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Self-aggregated nanoparticles of carboxylic curdlan-deoxycholic acid conjugates as a carrier of doxorubicin

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ABSTRACT

In this study, a new non-toxic, biodegradable, biocompatible and water-soluble carboxylic curdlan bearing the dissociable COOH group in 100% purity, which was prepared by 4-acetamido-TEMPO-mediated oxidation, was hydrophobically modified by deoxycholic acid (DOCA) to attain novel amphiphilic curdlan derivatives (CCDs) for the preparation of nano-carriers for antitumor drug doxorubicin (DOX). Under the effect of ultrasonication, the carboxylic curdlan derivatives in water were self-aggregated into spherical nanoparticles with diameters ranging from 214 nm to 380 nm. The critical aggregation concentrations decreased from 0.047 mg/mL to 0.016 mg/mL with increasing DS of DOCA. DOX-loaded CCD nanoparticles were prepared in an aqueous medium with dialysis method. The DOX-CCD nanoparticles exhibited pH- and dose-dependent drug release profiles during *in vitro* release experiments. Moreover, the drug transport mechanism was Fickian diffusion according to the Ritger–Peppas model. The CCD nanoparticles might be explored as potential carriers for hydrophobic drugs with controlled release and delivery functions.

1. Introduction

Cancer is one of most threatening and deadly human diseases and chemotherapy is a common clinical treatment for cancer patients. However, the chemotherapeutic agents often cause serious side effects to the patients due to their non-specific toxicity to both normal and cancer cells. Considerable effort has been put forward to developing more favorable anticancer agents with high selectivity and low cytotoxicity. In recent years, there has been a wide and increasing interest in the development and use of nanoparticles as the carriers for site-specific drug delivery of cancer drugs [1]. In particular, polymeric nanoparticles have been shown to accumulate preferentially at the tumor sites and can be promising drug carriers to improve drug efficacy and to reduce the side effects [2,3].

Polymeric amphiphiles, which are composed of hydrophilic and hydrophobic segments, can form micelles or nanoparticles spontaneously through intra- and/or inter-molecular interactions between hydrophobic segments in aqueous media. These polymeric micelles or self-aggregated nanoparticles, which consist of an inner core of hydrophobic segments and an outer shell of hydrophilic segments, have been widely used as reservoir for various hydrophobic drugs [2–4]. As natural biomaterials, polysaccharides are highly stable, non-toxic, hydrophilic, biodegradable, and abundant in nature. Most polysaccharides have numerous hydrophilic groups such as hydroxyl, carboxyl, and amino groups which can form non-covalent bonds with biological tissues for bioadhesion [5]. Nanoparticle carriers formed with bioadhesive polysaccharides can prolong the release of loaded agents. Recently, a large number of water-soluble polysaccharides and their derivatives such as chitosan, pullulan, dextran, and heparin have been hydrophobically modified to form the self-assembly of amphiphilic polysaccharides for their potential applications as nanoparticle drug delivery systems [6,7].

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Curdlan is an exopolysaccharide produced by a *Alcaligenes faecalis* bacterium and belongs to a linear β-1,3-glucan [8]. Curdlan derivatives with various structures have been prepared through chemical modifications to improve the water solubility and bioactivity of curdlan. For example, carboxymethylated curdlan (CM-curdlan) [9–11], a negatively charged curdlan derivative, has been widely used to synthesize amphiphilic CM-curdlan conjugates by hydrophobic modifications to prepare self-aggregated nanoparticles in aqueous media as cancer drug carriers [12]. However, the preparation of CM-curdlan usually depends on the use of the esterification reagent chloroacetic acid, which is potentially toxic to the environment. More recently, we prepared water-soluble carboxylic curdlan (CC) derivatives using 4-acetamido-TEMPO/NaClO/$NaClO_2$ system under mild conditions [13]. The results indicated that these CC derivatives were non-toxic, biodegradable and biocompatible, and exhibited more flexible chain conformations than that of CM-curdlan with rigid triple helical conformation in aqueous solution [14], and this can be more convenient for the preparation of amphiphilic conjugates. Furthermore, these CC derivatives have the potential to produce gene carriers, bio-nanomaterials, and other chiral nanowires through hydrogen bonding interactions, hydrophobic interactions, and electrostatic attraction. In particular, the easily changeable charge of CC in media of various pH may provide potential applications in nanoparticle carriers [15]. In addition, these derivatives produced from 4-acetamido-TEMPO-mediated oxidation have notable bioactivities and functional properties [13,16]. However, the introduction of hydrophobic segments such as deoxycholic acid (DOCA) into CC to form self-aggregated nanoparticles that act as novel carriers for hydrophobic antitumor drugs has not yet been reported.

This study was to synthesize self-aggregated nano-particles of CC-DOCA (CCD) conjugates and evaluate their functions as the antitumor drug carriers. CCD conjugates with various degrees of substitution (DS) were first synthesized by hydrophobic modification with DOCA, and their structural characteristics were determined by Fourier transform infrared (FT-IR) spectroscopy and X-ray diffraction (XRD) measurements. The physicochemical properties of the CCD self-aggregated nanoparticles prepared by probe sonication were characterized in an aqueous medium. Doxorubicin (DOX) was then used as a model drug and loaded onto the CCD nanoparticles and their *in vitro* drug release and transport mechanism were investigated.

2. Materials and methods

2.1. Materials and chemicals

Commercial curdlan (M_w 1.1×10^6 g/mol) and 4-acetamido-TEMPO were purchased from Wako Pure Chemical Corporation (Osaka, Japan). Sodium chlorite, 12% sodium hypochlorite solution, DOCA, 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide (EDC), N-hydroxyl succinimide (NHS), and pyrene were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). DOX·HCl was obtained from Dalian Meilun Biology Technology Co., Ltd., China. All other chemicals and solvents were of laboratory grade and used without further purification.

2.2. Preparation and characterization of carboxylic curdlan

Carboxylic curdlan (CC) bearing the β-1,3-polyglucuronic acid structure was prepared by 4-acetamido-TEMPO-mediated oxidation using a previously described method [13]. CC had a carboxylate content of 2.07 mmol/g by electric conductimetric titration and a weight-average molecular weight (M_w) of 5.7×10^5 g/mol by size-exclusion chromatography with multi-angle laser-light scattering analysis.

2.3. Synthesis of CC-DOCA conjugates

The carboxyl groups of DOCA (0.3 g) in 60 mL of anhydrous DMSO were activated by the addition of EDC (0.36 g) and NHS (0.18 g) at room temperature (25 °C) for 0.5 h. Different amounts of activated DOCA (50 mg to 150 mg) were added to 20 mL of anhydrous DMSO solution containing 0.2 g of CC. The resulting solutions were stirred at room temperature under nitrogen atmosphere for 48 h. The reaction mixture was precipitated by adding excess cold acetone, and the precipitate was collected by centrifugation (5000 rpm, 30 min) and washed three times with acetone and methanol to remove any excess DOCA. The precipitate was dissolved in deionized (DI) water and dialyzed (MWCO: 8000–12,000 g/mol) against DI water for 48 h, followed by lyophilization to obtain CCD conjugates. The CCD products attained were placed in a desiccator before use.

2.4. Characterization of CCD conjugates

The DS of DOCA was spectrophotometrically determined according to the procedure described by previous reports [17]. DS (mol%, expressed as mole DOCA/100 sugar residues of CC) was calculated according to the given equation, $DS = (c/M_{DOCA})/((m-c)/M_{CC})$, where c is the content of DOCA determined from the corresponding calibration curve, m the amount of modified polymers used in the experiment, M_{DOCA} the M_w of the DOCA residue and M_{CC} the M_w of the anhydroglucose units of CC.

The FT-IR spectra of curdlan and derivatives were determined using a Nexus 670 FT-IR spectrometer (Thermo Nicolet Co., USA) in the wave number range of 500 cm^{-1} to 4000 cm^{-1} with KBr pellets. The ^1H NMR spectra were measured in D_2O or D_2O/DMSO-d_6 (3:1, v/v) using a Bruker AV 400 instrument (Bruker Co., Germany) and the data were processed using MestRenova v6.1.0-6244 software (Mestrelab Research SL). Curdlan and derivatives were pressed into pellets for the wide-angle XRD analysis (Bruker Co., Germany); the XRD patterns with C_u K_α radiation ($\lambda = 0.15406$ nm) at 40 kV and 40 mA were recorded in the region of 2θ from 5° to 80° with a step speed of 4°/min.

2.5. Preparation of self-aggregated nanoparticles of CCD conjugates

The self-aggregated nanoparticles of CCD conjugates were prepared using an ultrasonic method [11]. The CCD conjugates (10 mg) were suspended in deionized water under gentle shaking at 37 °C for 24 h, followed by sonication three times using a probe horn (JY92-II Ultrasonic Processor, Xinzhi, Ningbo, China) at 100 W, 2 min at an interval of 2 s to minimize temperature rise. The self-aggregated nanoparticles obtained were then filtered through a 0.45 μm filter and stored at 4 °C in a refrigerator before analysis.

2.6. Measurement of fluorescence spectroscopy (pyrene)

The self-aggregation behavior and critical aggregation concentration (*cac*) of CCD conjugates were measured by probe fluorescence technique using pyrene as a hydrophobic probe according to Wilhelm et al. [18]. In brief, 10 μL of pyrene solution (3.0×10^{-4} mol/L) in absolute methanol was added to a series of centrifuge tubes, followed by evaporation to remove the methanol. Various concentrations (1.0×10^{-4} mg/mL to 1.0 mg/mL) of as-synthesized CCD conjugate suspension (5.0 mL) were added to centrifuge tubes and sonicated in an ultrasonic bath (HS6150,