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Amphiphilic star-block copolymers and supramolecular transformation of nanogel-like micelles to nanovesicles†

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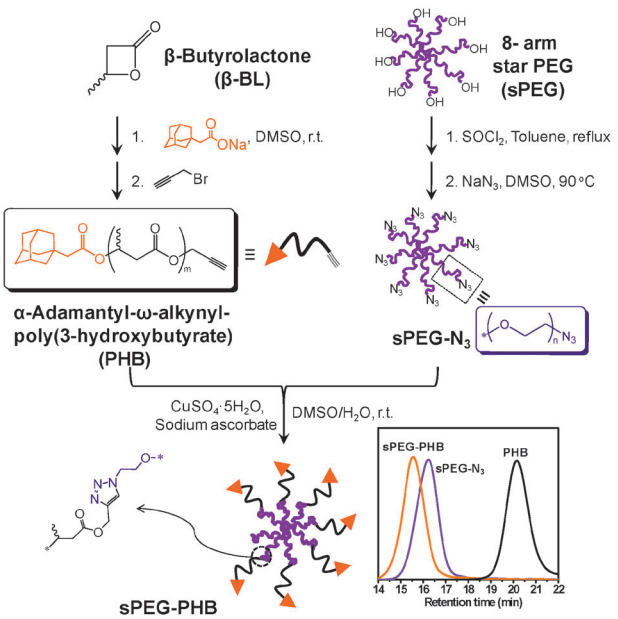
Amphiphilic star-block copolymers based on poly(3-hydroxybutyrate) with adamantyl end-functionalization were synthesized *via* anionic ring-opening polymerization and alkyne–azide "Click Chemistry" coupling. In aqueous medium, the copolymers self-assembled into nanogel-like large compound micelles, and transformed into vesicular nanostructures under the direction of host–guest interaction between the adamantyl end and dimethyl-β-cyclodextrin.

Nanostructures self-assembled from amphiphilic block copolymers have attracted great interests due to their unique properties and immense potential applications for nanofabrication, catalysis, and biomedicine.[1] To date, a variety of nanostructures of various shapes and intricacies have been reported.[2] These advancements were built upon a solid understanding on the segmental interactions of block copolymers with the surrounding environment[3] and the ability to create block copolymers with ever increasing complexity and functionality.[4] However, the relationship between polymer architecture and resultant self-assembled nanostructure is often not straightforward, especially with complex polymer architecture, and susceptible to variations caused by other supramolecular interactions.[5] Further understanding on these issues may open new opportunities into customized amphiphilic block copolymer architectures with well controlled nanostructured morphologies.

With a keen interest in biomedical applications, our attention was drawn to amphiphilic block copolymers containing biopolyester poly(3-hydroxybutyrate) (PHB) due to their inherent biodegradability and biocompatibility.[6] Efforts have been devoted to the synthesis of linear block copolymers *via* chemical modification of bacterial PHB, or chemosynthesis from the β-butyrolactone monomer.[7] The extremely hydrophobic nature of PHB has been exploited to fabricate stable micelles that has great potential in drug delivery application.[8] To our knowledge, reports on star-block copolymers of PHB are rare,[9] not to mention any examples on self-assembly behavior. The compact architecture of a star polymer with multiple polymer chains (arms) connected to a central core generally leads to smaller hydrodynamic size and more end-group functionality than its linear counterpart.[9c] In addition, due to the star architecture, the movement of each polymer chain during a self-assembly process is expected to be restricted and affected by other parts of the star polymer. These may potentially lead to self-assembled structures and properties not accessible by a linear counterpart.

Herein, we present a facile synthesis of a series of PHB-based amphiphilic star-block copolymers with adamantyl ends through a "coupling onto" method (Scheme 1). Interestingly, the copolymers self-assembled into nanogel-like large compound micelles (LCMs) in aqueous medium, while the self-assembly behaviors were modulated by heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) *via* host–guest interaction with the adamantyl ends, leading to the formation of nano-sized vesicles.



Scheme 1 Synthetic route of star PEG–PHB block copolymer (sPEG–PHB) with peripheral adamantyl moiety. On the lower right corner are GPC traces of sPEG–PHB (20–1.6) and its precursors.

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Heterofunctionalized PHB, with telechelic adamantyl moiety and alkynyl functionality, was first synthesized in a one pot fashion by anionic ring opening polymerization (ROP) of racemic β-butyrolactone. The ROP procedure proceeded with excellent control over molecular weight, molecular weight distribution and end-group fidelity (Fig. S1, ESI†). This has allowed facile incorporation of heterofunctionality, through a judicious selection of an anionic initiator and a nucleophilic capping agent, onto a PHB precursor with the desired molecular weight. Adamantaneacetate and propargyl bromide were chosen as the initiator and capping agent, respectively, to produce PHB that is able to participate in CD-binding as well as alkyne–azide conjugation. Two PHB polymer precursors with number-averaged molecular weight (M_n) of 1.60 and 3.07 kDa were synthesized and coupled to 8-arm star poly(ethylene glycol) (sPEG) cores with M_n of 9.50 and 19.9 kDa, respectively, through alkyne–azide coupling. The resultant four sPEG–PHB star-block copolymers are represented by the notation sPEG–PHB (x–y), where x and y denote the approximate M_n of the sPEG core and PHB arm in kDa, respectively (Table 1).

The successful syntheses of the sPEG–PHB block copolymers were first evidenced by shifts in molecular weight in gel permeation chromatography (GPC) analyses to heavier regions (inset of Scheme 1). Their moderately narrow molecular weight distributions imply an efficient and uniform PHB conjugation onto the sPEG cores.

The chemical structure of the obtained sPEG–PHB star-block copolymers was elucidated by ^{1}H NMR. A typical ^{1}H NMR spectrum of sPEG–PHB is shown in Fig. S2 (ESI†), where proton signals belonging to either PHB, PEG, linkage segment or adamantyl end group can be easily identified. In particular, the appearance of the triazole proton at δ 7.8 ppm (and the disappearance of azide functionality shown by FTIR in Fig. S3(a) (ESI†)) further attested to the successful PHB conjugation onto sPEG cores. The intensity ratios between the PHB's methylene proton at δ 2.3–2.8 ppm and PEG's methylene proton at δ 3.4–3.9 ppm were used to estimate PHB contents in the sPEG–PHB star-block copolymers and they show good agreement with the values estimated from thermal gravimetric analyses (TGA) (Table 1 and Fig. S3(b) (ESI†)). These values were further used to calculate the number of PHB arms on each sPEG core and M_n of the final sPEG–PHB star-block copolymers, as listed in Table 1. The M_n values derived from ^{1}H NMR estimation also corroborated well with data obtained from online GPC light scattering (GPC-LS) measurements.

Table 1 Molecular characteristics of sPEG–PHB star-block copolymers

	Molecular weight/kDa			PHB content/wt%		PHB arm number	
sPEG–PHB	M_n[a]	M_w[b]	PDI[c]	NMR[a]	TGA[d]	NMR[a]	TGA[d]
10–3.1	27.6	29.6	1.41	63.8	62.6	6	6
20–3.1	36.8	43.6	1.39	45.7	46.0	6	6
10–1.6	20.6	23.3	1.30	51.4	51.3	7	7
20–1.6	30.3	29.3	1.35	34.1	34.3	7	7

[a] Calculated from ^{1}H NMR spectroscopy. [b] Determined by GPC-LS. [c] Determined by GPC with refractive index detector. [d] Calculated from TGA.

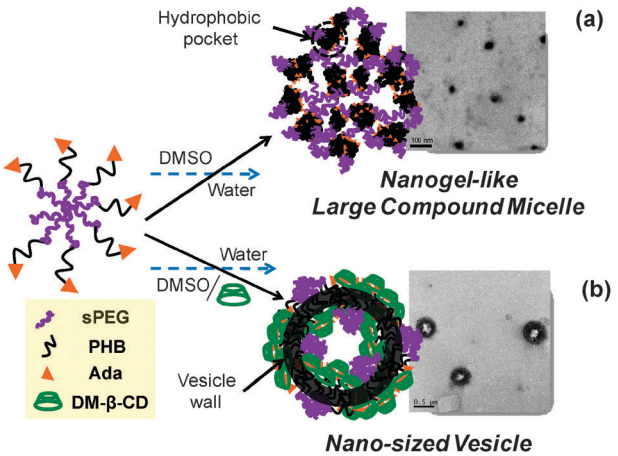

Fig. 1 Schematic representation of: (a) the self-assembly of sPEG–PHB and transmission electron microscopy (TEM) image of sPEG–PHB (10–3.1) micelles (scale bar = 100 nm); (b) the self-assembly of sPEG–PHB/DM-β-CD complexes and TEM of sPEG–PHB(10–3.1)/DM-β-CD aggregate (scale bar = 0.5 μm).

In aqueous solution, all of the synthesized sPEG–PHB star-block copolymers self-assembled into micelles (Fig. 1(a) and Fig. S4 (ESI†)). Hydrodynamic radii (R_h) of the micelles, as measured from dynamic light scattering (DLS) experiments (see Table 2), are found to be inversely proportional to PHB contents of the star copolymer and are very stable against dilution down to 1.0 mg L^{-1} (Fig. S4(d), ESI†). The excellent micelle stability demonstrated here could be due to the highly hydrophobic nature of the PHB chain and the adamantane end group.

In light of the data extracted from static light scattering (SLS) experiments, particularly their ratios of radii of gyration (R_g) to R_h notated as ρ, the sPEG–PHB micelles are thought to be of higher penetrability than non-draining hard-sphere micelles (ρ = 0.78).[10] On account of the ρ values as well as very large molecular weight of micellar aggregates ($M_{w,agg}$) and aggregation number (N_{agg}), the self-assembly of sPEG–PHB is thought to occur through the formation of nanogel-like large compound micelle (LCM).[11] During LCM formation, the entropic penalty associated with intramolecular PHB sequestration *via* chain looping[12] could be reduced by the more favorable intermolecular PHB aggregation, forming many hydrophobic PHB pockets that are interconnected by loose PEG chains within each LCM (Fig. 1(a)). Such a morphology allows deep penetration of water into the particles, akin to nanogel. Indeed, the large amount of water contained in the LCM can be inferred from the much smaller particle sizes as measured from TEM than DLS (refer to Table S1, ESI†). In addition, further assessment on the average particle density (ρ_p) of the LCMs revealed particle densities that are far lower than bulk polymer density (~1 g cm^{-3}).[13] This again points to a loosely packed morphology of sPEG–PHB LCMs. Previous studies on linear block copolymers micelles showed that N_{agg} increased sharply as the molecular weight of the hydrophobic block increased.[14] However, our LCMs displayed a different trend. For a fixed sPEG core, N_{agg} decreased with increased PHB length, while N_{agg} increased with larger sPEG core. The trend suggests an interplay of effects arising from sPEG size, which

Table 2 Micellar properties of sPEG–PHB micelles in aqueous solution

sPEG–PHB micelles	R_h[a]/ nm	R_g[b]/ nm	ρ[c]	$M_{w,agg}$[b]/ MDa	N_{agg}[d]	ρ_p[e]/g cm^{-3}	A_2[b] $\times 10^4$/ cm^3 mol g^{-2}
10–3.1	77	81	1.05	30.5	1030	0.026	−2.82
20–3.1	100	119	1.19	61.5	1411	0.024	−2.64
10–1.6	109	142	1.30	56.2	2412	0.017	−5.74
20–1.6	132	223	1.69	185	6314	0.032	−1.28

[a] Determined by DLS. [b] Determined by SLS. [c] Ratio of R_g/R_h. [d] Aggregation number $N_{agg} = M_{w,agg}/M_w$, M_w as determined from GPC-LS. [e] Average particle density $\rho_p = 3M_{w,agg}\langle R_h^{-1}\rangle/4\pi N_A$, N_A = Avogadro constant.

restricts PHB aggregation because of its star architecture, and PHB chain length on LCM formation. Larger sPEG, being more flexible, also provides more room for sPEG–PHB micellization, as evidenced by DLS measurements. In any case, the restrictive movement of sPEG resulted in an incomplete packing of PHB into LCM interior, causing the LCM surface to be slightly hydrophobic, as suggested by negative second virial coefficients (A_2) which allude to unfavorable polymer–solvent interaction.[15]

Interestingly, when sPEG–PHB star-block copolymers were self-assembled in the presence of DM-β-CD, they formed vesicles rather than LCMs (Fig. 1(b) and Fig. S5 (ESI†)). DM-β-CD is a highly hydrophilic derivative of β-CD. Its donut-shaped molecular structure has a hydrophobic cavity that binds strongly to adamantane (Ada) *via* host–guest interaction.[16] Hence, the vesicle formation observed here could be a manifestation of CD-binding onto the Ada-functionalized sPEG–PHB star-block copolymers that in turn shifts the hydrophilic/hydrophobic balance of the amphiphilic star polymers and hinders hydrophobic interaction between PHB chains. It has been reported that linear diblock copolymers self-assemble into vesicles only when the ratio of hydrophilic to total mass ($f_{hydrophilic}$) is around 35 ± 10%, while a micelle is formed when $f_{hydrophilic} > 45\%$.[2a] However, the sPEG–PHB/DM-β-CD complexes formed vesicles even when $f_{hydrophilic}$ exceeds 45%. The star architecture of sPEG–PHB which restricts chain movements is critical to such unique self-assembly behavior. This is demonstrated by the absence of vesicle formation in the self-assembly of the linear analogues of sPEG–PHB star-block copolymers, methoxy poly(ethylene glycol)-*block*-poly-(3-hydroxybutyrate) (MPEG–PHB), although these linear analogues are comparable to the star-block copolymers in terms of hydrophobic content, PEG arm length and CD-binding capability (Fig. S6, ESI†).

The vesicle diameters are in the range of 200–500 nm and are much larger than the corresponding sPEG–PHB LCMs. sPEG–PHB with longer PHB generally gives a thicker vesicle wall (refer to Table S2 (ESI†)). It should, however, be noted that the thickness of the vesicle wall ranged between 45–155 nm which is much larger than the length of one PHB chain. Considering both the restrictive nature of the star architecture and the hydrophobic properties of PHB, we propose a possible model for the supramolecular self-assembly of sPEG–PHB/DM-β-CD complexes, as illustrated in Fig. 1(b). The vesicle wall is formed by PHB aggregation with the possibility of embedded sPEG segments and DM-β-CD while its exterior and interior surfaces are covered with hydrophilic sPEG and DM-β-CD.

In summary, we present here the synthesis of novel amphiphilic PHB-based star-block copolymers with sPEG cores and PHB-adamantyl peripheries, and their unique self-assembly behaviors forming nanogel-like LCMs and the supramolecular transformation to nano-sized vesicles. The well-controlled ROP of β-butyrolactone into heterofunctionalized PHB precursors is key to the facile synthesis of the star-block copolymers. The amphiphilic star-block copolymers were found to self-assemble into very stable nanogel-like LCMs in aqueous medium. On the other hand, more remarkably, in the presence of DM-β-CD, the self-assembled particles took the form of nanovesicles. The favorable adamantane–CD host–guest interaction and the star architecture of sPEG–PHB star-block copolymers are critical factors leading to such unique self-assembly behaviors. The highly stable nanogel-like LCMs and sPEG–PHB/DM-β-CD nanovesicles are potential carrier materials for co-delivery applications of both hydrophilic and hydrophobic therapeutic agents. We also believe that the host–guest approach of nanoparticle modification demonstrated here encompasses a robust and modular strategy that could be adopted for synthesis of other functional nanostructures.

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