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Synthesis, anti-oxidant activity, and biodegradability of a novel recombinant polysaccharide derived from chitosan and lactose

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ABSTRACT

A novel recombinant polysaccharide (RP) based on polysaccharide–disaccharide was synthesized from oligo-chitosan (oligo-CS) and reducing lactose using Maillard reaction with the yield of 85.1%. Chemical structure and thermal stability of RP was characterized by Fourier transform infrared spectrum (FT-IR), solid-state nuclear magnetic resonance spectroscopy (CP/MAS ^{13}C-NMR), and thermo gravimetric analysis (TGA). The anti-oxidant activity of RP was preliminarily investigated by its scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Biodegradability of RP was also examined by the observation of growth status of *Aspergillus niger* colony. It was demonstrated that RP achieved excellent radical-scavenging efficiency (>80%) at high concentrations of DPPH and its scavenging ability was superior to that of CS, suggesting that anti-oxidant property of CS was remarkably promoted by chemical modification with reducing lactose via Maillard reaction. And biodegradation test revealed that RP had better biodegradability than CS.

1. Introduction

As one of the most promising materials, natural polymers have recently received increasing attentions due to their renewable, non-toxic, eco-friendly, and biodegradable benefits (Kim, Yun, & Ounaies, 2006; Malinconico, Cerruti, Santagata, & Immirzi, 2014; Thakur, Thakur, & Gupta, 2013). However, the unsatisfactory performance of naturally available polymers usually fails to meet the needs in different fields. In order to expand their range of applications, structure modification through semi-synthesis, whole synthesis, and combinatorial chemistry, is considered to be the effective ways in improving the performance of natural polymers (Beloshenko, Askadskii, & Varyukhin, 1998). As for these synthesis methods, small molecules tend to be grafted onto natural polymer chains. However, few restructuring studies of different natural polymers were reported due to the non-isotropic nature between their external structures.

Gene recombination refers to a process of two or more than two parents DNA that are combined to form new DNA molecular sequences (Jocelyn, Elliott, & Stephen, 2010), and is considered as one of the most important findings in the biological science. Its principle lies in the fact that extracted DNA fragments are covalently linked onto targeted DNA ones to obtain recombinant genes through the complementary pairing of base groups, independent distribution of genes, or exchange of linkage genes. Like DNA molecules consisting of different base units, polysaccharide is composed of monosaccharide unit. The similarity in the constructing rationale between polysaccharides and DNA has motivated us to consider whether the concept of gene recombination can be applied to the synthesis of recombinant polysaccharides (Wrodnigg & Stütz, 1999), which would be interesting from the viewpoint of designing new natural polymers.

Natural polysaccharides, such as chitosan (CS) and cellulose, are a class of very important polymers that have been widely utilized in a variety of fields. Chemical modification with functional molecules can impart improved performance to polysaccharides. Among various modification reactions, Maillard reaction is a chemical reaction

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between nucleophilic amino groups (—NH_2) and reactive carbonyl ones (—C=O) of reducing saccharides (Jing, Yap, Wong, & Kitts, 2009; Maillard, 1912; Rao, Chawla, Chander, & Sharma, 2011). CS contains the —NH_2 functional group, whereas semi-acetal groups of the saccharides can be converted to —C=O under the specific condition. Thus, Maillard reaction between CS and reducing saccharides can be utilized to synthesize recombinant polysaccharides (RP). Since Maillard reaction products (MPRs) exhibited some extent of oxidant resistance, the study of their anti-oxidation activity has been attracting considerable interests (Azlan & Wan, 2009; Cai et al., 2009; Harish Prashanth & Tharanathan, 2007; Ma, Chen, & Zhao, 2013; Zhang, Yesim, Parveen, Dachao, & Stuart, 2010). It has been demonstrated that the anti-oxidation properties of chitosan after the modification of Maillard reaction were remarkably improved as compared with chitosan or N-alkylation chitosan derivative. To date, the majority of studies focused on Maillard reaction of chitosan with such mono-saccharides as glucose (Rao et al., 2011; Weerakkody, Labbett, Cheng, & Kosaraju, 2011) and fructose (Dong et al., 2014; Ying, Xiong, Wang, Sun, & Liu, 2011). Because of different chemical structure between mono-saccharides and disaccharides, it was expected that the MRPs from disaccharide and chitosan would display varying antioxidant activity from those from mono-saccharide and chitosan. However, the use of CS and reducing disaccharide containing reactive carbonyl groups (e.g. lactose) is scarcely reported in the literature so far.

In this work, a novel recombinant polysaccharide (RP) was successfully synthesized from lactose and the oligo-polysaccharide from chemical degradation of CS in terms of Millard reaction, and its chemical structure and thermal stability were characterized by means of FT-IR and CP/MAS ^{13}C-NMR, and TGA. And anti-oxidation and biodegradability of the resultant RP were evaluated as well.

2. Experimental

2.1. Materials

Chitosan (degree of deacetylation ≥90.0%, $M_w = 1.43 \times 10^5$ Da) was purchased from Sinopharm Chemical Reagent Co., Ltd. Lactose (purity ≥ 97.0%) was provided by Hongrun technology Co., Ltd. Hydrogen peroxide solution with H_2O_2 content of ≥30.0% was supplied by Xilong chemical Co., Ltd. All other reagents were analytical grade and used without further purification.

2.2. Synthesis of oligo-chitosan (oligo-CS) and recombinant polysaccharide (RP)

Chitosan (CS) solution was prepared by dissolving chitosan (2.0 g) in 150 mL acetic acid solution(1.0 wt%)under vigorous stirring. And 80 mL of 5 wt% H_2O_2 was slowly added to the CS solution and then refluxed at 60 °C for 4 h. After cooling to room temperature, the pH value of the mixture was adjusted to 10 using 10 wt% NaOH solution, and then concentrated under reduced pressure. Afterward, 2–3 times volume of anhydrous ethanol was added into the concentrate to allow the precipitation of the products overnight. The precipitate, was finally filtered off and dried at 50 °C under vacuum to obtain oligo-CS.

Oligo-CS (2.3 g) was dissolved in 50 wt% ethanol and lactose (1.0 g) was separately dissolved in 1 wt% acetic acid. A certain amount of lactose–acetic acid solution was added dropwise to oligo-CS–ethanol solution under stirring at 85 °C and then reflux for 8 h. After the completion of reaction, an excessive amount of anhydrous ethanol was added into the above mixture under stirring. After standing for 8 h, RP was recovered as the precipitate using the centrifugation (4000 rpm and 15 min), then adequately washed using anhydrous ethanol, and finally dried until the constant weight (yield: 85.1%).

2.3. Characterization

Molecular weight and its distribution index (PD = M_w/M_n) of the sample were determined by PL GPC 50 plus Gel Permeation Chromatography equipped with differential refractive detectors and PL Guard Column (10 μm) with H_2O as an eluent and poly(ethylene oxide) as standard substances. Column temperature and flow rate were set at 30 °C and 0.80 mL min^{-1}, respectively. Infrared spectra of oligo-CS, lactose and RP were recorded using Prestige-21 Fourier transform infrared spectrometer (FT-IR) in the wave number range of 500–4000 cm^{-1}. CP/MAS ^{13}C NMR spectra of CS and RP were recorded with AVANCE II/400 MHz nuclear magnetic resonance spectrometer. Fracture surfaces of the sample films were coated with gold and then examined on a SS-550 scanning electron microscope (SEM, Shimadzu, Japan) with an accelerating voltage of 10 kV. Thermal stability of samples was studied on a Netzsch STA 200 PC thermal analyzer (NETZSCH, Germany) under nitrogen atmosphere with a flow rate of 25 mL min^{-1}. A certain amount of samples (9.2–10.4 mg) were heated from 25 to 600 °C at a heating rate of 20 °C min^{-1}.

Radical-scavenging activity of the products was measured in the calorimetric cylinder equipped with 5 mL anhydrous ethanol solution of 1×10^{-4} mol L^{-1} 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Hatano et al., 1989). A certain amount of the samples (0.5–2.5 g) were added into the cuvette filled with the above solution. After left to stand for 30 min at room temperature in a dark room, the absorbance (A_i) at 517 nm was measured using UV-Vis spectrometer (UV-2450, Shimadzu, Japan). The absorbance of deionized water was measured as A_0, while the absorbance is measured as A_j when DPPH was replaced by anhydrous ethanol.

$$\text{DPPH clearance} = (1 - (A_i - A_j)/A_o) \times 100\% \quad (1)$$

For the biodegradation test, the CS and RP samples were examined as the sole carbon source medium with *Aspergillus niger* as a degrading strain material. The composition of *Agar* culture-medium is 15 wt% sample powder, 0.05 wt% $MgSO_4 \cdot 7H_2O$, 0.1 wt% NH_4Cl, 0.0005 wt% $CaCl_2 \cdot 2H_2O$, 0.554 wt% KH_2PO_4, and 1.194 wt% $Na_2HPO_4 \cdot 12H_2O$. After pH value of the culture medium was adjusted to 6.8-7.0 using 0.01 mol L^{-1} NaOH, a certain amount of sample powder was evenly spread on the surface of the culture medium. The strain was inoculated using the sterile gun in the center of the culture medium, which was then put into the oven at 28 °C for the cultivation. Growth status of colony was observed at some intervals to evaluate the biodegradability of samples.

3. Results and discussions

3.1. Synthesis of recombinant polysaccharide

Due to the presence of strong hydrogen bonding networks, chitosan (CS) is difficult to dissolve in common solvents except diluted acid, thus restricting the broad use of CS in many applications. Usually, physical degradation, chemical degradation, and bio-degradation were often employed to improve the solubility of CS (Cai et al., 2011; Gryczka et al., 2009; Patwardhana, Satrio, Brown, & Shanks, 2009; Wasikiewicz & Yeates, 2013; Zawadzki & Kaczmarek, 2010). Among them, chemical degradation is considered to be an ideal method for the preparation of oligo-CS (Sun, Zhou, Xie, & Mao, 2007) because of simple and convenient advantages. It was found that the oligo-meglumine sugar synthesized from the hydrolysis of CS showed unique functional properties and physiological activities. Molecular weight of oligo-CS was much decreased with respect to that of CS, and so the intermolecular