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Hypolipidemic Effect of Glycosaminoglycans from the Sea Cucumber *Metriatyla scabra* in Rats Fed a Cholesterol-Supplemented Diet

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The purpose of this study was to determine the hypolipidemic effects of the glycosaminoglycans (GAGs) extracted from a sea cucumber, *Metriatyla scabra*. Using DEAE column chromatography, two major peaks containing GAGs were obtained: peak 1 (P-1) contained mainly GAGs (as hexuronic acid and hexosamine), whereas P-2 contained mostly free glycan (as fucose) with little hexuronic acid or hexosamine. Therefore, we used only the P-1 fraction (with molecular weights in the range 200−500 kDa) for evaluation of hypolipidemic effects. The lyophilized GAGs were administered orally to male Wistar rats at 5, 10, 20, and 50 mg/kg body weight for six consecutive weeks, during which the rats were fed ad libitum a basal laboratory diet with or without 1% cholesterol. The results show that the 1% cholesterol diet significantly increased plasma total cholesterol, LDL-cholesterol, and atherogenic index. Cholesterol supplementation also significantly increased hepatic TG, cholesterol, phospholipid, and liver weight. When rats fed the 1% cholesterol diet were supplemented orally with the sea cucumber GAGs, plasma levels of total cholesterol, LDL-cholesterol, and atherogenic index were significantly decreased, while HDL-cholesterol was significantly increased, although these effects of the GAGs were only dose-dependent at doses lower than 20 mg/kg b.w. Similarly, the GAGs significantly prevented the increase ($p < 0.05$) in hepatic contents of triglyceride, cholesterol, and phospholipid. Thus, the present study demonstrates that the sea cucumber GAGs have the potential of being used for reducing the risk of atherosclerosis and hyperlipoproteinemia.

KEYWORDS: Sea cucumber; glycosaminoglycan; cholesterol; hyperlipoproteinemia

INTRODUCTION

Sea cucumber belongs to Holothuroidea in biological classification and is distributed throughout the world seas. Sea cucumber is consumed in an ordinary diet as raw product and as beche-de-mer in Asia (*1*). It is rich in sulfated polysaccharides called glycosaminoglycans (GAGs), which are widely distributed in tissues of vertebrates and in the connective tissues of members of the Holothuroidea (such as sea cucumber and Ascidians) (*2*, *3*). GAGs are structurally related to heparin, heparan sulfate, chondroitin sulfate, and hyaluronic acid, all of which exist mainly in the cell surfaces and in the extracellular matrix and are important in the cell−cell interaction by binding and localizing biologically important proteins (*4*−*6*). GAGs are also known as proteoglycans because GAGs are often attached to a protein core in the animal tissues (*7*−*9*). GAGs influence various physiological functions, including enhancement of immune function (*10*, *11*), facilitation of gestation and childbirth in humans (*12*), facilitation of coagulation (*13*), and lipoprotein metabolism (*14*). The physiological activities of proteoglycans vary with the types of proteins coupled to them (*15*).

The body wall of various sea cucumber species has been shown to contain heterogeneous fucose-branched chondroitin sulfate, heparin, and heparan sulfate (*16*−*20*). The sea cucumber GAGs may be expected to affect various physiological functions based on the results obtained from GAGs from other sources. However, biological evaluations on the sea cucumber GAGs are few, and no report on lipid metabolism is available. We, therefore, extracted the GAGs from a commonly consumed sea cucumber, *Metriatyla scabra*, and administered them to rats fed a cholesterol-supplemented diet for six consecutive weeks to study the effects on cholesterol and lipid metabolism.

MATERIALS AND METHODS

Materials. Dried sea cucumber (*Metriatyla scabra*) was purchased from a local market in Taichung, Taiwan. Blue dextran (200 kDa), albumin (67 kDa), ovalbumin (43 kDa), chymotrypsinogen A (12 kDa), and ribounclease A (13.7 kDa) were purchased from Pharmacia (Uppsala, Sweden). DEAE-cellulose, Sephacryl S-400 column, hexosamine, and cholesterol were obtained from Sigma Chemical Co. (St. Louis, MO). All chemicals used were of reagent grade.

Extraction and Purification of Sea Cucumber GAGs. The sea cucumber GAGs were prepared according to the method described by Vieira et al. (*4*). Briefly, the body walls of the GAGs were immersed

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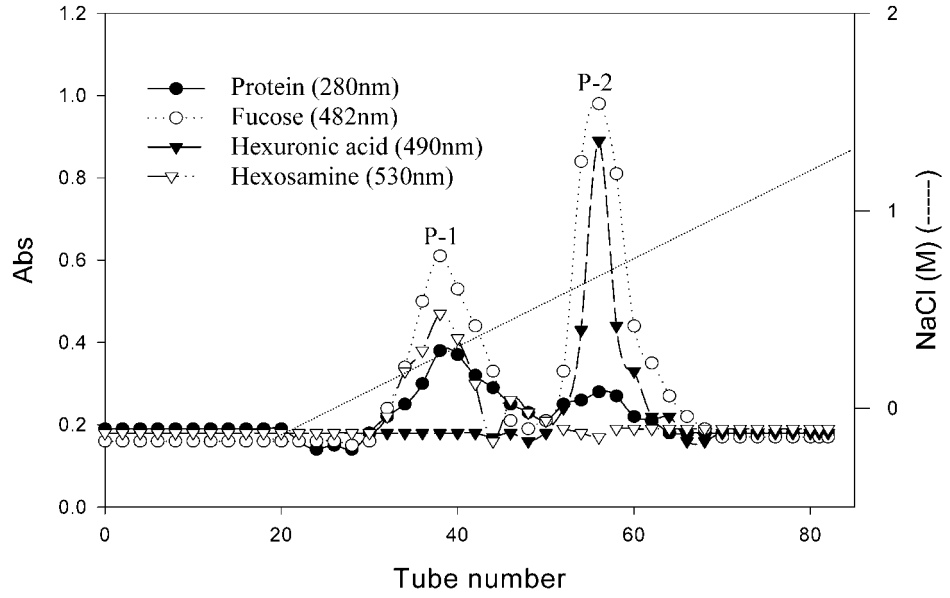

Figure 1. Fractionation of crude GAGs from a sea cucumber (*Metriatyla scabra*) on DEAE-cellulose chromatography. Crude GAGs were obtained by extraction at 60 °C for 24 h using 50 mM sodium acetate buffer (pH 6.0) containing 5 g of papain, 5 mM EDTA, and 5 mM cysteine. The lyophilized crude GAGs were further purified by DEAE column chromatography with elution using a linear gradient of NaCl (1∼1.2 M). P-1 (200–500 kDa) contained mainly GAGs (as hexuronic acid and hexosamine), whereas P-2 contained mostly free glycan (as fucose) with little protein, hexuronic acid, and hexosamine. Lyophilized P-1 was then used for biological evaluation.

Table 1. Chemical Composition of Glycosaminoglycans (GAG) from the Sea Cucumber (*Metriatyla scabra*) Body Wall

	protein[c] (μg/mL)	fucose (μg/mL)	hexuronic acid (μg/mL)	hexosamine (μg/mL)	MW (kDa)
crude GAG[a]	23 ± 2	162 ± 12	271 ± 18	289 ± 10	∼500
P-1[b]	24 ± 1	75 ± 4	230 ± 12	245 ± 13	200∼500
P-2[b]	2 ± 1	81 ± 7	10 ± 5	5 ± 11	40∼200

[a] Crude GAGs (lyophilized) were obtained by extraction at 60 °C for 24 h using 50 mM sodium acetate buffer (pH 6.0) containing 5 g of papain, 5 mM EDTA, and 5 mM cysteine. [b] P-1 and P-2 refer to the lyophilized fractions after DEAE column chromatography with elution using a linear gradient of NaCl (1∼1.2 M). [c] Protein was determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA).

immediately in acetone and kept for 24 h at 4 °C. The clean body wall (∼30 g, wet weight) was cut into small pieces and immediately immersed in 50 mM sodium acetate buffer, pH 6.0, containing 5 g of papain, 5 mM EDTA, and 5 mM cysteine, and incubated at 60 °C for 24 h. The mixture was centrifuged (2000*g* at 4 °C for 15 min), and the clear supernatant was precipitated with 2 vol 95% ethanol. After standing at −10 °C for 24 h, the precipitate was collected by centrifugation (2000*g* at 4 °C for 30 min), dissolved in distilled water, and lyophilized (crude GAG). The powder was then suspended in 50 mM sodium acetate buffer, pH 6.0, containing 50 mM EDTA, and used in DEAE chromatography. Elution was performed with a linear gradient of NaCl from 0 to 1.2 M in 50 mM sodium acetate buffer (pH 6.0) at a flow rate of 12 mL/h. The concentration of NaCl was determined by conductivity (**Figure 1**). The eluent was collected at 3 mL/tube for the determination of protein (measured at 280 nm), fucose (*21*), hexuronic acid (*22*), and hexosamine (*23*). Two major peaks appeared: one was sulfated glycan (P-1) and the other was primarily free glycan (P-2) (**Table 1**). The fractions of each peak were pooled, dialyzed in distilled water, and lyophilized. For estimation of molecular weights, the lyophilized sulfated glycan and free glycan were applied to a Sephacryl S-400 column (110 × 1.5 cm) and eluted with 50 mM sodium acetate buffer (pH 6.0) containing 8 M urea, 0.5 M NaCl, and 10 mM EDTA at a flow rate of 8 mL/h (1 mL/tube). Blue dextran (200 kDa), albumin (67 kDa), ovalbumin (43 kDa), chymotrypsinogen A (12 kDa), and ribounclease A (13.7 kDa) was used as molecular markers.

Animals. A total of 30 male Wistar rats, with an average weight of 180 g, were purchased from the Animal Center of National Taiwan University and randomly divided into six groups after one week of acclimation. One group of rats was fed a basal laboratory rodent diet (Purina Rat Chow 5001, Purina Mills, St. Louis, MO) (group B), and the other five groups of rats were fed the same diet supplemented with 1% cholesterol (group BC), prepared by gently grinding and mixing the laboratory diet with cholesterol. Among the five cholesterol-supplemented groups, four groups of rats were orally administered every day with the purified GAGs at 5 mg/kg b.w. (GAG-5), 10 mg/kg b.w. (GAG-10), 20 mg/kg b.w. (GAG-20), or 50 mg/kg b.w. (GAG-50). The experiments were carried out for 6 weeks, during which the rats were kept separately in a room maintained at 22 ± 2 °C with a relative humidity of 40−60% and a light/dark cycle of 12/12 h. Food and water were given ad libitum. The body weights of rats were recorded weekly. This animal study protocol was approved by the Animal Research Committee of NCHU.

Specimen Collection and Analysis. After feeding for 6 weeks, rats were starved for 12 h and anesthetized by CO_2. Blood taken from the abdominal cavity was collected into tubes with EDTA as anticoagulant. Plasma was obtained by centrifuging blood at 365*g* for 20 min. Levels of triglycerides and total cholesterol were determined enzymatically using the glycerol-3-phosphate oxidase reaction system (GPO-PAP) and the cholesterol oxidase reaction system (CHOD-PAP), respectively (*24*). Total phospholipid in plasma was measured according to the method of Murata et al. (*25*). High-density lipoprotein (HDL) in plasma was determined using phosphotungstic acid and magnesium to precipitate LDL and VLDL and analyzed by the CHOD-PAP method after centrifugation at 4000*g* for 15 min. Low-density lipoprotein (LDL) in plasma was determined using heparin and sodium citrate to precipitate LDL and analyzed by CHOD-PAP method after centrifugation at 4000*g* for 15 min.

Rat livers were removed by dissection, weighed, rapidly frozen in liquid nitrogen, and stored in −70 °C. After defrosting, the lipids of liver tissues were extracted using the method of Folch et al. (*26*). Hepatic triglyceride (*27*), total cholesterol, and total phospholipid were measured enzymatically, and total phospholipid was determined colorimetrically, as described elsewhere (*28*).

Statistical Analysis. Data, expressed as mean ± SD, were analyzed by one-way ANOVA using SAS system.

Table 2. Effects of the Sea Cucumber Glycosaminoglycans (GAGs) Administered Orally on the Body Weight, Feed Efficiency, and Liver Weight of Wistar Rats Fed a 1% Cholesterol Diet for 6 Weeks[a]

treatment	body weight (g)	feed efficiency[b] (%)	liver weight[c] absolute	liver weight[c] relative
B	472 ± 15	26.2 ± 0.8	114.5 ± 1.4a	3.27 ± 0.3
BC	485 ± 18	24.2 ± 1.1	116.2 ± 0.9b	3.42 ± 0.4
+GAG-5	480 ± 17	25.4 ± 1.2	116.5 ± 1.7ab	3.32 ± 0.4
+GAG-10	479 ± 16	24.6 ± 1.5	116.6 ± 1.9ab	3.42 ± 0.2
+GAG-20	488 ± 11	24.0 ± 1.0	115.9 ± 1.4b	3.34 ± 0.3
+GAG-50	475 ± 16	26.4 ± 1.8	115.0 ± 1.6a	3.34 ± 0.4

[a] B, basal laboratory diet; BC, basal diet supplemented with 1% cholesterol. The numbers following GAG represent the amounts of GAGs (mg/kg body weight) administered orally. [b] Feed efficiency is calculated as 100% × (body wt gain)/(feed intake). [c] Relative liver weight is obtained as liver wt/body wt × 100%. Values (means ± SD of 5 rats) in a column not sharing a common letter are significantly different ($p < 0.05$).

Table 3. Effects of Sea Cucumber Glycosaminoglycans (GAGs) Administered Orally on Total Cholesterol (C) and Lipoprotein Levels in Plasma of Wistar Rats Fed a 1% Cholesterol Diet for 6 Weeks[a,b]

	total C (mg/100 mL)	HDL-C (mg/100 mL)	LDL-C (mg/100 mL)	AI
B	110 ± 7d	34.2 ± 4.9a	37.5 ± 9.6b	1.15 ± 0.1b
BC	223 ± 29a	27.6 ± 3.2b	76.7 ± 7.7a	2.73 ± 0.3a
+GAG-5	181 ± 16b	34.8 ± 2.6a	32.2 ± 6.1bc	0.92 ± 0.1bc
+GAG-10	144 ± 17c	35.6 ± 4.6a	28.2 ± 4.2c	0.98 ± 0.2c
+GAG-20	113 ± 10d	37.4 ± 3.0a	27.3 ± 3.6c	0.92 ± 0.4c
+GAG-50	106 ± 9d	37.0 ± 4.1a	26.8 ± 7.6c	0.92 ± 0.1c

[a] B, basal laboratory diet; BC, basal diet supplemented with 1% cholesterol. The numbers following GAG represent the amounts of GAGs (mg/kg body weight) administered orally. [b] LDL and HDL are low and high-density lipoproteins. AI, Atherogenic index = LDL cholesterol/HDL cholesterol. Values (means ± SD of 5 rats) in a column not sharing a common letter are significantly different ($p < 0.05$).

RESULTS

Chemical Composition of Sea Cucumber PG. Figure 1 shows the profile of DEAE chromatography, where two peaks (P-1 and P-2) appeared, a result resembling those reported previously (*2*−*4*). Gel filtration on Sephacryl S-400 indicates that the average molecular weights for crude GAGs, P-1, and P-2 were ca. 500, 200−500, and 40−200 kDa, respectively (**Table 1**). P-1 contained mainly GAGs (as hexuronic acid and hexosamine), while P-2 contained mostly free glycan (as fucose) with little protein, hexuronic acid, and hexosamine. Therefore, we used only the lyophilized P-1 fraction for the following biological evaluation. The P-1 fraction accounted for 2.5% of the dried sea cucumber body wall (w/w).

Feed Efficiency and Body and Liver Weights. After six weeks of feeding, cholesterol supplementation resulted in hepatomegaly, as indicated by the significant increase in absolute liver weights, although the relative liver weights were not significantly different (**Table 2**). The GAGs administration had no significant effect on cholesterol-induced hepatomegaly. Neither cholesterol nor the SCGAGs significantly affected the feed efficiency and the body weight.

Lipid Contents in Plasma. As expected, total cholesterol, LDL-cholesterol, and atherogenic index (AI) in the plasma of rats from the BC group were significantly higher, while HDL-cholesterol was significantly lower, than those of rats fed the basal diet (**Table 3**). As compared to the BC group, the sea cucumber GAGs significantly decreased total cholesterol, LDL-cholesterol and the AI, and dose-dependent effects were evident when the sea cucumber GAGs were administered below 20 mg/kg b.w. Cholesterol supplementation significantly decreased plasma TG over the basal group, whereas feeding of the sea cucumber GAGs partially restored the plasma TG level, as compared to the BC group, but the effects of GAGs were not dose-dependent (**Table 4**). Cholesterol supplementation also significantly increased plasma total phospholipid, and this increase was prevented by feeding of the sea cucumber GAGs, although the effect of the latter was not dose-dependent.

Table 4. Effects of Sea Cucumber Glycosaminoglycans (GAGs) Administered Orally on Triglyceride (TG) and Phospholipid (PL) Levels in Plasma of Wistar Rats Fed a 1% Cholesterol Diet for 6 Weeks[a]

	total TG (mg/100 mL)	total PL (mg/100 mL)
B	174 ± 16a	92 ± 18b
BC	76 ± 15c	117 ± 9a
+GAG-5	127 ± 18b	103 ± 18ab
+GAG-10	119 ± 13 b	99 ± 11ab
+GAG-20	116 ± 16 b	98 ± 15b
+GAG-50	116 ± 18 b	98 ± 21b

[a] B, basal laboratory diet; BC, basal diet supplemented with 1% cholesterol. The numbers following GAG represent the amounts of GAGs (mg/kg body weight) supplemented orally. Values (means ± SD of 5 rats per group) in a column not sharing a common letter are significantly different ($p < 0.05$).

Table 5. Effects of Glycosaminoglycans (GAGs) Administered Orally on Hepatic Contents of Total Triglyceride (TG), Total Cholesterol (C), and Total Phospholipid (PL) in Wistar Rats Fed a 1% Cholesterol Diet for 6 Weeks[a]

	total TG (mg/g)	total C (mg/g)	total PL (mg/g)
B	97.4 ± 11.4c	40.7 ± 6.7d	43.2 ± 4.9b
BC	172 ± 12.2a	160 ± 21.6a	51.2 ± 7.6a
+GAG-5	110 ± 10.5b	103 ± 8.2b	43.8 ± 4.7b
+GAG-10	101 ± 5.0bc	79.8 ± 6.9c	39.3 ± 5.1b
+GAG-20	94.8 ± 5.2c	73.7 ± 9.1c	39.5 ± 4.2b
+GAG-50	94.2 ± 6.2c	69.9 ± 8.2c	38.9 ± 5.1b

[a] B, basal laboratory diet; BC, basal diet supplemented with 1% cholesterol. The numbers following GAG represent the amounts of GAGs (mg/kg body weight) supplemented orally. Values (means ± SD of 5 rats per group) in a column not sharing a common letter are significantly different ($p < 0.05$).

Lipid Contents in Liver. As compared to those of the B group, hepatic contents of total TG, total cholesterol, and total phospholipid in rats of the BC group increased 77% ($P < 0.05$), 293% ($P < 0.05$), and 19% ($P < 0.05$), respectively (**Table 5**). The sea cucumber GAGs significantly prevented these increases, but its effects were stronger at lower doses than at higher doses.

DISCUSSION

The GAGs from various sources have been shown to have several physiological functions, but very little is known of the functions of the GAGs from sea cucumber species. The molecular weight (200−500 kDa) of the GAGs from the sea cucumber *Metriatyla scabra* is similar to that of other sea cucumber species (*3*, *16*, *17*) but is much higher than that from various other nonsea-cucumber species (lower than 100 kDa) (*29*). In this study, we administered the GAGs from *Metriatyla scabra* to male rats fed a 1% cholesterol diet for 6 weeks to investigate the hypolipidemic effects of the GAGs in vivo. As expected, the 1% cholesterol diet led to markedly increased levels of hepatic TG and plasma cholesterol, a result that confirmed previous findings (*30*, *31*). By contrast, the 1%

cholesterol diet markedly decreased plasma TG levels. As reported by Liu et al. (*31*), the 1% cholesterol diet may hinder the metabolism of fatty acids through decreased activity of β-oxidation and may reduce excretion of hepatic TG to blood via VLDL (*31*). In addition, Sultan et al. (*32*) reported that the 1% cholesterol diet decreases the activity of hepatic lipase in both normal rats and genetically hypercholesterolemic rats, leading to decreased TG in the plasma. As a result of overproduction of TG in the liver, fatty liver may develop in rats fed a high cholesterol diet (*31*). In rats fed the 1% cholesterol diet (without the GAGs supplementation), we found not only markedly lowered plasma TG but also the formation of primary fatty liver (data not shown) and hepatomegaly; the latter findings confirmed those reported by Liu et al. (*31*).

Oral administration of the sea cucumber GAGs to rats for 6 weeks effectively prevented the changes in plasma and hepatic lipid contents induced by feeding of 1% cholesterol. The GAGs were most effective in restoring cholesterol and phospholipid levels, and these effects of the GAGs were dose-dependent between 5 and 20 mg/kg b.w. The decreased cholesterol by the GAGs may be attributed to its inhibition of HMG-CoA reductase (*33*) or to the binding of oxidized LDL by the polysaccharide moiety of the GAGs (*34*−*36*). The latter effect could increase lipoprotein lipase (LPL) activity, promote the decomposition of TG into fatty acid, glycerol, and water, and speed up the metabolism of cholesterol. Moreover, studies of the effects of GAGs on LPL have revealed that there are 3 LPL binding receptors, including a 220-kDa PG, a 116-kDa protein, and an 85-kDa protein, on the cell surface of artery vessel walls (*37*). Therefore, activity of LPL may be significantly elevated when GAGs are coupled to LPL via ion interaction (*37*).

When partially depolarized chondroitin sulfate from a sea cucumber was administered orally to rats, Imanari et al. (*38*) showed that these macromolecules are absorbed in the gastrointestinal tract. Thus, the GAGs used in the present study may also be absorbed in the gastrointestines before participation in lipid metabolism. Metabolism of GAGs in the gastrointestines to smaller molecular-weight glycan sulfate has been shown to have higher bioavailability (*39*) while retaining original physiological functions (*5*, *6*). Because we did not measure fecal cholesterol contents, we cannot exclude the possibility that the sea cucumber GAGs may exert their hypolipidemic effect by interfering with the absorption of cholesterol.

In summary, our results show that the sea cucumber GAGs administered orally to rats fed a high-cholesterol diet reduce lipid concentration in plasma and liver, possibly by accelerating in vivo metabolism of cholesterol. These effects of the GAGs were already evident when administered at low doses, i.e., 5−20 mg/kg b.w., which are equivalent to a daily consumption of 200 to 800 mg (dry wt) of the sea cucumber (*Metriatyla scabra*) body wall, based on a 2.5% yield of the GAGs (or equivalent to 220−880 mg of the wet sea cucumber, based on a 90% water content). Therefore, the sea cucumber GAGs may be potentially useful in reducing the incidence of cholesterol-related diseases such as atherosclerosis and cholesterol fatty liver.

LITERATURE CITED

(1) Conand, C.; Byrme, M. A review of recent developments in the world sea cucumber fisheries. *Mar. Fish Rev.* **1993**, *55*, 13−33.

(2) Mourão, P. A. S.; Bastos, I. G. Highly acidic glycans from sea cucumbers isolation and fractionation of fucose-rich sulfated polysaccharides from the body wall of *Ludwigothurea grisea*. *Eur. J. Biochem.* **1987**, *166*, 639−645.

(3) Vieira, R. P.; Pedrosa, C.; Mourão, P. A. S. Extensive heterogeneity of proteoglycans binding fructose-branched chondroitin sulfate extracted from the connective tissue sea cucumber. *Biochemistry* **1993**, *32*, 2254−2262.

(4) Vieria, R. P.; Mourão, P. A. S. Occurrence of a unique fucose-branched chondroitin sulfate in the body wall of a sea cucumber. *J. Biol. Chem.* **1988**, *263*, 18176−18183.

(5) Hardingham, T. E.; Fosang, A. J. Proteoglycans: many forms and many functions. *FASEB J.* **1992**, *6*, 861−870.

(6) Templeton, D. M. Proteoglycans in cell regulation. *Curr. Rev. Clin. Lab. Sci.* **1992**, *29*, 141−184.

(7) Mourão, P. A. S.; Ribeiro, A. C.; Vieira, R. P.; Barbra, M. A sulfated α-L-fucan from sea cucumber. *Carbohydr. Res*. **1994**, *255*, 225−240.

(8) Trotter, J. A.; Gillian, L.; Luna, D.; Koob, T. J.; Keene, D. R.; Atkinson, M. A. L. A glycoprotein from sea cucumber dermis that aggregates collagen fibrils. *Matrix Biol.* **1996**, *15*, 99−110.

(9) Trotter, J. A.; Thurmond, F. A.; Koob, T. J. Collagen and proteoglycan in a sea urchin ligament with mutable mechanical properties. *Cell Tissue Res.* **1989**, *258*, 527−539.

(10) Levy, D. E.; Horner, A. A.; Solomon, A. Immunoglobulin-sulfated polysaccharide interactions: binding of agaropectin and heparin by human IgG proteins. *J. Exp. Med.* **1981**, *153*, 883−896.

(11) Stevens, F. J.; Kisilevsky, R. Immunoglobulin light chains, glycosaminoglycans, and amyloidal. *Cell. Mol. Life Sci.* **2000**, *57*, 441−449.

(12) Fried, M.; Duffy, P. E. Maternal malaria and parasite adhesion. *J. Mol. Med.* **1998**, *76*, 162−171.

(13) Beijering, R. J. R.; Cate, H. Ten.; Cate, J. W. Clinical applications of new antithrombotic agents. *Ann. Hematol.* **1996**, *72*, 177−183.

(14) Kolset, S. O.; Salmivirta, M. Cell surface heparin sulfate proteoglycans and lipoprotein metabolism. *Cell. Mol. Life Sci.* **1999**, *56*, 857−870.

(15) Caterson, B.; Mahmoodian, F.; Sorrell, J. M.; Hardingham, T. E.; Bayliss, M. T.; Carney, S. L.; Ratcliffe, A.; Muir, H. Modulation of native chondroitin sulphate structures in tissue development and in disease. *J. Cell Sci.* **1990**, *97*, 411−417.

(16) Albano, R. M.; Mourão, P. A. S. Isolation, fractionation, and preliminary characterization of a novel class of sulfated glycans from the tunic of *Styela plicata* (*Chordata Tunicata*). *J. Biol. Chem.* **1986**, *261*, 758−765.

(17) Mulloy, B.; Ribeiro, A.-C.; Alves, A.-P.; Vieira, R. P.; Mourão, P. A. S. Sulfated fucans from echinoderms have a regular tetrasaccharide repeating unit defined by specific patterns of sulfation at the *o*-2 and *o*-4 positions. *J. Biol. Chem.* **1994**, *269*, 22113−22123.

(18) Mourão, P. A. S.; Perlin, A. S. Structural features of sulfated glycans from the tunic of *Sryela plicate (Chordata-Tunicata). Eur. J. Biochem.* **1987**, *166*, 431−436.

(19) Kariya, Y.; Watabe, S.; Kyogashima, M.; Ishihara, M.; Ishii, T. Structure of fucose branches in the glycosaminoglycan from the body wall of the sea cucumber *Stichopus japonicus*. *Carbohydr. Res.* **1997**, *297*, 273−279.

(20) Vieria, R. P.; Mulloy, B.; Mourão, P. A. S. Structure of a fucose-branched chondroitin sulfate from sea cucumber. Evidence for the presence of 3-*O*-sulfo-beta-glucuronosyl residues. *J. Biol. Chem.* **1988**, *266*, 13530−13536.

(21) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *J. Biol. Chem.* **1956**, *28*, 350−356.

(22) Dische, Z. New specific color reaction of hexuronic acids. *J. Biol. Chem.* **1947**, *167*, 189−198.

(23) Rondle, C. J. M.; Morgan, W. T. J. The determination of glucosamine and galactosamine. *J. Biol. Chem.* **1955**, *20*, 586−589.

(24) Richmond, W. Preparation and properties of a cholesterol oxidase from nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. *Clin. Chem.* **1973**, *19*, 1350−1356.

(25) Murata, M.; Imaizumi, K.; Sugano, M. Effect of dietary phospholipids and their constituent bases on serum lipids and apportion in rats. *J. Nutr. Sci. Vitaminol*. **1982**, *28*, 281−294.
(26) Folch, J.; Lees, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **1957**, *226*, 497−509.
(27) Soloni, F. G. A sensitive enzymatic method for the determination of free and esterified tissue cholesterol. *Clin. Chem*. **1971**, *17*, 529−534.
(28) Stewart, J. C. M. Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Anal. Biochem.* **1980**, *104*, 10−14.
(29) Oldberg, A.; Kjellen, L.; Hook, M. Cell-surface heparin sulfate. Isolation and characterization of a proteoglycan from rat liver membranes. *J. Biol. Chem.* **1979**, *254*, 8505−8510.
(30) Fungwe, T. V.; Cagen, L.; Wilcox, H. G.; Heimberg, M. Regulation of hepatic secretion of very low-density lipoprotein by dietary cholesterol. *J. Lipids Res.* **1992**, *33*, 179−191.
(31) Liu, C. H.; Huang, M. T.; Huang, P. C. Sources of triglycerol accumulation in livers of rats fed a cholesterol-supplemented diet. *Lipids* **1995**, *30*, 527−531.
(32) Sultan, F.; Sanclemente, D.; Lagrange, D.; Lutton, C.; Griglio, S. Lipoprotein lipase and hepatic lipase activities in a hypercholesterolemic (RICO) strain of rat. *Biochem. J*. **1990**, *266*, 349−353.
(33) Riessen, R.; Axel, D. I.; Fenchel, M.; Herzog, U. U.; Karsch, K. K. Effect of HMG-CoA reductase inhibition on extracellular expression in human vascular smooth muscles cells. *Basic Res. Cardiol*. **1999**, *94*, 322−332.
(34) Kaplan, M.; Avirum, M. Oxidized LDL binding to a macrophage secreted extracellular matrix. *Biochem. Biophys. Res. Commun.* **1997**, *237*, 271−276.
(35) Kaplan, M.; Aviram, M. Macrophage plasma membrane chondroitin sulfate proteoglycan bind oxidized low-density lipoprotein. *Atherosclerosis* **2000**, *149*, 5−17.
(36) Makoveichuk, E.; Lookene, A.; Olivecrona, G. Lipoprotein lipase increases interaction of both heparan sulfate-covered surfaces and cells with oxidized lipoproteins. *Atherosclerosis* **1997**, *130*, S34.
(37) Wölle, D.; Fergulson, E.; Devall, L. J.; Newton, R. S.; Saxena, U. Identification of a novel 85-kDa lipoprotein lipase binding protein on human aortic endothelial cell surface. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 906−912.
(38) Imanari, T.; Washio, T.; Huang, Y.; Toyoda, H.; Suzuki, A.; Toida, T. Oral absorption and clearance of partially depolymerized fucosyl chondroitin sulfate from sea cucumber. *Thrombosis Res.* **1999**, *93*, 129−135.
(39) Boneu, B.; Lindblad, B.; Matzsch, T. Glycosaminoglycans in prophylaxis against venous thromboembolism. *Adv. Exp. Med. Biol*. **1992**, *313*, 259−274.

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