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SHORT COMMUNICATION

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Self-assembled nanostructures of a biomimetic glycopolymer–polypeptide triblock copolymer

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Abstract The self-assembling behavior of a biomimetic glycopolymer–polypeptide triblock copolymer in aqueous solution was described and characterized by employing the hydrophobic dye solubilization method and transmission electron microscopy. The large spherical micelles can be easily generated from the dissolution of triblock copolymer in water. The morphology changes from sphere to lamellae, then to worm-like micelle, can be conveniently transformed by initial copolymer concentration. The multivalent interaction of lectins with lactose-installed polymeric aggregates was preliminarily investigated by UV-Vis spectra. Notably, this kind of aggregates may be useful as artificial polyvalent ligands in the investigation of carbohydrate–protein recognition and for the design of site-specific drug delivery systems.

Keywords Glycopolymer–polypeptide triblock copolymer · Self-assembly · Lactose-installed aggregate · Multivalent ligand · Drug delivery system

Introduction

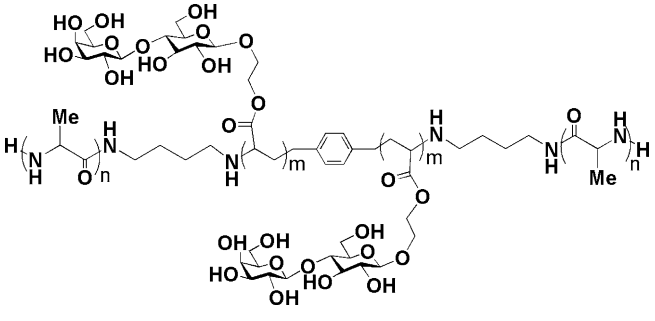
Amphiphilic block copolymers have been shown to yield a variety of hierarchical structures, for example, micelles, vesicles, tubules, cylinders, etc. [1–4]. The driving forces for the self-assembly come from both the hydrophobic blocks and the repulsive interactions of the hydrophilic blocks. Significantly, these efforts hold relevance for both the fabrication of hierarchical nanobiomaterials and drug delivery vesicles. For examples, it was reported that certain zwitterionic AB diblock copolymer could self-assemble in aqueous media to form both conventional micelles (with A block forming the micelle core) and reverse micelles (with B block forming the micelle core) [5–7]. A class of ''crew-cut'' aggregates was investigated in detail, which were generated from asymmetric coil–coil block copolymers [8–13]. For the peptide-based rod–coil block copolymers, such as polystyrene-*b*-poly(isocyano-L-alanine-L-alanine) and polybutadiene-*b*-poly(L-glutamates), which demonstrated collapsed vesicles coexisting with rod-like filaments, and hollow spherical vesicles, respectively [14, 15]. Interestingly, Nolte et al. [16–18] have developed a new class of giant amphiphiles consisting of an enzyme headgroup and a single covalently connected hydrophobic polymeric tail. For the self-assembly of biomimetic sugar-containing polymer (glycopolymer), the crew-cut aggregates surfaced by glucose were generated from a polystyrene-*b*-poly[2-(*β*-D-glucopyranosyloxy) ethyl acrylate] glycopolymer (PS-*b*-PGEA) [19]. Thoma et al. [20] reported that a novel glycodendrimer self-assembled to nanoparticle and its function as polyvalent ligand in vitro and in vivo. Very recently, Cowan and Davis et al. [21] synthesized a new class of ''glycodendriproteins'', which potently reduced the binding ability of pathogenic bacteria. Roy [22, 23] reviewed the development of glycodendrimer chemistry and its biological applications. However, the research on the synthesis and self-assembling properties of biomimetic polypeptide–glycopolymer and/or glycopeptide mimics is rare [24, 25].

In our recent publication [26], a biomimetic glycopolymer–polypeptide triblock copolymer, i.e., an amphiphilic [poly(L-alanine)-*b*-poly(2-acryloyloxyethyllactoside)-*b*-poly(L-alanine)] triblock copolymer composed of a hydrophilic lactose-bearing central block flanked by hydrophobic poly(L-alanine) endblocks (AGA, Scheme 1), was synthesized by the combination of atom transfer radical polymerization (ATRP) of a protected lactose-bearing monomer followed by ring opening polymerization (ROP) of L-alanine *N*-carboxyanhydride, which have controlled molecular weight and low polydispersity ($M_w/M_n = 1.06–1.26$, M_w: weight-average molecular weight, M_n: number-average molecular weight). Therefore, the work reported herein is motivated by taking advantage of two aspects, one in which glycopolymer as the shell of nanostructure will function as an artificial multivalent ligand utilized for the design of site-specific drug delivery and facilitate the investigation of the multiple carbohydrate–protein interactions occurring during these molecular recognition events, and the other in which polypeptide with the tunable secondary structures, as a biodegradable core, can be used to manipulate the size and shape of nanostructures. To our knowledge, this is the first report describing a novel class of lactose-installed polymeric nanostructures from the self-assembly of a biomimetic glycopolymer–polypeptide triblock copolymer in aqueous solution, which shows specific interactions with RCA_{120} lectin than ConA lectin. Furthermore, the morphology of the self-assembled nanostructures can be conveniently transformed by initial copolymer concentration.

Experimental section

Methods

Transmission electron microscopy (TEM) was performed using a JEOL 1210 TEM at a 90 kV accelerating voltage. Samples were deposited onto the surface of 200 mesh Formvar-carbon film-coated copper grids. Excess water was wicked away, and the grids were dried in a vacuum. The image contrast was enhanced by negative staining with uranyl acetate. Infrared spectra of powder sample were acquired using a BioRad FTS-60 Fourier transform infrared (FT-IR) spectrometer equipped with a wide band MCT detector. UV-Vis absorption spectra of samples were recorded at room temperature using a Cary 50 Bio UV-Visible spectrophotometer (Varian).



Scheme 1 The chemical structure of a biomimetic glycopolymer–polypeptide triblock copolymer [poly(L-alanine)-*b*-poly(2-acryloyloxyethyllactoside)-*b*-poly(L-alanine)] (AGA)

Measurement of copolymer critical aggregation concentration

The A9G52A9 and A22G52A22 triblock copolymers were used to determine the self-assembling behavior, in which the numbers indicate the number of repeating units in the blocks, and their M_n and M_w/M_n were 29,680 and 31,860, and 1.06 and 1.26, respectively. The critical aggregation concentration (CAC) of triblock copolymer in aqueous solution was determined employing the hydrophobic dye solubilization method using 1,6-diphenyl-1,3,5-hexatriene (DPH, Aldrich). DPH was dissolved in methanol to produce 0.5 mM DPH methanol solution. Copolymer aqueous solutions were prepared by dissolving 20 mg of polymer in 10 mL of distilled water to produce 2 mg/mL solution at room temperature and filtered through 200-nm inorganic membrane filter (Whatman International Ltd., England), and then diluted to the desired concentration. In a quartz cell, 0.5 mL copolymer solution and 5 μL of DPH solution were added and capped with Teflon, and then homogenized for 1–2 min at room temperature. To prevent the evaporation of water during the measurement, the sample cell was sealed with Teflon film and Parafilm. UV-Vis spectra of samples were recorded in the range of 200–500 nm range at room temperature.

Lectin recognition

Concanavalin A (Con A) and Agglutinin RCA_{120} were purchased from Sigma. The lectin recognition activity of the triblock copolymer aggregates solution was analyzed by changes in the transmittance with time at 500 nm and room temperature following the addition of the various concentrations of aggregates solution into lectin solution, in which its concentration is equal to 0.5 mg/mL.

Results and discussion

In this communication, we show the self-assembled nanostructures of a biomimetic glycopolymer–polypeptide triblock copolymer in water, the morphological changes induced by initial copolymer concentration, and

the multivalent interaction of lectins with the nanostructures. The amphiphilic [poly(L-alanine)-*b*-poly(2-acryloyloxyethyllactoside)-*b*-poly(L-alanine)] triblock copolymers (AGA), composed of a hydrophilic lactose-bearing central block flanked by hydrophobic poly(L-alanine) endblocks, were synthesized by sequential ATRP and ROP techniques, which have controlled molecular weight and low polydispersity ($M_w/M_n = 1.06–1.26$), as described in our recent publication [26]. The stable aggregates in aqueous solution were obtained by simple dissolution of triblock copolymer by stirring overnight, and the resulting solutions were optically transparent or slightly opalescent. First of all, we examined the CAC values of AGA triblock copolymer in aqueous solution employing the dye solubilization method described by Kim et al. [27]. 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe molecule was added to the copolymer aqueous solution, and the absorbance was measured by a UV-Vis spectrophotometer. The relationship of the absorbance intensity as a function of copolymer concentration at room temperature is shown in Fig. 1. It can be seen that the absorbance intensity values of DPH remained virtually constant below a certain concentration. Above that concentration, the intensity increased substantially, reflecting the incorporation of DPH in the hydrophobic region of aggregates. The CAC value was determined by intersecting the two straight lines, and a value of ~0.05 mg/mL was obtained for A9G52A9 copolymer. Similarly, the CAC value for A22G52A22 copolymer was determined to be ~0.03 mg/mL. The value for A22G52A22 is smaller than that for A9G52A9, which may reflect the effect of molecular weight of poly(L-alanine) segment.

The morphology of these aggregates was investigated by TEM using negative staining. Figure 2 shows four morphologies of the aggregates generated from triblock copolymer A22G52A22 with the concentration of 2.5–10 mg/mL. Figure 2a shows the large spherical micelles

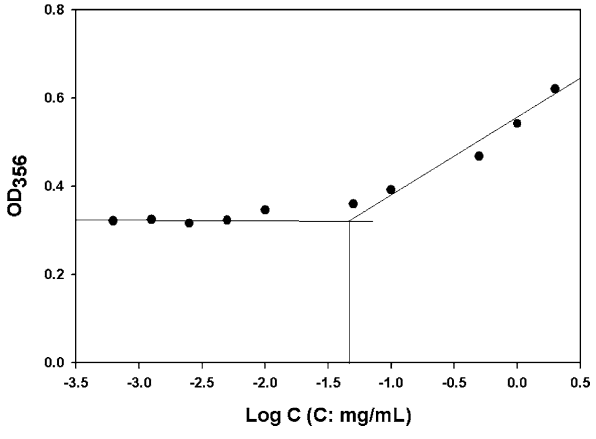
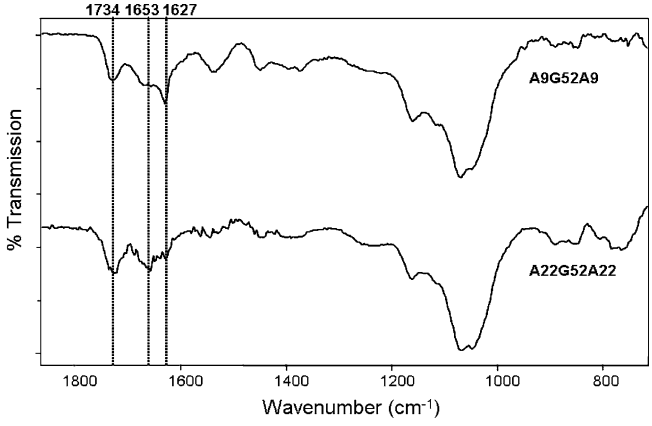


Fig. 1 The relationship of the absorbance intensity of DPH at 356 nm as a function of A9G52A9 copolymer concentration

(LSMs) with diameter of 400–600 nm when the copolymer concentration is equal to 2.5 mg/mL. This is different from the most common morphology reported for block copolymer micelles with diameter usually in the range of 10–100 nm, which is probably attributed to both the secondary molecular structure of poly(L-alanine) hydrophobic blocks and the sugar intermolecular interactions among the hydrophilic glycopolymer blocks. As the copolymer concentration increases to 5 mg/mL, the morphology changes from sphere to combined morphologies, which mainly consist of lamellae, with some collapsed vesicles in the right corner, as shown in Fig. 2b. Figure 2c indicates that the morphology again changes to inner-connected spheres, which looked like "worm" when the copolymer solution is formed at pH 6.69 with contrast to previous pH 2. This appears to be a result of the decreased repulsion among the corona chains, which is a general phenomenon for the self-assembly of amphiphilic block copolymer. Finally, Fig. 2d shows polydisperse particles from 10 mg/mL of the block copolymer. The above morphology changes induced by copolymer concentration are probably attributed to both the stretching of hydrophobic poly(L-alanine) blocks and the hydrogen-bond interactions of sugar molecules among the hydrophilic blocks. This is consistent with that of an amphiphilic glycopolymer-based block copolymer (PS77-*b*-PGEA6), i.e., the morphology changes from sphere to lamellae, and vesicle until large aggregate [28]. However, for the triblock copolymer A9G52A9 with different copolymer concentrations (5 and 10 mg/mL), Fig. 3 only shows the LSMs, and only a little difference in sphere size can be seen from TEM micrographs. Moreover, the micrograph of a section of the LSMs shows that it has an internal structure of gel-like micelles network, which is formed, probably, owing to the sugar molecular interactions (strong intermolecular hydrogen bonding) among the micelles. (The micelles were surfaced by lactose shell, which was demonstrated by the following UV-Vis analysis.) Notably, the different self-assembling behaviors of the triblock copolymers may be attributed to the different length of hydrophobic poly(*L*-alanine) block (i.e., hydrophilic/hydrophobic balance) and the secondary molecular structures of A22G52A22 and A9G52A9 copolymers, in which the former has the main structure of α-helix, however, the latter mainly has the β-sheet structure [29], which was verified by FT-IR spectra (see supporting information, Scheme 2).

Multivalent ligands can cluster soluble and cell-surface receptors, which govern many biological processes, including immune responses and growth factor signaling. Significantly, understanding the molecular features of a multivalent ligand would provide insight into how natural multivalent interactions are regulated [22, 30–32]. It is reported that Con A and RCA_{120} lectins recognize the mannose and galactose/lactose residues,



Scheme 2 The FT-IR spectra of A9G52A9 and A22G52A22 triblock copolymers

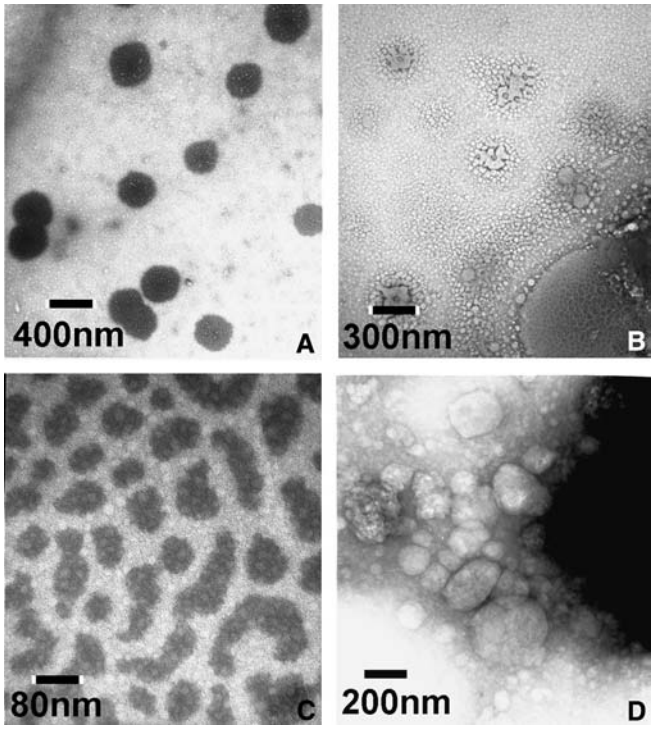


Fig. 2 TEM microphotographs of the aggregates from amphiphilic A22G52A22 triblock copolymer in water: **a** 2.5 mg/mL, pH 2; **b** 5 mg/mL, pH 2; **c** 5 mg/mL, pH 6.69; **d** 10 mg/mL, pH 2

respectively. Thus, the interaction of lectins with the lactose-installed aggregates was preliminarily characterized by the UV-Vis spectra, as shown in Fig. 4. It can be seen that the turbidity increases with the increase of the copolymer concentration after lectin addition, and the turbidity nearly has no apparent increase after the copolymer concentration is equal to 0.5 mg/mL. However, when Con A was added to the aggregates solution, no turbidity change was observed. This demonstrates that the aggregates had specific

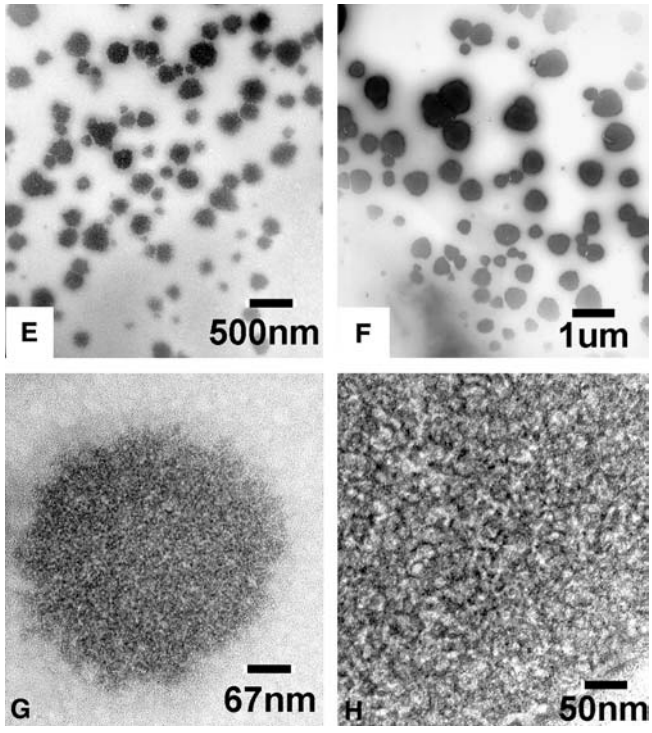


Fig. 3 TEM microphotographs of the aggregates from amphiphilic A9G52A9 triblock copolymer in water: **e** 5 mg/mL; **f** 10 mg/mL; **g** one LSM in samples **f**; **h** a section of **g** sample

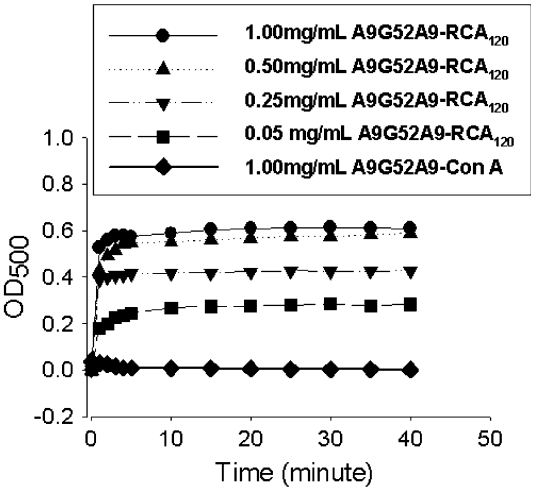


Fig. 4 The interaction of RCA_{120} (0.5 mg/mL) or Con A (0.5 mg/mL) with 0.05–1.0 mg/mL of A9G52A9 aggregates as measured by the absorption intensity at 500 nm

interaction with RCA_{120} lectins and the sugar-lectin complexes would be saturated after the copolymer aggregates were enough for lectin molecules. The above indirectly indicates that the aggregates were surfaced by lactose shell.

Conclusion

In summary, we have fabricated a novel class of lactose-installed polymeric nanostructures from a biomimetic glycopolymer–polypeptide triblock copolymer. Notably, this self-assembling system at least has two advantages. Firstly, the LSMs can be easily generated from dissolution of copolymer in water, which is of interest in an artificial tissue-like soft biomaterials and the controlled drug release because of the multiple concentric layers. Secondly, the aggregates may be useful as artificial polyvalent ligands in the study of carbohydrate–protein recognition and for the design of site-specific drug delivery systems, which are under investigation in our lab.

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