Structural Analysis of Unimer Nanoparticles Composed of Hydrophobized Poly(amino acid)s and Their Potential Application as Drug Carriers

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Statement of Purpose: In drug delivery system (DDS), many kinds of particulate drug carriers have been continuously proposed. One of the unique properties of the amphiphilic graft copolymers is their ability to form unimolecular (unimer) micelles or nanoparticles (NPs) with intra-polymer associations (Figure 1a). Various approaches for folding a single polymer chain to obtain unimer micelles or NPs in a dilute condition have been reported, for example thermo-induced folding (He J. Soft Matter. 2011;7:2380-2386.) as well as method requiring no external stimulus such as hydrophobic interaction (Yusa S. Macromolecules. 2002;35:5243-5249.). The fabrication of unimer NPs consisting of biodegradable polymer is one of the major challenges in polymer science and pharmaceuticals. In this study, we prepared biodegradable unimer NPs composed of poly(γ-glutamic acid) conjugated with L-phenylalanine (γ-PGA-Phe) as a hydrophobic group (Akagi T. Langmuir. 2012;28:5249-5256.). Furthermore, structural analysis and potential applications as drug carriers of unimer NPs of the unimer NPs were also investigated.

Methods: γ-PGA (M_w of 70, 140, and 220 kDa) were hydrophobically modified by Phe in the presence of carbodiimide. The degree of Phe grafting was controlled by changing the amount of carbodiimide. γ-PGA-Phe with 12-65 of Phe groups per 100 glutamic acid units of γ-PGA were synthesized. NPs of γ-PGA-Phe were subsequently prepared by directly dissolved the synthesized γ-PGA-Phe into PBS and/or by dialysis method depending their grafting degree. NPs were further characterized by means of dynamic and static light scattering (DLS and SLS), transmission electron microscopy (TEM), small-angle neutron scattering (SANS) as well as steady-state fluorescence measurements/quenching techniques in order to determine particle size, molecular weight, morphologies, hydrophobic associations and inner structures of NPs, respectively. In addition, the efficiency of drug absorption into unimer NPs compared with large-sized NPs was measured by UV-Vis using irinotecan and cisplatin as model hydrophobic drugs.

Results: Amphiphilic graft copolymers of γ-PGA-Phe having various lengths of γ-PGA main chain (M_w of 70, 140, and 220 kDa) self-assemble forming NPs when employing hydrophobic moieties of Phe groups (grafting degree 12-60%) in aqueous media. The aggregation number (N_{agg}) of which could be adjusted according to their physically molecular structures as well as preparative methods/conditions. Therefore, single chain state having hydrophobic domains, forming spherical core-shell structures, herein called unimer NPs, could obtain when employing M_w of γ-PGA higher than 140 kDa conjugated with Phe of 27-42% due to the balance of hydrophobicity/hydrophilicity along the single polymer chain. Their morphologies were confirmed by TEM and SANS measurements in which correlated well with data obtained from DLS and SLS. The number of hydrophobic domains in one NP (N_{domain}), estimated by means of fluorescence quenching techniques with the use of CPC as a quencher and rigidity of the inner particles detected by fluorescent measurement using dipyrene as a probe demonstrated that N_{domain} and the rigidity of NPs affected by particle sizes and preparative methods. Moreover, higher irinotecan absorption over than large-sized NPs has been observed from 10-nm unimer NPs (Figure 1b).

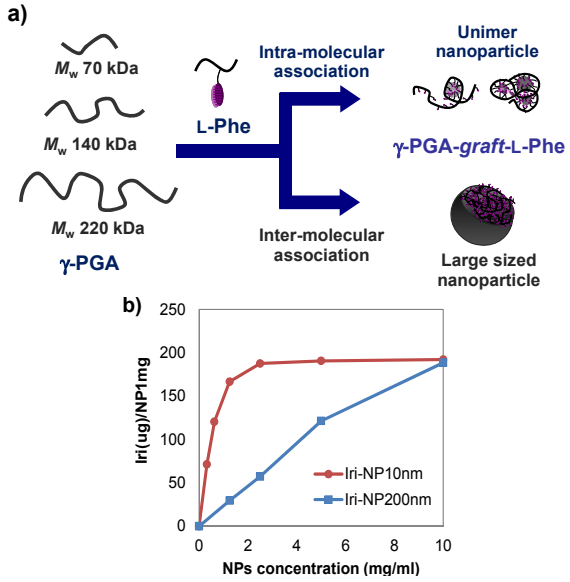

Figure 1. Illustration of the formation of unimer NPs composed of hydrophobized γ-PGA a), and the adsorption of irinotecan, 10-nm NPs shows higher absorption over large-sized NPs b).

Conclusions: 10-nm unimer NPs (single chain state forming hydrophobic domains) were obtained from γ-PGA-Phe when employing M_w of γ-PGA higher than 140 kDa, and grafting degree of Phe in the range of 27-42%. A key step in control the aggregation number for the formation of unimer NPs is to balance the hydrophobic-hydrophilic moieties along the polymer chain with the help of preparative methods in dilute condition in which salinity is also one factor that can induce the self-assembly. In addition, 10-nm unimer NPs shows advantages on drug absorption due to their large surface area over large sized NPs. The obtained unimer NPs are expected to offer possibility of biomedical applications, such as drug, gene, and vaccine delivery.