Carbohydrate Research, 142 (1985) 338–343
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

A bacteriophage-associated lyase acting on *Klebsiella* serotype K5 capsular polysaccharide

JAN E. G. VAN DAM*†, HERMAN VAN HALBEEK, JOHANNIS P. KAMERLING, JOHANNES F. G. VLIEGENTHART,
Department of Bio-Organic Chemistry, State University of Utrecht, Utrecht (The Netherlands)*

HARM SNIPPE, MARGRIET JANSZE, AND JAN M. N. WILLERS
Department of Immunology†, Laboratory of Microbiology, State University of Utrecht, Utrecht (The Netherlands)

(Received February 18th, 1985; accepted for publication, March 28th, 1985)

For the depolymerisation of polysaccharides, chemical[1] and enzymic[2] approaches are available. The enzymic methods are mainly based on the use of endo-glycanases[2], yielding oligosaccharides. Several polysaccharides containing 4-linked hexuronic acids can be cleaved with lyases, leading to oligosaccharides having terminal unsaturated hex-4-enuronic acid residues[2–4]. Bacteriophage-associated lyases have also been described[5–7].

For *Klebsiella* capsular polysaccharides, including serotype 5, bacteriophage-borne depolymerases have been studied extensively[2,8], but there has been no indication of the nature of the cleavage, *i.e.*, endo-glycanase *versus* lyase activity. We now describe a bacteriophage-associated lyase which cleaves the *Klebsiella* serotype 5 capsular polysaccharide. Part of these data have been reported in a preliminary communication[9].

Klebsiella serotype 5 capsular polysaccharide consists of a linear trisaccharide repeating-unit containing D-glucose, D-glucuronic acid, and D-mannose, with additional pyruvate and *O*-acetyl groups[10] (**1**).

→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→3)-β-D-Man*p*-(1→
(Glc*p* substituted at O-2 by OAc: 2 ← OAc; Man*p* substituted at O-4,6 by pyruvate acetal: Me/COOH)

1

Detailed conformational studies using o.r.d.[11], c.d.[11], and X-ray diffraction[12] have been published. The reported phage-associated depolymerase splits

0008-6215/85/$ 03.30 © 1985 Elsevier Science Publishers B.V

specifically the (1→4) linkage between Man and GlcA, as was established by identification of the reducing unit[8].

Incubation of the capsular polysaccharide of *Klebsiella* serotype 5 with bacteriophage $\phi 5'$ from sewage yielded a mixture of oligosaccharides, showing a strong absorption at $\lambda_{max}^{H_2O}$ 230 nm indicative of a conjugated double-bond system[13]. This mixture was fractionated on Sephadex G-25, and the fraction having the highest retention time was further fractionated on Bio-Gel P-4 to give a major and a minor fraction. The major fraction was purified by f.p.l.c. on Mono Q and shown to be an unsaturated trisaccharide.

The sugar analysis data of the major fraction, before and after borohydride reduction, are presented in Table I together with data for the native polysaccharide. Methanolysis cleaved only 30% of the pyruvate methyl ester group attached to the Man residue, as was established by g.l.c.–m.s. Therefore, the routine methanolysis procedure[14,15] was preceded by a hydrolysis. The major fraction contained equal amounts of Glc and Man, with Man as the reducing terminus (GlcA was not present), and had $\lambda_{max}^{H_2O}$ 230 nm, a value reported earlier for hex-4-enopyranuronic acid-containing oligosaccharides[13]. Unsaturated hexuronic acids are known to be degraded in the sugar analysis procedure used. Based on the reported structure of the native polysaccharide, these results suggest the major fraction to be an unsaturated trisaccharide of the core structure β-Hex-4-ene*p*A-(1→4)-β-D-Glc*p*-(1→3)-D-Man.

The structure of the *O*-deacetylated trisaccharide was obtained from the 500-MHz ^{1}H-n.m.r. spectrum (Fig. 1 and Table II). The *O*-deacetylation with ammonia was carried out because the ^{1}H-n.m.r. spectrum of the native oligosaccharide indicated only 20% *O*-acetylation (δ 2.054) and the peak patterns in the structural-reporter group region were complex.

In the anomeric region (δ 4.4–5.5) of the spectrum, there were four distinct signals at δ 5.202 ($J_{1,2}$ 1.8 Hz; α-Man H-1), 4.943 ($J_{1,2}$ 0.9 Hz; β-Man H-1; αβ-ratio ~3:2), 4.642 ($J_{1,2}$ 8.1 Hz; β-Glc H-1), and 5.136 ($J_{1,2}$ 5.9 Hz; Hex-4-ene*p*A H-1). The unsaturated nature of the last unit is indicated by the doublet for H-4 at δ 5.827 ($J_{3,4}$ 3.6 Hz)[16]. The $J_{1,2}$ value (5.9 Hz) observed for H-1 of Hex-4-ene*p*A points to quasi-diaxial orientation[16] of H-1,2, which accords with the β configuration expected from the structure of the native polysaccharide. Comparable chemical

TABLE I

SUGAR ANALYSIS DATA OF THE CAPSULAR POLYSACCHARIDE OF *Klebsiella* SEROTYPE 5 [K5(PS)] AND THE UNSATURATED TRISACCHARIDE K5($\phi 5'$) OBTAINED BY PHAGE-CLEAVAGE FROM K5(PS), BEFORE AND AFTER SODIUM BOROHYDRIDE REDUCTION

	GlcA	*Glc*	*Man*	*Man-ol*
K5(PS)	1.0	1.1	1.1	—
K5($\phi 5'$)	—	1.0	1.0	—
Reduced K5($\phi 5'$)	—	1.0	—	1.0

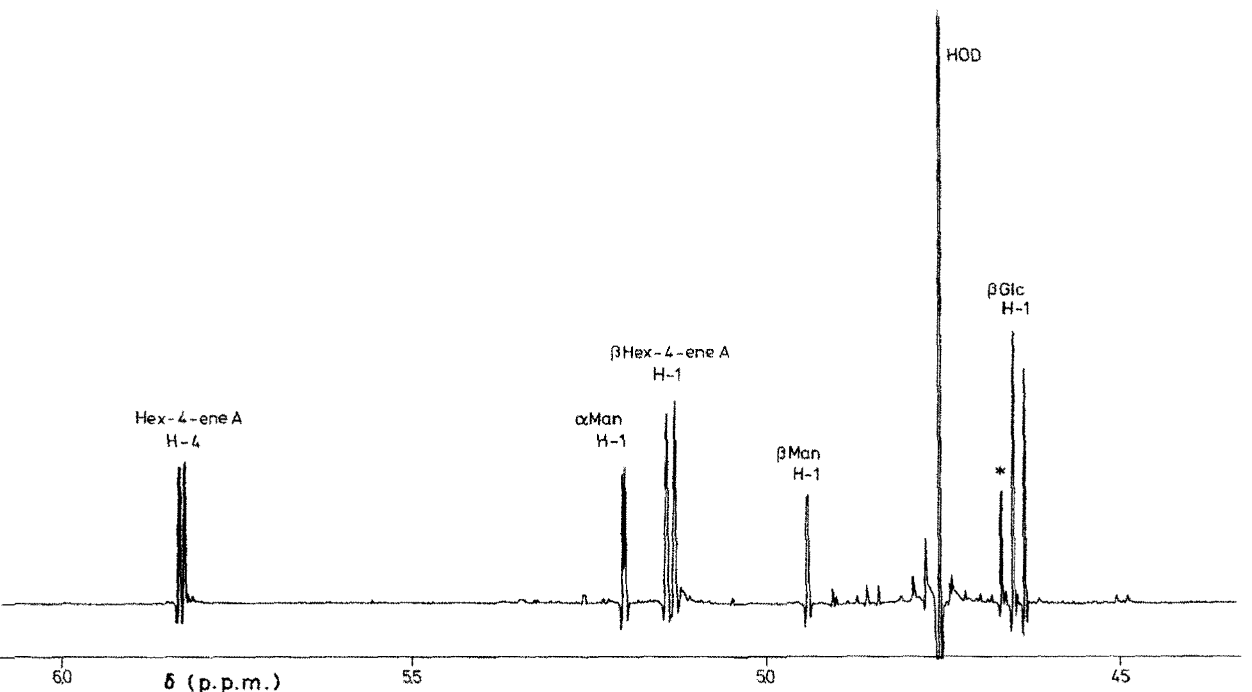

Fig. 1. Structural reporter-group region of the resolution-enhanced 500-MHz ^{1}H-n.m.r. spectrum of β-Hex-4-ene*p*A-(1→4)-β-D-Glc*p*-(1→3)-D-Man$p_4^6 \times _{Me}^{COOH}$ in D_2O at pD 7 and 27°, * signal of unknown origin.

TABLE II

^{1}H-N.M.R SPECTRAL DATA FOR THE *Klebsiella* CAPSULAR POLYSACCHARIDE TYPE K5-DERIVED, UNSATURATED, *O*-DEACETYLATED TRISACCHARIDE

	δ	J *(Hz)*
α-Man*p* H-1	5.202	1.8
β-Man*p* H-1	4.943	0.9
β-Glc*p* H-1	4.642	8.1
β-Hex-4-ene*p*A H-1	5.136	5.9
H-4	5.827	3.6
Pyruvate Me	1.478	—

shifts of the signals for H-1 and H-4 have been observed for β-Hex-4-ene*p*A (1→3)-linked to GalNAc in chondroitinase-degraded chondroitin sulfates, like the Swarm rat chondrosarcoma proteoglycan and the C6S-proteoglycan from human atherosclerotic aorta[17]. The α-Hex-4-ene*p*A-(1→4)-D-Gal*p*A, obtained from lyase-degraded pectin, gave a similar doublet for H-4 of α-Hex-4-ene*p*A at δ 5.788 ($J_{3,4}$ 3.5 Hz), and a doublet for H-1 of α-Hex-4-ene*p*A at δ 5.107 ($J_{1,2}$ 2.0 Hz). Finally, the trisaccharide contains a pyruvate group linked to C-4 and C-6 of Man; the pyruvate Me singlet is found at δ 1.478 in accordance with the *S* configuration[18].

Catalytic hydrogenation (Pd/C) of the trisaccharide gave a product having no u.v. absorption at 230 nm. Sugar analysis gave, in addition to Glc and Man, four unknown peaks at R_{Mannitol} 0.40, 0.43, 0.48, and 0.51 in the ratios 9:13:1:1. The

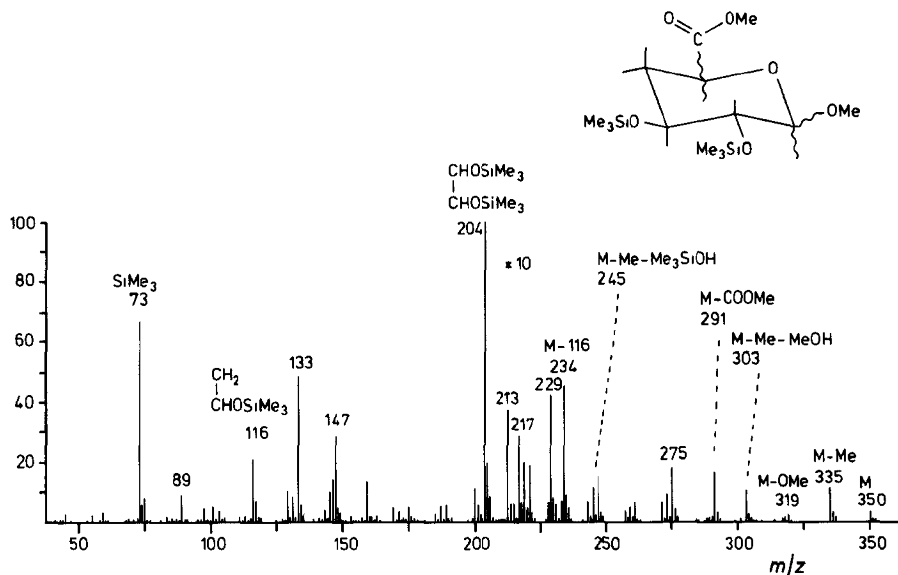

Fig. 2. E.i. mass spectrum (70 eV) of 4-deoxy-2,3-di-*O*-trimethylsilylhexuronic acid methyl ester methyl glycoside.

mass spectra of these four products were similar (Fig. 2) and indicated α,β-4-deoxyhexuronic acid methyl ester methyl glycosides. Sugar analysis of hydrogenated α-Hex-4-ene*p*A-(1→4)-D-Gal*p*A gave identical 4-deoxyhexuronic acid derivatives.

Thus, the *Klebsiella* K5 capsular polysaccharide depolymerase from bacteriophage $\phi 5'$ has to be classified as a lyase. Although many bacteriophage-associated polysaccharide-degrading enzymes act on polysaccharides possessing 4-*O*-substituted hexuronic acid residues, most of them have not been adequately characterised. In this context, it is interesting to note that the repeating unit of *Klebsiella* K22 capsular polysaccharide has been proposed to contain a naturally occurring Hex-4-ene*p*A residue[8].

EXPERIMENTAL

Klebsiella serotype K5 (strain NCTC 9660) was grown on Worfel Ferguson Agar (Difco Laboratories). The capsular polysaccharide K5 was isolated[19] by the phenol–water–Cetavlon method. Bacteriophage $\phi 5'$ was obtained from sewage following standard procedures[20]. High-titre phage stocks (1.10^{12} phages/mL) were prepared by DIAFLO ultrafiltration (HIP100, nominal molecular weight cut-off: 100,000; Amicon Corp.) and purified by subsequent isopycnic centrifugation through a continuous CsCl gradient[20].

K5 polysaccharide (92 mg) was depolymerised by incubation with $\phi 5'$ bacteriophage (1 mL; 1.10^{12} phages/mL) in aqueous 1% ammonium acetate buffer (pH 7.2–7.5) at 37° for 48 h. The resulting oligosaccharide mixture was eluted from a column (2.5 × 135 cm) of Sephadex G-25 (medium) with water (65–80% recovery). For the isolation of the unsaturated trisaccharide, additional fractionations were successively carried out on a column (2 × 120 cm) of Bio-Gel P-4 (−400 mesh), using water as eluent and refractive index detection, and on a Mono Q HR 5/5 column (f.p.l.c., Pharmacia), using a 0–0.5M NaCl gradient with u.v. detection (214 nm).

Sugar analyses were carried out by g.l.c. on a CPsil5 WCOT fused-silica capillary column (25 m × 0.32 mm i.d.), using a Varian Aerograph 3700 gas chromatograph[21]. Trimethylsilylated sugar derivatives were obtained by hydrolysis (4M HCl, 2 h, 100°) followed by methanolysis[15,21]. *O*-Deacetylation was effected with aqueous ammonia (pH 11) for 6 h at room temperature. The solution was then concentrated to dryness and the trisaccharide was obtained by gel filtration on Bio-Gel P-2.

500-MHz ^{1}H-N.m.r. spectroscopy was carried out after repeated dissolution of the trisaccharide in D_2O and lyophilisation. A Bruker WM-500 spectrometer (SON hf-n.m.r. facility, Department of Biophysical Chemistry, University of Nijmegen, The Netherlands), operating in the pulsed Fourier-transform mode at a probe temperature of 27° and equipped with a Bruker Aspect-2000 computer, was used. Chemical shifts (δ) are expressed in p.p.m. downfield from the signal for

internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate, and measured by reference to internal acetone (δ 2.225).

Hydrogenation was carried out in water over Pd/C (10%) for 2 h at room temperature.

U.v. absorptions (300–200 nm) were measured on a Hewlett–Packard 8450 A diode array spectrophotometer.

E.i. mass spectra (70 eV) of the trimethylsilylated methanolysis products were recorded on a Carlo Erba GC/Kratos MS 80/Kratos DS55 system [accelerating voltage, 2.7 kV; ionising current, 100 μA; ion-source temperature, 225°; CPsil5 WCOT fused-silica capillary column (25 m × 0.32 mm, i.d.)].

ACKNOWLEDGMENTS

The authors thank Mrs. Anca van der Kerk-Van Hoof for recording the mass spectra, and Mr. H. A. Schols (Landbouw Hogeschool Wageningen, The Netherlands) for kindly supplying the disaccharide originating from lyase-degraded pectin. This investigation was supported by a research grant from Centrascience B.V., Etten-Leur (The Netherlands), and by the Netherlands Foundation for Chemical Research (SON/ZWO).

REFERENCES

1 B. Lindberg, J. Lönngren, and S. Svensson, *Adv. Carbohydr. Chem. Biochem.*, 31 (1975) 185–240.
2 H. Geyer, K. Himmelspach, B. Kwiatkowski, S. Schlecht, and S. Stirm, *Pure Appl. Chem.*, 55 (1983) 637–653.
3 J. Kiss, *Adv. Carbohydr. Chem. Biochem.*, 29 (1974) 229–303.
4 G. E. Becker and A. M. Pappenheimer, *Biochim. Biophys. Acta*, 121 (1966) 343–348.
5 I. W. Davidson, C. J. Lawson, and I. W. Sutherland, *J. Gen. Microbiol.*, 98 (1977) 223–229.
6 H. Niemann, A. Birch-Andersen, E. Kjems, B. Mansa, and S. Stirm, *Acta Pathol. Microbiol. Scand., Sect. B*, 84 (1976) 145–153.
7 R. I. Hollingsworth, M. Abe, J. E. Sherwood, and F. B. Dazzo, *J. Bacteriol.*, 160 (1984) 510–516.
8 D. Rieger-Hug and S. Stirm, *Virology*, 113 (1981) 363–378.
9 J. E. G. van Dam, H. Snippe, M. Jansze, J. M. N. Willers, H. van Halbeek, J. P. Kamerling, and J. F. G. Vliegenthart, *Abstr. Int. Carbohydr. Symp., XIIth, Utrecht*, 1984, p. 373.
10 G. G. S. Dutton and M. I. Yang, *Can. J. Chem.*, 51 (1973) 1826–1832.
11 Ch. Wolf, U. Elsasser-Beile, S. Stirm, G. G. S. Dutton, and W. Burchard, *Biopolymers*, 17 (1978) 731–748.
12 D. H. Isaac, K. H. Gardner, Ch. Wolf-Ullish, E. D. T. Atkins, and G. G. S. Dutton, *Int. J. Biol. Macromol.*, 1 (1979) 107–110.
13 A. Linker, K. Meyer, and P. Hoffman, *J. Biol. Chem.*, 219 (1956) 13–25.
14 J. P. Kamerling, G. J. Gerwig, J. F. G. Vliegenthart, and J. R. Clamp, *Biochem. J.*, 151 (1975) 491–495.
15 J. P. Kamerling and J. F. G. Vliegenthart, *Cell Biol. Monogr.*, 10 (1982) 95–125.
16 A. S. Perlin, D. M. Mackie, and C. P. Dietrich, *Carbohydr. Res.*, 18 (1971) 185–194.
17 Unpublished results from the authors' laboratory.
18 P. J. Garegg, B. Lindberg, and I. Kvarnstrom, *Carbohydr. Res.*, 77 (1979) 71–79.
19 K. Jann, B. Jann, F. Ørskov, I, Ørskov, and O. Westphal, *Biochem. Z.*, 342 (1965) 1–22.
20 H. Thurow, H. Niemann, and S. Stirm, *Carbohydr. Res.*, 41 (1975) 257–271.
21 G. J. Gerwig, J. P. Kamerling, and J. F. G. Vliegenthart, *Carbohydr. Res.*, 129 (1984) 149–157.