance of pendants, S1, S2, and S3 were analyzed by $^1$H nuclear Overhauser effect (NOE) difference spectroscopy in CDCl$_3$ and D$_2$O at 30 °C (Figures 3, S8, and S9).

These polymers showed the aromatic protons of BzMA units at 7.4–7.3 ppm without overlapping with the other protons (Figure 3: S3 in CDCl$_3$). In general, the intensity of $^1$H NOE signals depends on the distance between the proton and an irradiated proton (NOE/No) of poly(ethylene glycol) methyl ether ($d$: $-CH_2(OCH_2CH_2)_n$: $r$, $e$: $-OCH_3$), dodecyl (h), and cross-linked (w) units in S1, S2, and S3 in CDCl$_3$ and D$_2$O at 30 °C.

**Figure 2.** (a, b) Pair-distance distribution function $P(r)$ of the SAXS profiles of (a) S1 and (b) a single-chain cross-linked polymer with a DMA core in DMF at 25 °C. (c, d) TEM images of (c) S1 and (d) S3 cast from the aqueous solutions ([S1 or S3] = 10 mg/mL) to carbon-coated grid. TEM samples were stained with 1% OsO$_4$ aqueous solution. Right top: magnified images of the circled parts.

**Figure 3.** (a) $^1$H NMR and (b) $^1$H NOE difference spectroscopies of S3 in CDCl$_3$ at 30 °C. (c, d) $^1$H NOE signal intensity for one proton (NOE/No) depends on the distance between the proton and an irradiated proton (NOE/No) of poly(ethylene glycol) methyl ether ($d$: $-CH_2(OCH_2CH_2)_n$: $r$, $e$: $-OCH_3$), dodecyl (h), and cross-linked (w) units in S1, S2, and S3 in CDCl$_3$ and D$_2$O at 30 °C.
NOE signal intensity for the dodecyl and PEG pendants of S1 was virtually identical to that of S2. Non-cross-linked P3-O hardly showed NOE signals of dodecyl and PEG chains in CDCl$_3$ (Figure S9). These results importantly indicate the following facts: (1) Local distance between benzyl pendant and dodecyl or PEG pendants within a single polymer turns close via intramolecular cross-linking. (2) Even after intramolecular cross-linking, dodecyl pendants in S1 and S2 are yet spatially separated from benzyl counterparts, in contrast to the close location of dodecyl and benzyl pendants in S3. (3) S1 has almost the same inner domain structure as S2 in spite of different synthetic processes.

In D$_2$O, similar tendency was observed for the signal intensity of the dodecyl pendants (Figure 3d). The absolute NOE signal intensity in D$_2$O was much larger than that in CDCl$_3$. This is owing to the shrunk structures of S1, S2, and S3 in water by hydrophobic effect; the compact structures in water were confirmed by SEC and DLS [Figure S10, e.g., S1: $R_g$ = 7.5 nm (H$_2$O), 8.9 nm (DMF)]. However, S1 and S2 still maintain remote position between dodecyl and benzyl pendants, compared with S3. In all of the samples, the absolute signal intensity for PEG chains (d, e) increased in D$_2$O whereas the intensity of the tip methyl group (e) close to that of the middle ethylene oxide segments (d). This result suggests two facts: (1) Inner PEG pendants, partially included within the hydrophobic spaces, become close to hydrophobic benzyl pendants by shrunk structure in water. (2) Outer PEG chains effectively cover the hydrophobic cores in water and all of the segments are uniformly close to inner benzyl units.

In contrast to S1–S3, an amphiphilic tadpole comprising a hydrophilic nanoparticle and a hydrophobic poly(BzMA) (P4, $M_n$ = 46 700, $M_w/M_n = 1.28$, DP of BzMA = 53) formed large aggregates in water ($R_g$ = 53 nm by DLS, Figure 4a). The TEM image showed unique spherical aggregates via self-assembly of multiple P4 (Figure 4b). The aggregates contain multiple S1 bearing a hydrophobic core as distinct compartments. Thus, living radical polymerization with single-chain cross-linked polymers as macroinitiators is effective to design multi-compartmentalized materials.

In conclusion, amphiphilic random block copolymers with distinct hydrophobic pendants successfully provided single-chain cross-linked polymers bearing double domains. The compartmentalization is effectively achieved via the phase separation of the hydrophobic pendants within self-folded polymers in water. This is indeed an innovative technique to create nanospaces within single polymer chains. LRP with a chloride-capped single-chain polymer nanoparticle as a macroinitiator was further effective for the design of multicompartmentalized materials. The precision compartmentalization technologies with amphiphilic random block copolymers, developed herein, would open new vistas in polymer, organic, bio, and material chemistries and related research fields. Distinct but closely connected nano-compartment materials would be useful to create biomimetic, cooperative functions, tandem catalysis, among many others.

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