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PRIMARY STRUCTURE OF *Klebsiella* SEROTYPE 6 CAPSULAR POLYSACCHARIDE

URSULA ELSÄSSER-BEILE*†, HORST FRIEBOLIN, AND STEPHAN STIRM

Max-Planck-Institut für Immunbiologie†, D-7800 Freiburg-Zähringen, and Institut für Organische Chemie der Universität, D-6900 Heidelberg (German Federal Republic)

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ABSTRACT

The primary structure of the *Klebsiella* serotype 6 capsular polysaccharide has been shown to consist of →3)-α-L-Fuc*p*-(1→3)-β-D-Glc*p*-(1→3)-β-D-Man*p*-(1→4)-α-D-GlcA*p*-(1→ repeating-units, substituted by pyruvate acetal on positions 4 and 6 of the mannosyl residue.

INTRODUCTION

In pursuit of an understanding of the substrate specificity of bacteriophage-borne glycanases[1–5], we are comparing the structures of different *Klebsiella* capsular heteropolysaccharides acted upon by single viral enzymes, with those that are not[6,7].

Of seventy-two *Klebsiella* capsular glycans of different K. serotypes[8], three, *viz.*, K6, K1, and K57, are depolymerized[9] by the hydrolase activity associated with particles of *Klebsiella* bacteriophage No. 6. Since only the primary structures of the type-1[10] and type-57[11] polysaccharides are known, we have now analyzed the type-6 glycan also.

MATERIAL AND METHODS

Bacteria. — *Klebsiella ozaenae* F5052 (O2:K6), the serological test strain for the *Klebsiella* K6 antigen[8], was used; it was kindly supplied by Dr. Ida Ørskov, WHO International Escherichia Center, Statens Seruminstitut, Copenhagen (Denmark).

Methods. — The o.r.d. spectra (185 to 500 nm) of isolated K6 constituent sugars (in water) were recorded with a Cary 60 spectropolarimeter.

For the selective hydrolysis of pyruvate acetal groups[12], type-6 polysaccharide (1%) in 0.01M aqueous trifluoroacetic acid was heated at 100° for 90 min, dialyzed against distilled water, and lyophilized; the yield was almost quantitative.

All other techniques have been described or cited previously[13,14].

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†To whom all correspondence should be addressed.

TABLE I

DENTIFICATION AND RATIOS OF *O*-ACETYL-*O*-METHYLALDITOLS OBTAINED FROM *Klebsiella* SEROTYPE 6 CAPSULAR POLYSACCHARIDE AND ITS DERIVATIVES

Per-O-acetyl derivative of[a]	T[b]		*Primary fragments found* (m/e)									1[c]	2	3
	Lit.	*Found*	*117*	*131*	*161*	*233*	*247*	*261*	*277*	*305*	*333*	*Ratio of peak integrals*		
2,4-FucOH	1.12	1.15	+	+		+	+					1	1	1
2,4,6-GlcOH	1.95	1.93	+		+	+			+			1.1	1.1	1.1
2,4,6-ManOH	2.10	2.13	+		+	+			+			—	—	1.0
2,3-GlcOH	5.39	5.39	+		+			(263)[d]		(307)[d]		—	1.0	—
2-ManOH	7.9	7.8[e]	+								+	1.0	1.2	—

[a]2,4-FucOH = 2,4-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 ECNSS-M[20,21]. [c]*1*, Type-6 polysaccharide, permethylated; *2*, type-6 polysaccharide, permethylated, and then reduced/dideuterated with calcium borodeuteride[14,22]; *3*, type-6 polysaccharide, selectively depyruvylated with aqueous trifluoroacetic acid, and then permethylated. [d]Dideuterated fragment found. [e]Co-chromatographing with authentic, peracetylated 2-ManOH on OV-225[21].

RESULTS

Klebsiella serotype 6 capsular polysaccharide was isolated from *Klebsiella ozaenae* F5052 by the phenol–water–cetyltrimethylammonium bromide procedure[13,15]; 30 g of dry bacteria, and thence 3.5 g of glycan (sodium salt), were obtained from 100 large nutrient-agar plates. The material had $[\alpha]_{578}^{20}$ +46° (*c* 1.0, water), and a sedimentation coefficient of $s^{\circ}_{20,PBS} = 11.4 \times 10^{-13}$ sec. It was found[16,17] to consist of glucose, mannose, fucose, glucuronic acid, and pyruvate in the molar ratios ~1:1:1:1:1, and not to contain appreciable amounts of *O*-acetyl (*i.e.*, ~0.3%, w/w). The glucose and the mannose could be assigned to the D series by enzymic determinations[14,18,19], and the glucuronic acid and the fucose to the D and the L series, respectively, by o.r.d. spectroscopy of isolated samples.

Type-6 polysaccharide, as well as its carboxyl-reduced (dideuterated) or selectively depyruvylated derivatives were subjected to methylation–g.l.c.–m.s.[20,21]. The results are summarized in Table I.

After partial hydrolysis of the glycan with acid, an aldobiouronic, aldotriouronic, and aldotetrauronic acid could be isolated by paper electrophoresis[13,23]; they were characterized as summarized in Table II.

The p.m.r. spectrum[14,31] of type-6 polysaccharide showed two signals for anomeric protons at δ ~5.2 and ~4.7, as well as the signals of pyruvate and fucose

TABLE II

ACIDIC OLIGOSACCHARIDES OBTAINED BY PARTIAL ACID HYDROLYSIS[a] OF *Klebsiella* SEROTYPE 6 CAPSULAR POLYSACCHARIDE

Determination	*H2*[b]	*H3*	*H4*
Approximate molar ratio of sugar components[c]:			
D-Glucose	—	—	+[d]
D-Mannose	—	1.1	+[d]
L-Fucose	0.7	0.7	+[d]
D-Glucuronic acid	1	1	+[d]
Reducing-end sugar[e]	Fuc	Fuc	Fuc
Mobility in paper electrophoresis[f] (relative to glucuronic acid)	0.71	0.49	0.32
Enzymic hydrolysis of carboxyl-reduced oligosaccharide[g] by:			
α-D-Glucosidase[h]	+	n.d.	−
β-D-Glucosidase[i]	−	n.d.	+

[a]4 h, or 50 min, respectively, in 0.5M aqueous trifluoroacetic acid at 100°, for an optimal yield of H2, or of H3 and H4. [b]H2, aldobiouronic acid, *etc.* [c]Hexoses by g.l.c. of the alditol acetates[16], hexuronic acid by the carbazole–sulfuric acid method[24]; all pyruvate was removed under the hydrolysis conditions used. [d]Present, no exact quantitative determination carried out. [e]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]; in all cases, fucose was the only reducing-end sugar found in this manner. [f]At pH 5.3[13,14,23]. [g]Before exposure to exo-glucosidases, the oligosaccharides were reduced[26–28] with carbodiimide(CMC)/$NaBH_4$. [h]α-D-Glucosidase from yeast[13,29]. [i]β-D-Glucosidase from sweet almonds[13,30].

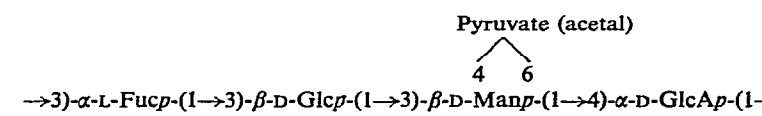
methyl protons at δ 1.5 and 1.2, with relative intensities approaching 2:2:3:3. The relative intensities of equatorial (δ ~5.2) and axial (δ ~4.6) anomeric protons in the aldobiouronic, aldotriouronic, and aldotetrauronic acids were ~1.4:0.6, ~1.3:1.7, and ~1.5:2.5, respectively.

DISCUSSION

The results of quantitative constituent analysis (*cf.* Ref. 32), of methylation–g.l.c.–m.s., of p.m.r. spectroscopy, as well as of bacteriophage degradation[9] show that the *Klebsiella* serotype 6 capsular polysaccharide (as isolated from *Klebsiella ozaenae* F5052) consists of tetrasaccharide repeating-units comprising 3-substituted L-fucose, 3-substituted D-glucose, 3,4,6-trisubstituted D-mannose, and 4-substituted D-glucuronic acid residues. The tetrasaccharide repeating-unit is substituted by a pyruvate acetal group at positions 4 and 6 of the mannose, as evidenced by methylation analysis before and after selective depyruvylation (Table I, columns 1 and 3).

The sequence of these constituents in the linear repeating-unit follows from the constituent and reducing-end sugar analyses of the type-6 oligosaccharides obtained by partial hydrolysis with acid (Table II).

From the p.m.r. spectrum of the polysaccharide, it is clear that the unit contains two α and two β linkages. The spectra of the oligosaccharides, as well as the results of exo-glucosidase action upon them (Table II), show that the glucuronic acid is α-linked, and that the glucose and the mannose are β-linked. Thus, the second α linkage must be assigned to the fucosyl residue. Therefore, the complete structure of the tetrasaccharide repeating-unit is:



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