Characterization of a *Bacteroides* species from human intestine that degrades glycosaminoglycans

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Abstract: Polysaccharide lyases that can degrade glycosaminoglycans (GAGs) were identified in an anaerobic strain living in the human intestine. The strain was isolated from the stool of a healthy male and identified as *Bacteroides* sp. strain HJ-15. A detailed taxonomical study indicated the species is a strain of *Bacteroides stercoris*. The isolate was cultured and the polysaccharide lyase activity was partially purified. This enzyme preparation could act on GAGs containing either glucosamine or galactosamine suggesting the presence of both heparinases and chondroitinases. Various GAGs were incubated with the partially purified enzyme and the products formed were analyzed by strong anion-exchange high performance liquid chromatography and proton nuclear magnetic resonance spectroscopy. These studies demonstrated the presence of at least two types of polysaccharide lyases: heparin lyase and chondroitin sulfate lyase. The eliminative mechanism of these lyase enzymes was confirmed through the isolation of unsaturated disaccharide products. The heparin lyase acted on both heparin and acharan sulfate, a GAG recently isolated from *Achatina fulica*. The *Bacteroides* chondroitin lyase, acted on chondroitin sulfates A, B (dermatan sulfate), and C, resembling chondroitin lyase ABC. The presence of a GAG-degrading organism in human intestine may pose problems for the effective oral administration of GAG drugs.

Key words: *Bacteroides stercoris*, glycosaminoglycan, nuclear magnetic resonance spectroscopy, polysaccharide lyase, heparinase, chondroitinase.

Résumé : Des polysaccharides lyases capables de dégrader des glycosaminoglycanes (GAGs) ont été identifiées dans une souche anaérobie vivant dans l'intestin humain. La souche a été isolée à partir d'excréments d'homme sain et identifiée à *Bacteroides* sp. HJ-15. Une étude taxonomique détaillée a permis d'établir que ces espèces appartenaient à la souche *Bacteroides stercoris*. Après isolement, cette souche a été cultivée et l'activité polysaccharide lyase a été partiellement purifiée. La préparation enzymatique était active sur des GAGs contenant des glucosamines ou galactosamines, suggérant la présence d'héparinases et de chondroïtinases. Différents GAGs ont été incubés en présence de l'enzyme partiellement purifiée et les produits formés analysés par chromatographie liquide échangeuse d'anions et par spectroscopie de résonance magnétique nucléaire du proton. Ces études ont démontré la présence d'au moins deux types de polysaccharides lyases, soit l'héparine lyase et la chondroïtine sulfate lyase. Le mécanisme d'élimination de ces enzymes lyases a été confirmé par isolement des produits disaccharides insaturés. L'héparine lyase était active sur l'héparine et l'acharane sulfate, un GAG récemment isolé d'*Achatina fulica*. La chondroïtine lyase de *Bacteroides* fut active sur les chondroïtines sulfates A, B (dermatane sulfate) et C. La présence d'un organisme dégradant les GAGs dans l'intestin humain est susceptible de poser un problème pour l'administration orale efficace de médicaments à base de GAGs.

Mots clés : *Bacteroides stercoris*, glycosaminoglycane, spectroscopie de résonance magnétique nucléaire, polysaccharide lyase, héparinase, chondroïtinase.
[Traduit par la rédaction]

Introduction

Glycosaminoglycans (GAGs) are a family of linear anionic polysaccharides. These macromolecules are found in many animal tissues and are typically isolated as proteoglycans, covalently bound to the core protein (Hardingham and Fosang 1992; Jackson et al. 1991; Kjellen and Lindahl 1991). Endogenous GAGs play a central role in many important biological processes including maintaining blood flow (Kjellen and Lindahl 1991; Linhardt and Toida 1997), the modulation of cell signaling through growth factors (Rapraeger 1995), and in the protection against and infection by human pathogens

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(Sawitzky 1996; Chen et al. 1997). Exogenous GAGs have also been administered clinically as anticoagulant and antithrombotic agents (Kjellen and Lindahl 1991; Linhardt and Toida 1997). Heparin is a GAG having a major repeating disaccharide structure of →4)-α-D-2-deoxy-2-amino glucopyranose-6-sulfate(1→4)-α-L-idopyranosyluronic acid-2-sulfate (1→. Its structure is also complicated by variable *N*- and *O*-sulfation and *N*-acetylation (Lindahl 1989; Linhardt and Toida 1997). Anticoagulation by heparin typically relies on intravenous or subcutaneous routes of administration (Linhardt and Toida 1997). Recent interest has focused on specially formulated heparin that is orally administered (Jaques et al. 1991; Engelberg 1995; Linhardt and Toida 1997) raising issues about both its bioavailability and its stability in the digestive tract.

Enzymes acting on GAGs have been useful in studying structural features of heparin and other GAGs, as well as their mechanisms of biological activity (Linhardt and Toida 1997). The substrate specificities of these enzymes have been interpreted based primarily on the structure of the resulting oligosaccharide products (Linhardt et al. 1986; Linhardt 1994). GAG-degrading enzymes are divided into hydrolases and lyases, depending on their mechanism of action (Linhardt 1994). GAG-degrading hydrolases are typically found in higher animals, while GAG-degrading lyases have only been isolated from microbial sources (Linhardt et al. 1986). There are three categories of GAG-degrading lyases: (*i*) the heparinases, acting on heparin, heparan sulfate, and structurally related GAGs; (*ii*) the chondroitinases acting primarily on chondroitin and dermatan sulfates; and (*iii*) the hyaluronidases acting primarily on hyaluronan (Linhardt et al. 1986; Linhardt 1994; Ernst et al. 1995; Sutherland 1995).

Heparin-degrading activity has only been observed in a small number of organisms including *Flavobacterium heparinum* (Payza and Korn 1956), *Flavobacterium* sp. (Böhmer et al. 1990), *Bacillus* sp. (Bellamy and Horikoshi 1992), and anaerobic *Bacteroides* sp. (Gesner and Jenkin 1961), *Bacteroides thetaiotaomicron*, *Bacteroides ovatus*, *Bacteroides eggerthii* (Saylers et al. 1977), and *Bacteroides uniformis* (Riley 1987). Only the heparin-degrading enzymes from *F. heparinum* have been conclusively demonstrated to be polysaccharide lyases (Linker and Hovingh 1972).

Acharan sulfate is a GAG recently isolated from the giant African snail, *Achatina fulica* (Kim et al. 1996). Acharan sulfate is structurally similar to heparin and heparan sulfate, having an uncomplicated repeating disaccharide structure of →4)-α-D-2-acetamido 2-deoxy-glucopyranose (1→4)-α-L-idopyranosyluronic acid-2-sulfate (1→. Thus, acharan sulfate represents a structurally simple model for heparin in studies on GAG catabolism. The current study focuses on the products of GAG catabolism by a bacteria isolated from human intestine.

Materials and methods

General anaerobic medium (GAM), containing glucose as the main carbon source and with a pH of 7.2 was purchased from Nissui Pharm Co. (Tokyo). Tryptic soy broth was obtained from Difco (Detroit, Mich.) and sodium thioglycolate and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, Mo.). Acharan sulfate was prepared as described previously (Kim et al. 1996). Heparin (porcine intestinal mucosa), chondroitin sulfate A (chondroitin 4-sulfate, bovine trachea), B (dermatan sulfate, porcine skin), and C (chondroitin 6-sulfate, shark cartilage) were from Calbiochem (La Jolla, Calif.). Standard disaccharides, ΔUA2S(1→4)-D-GlcNAcα,β (where ΔUA is 4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid, GlcN is 2-amino-2-deoxy-glucopyranose, Ac is acetate, and S is sulfate), ΔUA2S(1→4)-β-D-GlcNS6S, ΔUA2S(1→4)-β-D-GlcNS, and ΔUA(1→3)-D-GalNAc4Sα,β (where GalN is 2-amino-2-deoxy-galactopyranose), were purchased from Sigma Co. Hydroxylapatite (microcrystalline, 4% beaded in agarose), 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid (TSP), ^2H_2O, and Azure A dye (total dye content 75%) were also from Sigma Co. Bio-Gel P-2 (medium) was a product of Bio-Rad (Richmond, Calif.). The American Type Culture Collection (Rockville, Maryland) was the source of *Bacteroides stercoris* ATCC 43183.

Electron microscopy was performed using a H-600 electron microscope from Hitachi Co. (Tokyo). The sample embedded in epoxyresin was sectioned on a Sorvall MT-6000 ultramicrotome from Dupont (Wilmington, Del.). Sonication was performed on an Ultrasonic Processor VC501 (Eyela, Japan). HPLC used a Spectra Physics (San Jose, Calif.) system equipped with UV detector. The analytical strong anion-exchange (SAX) column (4.5 × 250 mm, 5-μm-particle size) was purchased from Phenomenex (Torrance, Calif). All ^{1}H-NMR spectra were performed using a GXS500A spectrometer at the operating frequency of 500 MHz of JEOL equipped with a VAX32 computer system, with a process controller and an array processor.

Screening of anaerobic strains

An aliquot (0.2 mL) of human feces was anoxically diluted 100-fold in 20 mM sodium phosphate buffer (pH 7.2) and inoculated on a GAM agar plate. The plate was anaerobically incubated at 37°C for 4 days and more than 200 colonies were selected. Each of them was cultivated anaerobically in 10 mL of GAM broth and the cells were collected by centrifugation. The distribution of acharan sulfate degrading activity in the cells was assayed directly by measuring the decrease in metachromasia with Azure A (Galliher et al. 1981; Klein et al. 1982). After the active isolates were identified, their activity towards heparin and chondroitin sulfate was determined. Among the twelve GAG-degrading strains, a single strain, HJ-15, showing the highest activity on acharan sulfate, heparin, and chondroitin sulfate, was isolated. This strain was subcultured three times until it was considered to be pure based on the presence of a single colony type. The morphological, cultural, and physiological properties of strain HJ-15 were examined in accordance with methods described in Bergey's Manual of Systematic Bacteriology (Holdeman and Moore 1984). Methyl carboxylic esters were prepared from bacterial cells and analyzed using gas chromatographic analysis by Microcheck Lab (Northfield Falls, Vt.). The resulting fatty acid profile was compared against a microbial library containing 57 *Bacteroides* species to determine relatedness. This organism is deposited in the Korean Culture Collection and has the International Depository Authority accession No. KCCM-10096.

Fermentation

Bacteroides sp. strain HJ-15 showing GAG-degrading activity was maintained on semisolid GAM media containing 0.05% v/v agar. It was cultured anaerobically under an atmosphere of 90% nitrogen and 10% carbon dioxide at 37°C in a 1-L flask (without shaking) containing 800 mL of GAM broth or tryptic soy broth (pH 7.2) containing 0.01% w/v sodium thioglycolate and 0.1% w/v ascorbic acid for 14 h, by which time the culture had reached stationary phase with a cell density of approximately 300 mg dry cell weight/L. The culture broth was centrifuged (5000 × *g*, 20 min) and the resulting cell pellet was collected and washed twice with saline containing 50 mM sodium phosphate (pH 7.0).

Scanning and transmission electron microscopy

Cells were fixed with 5% v/v glutaraldehyde in 100 mM sodium phosphate buffer (pH 7.4) and postfixed with osmium fixative. The

sample was embedded in epoxyresin and then sectioned on a ultramicrotome. Ultrathin sections were stained with 12.5% w/v uranyl acetate and 0.25% w/v lead citrate and examined under an electron microscope.

Partial purification of enzyme

Since the saline washes of the cell pellet contained little or no GAG-degrading activity, the harvested cells (6.0 g dry cell weight) were suspended in 150 mL of 20 mM sodium phosphate buffer (pH 7.0) and disrupted by sonication. The suspended pellet was sonicated for 10 min in 10-mL portions at 500 W using a 3-s pulse at 40% power. The sonicate was centrifuged (16 000 × *g*, 20 min), the pellet was collected and resuspended in 20 mM sodium phosphate buffer (pH 7.0), and the sonication procedure was repeated. After centrifugation, the combined supernatants were assayed and subjected to further purification. Fractional precipitation of the supernatant was performed by varying the concentration of ammonium sulfate. The precipitate (30–70% saturation) was recovered by centrifugation and dialyzed against three times against 1 L of 10 mM sodium phosphate buffer (pH 7.0). The GAG lyase activities of the crude enzyme, sonicate, and ammonium sulfate precipitate were measured using the Azure A assay (Galliher et al. 1981; Klein et al. 1982) according to the previously published methods (Linhardt 1994). The hydroxylapatite purified enzyme was determined by measuring the increase in absorbance at 232 nm (1 U = 1 μmol of ΔUA-containing product formed/min) using acharan sulfate, heparin, and chondroitin sulfate as substrates (Linker and Hovingh 1972; Linhardt 1994). Protein concentration was measured by Bradford's method (Bradford 1976) using bovine serum albumin as the standard. After concentration to 10 mL it was loaded onto a hydroxylapatite column (1.5 × 17 cm). A stepwise gradient of sodium phosphate buffer (pH 7.0; 100 mL each of 10, 50, 200, and 500 mM) was used to elute the GAG lyases. The fraction eluting in 200 mM sodium phosphate buffer (pH 7.0) contained most of the GAG lyase activity (40 mU/mg of heparin lyase, 4.1 mg of protein in 12 mL) and was used for enzymatic depolymerization of GAGs. GAGs (0.1 mg) in 1 mL of 0.02 M sodium phosphate buffer (pH 7.0) were depolymerized by treating with 10 mU of the enzyme for 8–48 h at 37°C. The reaction mixtures were boiled to inactivate the enzyme and stored at –70°C.

Analysis of depolymerization mixtures by SAX-HPLC

The composition of disaccharides and oligosaccharides produced from GAG on enzyme treatment was analyzed using SAX-HPLC (Linhardt et al. 1997). HPLC was performed under a linear gradient of NaCl (0.1–1.6 M, pH 3.5). The flow rate was 1.0 mL/min and detection was at 232 nm. Each peak eluting from SAX-HPLC was collected, desalted on a Bio-Gel P-2 column (2 × 45 cm), eluted with water and the salt-free product was freeze dried.

^{1}H-NMR spectroscopic analysis

Approximately 100 μg of each sample was exchanged three times with 1-mL portions of ^2H_2O (99.9%; Sigma), followed by in vacuo desiccation over P_2O_5. The thoroughly dried sample was dissolved in 0.5 mL of ^2H_2O (99.96%; Sigma) and transferred to the NMR tube. The operating conditions for one-dimensional (1D) spectra were a frequency of 500 MHz, a sweep width of 6 kHz, a flip angle of 90° (12.8 μs), and sampling points of 32 K. Chemical shifts are reported as parts per million from the signal of TSP as an internal standard. A small amount of acetone was used as the internal standard (2.225 ppm at 303 K). The water resonance was suppressed by selective irradiation during the relaxation delay.

Results and discussion

More than 200 bacterial isolates of human feces were screened for GAG-degrading enzymes. While 12 of the isolates were able to degrade heparin, a single isolate, HJ-15, was found to be most potent. Examination of this isolate showed it to be an obligate anaerobe producing small colonies (<0.5-mm diameter) on GAM agar plates. HJ-15 was determined to be a Gram-negative nonspore-forming rod-shaped organism, suggesting that it was a *Bacteroides* species. Transmission and scanning electron microscopy (Fig. 1) showed it to have the dimensions 2 × 0.5 μm.

Heparinase production by *Bacteroides* was first described by Gesner and Jenkin (1961). Saylers et al. (1977) reported strains of *Bacteroides ovatus* and *Bacteroides thetaiotamicron* from the human colon could also ferment GAGs. Two types of chondroitinase were also purified from *Bacteroides thetaiotaomicron* (Linn et al. 1983). Another anaerobic bacteria, *Bacteroides uniformis* from clinical specimens, also had heparinase activity (Riley 1987). A heparinase was also purified from the oral strain, *Bacteroides heparinolyticus*, but no other GAG-degrading enzymes were reported in this organism (Nakamura et al. 1988).

All the anaerobic *Bacteroides* species producing heparinase (Table 1) were indole-positive. Differences of utilization of various carbohydrates clearly differentiate *Bacteroides* sp. strain HJ-15 from other heparinase producing species (Table 1). *Bacteroides stercoris* was phenotypically identical to *Bacteroides* sp. strain HJ-15, except that acid production was reported when *Bacteroides stercoris* was fermented on raffinose. Gas chromatographic analysis of cellular fatty acid composition afforded a 95% similarity level to *Bacteroides stercoris*. The next closest similarity level of the 57 species of *Bacteroides* examined was for *Bacteroides uniformis* and *Bacteroides eggerthil* at 74 and 67%, respectively. These findings strongly suggest that isolate HJ-15 is a substrain of *Bacteroides stercoris*. To confirm this identification, *Bacteroides stercoris* ATCC 43183 was examined. This organism gave identical characteristics in our laboratory to those previously reported (Johnson et al. 1986; Citron et al. 1990) (see Table 1). When grown under the conditions used for HJ-15, the ATCC strain showed a level of heparinase activity comparable to our isolate.

No decrease in the heparinase or chondroitinase activity was observed even after HJ-15 was passed four times through GAM or soy broth culturing media in the absence of GAG and using a 1% inoculum, suggesting that these enzymes were constitutive. When heparin (0.1 mg/mL) or acharan sulfate (0.05 mg/mL) was added to the media, a four- to five-fold increase in heparin-degrading activity was observed. Thus, the addition of GAGs results in only a low level of enzyme induction. In contrast, GAGs are required for the induction of the GAG-degrading enzymes observed in *Bacteroides* sp. (Gesner and Jenkin 1961) and *Flavobacterium heparinum* (Galliher et al. 1981).

Next, we turned our attention to the partial purification of the GAG-degrading enzymes obtained from the fermentation of noninduced cultures of HJ-15. After sonication the supernatant contained 771 mg of protein having a specific activity towards heparin of 0.63 mU/mg corresponding to a total activity of 485 mU Analysis by reducing sodium dodecyl sulfate (SDS) – polyacrylamide gel electrophoresis (PAGE) with staining by Coomassie blue showed a smear of bands throughout the entire lane. Following hydroxylapatite chromatography using a stepwise gradient, a peak was obtained in 200 mM

Fig. 1. Scanning (A) and transmission (B) electron micrographs of *Bacteroides* sp. strain HJ-15. The procedures are described in Materials and methods.

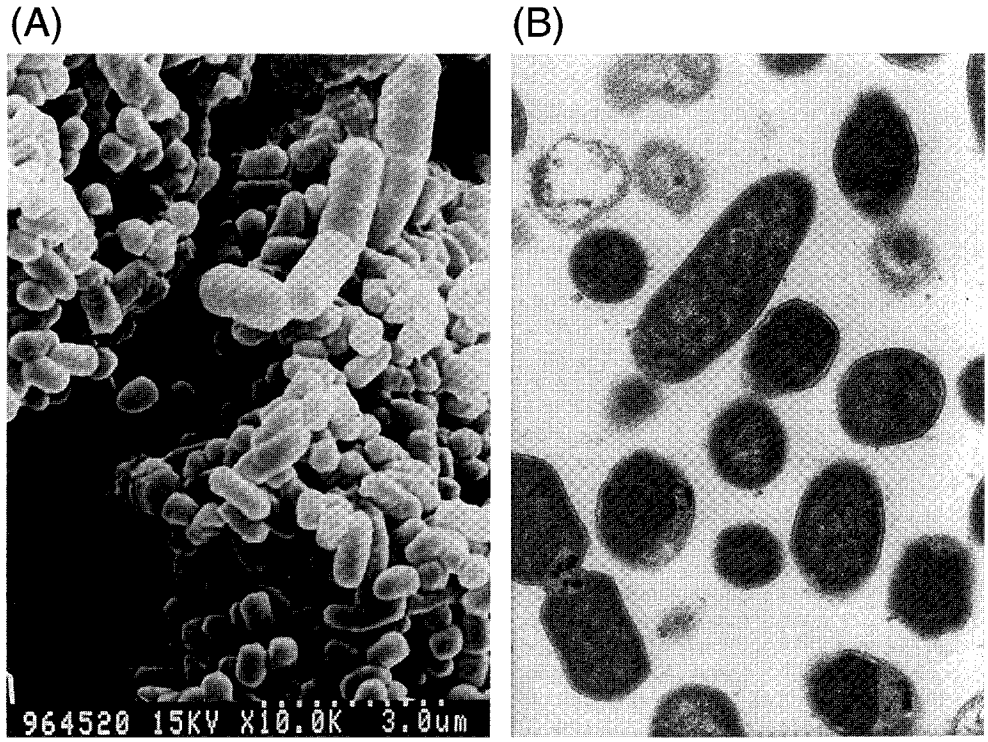

Table 1. Characteristics of the species of indole-positive *Bacteroides*.

Species	Trehalose	Rhamnose	Xylose	Salicin	Melezitose	Cellobiose	Raffinose
Bacteroides ovatus[a]	+	+	+	+	+	+	+
Bacteroides thetaiotaomicron[a]	+	+	–	–	+	+	+
Bacteroides uniformis[a]	V(W)	V(W)	V	+	–	+	+
Bacteroides heparinolyticus[a]	NR	NR	+	+	NR	+	–
Bacteroides stercoris[a]	–	+	+	–	–	–	+
Bacteroides sp. strain HJ-15	–	+	+	–	–	–	–

Notes: +, 90–100% of colonies are positive; –, 10% or less of colonies are positive; V, variable; W, weakly positive; NR, not reported.
[a]From the data of Johnson et al. (1986) and Citron et al. (1990).

sodium phosphate buffer (pH 7) containing 3.8 mg of protein and having a specific heparinase activity of 9.2 mU/mg, corresponding to a total activity of 35 mU. Reducing SDS–PAGE analysis of this partially purified heparinase showed three to four major bands and six to eight minor bands. Chondroitinase activity was similarly purified by stepwise elution from a hydroxylapatite column using 50 mM sodium phosphate buffer. The purification resulted in 14.6- and 8.8-fold enrichments of heparinase and chondroitinase, respectively. While the specific activities obtained were low when compared to the *Flavobacterial* lyases (Lohse and Linhardt 1992; Gu et al. 1995), this limited purification procedure afforded sufficient enzymatic activity to demonstrate that *Bacteroides* sp. strain HJ-15 produced enzymes acting on chondroitin sulfate and heparin through an eliminase mechanism.

The degradation of various GAGs, including heparin, acharan sulfate, and chondroitin sulfate A, by the partially purified enzyme preparation was examined using gradient PAGE and SAX-HPLC analysis. Gradient PAGE analysis (Linhardt et al. 1997) demonstrated that heparin, acharan sulfate, and chondroitin sulfate A were depolymerized to a mixture of disaccharides and higher oligosaccharides (not shown). SAX-HPLC analysis (Fig. 2) was performed next on these products. The depolymerization of heparin, a structurally complex GAG, with partially purified enzyme resulted in a major peak that on isolation and characterization by ^{1}H-NMR (Fig. 2*a* and Table 2) was assigned the structure ΔUA2S(1→4)-α-D-GlcNS6S. This disaccharide is derived from the major trisulfated disaccharide repeating unit in the heparin polymer (Merchant et al. 1985). The depolymerization of acharan sulfate, a structurally simple GAG comprised of a repeating disaccharide sequence of →4)-β-D-GlcNAc(1→4)-α-L-IdoA2S (1→ (Kim et al. 1996), produced a single major disaccharide of the structure ΔUA2S-(1→4)GlcNAcα,β (Fig. 2*b* and Table 2). Enzymatic treatment of chondroitin sulfate A produced a major disaccharide ΔUA(1→3)GalNAc4Sα,β (Fig. 2*c* and Table 2) consistent with the major repeating structure of →3)-β-D-GalNAc4S(1→4)-β-D-GlcA(1→ found in chondroitin

Fig. 2. HPLC-SAX analysis of GAGs after treatment with lyases from *Bacteroides* sp. strain HJ-15. (*a*) Heparin. (*b*) Acharan sulfate. (*c*) Chondroitin 4-sulfate. Each GAG was depolymerized for 48 h and the reaction mixture was injected into the HPLC. The peaks indicated by arrows were collected and their structures were analyzed by NMR.

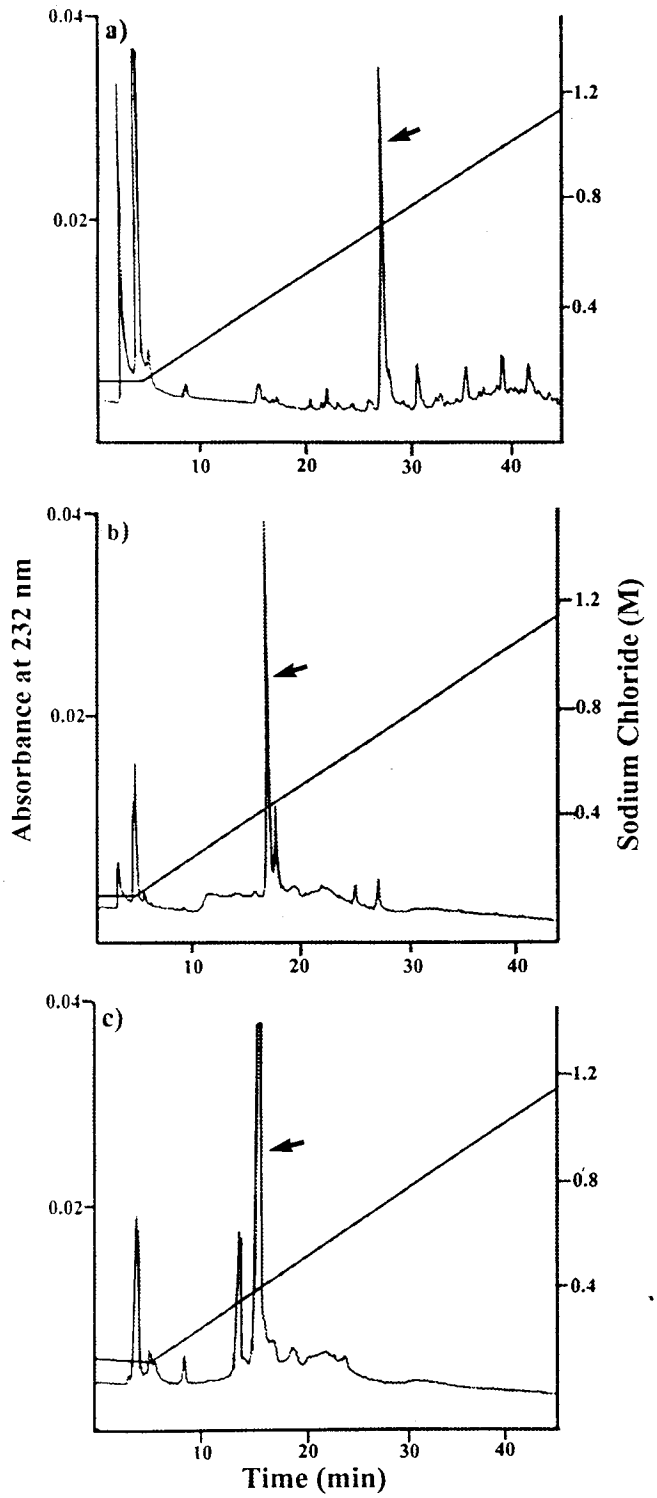

Table 2. Chemical shifts and coupling constants of the protons of the unsaturated disaccharides derived from heparin, acharan sulfate, and chondroitin 4-sulfate by the enzyme.

Sample	Saccharide	H-1 *J 1,2*		H-2 *J 2,3*		H-3 *J 3,4*		H-4 *J 4,5*		H-5 *J 5,6*		H-6a *J 5,6b*		H-6b *J 6a,6b*	
		CS	CC	CS	CC	CS	CC	CS	CC	CS	CC	CS	CC	CS	CC
Heparin disaccharide	GlcNS6S	5,456	3.4	3.272	9.8	3.763	9.2	3.835	9.6	4.167	4.4	4.355	1.8	4.217	–10.8
	ΔUA2S	5.515	3.8	4.566	2.2	4.343	4.6	5.991		–		–		–	
Acharan sulfate disaccharide[a]	GlcNAcα	5.192	2.6	3.851	6.5	3.851	8.6	3.771	6.7	3.945	5.2	3.828	3.4	3.824	–9.9
	GlcNAcβ	4.665	8.1	3.674	6.9	3.668	8.4	3.758	7.9	3.567	5.5	3.887	2.6	3.792	–10.6
	ΔUA2S attached to GlcNAcα	5.477	3.0	4.526	3.2	4.291	4.7	5.952		–		–			
	ΔUA2S attached to GlcNAc4Sβ	5.471	3.0	4.511	2.5	4.291	4.8	5.952							
Chondroitin sulfate A disaccharide[a]	GalNAcα	5.214	3.5	4.365	10.5	4.325	2.6	4.688	<1.5	4.291	4.4	3.773	8.5	3.707	–11.6
	GalNAcβ	4.791	8.6	4.068	10.6	4.157	2.6	4.621	<1.5	3.855	3.5	3.781	7.4	3.763	–10.9
	ΔUA attached to GalNAc4Sα	5.307	3.2	3.844	<1.5	3.959	4.6	3.971							
	ΔUA attached to GalNAc4Sβ	5.271	3.6	3.838	<1.5	5.969	4.6								

Notes: CS, chemical shifts (ppm); CC, coupling constants (Hz).

[a]Since the acharan sulfate and chondroitin sulfate A disaccharides are mixtures of α,β anomers, assignments are made for both forms. The heparin disaccharide is >95% α-anomer.

sulfate A (Yamada et al. 1992). The partially purified enzyme could also depolymerize chondroitin sulfate B and C (data not shown). The ^{1}H-NMR spectral data and the UV absorbance maxima at 232 nm conclusively demonstrate that the heparinase and chondroitinase enzymes isolated from HJ-15 act through an eliminative mechanism and are lyases (EC 4.2.X.X). Furthermore, these studies clearly demonstrate that the partially purified enzyme preparation is free of glycuronidases and sulfatases that could further act on the product(s) formed by the lyase (Galliher et al. 1982).

Flavobacterium heparinum is the only bacterium that produces both chondroitin and heparin lyases and in which these enzymes have been purified to homogeneity and well characterized (Lohse and Linhardt 1992; Gu et al. 1995; Godavati et al. 1996*a*, 1996*b*, 1996*c*; Su et al. 1996). Heparin lyases (and chondroitin lyases) can be further classified based on their specificity. The heparin lyase activity prepared from HJ-15 acts on heparin, heparan sulfate, and acharan sulfate, giving similar products to those observed with heparin lyase II from *F. heparinum*. It is also possible that the partially purified *Bacteroides* activity results from a mixture of heparin lyases having an overall specificity similar to the broad specificity exhibited by *Flavobacterium* heparin lyase II. A similar analysis can be made for the chondroitin lyase activity. The partially purified activity from (HJ-15) exhibits broad specificity most like chondroitin lyase ABC from *Proteus vulgaris* (Linhardt 1994). Again, this result could be explained either by a single *Bacteroides* chondroitin lyase ABC or a mixture of chondroitin lyase having the same broad specificity exhibited by this enzyme. Further purification and characterization studies are underway to answer these questions.

Studies in our laboratory on the chemical stability of GAGs showed that heparin was stable at temperatures <60°C, under acidities comparable to those found in the stomach and basicities greater than those found in intestinal tract (Jandik et al. 1996). Preliminary studies in our laboratory, on rats administered heparin through a stomach tube, demonstrated that heparin breaks down as it travels through the gastrointestinal tract. Similar results on the breakdown of orally administered heparin in rats have been reported by others (Larsen et al. 1986). While mammalian heparanases have been reported, these enzymes are hydrolyases (EC 3.2.X.X) that act at specific sites in GAGs affording products having relatively high molecular weights (Bame and Robson 1997). The isolation of a *Bacteroides* species from human intestine suggests that the catabolism of heparin observed in animal studies might also take place in man. Furthermore, the current study shows that these bacterial enzymes are lyases and produce predominantly small disaccharide products. While such low molecular weight oligosaccharides might be absorbed, it is not anticipated that they would exhibit significant anticoagulant activity (Toida et al. 1996). The current study begins to explain how orally administered heparin can result in the observation of small oligosaccharides in the urine (Larson et al. 1986) without resulting in systemic anticoagulation (Engelberg 1995). Furthermore, the failure of a number of studies to obtain consistent and reproducible data on heparin's oral bioavailability, even when using radiolabeled GAG (Linhardt and Toida 1997), may result from variability in the intestinal flora of the individuals being studied.

In conclusion, this is the first report that an anaerobe, *Bacteroides* sp. strain HJ-15, isolated from human intestine, produces heparinases and chondroitinases that can degrade GAGs including heparin, chondroitin sulfate, and acharan sulfate through the eliminative mechanism. Furthermore, HJ-15 has been identified as *Bacteroides stercoris*. Previous studies of *Bacteroides* strains from the human colon demonstrated that these strains fermented plant polysaccharides and some GAGs (Saylers et al. 1977), utilizing the polysaccharides as a source of carbon and energy. This study shows that *Bacteroides stercoris* (HJ-15) produces enzymes that degrade GAG chains. This may represent an important catabolic process that could interfere with the utilization of therapeutic GAGs administered orally (Linhardt and Toida 1997). More studies are required to understand the location of this organism with the intestinal tract, how widespread the occurrence of this organism is in man, and finally its importance in the in vivo catabolism of GAGs.

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