Carbohydrate Research, 73 (1979) 169–174

THE STRUCTURE OF THE CAPSULAR POLYSACCHARIDE OF *Klebsiella* K-TYPE 31

CHO-CHAK CHENG, SUI-LAM WONG, AND YUEN-MIN CHOY
Department of Biochemistry, The Chinese University of Hong Kong (Hong Kong)
(Received June 21st, 1978; accepted for publication in revised form, September 8th, 1978)

ABSTRACT

The structure of the *Klebsiella* K31 capsular polysaccharide has been elucidated by periodate oxidation, methylation analysis, characterization of oligosaccharides obtained by partial, acid hydrolysis, and proton magnetic resonance. The polymer consists of pentasaccharide repeating-units having the following structure (**1**).

→3)-β-D-Glc*p*-(1→3)-α-D-Glc*pA*-(1→3)-β-D-Gal*p*-(1→
4
↑
1
α-D-Man*p*
2
↑
1
β-D-Glc*p*
4 6
v
C
^
Me CO_2H

1

INTRODUCTION

The serological cross-reactions among different *Klebsiella* K antigens and between them and surface antigens of other bacteria have been studied[1–5]. Studies of bacteriophage and capsular antigen of *E. coli* serotype 29 showed that not only did *Escherichia coli* OK29 serum react with *Klebsiella* K31 capsular polysaccharide, but its bacteriophage also depolymerized this capsular glycan[6,7]. For this reason, we have analyzed the capsular polysaccharide of *Klebsiella* K-type 31. The polymer was found to have a structure similar to that of the capsular polysaccharide of *E. coli* K29 (see structure **1** and ref. 6). The side chains are identical to each other, whereas

the main chain of the former differs by having one D-mannose residue fewer and it varies in two anomeric linkages.

MATERIALS AND METHODS

General techniques. — These were as described for the polysaccharide[6] of *E. coli* serotype 29. Worfel–Ferguson Agar (supplied by Difco Laboratories, U.S.A.) was used for optimal capsular production. Optical rotations were measured at 25° on a Perkin–Elmer model 141 polarimeter. ^{1}H-n.m.r. spectra were recorded with a C-60 HL instrument, with tetramethylsilane as the external standard. Mass spectra, obtained on individual fractions collected from a gas chromatograph, were recorded with an RMS-4 instrument operating at 70 eV.

Isolation of capsular polysaccharide. — A culture of *Klebsiella* K-type 31 (6258) was grown on Worfel–Ferguson agar. After 3 days, the cells were collected, extracted with 1% phenol–water, and purified by Cetavlon precipitation[8].

Constituent analysis. — After hydrolysis (18 h at 110° in 2M hydrochloric acid), the aldoses were determined by g.l.c. of the alditol acetates[9]. Glucuronic acid was assayed qualitatively by high-voltage paper electrophoresis[10], and quantitatively by the carbazole–sulfuric acid method[11]. Pyruvic acid was determined[12] by examining the ^{1}H-n.m.r. spectrum of a 2% solution of the polysaccharide in D_2O at 95°. For preparing the K31 derivative, selective removal of the pyruvic acetal was attained by treating the original polysaccharide with 0.1M trifluoroacetic acid[13].

Smith periodate degradation. — For Smith degradation, the procedure of Goldstein *et al.* was followed[14]. Periodate consumption of the K31 polysaccharide was determined by the method of Avigad[15]. In a typical experiment, 100 mg of polysaccharide was used. After the Smith hydrolysis[14], the products were dialyzed against distilled water. The degraded polysaccharide (45 mg) remaining in the dialysis sac was again subjected to constituent analysis, ^{1}H-n.m.r. studies, Smith degradation, and methylation analysis.

Methylation analyses. — The K31 polysaccharide, its depyruvylated derivative, and degraded polysaccharide were methylated with methylsulfinyl carbanion in methyl sulfoxide[16]. Part of the methylated products was hydrolyzed and the methylated monomers identified by g.l.c.–m.s. of the alditol acetates (or occasionally, of the aldose acetates)[17,18]; for the rest of the methylated polysaccharides, the carboxyl groups were reduced with lithium borohydride and the reduced polysaccharides then hydrolyzed, and the methylated monomers analyzed as already described.

Isolation of oligosaccharides after partial acid hydrolysis. — The K31 polysaccharide) (102 mg) was hydrolyzed with 0.5M hydrochloric acid for 4 h. The partially hydrolyzed polysaccharide was fractionated by gel filtration on Sephadex G-15 and Amberlite IR-410 (HCO_3^-) columns to yield a neutral disaccharide (5 mg), an aldobiouronic acid (4 mg), and a mixture of oligosaccharides (23 mg). The disaccharides were then analyzed by enzymic and acid hydrolyses.

RESULTS

The acidic, capsular polysaccharide from *Klebsiella* K31, after purification by Cetavlon fractionation, had $[\alpha]_D^{25}$ +68.5° (*c* 0.7, water). The ^{1}H-n.m.r. spectrum of a 2% solution of the polysaccharide in D_2O showed a sharp singlet at δ 1.5, characteristic of pyruvate[12]. The anomeric region of the spectrum suggested that the repeating unit consists of five sugar residues, of which two are linked by α- and three by β-glycosidic bonds (Table I). Integration of the anomeric and pyruvate signals indicated one pyruvate group to five sugar residues.

Acid hydrolysis of the polysaccharide showed the rapid liberation of D-mannose D-glucose, and a neutral disaccharide. The molar composition of the polysaccharide, after complete acid hydrolysis, was 2:1:1:0.95 D-glucose:D-mannose:D-galactose:D-glucuronic acid. Partial acid hydrolysis yielded the same neutral disaccharide, an aldobiouronic acid, and a mixture of oligosaccharides. The neutral disaccharide was susceptible to cleavage by β-D-glucosidase (and not by α-D-glucosidase) yielding D-glucose and D-mannose in the ratio of 1.2:1. β-D-Glucosiduronase had no effect on the aldobiouronic acid, in which D-galactose was found to constitute the reducing end.

The periodate consumption of K31 capsular polysaccharide was found to be 1.9 mol per repeating unit. When the Smith-degradation products were separated into two portions by dialysis, the dialyzate was found to consist of glycerol and erythritol in the ratio of 1:1; the degraded polysaccharide remaining in the dialysis sac had $[\alpha]_D^{25}$ +50° (*c* 0.1, water). The ^{1}H-n.m.r. spectrum showed the presence of one α linkage and two β linkages (Table I). Constituent analysis showed the ratios 1:0.9:1 for D-glucose:D-galactose:D-glucuronic acid. The degraded polysaccharide, when subjected to further Smith degradation, did not consume periodate.

TABLE I

PROTON MAGNETIC RESONANCE OF *Klebsiella* K31 AND SMITH-DEGRADED POLYSACCHARIDES

Compound	*δ-Value*[a]	*Approximate ratio of integrals*	*Tentative proton assignment*
Klebsiella K31 polysaccharide	5.36	1	Equatorial, anomeric (α linkages)
	5.16	1	
	5.03[b]	1	Axial, anomeric (β linkages)
	4.78[b]	1	
	4.60[b]	1	
	1.50[b]	3	CH_3 of pyruvate
Smith-degraded polysaccharide	5.15	1	Equatorial, anomeric (α linkage)
	4.75[b]	1	Axial, anomeric (β linkages)
	4.61[b]	1	

[a]Spectra recorded at 60 MHz in D_2O with external tetramethylsilane ($\delta = 0$). [b]Signals ~6–9 Hz wide, but no distinct splitting, presumably because of the viscosity of the solution.

Methylation–g.l.c.–m.s.[17] of the K31 polysaccharide, the depyruvylated derivative, and the degraded polysaccharide gave the results shown in Table II.

DISCUSSION

The bacteriophage 29 of *Escherichia coli* was found by Stirm[7] to be highly specific. Using this bacteriophage to test with 82 other heterologous bacterial (mainly *Enteriobacteriaceae*), capsular glycans, he found only one enzymic cross-reaction, namely with *Klebsiella* K31 polysaccharide. The *Klebsiella* K31 polysaccharide is not only depolymerized by the *E. coli* 29 phage enzyme, but it also reacts with a serum of *E. coli* OK29—indicating that the *Klebsiella* K31 and *E. coli* K29 polysaccharides may be similar (S. Stirm, personal communication). Our present findings on the capsular polysaccharide of *Klebsiella* K31 seems to support this contention.

Constitutents of the repeating unit. — The molar composition of *Klebsiella* K31 polysaccharide already indicated that it is composed of pentasaccharide repeating-units (structure **1**), and this conclusion was confirmed by the results of methylation analysis (Table I). Rapid liberation of a neutral disaccharide by acid hydrolysis suggests that the polysaccharide consists of a two-unit side-chain of β-Glc→Man, as confirmed by acid and enzymic hydrolysis. Results on the aldobiouronic acid shows that it is α-GlcA→Gal.

Substitution pattern of the constituents. — Methylation analysis (Table II) shows that the pentasaccharide repeating-unit consists of 4,6- and 3-substituted Glc, 2-substituted Man, 3,4-substituted GlcA, and 3-substituted Gal, approaching molar

TABLE II

METHYLATION–GAS–LIQUID CHROMATOGRAPHIC–MASS SPECTROMETRIC ANALYSIS OF *Klebsiella* K31 (A), CARBOXYL-REDUCED[a] (B), DEPYRUVYLATED DERIVATIVE (C), AND SMITH-DEGRADED AND CARBOXYL-REDUCED[a] (D) POLYSACCHARIDES

Alditol derivative[b]	*T*[c]		*Approximate molar ratio*			
	Lit.	*Found*	*A*	*B*[a]	*C*	*D*[a]
2,3,4,6-Glc	1.00	1.00[c]			0.8	
2,4,6-Glc[d]	1.95					0.9
		1.98[d]	2[d]	2[d]	2[d]	
3,4,6-Man[d]	1.95					
2,4,6-Gal	2.28	2.30	0.9	0.8	1	1
2,3-Glc	5.39	5.39[c]	1	1	0.1	
2,4-Glc	5.10	5.20				1
2-Glc	7.90	8.10		0.8		

[a]The polysaccharide was methylated, reduced with lithium borohydride, and hydrolyzed to give the partially methylated monosaccharides. [b]2,3,4,6-Glc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, and so on. [c]Retention time[17] relative to 2,3,4,6-Glc (T = 1.00) and 2,3-Glc (T = 5.39) on an ECNSS-M column at 170°. [d]2,4,6-Glc and 3,4,6-Man are not separable as the alditol acetates. However, the two methylated sugars may be separated as the aldose acetates[18].

ratios of 1:1:1:1:1 (Table II). The 2,3-di-*O*-methyl-D-glucose cannot arise from a branch point, as no corresponding quantity of terminal residues was found. The appearance of 2,3,4,6-tetra-*O*-methyl-D-glucose and the disappearance of 2,3-di-*O*-methyl-D-glucose on methylation of the K31 derivative provides convincing evidence that the pyruvic acid must linked to D-glucose as a 4,6-acetal. These results are also in agreement with those of periodate oxidation — consumption of nearly 2 mol of periodate per repeating unit and production of glycerol and erythritol in the ratio of 1:1 upon Smith hydrolysis. Resistance to periodate oxidation of the degraded polysaccharide indicates that all of the three sugar residues in the main chain are 3-substituted, a conclusion confirmed by methylation analysis.

Sequence of constituents. — Our results with the aldobiouronic acid and the degraded polysaccharide establish that the main chain is composed of Glc→GlcA→Gal as repeating units. As a branch-terminal, pyruvyl glucose residue was indicated by methylation analysis, and as the neutral disaccharide was identified as Glc→Man, the foregoing trisaccharide is therefore substituted by pyruvate→Glc→Man→ branches. Methylation analysis and periodate oxidation of the degraded polysaccharide indicated that the side chain is (1→4)-linked to GlcA.

Anomeric configurations. — Besides the signal of the pyruvate acetal methyl protons, the ^{1}H-n.m.r. spectrum of the K31 polysaccharide gave tentative evidence of three β and two α linkages. With use of specific exoglycosidases, a β-D linkage could be allocated to the neutral disaccharide, and an α-D linkage tentatively to the aldobiouronic acid. As the degraded polysaccharide contained two β and one α linkage (see Table I), it followed that glucose and galactose in the main chain should be β-linked. The remaining α linkage must be attributed to mannose in the side chain.

In total, the results establish structure **1** as the repeating unit of the capsular polysaccharide of *Klebsiella* K31 and demonstrate that this polysaccharide is indeed quite similar to the capsular polysaccharide of *E. coli* K29. As with the polysaccharide of *E. coli* K29, the *E. coli* capsular bacteriophage 29 depolymerizes *Klebsiella* K31 polysaccharide, probably also by splitting the β-D-glucosyl-(1→3)-D-glucuronic acid bonds.

ACKNOWLEDGMENTS

We are grateful to Professor S. Stirm for a culture of *Klebsiella* K31 (6258). We thank the Takshing Investment Co., Ltd., Hong Kong, for financial support. Advice and comments from Professors L. Ma and S. Stirm are gratefully acknowledged.

REFERENCES

1 M. HEIDELBERGER, W F. DUDMAN, AND W. NIMMICH, *J. Immunol*, 104 (1970) 1321–1328.
2 M. HEIDELBERGER AND W. NIMMICH, *J. Immunol.*, 109 (1972) 175–182.
3 M. HEIDELBERGER AND G. G. S. DUTTON, *J Immunol*, 111 (1973) 857–859
4 J. ERIKSEN, *Acta Pathol. Microbiol. Scand*, 64 (1965) 527–533.
5 S. D. HENRIKSEN, *Acta Pathol. Microbiol. Scand.*, 26 (1949) 903–915.
6 Y.-M. CHOY, F. FEHMEL, N. FRANK, AND S. STIRM, *J. Virol*, 16 (1975) 581–590.

7 F. FEHMEL, U. FEIGE, H. NIEMANN, AND S. STIRM, *J. Virol.*, 16 (1975) 591–601.
8 J. E. SCOTT, *Methods Carbohydr. Chem.*, 5 (1965) 38–44.
9 J. S. SAWARDEKER, J. H. SLONEKER, AND A. JEANES, *Anal. Chem.*, 12 (1965) 1602–1604.
10 B. KICKKÓFEN AND O. WESTPHAL, *Z. Naturforsch.*, 76 (1952) 655–659.
11 Z. DISCHE, *Methods Carbohydr. Chem*, 1 (1962) 497–501.
12 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.
13 Y.-M. CHOY AND G. G. S. DUTTON, *Can. J. Chem.*, 52 (1974) 684–687.
14 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohydr. Chem.*, 5 (1965) 361–370.
15 G. AVIGAD, *Carbohydr. Res.*, 11 (1969) 119–123.
16 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–207.
17 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem.*, 82 (1970) 643–674.
18 G. M. BEBAULT, G. G. S. DUTTON, AND R. H. WALKER, *Carbohydr. Res.*, 23 (1972) 430–432.