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Research Paper

Novel cationic chitosan derivative bearing 1,2,3-triazolium and pyridinium: Synthesis, characterization, and antifungal property

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ABSTRACT

In this paper, novel cationic chitosan derivative possessing 1,2,3-triazolium and pyridinium groups was synthesized conveniently *via* cuprous-catalyzed azide-alkyne cycloaddition (CuAAC) and methylation. FTIR, ^{1}H NMR, and elemental analysis examined the structural characteristics of the synthesized derivatives. The antifungal efficiencies of chitosan derivatives against three plant-threatening fungi were assayed by hypha measurement *in vitro*. The determination showed that chitosan derivative bearing 1,2,3-triazolium and pyridinium displayed tremendously enhanced antifungal activity as compared with chitosan and chitosan derivative bearing 1,2,3-triazole and pyridine. Notably, the inhibitory indices of it against *Colletotrichum lagenarium* attained 98% above at 1.0 mg/mL. The results showed that *N*-methylation of 1,2,3-triazole and pyridine could effectively enhance antifungal activity of the synthesized chitosan derivatives. Besides, the prepared chitosan derivatives showed non-toxic effect on cucumber seedlings. This synthetic strategy might provide an effective way and notion to prepare novel cationic chitosan antifungal biomaterials.

1. Introduction

Chitosan, a deacetylated derivative from chitin, is the only readily available basic amino-polysaccharide in the nature (de Oliveira Pedro, Schmitt & Neumann, 2016; Wu et al., 2016). As one of the most promising biomaterials with good biocompatibility and biodegradability (Li, Duan, Huang & Zheng, 2016; Qian, Xu, Shen, Li & Guo, 2013), chitosan has wide application as wastewater treatment agent, a drug delivery system, and bactericidal or antibacterial agent in the medical industry (Cruz, Garcia-Uriostegui, Ortega, Isoshima & Burillo, 2017; Khan, Ullah & Oh, 2016). However, applications of chitosan are considerably limited by poor solubility in both organic and aqueous solvents (Chen et al., 2016; Qin et al., 2012). Chemical modification of chitosan can overcome this problem to some extent (Jia, Duan, Fang, Wang & Huang, 2016; Sun, Shi, Wang, Fang & Huang, 2017). Derivatization by introducing small functional groups to chitosan backbone can drastically increase the solubility of chitosan at neutral and alkaline pH values as well as strengthen its original bioactivities to broaden the industrial applications (Li, Duan, Huang & Zheng, 2016; Liu, Meng, Liu, Kan & Jin, 2017).

Cationic chitosan is one of the most important group of chitosan derivatives, which has many important physicochemical features such as water solubility and chemical stability as well as antimicrobial properties, due to the electrostatic interactions with anionic biomolecules at the cell surface (Moreno-Vasquez et al., 2017; Sun et al., 2017). Currently, cationic chitosan derivatives can be successfully produced by transforming amino groups into quaternary ammonium salts or by introducing cationic functional groups, such as ammonium, phosphonium, or sulfonium, to chitosan backbones *via* chemical reactions with primary amino and hydroxyl groups (Chen et al., 2016).

The cuprous-catalyzed azide-alkyne cycloaddition (CuAAC) leading to regioselective 1,4-disubstituted-1,2,3-triazoles (Saravanakumar, Ramkumar & Sankararaman, 2011) has received widespread attention in polysaccharide modification due to the merits of CuAAC, including wide in scope, high yield, modularity, tolerant to other functional groups, ready available starting materials, and be stereospecific (Su et al., 2017; Wang et al., 2017). And the application of CuAAC in polysaccharide modification has brought substantial progress

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(Elchinger et al., 2011; Meng & Edgar, 2016; Sahariah et al., 2015). Very recently, 1,2,3-triazolium cations prepared by *N*-alkylation of 1,2,3-triazoles with alkyl halides begin to gain attention because of their interesting properties and versatile applications in ionic liquids, polymers, and catalysis (Aizpurua et al., 2014; Mudraboyina, Obadia, Abdelhedi-Miladi, Allaoua, & Drockenmuller, 2015; Ohmatsu, Hamajima & Ooi, 2012; Sood et al., 2014). However, at the best of our knowledge, to date surprisingly there are no reports describing the preparation of chitosan derivatives containing 1,2,3-triazolium. Besides, 1,2,3-triazole or 1,2,3-triazolium can be also regarded as attractive bridge groups, which can effectively connect pharmacophores to give an innovative bioactive compound (Ruddarraju et al., 2016; Tang et al., 2016). Moreover, pyridine group can be also considered as an excellent reactive precursor to synthesize pyridinium cation by the *N*-alkylation reaction (Jia et al., 2016; Sajomsang, Ruktanonchai, Gonil & Warin, 2010). However, the effect of *N*-alkylation of 1,2,3-triazole and pyridine moieties on the bioactivity of cationic chitosan derivatives was still unknown.

The aim of our project was to synthesize the 1,2,3-triazolium and pyridinium functionalized chitosan derivative and investigate the effect of 1,2,3-triazolium and pyridinium charged units on biological activity of cationic chitosan derivatives. In this paper, we report the preparation and antifungal property of a functionalized chitosan bearing *N*-methyl-1,2,3-triazolium and *N*-methyl-pyridinium installed *via* efficient CuAAC reaction. These novel cationic chitosan derivatives were characterized in details by FTIR and ^{1}H NMR spectroscopy. The quantitative data on degree of substitution, thermal stability, and water solubility of the synthesized chitosan derivatives were also calculated. Three plant-threatening fungi, *Colletotrichum lagenarium (C. lagenarium)*, *Watermelon fusarium (W. fusarium)*, and *Fusarium oxysporum (F. oxysporum)*, were selected to evaluate the antifungal property by hypha measurement *in vitro*.

2. Experimental

2.1. Material

Chitosan with molecular weight of 200 kDa was purchased from Qingdao Baicheng Biochemical Corp. (Qingdao, China) and the degree of deacetylation of it was 0.83 calculated by elemental analysis (C: 43.42%, N: 7.98%, H: 6.30%, C/N: 5.44). Iodomethane, propargyl bromide, and 3-aminopyridine were purchased from the Sigma-Aldrich Chemical Corp (Shanghai, China). Hydrochloric acid, sodium nitrite, urea, sodium azide, diethyl ether, magnesium sulfate, *N*-methyl-2-pyrrolidone, sodium iodide, sodium hydroxide, dimethylsulfoxide, triethylamine, cuprous iodide, absolute ethanol, and acetone were purchased from Sinopharm Chemical Reagent Co.,Ltd (Shanghai, China).

2.2. Structural characterization of chitosan derivatives

2.2.1. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of compounds powder in transmission mode were recorded using a Jasco-4100 Fourier Transform Infrared Spectrometer (Japan, provided by JASCO Co., Ltd. Shanghai, China) under ambient conditions. The samples were prepared in the pellet form by mixing the powder with KBr by the ratio 1:100 and analyzed in the mid-infrared range (from 4000 to 400 cm^{-1}) at a resolution of 4.0 cm^{-1}.

2.2.2. ^{1}H nuclear magnetic resonance (NMR) spectroscopy

^{1}H NMR spectra of compounds were all collected on a Bruker AVIII-500 Spectrometer (Switzerland, provided by Bruker Tech. and Serv. Co., Ltd. Beijing, China) at room temperature operated at a resonance frequency of 500 MHz using 99.9% Deuterium Oxide (D_2O) or Dimethyl Sulfoxide-d_6 (DMSO-d_6) as the solvent. The concentration of the polymer solution was about 40–50 mg mL^{-1} and data were processed using MestReNova software. Chemical shifts were reported in parts per million (ppm) downfield from the tetramethylsilane resonance which was used as the internal standard.

2.2.3. Thermogravimetric analysis (TGA)

TGA measurements of samples were performed on a TA instrument Mettler 5MP (Mettler-Toledo, Switzerland) by heating the samples at a rate of 10 °C min^{-1} from 25 °C to 800 °C under nitrogen atmosphere.

2.2.4. Elemental analysis

The C, H, and N proportions in the native and derived chitosan derivatives were determined on a Vario EL III (Elementar, Germany). The degrees of substitution (DS) of chitosan derivatives were defined as the molar number of grafted functionalized groups per mol of monomeric unit of original chitosan and were calculated on the basis of the percentages of carbon and nitrogen according to the following formulas (Tan, Li and Dong et al., 2017):

$$DS_1 = \frac{n_1 \times M_C - M_N \times W_1}{n_2 \times M_C} \quad (1)$$

$$DS_2 = \frac{M_N \times (W_2 - W_1)}{n_3 \times M_C} \quad (2)$$

$$DS_3 = \frac{M_N \times (W_3 - W_2)}{n_4 \times M_C} \quad (3)$$

$$DS_4 = \frac{M_N \times (W_4 - W_3)}{n_5 \times M_C - n_6 \times M_N \times W_4} \quad (4)$$

$$DS_5 = \frac{(M_N + n_6 \times M_N \times DS_4) \times (W_5 - W_4)}{n_7 \times M_C} \quad (5)$$

where DS_1, DS_2, DS_3, DS_4, and DS_5 represent the deacetylation degree of chitosan, the degrees of substitution of *N*,*N*,*N*-trimethyl in chitosan derivatives, propargyl in chitosan derivative **a**, 1,2,3-triazole groups in chitosan derivatives **b**, and 1,2,3-triazolium groups in chitosan derivatives **c**; M_C and M_N are the molar mass of carbon and nitrogen, M_C = 12, M_N = 14; n_1, n_2, n_3, n_4, n_5, n_6, and n_7 are the number of carbon of chitin, carbon of acetamido group, carbon of trimethyl, carbon of propargyl group, carbon and nitrogen of 3-azidopyridine, and carbon of dimethyl groups, n_1 = 8, n_2 = 2, n_3 = 3, n_4 = 3, n_5 = 5, n_6 = 4, n_7 = 2; W_1, W_2, W_3, W_4, and W_5 represent the mass ratios between carbon and nitrogen in chitosan derivatives.

2.2.5. Inductively coupled plasma mass spectrometry (ICP-MS) analysis

In consideration of the biotoxicity of cuprum, the determination of cuprum content of chitosan derivatives **b** and **c** by ICP-MS were performed with an Elan DRC II instrument (America, provided by PerkinElmer) with dual detector mode. External Calibration was done as standard addition with commercial available cuprum standard solutions. The analytical curve was prepared using seven points at concentrations in the range of 0–200 μg L^{-1}. Results of ICP-MS analysis were received in μg L^{-1} and were converted into μg g^{-1} by taking the initial sample weight and the volume of used acid for the etching steps into account. The determination of Cu by ICP-MS was performed in triplicate.

2.3. Synthesis of chitosan derivatives

2.3.1. Synthesis of 3-azidopyridine

3-Aminopyridine (1.88 g, 20 mmol) was added to 24 mL of 2 N aqueous solution of HCl before the solution was heated to 55 °C while stirring until a clear solution was obtained and then chilled to 0 °C using an ice bath. A solution of sodium nitrite (1.67 g, 24 mmol) in 14 mL of deionized water was subsequently added dropwise. The reaction mixture was allowed to stir for 20 min at an ice bath, followed by the addition of urea (0.24 g, 4 mmol). Then a solution of sodium azide (1.56 g, 24 mmol) in 15 mL of deionized water was added dropwise