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Oxidation and pH responsive nanoparticles based on ferrocene-modified chitosan oligosaccharide for 5-fluorouracil delivery

Youqian Xu, Liang Wang, Ya-Kun Li, Cai-Qi Wang*

School of Chemistry and Chemical Engineering, University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Stimuli-responsive nanoparticles based on biodegradable and biocompatible saccharides are potentially superior carriers under different physical conditions. In this study, we present a detailed investigation on the oxidation and pH responses of ferrocene-modified chitosan oligosaccharide (FcCOS) nanoparticles for 5-Fluorouracil (5-FU) Delivery. The dispersion of FcCOS nanoparticles depends strongly on pH change. NaClO, H_2O_2 and oxygen, as oxidant models, in a weak acid solution displayed varying accelerations as the disassembly progressed. 5-FU, as a drug model, is efficiently uploaded in FcCOS nanoparticle (approximately 238 nm). The in vitro release of 5-FU from FcCOS nanoparticles studies show that the accumulative release increased with the decrease of pH under bubbled N_2. Interestingly, the sample under bubbled air has a higher accumulative release up to 59.64% at pH 3.8, compared with samples under bubbled N_2 just 49.02%. The results suggested that FcCOS nanoparticles disassembled faster and the release of drug molecules was accelerated because of the synergistic effect of oxidative agent and low pH. Thus, FcCOS can be developed as an effective pH and oxidation dual-responsive carrier to enhance drug efficacy for cancer treatment.

1. Introduction

Nanoparticles that are sensitive to redox potential, pH and temperature have attracted considerable attention in nanomedicine because their structures can be altered by small external changes (Angelatos, Radt, & Caruso, 2005; Chen et al., 2014; Cheng, Meng, Deng, Klok, & Zhong, 2013; Li & Keller, 2009; Motornov, Roiter, Tokarev, & Minko, 2010; Zhou, Du, Cui, & Zhang, 2013). In contrast to a single-stimulus system, a dual-stimuli system shows a synergistic effect, thereby enhancing the recognition efficiency for targeting tumors. Dual-stimuli responsive nanoparticles have shown distinct drug delivery and release behaviors, leading to superior anti-cancer efficacy (Kumar & De, 2014; Xuan, Han, Xia, & Zhao, 2014). The dual stimuli, redox potential and pH, have attracted attention because activities of oxidative and pH microenvironments are different between tumor and healthy cells in vivo (Ge & Liu, 2013). The pH of tumor internal environment is lower than that of normal physiological environment, which is advantageous in designing pH-responsive drug carriages. At the same time, reactive oxygen species (ROS) and other oxidants have important functions in several physiological processes, such as cell signaling and metabolism (Borrelli et al., 2014; Song, Ji, Du, & Li, 2013). Thus, nanocarriers that are responsive to the described bio-relevant stimuli or characteristic biochemical signals in pathologic tissues and cells may be utilized to enhance therapeutic efficacy.

Although much progress has been made on the assembly of nanoparticles stimulated by pH and oxidation, challenges for clinical application still exist (Ge & Liu, 2013). Nanoparticles designed for biomedical applications should be biocompatible, biodegradable, and non-toxic. However, currently available constructed nanocarriers are mainly based on organic/inorganic hybrid or blocked part biocompatible and biodegradable materials (Ge &

* Corresponding author at: School of Chemistry and Chemical Engineering, University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, PR China. Tel.: +86 10 88256920; fax: +86 10 88256093.
E-mail addresses: wang-caiqi@ucas.ac.cn, wang.caiqi@sohu.com (C.-Q. Wang).

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Liu, 2013; Salonen et al., 2005; Wang, Chen, Ding, & Yan, 2012; Xuan et al., 2014). Chitosan oligosaccharide (COS), the oligomer of chitosan (CS), has been utilized to fabricate amphiphilic biofunctional materials because COS possesses excellent biocompatibility, biodegradability, and desirable bioactivity for biological applications (Kim & Rajapakse, 2005; Xia, Liu, Zhang, & Chen, 2011). COS can also inhibit the growth of tumor cells by exerting immune enhancing effects via increased production of lymphokines (Xia et al., 2011). Thus, COS has attracted considerable interest in the preparation of drug or gene carriers for drug delivery applications. The responsive assembly of COS derivatives to temperature and pH have been investigated (Shi, Alves, & Mano, 2008; Sun, Shi, Xu, & Cao, 2013; Wei, Luo, Fu, Zhang, & Ma, 2012). However, the response of COS derivatives to oxidation receives relatively less attention.

Ferrocene (Fc) is an organometallic compound that consists of two cyclopentadienyl (Cp) rings bound on the opposite sides of a central metal atom, which is known for its redox properties and hydropobicity (Duan et al., 2013; Liu et al., 2012; Zhu, Shangguan, Sun, Ji, & Zheng, 2010). The interconversion between Fe(II) and Fe(III) states results in the hydrophobicity to hydrophilicity transition of the hydrophobic block of Fc-containing amphiphiles (Zhu et al., 2010). Therefore, Fc is exploited in designing stimuli-sensitive assemblies, which acted as both hydrophobic moiety and redox-responsive trigger (Fouda, Abd-Elzaher, Abdelsamaia, & Labib, 2007; Top et al., 2001). Many ferrocenyl derivatives have excellent effects as antitumor agents, and some derivatives are now in clinical trials (Fouda et al., 2007; Top et al., 2001). Therefore, Fc derivative assembly equipped with pH responsive characteristics has a unique advantage in the drug delivery system.

5-Fluorouracil (5-FU), a pyramidine analogue that interferes with thymidylate synthesis, has been employed extensively in clinical chemotherapy for the treatment of solid tumors (Wen, Li, & Wu, 2012). However, the use of 5-FU is still limited by its rapid metabolism, short biological half-life, non-uniform absorption, and nonselective action against healthy cells (Rejinold, Muthunarayanan, Chennazhi, Nair, & Jayakumar, 2011). CS is useful for the prevention of side effects, such as myelotoxicity, gastrointestinal toxicity, and immunotoxicity that are caused by 5-FU (Aydin & Pulat, 2012; Kimura & Okuda, 1999; Yang & Hon, 2009). Given these properties, COS can be the most suitable candidate for 5-FU delivery.

Recently, our group presented a novel and facile method for constructing micro-size "snowflake-like" assemblies that respond to pH and oxidation stimuli. The "snowflake-like" assemblies with micron size are formed via the self-organization of Fc-modified COS with substitution degree as 0.24 in an acid aqueous solution (Wang, Li, Xu, & Wang, 2014). However, the controlled release behavior of drug-load FcCOS assemblies under various pH buffers and bubbled oxygen was not concerned. In this work, oxidation and pH responsive nanoparticles based on FcCOS for 5-FU Delivery were detailedly investigated. 5-FU was loaded in FcCOS nanoparticles and the controlled release behavior under various pH buffers and bubbled oxygen in weak acid solutions was evaluated.

2. Experimental

2.1. Materials

Chitosan oligosaccharide (COS) (M_W < 3000, deacetylation degree 92%) was purchased from Dalian GlycoBio Co. Ltd. Ferrocene carboxaldehyde (FcCHO) (98%) and 5-fluorouracil (5-Fu) (99%) were obtained from J&K Chemical Ltd. Sodium hypochlorite (NaClO) (≥8.0%, analytical grade) was purchased from Xilong Chemical Co. Ltd. Hydrogen peroxide (H_2O_2) (≥30%, analytical grade) was obtained from Beijing Chemical Works. Sodium borohydride ($NaBH_4$) was purchased from Sinopharm Chemical Reagent Co. Ltd. All the other chemical reagents in the study were in analytical grade and utilized without further purification. Deionized water (specific resistance 18.25 MΩ) was used in all experiments.

2.2. Synthesis of Fc-modified COS

Fc-modified COS (FcCOS) was synthesized according to the previous literature (Garcia, Peniche-Covas, Chico, Simpson, & Villalonga, 2007). COS (1.93 g, 11 mmol -NH_2) was dissolved in 20 mL of aqueous acetic acid (3.0 wt%) and FcCHO (2.57 g, 12.01 mmol) solution in 20 mL methanol was added dropwise under the protection of nitrogen (N_2). The mixed solution was vigorously stirred at room temperature for 1 h, and $NaBH_4$ (1.36 g, 36 mmol) was added and left stirred for 4 h. 5.0% NaOH aqueous solution was instilled to quench the reaction until the pH of the system reached 10. The resulting yellowish-brown precipitate was detached by centrifugation and washed with 90% methanol repeatedly until the absence of FcCHO was confirmed by spectrophotometer in the elution. The solid products were dried under vacuum at ambient temperature.

2.3. The degree of substitution of ferrocene

The degree of substitution of ferrocene (DS_{Fc}) on COS was quantified by UV–vis spectrophotometer according to following equation:

$$DS_{Fc} = \frac{c_{Fc}V/M_{Fc}}{DD \times \left[m - (M_{Fc} - 16)(c_{Fc}V/M_{Fc})\right]/M_{avg}} = \frac{c_{Fc}M_{Fc}}{DD \times (c_0 M_{Fc} - c_{Fc}M_{Fc} + 16c_{Fc})} \quad (1)$$

where c_{Fc} is the ferrocene concentration of resultant determined by UV–vis spectrophotometer, mg/mL; DD is the deacetylation degree of COS; M_{avg} is the average molecular weight of COS unit, mg/mL; V is the volume of the sample, mL; c_0 is the concentration of the sample, mg/mL; M_{Fc} is the molecular weight of FcCHO, mg/mmol; 16 is the difference in molecular weight before and after modification.

2.4. Preparation of FcCOS nanoparticles

The nanoparticles of FcCOS were prepared in the acid aqueous solution of HAc (2 wt%), followed by sonication for 30 min (KQ-50B ultrasonic bath, Kunshan Ultrasonic Instrument Co., China, 50 W). The morphology and size distribution of the nanoparticles were tested by TEM and DLS respectively.

2.5. Determination of critical nanoparticle concentration (CMC)

The critical micelle concentration (CMC) was determined by surface tension. Contact angle of 5 μL water droplet on the tetrafluoroethylene platform was measured with intravenous drip contact angle instrument (JC2000C1, Shanghai Zhongchen Digital Technology Co., Ltd.) at 25 °C and repeated at least three times.

2.6. pH responses of the FcCOS nanoparticles

The nanoparticles solution was prepared with the concentration of 1 mg/mL. The pH of the solution was adjusted by HAc and NaOH to investigate the change of FcCOS nanoparticles. The performance at different pH values were determined by UV–vis spectrophotometer.