Worms, Beehives and Woodlice – Evolution of Multicompartment Micelles from New ABC Miktoarm-Star-Terpolymers

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INTRODUCTION

Both polymer architecture and composition play a crucial role for structure formation in block copolymer assemblies. Compared to AB diblock copolymers, the micellar structures observed in the solution state for ABC triblock terpolymers are far more complex[1, 2]. This can be further pronounced *via* the introduction of a central linking point for all three compartments, as shown for ABC miktoarm star terpolymers[3, 4].

In this contribution we investigate the aqueous solution self-assembly of amphiphilic ABC miktoarm star terpolymers. The material, µ-BVT, consists of polybutadiene (PB), poly(2-vinylpyridine) (P2VP) and poly(*tert*-butyl methacrylate) (P*t*BMA). It was synthesized *via* a combination of sequential living anionic polymerization, controlled radical methods (ATRP), and click chemistry. P2VP is pH-sensitive and can be quaternized to obtain a salt-sensitive polycation.

EXPERIMENTAL

Materials. Butadiene (Messer-Griesheim) was passed through columns filled with molecular sieves (4 Å) and basic aluminium oxide and stored over dibutylmagnesium. 2-Vinylpyridine (Aldrich) was degassed, stirred with triethylaluminium and condensed on a high vacuum line. THF (Sigma-Aldrich) was distilled from CaH_2 and Na/K alloy. *Tert*-butyl methacrylate (Sigma-Aldrich) was filtered over basic aluminium oxide. 1-(4-(*tert*-butyl-dimethylsilyl)ethynylphenyl)-1-phenyl-ethylene was synthesized from 1-(4-bromophenyl)-1-phenylethylene with a procedure adopted from literature[5]. For the preparation of the aqueous polymer solutions distilled desalinated water was used. All other chemicals were of analytical grade and used as received. The dialysis membrane was purchased from Roth and had a MWCO of 1000 g/mol.

Polymerization. First, butadiene was initiated with *sec*-butyllithium at -70 °C, then warmed to -20 °C and polymerized for 3 h. After lowering the temperature to -50 °C, the living polybutadiene chains were end-capped with 1-(4-(*tert*-butyl-dimethylsilyl)ethynylphenyl)-1-phenylethylene (1.5 eq.) for 2 h. Afterwards, 2-vinylpyridine was injected into the reactor *via* syringe at -70 °C and polymerized for 5 minutes. Finally the polymerization was terminated through the addition of 2 ml degassed isopropanol and the polymer was precipitated in water to yield $B_{109}V_{81}$ (as determined by a combination of MALDI-ToF MS and ^{1}H-NMR spectroscopy; subscripts denote the number of repeating units). The polymer exhibits a molecular weight of 14.700 g/mol and a monomodal narrow distribution with a PDI of 1.03 as determined by SEC with PS standards. For the subsequent deprotection of the alkyne-function the polymer was dissolved in THF (~0.1 g/ml) and degassed for 30 minutes. Then 10 equivalents tetrabutylammonium fluoride (1M solution in THF) corresponding to the alkyne functions were added at 0 °C and the mixture was stirred at this temperature for 2 h. After warming to room temperature, the solution was stirred over night. The polymer was precipitated in water, dialyzed against THF and then freeze-dried from dioxane.

In a second step, *tert*-butyl methacrylate was polymerized *via* ATRP following standard procedures[6]. To obtain a readily azido-functionalized P*t*BMA, bromo-isobutyric acid 3-azidopropylester was chosen as the initiator. This was synthesized according to literature[7].

The obtained polymer had a M_n of 7500 g/mol and a PDI of 1.13, as determined by SEC using a P*t*BMA calibration.

Finally, the alkyne-functionalized PB-*b*-P2VP diblock copolymer and the azido-P*t*BMA homopolymer were ligated by Huisgen 1,3-dipolar cycloaddition[8]. A mixture of the diblock copolymer and 1.1 eq. of the homopolymer were dissolved in THF at a concentration of ~20 mg/ml and degassed for 10 minutes. Then CuBr (1 eq.) was added and the solution degassed for further 15 minutes. PMDETA was added (1 eq.) and the reaction was followed by SEC. After 2 days, the resulting miktoarm star terpolymer was purified by passing the solution through a small column with basic alumina to remove copper.
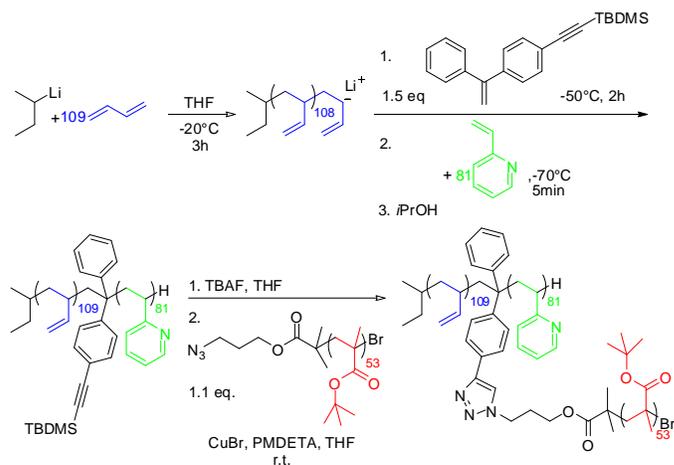
Figure 1. Reaction pathway for synthesis of µ-BVT.

Sample Preparation. For the preparation of aqueous solutions, the star terpolymer was dissolved in dioxane at a concentration of 2 mg/ml. After addition of a 10-fold excess of methyl iodide, the solution was allowed to stir for 3 days at room temperature. Excess quaternization agent was removed by dialysis against dioxane. Finally, the solution was diluted to 1 mg/ml and the solvent was subsequently exchanged against water.

Instrumentation. TEM images were taken with a Zeiss CEM 902 or 922 OMEGA electron microscope operated at 80 kV or 200 kV, respectively. Both machines are equipped with an in-column energy filter. For cryo-TEM studies, a drop (~2 µL) of the aqueous micellar solution (c ~ 0.4 – 0.7 mg/ml) was placed on a lacey carbon-coated copper TEM-grid (200 mesh, Science Services), where most of the liquid was removed with blotting paper, leaving a thin film stretched over the grid holes. The specimens were shock vitrified by rapid immersion into liquid ethane in a temperature-controlled freezing unit (Zeiss Cryobox, Zeiss NTS GmbH) and cooled to approximately 90 K. The temperature was monitored and kept constant in the chamber during all of the preparation steps. After freezing the specimens, they were inserted into a cryo-transfer holder (CT3500, Gatan) and transferred to the Zeiss EM922 OMEGA EFTEM instrument. Examinations were carried out at temperatures around 90 K. For investigation of the particle films the freeze dried polymer was embedded into a resin (EpoTek 301). 50 nm thin cuts were prepared with a Leica EM UC7 microtome equipped with a diamond knife and deposited onto TEM grids (copper, 200 mesh).

RESULTS AND DISCUSSION

With this modular approach we synthesized a well defined ABC miktoarm star terpolymer $B_{109}V_{81}T_{53}^{22.2}$ through a combination of living anionic polymerization, ATRP, and click chemistry (the indices denote degrees of polymerization and the superscript the molecular weight in kDa). The core molecule of this approach is the newly synthesized DPE derivative 1-(4-(*tert*-butyl-dimethylsilyl)ethynylphenyl)-1-phenyl-ethylene. The evolution of the star terpolymer could nicely be followed by taking samples during the click reaction and investigation *via* SEC. The position of the diblock shifted simultaneously with the consumption of PtBMA homopolymer. The star terpolymer exhibits a narrow molecu-

lar weight distribution with a PDI of 1.07. Nevertheless, a small shoulder at higher molecular weights could be detected. During the anionic polymerization, an excess of the functionalized DPE-derivative has been used. This might have also been incorporated into the second block, P2VP, to a certain extent. Therefore, the degree of alkyne functionalization as determined by UV was around 116%. Thus, the resulting star terpolymer might contain minor impurities with more than one chain of P*t*BMA attached.

To study the self-assembly behavior of this star terpolymer in water, the P2VP block was quaternized using methyl iodide thus transforming it into a strong polyelectrolyte. Dialysis of this solution against water yielded an opaque solution. Cryo-TEM measurements showed complex aggregates with an internal lamellar fine structure (Figure 2A). The shape of the aggregates is mostly ellipsoidal, but also some spheres were found. The typical size of the aggregates is 200-500 nm and the thickness of the internal lamellae is around 5 and 8 nm respectively as observed from cryo-TEM. Typically, the internal fine structure is only visible when the objects are aligned longitudinal to the plane of the vitrified film as verified by cryo-TEM tilt image series.
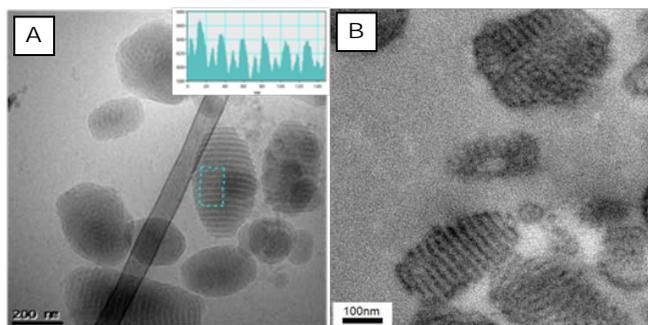
Figure 2. Cryo-TEM micrograph of micellar aggregates obtained from μ-BVqT after dialysis to water, 0.6 mg/ml (A) and TEM image of a 50 nm thick cut of the freeze dried and resin-embedded sample, OsO_4 stained (B).

Further insight into the aggregate morphology was achieved by a different way of sample preparation. The solution was freeze dried and the powder embedded within a resin. Afterwards, 50 nm thick cuts were prepared with a microtome and the corresponding TEM micrograph is shown in Figure 2B. Again, a lamellar structure can be observed, as shown for both the unstained images – where the contrast originates from the quaternized P2VP phase – and the samples after staining with OsO_4. This leads to the assumption that we have alternating lamella of P2VP and a mixed phase of P*t*BMA/PB ($\chi N \sim 8.1$). These structures are similar to the phase-separated lamellar droplets observed by Pochan et al.[9] An important difference is the fact that our assemblies exist as colloidally stable particles. This is supported by theoretical simulations from the group of Lin[10], which predict the presence of barrel shaped multicore micelles with two disklike compartments from terpolymers with a solvophilic middle block. Here, however, only one hydrophilic compartment is present (P2VPq) and we observe that this block forms the other lamella. This might be a result of two driving forces. First, incomplete quaternization can reduce the hydrophilicity of P2VPq and lead to some aggregation. Second, P2VPq can form charge transfer complexes with iodine, which have been shown to be of hydrophobic nature[11].
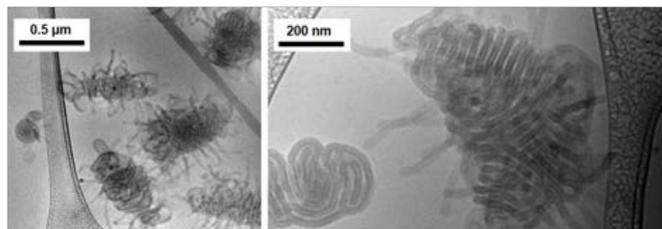
Figure 3. Cryo-TEM micrographs of intermediate wormlike structures obtained from μ-BVqT after dialysis to water, 0.4 mg/ml.

Recently, we were able to observe the intermediate formation of wormlike aggregates *via* the same sample preparation pathway (Figure 3). Tentatively, we propose that the core of these wormlike micelles consists of a mixed PB/PtBMA phase and the corona is formed by P2VPq. The wormlike micelles tend to wrap up into superstructures with time. Also here, we think that this is caused by the P2VPq corona. We further assume that these wormlike structures are pre-stages of the bicontinuous multicompartment micelles (woodlice) observed in Figure 2.

Although the exact formation pathway is not clear up to now, complex and compartmentalized self assembled structures could be prepared *via* the solution self-assembly of μ-BVqT star terpolymers. The internal aggregate structure seems to change over time, as intermediate wormlike structures were also identified. We are currently investigating the influence of these parameters in more detail.

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