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The structure of the capsular polysaccharide from *Klebsiella* K43

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Abstract

The structure of the capsular polysaccharide from *Klebsiella* type K43 has been investigated using sugar and methylation analysis, uronic acid degradation, and NMR spectroscopy on the native and the *O*-deacetylated polysaccharide. It is concluded that the polysaccharide is composed of pentasaccharide repeating units with the structure

$$\begin{array}{l} \rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \uparrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 1 \\ \qquad\beta\text{-D-Man}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A} \end{array}$$

The polysaccharide contains approximately 0.4 equiv of *O*-acetyl group per repeating unit, located at a primary position.

1. Introduction

The *Klebsiella* genus of the Enterobacteriaceae has been classified into ca. 80 serologically different types on the basis of their capsular polysaccharides [1,2]. Three further types have been established: K82 [3], K83 [4], and K21b [5]. The structures for most of the type-specific capsular polysaccharides have been determined. The structure for type K43, which contains glucuronic acid, galactose,

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mannose, and *O*-acetyl groups [1], was, however, not known. We now report structural studies of this polymer.

2. Results and discussion

Hydrolysis of the capsular polysaccharide from *Klebsiella* type 43 (K43) strain 2482 with trifluoroacetic acid yielded galactose and mannose in the ratio 1.0:2.3, and hydrolysis of the carboxyl-reduced K43 yielded these sugars and glucose in the proportions 1.0:2.6:0.2, thus indicating the presence of glucuronic acid. The low yield of glucose is attributed to the high viscosity of polysaccharide solutions, which rendered the carboxyl-reduction difficult. The absolute configurations were determined by GLC of the trimethylsilylated (+)-2-butyl glycosides [6,7], and were D for all the sugars.

Methylation analysis of *O*-deacetylated K43 revealed the presence of terminal D-mannose, 2-linked D-mannose, 3-linked D-galactose, and 2,3-linked D-mannose (Table 1, column A). Methylation analysis with carboxyl-reduction of the methylated polysaccharide also yielded 2,3-di-*O*-methyl-D-glucose deriving from 4-linked D-glucuronic acid (Table 1, column B). This indicates that K43 is composed of branched pentasaccharide repeating units.

The ^{1}H and ^{13}C NMR spectra (Figs. 1 and 2) were poorly resolved because of the viscosity of solutions. It was, however, possible to identify signals for five anomeric protons at δ 4.54, 4.66, 5.16, 5.23, and 5.32. Signals for 0.4 equiv of *O*-acetyl group at δ 2.18 (minor) and 2.16 (major) were also detected. Due to the partial substitution by *O*-acetyl groups, the ^{13}C NMR spectrum contained six anomeric signals, at δ 95.9, 100.6, 100.8, 101.3, 102.2, and 102.5. The presence of a carbon signal at δ 64.3 in the ^{13}C NMR spectrum of the native polysaccharide indicated that one *O*-acetyl group occupied a primary position. This was further substantiated by the absence in the anomeric region of signals from protons on

Table 1
Methylation analysis of K43 and of modified products [a]

Sugar [b]	Detector response %			
	T [c]	A	B	C
2,3,4,6-Man	1.00	27	29	7
3,4,6-Man	1.21	21	15	31
2,4,6-Man	1.33			4 [e]
2,4,6-Gal	1.27	26	20	30
4,6-Man	1.43	26	18	28
2,3-Glc	1.55		18 [d]	

[a] Key: A, Without carboxyl-reduction; B, with carboxyl-reduction of the methylated polysaccharide; C, uronic acid degradation. [b] 2,3,4,6-Man = 2,3,4,6-tetra-*O*-methyl-D-mannose, etc. [c] Relative retention time on a linear scale between 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (T = 1) and D-glucitol hexaacetate (T = 2) on an HP-5 capillary column, using the temperature program 180°C (1 min) → 250°C at 3°C/min. [d] Deuterium-labelled at C-6. [e] CD_3-group at C-2.

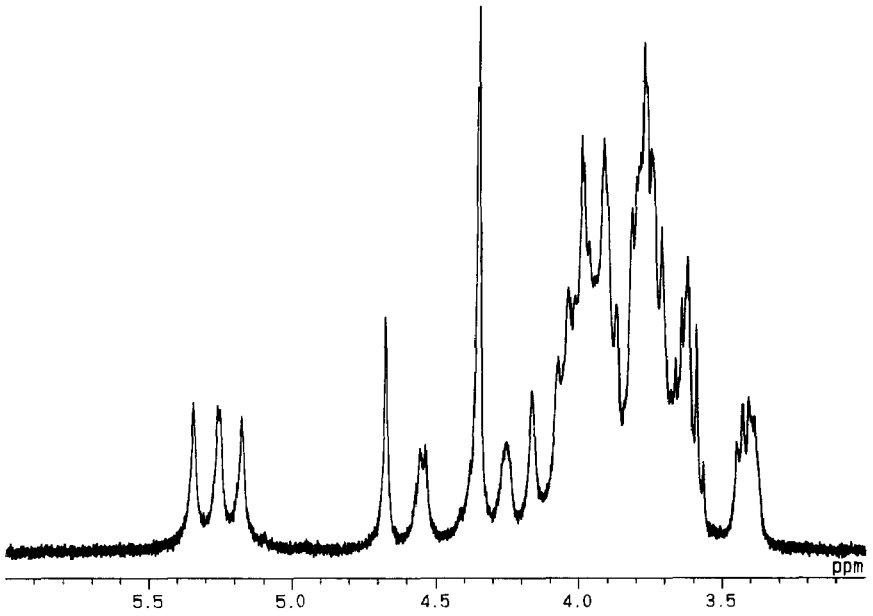

Fig. 1. ^{1}H NMR spectrum of the *O*-deacetylated K43 capsular polysaccharide.

acetoxylated secondary carbons. The NMR spectra of *O*-deacetylated K43 were better resolved and the ^{1}H NMR spectrum contained signals for five anomeric protons at approximately the same chemical shifts as for native K43. The ^{13}C NMR spectrum contained five signals for anomeric carbons, at δ 95.9, 100.6, 100.8, 102.3, and 102.5. The signals at δ 64.8 and 101.3 were absent and that at δ 100.8 had increased. Three signals were of lower intensity and broader, and were shown to be derived from sugars in the main chain, as discussed below.

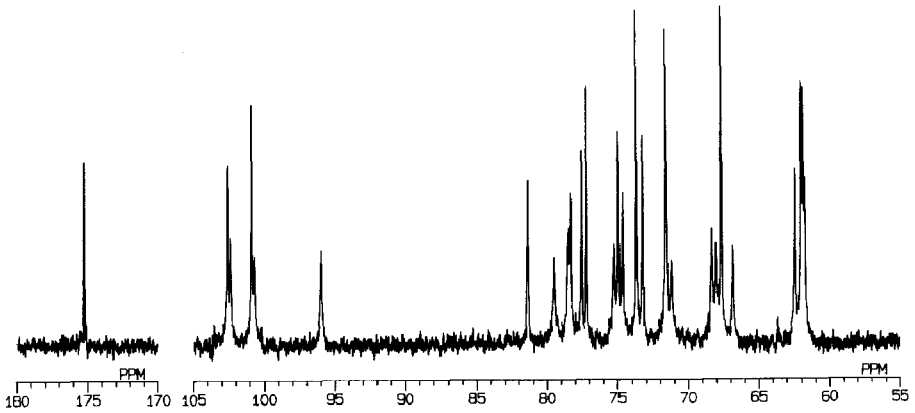

Fig. 2. ^{13}C NMR spectrum of the *O*-deacetylated K43 capsular polysaccharide.

Table 2
^{1}H NMR data for *O*-deacetylated *Klebsiella* type K43 polysaccharide

Sugar residue	Chemical shifts (δ) [a]				
	H-1	H-2	H-3	H-4	H-5
→ 2)-α-D-Man*p*-(1 →	5.34[n.r.]	4.07	4.01	3.57 [b]	3.90 [b]
A	(0.16)	(0.13)	(0.15)	(−0.11)	(0.08)
→ 3)-α-D-Gal*p*-(1 →	5.25[3]	3.88	3.98	4.16	4.24 [b]
B	(0.03)	(0.10)	(0.17)	(0.21)	(0.19)
→ 3)-α-D-Man*p*-(1 →	5.17[n.r.]	4.34	4.04	3.78	3.92 [b]
C 2 ↑	(−0.01)	(0.40)	(0.18)	(0.10)	(0.10)
β-D-Man*p*-(1 →	4.67[n.r.]	3.98	3.63	3.58	3.39
D	(−0.22)	(0.03)	(−0.03)	(−0.02)	(0.02)
→ 4)-β-D-Glc*p*A-(1 →	4.55[8]	3.43	3.64	3.81	3.76
E	(−0.10)	(0.13)	(0.12)	(0.27)	(0.05)

[a] Chemical shift differences compared to free glycopyranose monomers are given in parentheses and $J_{H\text{-}1,H\text{-}2}$ values (Hz) are given in square brackets; n.r., not resolved. [b] Tentative assignments.

In order to determine the anomeric configuration and the sequence of the sugar residues (**A**–**E**), the ^{1}H and ^{13}C NMR spectra were assigned by H,H- and C,H-correlated 2D NMR spectroscopy. The results are given in Tables 2 and 3, respectively. From a ^{1}H-detected HMQC experiment (heteronuclear multiple quantum coherence), it was possible to establish the $J_{H\text{-}1,C\text{-}1}$ values (Table 2). The small $J_{H\text{-}1,C\text{-}1}$ values, ~ 160 Hz, and the ^{1}H NMR chemical shifts of signals from anomeric protons of residues **D** and **E** showed that they had the β configuration, while the larger $J_{H\text{-}1,C\text{-}1}$ values, ~ 170 Hz, of the remaining residues showed that

Table 3
^{13}C NMR data for *O*-deacetylated *Klebsiella* type K43 polysaccharide

Sugar residue	Chemical shifts (δ) [a]					
	C-1	C-2	C-3	C-4	C-5	C-6
→ 2)-α-D-Man*p*-(1 →	95.9[171]	79.4	71.2	67.6	73.6	
A	(1.0)	(7.7)	(−0.1)	(−0.3)	(0.3)	
→ 3)-α-D-Gal*p*-(1 →	102.3[173]	68.3	75.2 [b]	66.8	71.5	
B	(9.1)	(−1.1)	(5.0)	(−3.5)	(0.2)	
→ 3)-α-D-Man*p*-(1 →	100.6[171]	78.4	78.2	67.2	74.6	
C 2 ↑	(5.6)	(6.7)	(7.0)	(−0.7)	(1.3)	
β-D-Man*p*-(1 →	100.8[161]	71.5 [b]	73.6	68.0 [b]	77.1	
D	(6.2)	(−0.6)	(−0.4)	(0.3)	(0.1)	
→ 4)-β-D-Glc*p*A-(1 →	102.5[163]	73.2	74.9	81.3	77.5	175.2
E	(5.7)	(−1.8)	(−1.7)	(8.6)	(0.5)	(−1.3)

[a] Chemical shift differences compared to free glycopyranose monomers are given in parentheses and $J_{C\text{-}1,H\text{-}1}$ values (Hz) are given in square brackets. [b] Tentative assignments.

they had the α configuration. The large $J_{H\text{-}1,H\text{-}2}$ value, ~ 8 Hz, and the chemical shift of the H-2 signal, δ 3.43, of residue **E** demonstrated that it was the 4-linked β-D-glucuronic acid residue. The chemical shift of the signal from the anomeric proton of residue **B**, δ 5.25, and the J value, ~ 3 Hz, demonstrated that it was the 3-linked α-D-galactose residue. Residues **A**, **C**, and **D** showed small $J_{H\text{-}1,H\text{-}2}$ values for H-1 signals as expected for D-mannose residues. The chemical shifts of the H-1–H-5 signals from residue **D** were similar to those of β-D-mannopyranose, and it was concluded that **D** was the terminal β-D-mannosyl group. Residues **A** and **C** could be assigned to the 2-linked and the 2,3-linked D-mannose residue, respectively, as the chemical shift for the H-2 signal from residue **C** was substantially higher (δ 4.34) than that for the corresponding signal from residue **A** (δ 4.07).

Methylated K43 was subjected to a uronic acid degradation [8,9], i.e., treatment with base followed by addition of trideuteriomethyl iodide and standard methylation analysis workup (Table 1, column C). During this treatment, the glycosidic linkage of the D-glucuronic acid should be cleaved and the hydroxyl group in the released sugar residue trideuteriomethylated. In addition, most of the sugar linked to its 4-position should be degraded. The new methyl ether, 2,4,6-tri-*O*-methyl-D-mannose with a trideuteriomethyl group at O-2, and the substantial loss of 2,3,4,6-tetra-*O*-methyl-D-mannose demonstrated structural element **1**.

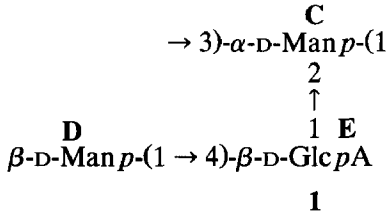

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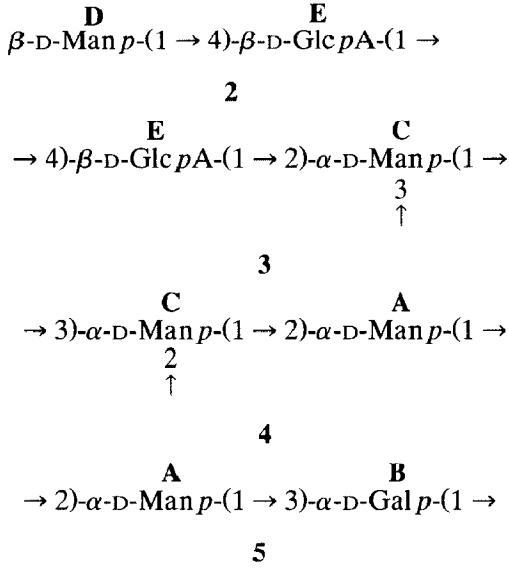
Table 4
Observed $^2J_{C,H}$ and $^3J_{C,H}$ connectivities in a ^{1}H-detected HMBC experiment from anomeric protons of *O*-deacetylated polysaccharide from *Klebsiella* K43

Anomeric proton		$J_{C,H}$ connectivities to ^{13}C-atom	
δ	residue	δ	residue, atom
5.34	→ 2)-α-D-Man*p*-(1 →	75.2	**B**, C-3
	A	73.6	**A**, C-5
		71.2	**A**, C-3
5.25	→ 3)-α-D-Gal*p*-(1 →	not observed	
	B		
5.17	→ 3)-α-D-Man*p*-(1 →	79.4	**A**, C-2
	C 2 ↑	74.6	**C**, C-5
4.67	β-D-Man*p*-(1 →	81.3	**E**, C-4
	D	71.5	**D**, C-2
4.55	→ 4)-β-D-Glc*p*A-(1 →	78.4	**C**, C-2
	E		

A ^{1}H-detected HMBC experiment (hetronuclear multiple bond connectivity), using a delay time of 60 ms, was employed in order to obtain sequential information. The cross-peaks of the anomeric protons were examined and, in addition to intra-residual connectivities, four inter-residual connectivities were found (Table 4). A correlation from δ 4.67, i.e., H-1 in residue **D**, to a carbon resonance at δ 81.3 is obtained. This latter signal is assigned to C-4 of residue **E**, thus confirming the structural element **2**. The anomeric protons of residues **E** (δ 4.55), **C** (δ 5.17), and **A** (δ 5.34) showed connectivities to C-2 (δ 78.4) of residue **C**, C-2 (δ 79.4) of residue **A**, and C-3 (δ 75.2) of residue **B**, respectively. This confirms partial structure **3** and establishes disaccharide elements **4** and **5**.

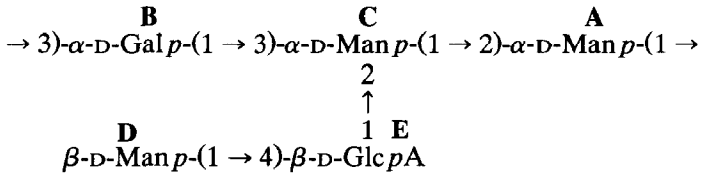


NOESY experiments using mixing times of 250 and 600 ms further confirmed structural elements **2–5** and established the disaccharide element → 3)-α-D-Gal*p*-(1 → 2)-α-D-Man*p*-(1 → (Table 5). For all elements, NOE contacts between the anomeric proton and the proton on the linkage carbon were observed. In addition, a cross-peak deriving from a contact between the anomeric proton and the neighbouring equatorial proton, H-4, was given by the disaccharide element **A–B** (**5**). This interaction, which is over five bonds, is commonly referred to as the γ-gauche effect [10,11]. A related stereochemical arrangement is present also in the disaccharide element **E–C** (**3**), and NOE was observed between H-1 of **E** and H-1 of **C**. The presence of these proton contacts changes the chemical shift of the C-1 signal of residue **A** to the unusual value δ 95.6. The chemical shift is only displaced 1.0 ppm from the corresponding value in α-D-mannopyranose. The γ-gauche interaction causes the signals from the corresponding carbons to shift upfield, i.e., for an anomeric carbon signal less downfield relative to the parent sugar. Thus, as H-1 in **A** is involved in a γ-gauche interaction to H-4 in **B**, it will only experience a small downfield shift. Analogously, signals for C-1 in **E** and **C** have a downfield shift smaller than the normal $\sim$ 7 ppm. From the combined

Table 5
Observed NOE contacts from anomeric protons of *O*-deacetylated polysaccharide from *Klebsiella* K43

Anomeric proton		NOE contacts to
5.34	→ 2)-α-D-Man*p*-(1 →	4.16 **B**, H-4
A		3.98 **B**, H-3
5.25	→ 3)-α-D-Gal*p*-(1 →	4.04 **C**, H-3
B		3.88 **B**, H-2
5.17	→ 3)-α-D-Man*p*-(1 →	4.55 **E**, H-1
C	2	4.34 **C**, H-2
	↑	4.07 **A**, H-2
4.67	β-D-Man*p*-(1 →	3.98 **D**, H-2
D		3.81 **E**, H-4
		3.63 **D**, H-3 (**E**, H-3)
		3.39 **D**, H-5
4.55	→ 4)-β-D-Glc*p*A-(1 →	5.17 **C**, H-1
E		3.76 **E**, H-5

evidence, it is concluded that the *Klebsiella* K43 capsular polysaccharide is composed of pentasaccharide repeating units with the structure **6**.



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3. Experimental

General methods.—Concentrations were performed under diminished pressure at < 40°C or under a stream of air or nitrogen. For GLC, a Hewlett–Packard 5890 instrument fitted with a flame-ionisation detector was used. GLC–MS (EI) was performed on a Hewlett–Packard 5970 MSD instrument.

Alditol acetates and partially methylated alditol acetates were analysed on an HP-5 capillary column (25 m × 0.20 mm), using the temperature program 180°C (1 min) → 250°C at 3°C/min. Analysis of the trimethylsilylated (+)-2-butyl glycosides were performed on the same column, but the temperature program 130°C (1 min) → 220°C at 3°C/min was used.

Gel permeation chromatography was performed on Bio-Gel P-2 and Sephadex G-50 columns, using water buffered with 0.07 M pyridinium acetate of pH 5.4 as eluent, and monitored by a differential refractometer.

Preparation of O-deacetylated polysaccharide.—The polysaccharide was dissolved in 0.1 M NaOH and kept at room temperature for 40 h. The *O*-deacetylated polysaccharide was recovered by gel filtration on a Sephadex G50 column (2.5 × 90 cm).

NMR spectroscopy.—NMR spectra of solutions in D_2O were recorded at 70°C with either a Jeol GSX-270 or Alpha-400 instrument. Chemical shifts are reported in ppm, using sodium 3-trimethylsilylpropanoate-d_4 (δ_H 0.00) or acetone (δ_C 31.00) as internal references. H,H-COSY, H,H-RCOSY, NOESY, and C,H-COSY were performed using Jeol standard pulse-sequences. H,H-COSY, using double-quantum filter, and H,H-HOHAHA experiments were performed in the phase-sensitive mode. The mixing times in the NOESY experiment were 250 (30°C) and 600 ms (70°C), and H,H-HOHAHA experiments were obtained using a mixing time of 30, 60, and 120 ms. The $^1J_{C\text{-}1,H\text{-}1}$ values were determined by an HMQC inverse-detected experiment, and the $^1H-^{13}C$ long-range couplings were investigated with an HMBC inverse-detected experiment using a delay time of 60 ms.

Sugar and methylation analysis.—Hydrolysis of native and methylated K43 was performed by treatment with 2 M CF_3CO_2H at 120°C for 2 h. The sugars in the hydrolysates were converted into alditol acetates and partially methylated alditol acetates. Carboxyl-reduction of methylated polysaccharide (1 mg in dry THF) was performed by treatment with 1 M lithium triethylborodeuteride in THF (0.20 mL) at 0°C for 1 h. The absolute configurations of the sugars were determined according to Gerwig et al. [6,7].

Uronic acid degradation [8,9].—Carefully dried methylated polysaccharide was dissolved in Me_2SO and treated with *p*-toluenesulfonic acid and 2,2-dimethoxypropane. Sodium methylsulfinylmethanide was generated in situ by the addition of butyl-lithium in hexane to the solution, which was kept at room temperature overnight. After cooling, trideuteriomethyl iodide was added, and the material was recovered and hydrolysed.

4. Acknowledgments

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