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Carbohydrate Polymers 71 (2008) 435–440
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Absorption and distribution of chitosan in mice after oral administration

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Received 3 April 2007; received in revised form 13 June 2007; accepted 15 June 2007
Available online 30 June 2007

Abstract

Four chitosan samples with different molecular weight M_w and the degree of deacetylation DD (HCS 7.60×10^5 and 85.5%, MCS 3.27×10^4 and 85.2%, COS 0.99×10^3 and 85.7%, WSC 3.91×10^4 and 52.6%) were prepared, and labeled by fluorescein isothiocyanate. These labeled samples were used to investigate the absorption and distribution in mice after oral administration. The results indicated that the absorption and distribution of chitosan was significantly influenced by its M_w and water-solubility. The absorption of chitosan molecules increased with the decrease of the M_w and the increase of the water-solubility. The absorbed chitosan molecules were distributed to all tested organs such as liver, kidney, spleen, thymus, heart and lung. The chitooligomer molecules were easily absorbed and metabolized. The absorbed chitosan molecules from water-soluble WSC in all tested tissues maintained high concentration for a long period. The results suggest that different chitosan may be employed for different functional food.
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Keywords: Chitosan; Molecular weight; Water-solubility; Intestinal absorption; Organ distribution

1. Introduction

Chitosan is a linear heteropolysaccharide composed of β-1–4-linked D-glucosamine (GlcN) and *N*-acetyl-D-glucosamine (GlcNAc) with various compositions of these two monomers, available largely in the exoskeletons of shellfish and insects. The US Food and Drug Administration approved chitosan as a feed additive in 1983. Chitosan has attracted tremendous attention as a potentially important renewable agricultural resource, and has been widely applied in the fields of agriculture, medicine, pharmaceuticals, functional food, environmental protection and biotechnology in the last 20 years (Dodane & Vilivalam, 1998; Harish Prashanth & Tharanathan, 2007; Hejazi & Amiji, 2003; Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Muzzarelli, 1996; Thanou, Verhoef, & Junginger, 2001).

Chitosan has many functions such as antitumor activity (Jeon & Kim, 2002; Qin, Du, Xiao, & Li, 2002; Suzuki et al., 1986; Tokoro et al., 1988), cholesterol-lowering effect (Gallaher, Munion, Hesslink, Wise, & Gallaher, 2000; Ormrod, Holmes, & Miller, 1998), immuno-enhancing effect (Peluso et al., 1994), antidiabetic effect (Hayashi & Ito, 2002), wound healing effect (Porporatto, Bianco, Riera, & Correa, 2003), antifungal activity and antimicrobial activity (Qin et al., 2006). Although numerous literatures are available on the aforementioned biological activities, the relationships of these activities with molecular weight and water-solubility of chitosan deserve to be investigated. It can be easily hypothesized that the biological properties of chitosan may be closely related to the molecular weight and water-solubility. As a preliminary study, in vivo absorption phenomena of different chitosan were investigated.

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doi:10.1016/j.carbpol.2007.06.016

2. Experimental

2.1. Materials

Crude chitosan was supplied by Golden-shell Biochemical Co., LTD (China). The crude chitosan was purified by HCl and NaOH to get HCS (Qin et al., 2002). Fluorescein isothiocyanate (FITC) was purchased from Sigma Chemical Co. (USA). Other reagents were of analytical grade. Kunming strain female mice (4 weeks old) weighing 20–24 g were purchased from Hubei Experimental Animal Center (China). The UF membranes (OSOO1C11, OMEGA) with NMWL 10 kDa, 5 kDa and 1 kDa were purchased from PallFiltron Corporation (USA).

2.2. Preparation of chitosans with different molecular weights

Fifty gram of crude chitosan was completely dissolved in 1000 ml 2% (v/v) acetic acid, the solution was placed in a water bath at 48 °C and 2.0 g cellulase was added to initiate the reaction.

After 2 h, half of the reaction mixture was taken out, boiled for 10 min to denature the enzyme, and filtered. The UF membrane of 10 kDa was used to separate out the product. The fraction with higher M_w was neutralized with 10% NaOH to pH 9. The precipitate was washed thoroughly with distilled water and ethanol. The sample MCS was collected after drying over phosphorus pentoxide *in vacuum*.

After 8 h, the left reaction mixture passed UF membrane of 5 kDa and 1 kDa to separate. The filtrate was concentrated by rotary evaporator, and neutralized to pH 9 and precipitated by adding ethanol. The precipitate was washed thoroughly with ethanol and collected after drying over phosphorus pentoxide *in vacuum* to gain sample COS.

2.3. Preparation of water-soluble chitosan

MCS (4.2 g) was dissolved in 120 ml 1% acetic acid solution. The solution was diluted with 100 ml absolute alcohol under stirring, and acetic anhydride (2.5 ml) was added to the solution (Qin et al., 2006). After stirring the solution at 20 °C for 12 h, the reaction was ended by adding 4% NaOH until the pH was 9. The solution was concentrated to about 1/5 with a rotary evaporator under diminished pressure, and precipitate by adding ethanol. The precipitate was repeatedly washed with ethanol. The product was dried over P_2O_5 in vacuum at room temperature for 24 h to obtain sample the product WSC.

2.4. Measurement of M_w

The weight average molecular weight (M_w) of samples was measured by a gel permeation chromatography (GPC). GPC system incorporated a TSP P1000 instrument. Two columns in series (TSK G5000-PW and TSK G3000-PW) were used. The eluent was 0.2 M CH_3COOH/0.1 M CH_3COONa. The flow rate was maintained at 1.0 ml min^{-1}. The temperature of the columns was maintained at 30 °C. The eluent was monitored by a RI 150 refractive index detector. The sample concentration was ca.0.4% (w/v). The standards used to calibrate the column were TOSOH pullulan. All data provided by the GPC system were collected and analyzed using the Jiangshen Workstation software package.

2.5. Estimation of the water-solubility

The pH dependence of water-solubility of chitosan was evaluated from turbidity. Chitosan (0.3 g) was completely dissolved in 100 ml 1% acetic acid. The pH of solution was adjusted to desired value by stepwise addition of 2 M NaOH solution. The transmittance of the solution was recorded on a Shimadzu UV-1601 spectrophotometer using a quartz cell with an optical path length of 1 cm at 600 nm.

2.6. FITC labeling of chitosan

To label the chitosan with FITC (Onishi & Machida, 1999), chitosan was completely dissolved in 1% (v/v) acetic acid solution, then DMSO was added to form DMSO/1%HAc co-solvent system (75/25, v/v), and a predetermined amount of FITC in acetone was added. The reaction mixture was vigorously stirred for 24 h at room temperature. After reaction, the FITC-labeled chitosan was obtained through precipitation of the reaction mixture in excess acetone. Then, the FITC-chitosan was washed with acetone for 6 times. Finally, the FITC-chitosan was obtained by lyophilization.

2.7. In vivo chitosan test

Female mice (20–24 g body weight) were fasted for 12 h before the administration of chitosan. They were administered FITC-labeled chitosan solutions through an oral gavage tube that was carefully passed down through the esophagus into the stomach (Chae, Jang, & Nah, 2005). The FITC-chitosan solutions were prepared in 1% (v/v) acetic acid solution at a concentration of 21 g/l. The total volume of the administrated chitosan was 0.50 ml (at the dose of 500 mg/kg). To measure the absorbed amount of chitosan, the blood, liver, kidney, thymus, spleen, lung and heart were collected serially after the mice were sacrificed at predetermined time. The blood sample was directly mixed with 3.50 ml 0.5 M hydrochloride, and suspended for a night at room temperature. Other samples were homogenized by glass homogenizer, then 4.00 ml 0.5 M HCl was added. The solution was centrifuged at 2400 r/min for 20 min to separate the insoluble solid. Then, 0.50 ml the solution of sample was taken out and added to 3.50 ml phosphorous buffer solution (PBS, pH 6.86). The fluorescence intensity of the sample was measured using a fluorescence plate reader. The emission (EM) wave-

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