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Bioactive vesicles from saccharide- and hexanoyl-modified poly(L-lysine) copolypeptides and evaluation of the cross-linked vesicles as carriers of doxorubicin for controlled drug release

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ABSTRACT

We report the synthesis and self-assembly of an amphiphilic glycopolypeptide through the conjugation of both lactobionolactone, a model targeted ligand to HepG2 liver cells, and hexanoyl groups onto poly(L-lysine) (PLL). The self-assembled glycopolypeptide vesicles were stabilized via genipin-cross-link and evaluated as cell-targeted carriers. The experimental data revealed that the saccharide and hexanoyl substitution can regulate the amphiphilic nature and chain conformation of the glycopolypeptide, and subsequently the size of the assembled vesicles. Taking the advantages of the glycopolypeptide vesicles including stable structure, pH-responsiveness, and cell-targeting ability, the glycopolypeptide was employed for drug encapsulation, *i.e.* doxorubicin (Dox). A high Dox loading level (45 wt.%) can be achieved with the aid of sonication as a pH gradient was applied between the outside and inside of the vesicles. The cross-linked, vesicles loaded with Dox exhibited noticeable pH-sensitive behavior with accelerated Dox release at acidic condition due to the protonation of amino groups and the release rate can be controlled by the genipin to amine feed ratio. The cytocompatibility of the polypeptide was improved upon grafting the saccharide group and cross-link. The Dox-loaded vesicles exhibited a comparable cytotoxicity with respect to free Dox against HepG2 liver cells. These results point to a potential of novel glycopolypeptide vesicles with cross-linkable membrane to be carriers for the delivery of bioactive agents.

1. Introduction

Polymer vesicles, so called polymersomes, are hollow spheres self-assembled from amphiphilic copolymers that currently have attracted great interest because of their analogous structure with cell membranes and potential applications as bionanoreactors or delivering vehicles [1–9]. Comparing with phospholipid-based vesicles, polymer vesicles exhibited improved stability, superior membrane properties, and versatile chemistry for functionalization [1,2,10]. The interior of the vesicles can encapsulate large quantities of cargoes such as bioactive agents and the cargoes can be released in a controlled manner by adjusting their permeability. For drug delivery application, the delivering vehicles are required to possess features including biodegradability, targetability, and stimuli-responsiveness [3,6–9,11–13]. Using polymers comprised of natural building blocks such as amino acids and saccharides is one way to avoid biodegradability issues. Polypeptide-based block and graft copolymers have been extensively studied for drug delivery application because of their biodegradability, biocompatibility, ordered chain conformation, stimuli-responsiveness, and possibly biological functions [7,14–16]. While amphiphilic polypeptide-based block copolymers can self-assemble to form vesicles, only a few of polypeptide-based

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graft copolymers reported thus far formed vesicular structures [17–20]. The self-assembled structures formed by graft copolymers can be controlled by readily tuning the side-chain properties such as the substitution degree and chain length. Moreover, diverse functionalities can be incorporated onto the polymer simply by conjugation. Previously, our group have demonstrated that poly(L-lysine) (PLL) conjugated with alkyl chain can self-assemble to form vesicles and the size can be tuned by the substitution degree [20]. PLL has been utilized as an important building block in graft copolymers, which has been shown to be potentially useful biomaterials in gene therapy, non-fouling coatings, and other applications [21–29]. By conjugating functional moieties onto PLL-based graft copolymers, it is expected that their self-assembled structures can be potential cell-targeted carriers for therapeutics. These carriers would be positively charged and pH-sensitive, which can promote the uptake by cells via proton sponge effect and facilitate the release of cargoes at acidic condition.

Meanwhile, some saccharine groups as well as oligosaccharides are well-known for their mediation of cell recognition, which are potentially useful for specific biomedical applications. To combine the advantages of peptides and saccharide moieties, the synthesis of so-called polypeptide–carbohydrate conjugates or glycopolypeptides have received increasing interest and the synthesis strategies rely mainly on the incorporation of saccharide moieties to existing polypeptides via amide linkages [30–33], click chemistry [15,34–37], or direct polymerization of glycosylated monomers [38,39]. This new class of biomaterials with versatile biological and structural functions can be designed and expected to be potentially useful as biomedical materials such as tissue engineering scaffolds or delivering vehicles.

One important issue in designing these materials for biomedical applications is their stability in solution. Previous studies have demonstrated that cross-linking one of the blocks within the self-assembled structures such as micelles and vesicles is a feasible approach to improve their stability [20,33,40–44]. Our group has studied the self-assembly of polypeptide-based block and graft copolymers and demonstrated that these assembled micelles and vesicles can be cross-linked to form stable structures [20,33,45,46]. In addition to stability, it is reasonable to assume that the permeability of vesicle membranes can be tuned via cross-link and subsequently the loaded cargoes can be released at a desired rate, which has sparsely addressed previously.

Here, we report the preparation and characterization of a glycopolypeptide, saccharide and alkyl chain modified poly(L-lysine), that self-assembled to form vesicles at acidic and neutral conditions in aqueous solution. The disaccharide, lactobionolactone, which is a model targeted ligand to HepG2 liver cells, was selected to conjugate onto PLL [47,48]. Previous studies have demonstrated that the attachment of cell-specific saccharide groups to polymer backbone is one promising way to enhance the efficacy of drug delivery and gene expression to target cells [48]. The influence of the hexanoyl and saccharide substitution on the secondary conformations adopted by the graft copolypeptides and the corresponding self-assembly behavior at different solution conditions were investigated by using light scattering (LS), circular dichroism (CD), electron microscopy, critical aggregation concentration (cac), and zeta potential. To evaluate the feasibility of using the glycopolypeptide vesicles as a cell-targeted carrier, the common anticancer drug, doxorubicin hydrochloride (Dox), was used as a model water-soluble drug to incorporate into the vesicles. In order to obtain stable structures and control the drug release, the drug-loaded vesicles were cross-linked by adding different amount of genipin. The loading efficiency and *in vitro* drug release of the cross-linked vesicles was evaluated. It was expected that the permeability of the membrane can be tuned by changing the degree of cross-link, which, in turn, can control the drug release.

2. Experimental section

2.1. Materials

THF (ACS Reagent, Merck) and diethyl ether (Anhydrous, ACS Reagent, J.T. Backer) were dried using Na metal (99.95%, in mineral oil, Aldrich). Hexane (ACS Reagent, ECHO) was dried using calcium hydride (95%, Aldrich). The amino acid used in this work N_{ε}-Z-L-lysine (~99%, Z-carboxybenzyl) was used as received from Sigma–Aldrich, as well as bis(1,5-cyclooctadiene) nickel (0) (98+%), 2,2′-bipyridyl (99+%), hexanoic/hexanoyl anhydride, lactobionic acid, genipin, and dithiodiglycolic acid. Doxorubicin hydrochloride (99.9%) and trifluoroacetic acid (TFA, 99%) were supplied by Alfa Aesar. Triphosgene (98%, Merck) was used as received, as were HBr (33 wt.% in acetic acid) from Fluka. Anhydrous methanol (99.9%) was purchased from Merck.

2.2. Synthesis of poly(L-lysine)-g-hexanoyl (PLH) and poly(L-lysine)-g-hexanoyl-g-lactobionolactone (PLHG)

Poly(Z-L-lysine) was synthesized using the nickel initiator, 2,2′-bipyridyl-Ni(1,5-cyclooctadiene) (BpyNiCOD), according to the previous reports [45,49]. Poly(L-lysine) (PLL) was obtained by deprotecting the Z group using HBr. PLHG can be successfully prepared by sequentially graft hexanoyl group and lactobionolactone onto PLL. Specifically, 1 g of freeze-dried poly(L-lysine) was dissolved in anhydrous methanol (10 mL) at room temperature and 0.71 g of hexanoyl anhydride was added to the solution in a glove box. The resulting solution was left to stir at room temperature for 1 day. Then the solution was dialyzed against DI water for 3 days using a cellulose membrane dialysis tube (Sigma, MWCO 12,000–14,000 g/mL) and freeze-dried to yield the product (PLH) as a white sponge material. Then, lactobionolactone was grafted onto PLH through the following steps. 0.53 g of lactobionolactone and 2.51 g of 1,1′-carbonyldiimidazole (CDI) were added to the solution with PLH (1 g) dissolved in anhydrous methanol (10 mL) in a glove box. Lactobionoic acid was converted to lactobionolactone based on the previous report [50]. The reaction mixture was left to stir for 1 day at room temperature. Subsequently, the product (PLHG)