Biosci. Biotechnol. Biochem., **64** (11), 2388–2394, 2000

Gordonan, an Acidic Polysaccharide with Cell Aggregation-Inducing Activity in Insect BM-N4 Cells, Produced by *Gordonia* sp.

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Received May 17, 2000; Accepted June 28, 2000

An acidic polysaccharide, termed gordonan, was isolated from the culture medium of *Gordonia* sp. as an inducer of cell aggregation in an insect cell line, BM-N4. Gordonan had an average molecular weight of 5×10^6 and its structure was identified as →3)-4-*O*-(1-carboxyethyl)-β-D-Man*p*-(1→4)-β-D-GlcA*p*-(1→4)-β-D-Glc*p*-(1→ mainly by acid hydrolysis experiments and NMR analysis. It induces cell aggregation at the concentration of 4 μg/ml. A partially hydrolyzed polysaccharide derived from gordonan with a molecular weight of 5×10^5 showed weak activity, while any fragment molecules with lower molecular weights prepared from gordonan showed no activity.

Key words: *Gordonia*; polysaccharide; cell aggregation; insect cell line

BM-N4 is an insect cell line established from an ovarian tissue of *Bombyx mori*.[1,2] Some insect hormones such as ecdysteroids and bombyxin-II and -IV[3] can induce characteristic morphological changes in BM-N4 cells when added to the culture medium at very low concentrations.[4] We have been continuing a search for bioactive compounds that induce morphological changes in the cell line to obtain useful probes to study insect cell growth or hormone signaling in insect cells.[5] During the screening of metabolites of microorganisms, we found that the culture medium of a bacterium strongly induced cell aggregation. This observation prompted us to isolate and characterize the active principle produced by the bacterium.

In this paper, we describe the isolation, characterization, and biological activity of the compound, termed gordonan, which turned out to be an acidic polysaccharide composed of trisaccharide repeating units.

Materials and Methods

General procedures. FABMS were measured on a JMX SX-102/SX102. ^{1}H- and ^{13}C-NMR spectra were recorded at 500 MHz and 125 MHz in D_2O on a JEOL α-500 at room temperature except for partially hydrolyzed polysaccharide (**1**), the spectra of which were measured at 60°C. Acetone δ_C 31.07 and δ_H 2.225 was used as an external standard.

Screening and bioassay. Screening and bioassays were done by the methods reported previously.[5] A sample of test-culture broth or crude gordonan was aseptically poured into each well of a microtiter plate (Iwaki 3860-096 Microplate). After drying the wells *in vacuo*, BM-N4 cells (7×10^3 cells in 100 μl of a medium) were added to each well and then cultivated at 25°C. The effects of each sample on the morphology of the cells were observed through a microscope every day for 7 days.

Identification of the microbe that produces gordonan. The producer of gordonan was a Gram-positive bacterium showing a coccus- or bacillus-form. It has a high GC content (67.7%) and uses MK-9 (H_2). The cell wall consists of arabinose, galactose, *meso*-diaminopimelic acid, alanine, and glutamine. From these data, the bacterium was identified as a *Gordonia* sp.

Fermentation and purification of gordonan. Gordonia sp. Y-102 was cultured for 2 days in a test tube containing 10 ml of a media (1% malt extract, 0.4% of yeast extract and 0.4% of glucose, pH 7.3) at 26.5°C. One ml of the culture was inoculated to a 500-ml Sakaguchi flask containing 100 ml of the same medium. After cultivation of the flask for 4 days at 26.5°C and 137 rpm on a rotary shaker, the supernatant was obtained by centrifugation. The super-

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Abbreviations: Glc*p*, glucopyranose; GlcA*p*, glucopyranouronic acid; Man*p*, mannopyranose; CE-Man, 4-*O*-(1-carboxyethyl)-mannose; CTAB, cetyltrimethylammonium bromide

natant (2 L) was concentrated to 200 ml *in vacuo* and partitioned with the solvent system of chloroform-methanol-water (8:4:3). The aqueous layer (600 ml) was concentrated to 200 ml *in vacuo*, and dialyzed against water with a molecular-porous membrane tubing (MWCO 12-14000, Spectrum) for 2 days. The dialyzate (400 ml) was mixed with CTAB to 5% saturation, and incubated for 4 hours at 37°C. After centrifugation of the suspension, the obtained precipitate was washed with EtOH (300 ml × 2) and 0.2 M NaCl (300 ml), successively. The precipitate was dissolved in 0.5 M NaCl (100 ml), followed by adding 400 ml of ethanol to precipitate polyanions. The precipitate was dissolved in 0.5 M NaCl (100 ml) and dialyzed against water for 2 days. The dialyzate was lyophilized and dissolved in 100 ml of 50 mM sodium acetate buffer (pH 4.7) containing 50% methanol. Then the solution was applied to a TSK-gel DEAE-5PW HPLC (7.5 × 75 mm, Tosoh, Tokyo, Japan) column. HPLC was done under the conditions described in Fig. 1. The active fraction from the HPLC was dialyzed and lyophilized to afford 104 mg of gordonan. The purified gordonan was stored in 50% methanol at 4°C.

Gordonan. The average molecular weight was estimated by gel filtration chromatography with Sephacryl S-500HR (Amersham Pharmacia Biotech, England, UK). $[\alpha]_D^{28}$ −46° (*c* 0.1, water). ^{1}H- and ^{13}C-NMR spectra are given in Fig. 2. NMR δ_C (D_2O): 18.7 (C-9″), 61.0, 61.6 (C-6, 6″), 68.5 (C-2″), 72.6, 73.8 (C-2, 2′, 4″), 75.0, 75.5 (C-3, 5, 3′, 5′, 5″), 77.9 (C-8″), 79.6 (C-4, 3″), 80.9 (C-4′), 99.5 (C-1), 100.4 (C-1″), 103.1 (C-1′), 175.5 (C-6′), 179.8 (C-7′).

Acid hydrolysis of gordonan and purification of **2**, **3**, *and* **5**. The purified gordonan (20 mg) was dissolved in 7.2 ml of 1 N-H_2SO_4 in a sealed tube, and hydrolyzed at 100°C for 4 h. The hydrolyzate was neutralized with barium carbonate, and filtered with a glass filter. The filtrate was passed through a Dowex-50 (H^+) column (6 × 200 mm) and the column was washed with 50 ml of water. The passed-through solution and the washing were combined and lyophilized. The residue was purified by HPLC (column-1: Pegasil ODS, 10 × 250 mm, Senshu Kagaku, Tokyo, Japan; mobile phase: distilled water; flow late: 1 ml/min; retention time of **2**; 8 min. column-2: NH_2-1151-N, 4.6 × 150 mm, Senshu Kagaku; gradient elution of 50–15% CH_3CN in distilled water in 15 min; flow rate: 1 ml/min; retention times of **3** and **5**: 4.5 min and 7 min) to afford 6 mg, 10 mg, and 1.5 mg of **2**, **3**, and **5**, respectively.

4-*O*-(1-carboxyethyl)-D-mannose (**2**). $[\alpha]_D^{28}$ −33° (*c* 0.1, water). FABMS (glycerol) *m/z*: 251 $(M-H)^-$, NMR δ_H (D_2O): 1.37 (3H, d, *J*=7 Hz, H-9β), 1.38 (3H, d, *J*=7 Hz, H-9α), 3.42 (1H, H-5β), 3.50 (1H, t, *J*=9.5 Hz, H-4β), 3.56 (1H, t, *J*=9.5 Hz, H-4α), 3.75 (1H, dd, *J*=2 Hz, 7.5 Hz, H-6β), 3.75–3.79 (2H, H-3β, H-6α), 3.82–3.87 (2H, H-5α, H-6α), 3.89 (1H, dd, *J*=2 Hz, 7.5 Hz, H-6β), 3.94–3.97 (3H, H-3α, H-2α, H-2β), 4.03 (1H, q, *J*=7 Hz, H-8β), 4.04 (1H, q, *J*=7 Hz, H-8α), 4.91 (1H, s, H-1β), 5.17 (1H, s, H-1α). δ_C (D_2O): 19.7 (C-9), 61.3 (C-6), 70.5 (C-2α), 71.1 (C-3α), 71.4 (C-2β), 72.6 (C-5α), 73.4 (C-3β), 76.6 (2C, C-4β, C-5β), 76.9 (C-4α), 79.2 (C-8), 94.6 (C-1), 182.7 (C-7). Key cross peaks (H-4 to C-8, H-8 to C-4) were observed in the HMBC spectrum.

β-D-GlcA*p*-(1→4)-D-Glc*p* (**3**). FABMS(glycerol) *m/z*: 355 $(M-H)^-$, NMR δ_H (D_2O): 3.03–3.74 (10H, H-2′, 3′, 4′, 5′, H-2, 3, 4, 5, 6,[6]), 4.29 (1H, d, *J*=7.5 Hz, H-1′), 4.44 (1H, d, *J*=8 Hz, H-1β), 4.99 (1H, d, *J*=2 Hz, H-1α). δ_C (D_2O): 60.8, 60.9 (C-6α, β), 70.9 (C-5α), 72.0 (C-2α), 72.2 (C-3α), 72.6 (C-4′), 73.8 (C-2′), 74.7 (C-2β), 75.2 (C-3β), 75.6 (C-5β), 76.1 (C-5′), 76.7 (C-3′), 79.7 (C-4), 92.6 (C-1α), 96.6 (C-1β), 103.1 (C-1′).

Trisaccharide (**5**). FABMS(glycerol) *m/z*: 589 $(M-H)^-$, NMR δ_H (D_2O): 1.05 (3H, d, *J*=6.5 Hz, H-9β), 1.06 (3H, d, *J*=6.5 Hz, H-9α), 3.12 (1H, t, *J*=8 Hz, H-2″), 3.17 (1H, t, *J*=8 Hz, H-2′), 3.20–3.35 (2H, t, H-3″, H-4″; 2H, H-5β, H-5′), 3.41 (1H, t, *J*=9 Hz, H-4β), 3.41 (1H, t, *J*=9 Hz, H-3′), 3.46 (1H, t, *J*=9 Hz, H-4′), 3.50 (1H, t, *J*=9.5 Hz, H-4α), 3.55 (1H, d, *J*=9 Hz, H-5″), 3.54–3.75 (7H, H-5α, 2H, H-6α, 2H, H-6β, 2H, H-6′), 3.81 (1H, H-3β), 3.82 (1H, s, H-2α), 3.86 (1H, d, H-2β), 3.97 (1H, H-3α), 4.12 (1H, q, *J*=6.5 Hz, H-8α), 4.15 (1H, q, J*J*=6.5 Hz, H-8β), 4.30 (1H, d, *J*=6 Hz, H-1″), 4.31 (1H, t, *J*=8 Hz, H-1′), 4.66 (1H, s, H-1β), 4.97 (1H, d, *J*=2 Hz, H-1α).

Glucose oxidase treatment. The purified gordonan (20 mg) was dissolved in 1.5 N-H_2SO_4 (5 ml) and hydrolyzed at 100°C for 4 h. After neutralization with barium carbonate, the hydrolyzate was passed through a Dowex-50 (H^+) (10 × 200 mm) column and the column was washed with 50 ml of water. The flow-through solution and the washing were combined and concentrated to 2 ml *in vacuo*. The solution was put on a column of Dowex-1 (Cl^-) (5 × 150 mm). The column was washed with water (20 ml), and eluted with 1 N acetic acid (20 ml). The passed-through solution and water fraction were combined and lyophilized. The residue was purified with HPLC (column: NH_2-1151-N, Senshu Kagaku, Tokyo; mobile phase: isocratic elution of 90% acetonitrile in distilled water; flow rate: 1 ml/min) to afford glucose. The 1 N acetic acid fraction was evaporated to dryness, and the residue was dissolved in a mixture of anhydrous pyridine (4 ml) and acetic anhydride (2 ml). After stirring for 14 h at room temperature, water (4 ml) was added to the reaction mixture, and the solution was evaporated to dryness. The residue was reacted with diazomethane in anhydrous diethyl ether (20 ml) on an ice bath. After addition of acetic

acid (1 ml) to the solution, the reaction mixture was evaporated to dryness. Lithium aluminum hydride (20 mg) was added to the diethyl ether solution (6 ml) of the residue and stirred for 1 h on an ice bath. After water was added (10 ml) to the solution, the reaction mixture was evaporated to obtain an aqueous solution (5 ml). The solution was passed through a Dowex-50 (H^+) column and the column was washed with water (50 ml). The passed-through solution and the washing were combined and evaporated to dryness to obtain crude *β*-D-Glc*p*-(1→4)-D-glucitol (9.9 mg). The residue was dissolved in 1 N-H_2SO_4 (1 ml) and kept at 100°C for 14 h. The hydrolysate was neutralized and passed through a Dowex-50 (H^+) column, and the flow-through solution was evaporated to dryness. The residue was purified with NH_2-1151-N column in the same condition to afford glucose. Each glucose sample obtained from the neutral and acidic fraction was dissolved in water (100 *μ*l) and digested with D-glucose oxidase (Nacalai Tesque, Kyoto, Japan) in 8 mM ammonium acetate buffer (pH 7.4). Digestion was monitored by loss of the spot of orcinol-slufuric acid reaction on silica gel thin layer chromatography (90% acetone in distilled water, R_f 0.4).

Hydrolysis of gordonan with a cation-exchange resin. A solution of gordonan (8 mg) in water (8 ml) was mixed with 2 ml of Dowex-50 (H^+) resin. The mixture was heated at 50°C for 1 h with stirring. The mixture was filtered and the resin was washed with 10 ml of water. The filtrate and washing were combined and lyophilized to afford 7.2 mg of partially hydrolyzed polysaccharide (**1**).

Partially hydrolyzed polysaccharide (**1**). The average molecular weight was estimated by gel filtration chromatography with Sephacryl S-500HR (Amersham Pharmacia Biotech). ^{1}H- and ^{13}C-NMR data of **1** are shown in Table 1.

Results and Discussion

Fermentation and isolation of gordonan

A microbe producing gordonan, strain Y-102, was newly isolated from soil in Narita, Chiba Prefecture, and identified as a *Gordonia* sp. Strain Y-102 was cultured in a 500-ml Sakaguchi flask containing 100 ml of a medium for 4 days at 26.5°C. Cell aggregation activity was observed in both culture medium and mycelial 80%-MeOH extract. Preliminary experiments suggested that the active substance was polyanionic and had a high molecular weight. Since the culture medium was expected to contain smaller amounts of other polyanionic compounds like DNA than the mycelial extracts, it was used for further purification.

The supernatant was concentrated and partitioned using a solvent system of $CHCl_3$-MeOH-H_2O (8:4:3). The aqueous layer was dialyzed against water and the dialyzate was mixed with CTAB to give 5% saturation. After this solution was left for 4 h at 37°C, the precipitate was collected by centrifugation, and washed with ethanol and 0.2 M NaCl successively. Then the residue was dissolved in 0.5 M NaCl, and 4 volumes of ethanol was added to the solution to precipitate the crude gordonan. The precipitate was finally purified by an anion-exchange HPLC to afford a single active fraction (Fig. 1). Dialysis of the active fraction and subsequent lyophilization of the dialyzate gave gordonan as a viscous solid in the yield of 104 mg from 2 L of culture broth. Gordonan was positive to a carbazole-sulfuric acid test. The molecular weight of gordonan was estimated to be around 5 $\times 10^6$ by gel filtration chromatography. Each signal observed in the ^{1}H- and ^{13}C-NMR (Fig. 2) spectra of gordonan was very broad, but the ^{1}H-NMR signals ranging from *δ*3.2 to 4.7 clearly suggested a polysaccharide nature of gordonan.

Structure analysis of gordonan

When gordonan was hydrolyzed with 4 N HCl at 100°C for 4 h, glucose was easily identified as a product on silica gel thin layer chromatography. But all other products were difficult to identify because of their low yields.

Next, gordonan was hydrolyzed with 1 N-H_2SO_4 at 100°C for 4 h, where uronic acid, if present, would be detected without decomposition.[6] In this case, two major products, **2** and **3**, other than glucose were obtained. Since the pseudomolecular ion of $(M-H)^-$ was observed at *m/z* 251 in the negative FAB mass spectrum, **2** was shown to have a molecular weight of 252. From NMR spectra, **2** was identified as 4-*O*-(1-carboxyethyl)-mannose (CE-Man), which was known

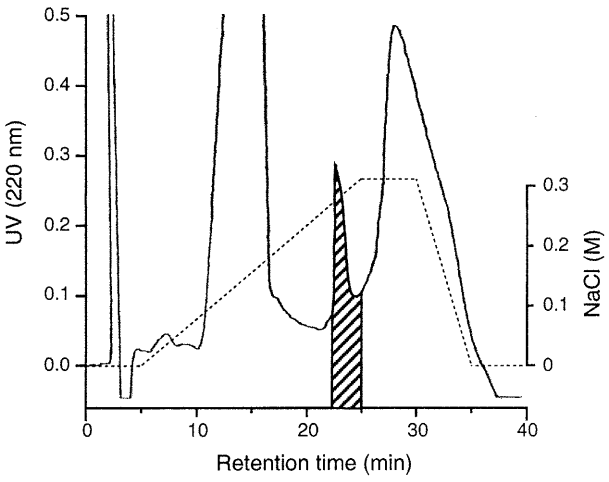


Fig. 1. Purification of Gordonan by HPLC.

HPLC conditions. Column: TSK-gel DEAE-5PW (7.5×75 mm, Tosoh). Mobile phase: gradient elution of NaCl 0–0.3 M in 50 mM sodium acetate buffer (pH 4.7) containing 50% methanol in 20 min. Flow rate: 1 ml/min. Detection: UV (220 nm). The activity was observed between 22 min and 25 min (shaded area).

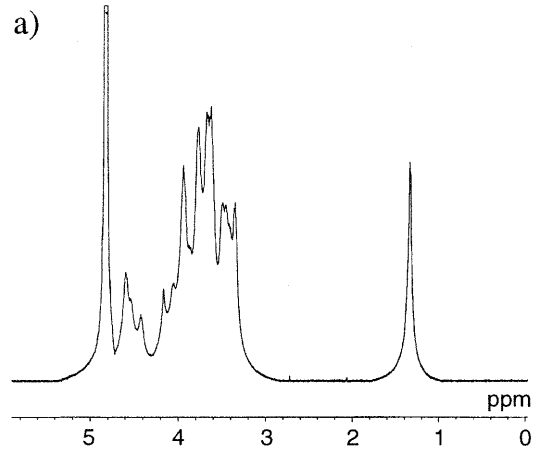
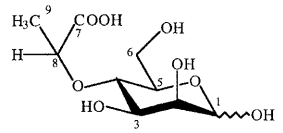
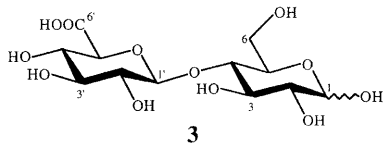


Fig. 2. ^{1}H- and ^{13}C-NMR Spectra of Gordonan in D_2O.
a) ^{1}H-NMR spectrum. b) ^{13}C-NMR spectrum.



2

Structure 2



3

Structure 3

Table 1. ^{1}H- and ^{13}C-NMR Data for Compound **1** in D_2O

C-No.	δ_C	δ_H	$^{3}J_{H,H}$ (Hz)
1	99.6	4.74 d	7
2	74.4	3.53 t	8
3	75.5	3.82 t	8.5
4	80.4	3.76 t	8.5
5	76.0	3.70 m	
6	61.7*	3.95, 4.11	
1′	103.5	4.75 d	7
2′	73.8	3.60 t	8.5
3′	75.2	3.88 t	9.5
4′	81.2	4.02 t	9
5′	74.6	4.27 d	9.5
6′	173.3		
1″	101.3	4.97 s	
2″	68.6	4.35 s	
3″	79.5	4.24 d	9.5
4″	73.8	3.90	
5″	76.0	3.65 m	
6″	62.0*	3.95, 4.11	
7″	178.5		
8″	77.9		
9″	19.1	1.55 d	7

* May be interchanged.

as a rare sugar found in some bacterial polysaccharides.[7] The pseudomolecular ion of $(M-H)^-$ was observed at m/z 355 in the negative FAB mass spectrum, then the molecular weight of **3** was shown to be 356. NMR spectra of **3** indicated that **3** was a disaccharide consisting of glucuronic acid and glucose in which the latter was present at its reducing end.[8,9] The β-1,4 linkage between the two residues was clarified by the J value of the anomeric proton at C-1′ (7.5 Hz) and the long range coupling between H-1′ and C-4 in the HMBC spectrum of **3**. Thus the structure of **3** was assigned as β-GlcA*p*-(1→4)-Glc*p*.

The absolute configuration of the glucose residue was assigned to D by D-glucose oxidase treatment of glucose in the hydrolysate of gordonan. The digestion of glucose was monitored on silica gel thin layer chromatography. The absolute configuration of glucuronic acid residue was also assigned to D by the same treatment of the glucose molecule derivatized from the glucuronic acid residue of **3** by acetylation and methyl esterification of **3**, followed by $LiAlH_4$ reduction and acid hydrolysis.

These data suggested that gordonan contained residues of D-glucose and **2**, and a unit of **3**. To analyze the total structure of gordonan, gordonan was hydrolyzed with a cation-exchange resin, Dowex-50 (H^+ type) resin in water at 50°C for 1 h, yielding a partially hydrolyzed polysaccharide (**1**). The average molecular weight of **1** was estimated to be around 5×10^5 by gel filtration chromatography. Since the signals observed in the ^{1}H- and ^{13}C-NMR spectra of **1** (Table 1) were not broadened, conventional 2 D NMR spectra of **1** were measured to identify its structure. Analyses of COSY, FGHMQC, and FGHMBC spectra of **1** indicated the presence of each residue of glucose, glucuronic acid, and CE-Man (**2**). Since all the signals in the ^{1}H- and ^{13}C-NMR spectra of **1** were involved in these three residues, it was strongly suggested that **1** had a trisaccharide repeating structure consisting of them. The J and δ values at each C-1 of the three residues ($J_{H,H}=7$ Hz, δ_H 4.74 and $^{1}J_{C,H}=158.0$ Hz for glucose, $J_{H,H}=7$ Hz, δ_H 4.75 and $^{1}J_{C,H}=158.1$ Hz for glucuronic acid, $J_{H,H}<2$ Hz, δ_H 4.97 and $^{1}J_{C,H}=156.3$ Hz for **2**) indicated that all of the

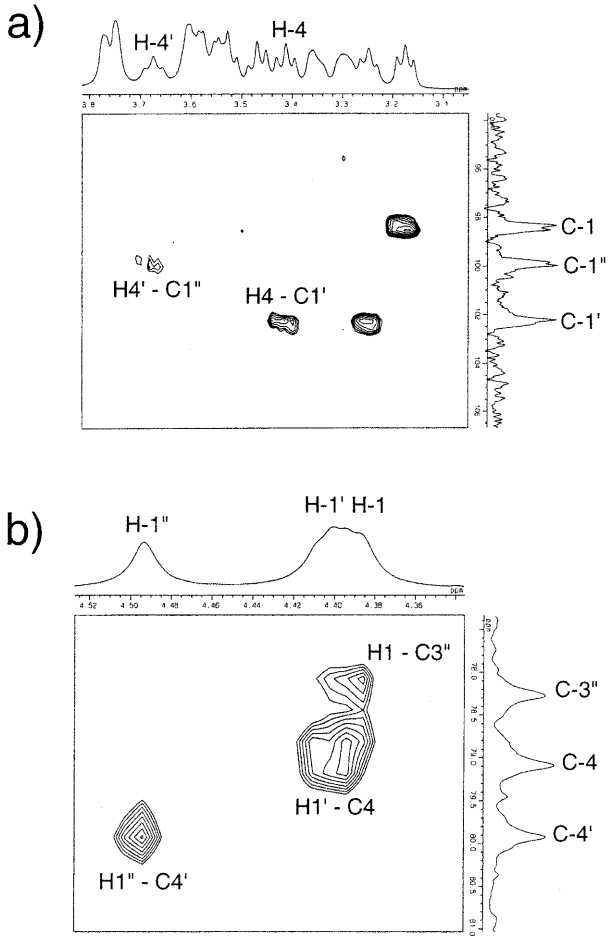


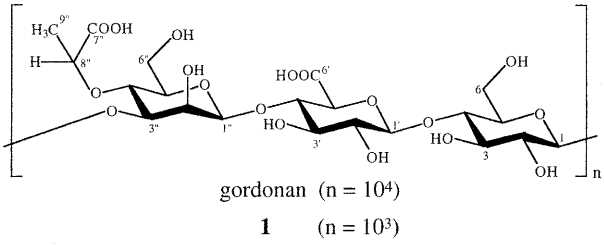
Fig. 3. Partial HMBC Spectra of Partially Hydrolyzed Polysaccharide (**1**).

(a) Cross peaks of H-4 to C-1′ and H-4′ to C-1″ are observed. (b) Cross peaks of H-1 to C-3″, H-1′ to C-4, and H-1″ to C-4′ are observed.

three residues had β configurations.[10] In the FGHMBC spectrum of **1** (Fig. 3), significant cross peaks were observed between H-1 of the Glc*p* residue and C-3 of the CE-Man residue, H-1 of the CE-Man residue and C-4 of the GlcA*p* residue, and H-1 of the GlcA*p* residue and C-4 of the Glc*p* residue. From these long range couplings, the glycosylation linkages of Glc*p*→**2**, **2**→GlcA*p*, and GlcA*p*→Glc*p* were assigned as 1→3, 1→4, and 1→4, respectively.

The transglycosidic NOE between H-1 of Glc*p* and H-2 of Man*p* observed in the NOESY spectrum of **1** indicated that the two residues have (D), (D)- or (L), (L)-configurations as reported by Wayne *et al.*[11] Since the Glc*p* residue has a D-configuration, the absolute configuration of the Man*p* residue was assigned to D-configuration.

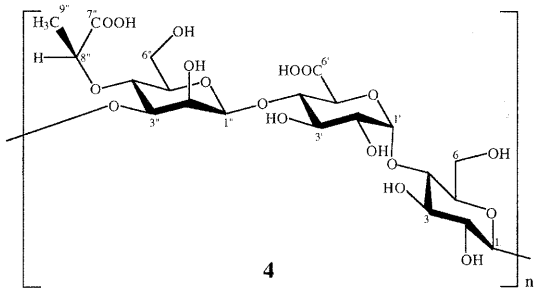
Thus, the structure of the partially hydrolyzed polysaccharide (**1**) was identified. The assignments of protons and carbons in the NMR spectra of **1** are summarized in Table 1. The ^{13}C-NMR spectrum of gordonan (Fig. 2) was essentially consistent with that of **1**. Therefore, it was concluded that gordonan had



gordonan (n = 10^4)

1 (n = 10^3)

Structure 1



4

Structure 4

the same trisaccharide repeating structure as **1**.

The structure of gordonan is very similar to that of an extracellular polysaccharide (**4**) from *Mycobacterium lacticolum* strain 121 reported by Kochetkov *et al.*[12] The polysaccharide (**4**) has almost the same structure as gordonan, but the anomeric configuration of the GlcA*p* residue is different from that of gordonan.

Biological activity

Gordonan induced the aggregation in BM-N4 cells at the concentration of more than 4 μg/ml as shown in Fig. 4. When gordonan was added at the beginning of cultivation, the cells began to adhere to each other and form clusters each consisting of 2–5 cells in several hours. After an overnight incubation, all cells in a well aggregated to form large colonies each consisting of 50–1000 cells. The aggregated cells were alive and proliferated to become confluent after further incubation.

All of the three monosaccharides which constitute gordonan showed no activity on BM-N4 cells at the concentration of 500 μg/ml (Table 2). And neither the disaccharide **3** nor the trisaccharide **5**, which was a minor product obtained by an acid hydrolysis of gordonan with 1 N-H_2SO_4, showed any activity even at 200 μg/ml (Table 2). However, the partially hydrolyzed polysaccharide **1** retained weak activity at 100 μg/ml. The activities of these fragment molecules of gordonan suggested that a polysaccharide nature with a high molecular weight of gordonan is essential for expression of its activity.

Activities of two types of dextran sulfate (1×10^4 and 5×10^5 Da) and hyaluronic acid (1.8×10^6 Da) on BM-N4 cells were also tested. Since those acidic poly-

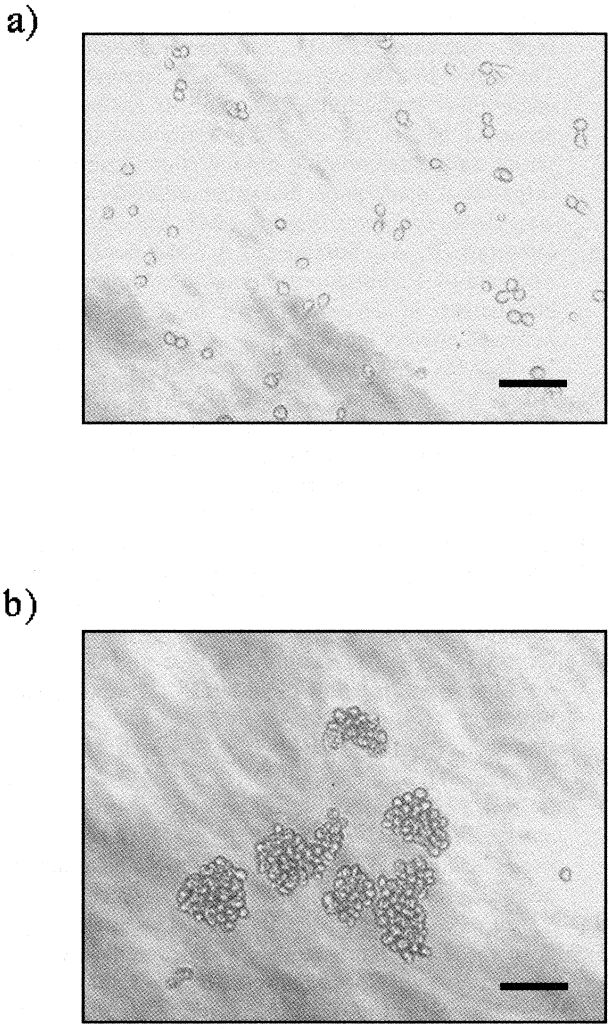


Fig. 4. BM-N4 Cells Cultured in Iwaki Microplates, Photographed under Phase Contrast with an Inverted Microscope (×100).

a) untreated cells after 2 days; b) cells treated with 10 μg/ml of gordonan for 2 days. The bar indicates 100 μm.

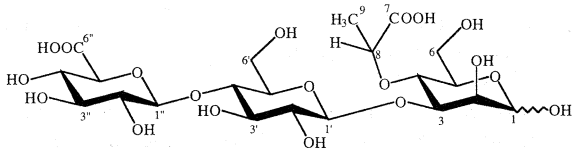
saccharides showed no activity even at the concentration of 1 mg/ml, the activity of gordonan was not derived only from its polyanionic nature.

In general, many species of bacteria produce extracellular polysaccharide in response to some environmental conditions. For instance, some pathogenic microorganisms such as *Staphylococcus aureus*,[13] *Staphylococcus epidermidis*,[14] and *Pseudomonas aeruginosa*[15] produce acidic polysaccharides including alginate as a main component to form sessile communities called biofilms.[16,17] This biofilm formation is thought to be concerned in the pathogenicity of the bacteria. *Gordonia* is a near genus to *Nocardia* and *Mycobacteria*, which cause tuberculosis or leprosy.[18] *Gordonia* is also a pathogenic microorganism found in patients suffering from bronchopulmonary disorders[19] or metastatic melanoma.[20] Gordonan might take part in the process of infection or fixation of *Gordonia* sp. to a host, as in the case of biofilms. Works to investigate the cell aggregation activity of gordonan against mammalian cells are now in progress.

Table 2. Cell Aggregation Activity of Gordonan and Related Compounds on BM-N4 Cells

Compound.	Conc. (μg/ml)	activity
gordonan	10	++[a]
gordonan	4	+[b]
1	100	+[c]
Glc	500	—[d]
GlcA	500	—
2	500	—
3	500	—
5	500	—

[a] All cells aggregated in a day.
[b] More than half cells aggregated in a day.
[c] All cells aggregated in 3 days.
[d] No change was observed.



5

Structure 5

Acknowledgments

We thank Dr. S. Imanishi of National Institute of Sericultural and Entomological Science for providing the BM-N4 cell line and Sankyo Co., Ltd. for fermentation. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan (No. 08556017) and a grant from JSPS Program for Research for the Future.

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