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Carbohydrate Research 277 (1995) 353–358

CARBOHYDRATE RESEARCH

Note

NMR reinvestigation of the capsular K27 polysaccharide (K27 antigen) from *Escherichia coli* O8:K27:H$^-$

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Received 3 April 1995; accepted 23 May 1995

Keywords: *E. coli*; K27 antigen; Capsular polysaccharide; Structure; NMR spectroscopy

The K27 polysaccharide was described in 1963 as one of the first capsular polysaccharides of *Escherichia coli* to be characterised [1]. It was later reported to be a branched polysaccharide, the main chain of which is made up from the trisaccharide glucosyl-(1 → 3)-glucuronyl-(1 → 3)-fucose, substituted at position 3 of the glucosyl residue with galactose [2]. Neither the linkage between the repeating tetrasaccharide units nor the anomeric configurations of the sugar constituents were reported. The K27 polysaccharide is structurally related to the capsular K54 polysaccharide of *Klebsiella aerogenes* which consists of a main chain of → 3)-β-D-Glc*p*-(1 → 4)-α-D-Glc*p*A-(1 → 3)-α-L-Fuc*p*-(1 → , substituted at position 4 of the main-chain glucose with β-D-Glc*p* [3]. A *Klebsiella* bacteriophage was isolated which enzymatically cleaved the fucosyl–glucose linkage with the formation of the tetrasaccharide repeating unit and its dimer [4,5]. This same bacteriophage-borne endo-fucosidase also cleaved the fucosyl–glucose linkage in the *E.coli* K27 polysaccharide [6]. Since this identical substrate-specificity is interesting and since the complete structure of the K27 polysaccharide remained unknown, we have reinvestigated the structure of the capsular K27 polysaccharide of *E.coli* by NMR spectroscopy.

The K27 polysaccharide was obtained from *E.coli* E56b (O8:K27:H$^-$), the test strain for the K27 antigen. The bacteria were grown overnight at 37°C on agar (1.5%)

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SSDI 0008-6215(95)00225-1

containing 1% tryptone, 0.1% yeast extract, 0.1% D-glucose, and 0.8% sodium chloride, and harvested with saline containing 2% (v/v) phenol. They were extracted with aqueous 45% phenol and, after dialysis, the aqueous phase was subjected to ultracentrifugation. The K27 polysaccharide was precipitated from the supernatant solution by precipitation with cetyltrimethylammonium bromide and purified by repeated precipitation with ethanol from a solution in 250 mM sodium chloride, as described [2,7]. It consisted of approximately equimolar amounts of glucose (Glc), galactose (Gal), glucuronic acid (GlcA), and fucose (Fuc), as determined by GLC. The absolute configuration of D-Glc was determined by reaction with glucose oxidase, specific for the D form. The polysaccharide was subjected to partial acid hydrolysis (0.1 M trifluoroacetic acid, 100°C, 30 min) and, after removal of CF_3CO_2H in vacuo, the hydrolysate was chromatographed on Bio-Gel P-2 with water as eluent. A disaccharide, consisting of GlcA and Fuc, and a trisaccharide, consisting of Glc, GlcA, and Fuc, were obtained. The ^1H and ^{13}C NMR spectra of the polysaccharide, the disaccharide, and the trisaccharide were recorded with a Bruker WM 300 spectrometer in D_2O at 70°C. Acetone served as internal standard (δ_H, 2.225; δ_C, 31.45). Standard Bruker software for Aspect 2000 was used for 2D homonuclear COSY, one- and two-step relayed coherence transfer COSY, and heteronuclear $^{13}C-^1H$ COSY, and for 1D NOE experiments (COSYHG, COSYRCT, COSYRCT2, XHCORR, and NOEMULT, respectively) [8,9].

The ^1H NMR spectrum of the disaccharide contained two signals at δ 5.24 ($J_{H\text{-}1,H\text{-}2}$ 3.5 Hz) and 5.20 ($J_{H\text{-}1,H\text{-}2}$ 3 Hz), characteristic of α-anomeric protons in the *gluco/galacto* configuration, and one signal at δ 4.59 ($J_{H\text{-}1,H\text{-}2}$ 7.5 Hz), characteristic of a β-anomeric proton in the *gluco/galacto* configuration. The spectrum of the trisaccharide had an additional signal at δ 4.51 ($J_{H\text{-}1,H\text{-}2}$ 8 Hz), indicative of a proton in the β configuration. Both spectra also contained a signal at δ 1.18–1.22 due to the methyl protons of a 6-deoxyhexose. The ^{13}C NMR spectra exhibited three signals characteristic of anomeric carbon atoms at δ 93.5, 97.4, and 102.0 (for the disaccharide, δ 101.8 for the trisaccharide), and an additional signal at δ 103.5 for the trisaccharide. Both also contained signals for C-6 of GlcA (at δ 175.5 for the disaccharide and at δ 175.0 for the trisaccharide) as well as for C-6 of a 6-deoxyhexose at δ 16.8 (in both spectra). The signal assignments for each oligosaccharide (Table 1) showed that the reducing sugar in each case was Fuc, occurring in both anomeric configurations. The NOE spectrum of the disaccharide, after irradiation of H-1 of GlcA, showed a response at H-3 of Fuc, and that of the trisaccharide, after irradiation of H-1 of Glc, showed a response at H-4 of GlcA. This indicated that the disaccharide was α-D-Glc*p*A-(1 → 3)-(α/β)-L-Fuc*p* and that the trisaccharide was β-D-Glc*p*-(1 → 4)-α-D-Glc*p*A-(1 → 3)-(α/β)-L-Fuc*p*.

The ^{13}C NMR spectrum of the K27 polysaccharide (Fig. 1) contained four signals in the anomeric region (δ 99.7–103.4), one signal characteristic of C-6 of a 6-deoxy sugar (δ 16.5), and one signal due to the carbon atom of a carboxyl group (δ 175.0). A gated decoupling spectrum revealed the presence of two β-anomeric carbon atoms ($J_{C\text{-}1,H\text{-}1}$ 163 Hz) and two α-anomeric carbon atoms ($J_{C\text{-}1,H\text{-}1}$ 172 and 173 Hz). An APT spectrum [10] showed that both CH_2OH groups present in the polysaccharide were unsubstituted. No low-field C-1 signals characteristic of furanoses [11] were detected.

In the ^1H NMR spectrum of the K27 polysaccharide (Fig. 1), the region of anomeric protons contained five signals, the visible multiplicity of one of which was a broadened

Table 1
^{1}H NMR and ^{13}C NMR data for the K27 di- and tri-saccharide

Residue	Proton	δ		Coupling		Carbon	δ	
		Disacch.	Trisacch.	$J_{H,H}$	Hz		Disacch.	Trisacch.
β-D-Glc*p*-	H-1		4.51	$J_{1,2}$	8	C-1		103.5
	H-2		3.30	$J_{2,3}$	9	C-2		74.4
	H-3		3.49	$J_{3,4}$	9	C-3		76.8
	H-4		3.40	$J_{4,5}$	9	C-4		70.8
	H-5		3.46	$J_{5,6a}$	< 2	C-5		77.4
	H-6a		3.92	$J_{6a,6b}$	12	C-6		61.9
	H-6b		3.72	$J_{5,6b}$	3			
α-D-Glc*p*A-	H-1	5.24	5.24	$J_{1,2}$	3.5	C-1	102.0	101.8
(or → 4)-α-D-Glc*p*A)	H-2	3.60	3.66	$J_{2,3}$	9.5	C-2	72.8	72.6
	H-3	3.82	3.92	$J_{3,4}$	9.5	C-3	73.8	72.6
	H-4	3.57	3.82	$J_{4,5}$	9.5	C-4	72.9	81.1
	H-5	4.26	4.36			C-5	73.1	72.2
						C-6	175.5	175.0
→ 3)-α-L-Fuc*p*	H-1	5.20	5.21	$J_{1,2}$	3	C-1	93.5	93.5
	H-2	3.94	3.97	$J_{2,3}$	-	C-2	68.6	68.6
	H-3	3.93	3.97	$J_{3,4}$	-	C-3	79.6	79.8
	H-4	3.92	3.97	$J_{4,5}$		C-4	73.2	73.1
	H-5	4.20	4.20	$J_{5,6}$	6.5	C-5	67.7	67.7
	H-6	1.18	1.18			C-6	16.8	16.8
→ 3)-β-L-Fuc*p*	H-1	4.59	4.59	$J_{1,2}$	7.5	C-1	97.4	97.4
	H-2	3.62	3.62	$J_{2,3}$	9.5	C-2	72.2	72.2
	H-3	3.72	3.72	$J_{3,4}$	3.5	C-3	82.8	82.9
	H-4	3.87	3.89	$J_{4,5}$	< 2	C-4	72.6	72.6
	H-5	3.79	3.80	$J_{5,6}$	6.5	C-5	72.1	72.1
	H-6	1.22	1.22			C-6	16.8	16.8

quartet, characteristic of H-5 of a 6-deoxyhexose in the α configuration [12]. The signals at δ 5.22 and 5.40 ($J_{H\text{-}1,H\text{-}2}$ 3.5 Hz) were characteristic of α protons in the *gluco / galacto* configuration, and the signals at δ 4.38 and 4.51 ($J_{H\text{-}1,H\text{-}2}$ 7.5 Hz) were characteristic of β protons in the *gluco / galacto* configuration. The spectrum also contained a doublet at δ 1.14, due to H-6 of a 6-deoxy sugar. The assignments of all signals in the ^{1}H and ^{13}C NMR spectra (Table 2) were performed as for the oligosaccharides. They were in agreement with those of the oligosaccharides (Table 1). The data also showed that, in the polysaccharide, Fuc was in the α configuration and linked to β-Glc*p* at position 4.

For a determination of the sugar and linkage sequence, a series of NOE spectra was performed with the K27 polysaccharide, after irradiation of the anomeric protons (Table 3). Irradiation of H-1 of β-Glc*p* (residue **A**) resulted in a response of H-4 of α-Glc*p*A (residue **B**) indicative of **A**-(1 → 4)-**B**. Preirradiation of H-1 of residue **B** affected H-3/H-4 of α-Fuc*p* (residue **C**), indicating a (1 → 3) or a (1 → 4) linkage between residues **B** and **C**. The results obtained with the disaccharide (Table 1), however, clearly showed that the linkage was **B**-(1 → 3)-**C**. Preirradiation of H-1 of residue **C** affected H-4 of residue **A** indicating a **C**-(1 → 4)-**A** linkage, and preirradiation of H-1 of β-Gal*p*

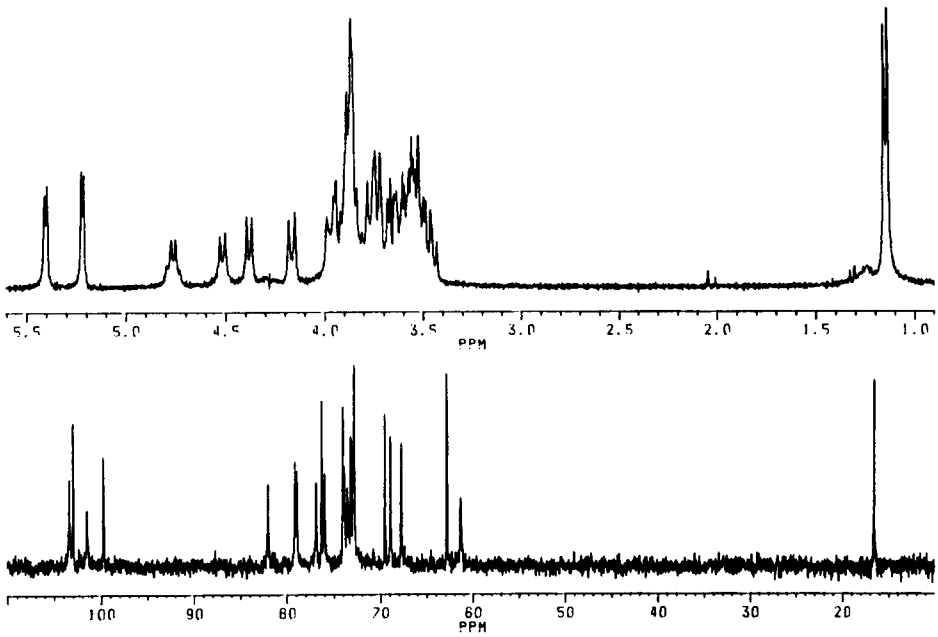


Fig. 1. 300-MHz ^{1}H NMR spectrum (top) and 75-MHz ^{13}C NMR spectrum (bottom) of the capsular K27 polysaccharide from *E.coli* O8:K27:H$^-$, recorded in D_2O (70°C) with acetone (δ_H, 2.225; δ_C, 31.45) as internal standard.

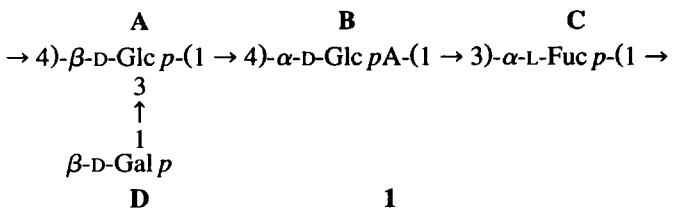
(residue **D**) induced a response of H-3 of residue **A**, which showed a **D**-(1 → 3)-**A** linkage. Thus the polysaccharide was branched at residue **A**, with vicinal substitutions at positions 3 and 4. This was also borne out by contacts of H-1 of residue **D** with H-2 and H-3/H-4 of residue **C**.

For a determination of the absolute configuration of the sugar constituents, the glycosylation effects, as given in Table 2, were calculated [13,14]. Thus, the large positive α-glycosylation effects for C-1 of residue **B** and C-3 of residue **C** as well as the small positive β-glycosylation effect for C-4 of residue **C** indicated opposite absolute configurations of residues **B** and **C**. Also, the significant negative glycosylation effect for C-6 of residue **A**, which is substituted by residue **C** at position 4, indicated opposite absolute configurations of residues **C** and **A** [14]. Further, the negative β-glycosylation effect for H-3 of residue **B** demonstrated the same absolute configurations of residues **A** and **B**, and the significant positive β-glycosylation effect for C-2 of residue **A**, substituted with residue **D** at position 3, confirmed the identical absolute configurations of **D** and **A**. Since the absolute configuration of β-D-Glc*p* (residue **A**) was established by its reactivity with D-glucose oxidase, these data indicated the presence of D-Glc*p*, D-Glc*p*A, L-Fuc*p*, and D-Gal*p* in the K27 polysaccharide which has structure **1**.

Table 2
^{1}H NMR and ^{13}C NMR data for the K27 polysaccharide

Residue	Proton	δ	Coupling		Carbon	δ	GE [a]	$J_{C1,H1}$ (Hz)
			$J_{H,H}$	Hz				
→ 4)-β-D-Glc *p*-(1 →	H-1	4.51	$J_{1,2}$	7.5	C-1	103.4		163
3	H-2	3.52	$J_{2,3}$	9	C-2	75.9	+1.5	
↑	H-3	3.75	$J_{3,4}$	9	C-3	78.9		
	H-4	3.87	$J_{4,5}$	9	C-4	73.8		
(A)	H-5	3.56			C-5	76.8		
	H-6a	3.96			C-6	61.3	−0.8	
	H-6b	3.78						
→ 4)-α-D-Glc *p*A-(1 →	H-1	5.22	$J_{1,2}$	3.5	C-1	101.5	+8.1	172
	H-2	3.62	$J_{2,3}$	9.5	C-2	72.7		
	H-3	3.89	$J_{3,4}$	9.5	C-3	72.7	−1.25	
(B)	H-4	3.71	$J_{4,5}$	9.5	C-4	82.0		
	H-5	4.16			C-5	73.5		
					C-6	175.0		
→ 3)-α-L-Fuc *p*-(1 →	H-1	5.40	$J_{1,2}$	3.5	C-1	99.7		173
	H-2	3.96	$J_{2,3}$	9.5	C-2	68.8		
	H-3	3.88	$J_{3,4}$	3.5	C-3	79.1	+8.8	
(C)	H-4	3.86	$J_{4,5}$	< 2	C-4	73.1	+0.3	
	H-5	4.77	$J_{5,6}$	6.2	C-5	67.7		
	H-6	1.14			C-6	16.5		
↑	H-1	4.38	$J_{1,2}$	7.5	C-1	103.0		163
1	H-2	3.46	$J_{2,3}$	9.5	C-2	72.7		
β-D-Gal *p*	H-3	3.58	$J_{3,4}$	3	C-3	74.0		
	H-4	3.87	$J_{4,5}$	< 2	C-4	69.5		
(D)	H-5	3.52	$J_{5,6a}$	7	C-5	76.3		
	H-6a,6b	3.73	$J_{6a,6b}$	12	C-6	62.8		
			$J_{5,6b}$	5				

[a] Glycosylation effect.



The structure of the capsular K54 polysaccharide of *K.aerogenes* is similar to that of the *E.coli* K27 polysaccharide reported here. The differences between these structures are the following. First, the K27 polysaccharide has β-D-Gal*p* as a side-chain sugar linked to position 3 of the main-chain (branch-point) glucose, whereas the K54 polysaccharide has β-D-Glc*p* linked to position 4 of the main-chain glucose. Second, the branch-point glucose is 4-substituted in the main chain in the K27 polysaccharide and 3-substituted in the K54 polysaccharide. Third, whereas the K27 polysaccharide has no non-carbohydrate substituent, the K54 polysaccharide is *O*-acetylated at position 2 of the fucose residue (the formyl substituent originally reported to be at position 4 of the

Table 3
NOE data for the K27 polysaccharide

NOE observed on		Preirradiated proton			
Residue	Proton	A,H-1	B,H-1	C,H-1	D,H-1
→ 4)-β-D-Glc *p*-(1 →	H-2	+			
3	H-3	+			+
↑	H-4			+	
(A)	H-5	+			
→ 4)-α-D-Glc *p*A-(1 →	H-2		+		
	H-3				
(B)	H-4	+			
	H-5				
→ 3)-α-L-Fuc *p*-(1 →	H-2			+	+
	H-3 + H-4		+		+
(C)					
↑					
1	H-2				+
β-D-Gal *p*	H-3				+
	H-5				+
(D)					

side-chain glucose was due to an artifact [15]). These structural differences seem to be tolerated by the bacteriophage-borne endo-fucosidase.

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