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A partial reductive-cleavage study of the capsular polysaccharide of *Escherichia coli* K57

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Abstract

Trideuteriomethylated and methylated derivatives of the capsular polysaccharide of *Escherichia coli* K57 were partially cleaved by Et_3SiH, using Me_3SiOSO_2Me and $Me_3SiOSO_2CF_3$ as catalysts, to produce oligosaccharide-anhydroalditols. The structures of the trideuteriomethylated trisaccharide- and tetrasaccharide-anhydroalditols isolated were established by FABMS and NMR spectroscopy. Although conditions for the selective production of the tetrasaccharide-anhydroalditol could not be established, oligosaccharide-anhydroalditols were isolated in sufficiently high yield to make this an attractive approach for the structural elucidation of the repeating units of bacterial polysaccharides.

Keywords: Reductive cleavage; *E. coli* K57; Trideuteriomethylated polysaccharide; FABMS; NMR spectroscopy

1. Introduction

Reductive cleavage of methylated polysaccharides was initially developed [1] as an alternative to the traditional methylation analysis in order to overcome the inability of the latter to distinguish between certain combinations of ring form and position of linkage. It subsequently became an integral part of a general strategy proposed for sequencing polysaccharides [2]. An important aspect of this strategy is the difference in susceptibility to reductive cleavage displayed by glycoside linkage-types depending on

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the catalyst or mixture of catalysts employed [3–8]. Thus, by judicious choice of catalyst, either total or selective reductive cleavage can be achieved. Analysis of the partially methylated anhyroalditols produced on total reductive cleavage identifies the monomeric residues present in the polymer, their ring forms, and their positions of linkage. Selective reductive cleavage on the other hand gives rise to small oligomers, the study of which provides information on the sequence of the residues and the configurations of selected glycoside linkages [5,6,9].

Partial, as distinct from selective, reductive cleavage has been employed where the susceptibility of glycosidic linkages to reductive cleavage is similar. In one such study of methylated β-cyclodextrin, the anhydroalditol-terminated oligosaccharides produced were separated by HPLC and were characterised by CIMS [10].

We now report on a reductive-cleavage study of the trideuteriomethylated and methylated derivatives of the capsular polysaccharide of *Escherichia coli* K57. The aim of this model study was to examine whether it was possible to produce selectively in high yield an oligosaccharide-anhydroalditol related to the repeating unit and to study its structure by FABMS and NMR spectroscopy.

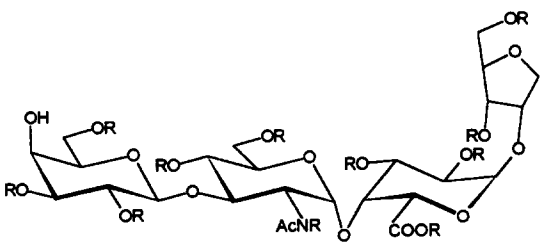
2. Results and discussion

The capsular polysaccharide of *E. coli* K57 [11] was chosen for study by partial reductive cleavage because of the presence in the repeating unit (**1**) of glycosidic linkages which were expected to display widely different susceptibilities to cleavage.

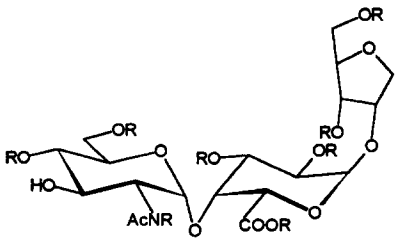
→ 4)-β-D-Gal*p*-(1 → 3)-α-D-Glc*p*NAc-(1 → 4)-α-D-Gal*p*A-(1 → 2)-β-D-Rib*f*-(1 →

1

Previous model studies [4,7] suggested that the ribofuranosyl linkages in the permethylated polysaccharide would be the most susceptible to reductive cleavage while the 2-acetamido-2-deoxy-α-glucopyranosyl linkages were expected to be resistant. Furthermore, the rapid cleavage of fructofuranosyl linkages and the relative stability of glucopyranosyl linkages in methylated inulin, when Me_3SiOSO_2Me was used as catalyst



2a R = CH_3
2b R = CD_3



3a R = CH_3
3b R = CD_3

[7], suggested that the ribofuranosyl linkages in methylated *E. coli* K57 capsular polysaccharide (**MP**) might be selectively cleaved to produce the tetrasaccharide-anhydroalditol **2a**.

Optimal conditions for the formation of **2a**, using Me_3SiOSO_2Me as catalyst, were determined by following the time course of reactions of **MP** with Et_3SiH by TLC. These conditions were then applied in a scaled-up experiment to trideuteriomethylated *E. coli* K57 polysaccharide (**TMP**), and the products were separated by preparative TLC to afford the trisaccharide- and tetrasaccharide-anhydroalditols **3b** and **2b** in 34 and 16% yield, respectively.

In a second series of experiments, **MP** was treated with $Me_3SiOSO_2CF_3$ as catalyst and the products were monitored by analytical GPC on Sephadex LH-20. The conditions established for the optimal production of **2a** were then applied in a scaled-up experiment as before, and **2a** and **3a** were isolated by preparative GPC in 46 and 38% yield, respectively.

Conditions could not be found for the selective formation of either **2a** or **2b**. Unreacted **MP** or **TMP** was isolated when the molecular equivalents of Et_3SiH or catalyst were halved.

Sequencing of **2b** *and* **3b** by *FABMS*. — The positive ion spectrum of **3b** showed a major signal for the protonated molecular ion at m/z 650 and a signal at m/z 255 for a "nonreducing" terminal HexNAc group containing one free hydroxyl group (HO-HexNAc). The positive ion spectrum of **2b** contained signals for the protonated and sodium-cationised molecular ion at m/z 863 (Hex higher than **3b**) and 885, respectively. A major fragment ion at m/z 468 corresponded to HO-Hex–HexNAc and arose from an A-type cleavage at HexNAc. Further evidence for the sugar sequence was provided by FAB monitoring of a time-course methanolysis of **2b** [12]. The FAB spectrum of the product after methanolysis for 20 min at 60°C contained major signals at m/z 514, 650, and 727 which corresponded to the molecular ion of HO-HexNAc–HexU-methyl glycoside, HO-HexNAc–HexU–APent (where APent is anhydropentitol), and HO-Hex–HexNAc–HexU-methylglycoside. These data indicated that HO-Hex and APent are the two terminal residues in **2b**. The FABMS data establish the sequence of **2b** as HO-Hex–HexNAc–HexU–APent and that of **3b** as HO-HexNAc–HexU–APent.

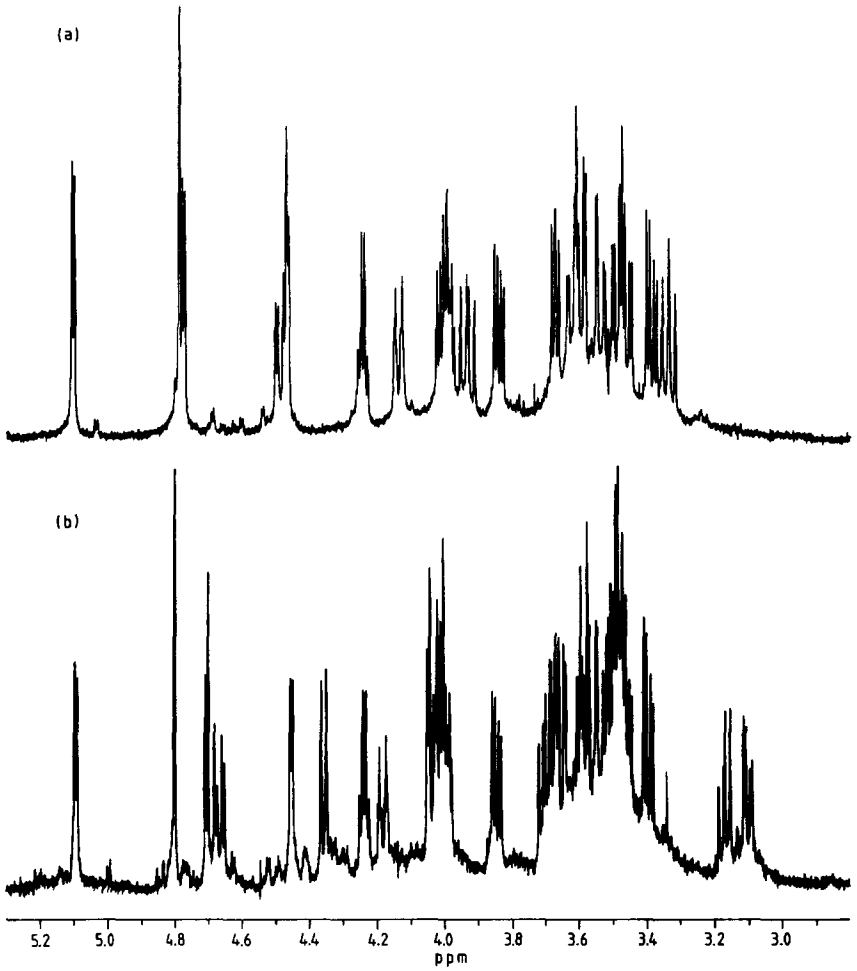


Fig. 1. Partial ^{1}H NMR spectra of **3b** (a) and **2b** (b).

NMR analysis of **2b** *and* **3b**.—The ^{1}H NMR spectra of **3b** and **2b** are shown in Figs. 1a and 1b, respectively. The chemical shifts of the ^{1}H resonances for each of the residues in **3b** and **2b** were established by COSY experiments and are collected in Table 1. ^{13}C Chemical shifts were also determined for **3b** from a $^{1}H-^{13}C$ correlation (HETCOR) experiment [13] and a 1D ^{13}C spectrum, and are shown in Table 2. The $^{1}H-^{1}H$ coupling constants were measured from resolution-enhanced 1D spectra.

The ^{1}H NMR data clearly establish the identity of the residues in **2b** and **3b**, and the configurations of their glycosidic linkages. They also establish the 1,4-anhydroribitol termini of the oligosaccharide-alditols. The positions of substitution of the residues are, however, not immediately apparent. These and the sequence of the residues in the oligomers can readily be established by further NMR experiments such as an HMBC [14] or a 2D NOE [15] experiment. However, since the sequence of the residues in **2b**

Table 1
Comparison of ^{1}H NMR data [a] for residues in **2b** and **3b** with methyl glycosides

Residue		H-1a	H-1b	H-2	H-3	H-4	H-5a	H-5b	H-6a	H-6b
β-Gal *p*-OMe [b]	δ	4.31		3.50	4.64	3.92	3.68		3.70	3.75
	^3J	8.0		10.0	3.8	0.8	7.6,4.4		−11.2 *	
$(CD_3)_3$-β-Gal *p*	δ	4.36		3.17	3.10	4.05	3.70		3.49	3.59
(2b)	^3J	7.4		9.5	3.3	<1	6.4,4.8		−9.5 *	
	Δδ			−0.33	−0.54	0.13	0.02		−0.21	−0.16
α-Gal *p*AMe-OMe [c]	δ	4.92		3.84	3.90	4.35	4.65			
	^3J	3.5		10.0	3.0	1.5				
→ 4)-α-$(CD_3)_2$-Gal *p*ACD_3	δ	5.09		3.46	3.58	4.45	4.80			
(2b)	^3J	3.8		10.3	3.2	1.1				
	Δδ			−0.38	−0.32	0.10	0.15			
→ 4)-α-$(CD_3)_2$-Gal *p*ACD_3	δ	5.10		3.46	3.60	4.47	4.79			
(3b)	^3J	3.3		10.3	2.8	1.1				
	Δδ			−0.38	−0.30	0.12	0.14			
α-Glc *p*NAc-OMe [d]	δ	4.76		3.92	3.71	3.49	3.67		3.78	3.88
	^3J	3.6		10.7	9.8	8.7	2.0,5.2		−12.3 *	
→ 3)-α-$(CD_3)_3$-Glc *p*NAc	δ	4.71		4.67	4.01	3.47	4.18		3.54	3.65
(2b)	^3J	4.0		10.7	9.8	10.2	2.3,2.3		−10.5 *	
	Δδ			0.75	0.30	−0.02	0.51		−0.24	−0.23
α-$(CD_3)_3$-Glc *p*NAc	δ	4.78		4.50	3.94	3.34	4.14		3.55	3.64
(3b)	^3J	3.5		10.7	9.5	10.4	2.0,2.0		−10.5 *	
	Δδ			0.58	0.23	−0.15	0.47		−0.23	−0.24
β-Rib *f*-OMe [e]	δ	4.89		4.02	4.14	4.00	3.59	3.78		
	^3J	0.8		4.6	7.0	3.2,6.4	−12.4 *			
→ 2)-$(CD_3)_2$-1,4ARib	δ	3.84	4.02	4.24	3.67	3.98	3.40	3.50		
(2b)	^3J	9.5,4.7	4.7	4.7	5.6	3.8,4.3	−10.5 *			
	Δδ			0.22	−0.47	−0.02	−0.19	0.28		
→ 2)-$(CD_3)_2$-1,4ARib	δ	3.84	4.02	4.25	3.67	3.98	3.39	3.50		
(3b)	^3J	9.5,4.8	4.8	4.8	5.8	3.8,4.5	−10.5 *			
	Δδ			0.23	−0.47	−0.02	−0.20	−0.28		

[a] Chemical shifts (δ) in ppm and ^{1}H–^{1}H (^3J) coupling constants in Hz. *^2J coupling constants in Hz. For the meaning of Δδ, see text. [b] Values obtained from Ref. 16; corrected for acetone at δ 2.23. [c] Values obtained from Ref. 17. [d] Values obtained from Refs. 18 and 19. [e] Values obtained from Ref. 20.

Table 2
^{13}C chemical shifts [a] for trisaccharide-anhydroalditol **3b**

Residue	C-1	C-2	C-3	C-4	C-5	C-6
→ 4)-α-$(CD_3)_2$-Gal*p*ACD_3	98.1	76.6	78.2	73.9	70.1	172.9
α-$(CD_3)_3$-Glc*p*NAc	99.1	56.4	68.9	80.7	70.9	70.7
→ 2)-$(CD_3)_2$-1,4ARib	70.8	78.0	81.1	79.7	72.6	

[a] Relative to chloroform at 77.0 ppm.

and **3b** is already known from the FABMS experiments, it is only necessary to establish the positions of substitution of the residues. These may be readily gleaned by comparing the ^1H NMR data for **2b** and **3b** with those for the appropriate methyl glycosides [16–20]. Such data are shown in Table 1. The value $\Delta\delta$ represents the ^1H chemical shift difference between a resonance in a methyl glycoside and the equivalent resonance in **2b** or **3b**. A negative difference implies an upfield shift for that resonance in **2b** or **3b**. The data show that the chemical shifts of all the protons attached to carbon atoms carrying OCD_3 groups are shifted upfield. These shifts range from −0.16 to −0.54 ppm with the exception of the H-4 resonance of the 3-linked α-Glc*p*NAc in **2b** which is shifted upfield by only 0.02 ppm. In contrast, the chemical shifts of all the other protons, viz. those attached to carbon atoms which are glycosylated or which carry a hydroxyl group or which are linked to the ring oxygen atom (excluding the anomeric carbon atom) or which are substituted by a deuteriomethylacetamido group, experience downfield shifts which vary from 0.02 to 0.75 ppm. The only exceptions are the H-4 resonances of the 1,4-anhydroribitol residues in **2b** and **3b** which show upfield shifts of 0.02 ppm. Since the ring size of each of the residues in **2b** and **3b** is established from the NMR data and since the location of the amide function is known from the ^{13}C NMR data (Table 2), it follows that C-4 of the β-Gal*p*, C-4 of the α-Gal*p*A, C-3 of the α-Glc*p*NAc, and C-2 of the 1,4-ARib must either be linkage positions or the location of hydroxyl groups. The linkage positions can now be established since it is known from the FABMS data that a free hydroxyl group is present in the HexNAc residue in **3b** and the Hex residue in **2b**. The ^{13}C NMR data for **3b** confirm the presence of a hydroxyl group at C-3 of the α-GlcNAc. All that remains to be established, in order to describe the structure of the repeating unit of *E. coli* K57 capsular polysaccharide, is the anomeric configuration of the linkage of the ribofuranosyl residue. This may be established by a comparison of the ^1H NMR spectra of **2b** and **TMP**. The latter shows a resonance at δ 5.20 ($J_{1,2}$ 0.8 Hz) which is not present in the spectrum of **2b**. This is consistent with the β configuration for the ribofuranosyl residues in the polysaccharide.

Although conditions could not be found for the selective cleavage of the ribofuranosyl linkages in either **MP** or **TMP**, oligosaccharides were nonetheless obtained in sufficiently high yield to make this approach attractive for determining the structures of the repeating units of bacterial polysaccharides. The preparation of the trideuteriomethylated, rather than the methylated, derivative of the polysaccharide ensures that both 1D and 2D NMR spectra of the derived oligosaccharide-anhydroalditols are neither swamped nor complicated by unwanted signals from methoxyl groups. It also preserves the identity of methoxyl groups which may be present in the native polysaccharide.

3. Experimental

General analytical methods.—E. coli K57 capsular polysaccharide was isolated as previously described [11]. The polysaccharide was methylated or trideuteriomethylated using potassium dimsyl [21] and methyl iodide or trideuteriomethyl iodide, respectively, in Me_2SO. Derivatised polysaccharide was purified on a column (1×15 cm) of Sephadex LH-20 gel with chloroform as eluent, and the completeness of the methylation and trideuteriomethylation processes was confirmed by IR spectroscopy. The ^1H and ^{13}C NMR spectra were recorded on a Bruker WM-500 spectrometer, using standard Bruker software. Spectra were recorded in $CDCl_3$ at 303 K and referenced to $CHCl_3$ at δ 7.24 for ^1H and 77.0 ppm for ^{13}C. FABMS was carried out on a VG Analytical ZAB-HF mass spectrometer equipped with an M-Scan FAB gun operated at 10 kV. The spectrum of **2b** was recorded in a thioglycerol matrix and that of **3b** in a matrix of *m*-nitrobenzyl alcohol. FAB monitoring of a time-course methanolysis of **2b** was carried out as previously described [12]. Samples were incubated at 60°C for 40 min and aliquot portions were removed at 2, 10, 20, and 40 min, and analysed.

Reductive cleavage of methylated (**MP**) *and trideuteriomethylated* (**TMP**) E. coli *K57 polysaccharide.—(a) Preliminary experiments.* In order to establish the optimal conditions for the production of oligosaccharide-anhydroalditols, samples of **MP** (5 mg) in CH_2Cl_2 (100 μL) under dry N_2 were treated sequentially with Et_3SiH (25 μL) and Me_3SiOSO_2Me (40 μL), and the reactions were allowed to proceed for 60 min. Aliquot portions were withdrawn at timed intervals and were quenched with MeOH prior to monitoring by TLC. In a second series of experiments, samples of **MP** (5 mg) in CH_2Cl_2 (25 μL) under dry N_2 were treated sequentially with Et_3SiH (10 μL) and $Me_3SiOSO_2CF_3$ (10 μL), and the reactions were allowed to proceed as before. Aliquot portions were withdrawn as before and were examined by GPC on Sephadex LH-20, using MeOH as eluent.

(b) Preparative experiments. **TMP** (51 mg) in CH_2Cl_2 (100 μL) under dry N_2 was treated with Et_3SiH (25 μL) and Me_3SiOSO_2Me (40 μL) for 20 min after which the mixture was quenched with MeOH, de-ionised with Amberlite MB-1 resin (column, 1×15 cm), evaporated to dryness, and separated by TLC on an aluminium-backed Kieselgel 60 F_{254} plate (20×5 cm), using 9:1 $CHCl_3$–MeOH as eluent; **2b** and **3b** were obtained in 16 and 34% yields, respectively. In a second preparative experiment, **MP** (50 mg) in CH_2Cl_2 (250 μL) under dry N_2 was treated with Et_3SiH (100 μL) and $Me_3SiOSO_2CF_3$ (100 μL) for 20 min; the mixture was worked up as before but, in this case, was separated by GPC on Sephadex LH-20 (column, 1.6×70 cm), using MeOH at a flow rate of 4 mL/h as eluent; **2a** and **3a** were isolated in 46 and 38% yields respectively.

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