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PRIMARY STRUCTURE OF THE *Klebsiella* SEROTYPE-51 CAPSULAR POLYSACCHARIDE

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ABSTRACT

The *Klebsiella* serotype-51 (K51) capsular polysaccharide consists of a tetrasaccharide repeating-unit comprising a →3)-α-D-Gal*p*-(1→3)-α-D-Gal*p*-(1→ chain substituted by an α-D-Glc*p*A-(1→6)-α-D-Glc*p*-(1→ branch at position 4 of one galactosyl residue.

INTRODUCTION

Of the approximately eighty *Klebsiella* capsular polysaccharides of different K serotypes[1–3], more than fifty have been subjected to analyses of primary structure (*e.g.* refs. 3–7). We now report on the structure of the serotype-51 glycan.

MATERIAL AND METHODS

Bacteria. — *Klebsiella* 4715/50 (O3:K51), the serological test-strain for the *Klebsiella* K51 antigen, was kindly supplied by Dr. Ida Ørskov (WHO International Escherichia Center, Statens Seruminstitut, Copenhagen).

All materials and methods used have been described or cited previously[8–10].

RESULTS

Klebsiella serotype-51 capsular glycan was isolated from *Klebsiella* 4715/50 (O3:K51). The sugar composition and some properties of the material are shown in Table I. The oligosaccharides obtained from the glycan by partial hydrolysis with

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TABLE I

COMPOSITION AND SOME PROPERTIES OF *Klebsiella* SEROTYPE-51 CAPSULAR POLYSACCHARIDE

Polysaccharide	*Weight percent of sugar residues (molar ratios)*[a]				s_{20}[c] *(10^{-13} sec)*	*Equivalent weight*[d]	$[\alpha]_{589}^{25}$[e] *(degrees)*
	D-*Glc*	*D-Gal*	D-*GlcA*	*Total*[b]			
Native[f]	17.2[g] (1.00)	38.1[h] (2.22)	19.8 (1.06)	94.5[b]	1) 3.6[c] 2) 6.1	645	+95
Alkali-treated[i]	18.5 (1.00)	39.2 (2.12)	20.2 (1.01)	n.d.	3.1[c]	n.d.	+107
Carboxyl-reduced[j]	31.3[k] (2.00)	38.6 (2.47)	6.5 (0.38)	n.d.	n.d.	n.d.	n.d.

[a]Hexoses determined by g.l.c. of the alditol acetates[11], and hexuronic acid by the carbazole–sulfuric acid method[12]. [b]Including 16.3% of water (loss of weight after 24 h at 50° *in vacuo* over P_2O_5), 0.3% of protein (determined after hydrolysis with an amino acid analyser), 0.2% of nucleic acid (estimated from the absorption at 260 nm, using yeast RNA as a standard), and 2.6% of sodium (calculated on the basis of the glucuronic acid value). Paper electrophoresis or g.l.c., after hydrolysis[13], and ^{1}H-n.m.r. spectroscopy indicated the absence of pyruvate acetal and *O*-acetyl substituents. [c]0.4% Solutions (w/v) in phosphate-buffered, physiological saline were centrifuged; like other capsular polysaccharides from Enterobacteriaceae[8,14], the material did not sediment uniformly before mild treatment with alkali (see footnote *i*). [d]Obtained by titration of the acidic form of the glycan[9]. [e]In water (*c* 0.3). [f]As extracted by the phenol–water–cetyltrimethylammonium bromide procedure[8,14]; 100 14-cm nutrient-agar plates yielded 21.5 g of dry bacteria and, from these, 2.2 g (10.2%) of K51 polysaccharide. [g]After optimum hydrolysis (32 h, 100°, 0.5M H_2SO_4); about the same value was obtained with fungal D-glucose oxidase[8]. [h]After an optimum hydrolysis time of 6 h; about the same value was obtained with D-galactose dehydrogenase from *Pseudomonas fluorescens*[8]. [i]For 4 h at 56° in 0.25M NaOH[8,14]. [j]Carboxyl-reduced by the method of Taylor *et al.*[15]. [k]About the same value was obtained with fungal D-glucose oxidase[8].

acid are listed in Table II, and the results of methylation–g.l.c.–m.s. are given in Table III. Table IV summarises the ^{1}H-n.m.r. data and the structural deductions.

DISCUSSION

As evidenced by the sugar composition (Table I) and the ^{1}H-n.m.r. data (Table IV), the *Klebsiella* serotype-51 capsular glycan consists of tetrasaccharide repeating-units, comprising one D-glucuronic acid, one D-glucose, and two D-galactose residues; it is not substituted by pyruvate acetal or by *O*-acetyl groups (Table I).

The data presented in Tables II and IV demonstrate that the component sugars occur in the sequence GlcA→Glc→Gal→Gal or GlcA→Glc→(Gal→)Gal, and that all anomeric configurations are α.

The results of methylation–g.l.c.–m.s. (Table III) show that GlcA is branch-terminal in the repeating unit of the polysaccharide (column 5), that it substitutes Glc at position 6 (column 1), and that one of the Gal residues is 3- and the other 3,4-di-substituted (branching point in the chain) (columns 4 and 5). From the

TABLE II

ACIDIC OLIGOSACCHARIDES OBTAINED FROM *Klebsiella* SEROTYPE-51 CAPSULAR POLYSACCHARIDE BY PARTIAL HYDROLYSIS WITH ACID

Determination	*A2*[a]	*A3*	*A4*[b]
Yield (%)	27	11	n.d.
Molar ratio of sugar components[c]			
D-Glucose	0.9	0.9	0.8
D-Galactose	—	1.0	1.8
D-Glucuronic acid	1.0	1.0	1.0
Reducing-end sugar[d]	Glc	Gal	Gal
Mobility in paper electrophoresis[e] (M_{GlcA})	0.67	0.54	0.45
R_{Glc} in paper chromatography[f]	0.52	0.21	0.08
Enzymic hydrolysis by exo-glycosidases after reduction[15] with carbodi-imide/$NaBH_4$			
α-D-Glucosidase[g]	+	+	+
β-D-Glucosidase[h]	—	—	—
α-D-Galactosidase[i] after α-D-glucosidase[j]	n.d.	n.d.	+
β-D-Galactosidase[k] after α-D-glucosidase[j]	n.d.	n.d.	—

[a]A2, aldobiouronic acid; A3, aldotriouronic acid; *etc.* Obtained from the polysaccharide by preparative, paper electrophoresis (see footnote *e*) after 60 min at 100° in 0.5M H_2SO_4 (or after 45 min at 98° in 2M trifluoroacetic acid). [b]A4 consists of a mixture of the two possible tetrasaccharides in the ratio ~1:3 (see Table III, column 3; Table IV; and Discussion). [c]Hexoses determined by g.l.c. of the alditol acetates[11], and hexuronic acid by the carbazole–sulfuric acid method[12]. [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 sugars[16]. [e]In pyridine–acetic acid–water (10:4:86; pH 5.3). [f]Descending p.c. with ethyl acetate–acetic acid–formic acid–water (18:3:1:4). [g]α-D-Glucosidase from yeast, using maltose and cellobiose as controls, and estimating the D-glucose liberated with fungal D-glucose oxidase[8]. [h]β-D-Glucosidase from sweet almonds, with the same controls and detection. [i]α-D-Galactosidase from green coffee-beans, using melibiose and lactose as controls, and determining the D-galactose liberated with D-galactose dehydrogenase from *Ps. fluorescens*[8]. [j]The tetrasaccharides were incubated for 24 h with a large excess of α-D-glucosidase, and the resulting disaccharide fraction was isolated by chromatography on Biogel P2 and then treated with the galactosidase. [k]β-D-Galactosidase from *Escherichia coli*[8] with the same controls and detection as in footnote *i*.

methylation analysis of the aldotriouronic acid (column 2) [*i.e.*, from its structure Glc*p*A-(1→6)-Glc*p*-(1→4)-Gal], it can be deduced that the branch in the repeating unit consists of α-D-Glc*p*A-(1→6)-α-D-Glc*p*-(1→, and that the chain comprises two →3)-α-D-Gal*p*-(1→ residues, one of which is substituted by the branch at position 4 (see Table IV for a summary). These structural conclusions are confirmed by the methylation results obtained with the mixture of tetraouronic acids, also isolated on partial hydrolysis of the glycan with acid (Table II; Table III, column 3; Table IV, footnote *b*).

TABLE III

IDENTIFICATION[a] AND RATIOS OF *O*-ACETYL-*O*-METHYLALDITOLS OBTAINED FROM *Klebsiella* SEROTYPE-51 CAPSULAR POLYSACCHARIDE AND ITS DERIVATIVES

Per-O-*acetyl derivative of*[b]	1[c]	2	3	4	5
	Ratio of peak integrals[d]				
2,3,4,6-GlcOH[e]	—	—	—	—	0.8[e]
2,3,4,6-GalOH	—	—	1.0	—	—
2,4,6-GalOH	—	—	0.4	1.0	1.0
2,3,6-GalOH	—	1.2	0.4	—	—
2,3,4-GlcOH	1.0	1.0	1.1	1.0	1.1
2,6-GalOH	—	—	1.3	1.1	1.1

[a]By combined gas chromatography–mass spectrometry employing columns packed with ECNSS-M[17,18], and capillary columns wall-coated with Silar 9CP or OV-101[10]. [b]2,3,4,6-GlcOH = 2,3,4,6-tetra-*O*-methyl-D-glucitol, *etc.* [c]*1*, aldobiouronic acid A2 (see Table II), permethylated; *2*, aldotriouronic acid A3, permethylated; *3*, mixture of the two possible tetraouronic acids (ratio ~1:3; see Table IV, footnote *b*, and Discussion), permethylated; *4*, K51 polysaccharide, permethylated; *5*, K51 polysaccharide, permethylated, carboxyl-reduced/dideuterated with calcium borodeuteride[19], and remethylated. [d]Detection by flame ionisation. [e]Dideuterated at position 6.

TABLE IV

^{1}H-N.M.R. SIGNALS OF ANOMERIC PROTONS IN *Klebsiella* SEROTYPE-51 OLIGOSACCHARIDES AND CAPSULAR POLYSACCHARIDE[a], AND SUMMARY OF STRUCTURES

Oligo- or poly-saccharide	*δ*	J *(Hz)*	*Approximate ratio of integrals*	*Proton assignment*
Aldobiouronic acid A2	5.20	~3	0.5	6)-α-Glc
α-D-Glc*p*A-(1→6)-D-Glc	4.90	~3	1.0	α-Glc*p*A
	4.63	7.5	0.5	6)-β-Glc
Aldotriouronic acid A3	5.29	3.0	0.5	4)-α-Gal
α-D-Glc*p*A-(1→6)-α-D-Glc*p*-(1→4)-D-Gal	4.96	~2.5	2.0	6)-α-Glc*p*
	4.96	~2.5		α-Glc*p*A
	4.63	7.0	0.5	4)-β-Gal
Polysaccharide	5.35	~3	1.0	3)-α-Gal*p*
α-D-Glc*p*A	5.20	~3	1.0	3,4)-α-Gal*p*
1	5.08	~3	1.0	6)-α-Glc*p*
↓	4.98	~3	1.0	α-Glc*p*A
6				
α-D-Glc*p*				
1				
↓				
4				
→3)-α-D-Gal*p*-(1→3)-α-D-Gal*p*-(1→				
↑[b] (↑)[b]				

[a]Solutions (0.5–3.0%, w/v) in deuterium oxide were run at 70° and 90 MHz[9]. [b]Formation of tetraouronic acids during partial, acid hydrolysis; preferential and minority cleavage-site (see Table III, column 3, and Discussion).

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