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Enhanced water-solubility, antibacterial activity and biocompatibility upon introducing sulfobetaine and quaternary ammonium to chitosan

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ABSTRACT

Chitosan (CS) has attracted much attention due to its good antibacterial activity and biocompatibility. However, CS is insoluble in neutral and alkaline aqueous solution, limiting its biomedical application to some extent. To circumvent this drawback, we have synthesized a novel *N*-quaternary ammonium-*O*-sulfobetaine-chitosan (Q_3BCS) by introducing quaternary ammonium compound (QAC) and sulfobetaine, and its water-solubility, antibacterial activity and biocompatibility were evaluated compare to *N*-quaternary ammonium chitosan and native CS. The results showed that by introducing QAC, antibacterial activities and water-solubilities increase with degrees of substitution. The largest diameter zone of inhibition (DIZ) was improved from 0 (CS) to 15 mm (*N*-Q_3CS). And the water solution became completely transparent from pH 6.5 to pH 11; the maximal waters-solubility was improved from almost 0% (CS) to 113% at pH 7 (*N*-Q_3CS). More importantly, by further introducing sulfobetaine, cell survival rate of Q_3BCS increased from 30% (*N*-Q_3CS) to 85% at 2000 μg/ml, which is even greater than that of native CS. Furthermore, hemolysis of Q_3BCS was dropped sharply from 4.07% (*N*-Q_3CS) to 0.06%, while the water-solution and antibacterial activity were further improved significantly. This work proposes an efficient strategy to prepare CS derivatives with enhanced antibacterial activity, biocompatibility and water-solubility. Additionally, these properties can be finely tailored by changing the feed ratio of CS, glycidyl trimethylammonium chloride and NCO-sulfobetaine.

1. Introduction

Chitosan (CS) has emerged as an important natural polymeric biomaterial in wound healing (Moura et al., 2014), drug delivery (Casettari & Illum, 2014; Xu, Strandman, Zhu, Barralet, & Cerruti, 2015), tissue engineering (Bondalapati, Ruvinov, Kryukov, Cohen, & Brik, 2014; Choi et al., 2015) due to its antibacterial activity (Regiel-Futyra et al., 2015; Sahariah et al., 2015), biocompatibility and biodegradability (Lu, Slomberg, & Schoenfisch, 2014). The antibacterial property of CS stems from its polycationic structure, as the amino groups can be protonated in weak acidic aqueous solutions. However, CS is insoluble in neutral and alkaline aqueous solution, owing to the linear aggregation of chain molecules and the formation of crystals due to intra- and intermolecular hydrogen bonding (Anitha et al., 2014). The poor solubility of CS in water restricts its biomedical application to some extent. Fortunately, CS contains one primary amino (NH_2) group and two hydroxyl (OH) groups in its constitutional unit, and these reactive sites can be easily synthetically modified to obtain derivatives with improved biological properties (Bondalapati et al., 2014; Sahariah et al., 2015) and water-solubility (Kim, Ryu, Lee, & Lee, 2013).

Turning NH_2 group into a quaternary ammonium salt (Wiarachai, Thongchul, Kiatkamjornwong, & Hoven, 2012), or introducing quaternary ammonium compounds (QACs) (Wan, Xu, Sun, & Li, 2013) or quaternary phosphonium compounds (Guo et al., 2014) to CS via chemical reactions with NH_2 or OH groups to form covalent bond are typical ways to improve its positive charge, leading to enhanced antibacterial activity (Guo et al., 2014; Wan et al., 2013) and water-solubility (Wen et al., 2015). But they could also increase their toxicity (Lv, Zhang, Wang, Cui, & Yan, 2006). Though its water-solubility (Krause et al., 2011) and biocompatibility (Wang et al., 2013) can be improved by introducing carboxyl groups, polyethylene glycol to CS, But they usually compromise

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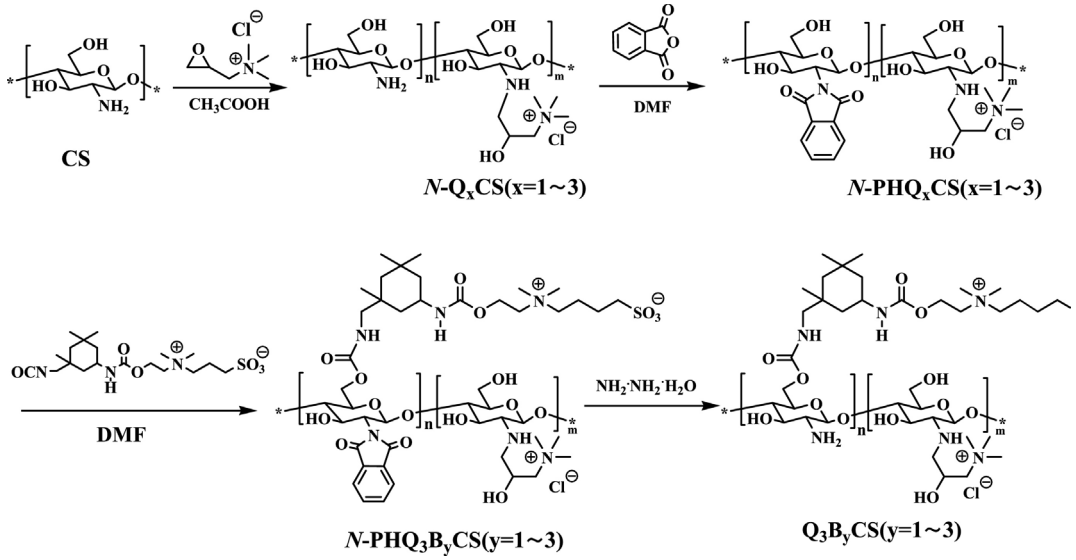

Scheme 1. Synthetic route of CS derivatives *N*-Q_xCS (x = 1–3) and Q_3B_yCS (y = 1–3).

its antibacterial activity (Xu, Xin, Li, Huang, & Zhou, 2010). This is an important problem having hindered CS for its widespread implementation.

Recently, zwitterionic compounds have attracted much attention owing to their excellent resistance to nonspecific protein adsorption (Shao & Jiang, 2015; Zhang et al., 2013), super-low platelet adsorption (Bai et al., 2014), good antibacterial activity (Chen et al., 2011a,b; Mi & Jiang, 2014) and hydrophilicity (Chen et al., 2011a,b). Herein, a novel reactive sulfobetaine was synthesized for the first time as shown in Scheme 1. QAC was introduced to improve antibacterial activity and water-solubility of CS, in the following way, biocompatible sulfobetaine were further introduced to *N*-Q_3CS to improve its biocompatibility, leading to novel CS derivatives with good antibacterial activity, water-solubility and biocompatibility. Furthermore, the zeta potential, antibacterial activity, water-solubility, cytotoxicity and hemolysis of these series CS derivatives were comprehensively evaluated compare to CS. This work may open a new avenue to design and synthesize CS derivatives as well as comprehensive utilize of CS.

2. Experimental

2.1. Materials

Chitosan with a viscosity-average molecular weight of 5.0×10^4 was purchased from Zhejiang Golden-Shell Pharmaceutical Co. Ltd. (China). Glycidyl trimethylammonium chloride (GTMAC) was purchased from Sigma Aldrich with a purity of 90%. Phthalic anhydride (99%), Isophorone diisocyanate (IPDI) (99%), *N*,*N*-Dimethylethanolamine (99%), Hydrazinium hydroxide (99.5%) and acetic acid (99.5%) were purchased from Energy Chemical (Shanghai, China) and used without further purification. 1,3-Propanesultone (1,3-PS, 98%) was purchased from Energy Chemical and recrystallized from ethyl acetate. All other solvents (analytical reagent) were purchased from Guangzhou Chemical Reagent Factory (China) and used as received. Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*, ATCC 6538), and gram-negative bacteria *Escherichia coli* (*E. coli*, ATCC 25922) were purchased from Guangdong Institute of Microbiology, and were incubated at 37 °C on a nutrient agar plate for 24 hours (h) before use. *Human skin fibroblast* (HSF, KCB 200537) were provided by Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China).

2.2. Synthesis of sulfobetaine containing isocyanate (NCO-sulfobetaine)

The reactive sulfobetaine containing isocyanate (NCO-sulfobetaine) was synthesized based on the reactive principle between NCO and OH group (Chen, Hu, Zhang, Li, & Rong, 2004; Chen et al., 2006) was well as ring-opening reaction of 1,3-propanesultone and tertiary amine (Chen et al., 2011a,b) (Scheme S1) presents the synthetic process of reactive sulfobetaine with isocyanate group (NCO-betaine). Briefly, 22.23 grams (g) of isophorone diisocyanate (IPDI, 0.1 mol), 8.91 g of *N*,*N*-dimethylethanolamine (0.1 mol) and dibutyltin dilaurate (0.1 ml) were added to a 250 ml three-neck round bottom flask equipped with a reflux condenser. The mixture was continuously stirred for 12 h at 60 °C, then, 12.20 g of 1,3-propanesultone (0.1 mol, soluble in 20 ml butanone) was dropped slowly to the mixture, and then large white precipitate was obtained after the mixture was continuously stirred for 5 h at 60 °C. The precipitate was further purified using vacuum filtering, and the filter residue was washed with diethyl ether and dried at 50 °C in vacuum oven for 24 h. Finally, the resulting *NCO-betaine* (white powder) was obtained in a yield of 90%. ^{1}H NMR (600 MHz, D_2O, 25, TMS, δ) (Fig. S2): 4.52 (s, 2*H*), 3.73 (s, 2*H*), 3.58 (d, 2*H*, *J* = 7.0 Hz), 3.20 (s, 6*H*), 3.00 (t, 2*H*, *J* = 5.2 Hz), 2.89 (d, 1*H*, *J* = 13.6 Hz), 2.28 (t, 2*H*, *J* = 7.89 Hz), 2.25 (s, 2*H*), 1.56 (s, 6*H*), 1.07 (m, 6*H*), 0.95 (m, 3*H*).

2.3. Synthesis of N-quaternary chitosan derivatives N-Q_xCS (x = 1–3)

In order to improve the antibacterial activity, *N*-quaternary chitosan derivatives *N*-Q_xCS (x = 1–3) were prepared according to previous references (Peng et al., 2010) (Scheme 1). In brief, 28.8 g of chitosan (CS, 180 mmol) was completely solubilized in 900 ml of aqueous solution of acetic acid (1 w/v%) under continuously stirring. Solution o f 9 g (60 mmol, 1 eq.), 18 g (120 mmol, 2 eq.), and 27 g (180 mmol, 3 eq.) of GTMAC in 40 ml of deionized water were added slowly into 300 ml of above CS/acetic acid aqueous solution (60 mmol, 1.0 eq.) under continuously stirring, respectively. After the mixtures were continuously stirred for 12 h at 50 °C, homogeneous and transparent yellow solutions were obtained. The yellow solutions were further purified using a dialysis tube (molecular weight cut off, MWCO 14,000 Da) against deionized water. The final *N*-Q_xCS (x = 1–3) were obtained by lyophilization at −80 °C for 48 h.