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Structural studies of the *Escherichia coli* O127 O-antigen polysaccharide

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ABSTRACT

The O-specific side-chain of the lipopolysaccharide from *Escherichia coli* O127a:H$^-$ (O127a:4932-53) has been investigated using 2D NMR spectroscopy, methylation analysis, and partial solvolysis with anhydrous hydrogen fluoride as the principal methods. It is concluded that the polysaccharide is composed of tetrasaccharide repeating-units having the following structure.

→2)-α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→3)-α-D-Gal*p*NAc-(1→

The polysaccharide contains approximately one mole of *O*-acetyl groups per repeating unit distributed over several positions.

INTRODUCTION

When Gram-negative bacteria are shown to possess human blood-group activity, this is often associated with the bacterial O-antigen[1] and some humans may be more prone to infection by these bacteria. The bacterial O-antigen of *Escherichia coli* O86 has high blood-group B activity[1] and the structure of its O-antigen polysaccharide was recently determined[2]. It was shown that the polysaccharide repeating-unit contains the trisaccharide determinant of the human B-type. *E. coli* O127 is an enteropathogenic *E. coli* (EPEC)[3] and has been shown to have blood-group H activity[1], thus it should contain the structural element α-L-Fuc*p*-(1→2)-β-D-Gal*p*. We now report on the structural determination of its O-antigen polysaccharide.

RESULTS AND DISCUSSION

The lipopolysaccharide (LPS) from the *E. coli* O127a:H$^-$ was obtained by phenol–water extraction[4]. The LPS was delipidated with acid under mild conditions to give the polysaccharide (O127 PS). An acid hydrolysate of O127 PS

TABLE I

Chemical shifts (ppm) of the signals in the ^1H and ^{13}C NMR spectra [a] of the *O*-deacetylated *E. coli* O127 O-polysaccharide

Sugar residue	H/C					
	1	2	3	4	5	6
→ 2)-α-L-Fuc*p*-(1 →	5.34 99.17 (174) [n.a.] [d]	3.95 71.59	3.85 70.73	3.69 73.27	4.29 67.68	1.21 16.12
→ 3)-α-D-Gal*p*NAc-(1 →	5.24 99.47 (174) [n.a.]	4.40 48.92	3.96 74.11	4.14 65.54	4.14 [c] 74.19 [c]	~3.77 62.06 [b]
→ 3)-α-D-Gal*p*NAc(1 →	5.10 94.03 (171) [n.a.]	4.27 49.70	3.97 75.66	4.24 69.73	3.77 [c] 71.92 [c]	~3.77 61.78 [b]
→ 2)-β-D-Gal*p*-(1 →	4.62 103.15 (165) [~8]	3.75 76.49	3.79 74.66	3.93 69.95	3.67 [c] 75.66 [c]	~3.77 61.67 [b]

[a] $J_{C\text{-}1,H\text{-}1}$ values in Hz in parenthesis and $J_{H\text{-}1,H\text{-}2}$ values in Hz in brackets. [b] May be interchanged. [c] Tentative assignments. [d] n.a., Not assigned.

contained, *inter alia*, L-fucose, D-galactose, and 2-amino-2-deoxy-D-galactose as the main components in the relative proportions 0.6 : 1 : 3. Mannose was also detected in the analysis, but this is believed to come from the growth media which contained, *inter alia*, yeast extract; no indication of mannose being a constituent of the O127 PS is seen from the NMR spectra. Determination of the absolute configuration of the sugars was performed using a modification of the method developed by Leontein et al.[5]. Methylation analysis of the O127 PS gave 3,4-di-*O*-methyl-L-fucose, 3,4,6-tri-*O*-methyl-D-galactose, and 2-deoxy-4,6-di-*O*-methyl-2-*N*-methylacetamido-D-galactose in the relative proportions 0.4 : 1 : 0.3. The ^1H and ^{13}C NMR spectra of O127 PS showed that the O-antigen contains approximately one mole of *O*-acetyl groups distributed over several positions. The assignments of ^1H and ^{13}C NMR spectra of the *O*-deacetylated O127 PS are given in Table I and the ^{13}C NMR spectrum is shown in Fig. 1. The NMR spectra of *O*-deacetylated O127 PS indicate that it is composed of tetrasaccharide repeating units, and the sugar and methylation analyses together with the NMR spectra show that it contains an L-fucose residue linked through O-2, a D-galactose residue linked through O-2, and two 2-amino-2-deoxy-D-galactose residues linked through O-3. From methylation analysis and spectral data of the *O*-deacetylated O127 PS, i.e., the chemical shifts and coupling constants of anomeric protons and carbons, it is deduced that the sugars are pyranosidic and that three have the α configuration and one has the β configuration. The residue with an anomeric proton resonance at δ 5.34 can, from the chemical shift of its H-6 resonance (δ 1.21), be assigned to the α-L-fucose residue. The residues with anomeric proton resonances at δ 5.24 and 5.10 can be assigned to the 2-amino-2-deoxy-α-D-galactose residues from the

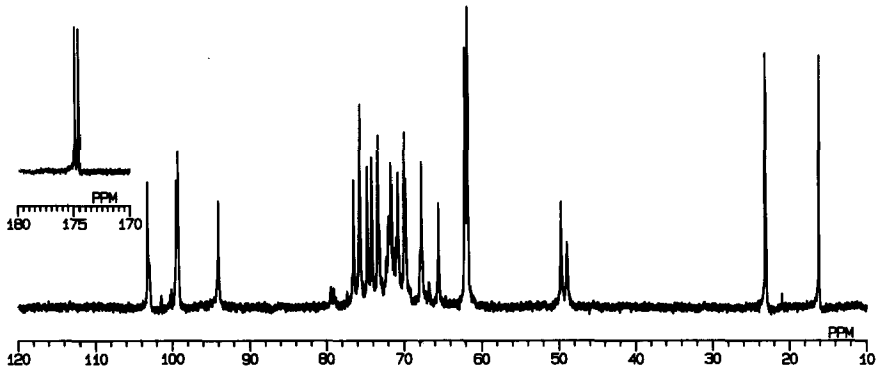

Fig. 1. The ^{13}C NMR spectrum at 67 MHz of the *O*-deacetylated *E. coli* O127 O-polysaccharide.

chemical shifts of their C-2 resonances. The last residue with an anomeric proton resonance at δ 4.62 can thus be assigned to the β-D-galactose residue. The amino sugars are demonstrated to be *N*-acetylated by the presence of signals at δ 2.07 and 2.08 in the ^1H NMR spectrum, and at δ 23.0, 23.2, 174.5, and 174.8 in the ^{13}C NMR spectrum.

The mutual order of the residues was determined from a NOESY experiment. A part of the spectrum is shown in Fig. 2. The anomeric proton (δ 5.34) of the 2-linked α-L-fucose residue showed an intraresidue NOE contact to its H-2 and an interglycosidic NOE to H-2 (δ 3.75) of the 2-linked β-D-galactose residue, thus establishing structural element **1**. All of the above data define the repeating unit **5** (*vide infra*).

$$\rightarrow 2)\text{-}\alpha\text{-L-Fuc}p\text{-}(1 \rightarrow 2)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow$$
$$\mathbf{1}$$

The anomeric proton (δ 5.24) of the 3-linked 2-acetamido-2-deoxy-α-D-galactose residue showed an intraresidue NOE contact to its H-2 and an interglycosidic NOE to H-2 (δ 3.95) of the 2-linked α-L-fucose residue; structural element **2** is therefore defined.

$$\rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{NAc-}(1 \rightarrow 2)\text{-}\alpha\text{-L-Fuc}p\text{-}(1 \rightarrow$$
$$\mathbf{2}$$

The anomeric proton (δ 5.10) of the other 3-linked 2-acetamido-2-deoxy-α-D-galactose residue showed an intraresidue NOE contact to its H-2 and interglycosidic NOEs to H-3 (δ 3.96) and H-4 (δ 4.14) of the 3-linked 2-acetamido-2-deoxy-α-D-galactose residue whose anomeric proton resonates at δ 5.24. Structural element **3** is thus defined.

$$\rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{NAc-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{NAc-}(1 \rightarrow$$
$$\mathbf{3}$$

Structural element **3** is also supported by a γ-gauche effect[6] between H-1 in the first and H-4 in the second of the 3-linked 2-acetamido-2-deoxy-α-D-galactose

residues. This is observed in the chemical shifts of the C-1 signal (δ 94.0) and the C-4 signal (δ 65.5) which show a small chemical shift difference and an upfield chemical shift, respectively, compared to the chemical shifts of the monosaccharide. The anomeric proton (δ 4.62) of the 2-linked β-D-galactose residue showed intraresidue NOE contacts to its H-3 and H-5, and an interglycosidic NOE to H-3 (δ 3.97) of the 3-linked 2-acetamido-2-deoxy-α-D-galactose residue whose anomeric proton signal is at δ 5.10. Thus, structural element **4** is defined.

$$\rightarrow 2)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{NAc-}(1 \rightarrow$$
4

Structural elements **1–4** can be combined into a unique structure which is the repeating unit of the *O*-deacetylated O127 PS and is given by structure **5**.

$$\rightarrow 2)\text{-}\alpha\text{-L-Fuc}p\text{-}(1 \rightarrow 2)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{NAc-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{NAc-}(1 \rightarrow$$
5

In the NOESY spectrum (Fig. 2), the anomeric proton (δ 5.34) of the 2-linked α-L-fucose residue shows an additional NOE, besides the intraresidue NOE contact to its H-2 and the interglycosidic NOE to H-2 (δ 3.75) of the 2-linked β-D-galactose residue, *viz.*, to a proton at δ 4.14. This is the chemical shift of the H-4 and H-5 signals from the 3-linked 2-acetamido-2-deoxy-α-D-galactose residue

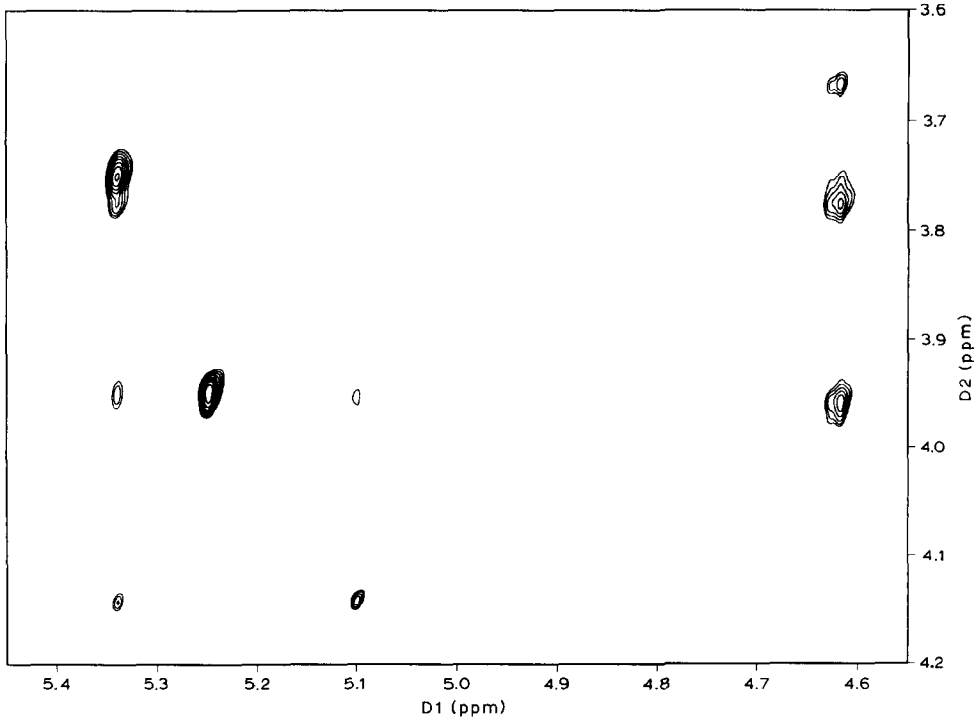

Fig. 2. Part of the NOESY spectrum of *O*-deacetylated O127 PS at 600 MHz, using a mixing time of 150 ms.

whose anomeric proton is at δ 5.24. A *qualitative* interpretation of this NOE contact can easily be obtained by molecular modelling. The energy-minimized pentasaccharide **6**, which to a first approximation can be regarded as a model for the repeating unit of *O*-deacetylated O127 PS, is shown in Fig. 3. The proton–proton distance from H-1 in the fucose residue to H-4 and H-5 in the preceding 2-acetamido-2-deoxy-α-D-galactose residue is 4.9 and 2.5 Å, respectively. This supports the view that, in the NOESY spectrum, the major part of the NOE to H-1 in the fucose residue originates from H-5 rather than from H-4 of the preceding residue.

α-D-Gal*p*NAc-(1 → 3)-α-D-Gal*p*NAc-(1 → 2)-α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc

6

Anhydrous hydrogen fluoride can be used for partial solvolysis[7]. This technique was recently used in the structural determination of the O-antigen polysaccharide from *E. coli* O78 (ref 8). An independent proof of the above structure of the *O*-deacetylated O127 PS was obtained from treatment of *O*-deacetylated O127 PS with anhydrous hydrogen fluoride at −50°C for 30 min. After reduction, an oligosaccharide was isolated by gel permeation chromatography, and methylation analysis showed the presence of 2-linked fucitol, terminal galactose, and 3-linked 2-acetamido-2-deoxygalactose in the relative proportions 2:49:49. Selected ^{1}H NMR chemical shifts are given in Table II and the presence of three anomeric protons shows that the component is a tetrasaccharide-alditol. The FABMS[9] spectrum of the underivatised tetrasaccharide-alditol, obtained in the positive

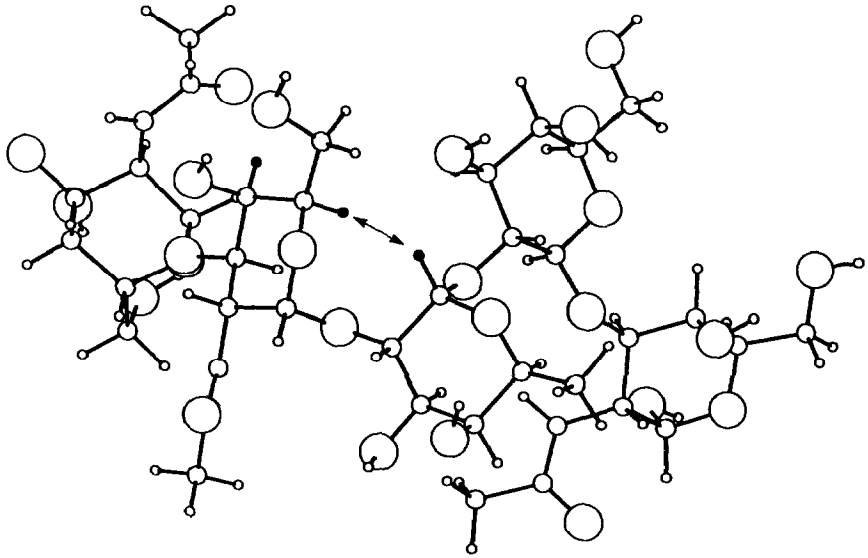

Fig. 3. Energy-minimized structure of pentasaccharide **6**. The proximity between H-1 in the α-L-fucose residue and H-5 in the preceding 2-acetamido-2-deoxy-α-D-galactose residue is indicated by an arrow.

TABLE II

Chemical shifts (ppm) of the signals in the ^{1}H NMR spectrum [a] of tetrasaccharide-alditol 7 derived from HF-solvolysis

Sugar residue	H-1	H-2	H-3	H-4
β-D-Gal*p*-(1 →	4.45	3.53	3.64	3.93
	(−0.08) [b]	(0.08)	(0.05)	(0.04)
→ 3)-α-D-Gal*p*NAc(1 →	5.10	4.44	4.01	4.22
	(−0.18)	(0.25)	(0.06)	(0.17)
→ 3)-α-D-Gal*p*NAc-(1 →	5.10	4.41	3.81	4.26
	(−0.18)	(0.22)	(−0.14)	(0.21)

[a] Recorded at 30°C. [b] Chemical shift differences with reference to free glycopyranose monomers (70°C) are given in parenthesis.

mode, showed a peak at *m/z* 735 attributed to $[M + H]^+$; upon addition of sodium ions, the peak moved to *m/z* 753 attributed to $[M + Na]^+$. A B/E linked scan experiment in the positive mode on *m/z* 735 gave, *inter alia*, the fragments *m/z* 366 (abA), 569 (abcA), 370 (cdB), and 573 (bcdB). These results show that the tetrasacchride-alditol has structure **7**, which is in agreement with structure **5** as the repeating unit of *O*-deacetylated O127 PS.

β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc-(1 → 3)-α-D-Gal*p*NAc-(1 → 2)-L-Fucitol
7

The H-type blood-group activity previously shown by *E. coli* O127 is thus supported by the structural element α-L-Fuc*p*-(1 → 2)-β-D-Gal*p* in the structure of the O-antigen polysaccharide.

EXPERIMENTAL

General methods.—Evaporations were performed under diminished pressure at < 40°C (bath) or by flushing with air. For GLC, a Hewlett–Packard 5890A instrument, fitted with a flame-ionisation detector, was used. Separations were performed on an HP5 fused-silica capillary column, using a temperature program 180°C (1 min) → 250°C at 3°C/min. GLC–MS was performed on a Hewlett-Packard 5790-5970 instrument, using the same phase. Hydrolysis of underivatised material was performed with 2 M HCl at 100°C for 2 h. Methylation analyses were performed as previously described[10,11]. The absolute configurations of fucose, galactose, and 2-acetamido-2-deoxygalactose were performed, essentially as described by Leontein et al.[5], by GLC of their glycosides, using optically active 2-butanol. A differential refractometer was used for monitoring the gel chromatography effluents. FABMS spectra in the positive mode were recorded on a JEOL SX 102 instrument, using Xe atoms (6 kV) and a matrix of glycerol, at a resolution of 1000. The B/E linked scan experiment used He as collision gas. Molecular

modelling was performed using Quanta/CHARMm[12] and the forcefield developed by Ha et al.[13].

NMR spectroscopy.—NMR spectra of solutions in D_2O were recorded at 70°C unless otherwise stated, using a JEOL GSX-270, JEOL ALPHA-400, or Varian Unity 600 instrument. Chemical shifts are reported in ppm relative to sodium 3-trimethylsilylpropanoate-d_4 (δ_H 0.00) and acetone (δ_C 31.07) as internal references. Double quantum COSY, relayed COSY, 2D-HOHAHA, and C,H-COSY experiments were used to assign signals, and were performed according to standard pulse sequences. The NOESY experiment used a mixing time of 150 ms.

Partial solvolysis of O127 PS.—The polysaccharide (22 mg) was treated with anhyd HF (2 mL) at −50°C for 30 min and the product was isolated by precipitation with Et_2O. After hydrolysis of the glycosyl fluorides with aq 50% AcOH at room temperature for 1 h and reduction of the material with $NaBH_4$, the mixture was fractionated on a Bio-Gel P-2 column. An oligosaccharide (3 mg) was isolated in the tetrasaccharide region.

Bacterial strain.—*E. coli* O127a:H$^-$ (O127a:4932-53) was obtained from the International Escherichia and Klebsiella Centre at Statens Seruminstitut, Copenhagen, Denmark.

Isolation and purification of the O-polysaccharide.—*E. coli* O127a:H$^-$ bacteria were grown in Ty medium (15-L culture). Bacteria were killed by the addition of formaldehyde (1% final concentration) and harvested by ultrafiltration. Lipopolysaccharide (LPS) was extracted by the hot phenol–water method[4]. The LPS was treated with aq 2% AcOH (pH 3.1) at 100°C for 2 h. Liberated lipid A was centrifuged, and the supernatant solution was neutralised, dialysed, and lyophilised. The product was further purified by column chromatography on a column (2.6 × 90 cm) of Bio-Gel P-10.

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