Consequences of Dispersity on the Self-Assembly of ABA-Type Amphiphilic Block Co-Oligomers

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Supporting Information

ABSTRACT: Intriguingly, little is known about the impact of dispersity on the crystallization driven self-assembly (CDSA) of amphiphilic block copolymers in aqueous media. Here, we investigate the influence of dispersity on the CDSA of ABA-type amphiphilic block co-oligomers (ABCOs). Two pairs of ABCOs are synthesized comprising discrete (Φ = 1.00) or disperse (Φ = 1.20) isotactic l-lactic acid 16-mers as the semicrystalline hydrophobic block and either oligo(ethylene glycol) methyl ether (MeOoEG) or oligo(tetraethylene glycol succinate) (oTEGSac) as the discrete hydrophilic block. Self-assembly studies in water with 10% THF reveal uniform nanofibers/2D sheets for the discrete oligomers, but such structural regularity is largely compromised in the disperse oligomers. The results are corroborated by sharp melting transitions in both solution and bulk for the discrete ABCOs, unlike their disperse analogues that show a lack of crystallization. Interestingly, the discrete MeOoEG-LLA oligomer reveals crystallization driven gelation, illustrating the contrasting differences between the discrete oligomers and their disperse counterparts.

Self-assembly of block copolymers is a topic of considerable interest in polymer science due to the tremendous potential for applications in both biomedical engineering and nanolithography.1,2 Although abiotic polymers synthesized via controlled polymerization techniques show great diversity in their structures and functions, they still suffer from significant molar mass distribution and cannot match up with the architectural purity, precision, and complexity displayed by biomacromolecules.3,4 In contrast, most biopolymers such as DNA, RNA, and polypeptides are monodisperse and sequence-specific, which is critical to their overall three-dimensional organization and thus their properties and functions. In the recent past, polymer research started focusing on both discrete (Φ < 1.000000)3−10 and sequence-specific polymers11−15 in an attempt to mimic these aspects of biomacromolecules.

Amphiphilic block copolymers (ABCPs) have been a topic of long-standing interest in biomedical research for their ability to form nanocarriers such as micelles, vesicles, nanorods, and other tailored shapes for drug delivery.2,16,17 Depending on the hydrophilic/hydrophobic block ratio, molecular weight of the polymer, and crystallinity of the hydrophobic core, the morphology and properties of these nanoparticles can be engineered. Surprisingly, the impact of the molar mass distribution (Φ) on nanoparticle formation, shape, structural uniformity, and efficacy for uptake and release of guest molecules has hardly been investigated in amphiphilic block copolymers. Comparative self-assembly studies between ultra-defined discrete ABCPs (Φ = 1.000) and their disperse counterparts could be extremely important in the fundamental understanding of the influence of dispersity on their properties after self-assembly in the aqueous phase. Contrasting differences between the discrete and disperse BCPs have been recently observed in the bulk phase. Our group has reported on the self-assembly of discrete diblock co-oligomers (BCOs) composed of oligolactic acid (oLA) and oligodimethylsiloxane (oDMS) obtained by iterative coupling-deprotection based synthetic strategies.16,19 Whereas the discrete polymer formed well-organized lamellar structures, its disperse counterpart revealed a lower extent of ordering with an increase of the domain spacing and greater stability of the phase-separated structures.19 In a complementary study, the group of Hawker observed similar differences in bulk between semidiscrete and disperse BCOs composed of oligomethyl methacrylate (oMMA) and oDMS.20

Intrigued by these results, we here aim to investigate the effect of dispersity in the aqueous phase, where the high mobility of the flexible polymers chains in solution presents an additional challenge. With this objective, we synthesized two pairs of ABA-type amphiphilic block co-oligomers (ABCOs) composed of discrete (Φ = 1.000) or disperse (Φ = 1.2)
isotactic oligo(L-lactic acid) (LLA) as the hydrophobic block and either oligoethylene glycol methyl ether (MeOoEG) or oligo(tetraethylene glycol succinate) (oTEGSuc) as the discrete hydrophilic block (Figure 1A). The rationale behind choosing these blocks is as follows: the self-assembly of polylactic acid-b-polyethylene glycol (PLA-b-PEG) has been explicitly studied in the context of drug delivery and regenerative medicine because these polymers are known to be biocompatible. oTEGSuc block was chosen as a biodegradable substitute to PEG, to generate fully biodegradable ABCOs. Discrete chains of oTEGSuc can be synthesized following the iterative synthetic approach presented in Scheme 1. The L-lactic acid 16-mer was selected as the hydrophobic core either as a discrete 16-mer of exact molecular weight (LLA16) or as a disperse one (LLA∼16) with Đ=1.20. Discrete LLA 16 is semicrystalline and forms ordered lamellae in the bulk. In the context of drug delivery, the crystallization driven self-assembly (CDSA) of amphiphilic block copolymers with a crystallizable hydrophobic core has been applied to fabricate nonspherical nanostructures in solution. However, as of yet there is no study on understanding the consequence of dispersity on block crystallinity in solution, which is investigated in the present work.

For the synthesis of discrete oTEGSuc, we followed a modified iterative coupling-deprotection route (Scheme 1a) recently reported by our group for monodisperse lactic acid oligomers. Succinic acid monobenzyl ester (1) was coupled with mono tert-butyl dimethylsilyl (TBDMS) ether protected tetraethylene glycol (TEG; 2) to obtain double-protected monomer (TEGSuc)1. Orthogonal deprotection of the TBDMS ether and the benzyl ester resulted in free hydroxyl and carboxylic acid containing 3 and 4, respectively. Carbodiimide-promoted coupling between the two afforded double-protected dimer (TEGSuc)2. By repetition of the deprotection and coupling steps, tetramer (TEGSuc)n was obtained. A stack plot of the MALDI-ToF spectra of the double-protected (TEGSuc)n oligomers from monomer to tetramer is shown in Scheme 1b. Single peaks corresponding to the mass of the desired species complexed with sodium ion and potassium ion indicate that precisely defined block lengths were obtained.

The synthesis of discrete telechelic LLA16 with free carboxylic acid moieties (HOOC-SA-LLA16-COOH, Scheme 1c) is based on the synthetic strategy previously reported by Hawker and co-workers. Disperse telechelic LLA16 was synthesized by ring-opening polymerization. The dispersity of LLA16 (Đ=1.2) was determined using size exclusion chromatography. Full synthetic details on the preparation of the hydrophobic blocks can be found in the Supporting Information. Subsequent ligation of the acid functionalized LLA block with two equivalents of hydroxyl functionalized discrete (TEGSuc)2-OH, or commercially available discrete MeOoEG11-OH resulted in the target ABA-type ABCOs P1 and P2 (Scheme 1c). The discrete ABCOs are designated as Px discrete and their disperse analogues are referred to as Px disperse. All the compounds were purified by automated column chromatography and fully analyzed by 1H NMR, 13C NMR, and matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry (Figures S1−S9). Despite similar degrees of polymerization based on 1H NMR, the MALDI-ToF spectra of the discrete and the disperse oligomers reveal a wide distribution in the chain length for disperse samples compared to a single peak for the discrete ones (Figure 1).

The thermal behavior and degree of ordering in the bulk of the discrete and disperse ABCOs was investigated using
The slope of disperse analogues (P2) this ABCO self-assembles into cylindrical micelles, whereas P2TEGSuc as compared to MeOoEG, water/THF mixtures with 10% THF. To prepare the in pure water. As a result, all the studies were performed in all, the differences in bulk properties of discrete and disperse ABCOs are in good correspondence to our previous results published on oLLA-oDMS block-co-oligomers, where we observed a significant loss in long-range order when dispersity was introduced into the oLLA block.

Subsequently, the self-assembly of the ABCOs was studied in aqueous media. Due to the more hydrophobic nature of TEGSuc as compared to MeOoEG, P2 could not be dissolved in pure water. As a result, all the studies were performed in water/THF mixtures with 10% THF. To prepare the solutions, each compound was dissolved in THF, and water was added dropwise to reach a 1:9 THF/water binary mixture at 1–5 mg ABCO per mL. The formation of the nanoparticles was studied with light and X-ray scattering (LS and SAXS), micro-DSC, and microscopy (cryoTEM and total internal reflection fluorescence (TIRF) microscopy). Diffusion coefficients were obtained from multiangle light scattering by fitting the decay rate (I) versus the scattering vector (q) plot (Figure S13). Using the Stoke–Einstein equation, the hydrodynamic radius (R_h) of the particles was calculated. After self-assembly in water, the R_h was found to be larger for the discrete variants (R_h = 74 nm for P1discrete and 125 nm for P2discrete) than for the disperse analogues (R_h = 42 nm for P1dispersive and 90 nm for P2dispersive). When comparing the LS data of P1 with P2, it appears that larger particles are for the P2 pairs. However, fitting the decay rate versus the scattering vector reveals some anisotropy in the structures, indicating that the particles are not spherical, and thus, the Stoke–Einstein equation does not apply. To get an indication of the shape of the particles formed, the scattering intensity (I) was plotted against q (Figure S14). The slope of −1 for both P1discrete and P1dispersive indicates that this ABCO self-assembles into cylindrical micelles, whereas P2discrete and P2dispersive self-assemble into vesicles or flat bilayers (slope of −2). The morphology of an ABCP is largely dependent on the hydrophobic/hydrophilic block ratio. For an invariant ratio, MeOoEG was replaced with a TEGSuc block of comparable molar mass. Possibly, the more hydrophobic nature of the TEGSuc block changes this balance, leading to the formation of vesicles or bilayers.

To substantiate the formation of cylindrical micelles by P1 in solution, SAXS measurements were performed. The SAXS profile obtained of P1discrete was best fitted with a flexible cylinder model (Figures 2a and S15a). The radius of 3.2 nm, the Kuhn length of 103 nm and the overall length of 1038 nm agrees well with the cryoTEM observations (Figure 3b), which confirms the formation of elongated thin fibers of consistent width. In contrast, the cryoTEM image (Figure 3a) of the P1dispersive reveals the coexistence of two populations. Next to the elongated thin fibers, bundles of shorter but much wider fibers are present. This bundling effect might be due to the coassembly of LLA blocks of varying lengths. The SAXS profile of P1dispersive could not be fit well, which is likely due to an overlay of the scattering of multiple species present in solution (Figures 2a and S15b). The results above clearly exemplify the pronounced impact of LLA block dispersity on the homogeneity of the self-assembled structures. In addition, the morphologies formed were highly stable over time. CryoTEM images of aged samples for both P1discrete and P1dispersive (Figure S16) retained the same morphologies even after keeping the solutions at room temperature for around 90 days.

Interestingly, the discrete oligomer P1discrete formed a transparent gel at 5 mg mL⁻¹ when the solution was heated and cooled back to room temperature (Figure 2a, inset). The gelation process was found to be reversible and the gel–sol transition (T_gel), as determined visually for multiple cycles, varied between 42 and 48 °C upon heating the sample. This transition is very close to the melting temperature (T_m
41 °C) of discrete LLA16 block measured in the bulk (Figure S10a), as well as the transition temperature of 43 °C measured in solution by micro-DSC (Figure 4, vide infra). Notably, no gelation was observed for P1\textsubscript{disperse} under identical conditions, although gelation in disperse PEG-PLLA-PEG based triblock copolymer is well reported via interdigitation of micelles through PEG chains.\textsuperscript{32} Such discrepancy in the gelation copolymer is well reported via interdigitation of micelles although gelation in disperse PEG-PLLA-PEG based triblock copolymer is indeed connected to the LLA16 crystallization. Further, close matching of the T\textsubscript{m} with the T\textsubscript{gel} values of P1\textsubscript{discrete} substantiates crystallization driven gelation. Interestingly, no phase transitions were observed for the discrete P2\textsubscript{discrete} (Figure S20), suggesting that the change in the hydrophilic block from MeOoEG to TEGSuc might influence the crystallization of the LLA16 core. This corroborates well with the amorphous nature of TEGSuc block as observed from its DSC profile (Figure S11a) in contrast to the semicrystalline PEG chain (Figure S11b). The very slow rate of crystallization of P2\textsubscript{discrete} in the bulk (Figure S10b,c) further supports our interpretation that the PEG chains aid the ordering of the LLA16 block unlike TEGSuc chains for discrete pairs.

No clear transitions were observed for P1\textsubscript{disperse} (Figure 4) or P2\textsubscript{disperse} (Figure S20). Such distinct differences between P1\textsubscript{discrete} and P1\textsubscript{disperse} reveal the negative impact of dispersity on the core crystallinity of the amphiphiles in the solution phase, leading to their varying self-assembly behavior. Possibly, the dispersity in the hydrophobic block does not allow effective packing of the LLA chains of varying length within the core of the nanoparticles in P1\textsubscript{disperse} unlike in P1\textsubscript{discrete}. Although there are multiple reports on crystallization driven self-assembly of block copolymer amphiphiles, this is the first demonstration of the consequence of dispersity on the crystallization mediated self-assembly of oligomeric amphiphiles in aqueous solution.

In summary, we have methodically manifested the effect of dispersity on the assembly behavior of two sets of discrete amphiphilic block co-oligomers by comparing their solution self-assembly behaviors with their disperse counterparts. The finding of this work reveals remarkable differences between the discrete and the disperse ABCOs not just in the bulk but also in the solution phase in terms of crystallinity, gelation, morphology, and homogeneity of the self-assembled structures. We anticipate that further fundamental studies on pharmaceutically relevant PEG-PLLA based block co-oligomers will pave the way for synthesis of tailor-made nanocarriers with more control over their structures, dynamics, and functions as delivery vehicles.

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.8b00168.

Experimental procedures, synthesis, and characterization data for all compounds, bulk characterization, and Figures S1–S20 (PDF).

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