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Enhanced water-solubility and antibacterial activity of novel chitosan derivatives modified with quaternary phosphonium salt

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ABSTRACT

Chitosan (CS) has been widely recognized as an important biomaterial due to its good antimicrobial activity, biocompatibility and biodegradability. However, CS is insoluble in water in neutral and alkaline aqueous solution due to the linear aggregation of chain molecules and the formation of crystallinity. This is one of the key factors that limit its practical applications. Therefore, improving the solubility of CS in neutral and alkaline aqueous solution is a primary research direction for biomedical applications. In this paper, a reactive antibacterial compound (4-(2,5-Dioxo-pyrrolidin-1-yloxycarbonyl)-benzyl)-triphenyl-phosphonium bromide (NHS-QPS) was synthesized for chemical modification of CS, and a series of novel polymeric antimicrobial agents, *N*-quaternary phosphonium chitosan derivatives (*N*-QPCSxy, x = 1–2,y = 1–4) were obtained. The water solubilities and antibacterial activities of *N*-QPCSxy against *Escherichia coli* and *Staphylococcus aureus* were evaluated compare to CS. The water solubility of *N*-QPCSxy was all better than that of CS at neutral pH aqueous solution, particularly, *N*-QPCS14 can be soluble in water over the pH range of 3 to 12. The antibacterial activities of CS derivatives were improved by introducing quaternary phosphonium salt, and antibacterial activity of *N*-QPCSxy increases with degree of substitution. Overall, *N*-QPCS14 represents a novel antibacterial polymer material with good antibacterial activity, waters solubility and low cytotoxicity.

1. Introduction

To satisfy our intensively increasing demand on health, an increasing number of studies have been devoted to numerous novel biomaterials for different biomedical applications [1]. Research on biomaterials has focused primarily on interactions between the material and biological samples, the production cost [2], and the performance of the material. Among all these materials, chitosan (CS), the *N*-deacetylation product of the natural biological polysaccharide chitin, has been widely applied in wound healing [3] [4], drug delivery [5–7], tissue engineering [8], gene delivery [9,10] due to its antimicrobial activity [11,12] and biocompatibility and biodegradability [13]. The application of CS for a polymeric antibacterial material is based on its polycationic structure as the amino groups (at the C-2 position) can be protonated in water at pH < 6.5. However, CS is insoluble in water in neutral and alkaline pH, due to the linear aggregation of chain molecules and the formation of crystallinity caused by its intra and intermolecular hydrogen bonds [14]. Therefore, improving the solubility of CS in neutral and alkaline aqueous solution is a primary research direction for biomedical applications. Fortunately, CS can be easily modified due to the existence of abundant reactive groups including hydroxyl groups (one primary hydroxyl at C-6 position and one secondary hydroxyl at C-3 position) and amino groups (at C-2 position). Therefore, chemical modifications of CS have been studied intensively in order to increase its water solubility and antibacterial activity [15,16].

Usually, antibacterial activity and solubility of CS have been improved by quaternary chitosan derivatives, which were synthesized by introducing a functional quaternary ammonium moiety to dissociate hydroxyl or amino groups [17,18]. Recently, quaternary phosphonium was reported to be a better antibacterial agent compared with quaternary ammoniums [15,16]. Therefore, it is assumed that introducing quaternary phosphonium to the CS structure by chemical modification is a better approach to increase antibacterial activity of CS.

In addition, the *N*-phosphonium chitosan derivatives modified by (2-carboxyethyl) triphenylphosphonium chloride showed better water solubility and stronger antibacterial activity than CS according to previous reports [15,16]. However, the degree of substitution is limited by steric hindrance. In addition, the relationship between the degree of substitution and antimicrobial activity has not been determined in previous studies.

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In this study, a novel approach to improve water solubility and antibacterial activity of CS is designed by chemical modification with a reactive quaternary phosphonium that was synthesized via two step process. The structures of the reactive quaternary phosphonium and CS derivatives were characterized. In addition, the water solubility and antibacterial activity of CS derivatives are evaluated from different aspects.

2. Experiment

2.1. Materials

Chitosan with a viscosity-average molecular weight of 10 kDa and degree of deacetylation higher than 95% was purchased from Zhejiang Golden-Shell Biochemical Co. Ltd. (Hangzhou, China). *N*-hydroxysuccinimide (NHS), 4-(bromomethyl) benzoic acid, Triphenylphosphine (PPh_3) and dicyclohexylcarbodiimide (DCC) were purchased from Energy Chemical (Shanghai, China), and they were used as received. The other reagents (Analytical grade, Guangzhou Chemical Reagent Factory, China) were 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 h before use.

2.2. Preparation of reactive quaternary phosphonium salt (NHS-QPS)

Scheme 1 presents the synthetic route of NHS-QPS. Briefly, NHS (0.05 mol), 4-(bromomethyl) benzoic acid (0.05 mol), DCC (0.05 mol) and dichloromethane (AR, 200 mL) were added to a 250 ml three-neck round bottom flask equipped with a reflux condenser. The mixture was continuous stirred for 36 h, and then the insoluble substance was removed by filtering. The *N*-hydroxysuccinimide-4-(bromomethyl) benzoic ester (white solid powder) was obtained after the supernatant was recrystallized in dichloromethane/petroleum ether solution. Then, the same molar equivalent of *N*-hydroxysuccinimide-4-(bromomethyl) benzoic ester and triphenylphosphine was kept to react in toluene (100 mL) at 50 °C for 72 h under continuous stirring [19]. The mixture was filtrated and the supernatant was removed, then the reactive quaternary phosphonium salt (4-(2,5-Dioxo-pyrrolidin-1-yloxycarbonyl)-benzyl)-triphenyl-phosphonium bromide (NHS-QPS) was obtained.

2.3. Preparation of N-quaternary phosphonium chitosan derivatives (N-QPCSxy)

The synthetic route of *N*-QPCSxy was shown as Scheme 1. In brief, 0.322 g CS (2 mmol of glycosyl units) was dissolved into 40 mL acetic acid/deionized water (*v*/*v*, 1%), then the desired amount (1 mmol, 2 mmol, 4 mmol, 8 mmol) of NHS-QPS in 40 mL dimethylformamide (DMF) was added, The pH of the mixture was adjusted to 8–9 using 3 M NaOH after the mixture were kept continuous reacted at 80 °C for 24 h under stirring. The product was further purified using dialysis tube (molecular weight cut off, MWCO 3500 Da) against deionized water. The final *N*-quaternary phosphonium chitosan derivatives (*N*-QPCSxy) were obtained by lyophilization at −80 °C. The synthetic process of *N*-QPCSxy(x = 1–2, y = 1–4) were repeated for some times to provide *N*-QPCSxy (x = 1–2, y = 1–4) enough for further characterization. xy means feed mole ratio that was defined as the glycosyl units of CS to NHS-QPS during synthetical process. The CS and *N*-QPCSxy were dissolved in acetic acid/deionized water (*v*/*v*, 1%) to prepare their films for further analysis by vacuum drying at 60 °C.

2.4. Structural characterization

^{1}H-NMR spectra of CS and *N*-QPCSxy were recorded on a 600 MHz spectrophotometer (AVANCE AV600 Bruker, Germany) at room temperature using D_2O/CD_3COOD as solvent and tetramethylsilane (TMS) as an internal standard.

FT-IR spectra of CS and *N*-QPCSxy were recorded on a Nicolet 6700 FT-IR spectrometer. Samples were prepared as KBr pellet and were scanned against a black KBr pellet background at wavelength range of 400–4000 cm^{-1} with 32 scans at 4 cm^{-1} resolution.

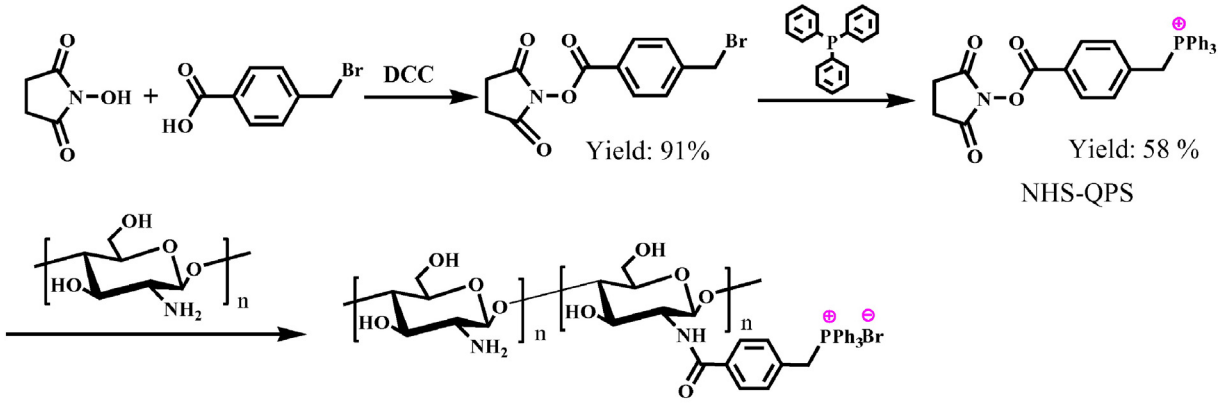
The crystalline structures of CS and *N*-QPCSxy were performed using X-ray diffraction (XRD) on an X-ray diffractometer (D-8 Advance diffractometer, Bruker, Germany) using Cu-Kα radiation (40 kV, 30 mA) with 5 °C/min scanning rate at room temperature. Diffraction was measured in a range of 2θ = 5–80 ° with an angular resolution of 0.0001°.

2.5. Water solubility

The solubility of chitosan and *N*-QPCSxy in distilled water at room temperature was calculated as reported [20]. Briefly, 1.0 g of sample was suspended in 20 mL distilled water and stirred 25 °C for 5 h to give a saturated solution and insoluble remains. The undissolved solids were then collected by gravity filtration, wash with acetone and dried at 40 °C under vacuum overnight. The solubility (% Sa) was calculated using Eq. (1):

$$\%Sa = \frac{1.0 - w_1}{1.0} \times 100 \quad (1)$$

where, w_1 is the weight of undissolved polymers (g). Thus, 100% Sa represents a solubility of almost 50 mg/mL.



Scheme 1. Synthetic route of *N*-quaternary phosphonium chitosan derivatives (*N*-QPCSxy).