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Completely disintegrable redox-responsive poly(amino acid) nanogels for intracellular drug delivery

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ABSTRACT

We report a disulfide cross-linked poly(amino acid) nanogel that is completely disintegrable in the intracellular reductive environment by cleavage of the disulfide cross-links. A short-poly(ethylene glycol)-grafted poly(succinimide) was synthesized as an amphiphilic prepolymer to prepare poly(amino acid) nanogels that consisted of a hairy PEG shell and a disulfide cross-linked poly(amino acid) core. The nanogels were prepared by chemical conversion of the hydrophobic core of the cross-linked micelles into a hydrophilic poly(aspartic acid) network. The nanogels disintegrated into fully water-soluble and biocompatible residues in the reductive condition. Post-endocytic disintegration of the nanogel improved nuclear translocation and efficacy of an anticancer drug.

Introduction

Stimuli-responsive polymeric nanoparticles are of great interest as drug carriers because they allow environmentally triggered drug release at target sites [1–3]. Such nanoparticle carriers also minimize side effects of, for example, anticancer drugs by stably encapsulating these drugs before reaching the targeted sites, and only releasing them at the tumor site.

Cross-linked nanostructures formed by chemical cross-linking with stimuli-cleavable linkages and/or physical cross-linking via stimuli-responsive non-covalent bonds provide improved protection of drugs due to the stability of cross-linked structures in the absence of destabilizing stimuli [3–5]. These nanostructures also allow stimuli-responsive release of the drugs at the target sites when they are destabilized by chemical or physical stimuli located specifically at the target site.

Among the various stimuli-responsive cross-links, the cleavage of covalent disulfide bonds using reduction reactions has been widely studied for post-endocytic degradation [6–10]. The disulfide bond is stable in the bloodstream and extracellular matrix due to low concentrations of reducing agents such as a glutathione (GSH) in these locations; in contrast, the concentration of reducing agents is 1000 times higher inside the cell than in the extracellular environment [8]. The concentration of GSH, in particular, in tumor tissue is four times higher than in normal tissue [11]. The large difference between intracellular and extracellular GSH concentrations leads to sudden destabilization of disulfide cross-linked carriers and consequent drug release after intracellular uptake. Though disulfide cross-linked polymeric micelles have shown post-endocytic drug release when these cross-links are cleaved, they maintain the micelle structure even after the disulfide cleavage, which impedes drug release; fully dissociable nanocarriers are therefore preferred [12].

Fully hydrophilic microgels and nanogels cross-linked by stimuli-cleavable bonds have shown structural destabilization after degradation, and consequent drug release [3,6,7,9,13]. However, only a few studies have demonstrated complete disintegration and generation of fully water-soluble or water-dispersible polymer residues by cleavage of cross-linkers [14–16]. Reported syntheses of stimuli-responsively degradable microgels/nanogels have also yielded non-optimally large particles for anticancer drug delivery, with diameters over 100 nm [13].

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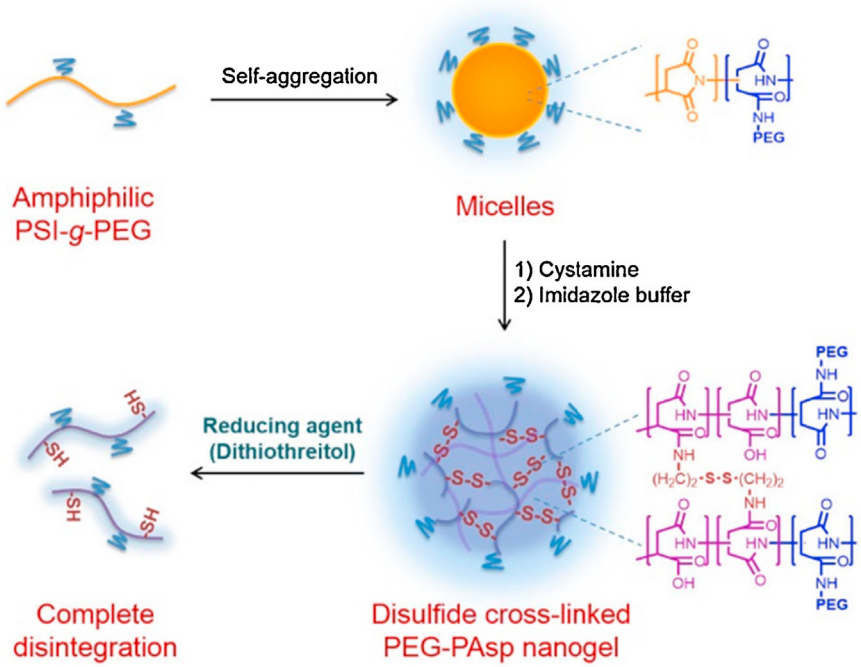

Fig. 1. Scheme of the synthesis of the disulfide cross-linked poly(amino acid) nanogel from the amphiphilic PSI-*g*-PEG and of its complete disintegration into hydrophilic polymers by reduction-sensitive decrosslinking.

Nanoparticles in a narrow size range of 10–100 nm generally lead to a high accumulation of carriers at the tumor site due to enhanced permeability and retention, and such nanoparticles generally show more efficient endocytic uptake than larger submicron particles [17–19].

In this paper, we report the simple synthesis of a disulfide cross-linked poly(amino acid) nanogel (<100 nm) that is completely disintegrable in a reducing environment, and report its efficient delivery of anticancer drugs to the nucleus. PEG-grafted poly(succinimide) (PSI-*g*-PEG) was synthesized as a diamine cross-linkable amphiphilic prepolymer to prepare poly(amino acid)-based nanogels by simple chemical conversion of the hydrophobic core of cystamine cross-linked PSI-*g*-PEG micelles into the hydrophilic PAsp (Fig. 1). Previously we demonstrated that PEG-PSI copolymers can readily produce cross-linked micelles and nanogels without surfactants, emulsions, or coupling agents [20–22]. However, this approach yielded nanogels with diameters larger than 100 nm. Hence, to produce nanogels with smaller diameters, in the present work we designed and synthesized a new copolymer, PSI-*g*-PEG. The hairy PEG shell of the nanogel is biocompatible and conceals the drug carriers and hence prolongs its circulation in the bloodstream [23]. The cross-linked poly(amino acid) core (PAsp) is biocompatible and biodegradable [24], and its negative charge provides effective electrostatically driven encapsulation of cationic anticancer drugs.

Experimental

Synthesis of poly(succinimide)-g-poly(ethylene glycol) (PSI-g-PEG)

PSI (M_n = 12,800, PDI = 1.32) was synthesized by acid-catalyzed thermal polycondensation of L-aspartic acid, as previously reported [25]. PEG-grafted PSI (PSI-*g*-PEG) was synthesized by reacting mPEG-NH_2 (2 g) with PSI (0.97 g) in *N,N*-dimethyl formamide (DMF; 20 mL) at 60 °C for 24 h. The resulting solution was dialyzed against deionized water using a dialysis membrane (MWCO = 6 ~ 8 kDa) to remove unreacted mPEG-NH_2, and the dried polymer was obtained by freeze-drying. For the calculation of the grafting efficiency, the unreacted PSI was converted into poly(2-hydroxyethyl aspartamide) (PHEA) by reaction with excess ethanolamine in dimethyl sulfoxide (DMSO).

Fluorescein-labeled PSI-*g*-PEG was prepared to analyze cellular uptake of nanogels. Fluoresceinthiocarbamyl ethylenediamine was synthesized by using the method of Pourfarzaneh et al. [26], and it was reacted with PSI-*g*-PEG in DMF for 24 h at 60 °C. The degree of substitution of fluorescein was less than 2%.

Preparation of poly(ethylene glycol)-poly(aspartic acid) nanogels

Spherical micelles of amphiphilic PSI-*g*-PEG were prepared by direct dissolution of the polymer in PBS buffer (10 mg/mL) with sonication for 20 min. Hexamethylenediamine or cystamine hydrochloride was added to the micelle solution as a cross-linking agent, and reacted for 48 h. The mole ratio of primary amine to succinimide was 0.5:1. To hydrolyze residual succinimide units, imidazole buffer was added to the reaction solution to be 0.1 M, and was stirred for 2 days. The resultant solution was neutralized by an HCl solution (1 M) and dialyzed against deionized water using a dialysis membrane (MWCO = 50 kDa) to remove unreacted cross-linking agents and uncross-linked PAsp-*g*-PEG, and the dried nanogels were obtained by freeze-drying. Nanogels cross-linked by hexamethylenediamine and cystamine are denoted as NHDA and NSS, respectively.

Preparation of doxorubicin-loaded PEG-PAsp nanogels and drug release analysis

PEG-PAsp nanogels were dissolved in deionized water and mixed with doxorubicin (DOX). The mole ratio of DOX to carboxylate was 1:8. The solution was titrated to pH 7 with a 0.1 M NaOH solution, and it was stirred for 6 h. The DOX-containing nanogels were washed with deionized water using a DOX-pre-treated centrifugal filter (MWCO = 10 kDa, Millipore), and the retentate was freeze-dried. To quantify the concentration of the encapsulated DOX drug, the amount of DOX in filtrate and