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Morphology transition of self-aggregates of poly(amino acid)-drug conjugates

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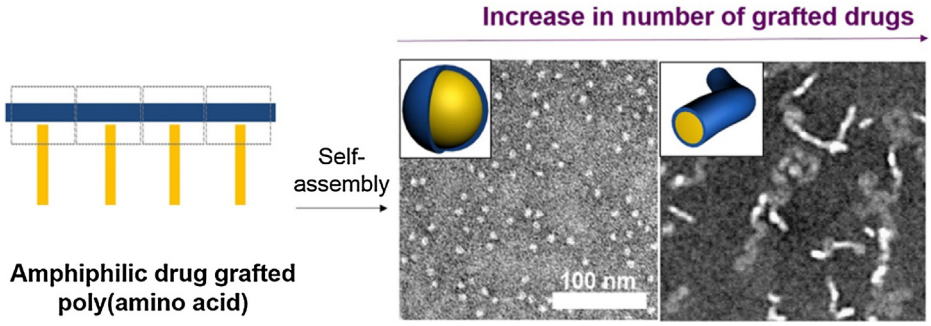
HIGHLIGHTS

- A series of amphiphilic phytosphingosine (PHS) grafted poly(amino acid)s were synthesized.
- Self-aggregation of the polymers were observed as functions of grafting degrees and types of poly(amino acid)s by small angle neutron scattering analysis.
- Morphology transitions of the self-aggregates from sphere to ellipsoid to cylindrical micelles were observed with increasing number of grafted phytosphingosines.
- The morphological change of PHS-grafted poly(amino acid) was interpreted with changes in the geometry of the building blocks.

GRAPHICAL ABSTRACT



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ABSTRACT

In this paper, we report morphology tunable self-aggregates of poly(amino acid)-drug conjugates. Amphiphilic drug-grafted polymers were synthesized by grafting hydrophobic phytosphingosine (PHS) onto two different hydrophilic poly(amino acid)s backbones, and the hydrophilic/hydrophobic ratio were controlled by varying the number of grafted drugs. The amphiphilic poly(amino acid)-*g*-PHS formed self-aggregates in aqueous solution, and morphology transitions of the self-aggregates from sphere to ellipsoid to cylindrical micelles were observed with increasing number of grafted phytosphingosines. Morphologies of self-aggregates were further confirmed by small angle neutron scattering, showing that the obtained self-aggregates consisted of a thin poly(amino acid) shell and a PHS core. The morphological changes of PHS-grafted poly(amino acid) were interpreted using geometrical changes of building blocks arising from the decrease of the hydrophilic part per PHS with increasing number of PHS grafts.

1. Introduction

Amphiphilic polymers composed of hydrophilic and hydrophobic parts in a single polymer chain exhibit a surface active property and form not only spherical self-aggregates but also ellipsoidal, worm-like, tubular, vesicular aggregates as well as many other

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types by spontaneous self-aggregation in selective solvents [1–4]. The self-aggregate morphologies depend on molecular geometry, the hydrophilic/hydrophobic ratio of amphiphiles, and many other preparation conditions. Modern chemical methods allow the tailored synthesis of block copolymers, and copolymers having precisely controlled hydrophilic and hydrophobic blocks were shown to form diverse aggregate morphologies [1,2,5]. However, morphology transition of amphiphilic graft polymers has not been investigated as much as block copolymers [6,7], even though graft polymers provide an advantage of multi-functionality based on multiple grafting of various substances in a single polymer [8,9].

Spherical self-aggregates of amphiphilic polymer have been widely studied as drug delivery carriers because the hydrophobic core can entrap and stabilize water-insoluble drugs, while the hydrophilic shell improves dispersion stability in the blood stream [10,11]. Polymeric self-aggregates are kinetically and thermodynamically more stable than micelles formed by surfactants, thus stably entrapping encapsulated payloads in a dilute blood stream [12].

Because drug carrier shape has recently received much attention as an important factor in drug delivery, transportation and cellular internalization of non-spherical particles have been investigated [13]. Cylindrical micelles are known to encapsulate twice the amount of drugs compared with the encapsulations provided by spherical micelles, and long cylindrical micelles showed prolonged circulation in the blood stream of up to one week, which is ten times longer than the circulation of spherical nanoparticles [14,15]. Targeted accumulation of spheres and elliptical discs were compared, and microscale discs showed higher accumulation than any spheres in the 100 nm to 10 μm diameter range [16]. Although the shape and size effect of metallic and hard particles have been widely studied, those of soft materials have not been investigated as much due to the difficulty of morphology control; the need to control morphology of self-aggregates from biopolymers or drug-conjugated polymers has been emphasized [17].

In this paper, we developed morphology tunable self-aggregates of drug-grafted poly(amino acid) which include sphere, ellipsoid and cylindrical micelles, and morphology transition of the self-aggregates was observed as a function of the number of grafted drugs. A series of amphiphilic graft polymers that consist of a hydrophilic poly(amino acid) backbone and phytosphingosine (PHS) grafts was synthesized via an simple aminolysis reaction, and grafting degrees of PHS were varied to induce morphology transition (Fig. 1). PHS is one of the natural sphingolipids which has anti-inflammatory, antifungal and anticancer activities, and micelles of PHS-conjugated polymers have successfully demonstrated intracellular delivery of PHS and its anticancer effect [18,19]. Two types of amphiphilic graft polymers with different hydrophilic and biodegradable poly(amino acid) backbones, poly(aspartic acid) (PAsp) and poly-α,β-(2-hydroxyethyl aspartamide) (PHEA), were synthesized, and their morphology changes were compared by small angle neutron scattering analysis.

2. Materials and methods

2.1. Materials

L-aspartic acid, mesitylene, tetramethylene sulfone, phosphoric acid, dimethyl sulfoxide (DMSO), *N,N*-dimethylformamide (DMF), and sodium hydroxide were purchased from Sigma-Aldrich and used as received unless otherwise stated. Phytosphingosine (>95%) was purchased from Doosan Biotech (South Korea) and D_2O was purchased from Cambridge Isotope Lab (USA).

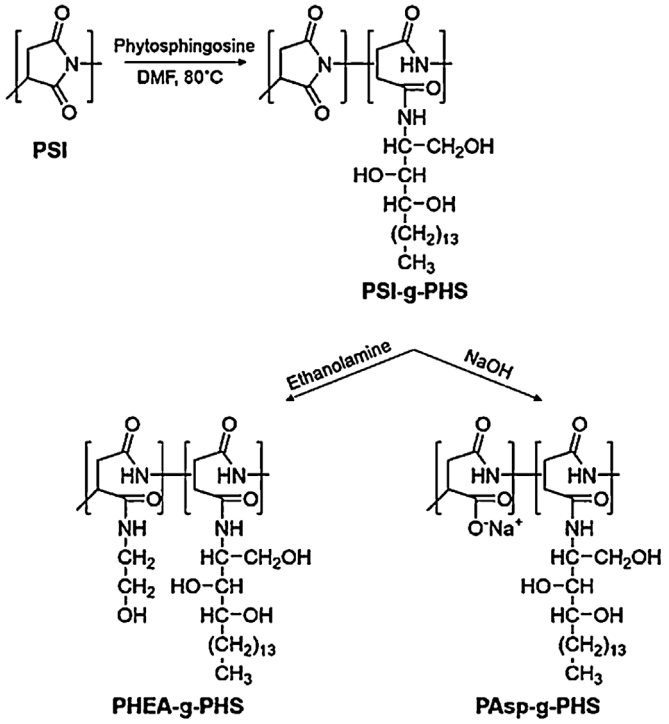

Fig. 1. Synthesis of phytosphingosine-grafted poly(2-hydroxyethayl aspartamide) (PHEA-*g*-PHS) and poly(aspartic acid) (PAsp-*g*-PHS).

2.2. Synthesis of poly(2-hydroxyethyl aspartamide)-g-phytosphingosine (PHEA-g-PHS)

Poly(succinimide) (PSI) was synthesized from L-aspartic acid via acid-catalyzed thermal polycondensation by following the previously reported method (M_n = 12.5 kDa, polydispersity = 1.4, by gel permeation chromatography (GPC) analysis, see Supplementary information) [20].

To synthesize PHS-grafted PSI (PSI-*g*-PHS), PSI was dissolved in *N,N*-dimethyl formamide (DMF) at 80 °C, and a desired amount of PHS was added to the solution, with the reaction then allowed to proceed for 3 days. The mixture was cooled down to 40 °C, and excess ethanolamine was added and reacted for 6 h. The polymer was precipitated in diethyl ether and dried in a vacuum oven. The polymer was dissolved in dimethyl sulfoxide (DMSO) and dialyzed against DMSO and deionized water using a dialysis membrane for 2 days, followed by free-drying. ^{1}H NMR spectra were obtained using a Bruker DRX 300 spectrometer (Germany). Polydispersities of the polymers obtained by GPC analyses were 1.4–1.8 (Supplementary information).

2.3. Synthesis of poly(aspartic acid)-g-phytosphingosine (PAsp-g-PHS)

Following the grafting reaction described, the synthesized PSI-*g*-PHS in DMF was cooled down to room temperature. A solution of NaOH was added to the reaction solution to obtain 0.1 M, and the residual PSI was hydrolyzed for 6 h. The polymer was precipitated in diethyl ether and dried in a vacuum oven. The polymer was dialyzed against DMSO and deionized water using a dialysis membrane for 2 days, followed by free-drying.

2.4. Preparation and characterization of self-aggregates

Self-aggregates were prepared by dissolving the PHEA-*g*-PHS or PAsp-*g*-PHS in deionized water, and the mixture was heated