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Inhibition of *Helicobacter pylori* adhesion to Kato III cells by intact and low molecular weight acharan sulfate

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Abstract We investigated the inhibitory activity of glycosaminoglycans (GAGs) in terms of growth, adhesion, and VacA vacuolation of *Helicobacter pylori*. Intact acharan sulfate (AS, MW:114 kDa) potently inhibited *H. pylori* adhesion to Kato III cells with IC_{50} value of 1.4 mg/mL, while other GAGs did not show any inhibitory activity except for heparin which is a well-known inhibitor of *H. pylori* adhesion. To investigate whether low molecular weight acharan sulfate (LMWAS) can inhibit *H. pylori* adhesion, we performed chemical depolymerization of AS by radical reactions to obtain LMWAS. Its physicochemical properties were characterized by high-performance size exclusion chromatography (HPSEC), agarose gel electrophoresis, disaccharide compositional analysis after digestion with heparinase II, and ^{1}H-NMR spectroscopy. The most potent molecular size of LMWAS was 3 kDa with IC_{50} value of 32 μg/mL, which is 44-fold more potent than intact AS. These results suggest that AS as well as other GAGs can be chemically depolymerized by free radicals and LMWAS compared to intact AS can be applied as a pharmaceutical candidate in order to inhibit *H. pylori* adhesion to Kato III cells.

Keywords Acharan sulfate · Low molecular weight · *Helicobacter pylori* · Chemical depolymerization · Free radicals · Vacuolating cytotoxin

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Introduction

Helicobacter pylori was isolated from the gastric antrum of chronic gastritis patients, which is a spiral-shaped and gram-negative bacterium [1]. *H. pylori* has been found in or beneath the mucus layer, and produces a virulence factor, vacuolating toxin (VacA) [2–5], which is able to induce apoptosis of gastric epithelial cells. The bacterial adhesion to gastric epithelial cells is a key step in initiating *H. pylori* colonization that leads to the development of gastritis and peptic ulcer [6]. It is now well established that cell surface carbohydrates mediate the cell-cell recognition through single or multiple interactions. The characteristic enzyme of *H. pylori* is a urease, which hydrolyses urea to generate ammonia and bicarbonate and it results in an elevation of stomach pH. Therefore, the inhibition of *H. pylori* adhesion, urease activity, and VacA toxin are important for the treatment of patients with *H. pylori* infection. During the course of antibiotic therapy for eradication, the occurrence of antibiotic-resistant strains, side effects, and high cost bring a need for other effective agents [7]. It is known that *H. pylori* heparan sulfate-binding proteins (HSBP) are involved in the adherence of *H. pylori* to HeLa S3 and Kato III cell lines [8]. The antibodies against these HSBP inhibited adherence of *H. pylori* to culture cell lines.

Sulfated carbohydrate polymers (heparin and dextran sulfate) were shown to inhibit heparan sulfate binding to *H. pylori* where carboxylated or non-sulfated compounds did not bind [9]. In addition, polysaccharides from plant sources, *Artemisia capillaries* [10] and *Panax ginseng* [11, 12] offer promising inhibitors of *H. pylori* infection.

Acharan sulfate (AS) is a novel GAG isolated from the giant African snail, *Achatina fulica* [13]. This polysaccharide has a repeating disaccharide structure of →4)-2-deoxy-2-acetamide-α-D-glucopyranose (1→4)-2-sulfo-α-L-idopyranosyluronic acid (1→) (Fig. 1). It is related to heparin and heparan sulfate but its structure is very distinctive from all known classes of GAGs. Many biological activities of AS have been reported including anti-angiogenic activity [14], anti-mitogenic activity [15], *in vivo* anti-coagulant activity [16], immunostimulating activity, hypoglycemic activity, anti-fatigue activity, and hypolipidemic activity [17]. Cell surface nucleolin was identified as one of AS-binding proteins with high affinity from mouse Lewis lung carcinomas [18, 19].

Studies of inhibitory activity of AS on infection and vacuolation of *H. pylori* have not been performed. Therefore, it would be worthwhile performing *in vitro* inhibitory activity of AS on *H. pylori* infection and VacA vacuolation. In addition, we compared inhibitory activity of intact AS with chemically-prepared low molecular weight AS (LMWAS).

Materials and methods

Materials

H. pylori strain ATCC 49503 and ATCC 43504 were purchased from American Type Culture Collection (USA). The giant African snails *Achatina fulica* were obtained from a greenhouse at Yong-In, Gyeonggi Province, Korea. AS was prepared from the soft body of snails according to the previous procedure [13]. Heparin (MW 13 kDa) from porcine intestine was purchased from New Zealand Pharmaceuticals (Palmerston North, New Zealand). Heparan sulfate from bovine kidney, chondroitin sulfate (CS) A from whale cartilage, and CS C from shark cartilage were obtained from Seikagaku Co. (Tokyo, Japan). Heparinase II (acharan sulfate lyase) was purified from *Bacteroides stercoris* HJ-15 [20]. Two CS (MW 40 and 15 kDa), three kinds of hyaluronic acid (MW 1390, 130, and 100 kDa) used as MW standard samples and unsaturated Chondro-Disaccharide Kit were obtained from SeikagakuBio Co (Tokyo, Japan). CS oligosaccharides (6-mer and 8-mer) and AS oligosaccharides (4-mer, 12-mer and 24-mer) were isolated by controlled enzymatic depolymerization as previously described [21]. Boric acid, tris[hydroxymethyl] aminomethane (Trizma® base), sodium chloride, sodium acetate, ethylenediaminetetraacetic acid (EDTA), azure A, and cetylpyridinium chloride were purchased from Sigma (St Louis, MO, USA). Agarose was from Cambrex Bio Science (Rockland, ME, USA) and other reagents were of the best grade available.

Chemical depolymerization of AS

Chemical depolymerization of AS was performed as previously described [22] with minor modifications. Briefly, AS (1 g), sodium acetate (0.5 g) and copper acetate monohydrate (0.46 g) were dissolved in 10 mL of water in a reaction vessel. The temperature was kept at 50°C and the pH was adjusted to 7.5 during the experiment. A 9% hydrogen peroxide solution was added at a rate of 1.2 mL/h and the reaction was lasted. Samples were collected at four time points (1, 2, 3, and 6 h). Pollutant copper from these products was removed by cation-exchange chromatography using a Duolite® GT-73 resin (Sigma, MO, USA). The pH of each fraction was adjusted to 6.0 and then freeze-dried. Dried samples were desalted by Sephadex G-10 (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) gel permeation chromatography.

Agarose gel electrophoresis

Agarose gel electrophoresis was performed as previously described [23] with minor modifications. Briefly, 1.2% agarose gel was prepared and 100 μg of samples including

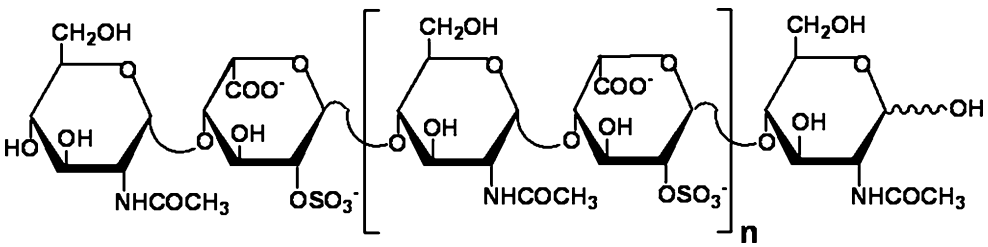

Fig. 1 Structure of AS (114 kDa, n-237)

LMWAS and AS octamer was loaded on the gel. The electrophoresis was performed at 100 V for 1 h at room temperature and the gels were stained with 0.5% azure A solution (in 1% acetic acid) for 10 min and visualized after destaining with water.

Average MW determination of intact AS and LMWAS

HPLC analysis was performed on the ÄKTA purifier system (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) equipped with a P-900 pump, a UV detector, and a fraction collector. UNICORN software version 5.01 (GE Healthcare Bio-Sciences) was used to control the apparatus and to collect data.

To determine the average MW of LMWAS, high-performance size exclusion chromatography (HPSEC) analysis was carried out on a TSK-G3000SW column (7.5×300 mm) and its guard column (7.5×75 mm) (Tosoh, Japan) as previously described [24, 25] with minor modifications. In the case of intact AS having high molecular weight, a TSK-G6000 PWXL column (7.5×300 mm) was used with different MW standard samples. The mobile phase was 0.1 M NaCl and UVabsorbance was measured at 206 nm. The flow rate was 1.0 mL/min and the system was operated at ambient temperature. The MW calibration curve for LMWAS was obtained by standard samples having known MWs such as CS (MW 15 kDa), AS-derived oligosaccharides (n=12, MW 5,436 Da; n=6, MW 2,718 Da), and CS-derived oligosaccharides (n=4, MW 2,013 Da; n=3, MW 1,510 Da). Otherwise, three kinds of hyaluronic acids (MW 1390, 130, and 100 kDa) and two CSs (MW 40 and 15 kDa) were used to calculate the average MW of intact AS. Samples and MW standards (200 μg) were injected to HPLC and the calibration curve was established by plotting the logarithm of MW versus each retention time of MW standards [24, 25].

Disaccharide compositional analysis of intact AS and LMWAS

To determine the disaccharide composition of intact AS and LMWAS, compositional analysis was performed. Briefly, 50 μL of each sample (10 mg/mL) mixed with 900 μL of 50 mM sodium phosphate buffer (pH 7.1). AS samples were depolymerized with 50 μL of heparinase II at 35°C for 12 h. After heating for 5 min and filtering on 0.45 μm filters (Millipore, Bedford, MA, USA), the depolymerization mixtures (100 μL) were injected to strong anion-exchange high-performance liquid chromatography (SAX-HPLC). SAX-HPLC was performed with a Hypersil SAX column (4.6×250 mm, 5 μm) from Thermo Hypersil-Keystone (Bellefonte, PA, USA). After sample injection, the column was washed with water (pH 3.5) for 4.155 min corresponding to one column volume (CV). Then, a linear gradient of 0~1.0 M NaCl (pH 3.5) for 41.55 min (10 CV) was used and the profile was monitored at 232 nm.

^{1}H-NMR analysis

Structural integrity of LMWAS was identified by ^{1}H-NMR analysis. Ten milligrams of each sample was exchanged with 1 mL of D_2O (Purity 99%, Sigma) followed by

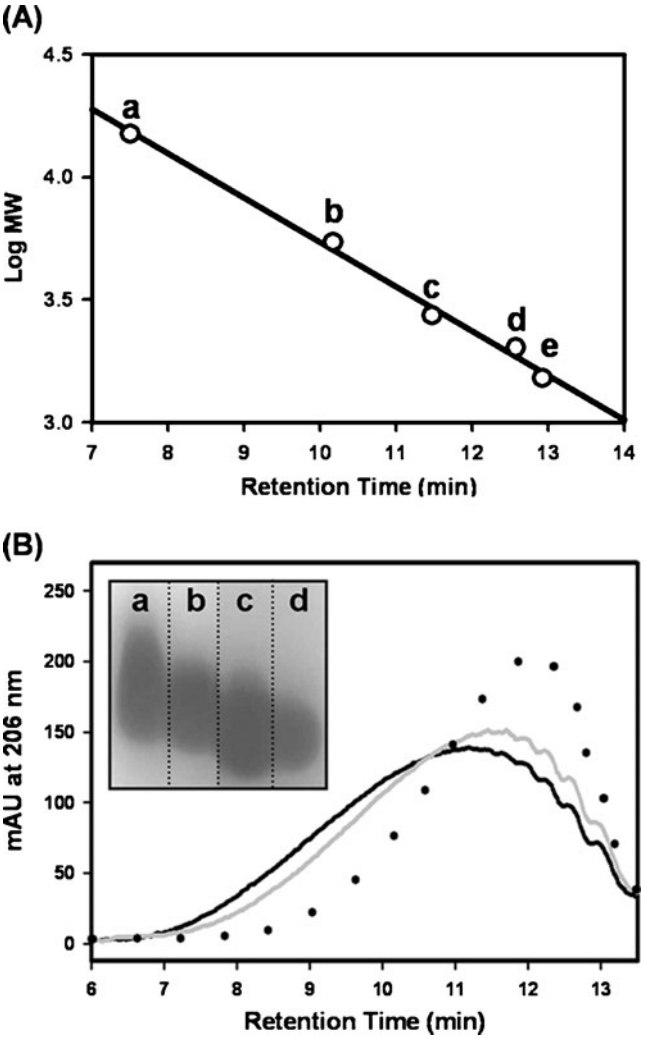

Fig. 2 Average MW determination of LMWAS by HPSEC **a** MW calibration curve for LMWAS: *a*, CS (15,000 Da) from Seikagaku; *b*, AS 24mer (5,436 Da); *c*, AS 12mer (2,718 Da); *d*, CS 8mer (2,013 Da); *e*, CS 6mer (1,510 Da). **b** HPSEC chromatogram of LMWAS ▬, LMWAS (3.3 kDa); ▬, LMWAS (3.0 kDa); •••, LMWAS (2.4 kDa). Inset shows the result of agarose gel electrophoresis. Lane *a*: LMWAS 1 h, lane *b*: LMWAS 2 h, lane *c*: LMWAS 3 h, lane *d*: LMWAS 6 h

Table 1 Determination of average MW and disaccharide composition of intact AS and LMWAS

Reaction time (hr)[a]	Average MW (Da)[b]	Disaccharide(%): tetrasaccharide(%)[c]
0	114,000	97:3
1	ND[d]	77: 23
2	3,300	75: 25
3	3,000	74: 26
6	2,400	74: 26

[a] The detailed reaction condition was explained in method section

[b] Average MW was determined by HPSEC

[c] The ratio (%) was calculated based on SAX-HPLC data in Fig. 3. Minor peaks were not taken into account

[d] Due to the size heterogeneity of sample, it was not amenable to determine the average MW

lyophilization. Dried samples were redissolved in 500 μL of D_2O and analyzed by a 300 MHz JEOL JNM-LA 300 spectrophotometer (Tokyo, Japan).

In vitro inhibitory effects of AS and its derivatives on *H. pylori* adhesion and VacA vacuolation

To investigate *H. pylori* vacuolation-inhibitory activity, HeLa cells were cultured as a monolayer in plastic flasks in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, 1% antibiotic-antimycological solution and 3.5 g/L sodium bicarbonate under 5% CO_2 at 37°C. Attached cells were released with trypsin/EDTA and seeded at a density of 7.0×10^3 cells / well in 96-well tissue culture plates 1 day before experiments.

The inhibitory activity of test agents on vacuolation by cytotoxin (VacA) in HeLa cells was measured by neutral red uptake assay [4]. Briefly, seeded HeLa cells were incubated for 16 h with *H. pylori* (ATCC 49503) VacA toxin (0.05 mg) partially purified according to the modified method of Cover and Blaser [4]. Serial dilutions of AS derivatives and GAGs were used in a microtiter assay [26]. To detect the vacuoles, cells were incubated for 8 min at room temperature with 100 μL of 0.05% neutral red in phosphate-buffered saline (PBS) and washed twice with 0.9% NaCl containing 0.1% BSA. After the addition of 100 μL of acidified ethanol solution (70% ethanol, 0.36% HCl), the absorbance was measured at 540 nm using a microtiter plate reader (Molecular Devices). All assays were performed in duplicate. VacA was purified according to the modified method of Cover and Blaser [4].

To evaluate *H. pylori* adhesion-inhibitory activity of GAGs, Kato III cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 1% antibiotic-antimycological solution and 2.2 g/L sodium bicarbonate under 5% CO_2 at 37°C. Cells were harvested with trypsin/EDTA for bacterial adhesion experiment [27]. Serial dilutions of AS derivatives and GAGs were

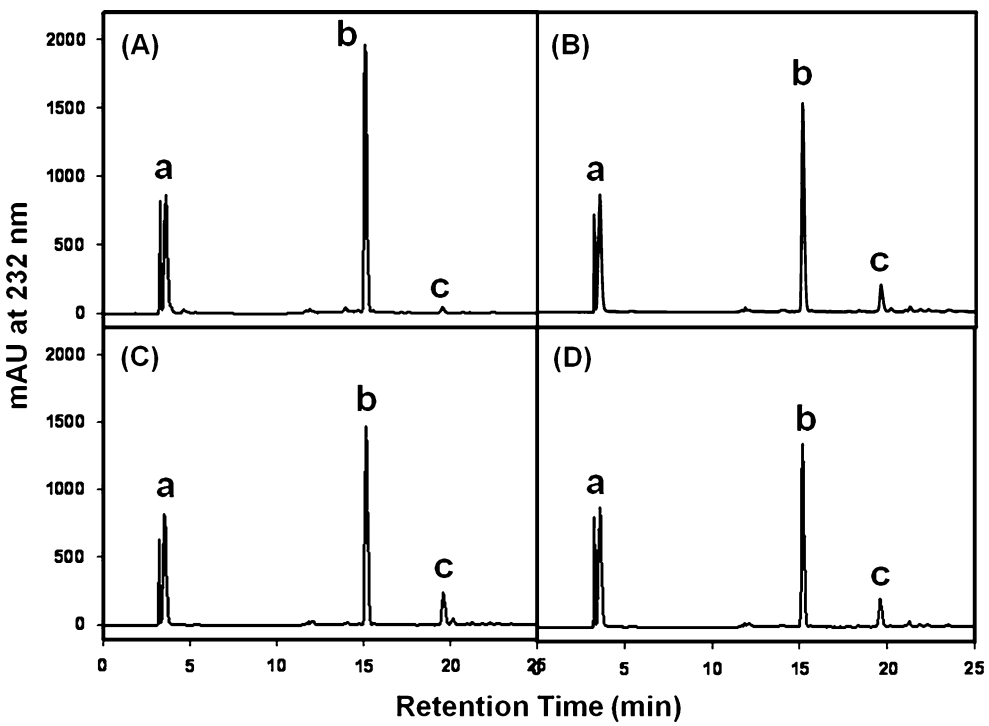

Fig. 3 Compositional analysis of intact AS and LMWAS **a** intact AS; **b** LMWAS (3.3 kDa); **c** LMWAS (3.0 kDa); **d** LMWAS (2.4 kDa) *a*, impurities derived from heparinase II solution; *b*, AS disaccharide (GlcNAc-IdoA2S); *c*, AS tetrasaccharide

incubated with the equal volume of *H. pylori* suspension in PBS for 30 min in a 37°C water bath and mixed with Kato III cells (5.0×10^6 cells/mL). After further 1 h incubation, incubation mixture was loaded on 15% sucrose, centrifuged and washed once in PBS. Subsequently, urease activity of the precipitated cells was determined by measuring the amount of ammonia released from urea in the phenol-hypochlorite urease assay as previously described [25].

Results

Average MW determination of intact AS and LMWAS

LMWAS was obtained and purified after chemical reaction (1, 2, 3, and 6 h, respectively) with hydrogen peroxide in the presence of copper salts. The result of agarose gel electrophoresis illustrates that as the reaction time with hydrogen peroxide with copper salts increased, the average MW of

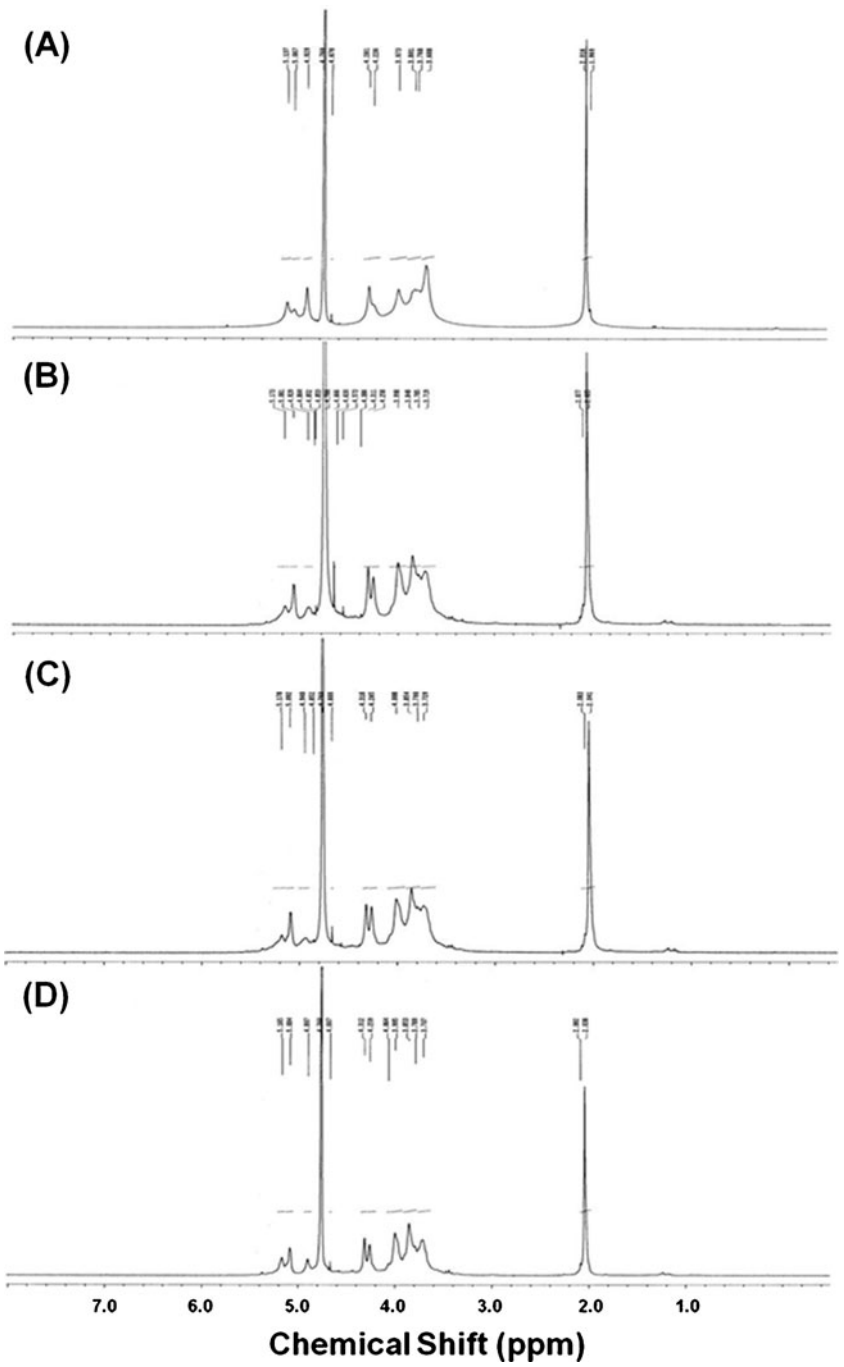

Fig. 4 ^{1}H-NMR of intact AS and LMWAS **a** intact AS; **b** LMWAS (3.3 kDa); **c** LMWAS (3.0 kDa); **d** LMWAS (2.4 kDa). LMWAS was prepared by chemical depolymerization as described in Materials and Methods

LMWAS was reduced (Fig. 2b, inset). Interestingly, AS was depolymerized more readily than CS under the same reaction conditions (data not shown).

The average MW of intact AS and chemically-depolymerized LMWAS at four different reaction times (1, 2, 3, and 6 h, respectively) was determined by HPSEC (Fig. 2b) with a MW calibration curve (Fig. 2a). As shown in Table 1, LMWAS with MW 2.4, 3.0, and 3.3 kDa were produced at 6, 3, and 2 h reaction time. In the case of LMWAS obtained after 1 h reaction, it was very heterogeneous due to the presence of high and low MW AS (data not shown).

Compositional analysis of LMWAS

Next, we depolymerized intact AS and LMWAS by heparinase II. The disaccharides and oligosaccharides derived by enzymatic depolymerization were analyzed by SAX-HPLC to compare the structural differences between intact and chemically depolymerized AS (Fig. 3 and Table 1). LMWASs contain the high portion (>22%) of tetrasaccharides, which are resistant to heparinase II. However, the intact AS has 96.76% of disaccharide and only 3.24% of tetrasaccharides. This result supports that the chemical reaction of AS with hydrogen peroxide in the presence of copper salts reduces the capacity of heparinase II to depolymerize intact AS into disaccharides. In additional experiments, the intact CS was completely depolymerized by chondroitinase ABC under our experimental conditions [23]. However, as the chemical reaction time was prolonged, the ratio of oligosaccharides, which are resistant to chondroitinase ABC increased up to 5.27% (data not shown).

Table 2 Inhibitory activity of GAGs on *H. pylori* adhesion, VacA toxin vacuolation, and *H. pylori* growth

Glycosaminoglycan	IC_{50} (mg/ml)[a]		MIC (mg/ml) [c]
	Adhesion	VacA vacuolation [b]	
Heparin	2.1	>0.1	>1
Heparan sulfate	>5	>0.1	>1
Intact acharan sulfate	1.4	>0.1	>1
LMWAS (3.0 kDa)	0.032	>0.1	>1
Chondroitin sulfate A	>5	>0.1	>1
Chondroitin sulfate C	>5	>0.1	>1
Ampicillin (control)	–[d]	–	0.001

[a] IC_{50}: The inhibitory concentration of glycosaminoglycan that causes 50% of the maximum inhibitions of adhesion and vacuolation

[b] VacA vacuolation: virulence factors in *H. pylori*-induced damage to human gastric epithelium

[c] MIC (minimal inhibitory concentration) means the lowest concentration of an antimicrobial agent at which there is complete inhibition of growth of a microorganism

[d] not detected

^{1}H-NMR analysis

The structural integrity of LMWAS was investigated by ^{1}H-NMR spectroscopy. The results of LMWAS were different from that of intact AS (Fig. 4a), suggesting that

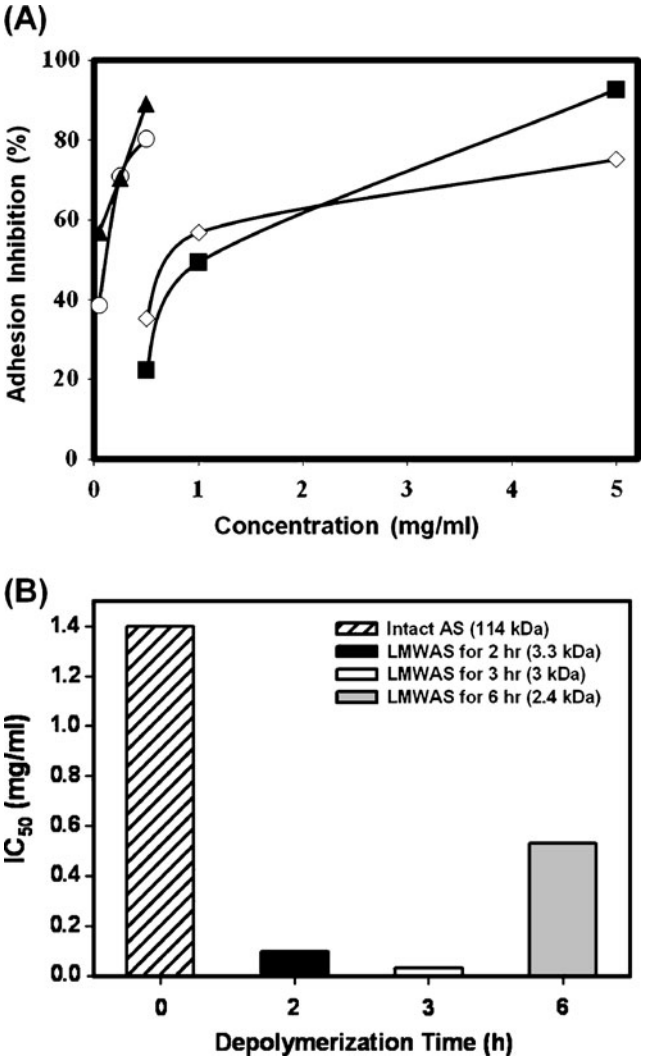

Fig. 5 Inhibitory activity of LMWAS on *H.pylori* adhesion into Kato III cells; Symbols in **a** indicate molecular weight of intact AS and LMWAS: ■, intact AS (114 kDa); ○, LMWAS (3.3 kDa); ▲, LMWAS (3.0 kDa); ◇, LMWAS (2.4 kDa). **b** shows IC_{50} (mg/ml) of LMWASs on *H.pylori* adhesion into Kato III cells. ▨, intact AS (114 kDa); ■, LMWAS (3.3 kDa); □, LMWAS (3.0 kDa); ▩, LMWAS (2.4 kDa). Inhibition %: 100 - (urease activity of precipitated cells mixed with test samples and *H. pylori*/total urease activity ×100). The experiment was performed in duplicate. The values are the average of duplicate results

decarboxylation on iduronic acid (IdoA) based on the signal of a triplet IdoA H-2 at 3.42 ppm and small signals of C-5 of IdoA at around 1.2 ppm. Furthermore, a deacetylated glucosamine H-2 signal at 3.38 ppm was seen (Fig. 4b–d).

In vitro inhibitory effects of AS on adhesion and VacA vacuolation of *H. pylori*

To evaluate anti-*H. pylori* activity of GAGs, their inhibitory activities against *H. pylori* growth and adhesion were measured (Table 2). GAGs did not inhibit *H. pylori* growth and vacuolation caused by VacA toxin. However, most of GAGs inhibited the *H. pylori* adhesion into Kato III cells. AS potently inhibited *H. pylori* adhesion with IC_{50} value of 1.4 mg/ml (Fig. 4b). Its adhesion-inhibitory activity was more potent than those of heparin and heparan sulfate, which are well-known inhibitors of *H. pylori* infection (Table 2). To measure the potent molecular size of AS for inhibiting *H. pylori* adhesion, we performed the controlled chemical depolymerization of AS by hydrogen peroxide in the presence of cupric ions. The purified LMWAS with different average MW (3.3, 3.0, and 2.4 kDa) were tested to measure its *H. pylori* adhesion-inhibitory activity. Among them, LMWAS with average MW 3.0 kDa showed the most potent inhibitory activity of *H. pylori* adhesion followed by 3.3 kDa (IC_{50} =32 μg/mL) (Fig. 5). LMWAS with average MW 3.0 and 3.3 kDa have the size of dodecasaccharide (12-mer) and tetradecasaccharide (14-mer). Thus, AS oligosaccharides with the defined size (12- and 14-mer) could be the promising inhibitor for *H. pylori* adhesion.

Discussion

H. pylori is a slow pathogen that can survive in the gastric mucosa of patients with chronic gastritis for decades. It was reported the binding of *H. pylori* to host receptors may be a strategy of the bacteria to survive immune surveillance, causing chronic infection [28]. It is an interesting notion that glycosylation patterns of soluble glycoconjugates, which are natural receptor analogues in secretions such as breast milk and saliva, may inhibit bacterial adhesion, acting as clearance factors. AS with the specific carbohydrate structure is likely to play an important role in this anti-adhesive activity. Previous studies showed that several acidic polysaccharides containing high content of uronic acid from the root of *Panax ginseng* [11], the leaves of *Artemisia capillaries* [10], and the leaves of *Camellia sinensis* [29] have inhibitory activity of *H. pylori* adhesion to host cells. In addition, heparin and heparan sulfate bind *H. pylori* cells [30, 31], indicating that acidic GAGs with sulfate groups, which are structurally similar to them could be candidates as *H. pylori* adhesion inhibitors. Especially, AS composed of $IdoA2SO_3^-$ and GlcNAc structurally resembles the heparin/heparan sulfate family of GAGs based on its alternating 1→4 linkage and its $IdoA2SO_3^-$ residue [18]. Because IdoA is a uniquely flexible in conformation, it appears to play a key role in the interactions of IdoA containing GAGs with a variety of proteins in cells [32].

In the present study, we have demonstrated that AS is useful for the prevention of chronic gastric injury. In addition, AS is ecologically safer than chemotherapeutic agents, which are widely used. Anti-adhesive agents that govern the susceptibility for bacterial adherence and colonization are now being developed, and this strategy for cell surface adhesion promises to facilitate the investigation of carbohydrate-mediated cell-cell interaction. Based on these findings, the LMWAS produced by controlled chemical depolymerization may inhibit the colonization of *H. pylori* in epithelial cells of stomach.

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References

1. Warren, J.R., Marshall, B.J.: Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet **1**, 1273–1275 (1983)
2. Boquet, P., Ricci, V., Galmiche, A., Gauthier, N.C.: Gastric cell apoptosis and *H. pylori*: has the main function of VacA finally been identified? Trends Mircobiol. **11**, 410–413 (2003)
3. Cover, T.L., Puryear, W., Perez-Perez, G.I., Blaser, M.J.: Effect of urease on HeLa cell vacuolation induced by *Helicobacter pylori* cytotoxin. Infect. Immun. **59**, 1264–1270 (1991)
4. Cover, T.L., Blaser, M.J.: Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. J. Biol. Chem. **267**, 10570–10575 (1992)
5. Kuck, D., Kolmerer, B., Iking-Konert, C., Krammer, P.H., Stremmel, W., Rud, J.: Vacuolating cytotoxin of *Helicobacter pylori* induces apoptosis in the human gastric epithelial cell line AGS. Infect. Immun. **69**, 5080–5087 (2001)
6. Tzouvelekis, L.S., Mentis, A.F., Makris, A.M., Spiliadis, C., Blackwell, C., Weir, D.M.: In vitro binding of *Helicobacter pylori* to human gastric mucin. Infect. Immun. **59**, 4252–4254 (1991)
7. Goodwin, C.S.: Gastric cancer and *Helicobacter pylori*: the whispering killer? Lancet **342**, 507–508 (1993)
8. Maria, A., Guzman, M., Edurado, R.B., Bow, H., Felipe, A.: Involvement of the heparan sulphate-binding proteins of *Helicobacter pylori* in its adherence to HeLa S3 and Kato III cell lines. J. Med. Microbiol. **50**, 320–329 (2001)
9. Utt, M., Wadstrom, T.: Identification of heparan sulphate binding surface proteins of *Helicobacter pylori*: inhibition of heparan sulphate binding with sulphated carbohydrate polymers. J. Med. Microbiol. **46**, 541–546 (1997)

10. Woo, J.S., Ha, B.H., Kim, T.G., Lim, Y., Kim, K.H.: Inhibition of *Helicobacter pylori* adhesion by acidic polysaccharide isolated from *Artemisia capillaries*. J. Microbiol. Biotechnol. **13**, 853–858 (2003)
11. Belogortseva, N.I., Yoon, J.Y., Kim, K.H.: Inhibition of *Helicobacter pylori* hemagglutination by polysaccharide fractions from roots of *Panax ginseng*. Planta Med. **66**, 217–220 (2000)
12. Lee, J.H., Park, E.K., Uhm, C.S., Chung, M.S., Kim, K.H.: Inhibition of *Helicobacter pylori* adhesion to human gastric adenocarcinoma epithelial cells by acidic polysaccharides from *Artemisia capillaris* and *Panax ginseng*. Planta Med. **70**, 615–619 (2004)
13. Kim, Y.S., Jo, Y.Y., Chang, I.M., Toida, T., Park, Y., Linhardt, R.J.: A new glycosaminoglycan from the giant African snail *Achatina fulica*. J. Biol. Chem. **271**, 11750–11755 (1996)
14. Ghosh, A.K., Hirasawa, N., Lee, Y.S., Kim, Y.S., Shin, K.H., Ryu, N., Ohuchi, K.: Inhibition by acharan sulphate of angiogenesis in experimental inflammation models. Br. J. Pharmacol. **137**, 441–448 (2002)
15. Wang, H., Toida, T., Kim, Y.S., Capila, I., Hileman, R.E., Bernfield, M., Linhardt, R.J.: Glycosaminoglycans can influence fibroblast growth factor-2 mitogenicity without significant growth factor binding. Biochem. Biophys. Res. Commun. **235**, 369–373 (1997)
16. Li, D.W., Lee, I.S., Sim, J.S., Toida, T., Linhardt, R.J., Kim, Y.S.: Long duration of anticoagulant activity and protective effects of acharan sulfate in vivo. Thromb. Res. **113**, 67–73 (2004)
17. Shim, J.Y., Lee, Y.S., Jung, S.H., Choi, H.S., Shin, K.H., Kim, Y. S.: Pharmacological activities of a new glycosaminoglycan, acharan sulfate isolated from the giant African snail *Achatina fulica*. Arch. Pharm. Res. **25**, 889–894 (2002)
18. Joo, E.J., ten Dam, G.B., van Kuppevelt, T.H., Toida, T., Linhardt, R.J., Kim, Y.S.: Nucleolin: acharan sulfate-binding protein on the surface of cancer cells. Glycobiology **15**, 1–9 (2005)
19. Joo, E.J., Yang, H., Park, Y., Park, N.Y., Toida, T., Linhardt, R.J., Kim, Y.S.: Induction of nucleolin translocation by acharan sulfate in A549 human lung adenocarcinoma. J. Cell. Biochem. **110**, 1272–1278 (2010)
20. Hong, S.W., Shin, H.Y., Kim, Y.S., Kim, D.H.: Purification and characterization of novel salt-active acharan sulfate lyase from *Bacteroides stercoris* HJ-15. Eur. J. Biochem. **270**, 3168–3173 (2003)
21. Chi, L., Munoz, E.M., Choi, H.S., Ha, Y.W., Kim, Y.S., Toida, T., Linhard, R.J.: Preparation and structural determination of large oligosaccharides derived from acharan sulfate. Carbohydr. Res. **341**, 864–869 (2006)
22. Volpi, N.: Characterization of heparins with different relative molecular masses (from 11,600 to 1600) by various analytical techniques. J. Chromatogr. **622**, 13–20 (1993)
23. Sim, J.S., Im, A.R., Cho, S.M., Jang, H.J., Jo, J.H., Kim, Y.S.: Evaluation of chondroitin sulfate in shark cartilage powder as a dietary supplement: Raw materials and finished products. Food Chem. **101**, 532–539 (2007)
24. Cho, S.Y., Sim, J.S., Jeong, C.S., Chang, S.Y., Choi, D.W., Toida, T., Kim, Y.S.: Effects of low molecular weight chondroitin sulfate on type II collagen-induced arthritis in DBA/1J mice. Biol. Pharm. Bull. **27**, 47–51 (2004)
25. Sim, J.S., Jun, G., Toida, T., Cho, S.Y., Choi, D.W., Chang, S.Y., Linhardt, R.J., Kim, Y.S.: Quantitative analysis of chondroitin sulfate in raw materials, ophthalmic solutions, soft capsules and liquid preparations. J. Chromatogr. **818**, 133–139 (2005)
26. Gutmann, I., Bergmeyer, H.U.: Methods of enzymatic analysis. Academic Press, Inc., New York, U.S.A. **4**, 791–1794 (1974)
27. Kamisago, S., Iwamori, M., Tai, T., Mitamura, K., Yazaki, Y., Sugano, K.: Role of sulfatides in adhesion of *Helicobacter pylori* to gastric cancer cells. Infect. Immun. **64**, 624–628 (1996)
28. Mahdavi, J., Sonden, B., Hurtig, M., Olfat, F.O., Forsberg, L., Roche, N., Angstrom, J., Larsson, T., Teneberg, S., Karlsson, K. A., Altraja, S., Wadstrom, T., Kersulyte, D., Berg, D.E., Dubois, A., Petersson, C., Magnusson, K.E., Norberg, T., Lindh, F., Lundskog, B.B., Arnqvist, A., Hammarstrom, L., Boren, T.: *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. Science **297**, 573–578 (2002)
29. Lee, J.H., Shim, J.S., Lee, J.S., Kim, J.K., Yang, I.S., Chung, M. S., Kim, K.H.: Inhibition of pathogenic bacterial adhesion by acidic polysaccharide from green tea (*Camellia sinensis*). J. Agric. Food Chem. **54**, 8717–8723 (2006)
30. Hirmo, S., Utt, M., Ringner, M., Wadstrom, T.: Inhibition of heparan sulphate and other glycosaminoglycans binding to *Helicobacter pylori* by various polysulphated carbohydrates. FEMS Immunol. Med. Microbiol. **10**, 301–306 (1995)
31. Ascencio, F., Fransson, L.A., Wadstrom, T.: Affinity of the gastric pathogen *Helicobacter pylori* for the *N*-sulphated glycosaminoglycan heparan sulphate. J. Med. Microbiol. **38**, 240–244 (1993)
32. Westergren-Thorsson, G., Onnervik, P.O., Fransson, L.A., Malmstrom, A.: Proliferation of cultured fibroblasts is inhibited by L-iduronate-containing glycosaminoglycans. J. Cell. Physiol. **147**, 523–530 (1991)