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Sulfation and biological activities of konjac glucomannan

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ABSTRACT

The sulfation of konjac glucomannan and its anti-HIV and blood anticoagulant activities were investigated. Konjac glucomannan is a polysaccharide occurring naturally in konjac plant tubers and has high molecular weights. Solubility in water is very low, and the aqueous solutions at low concentrations have high viscosity. Before sulfation, hydrolysis by diluted sulfuric acid was carried out to decrease the molecular weights of $\bar{M}_n = 19.2 \times 10^4 - 0.2 \times 10^4$. Sulfation with piperidine-*N*-sulfonic acid or SO_3-pyridine complex gave sulfated konjac glucomannans with molecular weights of $\bar{M}_n = 1.0 \times 10^4 - 0.4 \times 10^4$ and degrees of sulfation (DS) of 1.3–1.4. It was found that the sulfated konjac glucomannans had potent anti-HIV activity at a 50% effective concentration, (EC_{50}) of 1.2–1.3 μg/ml, which was almost as high as that of an AIDS drug, ddC, whose EC_{50} = 3.2 μg/ml, and moderate blood anticoagulant activity, AA = 0.8–22.7 units/mg, compared to those of standard sulfated polysaccharides, curdlan (10 units/mg) and dextran (22.7 units/mg) sulfates. Structural analysis of sulfated konjac glucomannans with negatively charged sulfated groups was performed by high resolution NMR, and the interaction between poly-L-lysine with positively charged amino groups as a model compound of proteins and peptides was measured by surface plasmon resonance measurement, suggesting that the sulfated konjac glucomannans had a high binding stability on immobilized poly-L-lysine. The binding of sulfated konjac glucomannan was concentration-dependent, and the biological activity of the sulfated konjac glucomannans may be due to electrostatic interaction between the sulfate and amino groups.

1. Introduction

The konjac glucomannan of konjac plant tubers is an abundant and easily available heteropolysaccharide with high molecular weights (Cescutti, Campa, Delben, & Rizzo, 2002; Chua, Baldwin, Hocking, & Chan, 2010; Dave & McCarthy, 1997). Konjac glucomannan has a linear structure composed of 1,4-β-linked D-glucopyranose and D-mannopyranose with a small number of branches and partially acetylated hydroxyl groups in the sugar units. A small amount of konjac glucomannan is soluble in water and gives a highly viscous solution; therefore, konjac glucomannan has been used in foods (Albrecht et al., 2011), food additives (Iglesias-Otero, Borderias, & Tovar, 2010), wrapping films when mixed with cellulose or curdlan (Lu, Zhang, & Xiao, 2004; Wu et al., 2012), cation-exchange resin supports (Zhou et al., 2012), and water-absorbent polymers (Li, Ji, & Li, 2012). Although studies on its biological activities are few, use as a carrier in a drug delivery system was reported among the application studies (Liu et al., 2012; Wang, Fan, Liu, & He, 2010).

Fundamental studies of konjac glucomannan have mainly focused on the structural analysis and gelation mechanisms. Katsuraya et al. (2003) reported in detail the structure of konjac glucomannan by methylation analysis and NMR spectroscopic measurements, indicating that a small proportion of branches existed at the C6 carbon of a glucosyl main chain with 1,6-β glucosyl branches. The ratio of glucose to mannose units in the main chain was about 2–1 and in the branches was about 8% glucose. Luo, Hu, and Lin (2011) described the gelation mechanism of konjac glucomannan in NaOH solution. Sodium hydroxide solution restrained expansion of the molecular chain and promoted gelation, probably due to the obvious effects of deacetylation, self-aggregation, and entanglement. On the other hand, Liu et al. (2012) developed a carrier for a pulsatile drug delivery system based on a highly impermeable capsule of konjac glucomannan. A 5-aminosalicylic acid drug was detected in plasma 5 h after oral administration in the capsule. The pulsatile capsule may have therapeutic potential for a colon-specific drug delivery system.

We have reported the synthesis, structural analysis, and biological activities of naturally occurring and synthetic polysaccharides obtained by a ring-opening polymerization of anhydro sugar monomers (Yoshida, 2001, 2005). Previously, we found that

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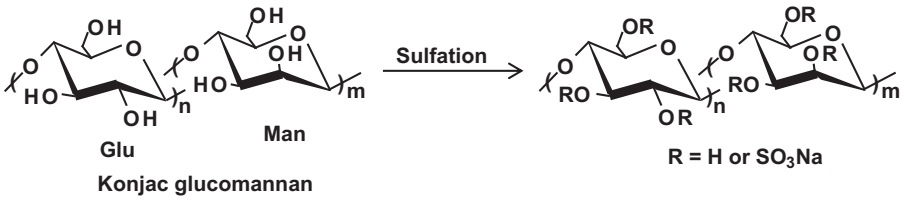

Scheme 1. Sulfation of konjac glucomannan.

sulfated polysaccharides had high anti-HIV and blood anticoagulant activities. In particular, curdlan sulfate, which was prepared by sulfation of curdlan, a naturally occurring polysaccharide, with a linear 1,3-β pyranoside structure, and produced by a bacterial strain, completely inhibited infection of MT-4 cells by HIV at concentrations as low as 3.3 μg/ml and with low cytotoxicity at concentrations as high as 1000 μg/ml (Yoshida et al., 1990). Therefore, an alkyl curdlan sulfate was prepared recently by ionic interaction between a positive didodecyldimethyl ammonium bromide and a negative sulfate group of curdlan sulfate, and then fixed on a membrane filter by a hydrophobic interaction with the long alkyl chain. The alkyl curdlan sulfate-coated membrane filters decreased the concentration of influenza A virus to below 1/4–1/32, suggesting that the membrane filter effectively removed influenza A virus by electrostatic interaction between negatively charged sulfate groups and the positively charged envelope protein of the viruses (Tegshi, Han, Kanamoto, Nakashima, & Yoshida, 2011).

Although there are many reports on the structure and applications of konjac glucomannan, few reports on the biological activities have been published. Sulfated polysaccharides are expected to have specific biological activities that are antiviral and heparin-like (Lane & Lindahl, 1989). In this paper, we report the sulfation of konjac glucomannan and its biological activities such as anti-HIV and blood anticoagulant activities. In addition, we describe the preliminary results on the interaction between the sulfated konjac glucomannan and poly-L-lysine as a model compound of proteins and peptides by using surface plasmon resonance (SPR) to elucidate the biological mechanisms.

2. Experimental

2.1. Measurement and materials

The ^{1}H NMR and ^{13}C NMR spectra were recorded with a JEOL ECM-400 spectrometer at 400 MHz and 100 MHz, respectively, in D_2O or 2.5% NaOH D_2O solution at 50 °C with 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) as an internal standard or in DMSO-d_6 at 60 °C. Infrared spectra were measured by a Perkin-Elmer Spectrum One FT-IR spectrometer using a KBr pellet. The molecular weight of hydrolyzed konjac glucomannan was determined by an aqueous phase GPC (column; Tosoh TSK-gel G2500PW$_{XL}$, G3000PW$_{XL}$, and G4000PW$_{XL}$, 7.6 mm × 300 mm × 3 mm eluted with 66.7 mM of phosphate buffer, pH = 6.86) with a Tosoh RI detector using pullulan as a standard. Optical rotation was measured by using a JASCO DIP-140 digital polarimeter in aqueous 2.5% NaOH solution at 25 °C in a water-jacketed 10 ml quartz cell. Elemental analysis was carried out with a CE-440 elemental analyzer (System Engineering Inc.). A surface plasmon resonance (SPR) spectrum was taken on a Biacore X100 instrument at 25 °C using a CM5 sensor chip.

Konjac glucomannan was obtained from Chengdu Root Industry (China). Poly-L-lysine with $\bar{M}_w = 1000 - 5000$ and anhydrous dimethyl sulfoxide were purchased from Sigma, Inc. Piperidine-*N*-sulfonic acid was prepared from piperidine and chlorosulfonic acid according to the method of Nagazawa and Yoshidome (Nagasawa & Yoshidome, 1969).

2.2. Hydrolysis of konjac glucomannan

A typical procedure for hydrolysis of konjac glucomannan is as follows. Konjac glucomannan (0.5 g) was added into 90 ml of deionized water and then stirred vigorously for 3 h at 70 °C. Sulfuric acid (25%, 10 ml) was added dropwise to the viscous konjac glucomannan solution (the final concentration of sulfuric acid in solution was 2.5%), and the mixture was further stirred for 2 h at 70 °C. After cooling to room temperature, the reaction mixture was neutralized by saturated $NaHCO_3$ solution, dialyzed against deionized water for 24 h, and then freeze-dried to give 0.41 g of a low molecular weight konjac glucomannan ($\bar{M}_n = 3.7 \times 10^4$).

2.3. Sulfation of konjac glucomannan and its biological activities

Konjac glucomannan was sulfated by piperidine-*N*-sulfonic acid or SO_3-pyridine complex (Scheme 1). Typical methods are as follows.

For the sulfation by piperidine-*N*-sulfonic acid (no. 2 in Table 2), konjac glucomannan (0.25 g, 1.5 mmol, $\bar{M}_n = 0.8 \times 10^4$) was dissolved in anhydrous DMSO (25 ml) solution at 85 °C and then piperidine-*N*-sulfonic acid (1.0 g, 6.1 mmol) was added. The mixture was stirred for 2 h at 85 °C, then cooled and neutralized by a 5% NaOH solution, and then the alkaline solution was dialyzed against deionized water for 2 d. The dialysate was freeze-dried to give 0.26 g of sulfated konjac glucomannan with the number-average molecular weight of $\bar{M}_n = 0.7 \times 10^4$. Found for C: 19.7%, H: 3.0%, S: 14.7%.

For the sulfation by SO_3-pyridine complex (no. 3 in Table 2), konjac glucomannan (0.25 g, 1.5 mmol, $\bar{M}_n = 0.8 \times 10^4$) was dissolved in anhydrous DMSO (25 ml) with stirring at 60 °C and then SO_3-pyridine complex (1.5 g, 9.4 mmol) was added. The mixture was stirred for 45 min further at 60 °C. After cooling to room temperature, the mixture was neutralized with saturated $NaHCO_3$ solution, dialyzed against deionized water for 2 d, and the dialysate was freeze-dried to give 0.57 g of sulfated glucomannan with the number-average molecular weight of $\bar{M}_n = 0.8 \times 10^4$. Found for C: 17.2%, H: 2.9%, S: 17.3%.

2.4. Biological activities

The anti-HIV activity was assayed in vitro by the MTT method (Pauwels et al., 1988). The activity was evaluated at an EC_{50} value, which is the half maximal inhibitory concentration of sulfated konjac glucomannan to prevent the infection of MT-4 cells by HIV. The cytotoxicity was determined as the 50% cytotoxic concentration (CC50) value, of sulfated konjac glucomannan on MT-4 cells.

The blood anticoagulant activity was measured using bovine plasma according to the modified method of the U.S. Pharmacopeia (U.S. Pharmacopoeia National Formulary, 1985), and the activity was calculated in comparison with that of a standard dextran sulfate (H-39) at 22.7 unit/mg.