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Disulfide and β-sheet stabilized poly(amino acid) nanovesicles for intracellular drug delivery

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ABSTRACT

We report the fabrication of redox-responsive poly(amino acid) vesicles containing bilayers highly stabilized by disulfide cross-links, β-sheets, and hydrophobic interactions. Tailor-made amphiphilic poly(amino acid)s, specifically poly(2-hydroxyethyl aspartamide)-*g*-oligo(L-cysteine) (P-*g*-OC), were used as building blocks for the formation of vesicles. The stable bilayer prematurely released hardly any of an encapsulated anticancer drug, doxorubicin, and the drug release was triggered only by millimolar concentrations of reducing agent, which mimic intracellular conditions. The vesicles demonstrated successful delivery of the drug to the inside of cells, triggered release of the drug after cellular uptake, and translocation of the drug to nuclei in cancer cells.

Introduction

The use of vesicles has attracted increasing interest in the biomedical field due to their having hollow structures enclosed by bilayers of lipids or amphiphilic polymers [1–3]. Because vesicles have an aqueous cavity as well as a hydrophobic shell, hydrophilic and hydrophobic cargo materials can be encapsulated simultaneously [1]. Vesicles used as anti-cancer drug delivery carriers should protect their encapsulated drugs stably during systemic circulation, and the release of their drug cargo should be triggered at the target site. To achieve these goals, vesicles should (i) display high structural stability in order to avoid as much as possible prematurely releasing any encapsulated drug into the blood stream, (ii) be triggered to release their drug cargo into the cytoplasm, with the drug translocated to target cellular compartments, (iii) be composed of polymers that degrade to safe and nontoxic species, (iv) have controllable, well-defined, and highly reproducible sizes and shapes, and (v) be able to encapsulate drugs easily [4,5]. In addition, the particle size of vesicles should be nanosized to achieve increased accumulation of the drug carriers in tumor tissue by passive tumor targeting based on the enhanced extravasation of nanoparticles through porous blood vessels in majority tumors [6].

Polymer vesicles have been extensively studied due to their enhanced mechanical and chemical stability [2,7]. Such vesicles can be formed by the self-assembly of block copolymers or graft copolymers in selective solvents [2,7]. There has, however, been increasing concerns about the biodegradability and biocompatibility of polymer vesicles for biomedical applications. To address these concerns, poly(amino acid)s have garnered increasing notice, as they are considered to be suitable building blocks for the fabrication of biocompatible and biodegradable vesicles [4,8–10]. A poly(amino acid)-based vesicle was first fabricated by Fujita et al., and such vesicles may be formed from peptides or copolymers containing poly(amino acid) blocks [8]. Poly(amino acid)s can be synthesized using many different types of natural or non-natural amino acids, and display modulatory functions such as a stimuli-responsivity [11]. Notably, poly(amino acid)s form secondary structures such as α-helices and β-sheets that are stabilized by hydrogen bonds, and these relatively rigid structures have been shown to improve the structural stability of poly(amino acid)-based aggregates [4,9,10].

Chemical or physical cross-linking of structures can also increase their stability [12–15], and hence breaking these crosslinks in specified conditions can be used to destabilize or even break up a structure in targeted environments. In particular, redox-responsive disulfide cross-linked carriers have been shown

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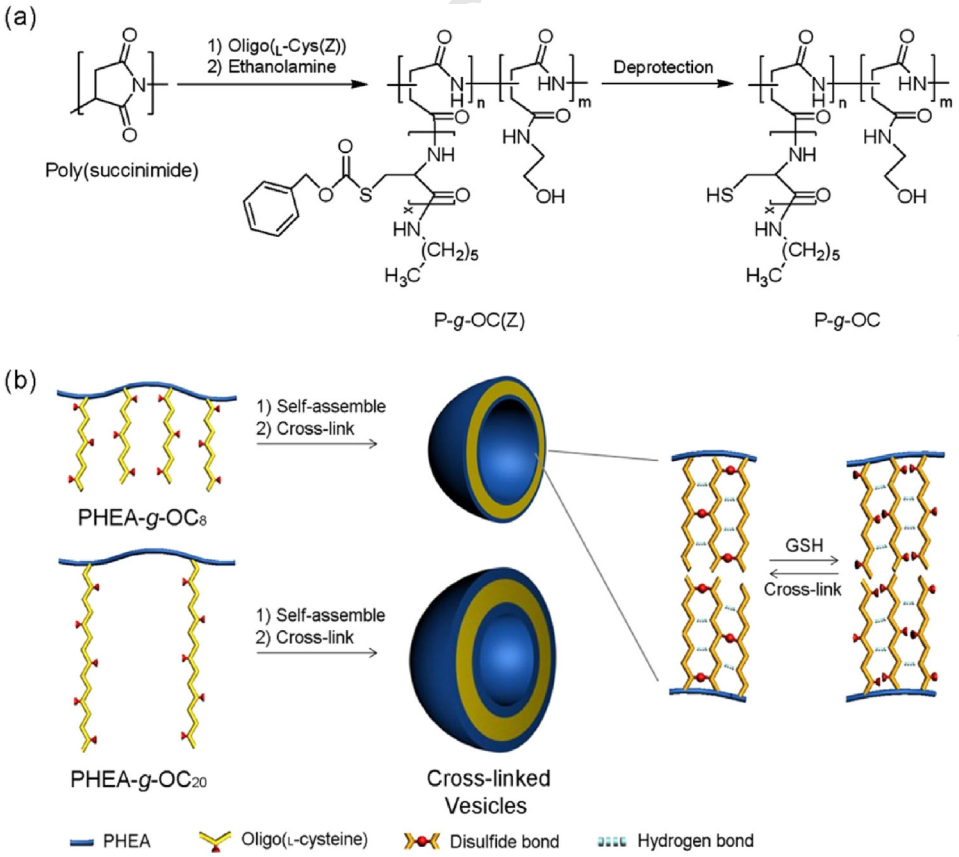
to accelerate drug release after cellular uptake because disulfide bonds tend to be cleaved inside the cell [15–18]. Here, the covalent disulfide bonds form by self-oxidation of thiol residues, and are cleaved in a reductive environment [19]. In the bloodstream or extracellular matrices, disulfide bonds are stable due to a low concentration of reducing agents such as glutathione (GSH). In contrast, the concentration of GSH in the cytosol is about a thousand times higher than that in the plasma — and at least four-fold higher in tumor tissues than in normal tissues [5,19]. Consequently, the large difference of GSH concentration between the intracellular and extracellular environments allows for a targeted release of drugs from structures whose stability is sensitive to the redox potential. For these reasons, cysteine, an amino acid containing a free thiol residue, has been receiving considerable attention for its use as a building block in the synthesis of self-crosslinkable and redox-responsive poly(amino acid) drug carriers [20–23]. Block and graft polymers containing poly(cysteine) and poly(ethylene glycol) have been successfully used to generate shell or core micelles cross-linked by disulfide bonds. However, cysteine-based vesicles have not yet been successfully investigated. Because vesicle structure has an aqueous cavity as well as a hydrophobic shell, the vesicle can stably encapsulate hydrophilic drugs unlike micellar structures. By taking advantages of the GSH sensitivity of poly(cysteine) and the vesicle structure, it would be possible to develop an ideal stimuli-responsive drug carrier for hydrophilic anticancer drugs.

Herein, we report the synthesis of disulfide cross-linked poly (amino acid) vesicles additionally stabilized by the β-sheet conformation and their triggered release of anti-cancer drugs in response to cellular GSH. We hypothesized that poly(amino acid) vesicles utilizing three common peptide-stabilization features found in nature – namely secondary structures, hydrophobic interactions, and cysteine disulfide cross-links – would serve as a stable yet GSH-responsive carrier system (Scheme 1). To prove this hypothesis, we designed and synthesized the amphiphilic poly(amino acid) poly(2-hydroxyethyl aspartamide)-*g*-oligo(L-cysteine) (P-*g*-OC) (Scheme 1). Poly(2-hydroxyethyl aspartamide) (PHEA) is a well-known water-soluble poly(amino acid) used as a drug delivery carrier due to its biodegradability, biocompatibility, nontoxicity and nonimmunogenicity [24,25]. Oligo(L-cysteine) is a hydrophobic polypeptide containing thiol residues, and preferentially forms the β-sheet secondary structure [23,26]. P-*g*-OC thus formed a "triply stabilized" vesicle structure in aqueous solution, and the shell thickness and particle size of this vesicle could be easily controlled by modifying the polymer. Because of the high stability of these vesicles, hardly any of the encapsulated anticancer drug doxorubicin (DOX) was prematurely released. However, the cross-linked vesicle abruptly released DOX upon encountering a reductive environment, which destabilized the cross-link. The P-g-OC vesicles demonstrated a successful triggered release of DOX inside cancer cells and the translocation of the drug to the nuclei of these cells, and the carrier system potentiated an anti-proliferation effect of the anticancer drug. To the best of our knowledge, this report is the first one describing a carrier system stabilized by both β-sheets and disulfide links.

Experimental methods

Synthesis of oligo(L-cysteine(Z)-OH)

The *N*-carboxyanhydride of L-cysteine(Z)-OH (C(Z)-NCA) was synthesized by the Fuchs-Farthing method using triphosgene [27]. Dried C(Z)-NCA (1 g) was dissolved in *N,N*-dimethylformamide (DMF, 7 mL) and heated to 60 °C in a nitrogen atmosphere.



Scheme 1. (a) Scheme followed to synthesize P-*g*-OC. (b) Illustration of the scheme followed to prepare a β-sheet-stabilized disulfide cross-linked vesicle.

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