ELSEVIER

www.elsevier.com/locate/carres

Carbohydrate Research 335 (2001) 45–54

CARBOHYDRATE RESEARCH

The structure of a polysaccharide from infectious strains of *Burkholderia cepacia*

Alfred Linker,[a,b,*] Leigh R. Evans,[a] Giuseppe Impallomeni[c]

[a] *Veterans Affairs (VA), Salt Lake City Health Care System, 500 Foothill Boulevard (151E), Salt Lake City, UT 84124, USA*

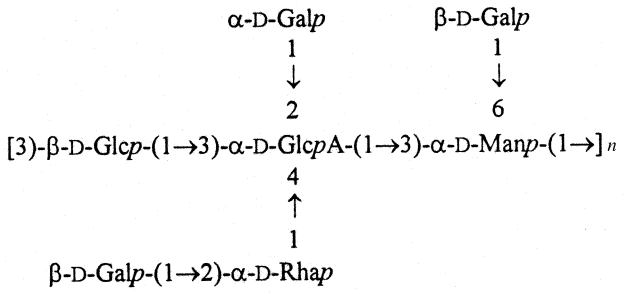
[b] *Departments of Pathology and Biochemistry, University of Utah, Salt Lake City, UT 84148, USA*

[c] *Istituto per la Chimica e la Tecnologia dei Materiali Polimerici, Consiglio Nazionale delle Ricerche, Viale A. Doria 6, I-95125 Catania, Italy*

Received 2 April 2001; accepted 5 July 2001

Abstract

The structure of an acidic exopolysaccharide (EPS) from eight strains of *Burkholderia cepacia* has been investigated by methylation and sugar analysis, periodate oxidation–Smith degradation, and partial acid-hydrolysis. An enzyme preparation obtained from the same organisms producing the EPS was also used to depolymerize the polysaccharide. Detailed NMR studies of the chemical and enzymatic degradation products showed that this EPS consists of a highly branched heptasaccharide-repeating unit with the following structure:



About three *O*-acetyl groups per repeating unit are present at undetermined positions.

Keywords: *Burkholderia cepacia*; Opportunistic pathogen; Enzymatic degradation; Exopolysaccharide, structure; Assignments, NMR

1. Introduction

Burkholderia cepacia (formerly *Pseudomonas*) is an opportunistic pathogen causing infection mainly in patients with cystic fibrosis and in immune defective or suppressed individuals. This organism, originally associated with rot in onions, has become increasingly prevalent and is aggressive and resistant to treatment causing a form of infection resulting in fairly rapid deterioration and death.[1,2] Detailed studies on mode of transmission have been carried out and have indi-

* Corresponding author. Tel.: +1-801-5821565; fax: +1-801-5839624.

0008-6215/01/$ - see front matter
PII: S0008-6215(01)00205-1

cated the prevalence of nosocomial infection by person-to-person contact and by contaminated equipment or solutions.[3–6] The mucoid forms of *B. cepacia* produce copious amounts of a highly viscous exopolysaccharide (EPS) when grown in culture. In analogy with the *P. aeruginosa* polymer and other slime or capsular polysaccharides, this EPS may well contribute to the organism's ability to survive adverse conditions and to its resistance to host defenses and antibiotic therapy.[7]

In previous preliminary studies, we have shown that strains of *B. cepacia* isolated from a variety of sources, patients as well as soil samples, produce the same characteristic polysaccharide containing galactose, glucose, mannose, rhamnose, and glucuronic acid.[8] This paper deals with a more detailed structural analysis of this complex and interesting polymer.

2. Experimental

Materials.—Bovine liver β-D-glucuronidase was obtained from Mann Research Laboratories (New York, NY). Glucuronidase from limpets and D-glucose oxidase of *Aspergillus* were obtained from Sigma (St. Louis, MO). D-Galactose oxidase was purchased from Worthington (Freehold, NJ) and *Escherichia coli* β-D-glucuronidase from Boehringer–Mannheim (Indianapolis, IN). Bacterial cultures were obtained as shown in Table 1.

Table 1
Bacterial cultures [a]

Strain	Obtained from	Original source
No. 542	Dr J. Mattssen (Univ. of Utah) [b]	cystic fibrosis patient
No. 88A3019	Dr J. Mattssen (Univ. of Utah) [b]	cystic fibrosis patient
No. 382	Berkeley Collection [b]	soil isolate
No. 249	ATCC 1716 [b]	soil isolate
No. 13945	ATCC	endocarditis patient
No. 25416	ATCC	onions
No. 25609	ATCC	bronchitis patient
No. 27515	ATCC	bone fracture patient

[a] Identified as *Pseudomonas cepacia* (now *Burkholderia*) by clinical laboratories or ATCC.

[b] Provided by Dr T.G. Lessie (University of Massachusetts at Amherst).

General methods.—The organisms were grown and the EPSs isolated as described previously.[8] Hexoses were determined colorimetrically by the anthrone reaction,[9] uronic acid by carbazole and by a metahydroxybiphenyl reagent.[10,11] Optical rotation was measured with a Zeiss polarimeter. Analysis for constituent monosaccharides was carried out by hydrolysis of polysaccharide with 2.0 M CF_3CO_2H for 14 h at 100 °C and removal of the acid in vacuo. Paper chromatography and HPLC were used for identification and quantitation. Paper chromatography was performed on Whatman No. 1 paper using the following solvent systems: **A**, 2:3:1 BuOH–HOAc–1.0 M NH_4OH; **B**, 5:16:3 pyridine–EtOAc–water; **C**, 9:2:2 EtOAc–HOAc–water. Papers were stained with an $AgNO_3$ reagent.[12]

HPLC was performed with a Waters model 6000A chromatography system with an HPX-87P Bio-Rad column at 70 °C using water as eluant. This column separated the neutral hexoses well but could not be used for uronic acid analysis, therefore a HPX-87H column eluted with 0.01 M H_2SO_4 was also used, although it did not separate galactose mannose and rhamnose adequately. A Waters refractometer was used to monitor elution. In addition, a Dionex series 4500 high-performance anion-exchange chromatography (HPAEC) system with pulsed amperometric detection (PAD) and a CarboPAc PA-1 column eluted with NaOAc–NaOH was used for better separation of some of the neutral hexoses.

Gel-permeation chromatography was carried out on columns of Sepharose CL-6B (Pharmacia) using 0.1 M NaCl in 10% EtOH as eluant and on Bio-Gel P-2 and P-4 extra fine (BioRad) using the volatile buffer 500:5:2 water–pyridine–HOAc. Elution was monitored by the anthrone[9] and the *m*-hydroxybiphenyl[11] reagents.

Periodate oxidation–Smith degradation.—For analytical purposes, 3.5 mg of deacetylated polysaccharide (DA-EPS) were dissolved in 0.75 mL of water and 0.75 mL of 0.1 M

$NaIO_4$ were added. The solution was kept in the dark at rt, 50 μL aliquots were withdrawn at 15 min intervals, diluted to 10 mL with water and read in a spectrophotometer at 223 nm.[13] For larger scale experiments, 100 mg of sample was dissolved in 25 mL of water and 25 mL of 0.1 M solution of $NaIO_4$ was added. The solutions were then kept for 48 h at 5 °C in the dark. Ethylene glycol (20 mL) was added and after 30 min at rt, the solution was dialyzed for 66 h against distilled water. Sodium borohydride (250 mg) was added slowly to the retentate, the mixture was left for 4 h at rt and for 1 h at 5 °C, neutralized to pH 5.0 with HOAc, dialyzed for 48 h against distilled water, reduced in volume in vacuo, and lyophilized. The yield was 90 mg.

For Smith degradation, 50 mg of the periodate product were dissolved in 10 mL of 0.5 M CF_3CO_2H and the solution kept for 23 h at 37 °C. The volume was then reduced to 2.0 mL in vacuo, and the solution loaded onto a Sephadex G-50 column (1 × 100 cm). Elution was monitored by the anthrone and orcinol reactions. The excluded peak was desalted on a column of Sephadex G-10 and the product (SD-DA-EPS) recovered by lyophilization. The yield from the excluded peak was 22 mg of material.

Acid hydrolysis.—Oligosaccharides were prepared by dissolving native polysaccharide (300 mg) in 300 mL of 0.025 M CF_3CO_2H and heated for 36 h at 100 °C. The hydrolyzate was loaded onto a Bio-Gel P-4 extra-fine column (BioRad) 1 × 120 cm. Elution was carried out with 5:2:500 pyridine–HOAc–water and monitored by the anthrone reaction.[9] Peak fractions were combined, desalted on Bio-Gel P-2 extra-fine columns, and lyophilized. Contents of peak fractions were examined by paper chromatography in solvents **B** and **C**.

Deacetylation and depolymerization for methylation and NMR analysis.—Deacetylation was carried out by treatment with 0.1 M NaOH for 1 h at 25 °C. The polysaccharide (DA-EPS) was recovered by chromatography on Sepharose CL6B. Native, very viscous polysaccharide was treated with 0.1 M CF_3CO_2H for 1.0 h at 100 °C for NMR analysis.

Linkage analysis.—Methylation was carried out by the CCRC (Athens, GA) by a modified Hakomori procedure[14] and products were identified by gas chromatography–mass spectrometry (GC–MS). Analysis of the native EPS showed incomplete methylation, due to the high viscosity in the Me_2SO solution, therefore linkage analysis was performed on DA-EPS which yielded less viscous solutions.

Preparation and use of enzymes.—A crude enzyme was prepared from the same organisms producing the exopolysaccharide. *B. cepacia* strain no. 249 from a stock culture was inoculated onto 62 plates containing 2% DAY agar and inoculated at rt for 5 days. The plates were then scraped into 1.5 L of a sterile 0.85% solution of NaCl. The suspension was stirred for 10 min at high speed and centrifuged at 14,000 rpm for 2 h at 5 °C. The supernatant was used for the isolation of polysaccharide. The precipitate, namely, the cellular material, was suspended in 37 mL of 1.0 M NaOAc and sonicated at full power in a closed chamber for 7 min in an ice–alcohol bath. The suspension was then centrifuged at 20,000 rpm for 2 h at 5 °C. The supernatant was dialyzed against 0.1 M NaOAc, pH 7.0 and redialyzed against 0.05 M NaOAc for 5 h at 5 °C. The retentate was lyophilized, yielding 647 mg of material containing enzymatic activity against the polysaccharides as shown by viscosimetry.

The uronic acid-containing disaccharide (1.0 mg) isolated after acid hydrolysis (see Section 3) was incubated with 1.0 mg of β-glucuronidase from bovine liver or from *E. coli* in 0.1 mg of 0.4 M NaOAc, pH 4.0 for 24 h at 37 °C. Alternatively, 2.0 mg of disaccharide or the acidic oligosaccharide mixture was incubated with 5.0 mg of limpet enzyme (containing α- and β-glucuronidase) in 0.5 mL of 0.1 M NaOAc pH 3.8 for 24 h at 37 °C. Products were determined by paper chromatography in solvent **B**.

In order to determine the absolute configuration of glucose and galactose, D-glucose and D-galactose oxidase were used according to the manufacturer's directions on acid hydrolyzates (2.0 M CF_3CO_2H for 7 h at 100 °C) of polysaccharide samples.[15]

For EPS enzymatic depolymerization, DA-EPS (90 mg) was dissolved in 2.7 mL of water and 0.3 mL of 1.0 M NaOAc, pH 7.0 and 45 mg of crude enzyme preparation was added. The solution was incubated for 24 h at 28 °C. All viscosity was lost after 2 h. The solution was then centrifuged, the small precipitate discarded and 4 mL of 95% EtOH were added. After 24 h, the suspension was centrifuged, the precipitate isolated, redissolved as before, and incubated with 30 mg of crude enzyme. After 24 h at 28 °C the solution was heated in a boiling-water bath for 30 min and centrifuged. The precipitate was discarded, the supernatant lyophilized and the degradation product was chromatographed on Sephadex G-25, material in the major elution peak was purified further on Biogel P-4. Enzyme degraded (ED-DA-EPS, 15 mg) material was obtained.

NMR spectroscopy.—NMR spectra were run by the CCRC (Athens, GA). Samples were prepared by exchanging lyophilized material twice with D_2O, followed by dissolving in 0.6 mL 99.96% D_2O. Chemical shifts were referenced to internal acetone at δ 2.225 and 31.55, for H-1 and C-1, respectively. Spectra of the native EPSs were recorded at 50 °C on a UNITY INOVA Varian spectrometer operating at 500 MHz. Spectra of SD-DA-EPS were recorded on a Bruker AMX500 MHz spectrometer at 25 and 35 °C. Spectra of ED-DA-EPS and of a sample with decreased viscosity were recorded on a Bruker DRX 600 MHz spectrometer at 25 °C. Standard DQF-COSY,[16] TOCSY,[17,18] and NOESY[19] data were collected in phase-sensitive mode using the TPPI[20] method. In these experiments, low-power presaturation was applied to the residual HDO signal. A phase-sensitive gradient HSQC[21,22] was also collected. For the TOCSY and NOESY experiments, 512 FIDs of 1024 complex data points were collected, with eight scans per FID. In the DQF-COSY data set, 1024 FIDs of 2048 complex data points were collected, with eight scans per FID. The spectral width was set to 1501 Hz at 500.132 MHz, and 1750 Hz at 600.13 MHz; the carrier placed at the residual HDO peak. The TOCSY pulse program contained a 90 ms MLEV17 mixing sequence.[23] For the HSQC spectrum, 512 FIDs of 2048 complex points were acquired with 32 scans per FID. The spectral frequency and width in the carbon dimension were 150.9 MHz and 7545 Hz, respectively. The GARP[24] sequence was used for ^{13}C decoupling during acquisition. Data were processed typically with a Lorentzian-to-Gaussian weighing function applied to t^2 and a shifted squared sinebell function and zero-filling applied to t^1. Processing and plotting were done with FELIX software (Molecular Simulations Inc.).

3. Results and discussion

Polysaccharide composition.—As previously reported for strains no. 249 and 542,[8] all of the other strains listed in Table 1 showed the presence of galactose, glucose, mannose, rhamnose, and glucuronic acid in the approximate ratio of 3:1:1:1:1, as determined at first by paper chromatography of acid hydrolyzates. The neutral sugars were quantitated for strain no. 542, 382, 249, and 88A3019 by standard HPLC using an HPX-87P column and by a Dionex PAD system. The ratios for Gal, Glc, Man, and Rha were 3:1:0.6:0.7. As will be shown, the low mannose value is due to a resistant uronic acid to mannose linkage. Glucuronic acid and its lactone were identified by paper chromatography and by HPLC using the HPX-87H column. As hydrolysis is not quantitative due to the loss of uronic acid, colorimetric analysis[10] showed it to be present in a ratio of 1:1 to Glc. The ^1H NMR spectra of the eight EPSs listed in Table 1 were almost identical, with only very minor differences due to slightly different linewidth of the spectra and to minor contamination by NaOAc. Although the broad linewidth of the spectra did not allow to extract much structural information, high field signals at $\sim$2.1 and 1.2 ppm could be assigned to the methyl group of *O*-acetyl groups and of Rha; from peak areas the presence of approximately three acetyl groups per repeating unit may be deduced. No difference in the acetyl signals was discernible among these samples. The optical rotation of the EPS was $[\alpha]_D$ +100°. The absolute configuration of all monosaccharides was found to be D by the use of D-glucose and

Table 2
Methylation analysis of SD-DA-EPS [a] from *B. cepacia*

Glycosyl residue	Ratio of products [b]	
	I	II
3-Linked glucose	1.58	1.36
3-Linked mannose	1.00	1.00
3-Linked glucuronate [c]		0.39

I, polysaccharide core remaining after HIO_4 treatment; II, carboxyl reduced[14] polysaccharide core, remethylated.
[a] Deacetylated, Smith degraded EPS.
[b] Ratios of the GC peak areas with 3-linked mannose set to 1.0.
[c] Determined as 3-linked glucose.

D-galactose oxidases on hydrolyzates[15] for Glc and Gal, and by the method of Gerwig et al.[25] for Rha, Man, and GlcA. The EPSs from strain no. 542, 249 and 382 were used interchangeably for the detailed studies.

Periodate oxidation–Smith degradation.— The native polysaccharide showed insignificant HIO_4 uptake, while the deacetylated polymer (DA-EPS) was readily oxidized. In preparative-scale experiments using DA-EPS, the products obtained after 48 h of periodate treatment were isolated in a yield of 90 mg from 100 mg of starting material. Paper chromatography of an acid hydrolyzate showed the presence of glucose, mannose, glycerol, and glucuronic acid. Galactose and rhamnose were absent. It should be noted that galactose could not be detected as soon as 45 min after periodate oxidation, indicating a terminal position. For Smith degradation, 50 mg of the periodate-oxidized polymer were used. Products were chromatographed on a Sephadex G-50 column and material present in the excluded peak representing the polysaccharide core (SD-DA-EPS) was hydrolyzed and examined by paper chromatography. Glucose, mannose, and glucuronic acid were present in about equal amounts. By HPLC analysis, the ratio found was 1:0.6:1.0 (by colorimetry for the uronic acid). The low mannose value indicated a uronic acid to mannose linkage that is resistant to hydrolysis. Retreatment of the core with periodate did not cause any further degradation, indicating that the linkages themselves were resistant, namely, not protected by branches. Linkage analysis of S-DA-EPS (Table 2) showed that Glc, Man, and GlcA are 3-linked. Finally, NMR analysis suggested the following structure:

3)-β-D-Glc*p*-(1→3)-α-D-Glc*p*A-(1→3)-α-D-Man*p*(1→

determined as follows. In Fig. 1 the 1D proton spectrum shows the three anomeric protons. Fig. 2 is a region of the NOESY spectrum, in

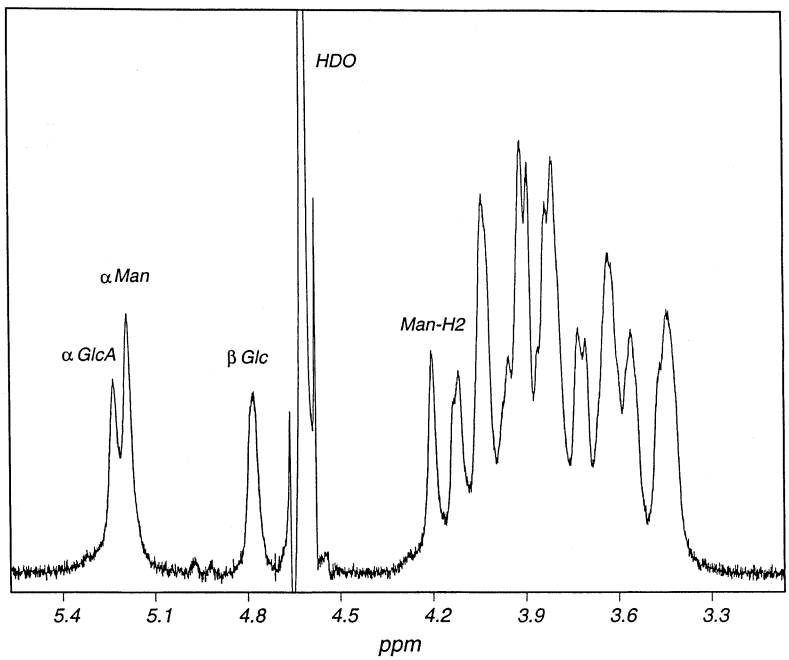


Fig. 1. 500 MHz ^1H NMR spectra of the polysacchaaride core (SD-DA-EPS) obtained after periodate treatment.

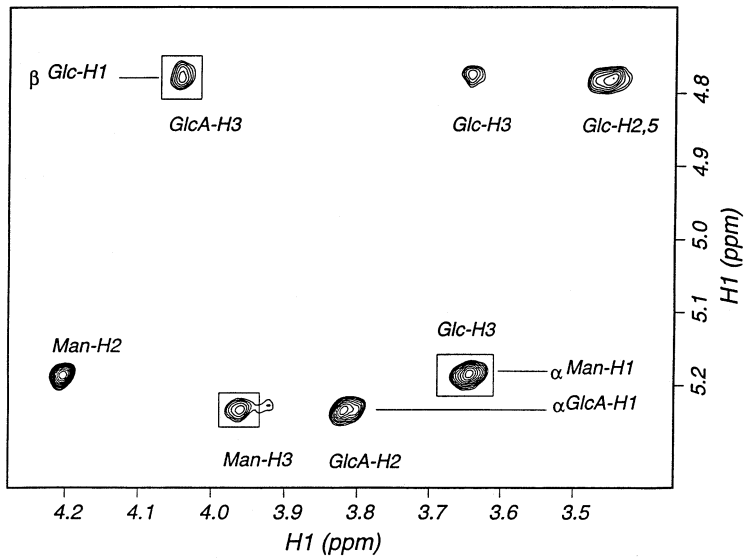


Fig. 2. 500 MHz ^{1}H NOESY spectrum of the polysaccharide core (SD-DA-EPS) obtained after periodate treatment showing NOE crosspeaks across the glycosidic linkage. The boxed crosspeaks are the interglycosidic NOEs.

Table 3
^{1}H chemical shifts for the trisaccharide repeating unit of the SD-DA-EPS from *B. cepacia* [a]

Residue	1	2	3	4	5	6a, 6b
A α-Man	5.196	4.205	3.964	3.917 [b]	4.042 [b]	nd
B α-GlcA	5.236	3.835	4.049	3.615	4.137	
C β-Glc	4.791	3.431	3.654	3.563	3.466	3.915, 3.727

[a] Deacetylated, Smith-degraded EPS chemical shifts are in ppm from DSS, based on internal acetone set to δ 2.225.
[b] Assignments are not confirmed.

which NOE crosspeaks between the anomeric protons of one residue and the proton at the corresponding glycosidic linkage site are indicated. The assignment of these crosspeaks was obtained from a TOCSY dataset (data not shown) and are listed in Table 3.

Graded acid hydrolysis of native polysaccharide.—Products of hydrolysis with 0.025 M CF_3CO_2H for 36 h at 100 °C were fractionated on columns of Bio-Gel P-4. Material eluted in the lower molecular-weight region was desalted on Sephadex G-10 and chromatographed on an anion-exchange column. Bio-Gel P-2 chromatography of the material eluted with an NaCl gradient from this column yielded a disaccharide as a major component, as determined by paper chromatography and HPLC (HPX-87H column). The disaccharide was further characterized. After hydrolysis with 2.0 M CF_3CO_2H for 6 h at 100 °C, the presence of glucuronic acid and mannose was detected by paper chromatography and HPLC. No degradation was observed when the disaccharide was incubated with β-D-glucuronidase from beef liver or from *E. coli*. Incubation with a crude glucuronidase preparation from limpets, containing both β- and α-D-glucuronidase yielded about equal amounts of glucuronic acid and mannose as products. The optical rotation, $[\alpha]_D$, of the disaccharide was + 73°. The data indicate that the disaccharide is an aldobiouronic acid, that is α-D-GlcA → Man. This result is in accord with the trisaccharide repeating-unit for the polysaccharide backbone already determined. Methylation analysis of a deacetylated, reduced-viscosity sample of EPS is shown in Table 4. These data are consistent with the presence of a heptasaccharide repeating unit containing the backbone structure already shown and three branches linked (1 → 6) to mannose and (1 → 4) and (1 → 2) to glucuronic

Table 4
Methylation analysis of DA-EPS from *B. cepacia* [a]

Glycosyl residue	Ratio of products [a]
2-Linked rhamnose	1.0
Terminal galactose	2.4
3-Linked glucose [b]	1.1
2,3,4-Linked glucuronic acid [c]	0.2
3,6-Linked mannose	0.6
3-Linked mannose [b]	0.1
3-Linked galactose	0.3

[a] DA-EPS is deacetylated EPS. Ratios of the GC peak areas with 2-linked Rha set to 1.0.

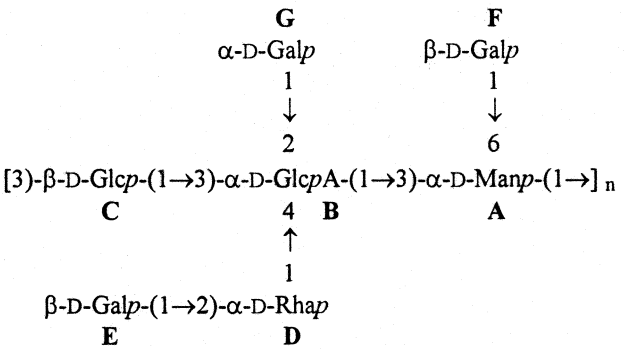
[b] The SP2330 column did not resolve 3-linked glucose from 3-linked mannose, therefore a DB1 column was used for these derivatives.

[c] Measured as a glucose derivative after reduction.[14]

acid, respectively. The high terminal galactose values indicated that these branches are terminated with galactose, with one of them containing a 2-linked rhamnose.

Enzymatic degradation.—As the high viscosity of EPS solutions did not allow a detailed NMR analysis, attempts were made to obtain lower molecular-weight samples. A crude enzyme preparation was unable to depolymerize native EPS, but was effective on the deacetylated sample. The enzyme-degraded polysaccharide (ED-DA-EPS) had a much lower viscosity than the native EPS and yielded useful NMR spectra.

The proposed structure for the heptasaccharide repeating unit obtained from the enzyme digest sample is shown here and supported by the data which follow.



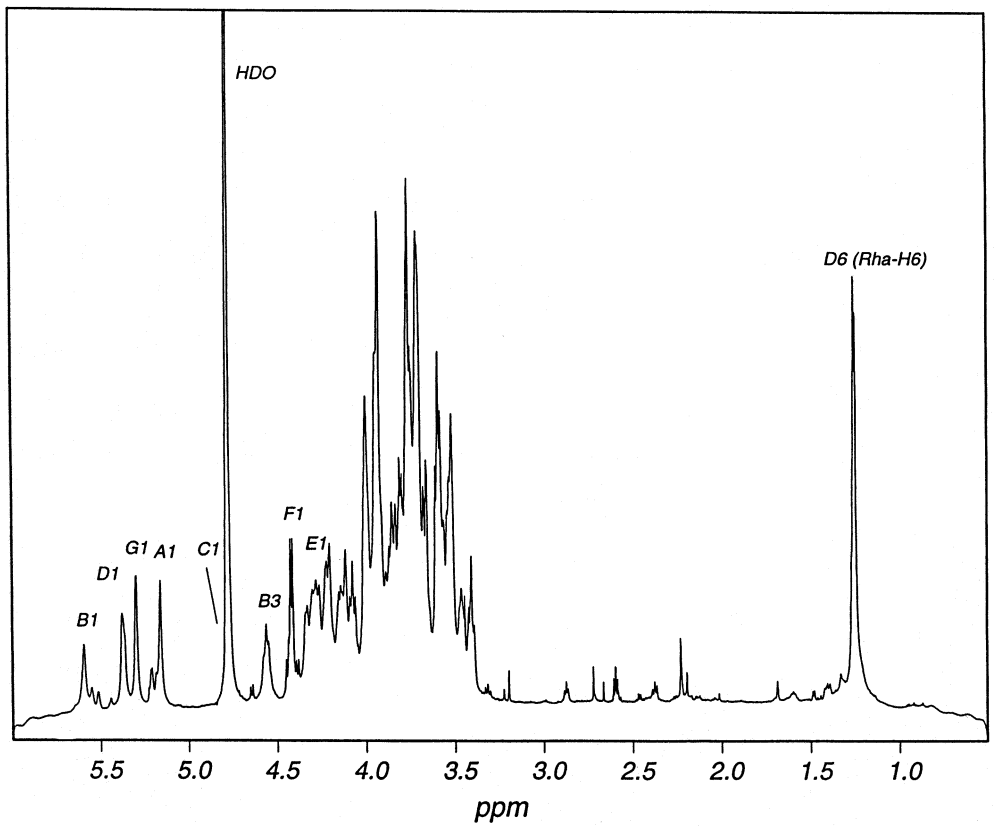


Fig. 3. 600 MHz ^{1}H NMR spectrum of the enzyme degraded EPS (ED-DA-EPS) at 25 °C. The anomeric protons of the repeating heptasaccharide unit are labeled along with some other resonances.

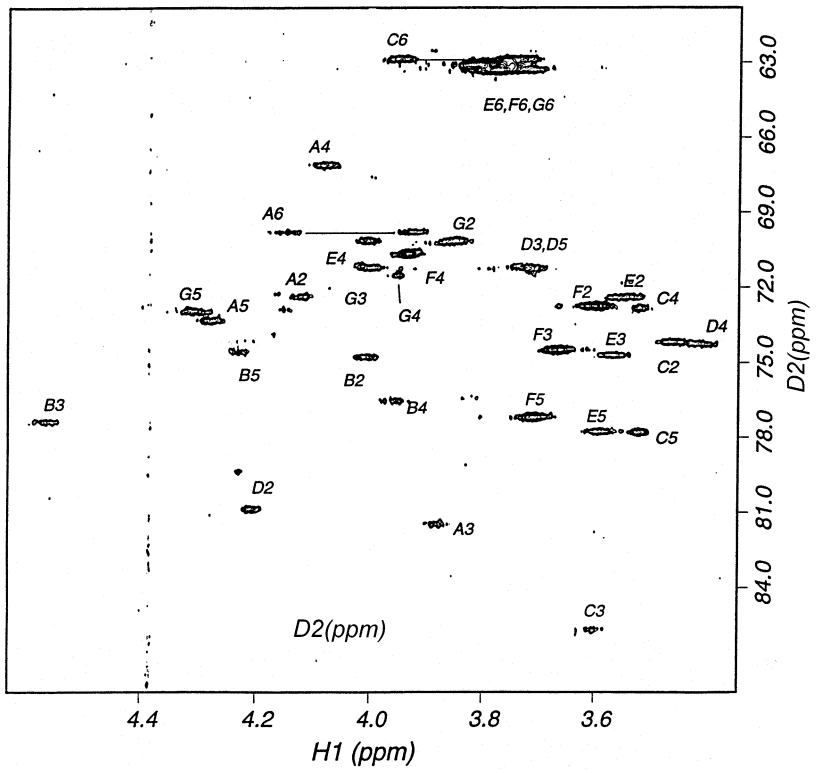


Fig. 4. Region of an HSQC spectrum of the enzyme degraded EPS (ED-DA-EPS) at 25 °C showing the ring proton/carbon correlations.

Fig. 3 reports the 1D proton spectrum for the ED-DA-EPS, indicating the seven anomeric protons and confirming the presence of a rhammosyl residue (for comparison, see Fig. 1 for the 1D proton spectra of the intact polysaccharide). A combination of DQF-COSY and TOCSY data, aided by a NOESY spectrum, allowed the assignment of most protons. The three residues of the core trisaccharide could be identified in the new spectra, although, as expected, various chemical shifts had changed owing to the addition of new sugars. The DQF-COSY and TOCSY data gave additional chemical-shift patterns characteristic of two β-galactosyl residues,[26] as well as an α-D-rhamnosyl residue. The chemical composition and linkage analysis suggested a 3:1 galactose to glucose ratio, so the remaining anomeric peak at 5.3 was proposed to be an α-galactosyl residue. This was confirmed by tracing the TOCSY data to obtain chemical shifts for H-1–H-4 consistent with that residue.[27] The carbon assignments and some additional proton assignments were then obtained from the HSQC spectrum shown in Fig. 4. The complete proton and carbon chemical shifts are reported in Table 5. In Fig. 5, a region of the NOESY spectrum is shown which highlights the NOE crosspeaks across the glycosidic linkages. In addition to the NOE data, these resonances have proton and carbon chemical shifts consistent with their assignment as linkage positions. For example, in Fig. 4 both the H-6s and C-6 of mannosyl residue **A** are shifted relative to unsubstituted examples.[26] Similarly, C-3 of residue **A** is seen at δ 81.5 due to its substitution by the backbone glucuronosyl residue **B**. Other residues have chemical shifts that corroborate the NOESY analysis.[28] In some cases, due to the highly substituted backbone, there are multiple NOE crosspeaks between residues. For example, H-1 of glucosyl residue **C** shows NOE crosspeaks to both H-2 and H-3 of glucuronosyl residue **B**. However, α-galactosyl residue **G** also shows a strong crosspeak to H-2 of B, and so by default residue **C** is linked at the 3-position, as was proposed in the analysis of the trisaccharide backbone. In another example, sidechain rhamnosyl residue **D**

shows a strong NOE crosspeak to H-4 of backbone residue **B**, but also a weaker one to H-1 of galactosyl residue **E**, which is linked 1→2 to **D**.

A sample of the polysaccharide with decreased viscosity, obtained by mild-acid hydrolysis, was also analyzed. The spectra indicated a homogeneous sample, however, detailed analysis was problematic due to the still high molecular weight. Nonetheless, the data were very similar to those obtained from the ED-EPS, confirmed the assignments and proposed structure.

4. Conclusions

B. cepacia has become an exceptionally troublesome infectious agent in debilitated patients and patients with cystic fibrosis. It is

Table 5
^{1}H and ^{13}C chemical shifts for the heptasaccharide-repeating unit of ED-DA-EPS from *B. cepacia* [a]

Residue	1	2	3	4	5	6a, 6b
A α-Man	5.158	4.118	3.873	4.074	4.272	4.143, 3.918
	(103.16)	(72.36)	(81.51)	(67.12)	(73.30)	(69.78)
B α-GlcA	5.594	4.006	4.562	3.957	4.227	
	(99.45)	(74.78)	(77.41)	(76.55)	(74.58)	
C β-Glc	4.771	3.463	3.600	3.521	3.520	3.942, 3.729
	(104.22)	(74.18)	(85.70)	(72.83)	(77.82)	(62.86)
D α-Rha	5.374	4.205	3.708	3.406	3.717(	1.252
	(101.45)	(80.90)	(71.25)	(74.27)	71.25)	
E β-Gal	4.335	3.540	3.568	4.002	3.586	3.77 [b]
	(105.66)	(72.40)	(74.71)	(70.13)	(77.78)	(63.3)
F β-Gal	4.419	3.590	3.663	3.928	3.712	3.81 [b]
	(105.41)	(72.74)	(74.49)	(70.66)	(77.21)	(63.1)
G α-Gal	5.297	3.846	3.996	3.943	4.312	3.72–3.75 [b]
	(95.67)	(70.15)	(71.22)	(71.53)	(72.94)	(63.2)

[a] ED-DA-EPS is deacetylated and enzyme degraded EPS. Chemical shifts are in ppm from DSS, based on internal acetone set to (δ 2.225 for ^{1}H and δ 31.55 for ^{13}C). ^{13}C shifts are in parenthesis.
[b] Assignments may be interchanged.

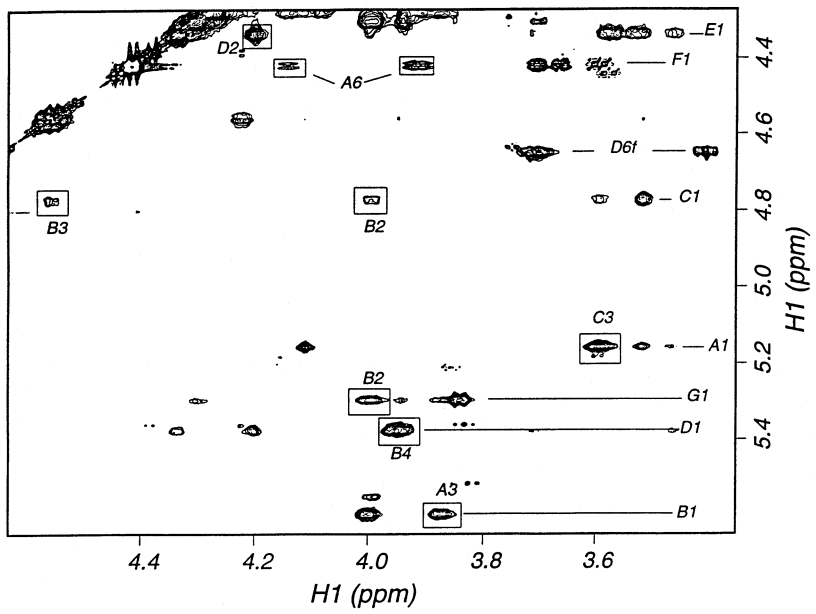


Fig. 5. Region of a NOESY spectrum of the enzyme degraded EPS (ED-DA-EPS) at 25 °C. The labeled boxes indicate crosspeaks between anomeric protons (A1–G1) and glycosidically linked ring protons.

difficult to eradicate due to its unusual resistance to host defenses and antibiotics. This organism, whether isolated from soil samples or from clinical specimens, produces a highly viscous exopolysaccharide with a very complex structure. This polysaccharide, like other known capsular or exopolysacchrides, may in part be responsible for the survival of *B. cepacia* in unfavorable environments; and, in addition, it is very likely that it may play an important role in biofilm formation,[29] which presents an additional obstacle to eradication by antibiotics or immune defenses. It is also of interest that *B. cepacia* represents another example of opportunistic pathogens which cause infection in both plants and animals,[30] implying a common mechanism of action.

The data presented here are in good agreement with those in recent publications of Cérantola et al.[31,32] and of Cescutti et al.[33] describing the structure of an exopolysaccharide produced by different strains of *B. cepacia* of various origin. The present work, reporting data from eight different strains of *B. cepacia*, adds strength to the hypothesis of Cérantola et al.[32] that this EPS may indeed be representative of this microbial species.

Acknowledgements

This material is based upon work supported by the Research and Development Service Center, Department of Veterans Affairs (VA), Salt Lake City Health Care System, Salt Lake City, UT 84148, USA.

References

1. Goldman, D. A.; Klinger, J. D. *J. Pediat.* **1986**, *108*, 806–812.
2. Hearst, J. E.; Elliott, K. E. *Nature Med.* **1995**, *1*, 626–627.
3. Sun, L.; Jiang, R.-Z.; Steinbach, S.; Holmes, A.; Campenelli, C.; Forstner, J.; Sajjan, U.; Tan, Y.; Riley, M.; Goldstein, R. *Nature Med.* **1995**, *1*, 661–666.
4. Hammill, R. J.; Houston, E. D.; Georghiou, P. R.; Wright, C. E.; Koza, M. A.; Cadle, R. M.; Goepfert, P. A.; Lewis, D. A.; Zenon, G. J.; Clarridge, J. E. *Ann. Intern. Med.* **1995**, *122*, 762–766.
5. Ouchi, K.; Abe, M.; Karita, M.; Aguri, T.; Igari, J.; Nakazawa, T. *J. Clin. Microbiol.* **1995**, *33*, 2353–2357.
6. Ledsom, M. J.; Gallagher, M. J.; Corkill, J. E.; Hart, C. A.; Walshaw, M. J. *Thorax* **1998**, *53*, 432–436.
7. Roberts, I. S. *Microbiology* **1995**, *141*, 2023–2031.
8. Sage, A.; Linker, A.; Evans, L. R.; Lessie, T. G. *Curr. Microbiol.* **1990**, *20*, 191–198.
9. Seifter, S.; Dayton, S.; Novice, B.; Muntwyler, E. *Arch. Biochem.* **1950**, *25*, 191–200.
10. Dische, Z. *J. Biol. Chem.* **1947**, *167*, 189–198.
11. Blumenkrantz, N.; Asboe-Hansen, G. *Anal. Biochem.* **1973**, *54*, 484–489.
12. Trevelyan, W. E.; Procter, D. P.; Harrison, J. S. *Nature* **1950**, *166*, 444–445.
13. Guthrie, R. D. *Methods Carbohydr. Chem.* **1962**, *1*, 435–441.
14. York, W. S.; Darvill, A. G.; McNeil, M.; Stevenson, T. S.; Albersheim, P. *Methods Enzymol.* **1985**, *18*, 3–40.
15. Aman, P.; McNeil, M.; Franzen, L.-E.; Darvill, A. G.; Albersheim, P. *Carbohydr. Res.* **1980**, *95*, 263–282.
16. Piantini, U.; Sorenson, D.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800–6801.
17. Brawnschweiler, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521–528.
18. Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355–360.
19. Macura, S.; Huang, Y.; Suter, D.; Ernst, R. R. *J. Magn. Reson.* **1981**, *43*, 259–281.
20. Marion, D.; Wuthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967–974.
21. Bodenhausen, G.; Ruben, D. J. *Chem. Phys. Lett.* **1980**, *69*, 185–186.
22. Davis, A. L.; Laue, E. D.; Keeler, J.; Moskau, D.; Lohman, J. *J. Magn. Reson.* **1991**, *94*, 637–644.
23. Davis, D. G.; Bax, A. *J. Am. Chem. Soc.* **1985**, *107*, 2820–2821.
24. Shaka, J. A.; Barker, P. B.; Freeman, R. *J. Magn. Reson.* **1985**, *64*, 547–552.
25. Gerwig, G. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1979**, *77*, 1–7.
26. Bock, K.; Thogerson, H. *Ann. Rep. NMR Spectrosc.* **1982**, *13*, 1–57.
27. Wieruszeski, J. M.; Michalski, J. C.; Strecker, G. *Glycoconjugate J.* **1990**, *7*, 13–26.
28. Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.
29. Costerton, J. W.; Stewart, P. S.; Greenberg, E. P. *Science* **1999**, *284*, 1318–1322.
30. Rahme, L. G.; Stevens, E. J.; Wolfort, S. W.; Shao, J.; Tompkins, R. G.; Asubel, F. M. *Science* **1995**, *268*, 1899–1902.
31. Cérantola, S.; Lemassu-Jacquier, A.; Montrozier, H. *Eur. J. Biochem.* **1999**, *260*, 373–383.
32. Cérantola, S.; Bounéry, J.-D.; Segonds, C.; Marty, N.; Montrozier, H. *FEMS Microbiol. Lett.* **2000**, *185*, 243–246.
33. Cescutti, P.; Bosco, M.; Picotti, F.; Impallomeni, G.; Leitão, J. H.; Richau, J. A.; Sá-Correia, I. *Biochem. Biophys. Res. Commun.* **2000**, *273*, 1088–1094.