JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Structural and Biological Characterization of Aminated-Derivatized Oat β-Glucan

MIN SU SHIN,† SUYONG LEE,‡ KWANG YEON LEE,† AND HYEON GYU LEE*,†

Department of Food and Nutrition, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul, 133-791, Korea, and Cereal Products & Food Science Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois 61604

β-Glucan extracted from oats was subjected to reductive amination, producing a cationic β-glucan derivative of which physiological properties were characterized. The degree of substitution was obtained from elemental analysis, which was 0.48. In addition, the distribution of amino groups in the β-glucan derivative was investigated by FT-IR analysis. In vitro bile acid binding capacity of the aminated β-glucan was examined, showing significantly higher bile acid binding activity than native β-glucan. Moreover, the β-glucan derivative showed pronounced antimicrobial effects against *Escherichia coli* and *Bacillus subtilis*, and ACE (angiotensin-converting enzyme) inhibition activities which were dependent on its concentration. Furthermore, bronchoalveolar lavage (BAL) experiments demonstrated that the β-glucan derivative stimulated the synthesis of nitric oxide. The improved functionalities of the derivative could be explained by its polycationic characteristics.

KEYWORDS: β-Glucan derivative; reductive amination; bile acid; antimicrobial activity; ACE (angiotensin-converting enzyme); nitric oxide

INTRODUCTION

Healthful diets containing functional materials or nutraceuticals are currently impacting food industries. A number of efforts have been made to search for new sources with beneficial health effects, and some of them already are on the market. These efforts may be mainly due to the increased insight of consumers into the relationship between diets and health as well as the increased cost of health care.

This recent trend has intensified the interest and importance in dietary fibers as health-enhancing materials. In particular, much attention has focused on β-glucan because of its physiological effectiveness in lowering cholesterols (*1*), controlling blood glucose levels (*2*, *3*), and reducing the risk of colon cancer (*4*). Moreover, it is allowed by the FDA to claim health benefits for oat products when 0.75 g of β-glucan is consumed in a serving portion (*5*).

In addition to investigating the intrinsic physiological properties of β-glucan mentioned above, it would be worthwhile to develop new functional properties of β-glucan through physical or chemical modifications. Incorporation of new functionalities may impart new or better physical or chemical properties to β-glucan. Even though these physical or chemical modifications have been widely applied to a variety of polysaccharides (*6*−*9*), there has been limited research on β-glucan modifications. Previously, the effect of depolymerizaton of β-glucan by ultrasonic irradiation on antitumor activity was studied, showing no significant differences between native and degraded β-glucan (*6*). Also, oat β-glucan was partially hydrolyzed by three different hydrolysis methods and their structural and rheological changes were presented (*10*). For chemical modifications, the hydroxyl groups of β-glucan allow the potential to graft new functional groups on the backbone. The addition of functional groups is able to provide extended chemical modifications for practical applications in a variety of fields. Previously, several β-glucan derivatives were prepared by carboxymethylation, carboxyethylation, hydroxyethylation, and sulfoethylation and then their mitogenic activities were assessed in terms of solubility, degree of substitution, and molecular weight distribution (*9*). The β-glucan isolated from *Poria cocos* sclerotium was also sulfated, carboxymethylated, methylated, hydroxyethylated, and hydroxypropylated, and their structures were correlated to antitumor activities (*11*).

The overall goals of this study were to prepare a β-glucan derivative by chemical modification, specifically, reductive amination, and then to characterize its structural and physiological properties.

MATERIALS AND METHODS

Extraction and Purification of Oat β-Glucan. The procedures to extract and purify β-glucan in oats were based on the previous method by Kim et al. (*12*). The oats (*Avena sativa* L.) used in this study were obtained from Korean National Institute of Crop Science (Suwon, Korea). They were dehulled and ground to pass through a 50-mesh

* To whom all correspondence should be addressed. Phone: 82-2-2220-1202. Fax: 82-2-2292-1226. E-mail: hyeonlee@hanyang.ac.kr.
† Hanyang University.
‡ United States Department of Agriculture.

10.1021/jf050273j CCC: $30.25
Published on Web 06/18/2005

screen in a miller (Jinyoung, Korea). The obtained oat powder (50 g) was suspended in distilled water (500 mL), and the pH was adjusted to 10.0. After softening for 20 h at room temperature, the pH of the suspension was readjusted to 6.0. Then, α-amylase (0.5 mL, Thermamyl 120L, Novo, Denmark) was added to the suspension, which was then kept at 95 °C for 2 h. Also, after the pH was adjusted to 4.5, amyloglucosidase (AMG 300L, Novo, Denmark) was added and the enzyme reaction was carried out at 60 °C for 4 h. After the enzymes were inactivated in a boiling water bath, the suspension was centrifuged and then ethanol was added to the supernatant, which was placed overnight at room temperature. After centrifugation, the resulting precipitates were collected and freeze-dried. This purification procedure was repeated again to increase the purity of β-glucan. The content of the obtained β-glucan was determined by an enzymatic procedure (*13*) to be 95.0% ± 1.1.

Reductive Amination of β-Glucan. The extracted β-glucan was subjected to reductive amination to produce a β-glucan derivative according to the method of Yalpani (*14*). Two grams of paraformaldehyde was added to 1 g of β-glucan in 40 mL of dimethyl sulfoxide (DMSO), which then was held at 90 °C for 3 h, 125 °C for 1 h, and 135 °C for 1 h and finally cooled at room temperature. After addition of 16 mL of acetic anhydride, the reaction mixture was kept in the dark for 20 h, followed by treatment with 500 mL of methanol overnight. The resulting precipitates, which were oxidized β-glucan, were treated with 100 mL of DMSO, 3.6 g of sodium acetate, and 2.2 g of sodium cyanoborohydride at room temperature for 7 days. The material was then dialyzed for 4 days and dried, yielding aminated β-glucan.

Structural Characterization. *Elemental Analysis.* Nitrogen analysis was performed with an elemental analyzer (EA1110, CE Instrument, Italy) in order to investigate the degree of substitution of aminated β-glucan.

FT-IR Spectrometer. FTIR (MAGNA-IR 760 E.S.P, Nicolet Instrument Corp.) was utilized to identify amino groups from the β-glucan derivative. Samples were mixed with potassium bromide (KBr) at a ratio of 1:20 and pulverized. This powder was then compressed into a thin pellet, which was used for analysis.

Physiological Property Measurements. *Bile Acid Binding Capacity.* As one of the experiments to elucidate the physiological properties of the β-glucan derivative, the test of in vitro bile acid binding was carried out by modifying the method of Boyd et al. (*15*) and Camire et al. (*16*). After β-glucan samples were added to 0.01 M sodium phosphate buffer (pH 7.0) containing 250 μM bile acid to yield 2.5 mg/mL, they were treated at 37 °C for 2 h and then filtered (0.2 μm syringe filter, Waters Co.). The resulting samples (0.2 mL) were treated with 70% sulfuric acid (1 mL) for 5 min, and then 25% furfural (0.2 mL) was added. After 1 h, absorbance was measured at 510 nm.

Antimicrobial Effect. The antimicrobial activities of aminated β-glucan against *E. coli* and *B. subtilis* were examined (*17*). *E. coli* and *B. subtiilis* were inoculated in MRS broth (pH 7.0) and incubated at 37 °C for 24 h. β-Glucan samples (90 μL) were added to the culture broth (10 μL), which was incubated at 37 °C for 18 h. Then, its absorbance was measured at 540 nm.

Antihypertensional Activity. ACE (angiotensin-1 converting enzyme, Sigma-Aldrich, St. Louis, MO) inhibitory activity was measured according to the method of Cushman and Cheung (*18*) and Hong et al. (*19*). A 0.15 mL amount of 6.5 mM HHL (hippuryl-L-histidyl-L-leucine) was dissolved in 0.1 mL of 0.1 M potassium phosphate buffer (pH 8.3) containing 0.3 M NaCl and preincubated at 37 °C. β-Glucan samples (0.1 mL) and ACE (0.1 mL) were mixed in the HHL buffer at 37 °C, and 1 N HCl (0.25 mL) was added after 30 min. Then, the resulting material was mixed with 2 mL of ethyl acetate, stirred for 15 s, and centrifuged. After 1 mL of the supernatant was dried, it was dissolved in 2 mL of distilled water, and then its absorbance was measured at 228 nm.

Bronchoalveolar Lavage (BAL). BAL was performed as described in the literature (*20*). After SPF Sprague−Dawley rats purchased from Semtaco (Kyungkido, Korea) were euthanized, their lungs and bronchi were removed by a surgical procedure and tracheas were incised to 1/2, which were fixed with an 18-gauge cannula. After injection of 3 mL of PBS (37 °C) into the lungs six times, cells in the lungs were collected. The harvested cells were fixed on a slide glass (0.5×10^5) using a Cytospin 3 cytocentrifuge (Shandon, Pittsburgh) and stained with Diff-Quick (Dade Behring Inc., Newark). Using a microscope, cells without inflammation were selected and used for subsequent culture and assays. The selected cells were placed into wells of 12-well tissue culture plates (Greiner, Germany) (3×10^5/well), incubated in a 37 °C incubator with an atmosphere of 5% CO_2 for 2 h, and exposed to β-glucan samples in order to determine nitric oxide formation. Then 100 μL of the culture was mixed with the same volume of the Griess reagent (1% sulfanilamide/0.1% naphthylene diamine dihydrochloride/2.5% H_3PO_4) and placed at room temperature for 2 min (*21*). Absorbance was measured at 540 nm using an ELISA reader (Biotech Instrument Inc.).

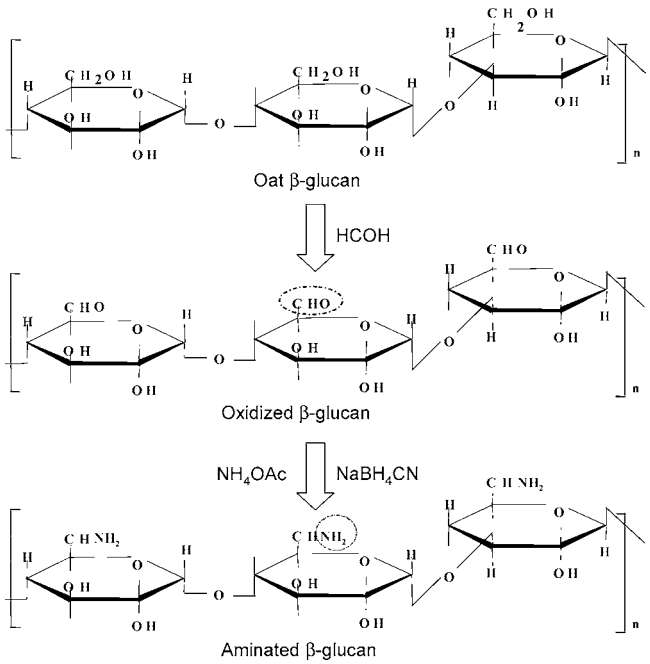

Figure 1. Scheme of reductive amination of β-glucan.

Statistical Analysis. All experiments were carried out in triplicate. Statistical analysis was performed with one-way analysis of variance to decide a significance of difference among samples at the level of 5%.

RESULTS AND DISCUSSION

Figure 1 displays the scheme of the reductive amination of β-glucan. It shows that hydroxyl groups were replaced with amino groups, yielding NH_2-β-glucan.

Degree of substitution (DS) of the aminated β-glucan per glucose unit was determined on the basis of measured % N obtained from elemental analysis, according to the following equation:

$$\mathrm{DS} = \frac{162 \times (N\%/14)}{100 - [(13/14) \times N\%]}$$

The elemental analysis of the β-glucan derivative gave the following results: C, 37.94%; H, 5.84%; and N, 4.19%, and the calculated DS was 0.50.

Figure 2 displays the FT-IR spectrum of aminated β-glucan which was compared with that of native β-glucan. First of all, two broad and intense bands appear in both spectra. A band at 3447 cm^{-1} would be associated with hydroxyl groups (OH), and the other observed band between 1020 and 1070 cm^{-1} would be attributed to CO bond stretching. It is interesting to note that the β-glucan derivative exhibited new absorption bands at 1250−1300 cm^{-1} and 700−800 cm^{-1}. They could be

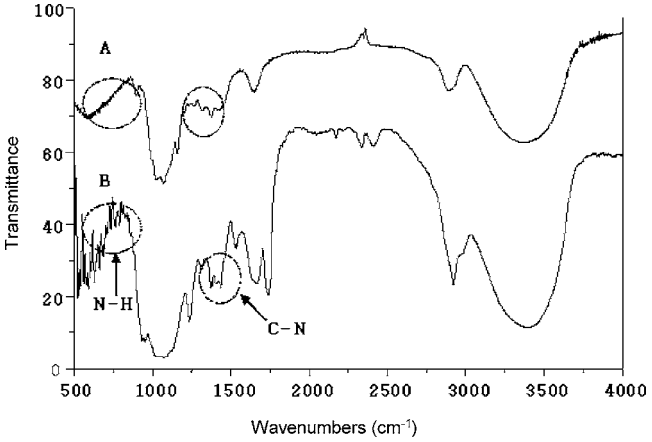

Figure 2. FT-IR spectra of native (**A**) and aminated β-glucan (**B**).

Table 1. In Vitro Bile Acid Binding by Native and Aminated β-glucan[a]

	bile acid binding (μM/mg, dry matter)	binding relative to cholestyramine (%)
native β-glucan	4.20 c	13.3 c
aminated β-glucan	21.89 b	69.3 b
cholestyramine	31.59 a	100.0 a

[a] Values followed by a different letter in the same column are significantly different at the 5% level, Duncan's multiple range test.

characteristic of C−N stretching and out of plane N−H wagging, respectively, according to the previous study on the structure of aminated chitosan (*22*). Thus, the FT-IR results supported the distribution of amino groups in the β-glucan derivative. The peak by NH_2 groups was not clearly observed in the FT-IR spectrum because it might overlap the peak of hydroxyl groups.

Bile acids are steroid carboxylic acids synthesized in liver from cholesterol, and the primary bile acids are cholic and chenodeoxycholic acids. Binding of bile acids and subsequent excretion in feces have been recognized as a significant mechanism to eliminate excess cholesterol (*23*−*25*). Therefore, high binding capacity of bile acids suggests a possible ability to lower cholesterol in the body.

It is well-known that β-glucan reduces blood cholesterol levels. Ingested β-glucan increases the intestinal viscosity and decreases the absorption of cholesterol and bile acids in the body, consequently promoting their excretion (*24*). It is thought to be one of the major mechanisms of the cholesterol-lowering activity of β-glucan.

Table 1 presents the bile acid binding capacities of native and aminated β-glucan. The results show that both β-glucans had bile acid binding activity. One striking feature is that there was significantly higher binding capacity of bile acids with the β-glucan derivative than with the native β-glucan ($P < 0.05$), implying more cholesterol-lowering effects. Furthermore, both β-glucans were compared with cholestyramine in Table 1. Because cholestyramine has been clinically proven to reduce the levels of cholesterol in blood (*26*), the effectiveness of the β-glucan derivative in lowering cholesterol could be readily evaluated by comparison with cholestyramine. Considering that cholestyramine binds 100% of bile acids, relative binding capacities of native β-glucan and the derivative were 13.3% and 69.3%, respectively.

The bile acid binding effect of cholestyramine is involved in an ionic interaction between cholestyramine and bile acids (*26*). In a similar way, the cationic amino groups in the aminated

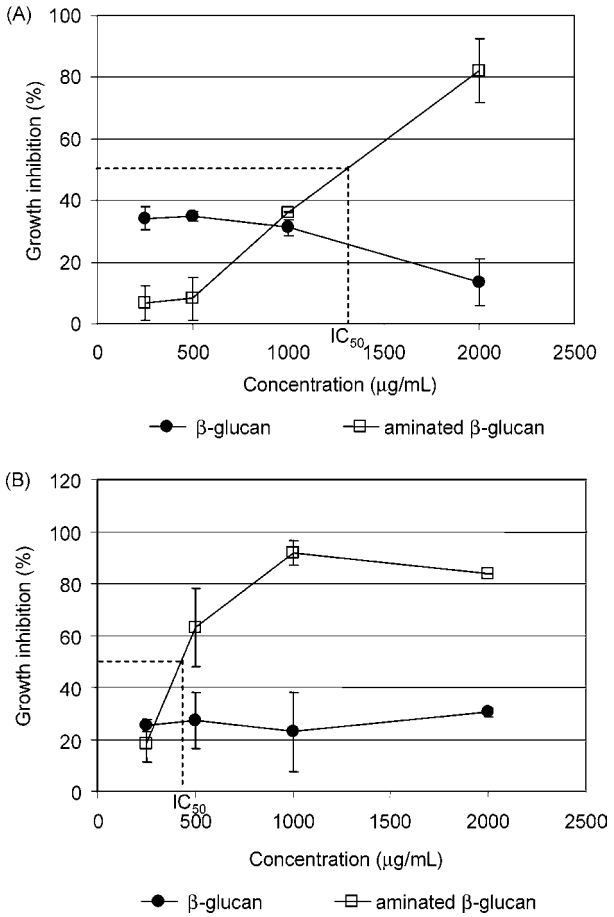

Figure 3. Antimicrobial activities of underivatized and derivatized β-glucan against *E. coli* (**A**) and *B. subtilis* (**B**) as a function of their concentration.

β-glucan were expected to enhance the bile acid binding capacity, which was experimentally demonstrated. Similar results were observed in a previous study where the effect of the reductive amination on the bile acid binding capacity of chitosan was investigated (*22*).

Antimicrobial effects of β-glucan samples against *E. coli* and *B. subtilis* were evaluated (**Figure 3**). It is shown in **Figure 3** that underivatized β-glucan had inhibitory effects on both *E. coli* and *B. subtilis* up to around 35% depending on their concentration, whereas aminated β-glucan significantly inhibited their growth up to 80% at a concentration of 2000 μg/mL. It implies that the amination of β-glucan stimulated antimicrobial effects. Moreover, the β-glucan derivative inhibited the growth of *E. coli* up to 50% at a concentration of 1290 μg/mL. On the other hand, 50% of *B. subtilis* was suppressed only with 400 μg/mL of the β-glucan derivative. The difference in the antimicrobial effect between *E. coli* (Gram-negative) and *B. subtilis* (Gram-positive) could be explained by the structure of cell walls because Gram-positive bacteria have a simpler cell wall structure, which is readily susceptible to attacks by foreign molecules (*27*).

Overall, the β-glucan derivative was more effective than underivatized β-glucan in preventing the growth of *E. coli* and *B. subtilis*. It could be attributed to the cationic nature of the derivative. The polycations interact with negative microbial surfaces, causing a change in membrane permeability of cells and consequently inhibiting microbial growth. The antimicrobial effects of polycations against Gram-positive and Gram-negative bacteria have been reported in the literature (*22*, *28*−*30*).

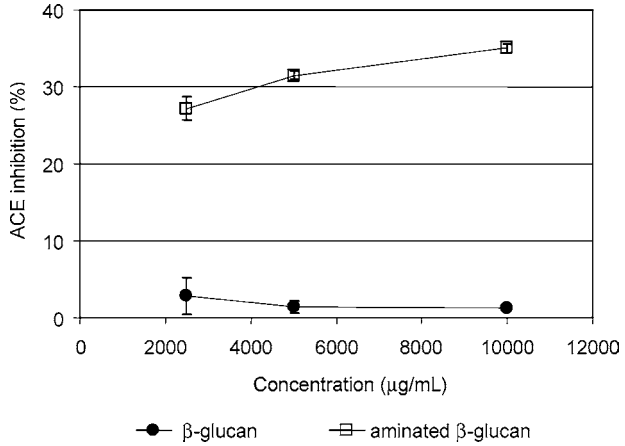

Figure 4. Effect of the concentration of native and aminated β-glucan on ACE inhibition.

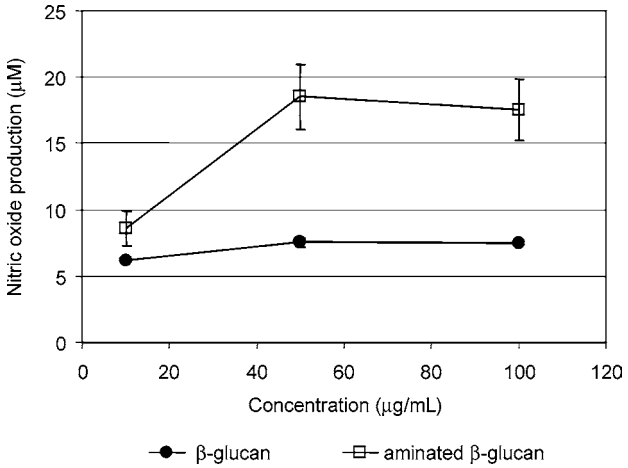

Figure 5. Production of nitric oxide by BAL cells treated with native and aminated β-glucan.

Inhibition of angiotensin-converting enzyme (ACE) by aminated β-glucan was assessed (**Figure 4**). It is interesting to note that the derivative demonstrated a pronounced inhibitory effect on ACE in a concentration-dependent manner while native β-glucan hardly showed an inhibitory effect on ACE.

The ACE converts angiotensin I, which is inactive by itself, into angiotensin II, which narrows blood vessels, causing an increase in blood pressure. Therefore, much effort has been made to lower the activity of ACE as a way to control blood pressure. It is reported that ACE activity increases with Cl^- (*31*, *32*). Therefore, one possible explanation might be that the β-glucan derivative appeared to interact with Cl^- as an anion exchange resin, consequently reducing the ACE activity.

Nitric oxide is a free radical generated from L-arginine by bronchoalveolar lavage cells in response to inflammatory exposures (*33*). As an immune response mediator, it plays an important role in host defense system. **Figure 5** displays the effect of native and derivatized β-glucans on the production of nitric oxide. Significant differences were detected in the amount of nitric oxide produced in bronchoalveolar laves among samples ($P < 0.05$). The β-glucan derivative generated significantly more nitric oxide than the underivatized. Specifically, the derivative generated around 18 μM of nitric oxide at a concentration of 50 μg/mL while only 7 μM was produced by the native β-glucan.

It was previously reported that specific receptors for β-glucan are present on the macrophage surface and that the interaction of β-glucan with the receptors enhances nitric oxide synthesis (*34*, *35*). Therefore, our data demonstrate that the substitution of hydroxyl groups by amino groups in β-glucan contributes to increased generation of nitric oxide by the derivative. Further studies are necessary to establish a full understanding of the nitric oxide production by the β-glucan derivative. The results present, however, the possibility of administering aminated β-glucan to a human body for stimulating immunological activities.

In conclusion, a β-glucan derivative carrying polycations was prepared from oat β-glucan by reductive amination, which imparted new physiological characteristics to the β-glucan. Our results revealed the increased in vitro bile acid binding capacity and antimicrobial effect of aminated β-glucan, compared to native β-glucan. In addition, the β-glucan derivative exhibited more ACE inhibition and produced more nitric oxide. Therefore, the aminated β-glucan would be positively expected to have several improved health benefits including reduction of cholesterol and blood pressure. However, research should be further focused on in vivo tests and toxicological evaluations for clinical or food applications.

ACKNOWLEDGMENT

This work was supported by an Agricultural Research Promotion Program under the Ministry of Agriculture and Forestry of the Republic of Korea.

LITERATURE CITED

(1) Newman, R. K.; Newman, C. W.; Graham, H. The hypercholesterolemic function of barley beta-glucans. *Cereal Foods World* **1989**, *34*, 883−886.
(2) Klopfenstein, C. F. The role of cereal beta-glucans in nutrition and health. *Cereal Foods World* **1988**, *33*, 865−869.
(3) Wood, P. J.; Anderson, J. W.; Braaten, J. T.; Cave, N. A.; Scott, R. W.; Vachon, C. Physiological effects of beta-D-glucan rich fractions from oats. *Cereal Foods World* **1989**, *34*, 878−882.
(4) Malkki, Y.; Virtanen, E. Gastrointestinal effects of oat bran and oat gum. A review. *Lebensm.-Wiss. Technol.* **2001**, *34*, 337−347.
(5) FDA Food labeling: Health claims; oats and coronary heart disease. *Fed. Regist.* **1997**, *62*, 3584−3601.
(6) Tabata, K.; Ito, W.; Kojima, T. Ultrasonic degradation of schizophyllan, an antitumor polysaccharide produced by *Schizophyllum commune* Fries. *Carbohydr. Res.* **1981**, *89*, 121−135.
(7) Osman, S. F.; Hoagland, P. D. A sequencing method for the reductive-amination derivative of oligo- and poly-saccharides. *Carbohydr. Res.* **1984**, *128*, 361−365.
(8) Cho, H. T.; Lee, J. D.; Cho, J. J.; Kim, N. S. Cationization of cellulose and its applications (1)-cyanoethylation of cellulose and aminization by reduction. *J. Korean Fiber Soc.* **1988**, *25*, 465−472.
(9) Sanjula, J.; Machova, E. Mitogenic activity of particulate yeast beta-(1-3)-D-glucan and its water-soluble derivatives. *Int. J. Biol. Macromol.* **1995**, *17*, 323−326.
(10) Tosh, S. M.; Wood, P. J.; Wand, Q.; Weisz, J. Structural characteristics and rheological properties of partially hydrolyzed oat beta-glucan: the effects of molecular weight and hydrolysis method. *Carbohydr. Polym.* **2004**, *55*, 425−436.
(11) Wang, Y.; Zhang, L.; Li, Y.; Hou, X.; Zeng, F. Correlation of structure to antitumor activities of five derivatives of a beta-glucan from *Poria cocos* sclerotium. *Carbohydr. Res.* **2004**, *339*, 2567−2574.
(12) Kim, M. O.; Cha, H. S.; Koo, S. J. Studies on rheological characterization of barley beta-glucan [mixed-linked (1-3),(1-4)-beta-D-glucan]. *Korean J. Food Sci. Technol.* **1993**, *25*, 15−21.

(13) McCleary, B. V.; Codd, R. Measurement of (1-3), (1-4)-beta-D-glucan in barley and oats: A streamlined enzymic procedure. *J. Sci. Food Agric.* **1991**, *55*, 303−312.
(14) Yalpani, M. Processes for the preparation branched, water soluble cellulose products. US Patent Number 4,531,000, 1985.
(15) Boyd, G. S.; Eastwood, M. A.; MacLean, N. Bile acids in the rat: studies in experimental occlusion of the bile duct. *J. Lipid Res.* **1966**, *7*, 83−87.
(16) Camire, M. E.; Zhao, J.; Violette, D. A. *In vitro* binding of bile acids by extruded potato peels. *J. Agric. Food Chem.* **1993**, *21*, 2391−2394.
(17) Seo, K. L.; Kim, D. Y.; Yang, S. I. Studies on the antimicrobial effect of Wasabi extracts. *Korean J. Nutr.* **1995**, *28*, 1073−1077.
(18) Cushman, D. W.; Cheung, H. S. Spectrophotometric assay and properties of the angiotensin converting enzyme of rabbit rung. *Biochem. Pharmacol.* **1971**, *20*, 1637−1640.
(19) Hong, S. P.; Kim, M. H.; Oh, S. W. ACE inhibitory and antihypertensive effect of chitosan oligosaccharides in SHR. *Korean J. Food Sci. Technol.* **1998**, *30*, 1476−1479.
(20) Kagan, E.; Oshiso, Y.; Hartmann, D. P. The effects of chrysotile and crocidolite asbestos on the lower respiratory tract: analysis of bronchoalveolar lavage constituents. *Environ. Res.* **1983**, *32*, 382−397.
(21) Tomas, G.; Ramwell, P. W. Vasodilatory properties of mono-L-arginine containing compounds. *Biochem. Biophys. Res. Commun.* **1988**, *154*, 332−338.
(22) Lee, J. K. Structural modification of polysaccharide for the improvement of functionality. Ph.D. Thesis, Korea Advanced Institute of Science and Technology, 1998.
(23) Jenkins, D. J. A.; Newton, C.; Leeds, A. R.; Cumings, J. H. Effect of pectin guar gum, and wheat fiber on serum-cholesterol. *Lancet* **1975**, *1*, 1116−1117.
(24) Lund, E. K.; Gee, J. M.; Brown, J. C.; Wood, P. J.; Johnson, I. T. Effect of oat gum on the physical properties of the gastrointestinal contents and on the uptake of D-galactose and cholesterol by rat small intestine *in vitro*. *Br. J. Nutr.* **1989**, *62*, 91−101.
(25) Kahlon, T. S.; Woodruff, C. L. *In vitro* binding of bile acids by rice bran, oat bran, barley and beta-glucan enriched barley. *Cereal Chem.* **2003**, *80*, 260−263.
(26) Daggy, B. P.; O'Connell, N. C.; Jerdack, G. R.; Stinson, B. A.; Setchell, K. D. R. Additive hypocholesterolemic effect of psyllium and cholestyramine in the hamster: influence on fecal sterol and bile acid profiles. *J. Lipid Res.* **1997**, *38*, 491−502.
(27) Franklin, T. J.; Snow, G. A. *Biochemistry of antimicrobial action*; Chapman & Hall: London, 1981.
(28) Kanazawa, A.; Ikeda, T.; Endo, E. Polymeric phosphonium salts as a novel class of cationic biocides. IV. Synthesis and antibacterial activity of polymers with phosphonium salts in the main chain. *J. Polym. Sci., Part A: Polym. Chem.* **1993**, *31*, 3031−3038.
(29) Young, D. H.; Kohle, H.; Kauss, H. Effect of chitosan on membrane permeability of suspension-cultured *Glycine max* and *Phaseolus vulgaris* cells. *Plant Physiol.* **1982**, *70*.
(30) Fang, S. W.; Li, C. F.; Shih, D. Y. C. Antifungal activity of chitosan and its preservative effect on low-sugar candied kumquat. *J. Food Prot.* **1994**, *56*, 136−140.
(31) Jeon, Y. J.; Lee, Y. H.; Kim, S. K. Bioactivities of chitin and chitosan (1) − antimicrobial function, hypertension control function and cholesterol control function. *Korean J. Chitin Chitosan* **1996**, *1*, 4−13.
(32) Liu, X.; Fernandez, M.; Wouters, M. A.; Heyberger, S.; Husain, A. Arg^{1098} Is critical for the chloride dependence of human angiotensin I-converting enzyme c-domain catalytic activity. *J. Biol. Chem.* **2001**, *276*, 33518−33525.
(33) Lowenstein, C. J.; Snyder, S. H. Nitric oxide, a novel biologic messenger. *Cell* **1992**, *70*, 705−707.
(34) Jung, K.; Ha, Y.; Ha, S. K.; Han, D. U.; Kim, D. W.; Moon, W. K.; Chae, C. Antiviral effect of *Saccharomyces cerevisiae* beta-glucan to swine influenza virus by increased production of interferon-gamma and nitric oxide. *J. Vet. Med. B* **2004**, *51*, 72−76.
(35) Czop, J. K.; Kay, J. Isolation and characterization of beta-glucan receptors on human mononuclear phagocytes. *J. Exp. Med.* **1991**, *173*, 1511−1520.

Received for review February 5, 2005. Revised manuscript received April 26, 2005. Accepted May 17, 2005.

JF050273J