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RESEARCH NOTE

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Anticoagulant Activity of Sulfated Barley β-Glucan

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Abstract Barley β-glucan was subjected to chemical modification and the anticoagulant activity of the derivative was investigated. The barley β-glucan was successfully sulfated, showing the degree of substitution calculated by elemental analysis of 0.40. In addition, the Fourier transform infrared (FT-IR) spectra of the derivative confirmed the sulfation, which generated two new absorption bands at 1,250/cm (S=O) and 810/cm (C-O-S) compared to the native. Specially, the anticoagulant activity of barley β-glucan was created by sulfation, which increased in a concentration-dependent manner. This result demonstrated that the incorporation of sulfate groups into the β-glucan structure added a blood clotting prevention effect.

Keywords: barley β-glucan, sulfation, anticoagulant activity

Introduction

β-Glucan, which is rich in the cell wall of endosperm and alleurone layer of cereals, has been used as a food additive, more specifically, as a gelling agent and thickener, owing to its high viscosity and gelling ability (1). In addition to its physical properties, much attention has focused on the β-glucan's physiological activities (2). β-Glucan is generally considered to be one of useful polysaccharides in barley and oat, providing valuable effects on hypocholesterolemia (3) and hypoglycemia (4,5). Recently, Food and Drug Administration (FDA) claimed that the products containing cereal β-glucans have health benefits, such as prevention of coronary heart disease by controlling blood cholesterol level (6).

Chemical modification can improve and/or create new functional properties besides the intrinsic biological activities of β-glucan, through the incorporation of the functional groups into structural chains (7). In particular, sulfated derivatives exhibited diverse effects on the physiological functions such as anti-coagulant, anti-tumor, and anti-HIV infection activities (8,9). Zhang *et al.* (10) reported that sulfation increased or developed the biological activities of the (1-3)-β-glucans isolated from the sclerotia of *Pleurotus tuber-regium*. Moreover, anti-coagulant and anti-thrombotic effects were improved by the sulfation on the (1-3)-β-glucan from the soil bacterium *Alcaligenes faecalis* var. *myxogene* (11,12) and (1-6)-β-glucan from the lichenized fungus *Parmotrema mantiqueirense* Hale (13). Previously, oat β-glucan has been chemically modified with sulfation, developing the anticoagulant activity (14). However, the effect of chemical modification on barley β-glucan has not been reported yet. Therefore, the aims of this study were to sulfate the β-glucan extracted from barley and to investigate its anticoagulant activity.

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Materials and Methods

Isolation of barley β-glucan Barley β-glucan was extracted and purified according to the method of Kim *et al.* (15). Barley (*Hordeum vulgare*) was dehulled, ground, and passed through a 50-mesh sieve. The barley powder suspension in distilled water (10%, pH 10.0) was placed at room temperature for 20 hr and its pH was adjusted to 6.0, followed by the addition of α-amylase (0.5 mL, Termamyl 120 L; Novozymes, Bagsvaerd, Denmark) at 95°C for 2 hr. The resulting solution's pH was readjusted to 4.5 and amyloglucosidase (200 μL, AMG 300L; Novozymes) was added at 60°C for 4 hr. For the enzyme inactivation, the mixture was boiled and centrifuged at 1,500×g for 5 min. Three volumes of ethanol (95%, v/v) were added into the supernatant, which was stored overnight at room temperature and the precipitated β-glucan was recovered by centrifugation (1,500×g, 5 min). This procedure was repeated again in order to increase the purity of β-glucan, which was determined to be 98%, and the precipitate was freeze-dried for further analysis.

Preparation of sulfated derivative Barley β-glucan (15 g) was mixed with formamide (150 mL) and chlorosulfonic acid (100 mL) at 80-90°C for 4 hr and propylene oxide (500 mL) was then added. The precipitate was resuspended in distilled water, followed by the adjustment of its pH to 10.0-11.0. The resulting samples were dialyzed against distilled water for 24 hr and were lyophilized (16).

Structural characterization The sulfated derivative was analyzed by using an elemental analyzer (EA1110; CE Instruments, Milan, Italy) and its degree of substitution was calculated by the following formula (10,17).

$$DS = \frac{162 \times S\% / 32}{100 - (80/32 \times S\%)}$$

The sulfated samples were ground with potassium bromide (KBr) at a ratio of 1:20 and pressed into a thin pellet for Fourier transform infrared (FT-IR) analysis (MAGNA-IR 760 E.S.P; Nicolet Instrument Corp., Madison, WI, USA).

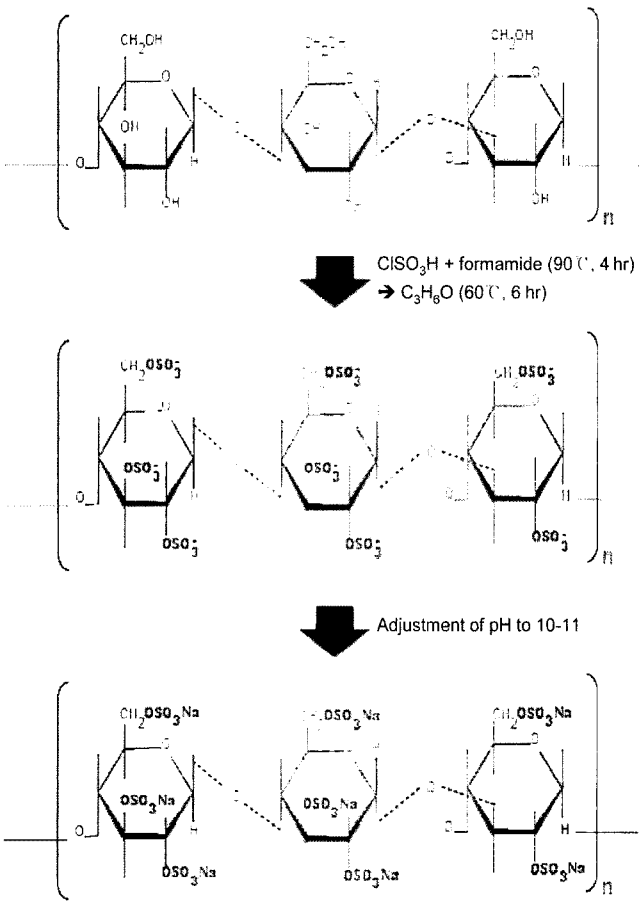

Fig. 1. Scheme of the sulfation of β-glucan.

Anticoagulant activity Platelet rich blood was obtained from Sprague-Dawley rats (Samtako, Korea). The blood was mixed with 3.13% sodium oxalate as a ratio of 10:1 and the platelet was then settled at 1,600×g for 25 min. The derivative sample (50 μL), which was dissolved in saline at different concentrations (10, 20, and 40 μg/mL), was added to the plasma (100 μL). After incubation at 37℃ for 3 min, 5 mM $CaCl_2$ (100 μL) was then added to the resulting solution. The clotting time (a fibrin clot appeared) was recorded and was compared with that of heparin (Choongwae Pharma Co., Seoul, Korea). All measurements were performed in triplicate.

Results and Discussion

Identification of sulfated barley β-glucan Barley β-glucan was subjected to sulfation where hydroxyl groups were replaced with sulfate groups as shown in Fig.1.

Degree of substitution (DS) of the sulfated barley β-glucan per anhydroglucose unit was calculated based on the sulfur content by elemental analysis. The sulfur content of the derivative was 6.66% and the estimated DS was 0.40.

The structure of the β-glucan derivative was also characterized by FT-IR. The characteristic vibrations at 1,250 (C-O-S) and 810 (S=O) 1/cm were observed in Fig. 2, showing the existence of sulfate groups in the derivative (16). Therefore, the FT-IR spectra confirmed that the barley β-glucan was successfully sulfated.

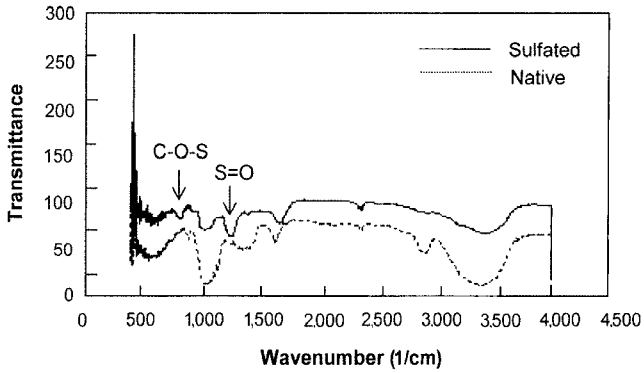

Fig. 2. FT-IR spectra of native and sulfated barley β-glucans.

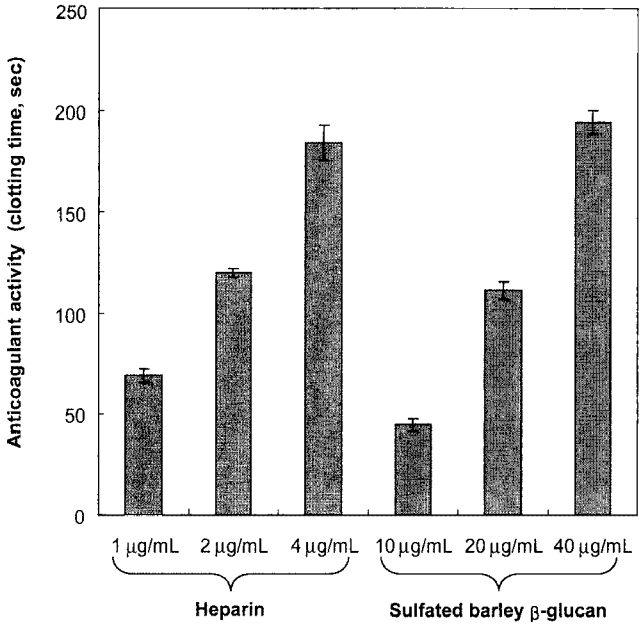

Fig. 3. Anticoagulant activity of sulfated barley β-glucan.

The effect of sulfation on anticoagulant activity The anticoagulant activities of the sulfated barley β-glucan were investigated and compared with heparin which is a commonly used anticoagulant medicine. As presented in Fig. 3, the sulfated β-glucan showed the anticoagulant effects. It implies that the incorporation of sulfate groups into the β-glucan structure is responsible for the anticoagulant activity because native barley β-glucan did not exhibit anticoagulant effect (data not shown). In addition, the sulfated derivative prolonged blood clotting time in a concentration-dependent manner. In the previous literature (14), a similar anticoagulant effect was observed in sulfated β-glucan from oats. It would be mainly due to the anionic properties of the sulfated β-glucan which interacted with the positive charges of coagulant proteins, thus playing an important role in anticoagulant activity (18). In addition, this anticoagulant action was enhanced with increasing sulfate group content and molecular weight (18,19).

In summary, the anticoagulant activity of barley β-glucan could be developed by sulfation through the introduction of sulfate groups into the β-glucan chains. The β-glucan derivative exhibited the anticoagulant effect which was estimated at around 1/10 of heparin. This result confirmed

that chemical modifications including sulfation could be a useful tool to provide new physiological properties for polysaccharides.

Acknowledgments

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