ELSEVIER

Carbohydrate Research 269 (1995) 295–302

CARBOHYDRATE RESEARCH

The structure of the capsular polysaccharide of *Escherichia coli* O8:K43:H11

Y.M. Choy [a], G.G.S. Dutton [b], M. Ruth Leslie [c], Haralambos Parolis [c,*], Lesley A.S. Parolis [c]

[a] *Department of Biochemistry, The Chinese University of Hong Kong, Shatin, Hong Kong*
[b] *Department of Chemistry, University of British Columbia, Vancouver, V6T 1Z1, Canada*
[c] *School of Pharmaceutical Sciences, Rhodes University, Grahamstown, 6140, South Africa*

Received 21 September 1994; accepted 11 November 1994

Abstract

The primary structure of the acidic capsular polysaccharide of *Escherichia coli* O8:K43:H11 was shown by monosaccharide analysis, methylation analysis, β-elimination, and by 1D and 2D ^{1}H and ^{13}C NMR spectroscopy to be composed of branched pentasaccharide repeating units with the structure

→4)-α-D-GlcpA-(1→3)-β-D-Manp-(1→4)-β-D-Manp-(1→3)-β-D-GlcpNAc-(1→
2
↑
1
β-D-GlcpNAc

Keywords: Escherichia coli; Capsular polysaccharide; K43 antigen; Structure; NMR spectroscopy

1. Introduction

More than 40% of the known capsular (K) polysaccharide antigens of *E. coli* occur in association with only two O-antigens, viz., O8 and O9 [1]. The structures of the repeating oligosaccharide units of all sixteen of the K-antigens associated with O9 and

* Corresponding author.

SSDI 0008-6215(94)00366-1

all but two (K41 and K43) of the sixteen K-antigens associated with O8 have been determined. In this paper we report the sructure of the repeating unit of the capsular antigen of *E. coli* O8:K43:H11.

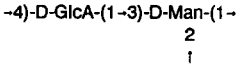
2. Results and discussion

Isolation, purification, and composition of the capsular polysaccharide.—*E. coli* O8:K43:H11 bacteria were grown on Mueller–Hinton agar and the harvested bacterial slime was diluted with an equal volume of aqueous 2% phenol and the cells were removed by ultracentrifugation. The acidic capsular polysaccharide was separated from the neutral material as the cetyltrimethylammonium complex. The recovered capsular polysaccharide was found to be lipid-bound and was released by treatment with hot aqueous 1% acetic acid. After removal of the lipid by ultracentrifugation the capsular polysaccharide was further purified by chromatography on DEAE-Sepharose CL-6B. GLC examination of the alditol acetates derived from an acid hydrolysate of the purified polysaccharide (**PS**) showed the presence of Man and GlcN. Prior methanolysis of the **PS**, reduction of the methoxycarbonyl groups formed, and GLC examination of the derived alditol acetates revealed the presence of Glc in addition to Man and GlcN thereby establishing GlcA as the acid component of the repeating unit. The D configuration was established for all the constituent monosaccharides by GLC examination of the derived acetylated (−)-2-octyl glycosides [2].

1D NMR studies of the **PS**.—The ^{1}H NMR spectrum of the **PS** (Fig. 1) contained discrete H-1 signals at δ 5.352 ($^{3}J_{H,H}$ 4.2 Hz) and 4.689 ($^{3}J_{H,H}$ 8.2 Hz) for an α- and a β-linked residue, respectively, and three overlapping H-1 signals between δ 4.71 and 4.75. In addition, signals for the methyl protons of two NAc groups at δ 2.055 and 2.070 were present. The ^{13}C NMR spectrum (Figs. 2a and 2b) of the **PS** had C-1 signals at 102.05, 102.00, 101.64, 101.50, and 100.42 ppm, signals at 23.33 and 23.19 ppm for the methyl carbons of NAc groups, at 55.50 and 56.65 ppm for acetamido substituted carbons, for the carbonyl carbons of NAc groups at 175.13 and 174.92 ppm, and a signal at 173.95 ppm for the carbon of a carboxyl group. These and the other results indicated that the repeating unit of the **PS** consisted of a pentasaccharide composed of GlcNAc: Man:GlcA in the ratios 2:2:1.

Methylation and β-elimination analyses.—Methylation of the **PS** by a modified Hakomori procedure [3], followed by Kuhn methylation [4], and GLC analysis of the partially methylated alditol acetates derived from an acid hydrolysate of the methylated **PS** showed the presence of 4,6-di-*O*-methylmannose, 2,3,6-tri-*O*-methylmannose, 2,3-di-*O*-methylglucose (after carboxyl reduction), 2-deoxy-4,6-di-*O*-methyl-2-methylacetamidoglucose, and 2-deoxy-3,4,6-tri-*O*-methyl-2-methylacetamidoglucose. The methylation results thus established the presence of 2,3- and 4-linked Man, 4-linked GlcA, and 3-linked and terminal GlcNAc. Treatment of the methylated **PS** with sodium methylsulphinylmethanide followed by remethylation of the product and GLC examination of the derived partially methylated alditol acetates showed the appearance of 3,4,6-tri-*O*-methylmannose and a concommitant decrease in the amount of 4,6-di-*O*-methylmannose. These results indicated that the GlcA is linked to *O*-3 of the 2,3-linked

Man in the repeating unit of the **PS**. The results thus far accord with the following partial structure for the pentasaccharide repeating unit of the **PS**.



The full sequence of the residues in the repeating unit of the **PS** was established by 2D NMR experiments.

2D NMR studies of the **PS**.—The identity of the residues in the repeating unit, the configurations of the glycosidic linkages, and the glycosylation sites were established by $^{1}H-^{1}H$ correlation spectroscopy (COSY [5], HOHAHA [6], and NOESY [7]) and by $^{1}H-^{13}C$ correlation spectroscopy (HMQC [8]). The residues in the repeating unit were denoted **a**–**e** in order of decreasing chemical shift of the H-1 resonances. The ^{1}H and ^{13}C chemical shifts are listed in Table 1. The ^{1}H chemical shifts of residues **a**, **d**, and **e** were established from the COSY and HOHAHA spectra. In the case of residues **b** and **c** H-1/H-2 cross-peaks were not observed in the COSY and HOHAHA spectra. The ^{1}H chemical shifts for these resonances were obtained from the H-1/H-2 NOEs observed in the NOESY spectrum. The remaining ^{1}H resonances for residue **b** could then be traced in the HOHAHA spectrum. The chemical shifts for H-3 and H-5 for residue **c** were

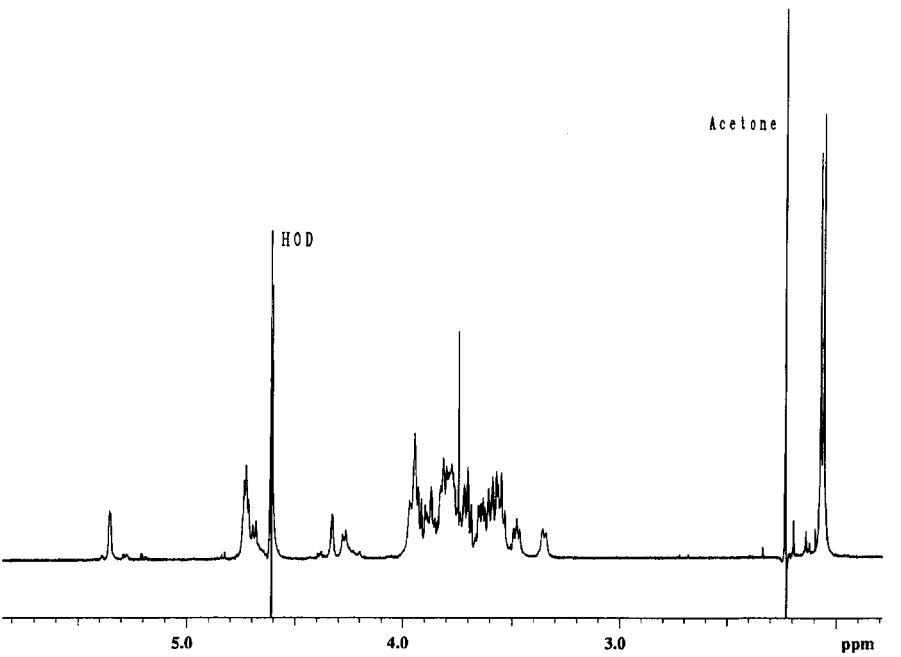


Fig. 1. ^{1}H NMR spectrum of *E. coli* K43 capsular polysaccharide.

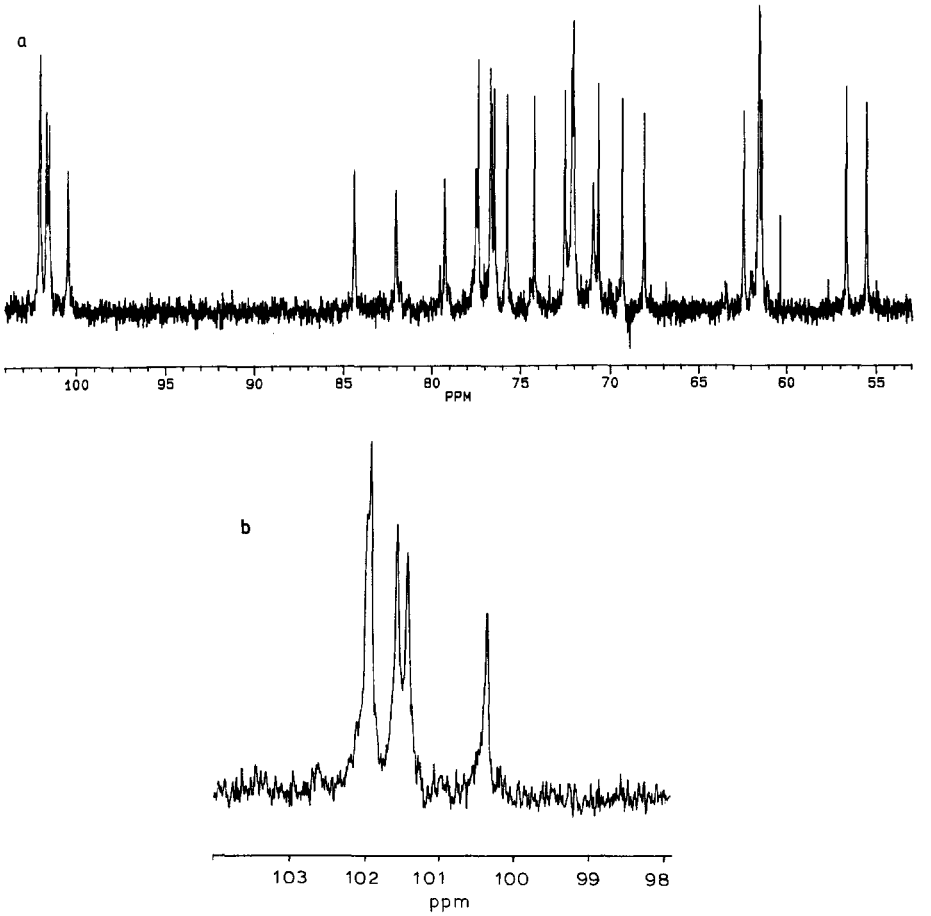


Fig. 2. (a) Partial ^{13}C NMR spectrum of *E. coli* K43 capsular polysaccharide. (b) Expansion of the ^{13}C NMR spectrum of *E. coli* K43 capsular polysaccharide showing the anomeric carbon resonances.

established from the H-1/H-5 and H-3/H-5 NOEs present in the NOESY spectrum. The ^{1}H chemical shifts for the remaining resonances were traced in the COSY spectrum.

Comparison of the ^{1}H and ^{13}C chemical shifts for residues **a**–**e** with literature values for methyl glycosides [9–11] identified the residues in the repeating unit as indicated in Table 1. The anomeric linkage of residue **a** was established as α from its H-1 and C-1 chemical shifts, its H-1/H-2 coupling constant, and from the intramolecular H-1/H-2 NOE observed in the NOESY spectrum. Residues **d** and **e** were shown to be β from their H-1 chemical shifts, their H-1/H-3, H-5 intramolecular NOEs, and the size of their H-1/H-2 coupling constants. The β anomeric configuration was established for the linkages of residues **b** and **c** from the chemical shifts of their C-5 resonances and from the intramolecular NOEs observed between H-1 and H-3 and H-1 and H-5 for residue **b** and between H-1 and H-5 for residue **c**. In agreement with the methylation results the

Table 1
NMR data [a] for *E. coli* K43 polysaccharide

Residue		Proton or carbon						
		1	2	3	4	5	6a	6b
a	H	5.352	3.642	3.917	3.778	4.274		
→ 4)-α-D-Glc*p*A	C	100.42	72.06	72.14	82.03	72.06	173.95	
b	H	4.735	4.332	3.823	3.704	3.479	3.628	3.961
→ 2,3)-β-D-Man*p*	C	102.00 [b]	76.73	77.48	68.09	77.36	62.40	
c	H	4.726	3.943	3.836	3.723	3.583	3.887	3.758
→ 4)-β-D-Man*p*	C	102.05 [b]	70.94	72.52	79.25	75.73	61.58 [c]	
d	H	4.724	3.701	3.571	3.549	3.351	3.804	3.940
β-D-Glc*p*NAc	C	101.64	56.65	74.22	70.65	76.61	61.40	
e	H	4.689	3.870	3.787	3.608	3.548	3.789	3.959
→ 3)-β-D-Glc*p*NAc	C	101.50	55.50	84.38	69.33	76.46	61.56 [c]	

[a] Chemical shifts in ppm with acetone as internal standard, δ 2.230 and 31.07 ppm for ^{1}H and ^{13}C, respectively.
[b,c] Values may have to be exchanged.

glycosylation sites were established as C-4 for **a**, C-2 and C-3 for **b**, C-4 for **c**, and C-3 for **e** by the significant deshielding experienced by these carbons.

The sequence of the residues in the repeating unit was established from the NOESY experiment. The intermolecular NOEs observed are collected in Table 2. Assignment of the NOEs was greatly facilitated by the PRONTO software program [12] which permitted simultaneous viewing and interrogation of selected expansions of all the 2D NMR contour plots. The NOEs from H-1 of the β-linked residues in the repeating unit of the **PS** are shown in Fig. 3.

Table 2
NOE data for K43 **PS**

Residue	Proton	Correlation to
a	H-1	3.823 (**b3**), 3,642 (**a2**)
→ 4)-α-D-Glc*p*A	H-3	4.274 (**a5**)
b	H-1	3.723 (**c4**), 4.332 (**b2**), 3.823 (**b3**), 3.479 (**b5**)
→ 2,3)-β-D-Man*p*	H-2	4.274 (**a5**), 3.823 (**b3**)
	H-5	3.823 (**b3**)
	H-6a	3.961 (**b6b**)
c	H-1	3.787 (**e3**), 3.943 (**c2**), 3.583 (**c5**)
→ 4)-β-D-Man*p*	H-5	3.836 (**c3**)
	H-6a	3.758 (**c6b**)
d	H-1	4.332 (**b2**), 3.571 (**d3**), 3.351 (**d5**)
β-D-Glc*p*NAc	H-3	3.351 (**d5**)
	H-6a	3.940 (**d6b**)
e	H-1	3.778 (**a4**), 3.787 (**e3**), 3.548 (**e5**)
→ 3)-β-D-Glc*p*NAc	H-6a	3.959 (**e6b**)

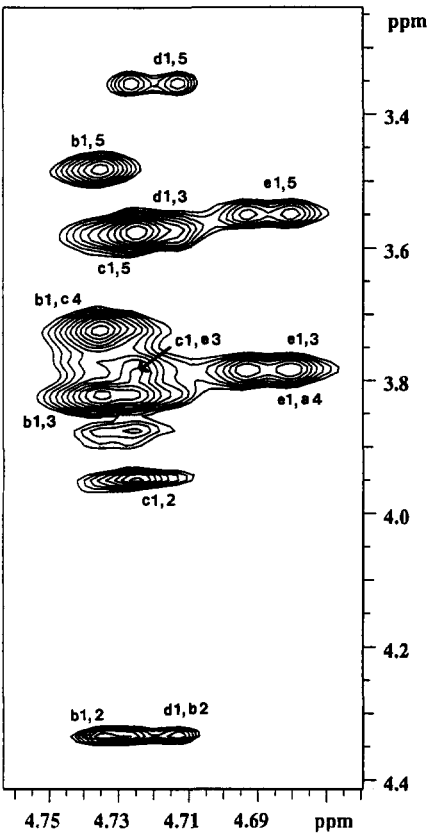
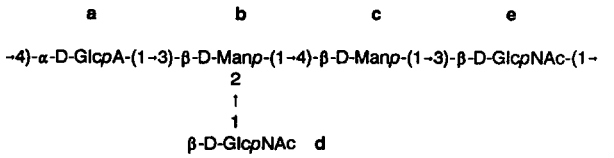


Fig. 3. Partial NOESY contour map of the K43 capsular polysaccharide showing the NOEs from H-1 of the β-linked residues (**b**–**e**). **b**1,2 connotes the cross peak between H-1 and H-2 of residue **b** while **b**1,**c**4 connotes the cross peak between H-1 of residue **b** and H-4 of residue **c**, etc.

The combined chemical and NMR results support the following structure for the repeating unit of the capsular polysaccharide of *E. coli* K43:



3. Experimental

General methods.—Analytical GLC was performed on a Hewlett–Packard 5890A gas chromatograph, fitted with flame-ionization detectors and a 3392A recording

integrator, with helium as carrier gas. A J&W Scientific fused-silica DB-17 bonded-phase capillary column (30 m × 0.25 mm, film thickness 0.25 μm) was used for separating partially methylated alditol acetates (programme I), alditol acetates, and acetylated octyl glycosides (programme II). A J&W Scientific fused-silica DB-225 bonded-phase capillary column (30 m × 0.25 mm, film thickness 0.25 μm) was also used for separating acetylated octyl glycosides (130 kPa, 240°C isothermal). The temperature programmes used were: I, 180°C for 2 min, then 3°C.min^{-1} to 240°C, 100 kPa; II, 180°C for 2 min, then 2°C.min^{-1} to 240°C, 100 kPa. The identities of all derivatives were determined by comparison with authentic standards and confirmed by GLC–MS on a Hewlett–Packard 5988A instrument, using the appropriate column. Spectra were recorded at 70 eV and an ion-source temperature of 200°C. Gel permeation ion-exchange chromatography was performed on a DEAE-Sepharose CL-6B column (2.6 × 27 cm) using gradient elution with 0–1 M NaCl in 0.01 M Tris-HCl (pH 8.50). Fractions were assayed for carbohydrate using the phenol–H_2SO_4 reagent [13].

Polysaccharide samples were hydrolysed with 4 M CF_3CO_2H for 1 h at 125°C. Alditol acetates were prepared by reduction of the products in acid hydrolysates with $NaBH_4$ for 1 h followed by acetylation with 2:1 Ac_2O–pyridine for 1 h at 100°C. Samples were methanolysed by refluxing with methanolic 3% HCl for 16 h. Methoxycarbonyl groups formed during methanolysis of native and methylated polysaccharides were reduced with $NaBH_4$ in dry CH_3OH. Methylations were carried out on the acid form of the polysaccharide, using potassium dimsyl [3] and CH_3I in Me_2SO, followed by a Kuhn methylation (72 h) in DMF with Ag_2O and CH_3I [4].

Preparation of E. coli *K43 capsular polysaccharide.*—An authentic culture of *E. coli* O8:K43:H11 was obtained from Dr. I. Ørskov (Statens Seruminstitut, Copenhagen) and propagated on Mueller–Hinton agar (9 stainless steel trays 30 × 60 cm, each inoculated with 10 mL of a 5 h culture in nutrient broth). Polysaccharide material was extracted from the cells with aq 1% phenol, the cells and debris were removed by ultracentrifugation, and the polysaccharide was isolated by precipitation into ethanol. The acidic capsular polysaccharide (460 mg) was separated from neutral material as its cetyltrimethylammonium complex. The recovered capsular polysaccharide was delipidated by treatment with aq 1% acetic acid (100°C, 1 h), after which it was converted to the acid form by passage down a column of Amberlite IR 120 [H^+] resin and was purified by gel permeation ion-exchange chromatography on DEAE-Sepharose CL-6B.

NMR Spectroscopy.—Polysaccharide samples were deuterium-exchanged by freeze-drying several times from 99.5% D_2O and then examined in 99.99% D_2O containing a trace of acetone as internal standard (δ 2.23 for ^1H and 31.07 ppm for ^{13}C). Spectra were recorded at 316 K on a Bruker AMX 600 spectrometer. The 2D NMR pulse programmes used were as follows: (a) Phase-sensitive COSY using TPPI with double quantum filter and presaturation during relaxation delay; (b) phase-sensitive NOESY using the method of States et al. [14] with presaturation during relaxation delay and mixing time (200 ms); (c) HOHAHA using the method of States et al. [14] with presaturation during relaxation delay and employing the DIPSI-2 sequence for mixing (mixing time 62.6 ms); (d) HMQC using the States-TPPI method with presaturation during the relaxation delay and the BIRD pulse delay and using GARP1 decoupling during acquisition. The data matrices for a, b, and c were 1024 × 4096, were zero filled

in both dimensions, and a shifted sine squared window function was applied prior to Fourier transformation. For the HMQC the data matrix was 512×4096, was zero filled in both dimensions, and sine squared (t_2) and sine (t_1) window functions were applied prior to Fourier transformation.

Acknowledgements

We thank Dr. I. Ørskov (Copenhagen) for the test strain of E. coli O8:K43:H11, the Foundation for Research Development (Pretoria) for financial support (to H.P.), and Mr. B.O. Petersen (Carlsberg Laboratory, Valby) for recording the NMR spectra.

References

[1] I. Ørskov, F. Ørskov, B. Jann, and K. Jann, *Bacteriol. Rev.*, 41 (1977) 667–710.
[2] K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res.*, 62 (1978) 358–362.
[3] L.R. Phillips and B.A. Fraser, *Carbohydr. Res.*, 90 (1981) 149–152.
[4] R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, 67 (1955) 32.
[5] U. Piantini, O.W. Sørensen, and R.R. Ernst, *J. Am. Chem. Soc.*, 104 (1982) 6800–6801.
[6] S.P. Rucker and A.J. Shaka, *Mol. Phys.*, 68 (1989) 509–517.
[7] R. Baumann, G. Wider, R.R. Ernst, and K. Wüthrich, *J. Magn. Reson.*, 44 (1981) 402–406.
[8] A. Bax and S. Subramanian, *J. Magn. Reson.*, 67 (1986) 565–569.
[9] P.A.J. Gorin and M. Mazurek, *Can. J. Chem.*, 53 (1975) 1212–1223.
[10] K. Bock and H. Thøgersen, *Annu. Rep. NMR Spectrosc.*, 13 (1982) 1–57.
[11] K. Izumi, *Carbohydr. Res.*, 170 (1987) 19–25.
[12] M. Kjaer, K.V. Andersen, and F.M. Poulsen, *Methods Enzymol.*, (1994) in press.
[13] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
[14] D.J. States, R.A. Haberkorn, and D.J. Ruben, *J. Magn. Reson.*, 48 (1982) 286–292.