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Structure–antioxidant relationships of sulfated galactomannan from guar gum

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ABSTRACT

Sulfated polysaccharides exerted potential biological property which was relative to degree of sulfation (DS), M_w, substitution position and chain conformation. In the present study, commercial guar gum was purified and its sulfated derivates with different DS and M_w were synthesized. FT-IR and ^{13}C NMR analysis indicated that C-6 substitution was predominant in sulfated samples compared with other positions. In the sulfation reaction, a sharp decrease in M_w was observed. The d_f values from 1.92 to 2.85 indicated that the $-SO_3H$ groups led to the relatively expanded conformation of sulfated polysaccharides. Antioxidant assays showed that sulfated polysaccharides had better antioxidant activities. The data obtained in in vitro models indicated that high DS and low M_w showed the best antioxidant capacities.

1. Introduction

Biological activities of polysaccharide depend on its molecular structure including sugar unit, glycosidic bond of the main chain, the types and polymerization degree of the branch, flexibility and configuration of the chains [1]. Therefore, molecular modification and structure improvement of polysaccharide arouse wide concern. Most studies have demonstrated that biological activities of polysaccharide are greatly increased by molecular modification [2]. Recently, chemical modifications of polysaccharides by esterification, oxidation and hydroxypropylation are generally done for preparing custom-made derivatives with desirable functionality attributes [3]. Sulfated polysaccharides comprise a complex group of macromolecules with a wide range of important biological properties. The sulfation of polysaccharides could not only enhance the water solubility but also change the chain conformation, resulting in the alteration of their biological activities [4]. Many studies have confirmed that the sulfated polysaccharides exerted potential biological properties in comparison with non-sulfated polysaccharides, such as anti-coagulant, anti-virus, antioxidant and antitumor activities [5].

Guar gum (GG) is a natural nonionic high molecular weight (100–1000 kDa) galactomannan. It consisted of (1 → 4)-linked β-D-mannopyranose units with α-D-galactopyranose units connected to the mannose backbone through (1 → 6) glycosidic linkages. The main chain is randomly substituted by galactose units at a mannose to galactose ratio of 1.8:1.0 [6]. The repeating unit of guar gum is shown in Fig. 1. GG is a widely applied polysaccharide in industry. It is very important to control the release and colon of targeted drugs in the gastrointestinal tract. It shows anti-cancer activities in the treatment of colorectal cancer and for oral rehydration solutions in the treatment of cholera in adults [7].

It was reported that sulfated GG (composition of GG: 85.2% of carbohydrates and 7.1% of protein) was prepared using SO_3/formamide complex in the presence of chlorosulfuric acid (CSA) [8]. It was found to have anti-inflammatory properties. For the nature of the substitution, the least substituted sections of the GG molecules showed the greatest tendency to associate [6]. However, their results were not related to any aspects of the structure information on sulfated GG.

In the present study, sulfated GGs (SGG) with different degrees of substitution (DS) and molecular weight were prepared with chlorosulfuric acid (CSA)/pyridine in formamide. The structure of SGG was analyzed by FT-IR and ^{13}C NMR spectroscopy. Size-exclusion chromatograph combined with multi-angle laser photometer (SEC-LLS) was employed to determine the molecular weight, molecular weight distribution and chain conformation. The antioxidant properties of GG and its sulfated derivatives were eval-

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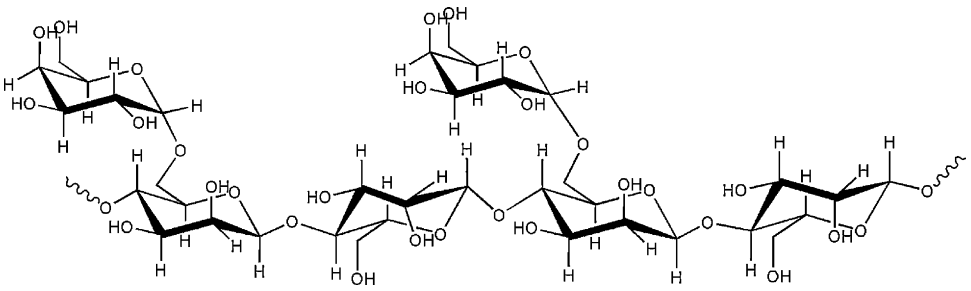

Fig. 1. The monomeric structure of guar gum.

uated and compared for the purpose of assessing the relationship between structure and bioactivity. Antioxidant properties were assayed in terms of antioxidant activities in vitro, by scavenging abilities on hydroxyl radicals, superoxide radicals, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and chelating ability on ferrous ions and reducing power.

2. Materials and methods

2.1. Purification of GG

Deproteinization of commercial GG (protein content was 5%, number average molecular weights was 2.5×10^5 g/mol) was made by Sevage method joined papain according to the reported method [9]. The purity of GG collections was monitored under ultraviolet (UV) light at 280 nm.

2.2. Sulfated modification of GG

2.2.1. Preparation of sulfating reagent

Chlorosulfuric acid (CSA) was dropped one by one in anhydrous pyridine filled in three-necked flask, under agitating and cooling condition in ice water bath [5]. All determinations were completed in 40 min.

2.2.2. Sulfation reaction

GG (500 mg) was suspended in anhydrous formamide (20 mL) at room temperature with stirring for 30 min, and the sulfating reagents were added dropwise. The mixture was stirred for 3 h at 60 °C. After the reaction, the mixture was cooled to room temperature and the pH value was adjusted to 7–8 with 2 mol/L NaOH solution. The mixtures were precipitated with EtOH (95%), washed, redissolved in water, and then dialyzed (molecular weight cutoff 8–12 kDa) against tap water for 48 h and distilled water for 24 h to remove pyridine, salt and potential degradation products. Five sulfated GGs (SGG-1 to SGG-5) with different DS were collected after lyophilizing and kept in dryness box.

The sulfur contents of SGG were determined by reported method [5]. A calibration curve was constructed with sodium sulfate as standard. The degrees of substitution (DS) was calculated according to the following equation:

$$\mathrm{DS} = \frac{1.62 \times S\%}{32 - 1.02 \times S\%} \quad (1)$$

2.3. Characterization of sulfated GG

2.3.1. FT-IR analysis

FT-IR spectra were recorded with KBr pellets on Nicolet NEXUS 670 FT-IR. Sixteen scans at a resolution of 4 cm^{-1} were averaged and referenced against air.

2.3.2. NMR spectroscopy

^{13}C NMR experiments were recorded on a Bruker Avance DPX-400 spectrometer (operating frequency of 100.593 MHz). Samples were deuterium-exchanged several times by freeze-dried from D_2O, and then examined in D_2O at 25 °C. The chemical shifts were expressed in ppm relative to the resonance of the internal standard Me_4Si.

2.3.3. Molecular weight determination

HPSEC-LLS measurements were carried out on size-exclusion chromatograph combined with multi-angle laser photometer (MALLS, $\lambda = 690$ nm; DAWN EOS, Wyatt Technology Co., USA). Ultrahydrogel™ column (7.8 mm × 300 mm, Waters, USA) was used as SEC instrument. An optilab refractometer (DAWN, Wyatt Technology Co., USA) was simultaneously connected. The polysaccharide samples with desired concentrations were prepared and optical clarification of the samples was achieved by filtration into a scattering cell. The injection volume was 50 μL and the flow rate was 0.5 mL/min. The refractive index increment (dn/dc) value of the sample was determined by an optilab refractometer at 690 nm (25 °C) to be 0.145 mL/g. The basic light scattering equation is as follows:

$$\frac{K_c}{R_\theta} = \frac{1}{M_\mathrm{w}}\left(1 + \frac{16\pi^2\langle S^2\rangle_z}{3\lambda^2}\sin^2\left(\frac{\theta}{2}\right)\right) + 2A_2C \quad (2)$$

where K is an optical constant equal to $[4\pi^2 n^2(\mathrm{d}n/\mathrm{d}c)^2]/(\lambda^4 N_\mathrm{A})$; C, the polysaccharide concentration in mg/mL; R_θ, the Rayleigh ratio; k, the wavelength; n, the refractive index of the solvent; dn/dc, the refractive index increment; N_A, the Avogadro' number; A_2, the second virial coefficient. As the column separated the polymer according to molecular weight, each fraction was led to the light scattering detector for instantaneous measurement of the scattering intensities. The refractive index detector connected in series gave the polymer concentration. In chromatography mode, we had a single and sufficiently low concentration at a particular slice. The dilute injected sample was further dilution by the SEC column.

2.4. Assay for antioxidant activities

2.4.1. Hydroxyl radical scavenging assay

The hydroxyl radical assay was measured by the method of Ghiselli [10] with a minor modification. Polysaccharides were dissolved in deionized water at the concentration of 0.1–5 mg/mL. The sample solution (0.1 mL) was mixed with 0.6 mL of reaction buffer [20 mM phosphate buffer (pH 7.4), 2.67 mM deoxyribose, and 100 μm EDTA], 0.2 mL of 0.4 mmol ferrous ammonium sulfate, 0.05 mL of 2.0 mM Vc, and 0.05 mL of 10 mM H_2O_2 was then added to the reaction solution. The reaction solution was incubated for 15 min at 37 °C and then 1 mL of 1% thiobarbituric acid (TBA) and 1 mL of 2% trichloroacetic acid (TCA) were added to terminate the reaction. The mixture was boiled for 15 min and cooled to room temperature. The absorbance of the mixture was measured at