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Note

Structure of the O-polysaccharide of *Escherichia coli* O112ab containing L-iduronic acid

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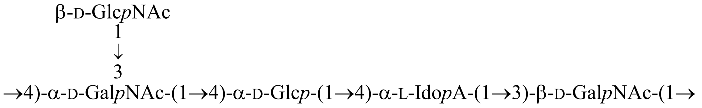
Abstract—An acidic O-polysaccharide was isolated by mild acid degradation of the lipopolysaccharide of *Escherichia coli* O112ab and studied by sugar analysis along with ^{1}H and ^{13}C NMR spectroscopy. The O-polysaccharide was found to contain a rarely occurring sugar component, L-iduronic acid (L-IdoA), and the following structure of the branched pentasaccharide repeating unit was established:



$$\begin{array}{l} \qquad\quad \beta\text{-D-Glc}p\text{NAc} \\ \qquad\qquad\quad 1 \\ \qquad\qquad\quad \downarrow \\ \qquad\qquad\quad 3 \\ \rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{NAc-}(1\rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-L-Ido}p\text{A-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{NAc-}(1\rightarrow \end{array}$$

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Keywords: Escherichia coli; O-polysaccharide; O-antigen; Bacterial polysaccharide structure; L-Iduronic acid; NMR spectroscopy

Escherichia coli is an important component of the human intestinal microflora and one of the most common causes of diarrhoeal diseases. *E. coli* strains are designated as O:K:H serotypes, where O refers to the O-antigen. Structural data on the O-antigens (O-polysaccharides) provide the molecular basis for classification of *E. coli* strains and are necessary for understanding the O-antigen genetics and biosynthesis pathways. *E. coli* species has at least 166 O-antigen forms, and the structures of only about half of them have been elucidated.[1–14] Now we report on a new structure of the O-polysaccharide (OPS) of *E. coli* O112ab containing a rarely occurring acidic sugar, L-iduronic acid.

The OPS was obtained by mild acid degradation of the lipopolysaccharide isolated from dried cells of *E. coli* O112ab by the phenol–water procedure.[15] It was separated from low-molecular-mass compounds, including a core oligosaccharide, by GPC on Sephadex G-50. GLC analysis of alditol acetates after full acid hydrolysis of the OPS revealed Glc, GlcN, and GalN in the ratios ∼1:1:2, respectively. Analysis of uronic acids in the OPS hydrolysate using a sugar analyzer demonstrated an acidic monosaccharide, which has a retention time different from those of glucuronic, mannuronic, galacturonic, and guluronic acids but identical to that of iduronic and altruronic acid, which could not be separated on the Dionex A × 8 anion-exchange resin used (see also published data[16]). These two components could be

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separated as acetylated methyl esters methyl glycosides, the derivatives from the OPS being identical in GLC–MS to those from iduronic acid and different from the derivatives from altruronic acid. Hence, the OPS contains iduronic acid (IdoA), which was also confirmed by NMR spectroscopy data given below. GLC analyses of the acetylated (*S*)-2-(+)-octyl glycosides demonstrated the D configuration of Glc, GlcN, and GalN. The L configuration of IdoA was determined by the analysis of the ^{13}C NMR chemical shifts in the OPS using known regularities in glycosylation effects (see below).

The ^{13}C NMR spectrum of the OPS (Fig. 1) showed signals for five monosaccharide residues, including those for five anomeric carbons at δ 97.6–104.9, four HO*C*H$_2$-C groups (C-6 of monosaccharides) at δ 61.8–62.6, three nitrogen-bearing carbons (C-2 of amino sugars) at δ 50.2–57.2, 17 oxygen-bearing sugar ring carbons in the region δ 69.7–81.0, four CO groups, including those of three NAc groups and C-6 of IdoA at δ 175.0–176.5, and three CH$_3$ groups of NAc at δ 23.6–23.8. The ^{1}H NMR spectrum of the OPS contained signals for six protons in a low-field region of δ 4.55–5.30, including those for five anomeric protons and H-5 of IdoA, three NAc groups at δ 2.03–2.07, and other signals at δ 3.44–4.45. These data indicate that the OPS has a pentasaccharide repeat containing one residue each of D-Glc, D-GlcNAc, L-IdoA, and two residues of D-GalNAc.

The ^{1}H and ^{13}C NMR spectra of the OPS were assigned using 2D COSY, TOCSY, ROESY, proton-detected ^{1}H,^{13}C HSQC, and HMQC–TOCSY experiments (Table 1). The TOCSY spectrum demonstrated correlations of H-1 with H-2 to H-6 for the *gluco*-configurated monosaccharides (Glc and GlcNAc), H-1 with H-2 to H-4 for the *galacto*-configurated monosaccharides (GalNAc$^{\mathrm{I}}$ and GalNAc$^{\mathrm{II}}$), and H-1 with H-2 to H-5 for IdoA. The assignment for the GalNAc residues was completed making use of correlations of H-4 with H-5 and H-6b for GalNAc$^{\mathrm{I}}$ and H-4 with H-5 for GalNAc$^{\mathrm{II}}$ in the ROESY spectrum as well as H-6 with C-6 for both in the HSQC spectrum. Identification of the spin systems of GlcNAc, GalNAc$^{\mathrm{I}}$, and GalNAc$^{\mathrm{II}}$ was confirmed by correlations of protons at the nitrogen-bearing carbons with the corresponding carbons at δ 3.82/57.2, 4.20/50.2, and 4.14/52.7. The *ido* configuration of the uronic acid was corroborated by ^{3}J coupling constants, which were all small (<4 Hz) and thus enabled excluding all configurations but *ido* and *talo*.

Relatively small $J_{1,2}$ coupling constant values of <4 Hz indicated that Glc and GalNAc$^{\mathrm{I}}$ are α-linked, whereas significantly larger $J_{1,2}$ values of 7–8 Hz showed that GlcNAc and GalNAc$^{\mathrm{II}}$ are β-linked. The α configuration of IdoA was inferred based on a strong intra-residue H-1,H-2 correlation at δ 5.04/3.63 with no significant H-1,H-5 correlation in the ROESY spectrum and confirmed by the C-5 chemical shift of δ 69.7 (compare published data δ 68.6). The pyranose form of all monosaccharides was confirmed by the absence from the ^{13}C NMR spectrum of any signals for non-anomeric sugar carbons at a lower field than δ 81.[17]

Relatively low-field positions of the signals for C-3 and C-4 of GalNAc$^{\mathrm{I}}$, C-3 of GalNAc$^{\mathrm{II}}$, C-4 of Glc, and C-4 of IdoA at δ 77.7, 75.8, 81.0, 76.7, and 76.0, respectively, in the ^{13}C NMR spectrum of the OPS, as compared with their positions in the corresponding

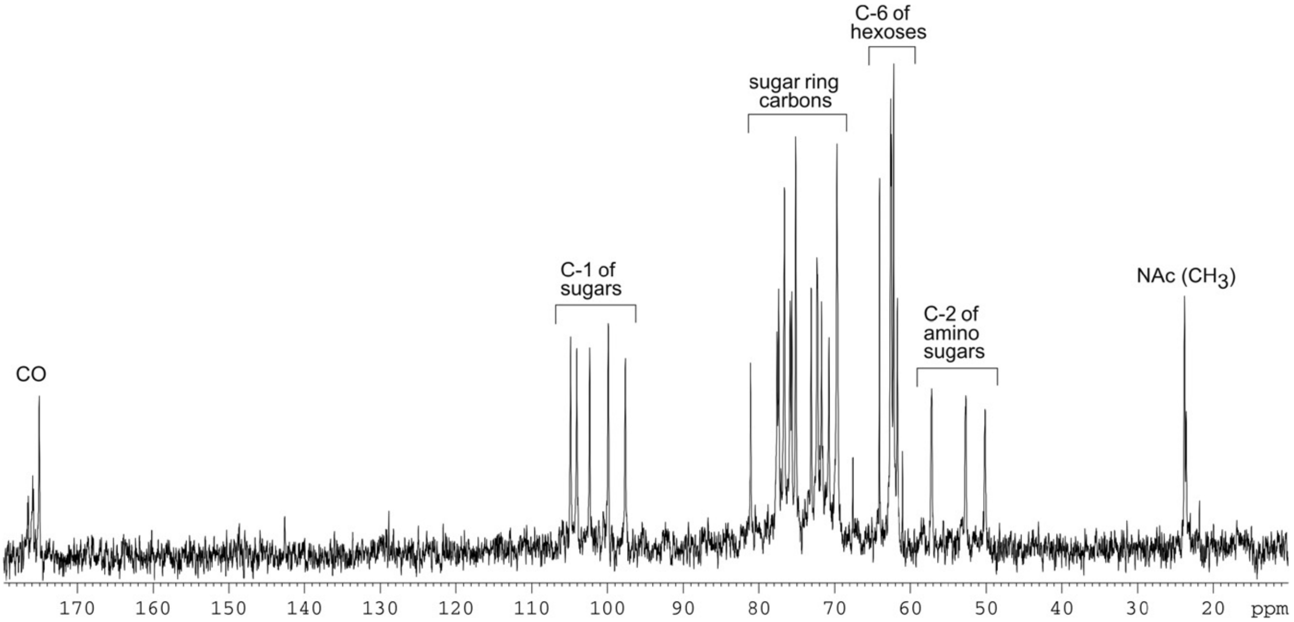


Figure 1. ^{13}C NMR spectrum of the OPS from *E. coli* O112ab.

Table 1. ^{1}H and ^{13}C NMR data of the OPS from *E. coli* O112ab (δ, ppm)

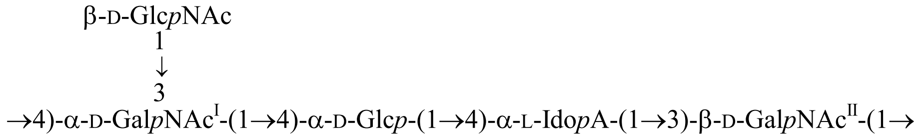
Sugar residue	Nucleus	1	2	3	4	5	6 (6a,6b)
β-D-Glc*p*NAc-(1→	^{1}H	4.55	3.82	3.55	3.44	3.45	3.79; 3.94
	^{13}C	104.1	57.2	75.2	71.8	77.4	62.5
→3,4)-α-D-Gal*p*NAc^{I}-(1→	^{1}H	5.30	4.20	4.00	4.45	4.06	3.78; 3,81
	^{13}C	99.9	50.2	77.7	75.8	72.3	61.8
→4)-α-D-Glc*p*-(1→	^{1}H	5.20	3.50	3.83	3.62	3.75	3.70; 3.81
	^{13}C	97.6	73.1	75.2	76.7	72.3	62.2
→4)-α-L-Ido*p*A-(1→	^{1}H	5.04	3.63	3.99	4.15	4.75	
	^{13}C	104.9	70.9	69.7	76.0	69.7	175.0
→3)-β-D-Gal*p*NAc^{II}-(1→	^{1}H	4.97	4.14	3.86	4.00	3.67	3.75; 3.77
	^{13}C	102.4	52.7	81.0	69.7	76.7	62.6

The chemical shifts for NAc are δ_{H} 2.03 (6H) and 2.07 (3H); δ_{C} 23.6, 23.8 (2C) (all Me), 175.0, 175.8, and 176.5 (all CO).

non-substituted monosaccharides,[17–19] demonstrated the modes of monosaccharide glycosylation. A 2D ROESY experiment revealed interresidue cross-peaks between the anomeric protons and protons at the linkage carbons at δ 4.55/4.00; 5.30/3.62; 5.20/4.15; 5.04/3.86, and 4.97/4.45, which were assigned to GlcNAc H-1,GalNAc^{I} H-3; GalNAc^{I} H-1,Glc H-4; Glc H-1, IdoA H-4; IdoA H-1,GalNAc^{II} H-3, and GalNAc^{II} H-1, GalNAc^{I} H-4 correlations, respectively. These data are in agreement with the glycosylation pattern revealed by the ^{13}C NMR chemical shift data and established the sequence of the monosaccharide residues in the repeating unit of the OPS. In addition to the Glc H-1,IdoA H-4 cross-peak, a strong Glc H-1,IdoA H-3 cross-peak was observed in the ROESY spectrum, which indicates the equatorial orientation of IdoA H-3[20] and, hence, the $^{1}C_{4}$ conformation of L-IdoA. This finding enabled also excluding the *talo* configuration of the uronic acid as L-TalA exists in the $^{1}C_{4}$ conformation with the axial H-3.

The absolute configuration of IdoA was determined by analysis of the glycosylation effects on ^{13}C NMR chemical shifts and NOE correlations in IdoA-containing disaccharide fragments of the OPS. The β-effect on C-4 of GalNAc^{II} in the α-Ido*p*A-(1→3)-D-Gal*p*NAc^{II} disaccharide was positive (0.6 ppm) and no significant IdoA H-1,GalNAc^{II} H-4 cross-peak was observed in the ROESY spectrum. These features are characteristic of different absolute configurations of IdoA and GalNAc (in case of the same absolute configuration, the β-effect on C-4 of GalNAc^{II} would be negative and >2 ppm by the absolute value and a NOE on H-4 of GalNAc^{II} would not be weaker than on H-3 at the linkage carbon).[20,21] Furthermore, as typical of different absolute configurations of the monosaccharide constituents in the α-D-Glc*p*-(1→4)-Ido*p*A disaccharide,[21] the effect on C-1 of Glc was relatively small (4.3 ppm) and the Glc H-1,IdoA H-3 cross-peak in the ROESY spectrum was about twice as intense as the Glc H-1,IdoA H-4 cross-peak. Therefore, as GalNAc^{II} and Glc have the D configuration, IdoA has the L configuration. To the best of our knowledge, L-iduronic acid has been previously found only once in lipopolysaccharides, namely in the O-polysaccharide of a marine bacterium *Pseudoalteromonas haloplanktis* KMM 223 (44-1).[16] Iduronic acid of unknown absolute configuration has been reported as a component of surface polysaccharides of *Clostridium perfringens*.[22,23]

Based on the data obtained, it was concluded that the OPS of *E. coli* O112ab has the following structure:



$$\begin{array}{l} \qquad\qquad\beta\text{-D-Glc}p\text{NAc} \\ \qquad\qquad\qquad 1 \\ \qquad\qquad\qquad \downarrow \\ \qquad\qquad\qquad 3 \\ \rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{NAc}^{I}\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-L-Ido}p\text{A-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{NAc}^{II}\text{-}(1\rightarrow \end{array}$$

This structure shows a significant similarity to that of the O-polysaccharide of *Shigella dysenteriae* type 2,[24] which, according to our preliminary data, requires a revision.

1. Experimental

1.1. Bacterial strain and isolation of the lipopolysaccharide

E. coli O112ab type strain G1250 was obtained from the Institute of Medical and Veterinary Science (IMVS), Adelaide, Australia. Bacteria were grown to late log phase in 8 L of LB using a 10-L fermentor (BIOSTAT C-10, B. Braun Biotech International, Germany) under constant aeration at 37 °C and pH 7.0. Bacterial cells were washed and dried as described.[25]

The lipopolysaccharide (300 mg) was isolated from dried cells (13.8 g) by the phenol–water method[15] and purified by precipitation of nucleic acids and proteins using aq 50% CCl_3CO_2H as described.[26]

1.2. Degradation of the lipopolysaccharide

Delipidation of the lipopolysaccharide (145 mg) was performed with aq 2% HOAc (6 mL) at 100 °C until precipitation of lipid A. The precipitate was removed by centrifugation (13,000*g*, 20 min), and the supernatant fractionated by GPC on a column (56 × 2.6 cm) of Sephadex G-50 (S) (Amersham Biosciences, Sweden) in 0.05 M pyridinium acetate buffer, pH 4.5 monitored with a Knauer differential refractometer (Germany). A high-molecular-mass OPS was obtained in a yield of 16% of the lipopolysaccharide weight.

1.3. Chemical analyses

The OPS was hydrolysed with 2 M CF_3CO_2H (120 °C, 2 h). Uronic acids were analysed using a Biotronik LC-2000 sugar analyser (Germany) on a column (0.4 × 15 cm) of Dionex A × 8 anion-exchange resin at 70 °C using 0.04 M sodium phosphate buffer, pH 2.4. Neutral and amino sugars were identified by GLC of the alditol acetates[27] on a Hewlett–Packard 5890 chromatograph equipped with an Ultra-1 capillary column (Supelco) using a temperature gradient of 150–290 °C at 5 °C min^{-1}. For identification of iduronic acid, the OPS hydrolysate was subjected to methanolysis with 1 M HCl in MeOH at 80 °C for 16 h, products were acetylated and analysed by GLC–MS on a Hewlett–Packard model 5890 Series II instrument equipped with a SPB-5 capillary column (Supelco) using a temperature gradient of 150–320 °C at 5 °C min^{-1}. The authentic samples of IdoA and AltA were derived from the O-polysaccharides of *Pseudoalteromonas haloplanktis* KMM 223 (44-1)[16] and *Proteus mirabilis* O10,[28] respectively. The absolute configurations of the monosaccharides were determined by GLC of the acetylated (*S*)-2-octyl glycosides as described.[29]

1.4. NMR spectroscopy

Samples were deuterium-exchanged by freeze-drying twice from D_2O and then examined as solutions in 99.96% D_2O at 35 °C. NMR spectra were recorded on a Bruker DRX-500 spectrometer (Germany) using internal acetone (δ_H 2.225, δ_C 31.45) as standard. 2D NMR spectra were obtained using standard Bruker software, and Bruker XWINNMR 2.6 program was used to acquire and process the NMR data. Mixing times of 200 and 100 ms were used in TOCSY and ROESY experiments, respectively.

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