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Heterogeneous Structure of O-Antigenic Part of Lipopolysaccharide of *Salmonella* Telaviv (Serogroup O:28) Containing 3-Acetamido-3,6-dideoxy-D-glucopyranose*

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Abstract—The O-polysaccharide of *Salmonella* Telaviv was obtained by mild acid degradation of the lipopolysaccharide and studied by chemical methods (sugar and methylation analyses, Smith degradation, de-*O*-acetylation) and NMR spectroscopy. The structure of the O-polysaccharide was established. The repeating units that are proximal to the lipopolysaccharide core region mostly have a digalactose side chain and lack glucose, whereas those at the other end of the chain mostly do bear glucose but are devoid of the disaccharide side chain. This is the first structure established for the O-polysaccharide of a *Salmonella* serogroup O:28 (formerly M) strain characterized by subfactors $O28_1$ and $O28_2$. Knowledge of this structure and the structure of the O-polysaccharide of *Salmonella* Dakar ($O28_1$, $O28_3$) established earlier is crucial for determination of the exact structures associated with subfactors $O28_1$, $O28_2$, and $O28_3$ and elucidation of the genetic basis of the close relationship between *Escherichia coli* O71 and *S. enterica* O:28 O-antigens.

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Key words: *Salmonella* Telaviv, O-antigen, structural analysis, 3-acetamido-3,6-dideoxy-D-glucose

The genus *Salmonella* is a typical representative of the family Enterobacteriaceae. It consists of 2579 serologically distinct types listed in the Kauffmann–White scheme, later modified by Popoff and Le Minor [1]. The serotyping of *Salmonella* strains is based on the immunoreactivity of three different types of antigens: somatic O, flagellar H, and capsular Vi [2, 3]. The somatic O-antigen (O-polysaccharide, OPS), together with the core region, is the polysaccharide chain of the lipopolysaccharide (LPS), which is a major component of the outer membrane of smooth-type Gram-negative bacteria [4]. The OPS protrudes from the bacterial cell to the environment and consists of several (up to about 20) repeating units usually made up of 2-8 sugar residues.

Variations among O-antigen structures are important not only for the serological classification of *Salmonella* but are also connected to pathogenicity [5, 6]. *Salmonella* strains figure predominantly as one of the leading causes of bacterial food-borne diseases [7] commonly described as salmonellosis. The *Salmonella* serotyping is essential for human disease surveillance and outbreak detection [8] as well as for the monitoring of the microorganism in food [9]. Knowledge of O-antigen structures is crucial for identifying the antigenic factors involved in the serological specificity and uncovering phylogenetic relations between Gram-negative bacteria [10-12].

Salmonella enterica subsp. *enterica* serovar Telaviv (*S.* Telaviv) has been classified in serogroup O:28 (formerly M) of the Kauffmann–White scheme [1]. Strains from this serogroup (107 serovars) are characterized by epitope O28, which is divided into three subfac-

Abbreviations: LPS, lipopolysaccharide; OPS, O-polysaccharide.

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tors: $O28_1$, $O28_2$, and $O28_3$ [3]. *S.* Telaviv has subfactors $O28_1$ and $O28_2$, whereas *S.* Dakar from the same serogroup is characterized by subfactors $O28_1$ and $O28_3$. As yet, the structural information about the LPSs and OPSs of bacteria belonging to serogroup O:28 is limited [2, 3, 13], and structural differences between the O28 subfactors have not been elucidated. The structure of the OPS of *S.* Dakar has only recently been established [14].

Salmonella Telaviv cross-reacts serologically with *S.* Mara (serogroup O:39) and *Citrobacter freundii* 869 but, as opposite to *S.* Dakar, not with *C. freundii* 8090 [13]. A close relationship between *Escherichia coli* O71 and *Salmonella* O28 O-antigens (represented by *S.* Dakar) has also been demonstrated [11]. The serogroup-specific genes of *E. coli* O71 and *S. enterica* O28 have been identified, and a structural similarity of the O-antigens of *E. coli* O71 and *S.* Dakar has been documented. Although *S.* Dakar ($O28_1$, $O28_3$) and *S.* Pomona ($O28_1$, $O28_2$) belong to the same O serogroup, their O-antigen gene clusters are quite different [12]. These finding and differences in the immunological behavior of *S.* Dakar and *S.* Telaviv suggested that their OPSs should be structurally different too. This paper describes elucidation of the O-antigen structure of *S.* Telaviv, which was found to be distinct from that of *S.* Dakar indeed.

MATERIALS AND METHODS

Bacterial strain, cultivation of bacteria, and isolation of the lipopolysaccharide. *Salmonella enterica* subsp. *enterica* serovar Telaviv (I 28:y:e,n,z_{15}), strain KOS No. 106, was obtained from the National Salmonella Centre of Poland, KOS collection (Gdansk, Poland). Cultivation of bacteria, isolation of the LPS, and purification from nucleic acids were performed as described for *S.* Dakar [14], and a pure LPS preparation (213 mg) was obtained.

Isolation of the OPS from the LPS. An LPS sample was hydrolyzed with 1% CH_3CO_2H and, following removal of the precipitated lipid A, the water-soluble products were fractionated by gel-permeation chromatography (GPC) on a Bio-Gel P-10 (200-400 mesh; BioRad, USA) column (100 × 0.9 cm) in pyridine–acetic acid buffer (pyridine–CH_3CO_2H–water 2 : 5 : 493 v/v) at a flow rate of 3.6 ml/h and monitoring using a RIDK 101 differential refractometer (Czech Republic). As a result, a high-molecular-mass OPS was obtained.

The products of hydrolysis of the LPS were fractionated also by GPC on a Bio-Gel P-100 (200-400 mesh) column using water as mobile phase at a flow rate of 4.6 ml/h and monitoring with a RI 2300 differential refractometer (Knauer, Germany) to give three fractions differing in molecular mass (OPSI, OPSII, and OPSIII).

Structure of the OPS was studied according to the scheme presented in Fig. 1, and experimental details are given below.

Sugar analysis was carried out for OPS, its fractions, and the major Smith degradation product from the OPS. Briefly, samples were hydrolyzed with 2 M CF_3CO_2H (120°C, 2 h), and the resulting monosaccharides were analyzed by GLC as the alditol acetates on a Clarus 500 (Perkin Elmer Instruments, USA) gas chromatograph equipped with a Rtx-5 capillary column (30 m, 0.25 mm ID, 0.25 μm film thickness; Restek Co., USA) using a linear temperature program from 120 to 270°C at 2°C/min followed by 20 min at 270°C. GLC–mass spectrometry analysis was performed using an SSQ710 instrument (Finnigan MAT, UK) with electron impact ionization energy of 70 eV, coupled with a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, USA).

Absolute configuration of sugars was determined by GLC of the acetylated 2-butyl glycosides according to the published method [15, 16]. The OPS was hydrolyzed with 2 M CF_3CO_2H as above, *N*-acetylated (400 μl 1% aqueous $NaHCO_3$, 100 μl $(CH_3CO)_2O$, 20°C, 1 h), subjected to 2-butanolysis (400 μl (*S*)-(+)-2-butanol, 35 μl CF_3CO_2H, 6 h, 105°C), and acetylated (400 μl $(CH_3CO)_2O$, 0.5 mg CH_3CO_2Na, 120°C, 2 h). GLC was performed on a DB-23 capillary column (60 m, 0.3 mm ID, 0.15 μm; J & W Scientific, USA) using a temperature program from 120 to 256°C at 2°C/min followed by 30 min at 256°C. The GLC peaks were assigned by co-injection with the authentic samples and the corresponding derivatives obtained from the OPS of *S.* Dakar [14].

Methylation analysis. Methylation of the OPS, its fractions, and the oligosaccharide obtained by Smith degradation of the OPS was performed according to the Hakomori procedure [17]. The permethylated products were purified by dialysis on a microscale against distilled water and then recovered using a Sep-Pak C18 cartridge [18]. Partially methylated monosaccharides were derived by hydrolysis with 2 M CF_3CO_2H, converted into the alditol acetates as above and analyzed by GLC and GLC–mass spectrometry using a Rtx-5 capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness; Restek) and a temperature program from 100 to 260°C at 4°C/min followed by 20 min at 260°C.

De-*O*-acetylation of the OPS. An OPS sample (7 mg) was treated with 1.4 ml 12% aqueous ammonia (25°C, 18 h), dialyzed against water (molecular mass cut off 1.0 kDa; Spectrum Laboratories, USA), and lyophilized.

Smith degradation. An OPS sample was oxidized with 40 mM $NaIO_4$ (Lancaster, Germany) in the dark at 4°C for 120 h according to the Pritchard and Rener procedure [19]. The reaction was terminated by adding ethylene glycol, and the product was dialyzed against distilled water (molecular mass cut off 1.0 kDa; Spectrum Laboratories) and lyophilized. After reduction with $NaBH_4$, the material was dialyzed again, lyophilized, and hydrolyzed with 2% CH_3CO_2H (100°C, 2 h). The solution was cooled and evaporated to dryness under reduced

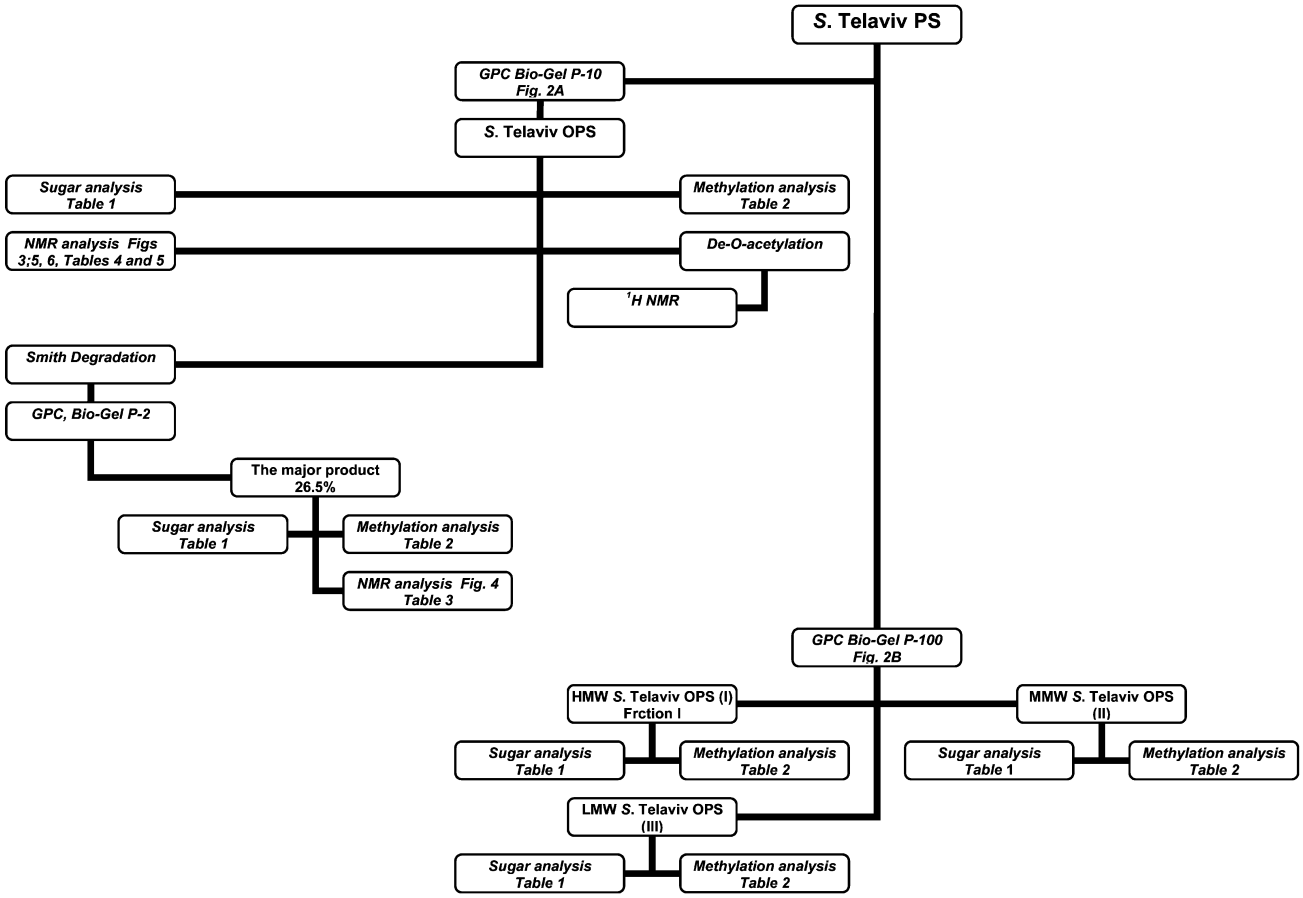


Fig. 1. Flow diagram of the structural study of the O-polysaccharide of *S.* Telaviv.

pressure; small portions of methanol were added before repeated evaporation. The main oligosaccharide fraction was isolated by GPC on a Bio-Gel P-2 (200-400 mesh) column (100 × 0.9 cm) in pyridine-acetic acid buffer at a flow rate of 3.3 ml/h.

NMR spectroscopy. Samples of the OPS, de-*O*-acetylated OPS (18.0 mg), and the major Smith degradation product (2.2 mg) were lyophilized twice from D_2O, and then dissolved in 0.7 ml of 99.95% D_2O (Deutero GmbH, Germany). NMR spectra were recorded with a Varian Mercury 400 MHz spectrometer (Varian, USA) at 50°C using internal acetone (δ_H 2.225, δ_C 31.45) as reference. In addition to one-dimensional ^1H and ^{13}C NMR spectra, the following two-dimensional NMR experiments were carried out: correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear single-quantum coherence (^1H,^{13}C HSQC), heteronuclear multiple-bond correlation (^1H,^{13}C HMBC), and nuclear Overhauser enhancement spectroscopy (NOESY). Mixing times of 100 and 300 msec were used in the TOCSY and NOESY experiments, respectively. In addition, ^1H,^{13}C HSQC-TOCSY and HMQC-TOCSY experiments were performed for the OPS on a Bruker 500 MHz spectrometer.

RESULTS AND DISCUSSION

The LPS was extracted using the phenol/water method from *Salmonella* Telaviv cells grown on an enriched agar medium containing glucose. Mild acid hydrolysis of the purified LPS afforded a lipid sediment (18.5% of LPS) and a water-soluble carbohydrate portion, which was fractionated by GPC on Bio-Gel P-10 to give a high-molecular-mass OPS (63.1% of the polysaccharide fraction). The asymmetric pattern of the chromatographic peak of the OPS suggested heterogeneity of the product (Fig. 2a). Therefore, the water-soluble substance was fractionated by GPC on Bio-Gel P-100 (Fig. 2b) to give high- (OPSI), medium- (OPSII), and low-molecular-mass (OPSIII) fractions (6.77, 9.72, and 44.98% of the polysaccharide fraction, respectively).

Monosaccharide analysis by GLC of the alditol acetates obtained after complete acid hydrolysis of the OPS (Table 1) revealed five sugars: ribose (Rib), 3-amino-3,6-dideoxyglucose (Qui3N), glucose (Glc), galactose (Gal), and galactosamine (GalN), in the respective molar ratios 0.73 : 0.92 : 1.0 : 1.90 : 0.94. The presence of these sugars in the *S.* Telaviv LPS has been reported earlier by Luderitz et al. [13] but their ratios have not been estab-

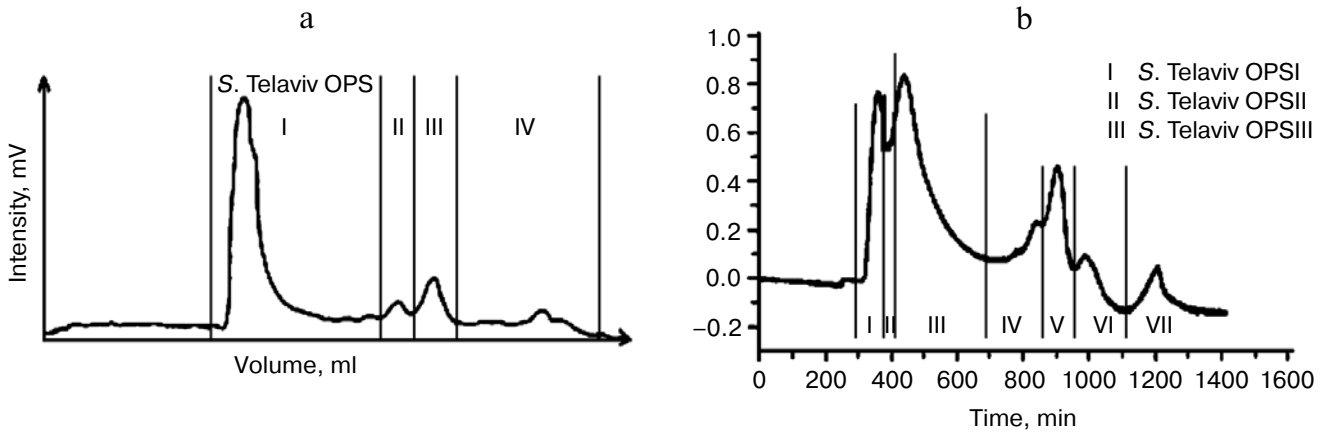


Fig. 2. Gel chromatography profiles on Bio-Gel P-10 (a) and Bio-Gel P-100 (b) of the water-soluble carbohydrate products obtained by mild acid degradation of the LPS of *S.* Telaviv.

lished. Determination of the absolute configuration by GLC of the acetylated glycosides with a chiral alcohol [15, 16] indicated that all the sugars have the D configuration.

Methylation analysis of the OPS, including GLC of the partially methylated alditol acetates, revealed derivatives from 3-substituted Rib, terminal Glc, terminal, 4-substituted, 3-substituted and 3,4-disubstiuted Gal, 4-substituted Qui3N, 3-substituted and 3,4-disubstituted GalN in the molar ratios 1.00 : 1.25 : 0.25 : 1.43 : 0.27 : 0.24 : 0.40 : 0.15 : 0.27, respectively. These data (ratios of components) confirmed structural heterogeneity of the OPS of *S.* Telaviv.

The presence of two terminal and two disubstituted sugar residues indicated a branched polysaccharide chain with two branching points. Similar relative contents of terminal, 3-substituted and 3,4-disubstituted Gal (0.25 : 0.27 : 0.24, respectively) enabled a suggestion that terminal and 3-substituted Gal form a side chain connected to 3,4-disubstituted Gal, whereas terminal Glc is attached to 3,4-disubstituted GalN. Moreover, the presence of 4-substituted and 3,4-disubstituted Gal in the molar ratio 1.43 : 0.24 suggested that the disaccharide branch is linked to position 3 of the Gal residue, whereas the 1→4-linkage to the Gal residue occurs in the main chain. The molar ratio (0.15 : 0.27) of 3-substituted and 3,4-disubstituted GalN showed that terminal Glc is connected to GalN by the 1→4-linkage and the content of the glucosylated repeating units is higher than non-glucosylated units. Methylation analysis of the OPS also revealed the pyranose form of terminal Glc, terminal Gal and 3-substituted Gal, and the furanose form of 3-substituted Rib. The pyranose from of the other sugar residues was inferred from NMR spectroscopic data (see below).

The OPS was treated with 12% ammonia solution to eliminate possible *O*-acetyl groups. The ^{1}H NMR spectrum of the product (not shown) was identical to the spectrum of the intact OPS (Fig. 3). Therefore, the OPS of *S.* Telaviv is not *O*-acetylated. The signals at δ 2.17, 2.10, and a much less intense signal at 2.19 in the ^{1}H NMR spectrum (Fig. 3) confirmed the *N*-acetylation of Qui3N and GalN.

Table 1. Sugar analysis data

Sugar residue	Molar ratio				
	OPS	OPSI	OPSII	OPSIII	Major Smith degradation product
Rib	0.73	0.48	0.57	0.81	0.99
Qui3NAc	0.92	0.78	0.95	1.01	1.00
Glc	1.00	1.00	1.00	1.00	0.07
Gal	1.90	1.55	1.66	2.05	0.08
GalNAc	0.94	0.90	1.14	1.37	1.41
GlcNAc*	0.03	0.01	0.01	0.05	–
Heptose*	0.04	0.01	0.03	0.08	–

* Components of LPS core.

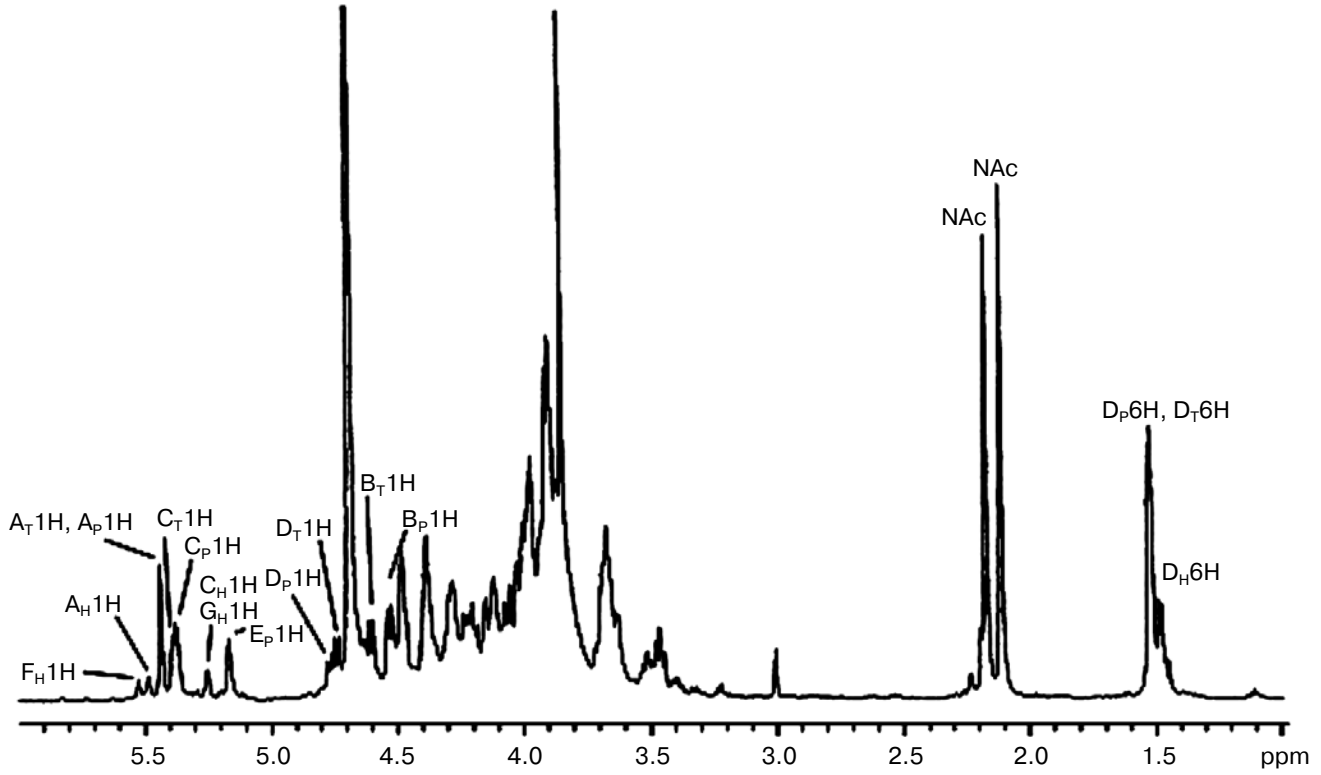


Fig. 3. ^{1}H NMR spectrum of the O-polysaccharide of *S.* Telaviv. Arabic numerals refer to protons in sugar residues denoted by capital letters as shown in Tables 4 and 5.

Further structural information was obtained using Smith degradation of the OPS. The major oligosaccharide product (26.5% of all products obtained) was isolated by GPC on Bio-Gel P-2 and subjected to sugar, methylation, and NMR analyses. Sugar analysis revealed the presence of threitol (derived from the degraded 4-substituted Gal), Rib, Qui3NAc, and GalNAc in the molar ratios 1.20 : 0.99 : 1.00 : 1.41 as well as trace amounts of Glc and Gal (0.07 and 0.08, respectively) (Table 1). Methylation analysis revealed the presence of 3-substituted Rib, terminal GalNAc, 4-substituted Qui3NAc, and 2-substituted threitol (Table 2).

The ^{1}H NMR spectrum of the Smith degradation product (not shown) displayed resonances for three anomeric protons at δ 5.36, 5.32, and 4.73, two *N*-acetyl groups at δ 2.18 and 2.11, and one methyl group (H6 of Qui3NAc) at δ 1.50. The ^{13}C NMR spectrum contained signals for three anomeric carbons at δ 106.94, 103.42, and 97.45, four HO*C*H$_2$–C groups (C5 of Rib, C6 of GalNAc, C1 and C4 of threitol) at δ 62.33, 61.60, 62.84, and 60.91, one *C*H$_3$–C group (C6 of Qui3NAc) at δ 18.82, two nitrogen-bearing carbons (C2 of GalNAc and C3 of Qui3NAc) at δ 49.73 and 57.14, eleven non-anomeric oxygen-bearing sugar-ring carbons at δ 66.0-78.0, and two *N*-acetyl groups (CO at δ 174.96 and 174.57, CH$_3$ at δ 22.85 and 22.66).

The spin systems of the three sugar residues and threitol were assigned (Table 3) according to the published methodology [20, 21] using two-dimensional ^{1}H,^{1}H COSY, TOCSY, NOESY, ^{1}H,^{13}C HSQC (Fig. 4), and HMBC experiments. The spin system of Qui3NAc was distinguished from GalNAc by correlations from H1 to H6 at δ 1.50 in the TOCSY spectrum. A comparison of the chemical shifts for Rib ($^3J_{H1,H2}$ < 2 Hz) with published data [22] confirmed the presence of a 3-linked β-ribofuranose residue. The absence of signals at δ 84-88 in the ^{13}C NMR spectrum of the OPS demonstrated the six-membered ring in both GalNAc and Qui3NAc [23]. The lack of down-field carbon signals for GalNAc (Table 3) indicated the terminal position of this residue, whereas relatively low-field positions of the signals for C4 of Qui3NAc (δ 76.54) and C2 of threitol (δ 79.15) demonstrated the glycosylation pattern of these monosaccharides [23]. These data were in agreement with the results of methylation analysis (Table 2).

The presence of α-GalNAc and the β-Qui3NAc was established by the chemical shifts of the anomeric protons and $^3J_{H1,H2}$ coupling constants (δ 5.36 and 4 Hz for GalNAc, δ 4.73 and 7.6 Hz for Qui3NAc). The sequence of the sugar residues was determined by NOESY and HMBC experiments. The NOESY spectrum revealed the following correlations between the anomeric protons and protons at the linkage carbons: GalNAc H1/Qui3NAc H4 at δ 5.36/3.66 (A1/B4) and Rib H1/threitol H2 at δ 5.32/3.88 (C1/D2). In the HMBC spectrum, there were

Table 2. Methylation analysis data

Sugar residue	Molar ratio				
	OPS	OPSI	OPSII	OPSIII	Major Smith degradation product
3-Rib	1.00	1.00	1.00	1.00	1.00
Terminal Glc	1.25	1.79	1.31	1.13	–
Terminal Gal	0.25	0.03	0.12	0.17	–
4-Gal	1.43	1.55	1.45	1.26	–
3-Gal	0.27	0.09	0.18	0.28	0.05
3,4-Gal	0.24	0.11	0.12	0.15	–
4-Qui3NAc	0.40	0.40	0.45	0.35	0.28
3-GalNAc	0.15	0.17	0.13	0.17	–
3,4-GalNAc	0.27	0.32	0.22	0.20	–
Terminal GalNAc	–	–	–	–	0.41

Note: 3-Rib stands for 3-substituted Rib, etc.

Table 3. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR chemical shifts (δ) of the major product of Smith degradation of the OPS of *S.* Telaviv

Sugar residue	H1 C1	H2 C2	H3 C3	H4 C4	H5 C5	H6 C6
A α-D-Gal*p*NAc-(1→	5.36 97.45	4.33 49.73	3.97 67.91	4.15 68.92	3.95 71.58	3.86 61.60
B →4)-β-D-Qui*p*3NAc-(1→	4.73 103.42	3.47 72.61	4.17 57.14	3.66 76.54	3.80 72.74	1.50 18.82
C →3)-β-D-Rib*f*-(1→	5.32 106.94	4.38 75.03	4.48 79.08	4.27 81.37	3.97 62.33	
D →2)-threitol	3.83, 3.74 62.84	3.88 79.15	4.15 72.03	3.80 60.91		

Note: Additional chemical shifts for NAc are δ_H 2.18 and 2.11 (CH_3); δ_C 22.85, 22.66 (both CH_3), 174.96 and 174.57 (both CO).

correlations between H1 of Rib and C2 of threitol at δ 5.32/79.15 (C H1/D C2) as well as between H1 of Qui3NAc and C3 of Rib at δ 4.73/79.08 (B H1/C C3).

The data allowed establishing the following structure of the major Smith degradation product from the OPS:

α-D-Gal*p*NAc-(1→4)-β-D-Qui*p*3NAc-(1→3)-β-D-
A B

Rib*f*-(1→2)-threitol,
C D

and, taking into account methylation data, the following structure of the main chain of the *S.* Telaviv OPS:

→3)-α-D-Gal*p*NAc-(1→4)-β-D-Qui*p*3NAc-(1→3)-β-D-Rib*f*-(1→4)-D-Gal*p*-(1→.

The data were useful for the interpretation of the NMR spectra of the OPS. The anomeric region of the ^{1}H NMR spectrum (Fig. 3) contained both high-intensity (δ 5.43, 5.38, 5.36, 5.16, 4,74, 4.59, 4.52, 4.47) and low-intensity (δ 5.52, 5.48, 5.25) signals. In addition to

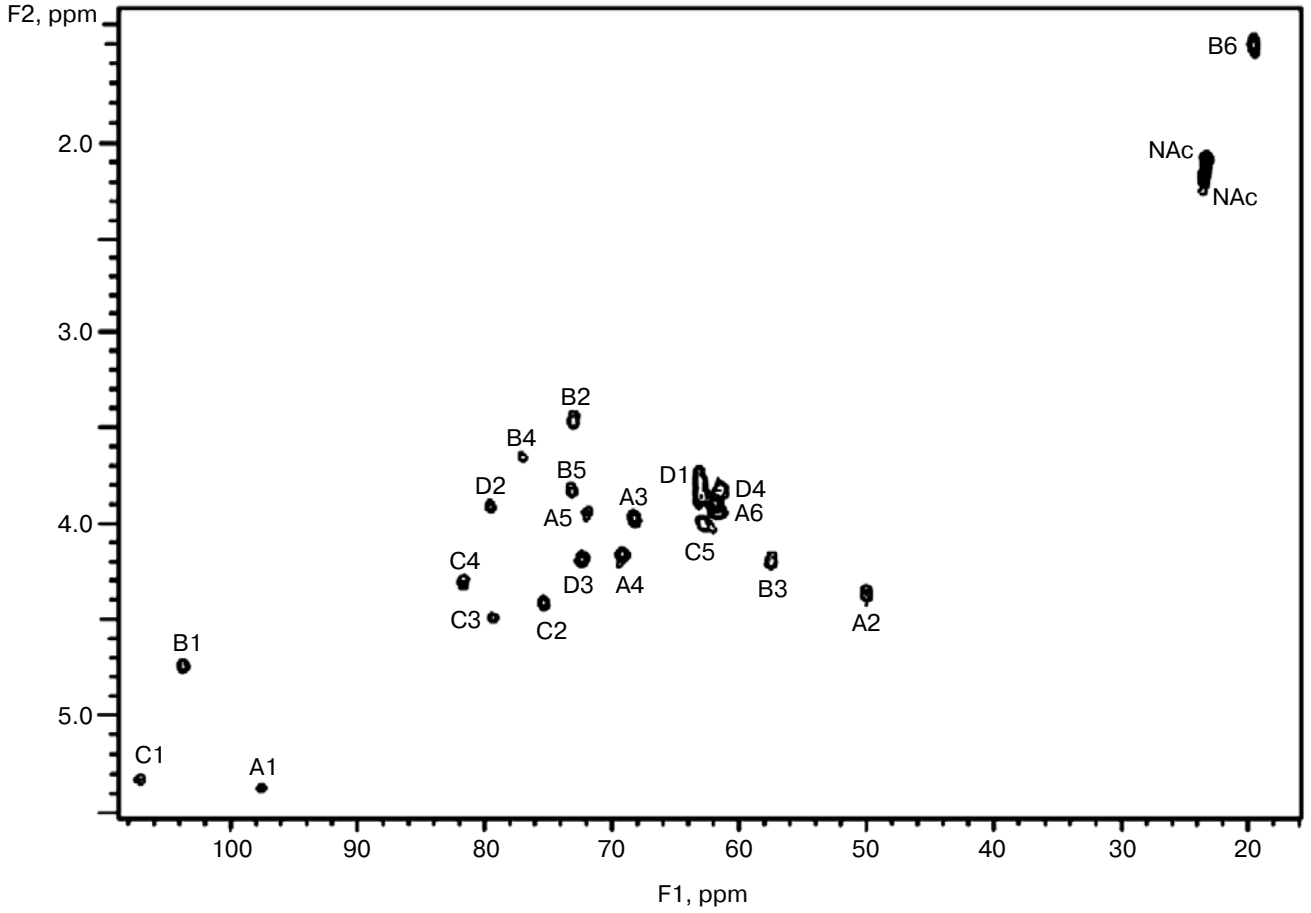


Fig. 4. Two-dimensional $^{1}H,^{13}C$ HSQC spectrum of the main product obtained by Smith degradation of the O-polysaccharide of *S.* Telaviv. Arabic numerals refer to cross-peaks of sugar residues denoted by capital letters as shown in Table 3.

the sugar ring proton signals at δ 3.3-4.3, there were signals at δ 1.51 (higher intensity) and δ 1.47 (lower intensity) for two methyl groups (H6 of Qui3NAc), as well as high-intensity signals at δ 2.17 and 2.10 and a less intense signal at δ 2.19 for three *N*-acetyl groups. These data confirmed that the OPS has an irregular structure and indicated that it contains at least two types of repeating units.

The ^{13}C NMR spectrum of the OPS (Fig. 5) showed seven high-intensity and a number of low-intensity signals for anomeric carbons in the region δ 94-110 as well as signals for at least 25 non-anomeric oxygen-bearing

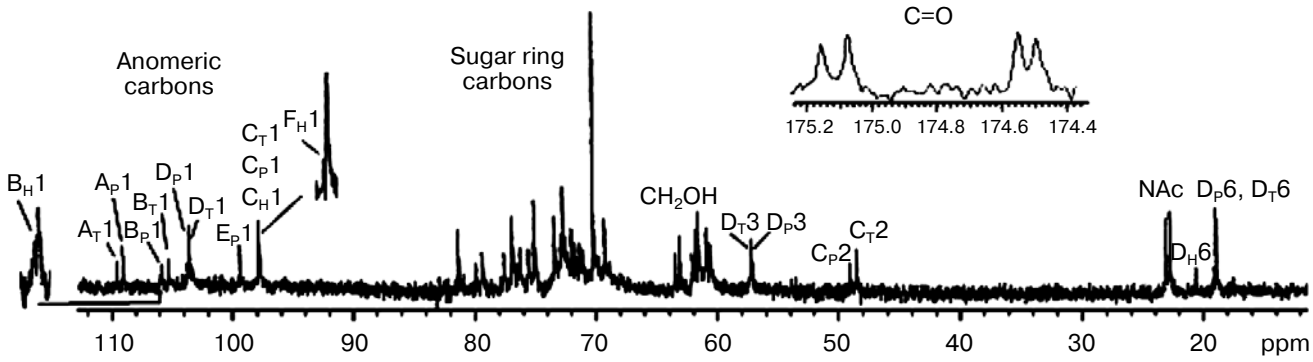


Fig. 5. ^{13}C NMR spectrum of the O-polysaccharide of *S.* Telaviv. Arabic numerals refer to carbons in sugar residues denoted by capital letters as shown in Tables 4 and 5.

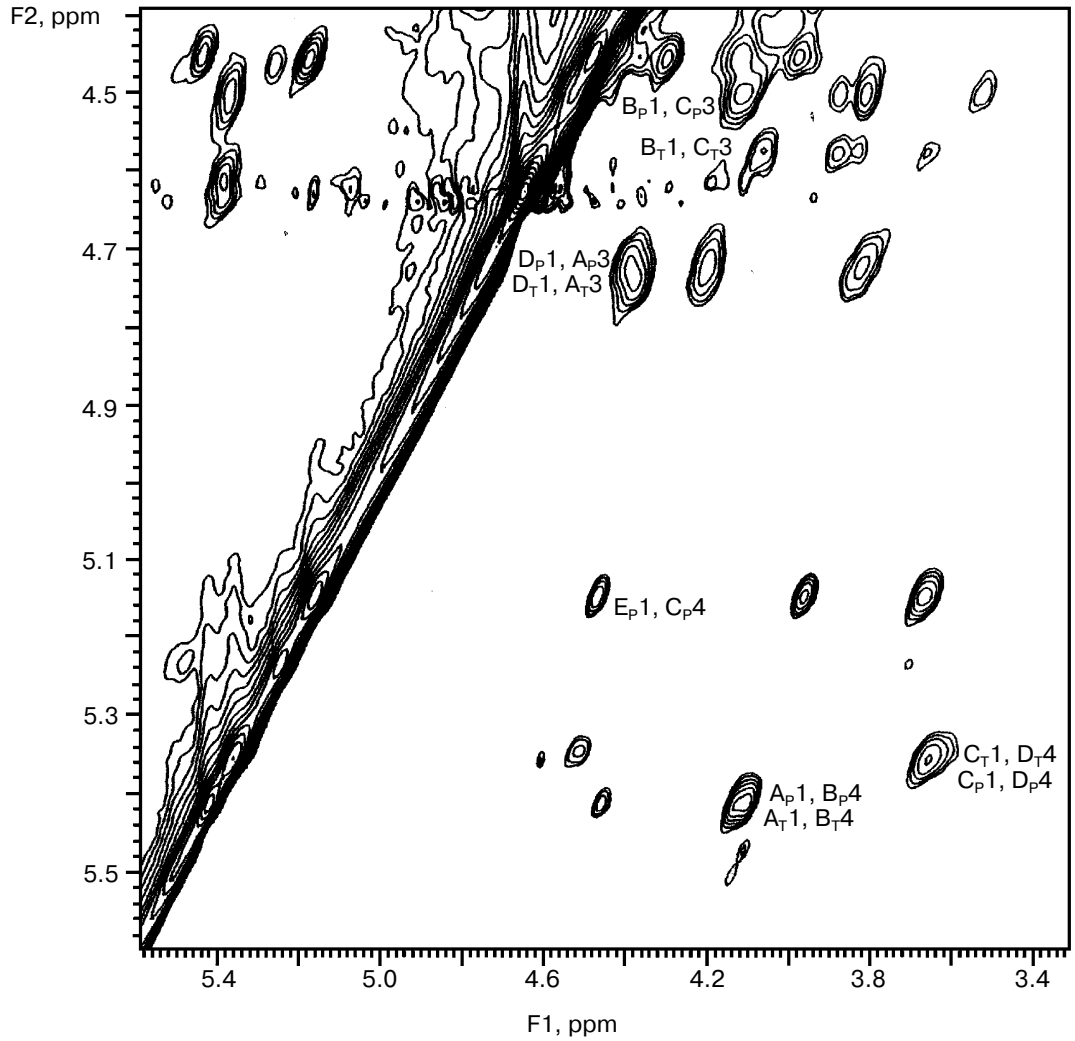


Fig. 6. Part of a NOESY spectrum of the O-polysaccharide of *S.* Telaviv. Arabic numerals refer to cross-peaks for sugar residues denoted by capital letters as shown in Tables 4 and 5.

sugar-ring carbons at δ 70.0-82.00; seven HO*C*H_2–C groups (C6 of Gal, Glc and GalN, and C5 of Rib) at δ 60.5-63.5; four nitrogen-bearing carbons (C2 of GalN and C3 of Qui3N) at δ 48.45 and 49.01, 57.14, and 57.17; two *C*H_3–C groups (C6 of Qui3N) at δ ~18.85 (higher intensity) and 20.54 (lower intensity); and four *N*-acetyl groups (CO at δ 174.49, 174.55, 175.08, and 175.15; CH_3 at δ 23.08, 23.00, 22.67, and 22.60). The absence of signals at δ 84-88 indicated the pyranose form of the 4-substituted (Gal and Qui3NAc) and 3,4-disubstituted (Gal and GalNAc) monosaccharide residues [23].

The structures of penta- and tetrasaccharide repeating units of the OPS were established by two-dimensional NMR techniques essentially as described [20, 21] (Figs. 3, 5, and 6; Tables 4 and 5). The TOCSY and $^1H,^{13}C$ HSQC-TOCSY spectra clearly revealed spin systems for eleven sugar residues, nine of which belonged to penta- and tetrasaccharide units. They were designated as A_P–E_P (in the pentasaccharide unit) and A_T–D_T (in the tetrasaccharide unit) according to their sequence established later. The COSY spectrum enabled differentiation between protons within each spin system (Table 4). The spin systems of Rib (A_P, A_T), GalNAc (C_P, C_T), and Qui3NAc (D_P, D_T) were determined as described for the major product of Smith degradation of the OPS. The spin systems of Glc (E_P) and Gal (B_P, B_T) were identified on the basis of the characteristic chemical shifts and $^3J_{H,H}$ coupling constants of the sugar ring protons using published data on the respective pyranosides [24, 25].

The β-configuration of the glycosidic linkages of Gal (B_P, B_T), GalNAc (C_P, C_T), and Qui3NAc (D_P, D_T) was established by the $^3J_{H1,H2}$ coupling constants of 7.6-8.0 Hz and the chemical shifts of the anomeric carbons and protons (Tables 4 and 5). The β-configuration of Rib*f* (A_P, A_T) followed from the C1 chemical shifts of δ 108.99 and 109.52. The α-configuration of GalNAc (C_P, C_T) was

Table 4. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR chemical shifts (δ) for the penta- and tetrasaccharide repeating units of the OPS of *S.* Telaviv

Sugar residue	H1 C1	H2 C2	H3 C3	H4 C4	H5 C5	H6 C6
Pentasaccharide unit						
A_P →3)-β-D-Rib*f*-(1→	5.43 108.99	4.47 74.99	4.38 79.40	4.27 81.31	3.85, 4.00 63.05	
B_P →4)-β-D-Gal*p*-(1→	4.52 105.81	3.52 71.00	3.86 73.38	4.09 77.54	4.04 77.54	4.02 61.53
C_P →3,4)-α-D-Gal*p*NAc-(1→	5.38 97.76	4.64 49.01	4.11 76.22	4.47 75.59	4.08 72.21	3.89, 3.83 61.53
D_P →4)-β-D-Qui*p*3NAc-(1→	4.76 103.42	3.50 72.43	4.20 57.14	3.68 76.85	3.82 72.60	1.51 18.82
E_P D-α-Glc*p*-(1→	5.16 99.35	3.65 72.43	3.98 73.38	3.69 70.11	4.05 71.93	3.96 60.86
Tetrasaccharide unit						
A_T →3)-β-D-Rib*f*-(1→	5.43 109.52	4.47 74.99	4.38 79.87	4.27 80.91	3.85, 4.00 63.42	
B_T →4)-β-D-Gal*p*-(1→	4.59 105.22	3.65 71.00	3.86 73.38	4.14 75.53	3.83 74.99	3.88 62.05
C_T →3)-α-D-Gal*p*NAc-(1→	5.36 97.76	4.52 48.45	4.05 77.54	4.37 69.65	4.23 71.61	3.88 61.77
D_T →4)-β-D-Qui*p*3NAc-(1→	4.74 103.46	3.46 72.43	4.20 57.17	3.66 76.85	3.82 72.60	1.51 18.85

Note: Additional chemical shifts for NAc are δ_H 2.19, 2.17, and 2.10 (CH_3); δ_C 23.08, 23.00, 22.67, 22.60 (all CH_3), 175.15, 175.08, 174.55, and 174.49 (all CO).

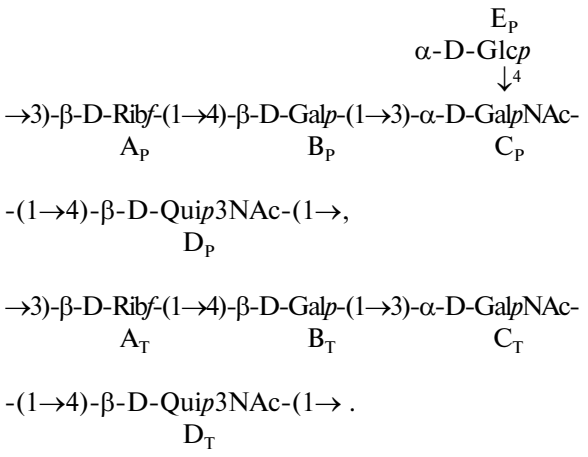
Table 5. $^{3}J_{H1,H2}$ coupling constants for the anomeric protons determined from the ^{1}H NMR spectrum and inter-residue correlations in the NOESY spectrum for the penta- and tetrasaccharide repeating units of the OPS of *S.* Telaviv

Sugar residue	δ_{H1}, ppm	$^{3}J_{H1,H2}$, Hz	NOE on proton (δ_H), ppm	Connectivity to
Pentasaccharide unit				
A_P β-D-Rib*f*-(1→	5.43	<2	H4 (4.09)	B_P →4)-β-D-Gal*p*
B_P β-D-Gal*p*-(1→	4.52	8.0	H3 (4.11)	C_P →3,4)-α-D-Gal*p*NAc
C_P α-D-Gal*p*NAc-(1→	5.38	4.0	H4 (3.68)	D_P →4)-β-D-Qui*p*3NAc
D_P β-D-Qui*p*3NAc-(1→	4.76	7.2	H3 (4.38)	A_P →3)-β-D-Rib*f*
E_P α-D-Glc*p*-(1→	5.16	4.0	H4 (4.47)	C_P →3,4)-α-D-Gal*p*NAc
Tetrasaccharide unit				
A_T β-D-Rib*f*-(1→	5.43	<2	H4 (4.14)	B_T →4)-β-D-Gal*p*
B_T β-D-Gal*p*-(1→	4.59	7.6	H3 (4.09)	C_T →3)-α-D-Gal*p*NAc
C_T α-D-Gal*p*NAc-(1→	5.36	4.0	H4 (3.66)	D_T →4)-β-D-Qui*p*3NAc
D_T β-D-Qui*p*3NAc-(1→	4.74	7.2	H3 (4.38)	A_T →3)-β-D-Rib*f*

inferred from the $^3J_{H1,H2}$ coupling constant (4 Hz) and the chemical shifts of the anomeric carbons and protons (Tables 4 and 5). Relatively low-field positions of the signals for C3 of units A_P and A_T at δ 79.40 and 79.87, C4 of units B_P and B_T at δ 77.54 and 75.53, C3 and C4 of unit C_P at δ 76.22 and 75.59, respectively, C3 of unit C_T at δ 77.54, and C4 of units D_P and D_T at δ 76.85 both, demonstrated the glycosylation patterns in the penta- and tetrasaccharide repeating units. The lack of downfield carbon signals for Glc (E_P) (Table 4) indicated the terminal position of this sugar residue.

In the NOESY spectrum (Fig. 6), inter-residual NOE correlations in the pentasaccharide unit were observed between A_P H1/B_P H4 at δ 5.43/4.09, B_P H1/C_P H3 at δ 4.52/4.11, C_P H1/D_P H4 at δ 5.38/3.68, D_P H1/A_P H3 at δ 4.76/4.38, and E_P H1/C_p H4 at δ 5.16/4.47. Inter-residual NOE correlations in the tetrasaccharide unit were also seen between A_T H1/B_T H4 at δ 5.43/4.14, B_T H1/C_T H3 at δ 4.59/4.05, C_T H1/D_T H4 at δ 5.36/3.66, and D_T H1/A_T H3 at δ 4.74/4.38 (Table 5). The sequence of the monosaccharides thus established was in agreement with the structure of the major product of Smith degradation of the OPS.

Therefore, the penta- and tetrasaccharide repeating units of the OPS of *S.* Telaviv have the following structures:

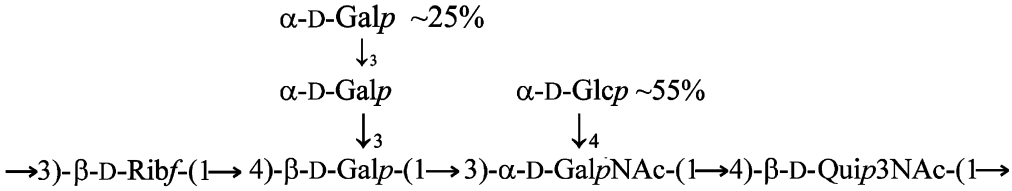


A comparison of the intensities of the H1 signals of Glc and other monosaccharides in the tetra- and pentasaccharide units (Fig. 3) indicated that the OPS consists of ~55% glucosylated and ~45% non-glucosylated repeating units.

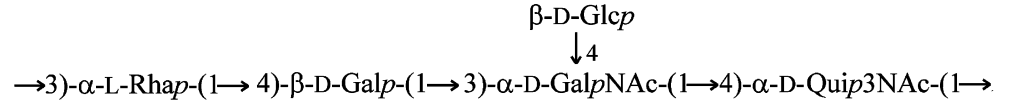
As already mentioned, apart from the signals belonging to the penta- and tetrasaccharide units, anomeric signals with lower intensities were observed in the ^{1}H and ^{13}C NMR spectra of the OPS (Figs. 3 and 5). Using the approach described above, the H1 signal at δ 5.52 was assigned to terminal α-Gal*p* (F_H) (C1 at δ 98.60, $^3J_{H1,H2}$ < 4 Hz), that at δ 5.48 to 3-linked β-Rib*f* (A_H) (C1 at δ 108.00), and the double signal at δ 5.25 to 3-linked α-Gal*p* (G_H) and 3-linked or 3,4-linked α-Gal*p*NAc (C_H) (C1 of both at δ 97.7, $^3J_{H1,H2}$ = 4 Hz). The relative intensities of the H1 signals (0.28 : 0.40 : 0.53, respectively) indicated that the digalactose side chain occurs in ~25% of the repeating units of the OPS. This finding is in agreement with the methylation analysis data of the OPS and its fractions (see below) (Tables 1 and 2).

Based on these data, it was concluded that the OPS of *S.* Telaviv consists of two major types of repeating units: tetrasaccharide (~45%) containing Gal, GalNAc, Qui3NAc, and Rib, and pentasaccharide (~55%) additionally containing lateral Glc. Furthermore, both tetra- and pentasaccharide units may be substituted by a digalactose side chain (totally ~25%) giving rise to hexa- and heptasaccharide repeating units (Fig. 7).

Three fractions obtained by GPC on Bio-Gel P-100 (Fig. 2b) were subjected to sugar and methylation analysis. They were shown to contain the same monosaccharides as the OPS but in different molar ratios (Table 1). The proportion of Gal in the OPSI was the lowest (1.55) in comparison to the OPSII (1.66) and OPSIII (2.05). The increased amount of Gal in the low-molecular-mass OPSIII is due not only to a higher content of the core oligosaccharide in this fraction (as judged by the determination of other sugar core components, GlcNAc and



Salmonella Telaviv ($O28_1$, $O28_2$)



Salmonella Dakar ($O28_1$, $O28_3$) [14]

Fig. 7. Structures of the O-polysaccharides of *Salmonella* serogroup O:28. D-Qui*p*3NAc indicates 3-acetamido-3,6-dideoxy-D-glucopyranose.

heptose) but also resulted from the presence of the digalactose side chain in the repeating unit. The opposite trend was observed for the content of Glc, which is higher in the high-molecular-mass OPSI.

Methylation analysis (Table 2) showed that the content of terminal Glc decreased from the OPSI (1.79) to OPSIII (1.13) corroborating the results of sugar analysis. The same trend (a content reduction from 0.32 to 0.20) was observed for 3,4-disubstituted GalNAc. This indicated that most terminal glucose residues occur in the longer OPS chains at some distance from the core region. The opposite results were obtained for terminal and 3-substituted Gal: their contents in the OPSI (0.03 and 0.09) were lower in comparison to the OPSIII (0.17 and 0.28, respectively). Therefore, the repeating units that are proximal to the core carry mostly a digalactose side chain, whereas the glucosylated units are present mostly at the other side of the longer OPS chains and bear no galactose disaccharide.

This is the first structure established for the OPS from a *Salmonella* strain belonging to serogroup O:28 in the Kauffmann–White classification and possessing subfactors $O28_1$ and $O28_2$. As already mentioned, only the structure of the OPS of *S.* Dakar, characterized by subfactors $O28_1$ and $O28_3$, has yet been determined (Fig. 7) [14]. The OPSs of *S.* Telaviv and *S.* Dakar differ both in the main chain and side chain structures. In the main chain of the former, a β-D-Qui*p*3NAc residue is present in place of α-D-Qui*p*3NAc and 3-substituted Rib*f* in place of 3-substituted rhamnopyranose (Rha*p*) in the latter. In the OPS of *S.* Telaviv, only ~55% of 3-substitttuted GalNAc is α-D-glucosylated at position 4, whereas in the OPS of *S.* Dakar, 3-substituted GalNAc is completely β-D-glucosylated at the same position. Additionally, ~25% of the repeating units of *S.* Telaviv OPS contained the α-D-Gal*p*-(1→3)-α-D-Gal*p* digalactose side chain linked at position 3 of 4-substituted β-D-Gal*p*.

This concurs with the results of Clark et al. [12], who found that the O-antigen gene cluster of *Salmonella* $O28_1$,$O28_2$ represented by *S.* Pomona is different from that of *Salmonella* $O28_1$,$O28_3$ represented by *S.* Dakar. Their suggestion that the OPSs of serogroup O:28 strains characterized by subfactors $O28_2$ or $O28_3$ should be structurally different was confirmed in the present study. Knowledge of the structures of the OPSs is crucial for determination of the exact structures associated with subfactors $O28_1$, $O28_2$, and $O28_3$ and elucidation of the genetic basis for the close relationship between *E. coli* O71 and *S. enterica* O28 O-antigens [11].

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REFERENCES

1. Grimont, P. A. D., and Weill, F.-X. (2007) *Antigenic Formulae of the Salmonella Serovars*, 9th Edn., WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur, Paris.
2. Luderitz, O., Staub, A. M., and Westphal, O. (1966) *Bacteriol. Rev.*, **30**, 192-255.
3. Lindberg, A. A., and Le Minor, L. (1984) Serology of *Salmonella*, in *Methods in Microbiology*, Vol. 15 (Bergan, T., ed.) Academic Press, London, pp. 1-64.
4. Caroff, M., and Karibian, D. (2003) *Carbohydr. Res.*, **338**, 2431-2447.
5. Lerouge, I., and Vanderleyden, J. (2001) *FEMS Microbiol. Rev.*, **26**, 17-47.
6. Wang, W., Perepelov, A. V., Feng, L., Shevelev, S. D., Wang, Q., Senchenkova, S. N., Han, W., Li, Y., Shashkov, A. S., Knirel, Y. A., Reeves, P. R., and Wang, L. (2007) *Microbiology*, **153**, 2159-2167.
7. D'Aoust, J.-Y. (1994) *Int. J. Food Microbiol.*, **24**, 11-31.
8. Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R., and Swaminathan, B. (2000) *J. Clin. Microbiol.*, **38**, 2465-2467.
9. Gast, R. K. (2007) *Avian Dis.*, **51**, 817-828.
10. Wang, L., and Reeves, P. R. (2000) *J. Bacteriol.*, **182**, 5256-5261.
11. Hu, B., Perepelov, A. V., Liu, B., Shevelev, S. D., Guo, D., Senchenkova, S. N., Shashkov, A. S., Feng, L., Knirel, Y. A., and Wang, L. (2010) *FEMS Immunol. Med. Microbiol.*, **59**, 161-169.
12. Clark, C. G., Grant, C. C. R., Trout-Yakel, K. M., Tabor, H., Ng, L.-K., Rahn, K., Franklin, K., and Kropinski, A. M. (2010) *Int. J. Microbiol.*, **2010**, 209291.
13. Luderitz, O., Ruschmann, E., Westphal, O., Raff, R., and Wheat, R. (1967) *J. Bacteriol.*, **93**, 1681-1687.
14. Kumirska, J., Szafranek, J., Czerwicka, M., Paszkiewicz, M., Dziadziuszko, H., Kunikowska, D., and Stepnowski, P. (2007) *Carbohydr. Res.*, **342**, 2138-2143.
15. Gerwing, G. J., Kamerling, J. P., and Vliegenthart, J. F. G. (1979) *Carbohydr. Res.*, **77**, 1-7.
16. Leontein, K., Lindberg, B., and Lönngren, J. (1978) *Carbohydr. Res.*, **62**, 359-362.
17. Hakomori, S.-I. (1964) *J. Biochem. (Tokyo)*, **55**, 205-208.
18. Waeghe, T. J., Darvill, A. G., McNeil, M., and Albersheim, P. (1983) *Carbohydr. Res.*, **123**, 281-304.
19. Pritchard, D. G., and Rener, B. P. (1988) *Carbohydr. Res.*, **173**, 255-262.
20. Hounsell, E. F. (1995) *Prog. Nucl. Magn. Reson. Spectr.*, **27**, 445-474.
21. Duus, J. O., Gotfredsen, C. H., and Bock, K. (2000) *Chem. Rev.*, **100**, 4589-4614.
22. Urbina, F., Nordmark, E.-L., Yang, Z., Weintraub, A., Scheutz, F., and Wildman, G. (2005) *Carbohydr. Res.*, **340**, 645-650.
23. Bock, K., and Pedersen, C. (1983) *Adv. Carbohydr. Chem. Biochem.*, **41**, 27-66.
24. Perry, M. B., Bundle, D. R., MacLean, L., Perry, J. A., and Griffith, D. W. (1986) *Carbohydr. Res.*, **156**, 107-122.
25. Brandbury, H. J., and Jenkins, G. A. (1984) *Carbohydr. Res.*, **126**, 125-156.