Carbohydrate Research, 59 (1977) 525–530

PRIMARY STRUCTURE OF THE *Klebsiella* SEROTYPE 16 CAPSULAR POLYSACCHARIDE

AJIT K. CHAKRABORTY*, HORST FRIEBOLIN, HEINER NIEMANN, AND STEPHAN STIRM
*Max Planck-Institut für Immunbiologie**, *Freiburg, and*
Institut für Organische Chemie der Universität, Heidelberg (German Federal Republic)
(Received February 15th, 1977; accepted for publication, February 21st, 1977)

ABSTRACT

The primary structure of the *Klebsiella* serotype 16 capsular polysaccharide consists of tetrasaccharide repeating-units comprising a→3)-α-D-Glc*p*-(1→4)-β-D-GlcA*p*-(1→4)-α-L-Fuc*p*-(1→ chain with a β-D-Gal*p*-(1→ branch at position 4 of the D-glucosyl residue.

INTRODUCTION

Of the 81[1] different capsular heteropolysaccharides[2] carrying the serological determinants for the *Klebsiella* K antigens[3], 55 have been (*e.g.*, Refs. 4–6), or are being (*e.g.*, Refs. 7 and 8), subjected to primary structural analyses. One of the remaining polysaccharides is the serotype-16 glycan, and we now report on its analysis.

MATERIAL AND METHODS

With the exception of those given in the following sections, all materials and methods have been described previously[6,9].

Bacteria and polysaccharide. — *Klebsiella* 2069/49, the serological test-strain for the K16 capsular antigen[2,3,10], was kindly supplied by Dr. Ida Ørskov, WHO International Escherichia Centre, Statens Seruminstitut, Copenhagen; 30 g of dry bacteria were obtained[6,9] from 100 large nutrient-agar plates, and thence 2.4 g (8%, w/w) of type-16 polysaccharide.

Reduction of carboxyl groups in the polysaccharide, and in oligosaccharides. — Type-16 polysaccharide was reduced with 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (CMC) and sodium borohydride, as detailed by Taylor *et al.*[11,12]. The same procedure was used for the reduction of the oligosaccharides isolated after partial hydrolysis of the glycan with acid (see Table III). In the latter case, however, the solutions obtained after reaction with $NaBH_4$ and

*To whom all correspondence should be addressed.

mild acidification were concentrated to about one-fifth volume, and subjected to paper electrophoresis[13] (45 V/cm; 2–3 h) on Schleicher & Schüll (Dassel, GFR) paper no. 2043b with a volatile pyridine–glacial acetic acid–water (10:4:86, pH 5.3) buffer. The pure neutral products were recovered by elution of the base-line strips and lyophilization.

RESULTS

The sugar composition and some properties of the *Klebsiella* serotype 16 capsular polysaccharide are recorded in Table I. As evidenced by paper electrophoresis after acid hydrolysis[9] and by the p.m.r. spectrum, or by g.l.c. after alkaline hydrolysis[14,15], respectively, the material does not carry pyruvate acetal, or *O*-acetyl substituents.

TABLE I

SUGAR COMPOSITION AND SOME PROPERTIES OF *Klebsiella* SEROTYPE 16 CAPSULAR POLYSACCHARIDE

Polysaccharide	*Molar ratios of constituent sugars*[a]				$s^{c}_{20,\,solvent}$[e] *(10^{-13} sec)*	*Equivalent weight*[f]	$[\alpha]^{20}_{589}$[g] *(degrees)*
	D-*Glc*[b]	D-*Gal*[c]	L-*Fuc*[d]	D-*GlcA*[b]			
Native[h]	1.00[i]	0.91[i]	0.85[i]	1.01	1) 1.8[e] 2) 2.2	n.d.	+65
Alkali-treated[j]	1.00	0.89	0.81	0.99	1.7[e]	605	+72
Carboxyl-reduced[k]	2.00[b]	0.95[c]	0.82	0.15	n.d.	n.d.	n.d.

[a]Hexoses determined by g.l.c. of the alditol acetates[16], and hexuronic acid by the carbazole–sulfuric acid method[17]. [b]About the same amount of D-glucose was determined in the hydrolyzate of the carboxyl-reduced polymer by using fungal D-glucose oxidase[18]. [c]About the same amount of galactose was determined with D-galactose dehydrogenase from *Pseudomonas fluorescens*[19]. [d]A syrupy sample of the fucose was levorotatory. [e]0.42% (w/v) solutions in phosphate-buffered, physiological saline were centrifuged; like other *Enterobacteriaceae* capsular polysaccharides (see Ref. 9), the glycan did not sediment uniformly before mild alkali-treatment (see footnote *j*). [f]Obtained by titration of the acidic form of the polysaccharide[6]. [g]In water (*c* 0.5). [h]As extracted from *Klebsiella* 2069/49 by the phenol–water–cetyltrimethylammonium bromide procedure[9,20]. [i]Maximum amounts analyzed after hydrolysis for 15 h (Glc), 12 h (Gal), or 16 h (Fuc) with 0.5M H_2SO_4 at 100°. [j]Treated with 0.25M aqueous NaOH at 56° for 2 h[9]. [k]Carboxyl-reduced by the method of Taylor *et al.*[11,12].

As summarized in Table II, type-16 polysaccharide and the repeating-unit tetrauronic acid obtained therefrom (Table III) were permethylated[21], and the methylated monomers were analyzed by g.l.c.–m.s. of the alditol acetates[22,23] — also after a subsequent reduction–dideuteration of the uronic acid methyl ester groups[6,24].

Furthermore, the acidic oligosaccharides obtained by partial hydrolysis of the glycan with acid were purified by paper electrophoresis and p.c.[6,9], and analyzed as shown in Table III.

TABLE II

IDENTIFICATION AND RATIOS OF *O*-ACETYL-*O*-METHYLALDITOLS OBTAINED FROM *Klebsiella* SEROTYPE 16 CAPSULAR POLYSACCHARIDE AND REPEATING-UNIT TETRASACCHARIDE

Peracetate of[a]	T[b]		*Primary fragments found* (m/e)								*I*[c]	*II*	*III*	*IV*
	Lit.	*Found*	*45*	*117*	*161*	*203*	*205*	*233*	*261*	*305*	*Ratio of peak integrals*			
2,3-FucOH	1.18	1.16		+	+	+					1.0	1.1	0.8	0.8
2,3,4,6-GalOH	1.25	1.24	+	+	+		+				0.9	0.8	1.0	1.0
2,3,6-GlcOH	2.50	2.52	+	+	(+)			+			—	—	1.1	1.1
2,6-GlcOH	3.83	3.77	+	+						+	1.0	1.1	—	—
2,3-GlcOH	5.39	5.39		+	+				(263)[d]		—	1.0	—	0.8

[a]2,3-FucOH = 2,3-di-*O*-methylfucitol, *etc.* [b]Retention time, relative to peracetylated 2,3,4,6-GlcOH (*T* 1.00) and 2,3-GlcOH (*T* 5.39) in g.l.c. on an ECNSS-M column[22,23]. [c]I, type-16 polysaccharide, permethylated; II, type-16 polysaccharide, permethylated, and reduced with calcium borodeuteride[6,24]; III, type-16 repeating-unit tetrasaccharide, as obtained by partial hydrolysis with acid (see Table III), permethylated; IV, type-16 tetrasaccharide, permethylated, and reduced with calcium borodeuteride. [d]Dideuterated fragment found instead of the normal one.

TABLE III

ACIDIC OLIGOSACCHARIDES OBTAINED BY PARTIAL HYDROLYSIS[a] OF *Klebsiella* SEROTYPE 16 CAPSULAR POLYSACCHARIDE WITH ACID

Determination	*H2*[b]	*H3*	*H4*
Yield (% of polysaccharide)	6.1	7.5	24.9
Approximate molar ratio of sugar components[c]:			
D-Glucose	—	1.0	1.0
D-Galactose	—	—	0.8
L-Fucose	0.8	0.9	1.0
D-Glucuronic acid	1.0	1.0	1.0
Reducing-end sugar[d]	Fuc	Fuc	Fuc
Ratio reducing/non-reducing hexoses[e]	—	1:0.85	1:1.82
Mobility in paper electrophoresis[f] (relative to glucuronic acid)	0.69	0.50	0.38
R_{GLC} in p.c.[g]	0.64	0.30	0.12
Enzymic hydrolysis by exo-glycosidases[h]:			
α-D-Glucosidase[i]	n.d.	+	—
β-D-Glucosidase[j]	n.d.	—	—
α-D-Galactosidase[k]	n.d.	n.d.	—
β-D-Galactosidase[l]	n.d.	n.d.	+
β-D-Glucuronidase[m]	+	n.d	n.d.

[a]50 min at 100° in 0.5M H_2SO_4 (optimal conditions, *cf.* Refs. 6 and 9). [b]H2, aldobiouronic acid; H3, aldotriouronic acid; H4, aldotetrauronic acid. [c]Hexoses by g.l.c. of the alditol acetates[16]; hexuronic acid by the carbazole–sulfuric acid method[17]. [d]Identified by g.l.c. as the alditol acetate, after reduction with $NaBH_4$, hydrolysis, and preparation of the acetylated aldononitriles from the other constituents[25]. [e]Ratio of acetylated alditol/aldononitriles (see footnote *d*); the GlcA derivative is not recorded in the g.l.c. analysis employed[25]. [f]At pH 5.3[6,9,13]. [g]Descending p.c. with ethyl acetate–glacial acetic acid–formic acid–water (18:3:1:4). [h]Before exposure to exo-glycosidases, the oligosaccharides were reduced with carbodiimide (CMC)$NaBH_4$ according to Taylor *et al.*[11,12] (see also Material and Methods). [i]α-Glucosidase from yeast[9,26]. [j]β-Glucosidase from sweet almonds[9,27]. [k]α-Galactosidase from green coffee-beans[9,28]. [l]β-Galactosidase from *Escherichia coli*[9,29]. [m]β-Glucuronidase from *Helix pomatia*[9,30].

Finally, Table IV shows the results of p.m.r. spectroscopy of the acidic oligosaccharides, and of type-16 glycan.

TABLE IV

P.M.R. SIGNALS OF ANOMERIC PROTONS AND FUCOSE METHYL PROTONS IN *Klebsiella* SEROTYPE 16 OLIGOSACCHARIDES AND CAPSULAR POLYSACCHARIDE[a]; SUMMARY OF STRUCTURES

Oligo- or poly-saccharide	*δ*	*J (Hz)*	*Approximate ratio of integrals*	*Proton assignment*
Aldobiouronic acid H2[b]	5.25	2.5	0.4	α-L-Fuc
β-D-GlcA*p*-(1→4)-L-Fuc	4.60	7.0	1.7	β-L-Fuc
	4.54	6.5		β-D-GlcA*p*
	1.30	7.0	3.0	CH_3 of L-Fuc
Aldotriouronic acid H3[b]	5.47	3.5	1.0	α-D-Glc*p*
α-D-Glc*p*-(1→4)-β-D-GlcA*p*-(1→4)-	5.27	2.3	0.4	α-L-Fuc
L-Fuc	4.56	7.0	0.6	β-L-Fuc
	4.48	6.5	1.0	β-D-GlcA*p*
	1.34	6.8	3.0	CH_3 of L-Fuc
Aldotetrauronic acid H4[b]	5.46	3.5	1.0	α-D-Glc*p*
β-D-Gal*p*	5.24	3.5	0.4	α-L-Fuc
$^{1}\downarrow_{4}$				β-L-Fuc
α-D-Glc*p*-(1→4)-β-D-GlcA*p*-(1→4)-	4.60	7.0	2.6	β-D-Gal*p*
L-Fuc	4.51	8.0		β-D-GlcA*p*
	1.30	7.0	3.0	CH_3 of L-Fuc
Type-16 polysaccharide	5.44	3.5	1.0	α-D-Glc*p*
β-D-Gal*p*	5.28	3.0	1.0	α-L-Fuc*p*
$^{1}\downarrow_{4}$	4.61	7.5	1.0	β-D-Gal*p*
→3)-α-D-Glc*p*-(1→4)-β-D-GlcA*p*-	4.47	8.0	1.0	β-D-GlcA*p*
(1→4)-α-L-Fuc-(1→	1.28	6.8	3.0	CH_3 of L-Fuc

[a] 1–4% (w/v) solutions in absolute deuterium oxide were run at 70° and 90 MHz[6]. [b] Compare Table III.

DISCUSSION

In agreement with the results of Nimmich[2], the *Klebsiella* serotype 16 capsular polysaccharide was found to contain D-glucose, D-galactose, L-fucose, and D-glucuronic acid (Table I).

The quantitative constituent analyses (Table I), the results of methylation–g.l.c.–m.s. (Table II) and of p.m.r. spectroscopy of the polymer (Table IV, bottom), as well as the isolation of tetrasaccharide H4 by partial hydrolysis with acid (Table III), show that the type-16 glycan consists of tetrasaccharide repeating-units containing one residue each of unsubstituted galactose, 3,4-disubstituted glucose, 4-substituted glucuronic acid, and 4-substituted fucose. From these data, it follows that the repeating unit must be branched, with a chain glucosyl residue carrying the branch that terminates in a galactosyl unit.

As summarized in Table IV, the sequence of these constituents in the repeating unit can be deduced from the analyses of the type-16 aldobiouronic, aldotriouronic.

and aldotetrauronic acids recorded in Table III, and from the results of methylation–g.l.c.–m.s. of the aldotetrauronic acid (Table II, columns III and IV), which show that the glucosyl residue carries the branch galactosyl unit at position 4, and hence the chain fucosyl residue at position 3.

The anomeric configurations of the glycosidic linkages follow from the results of exo-glycosidase treatment of the oligosaccharides (Table III), and from the p.m.r. data[31] (Table IV).

The complete primary structure of the *Klebsiella* serotype 16 capsular polysaccharide is shown at the bottom of Table IV.

ACKNOWLEDGMENTS

We thank Mrs. Hannelore Thoma (isolation of polysaccharide), Miss Helga Kochanowski (analytical ultracentrifugation), and Mr. D. Borowiak (g.l.c.–m.s.) for excellent technical assistance. This project was supported by the Max Planck-Gesellschaft and by Fonds der Chemischen Industrie.

REFERENCES

1 W. NIMMICH AND W. MÜNTER, *Z. Allg. Mikrobiol.*, 15 (1975) 127–129.
2 W. NIMMICH, *Z. Med. Mikrobiol. Immunol.*, 154 (1968) 117–131.
3 P. R. EDWARDS AND W. H. EWING, *Identification of* Enterobacteriaceae, Burgess, Minneapolis, 1966.
4 Y.-M. CHOY AND G. G. S. DUTTON, *Can. J. Chem.*, 52 (1974) 684–687.
5 C. ERBING, L. KENNE, B. LINDBERG, J. LÖNNGREN, AND I. W. SUTHERLAND, *Carbohydr. Res.*, 50 (1976) 115–120.
6 H. NIEMANN, N. FRANK, AND S. STIRM, *Carbohydr. Res.*, 59 (1977) 165–177.
7 G. G. S. DUTTON, personal communication.
8 B. LINDBERG, personal communication.
9 H. THUROW, Y.-M. CHOY, N. FRANK, H. NIEMANN, AND S. STIRM, *Carbohydr. Res.*, 41 (1975) 241–255.
10 F. KAUFFMANN, *The Bacteriology of* Enterobacteriaceae, Munksgaard, Copenhagen, 1966.
11 R. L. TAYLOR AND H. E. CONRAD, *Biochemistry*, 11 (1972) 1383–1388.
12 R. L. TAYLOR, J. E. SHIVELY, AND H. E. CONRAD, *Methods Carbohydr. Chem.*, 7 (1976) 149–151.
13 B. KICKHÖFEN AND O. WESTPHAL, *Z. Naturforsch.*, 7 (1952) 655–659.
14 D. M. OTTENSTEIN AND D. A. BARTLEY, *J. Chromatogr. Sci.*, 9 (1971) 673–681.
15 I. FROMME AND H. BEILHARZ, *Anal. Biochem.*, in press.
16 J. S. SAWARDEKER, J. H. SLONEKER, AND A. JEANES, *Anal. Chem.*, 12 (1965) 1602–1604.
17 Z. DISCHE, *Methods Carbohydr. Chem.*, 1 (1962) 497–501.
18 H. H. SCHLUBACH AND K. REPFNNING, *Angew. Chem.*, 71 (1959) 193.
19 K. WALLENFELS AND G. KURZ, *Biochem. Z.*, 335 (1962) 559–572.
20 K. JANN, B. JANN, F. ØRSKOV, I. ØRSKOV, AND O. WESTPHAL, *Biochem. Z.*, 342 (1965) 1–22.
21 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–207.
22 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem.*, 82 (1970) 643–674.
23 B. LINDBERG, *Methods Enzymol.*, 28 (1972) 178–195.
24 J. KOLLONITSCH, O. FUCHS, AND V. GÁBOR, *Nature (London)*, 175 (1955) 346.
25 I. M. MORRISON, *J. Chromatogr.*, 108 (1975) 361–364.
26 H. HALVORSON, *Methods Enzymol.*, 8 (1966) 559–562.
27 S. HESTRIN, D. S. FEINGOLD, AND M. SCHRAMM, *Methods Enzymol.*, 1 (1955) 231–257.
28 P. M. DEY AND J. B. PRIDHAM, *Adv. Enzymol.*, 36 (1972) 91–131.
29 K. WALLENFELS AND O. P. MALHOTRA, *Adv. Carbohydr. Chem.*, 16 (1961) 239–298.
30 W. H. FISHMAN, in H. U. BERGMEYER (Ed.), *Methoden der enzymatischen Analyse*, Verlag Chemie, Weinheim, 1970, pp. 885–900.
31 G. M. BEBAULT, Y.-M. CHOY, G. G. S. DUTTON, N. FUNNELL, A. M. STEPHEN, AND M. T. YANG, *J. Bacteriol.*, 113 (1973) 1345–1347.