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Redox-stimuli responsive micelles from DOX-encapsulating polycaprolactone-*g*-chitosan oligosaccharide

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ABSTRACT

Chitosan-based amphiphilic graft copolymers are commonly obtained by modification of chitosan backbones with synthetic polymers hampering both bioactivity and biodegradability. In this work, we report the preparation of a series of chitosan oligosaccharide-grafted copolymers (PCL-*g*-COs) from coupling reactions between azide-pendent polycaprolactones (PCL-N_3) and reducing-end alkynyl-modified chitosan oligosaccharides (COs-alkynyl). The resulting PCL-*g*-COs self-organized in water into nanoscale micelles (R_h < 20 nm) having a COs shell and a PCL core. Locking of the core-micelles structure employing a disulfide-containing bis-alkyne cross-linker resulted in the formation of nano-vehicles which can be degraded in response to physiological (redox) stimuli. This feature was advantageously exploited to preferentially release an anticancer drug, doxororubicin, in response to the intracellular glutathione level.

1. Introduction

The development of biodegradable and biocompatible stimuli-responsive drug delivery systems has been extensively raised over the past decades. A large library of "smart" polymer micelles have already been developed in order to effectively deliver a drug to a target site and thus increase the therapeutic benefit while minimizing side effects (Kaditi, Mountrichas, Pispas, & Demetzos, 2012; Mura, Nicolas, & Couvreur, 2013; Zhang, Koa, & Oh, 2012). Internal stimuli-responsive (pH, ions, glucose, enzymes, temperature, redox, etc.) systems have gained wider attention compared to systems responding to external stimuli (light, ultrasound, magnetic and electric field, etc.) due to their self-controlled drug release and facile application in clinical settings.

Drug delivery systems employing a redox cleavage of disulfide bonds are of particular relevance because cytosol and cell nuclei contain 100–1000 times higher concentration of reducing glutathione (GSH) tripeptide than body fluids including blood and extracellular medium (0.5–10 mM *versus* 2–20 μM GSH) (Lee et al., 2013; McCarley, 2012) and because cancer cells often exhibit even higher levels of glutathione (Manickam et al., 2010). Such redox-sensitive nanovehicles are generally prepared from self-association of amphiphilic block or graft copolymers, containing hydrophobic and hydrophilic segments, linked or cross-linked through a disulfide bridge (Haradaa & Kataoka, 2006).

A wide variety of biodegradable redox-sensitive amphiphilic copolymers has been described to incorporate and release drugs. Hydrophobic polymers such as poly(caprolactone) (PCL), poly(lactic-*co*-glycolic acid), poly(lactic acid), polyphosphoesters and polypeptides have been coupled, for example, with hydrophilic poly(ethylene glycol) (Khorsand, Lapointe, Brett, & Oh, 2013; Wang, Wang, Sun, & Wang, 2011) or polysaccharides (Huanli et al., 2010; Minghui et al., 2013). Polysaccharides are of particular interest due to their renewability, non-toxicity, biodegradability, and for some of them, their biological and physicochemical properties (Gref, Rodrigues, & Couvreur, 2002).

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Chitosan, a linear copolymer of *N*-glucosamine and *N*-acetylglucosamine with β-1,4 linkage derived from partial de-*N*-acetylation of chitin, is certainly one of the most studied polysaccharides in the biomedical and pharmaceutical fields (Shukla, Mishra, Arotiba, & Mamba, 2013), particularly in drug delivery (Meng et al., 2013; Sanyakamdhorn, Agudelo, & Tajmir-Riahi, 2013) and gene transfection (Richard, Thibault, De Crescenzo, Bushmann, & Lavertu, 2013; Zonghua, Ziyong, Changren, & Yanpeng, 2010). The significant attention paid to chitosan-based materials stems from the attractive biological properties of this polysaccharide including analgesic, antitumor, hemostatic, hypocholesterolemic, antimicrobial and antioxidant activities. Chitosan also acts as a permeation enhancer by opening epithelial tight junctions in acidic environments (Artusso, Lindmark, Davis, & Illum, 1994) and strongly interacts with mucus or negatively charged mucosal surfaces (Sogias, Williams, & Khutoryanskiy, 2008). These features are mostly related to the ionizable character of chitosan due to the presence of amino functions at C-2, and to a lesser extent, to the molecular weight of the polysaccharide chains.

Aiming at generating nano-scale chitosan-based drug delivery systems, numerous studies have described the preparation of amphiphilic chitosan-based copolymers able to self-assemble in water. In this context, amphiphilic chitosan-based graft copolymers containing a chitosan backbone and hydrophobic pendent blocks have been extensively studied owing to their straightforward preparation (Novoa-Carballal, Riguera, & Fernandez-Megia, 2012). For instance, biodegradable polyesters have been anchored on chitosan chains through "grafting from" procedures (Qu, Wirsen, & Albertsson, 1999) or through "grafting onto" procedures by amidation, esterification or urethanization reactions with the numerous reactive amine or hydroxyl groups of the polysaccharides, respectively (Feng & Dong, 2006; Gao, Li, Lu, Wu, & Fuhrhop, 2008; Haijun, Wenshou, Xuesi, Chao, & Xiabin, 2006; Moyuan et al., 2012). These grafting strategies are undeniably of interest to prepare biohybrids but unfortunately are surely inadequate to maintain the original biological and biodegradable properties of chitosan; in order to do so it may be necessary that the chitosan backbone remains intact. Obviously, the preparation of bioactive chitosan-based copolymers should thus rely on the (more challenging) chemical modification of chitosan chain ends.

We recently reported an effective route to reducing end-functionalized chitosan oligosaccharides (COs) through aniline-catalyzed reductive amination (Guerry et al., 2013). It was shown that aniline derivatives such as 4-propargyloxyaniline readily react with chitosan reducing end groups to generate "clickable" COs in high isolated yield. A brief study showed the successful grafting of COs-alkynyl onto one well-defined PCL-N_3 backbone.

In this work, we take advantage of these preliminary findings to expand our study on a series of chitosan oligosaccharide-grafted polycaprolactones which are prone to self-assemble in aqueous environment as characterized by diffusion light scattering and transmission electron microscopy pictures. *In situ* core cross-clicking with a disulfide-containing cystamine linker allowed the study of the encapsulation and the release properties of doxorubicin (DOX)-loaded redox stimuli-sensitive micelles.

2. Experimental

2.1. Materials

Chitosan oligosaccharides (trade name FACOS) exhibiting low molecular weight (inferior to 2 kDa and DP = 4.8 estimated by ^{1}H NMR) (Guerry et al., 2013) and having an acetylation degree of 10% were kindly provided by Kitto Life Co., Ltd. The catalyst CuI·P(OEt)$_3$ was synthesized as reported in the literature (Ziegler, Fowler, Rodgers, & Wester, 1987). All other chemicals were obtained from commercial suppliers and used without further purification. Water was purified by a Milli-Q water purification system (Billerica, MA, USA). Dialysis membranes (Spectra/Por® – MWCO: 10,000 and 1000) were purchased from Spectrum Laboratories, Inc. 2,2-dibutyl-2-stanna-1,3-dioxepane (DSDOP) (Kricheldorf & Eggerstedt, 1998), reducing-end alkynyl-modified chitosan oligosaccharides (COs-alkynyl; DP = 4 estimated by ^{1}H NMR) (Guerry et al., 2013) and poly(α-azido-ε-caprolactone-*co*-ε-caprolactone) (PCL-N_3) were synthesized as reported in the literature (Riva, Schmeits, Jérôme, Jérôme, & Lecomte, 2007). The synthesis of the disulfide-containing bis-alkyne cross-linker, bis[(propargyl carbamate)ethyl] disulfide (Cys-alkynyl) (Liu et al., 2010), was adapted from a previously reported procedure. (Sinha, Ilankumaran, & Chandrasekaran, 1999). Briefly, to a solution of cystamine dihydrochloride (1 g, 4.4 mmol) dissolved into 12 mL dioxane was added aq. NaOH (0.01 M, 8 mL) to reach pH 9, and 4 equivalents of propargyl chloroformate (1.73 mL, 17.6 mmol) were dropwise added at r.t. and allowed to react for 2 h. The obtained Cys-alkynyl was purified by chromatography on silica gel (eluent: ethyl acetate/petroleum ether, 2/8, v/v) to give a colorless oil (1.2 g, 81% yield).

2.2. Instrumentation

^{1}H NMR spectra were recorded using 400 MHz Bruker Avance DRX400. Size exclusion chromatography (SEC) was performed at 40 °C using a Agilent 390-MDS system (290-LC pump injector, ProStar 510 column oven) equipped with a Varian 390-LC refractive index detector and a Knauer Smartline UV detector 2500 and two Agilent PolyPore PL1113–6500 columns (linear, 7.5 mm × 300 mm; particle size, 5 μm; exclusion limit, 200–2,000,000) in DMF/LiCl (0.01 M) at the flow rate of 1.0 mL min^{-1}. Infrared (IR) spectra were recorded using a Perkin-Elmer Spectrum RXI FTIR spectrometer. Transmission electron microscopy (TEM) was carried out using a CM200 Philips microscope. TEM images of the micelles were conducted using a CM200 Philips transmission electron microscope operating at 80 kV with Kodak SO163 film. One drop of micelle suspension was placed on a copper mesh covered with nitrocellulose membrane and dried at room temperature before being stained with uranyl acetate solution (2%, w/v in water). Scattering measurements were performed using an ALV laser goniometer, which consists of a 35 mW HeNe linearly polarized laser operating at a wavelength of 632.8 nm, an ALV/LSE-5004 multiple τ digital correlator with a 125 ns initial sampling time, and a temperature controller. Data were collected typically for a counting time of 300 s at 90° using the digital ALV correlator control software. Each sample was analyzed in triplicate. The aqueous solutions of PCL-*g*-COs were directly filtered into the glass cells through a 0.45 μm Millipore Millex MCE (Mix Cellulose Ester) filter. The relaxation time distribution was obtained using CONTIN analysis of the autocorrelation functions $C(q,t)$. All fluorescence measurements were performed using a Perkin Elmer LS50B spectrofluorometer. Excitation and emission were set at 480 nm and 520 nm respectively with bandwidths of 2.5 and 10 nm, respectively.

2.3. Methods

2.3.1. Synthesis of PCL-g-COs

Typically, 50 mg of PCL-N_3 was dissolved into 5 mL of DMF and allowed to react with 100 mg of COs-alkynyl in the presence of 58 mg of CuI·P(OEt)$_3$ under argon atmosphere. 300-500 μL of water was added to facilitate the COs-alkynyl dissolution. The solution was stirred at 40 °C for 36 h. The reaction mixture was poured into 80 mL of water containing 200 μL of acetic acid to precipitate unreacted PCL-N_3 and centrifuged at 8000 rpm during 20 min at 4 °C. The