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Note

The structure of a core oligosaccharide component from *Hafnia alvei* strain 32 and 1192 lipopolysaccharides

Wojciech Jachymek [a], Czeslaw Lugowski [a,*], Elzbieta Romanowska [a], Danuta Witkowska [a], Carl Petersson [b] and Lennart Kenne [b]

[a] *L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Czerska 12, 53-114 Wroclaw (Poland)*
[b] *Swedish University of Agricultural Sciences, P.O. Box 7015, S-750 07 Uppsala (Sweden)*

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Hafnia alvei is an opportunistic pathogen found in some incidents of nosocomial infections[1]. Preliminary chemical characterization of lipopolysaccharides isolated from 33 strains of this species has been reported[2]. Recently, the structures of O-specific polysaccharides from *H. alvei* strains ATCC 13337, 2, 38, 39, 1198, 1205, and 1211 have been elucidated[3–8].

The detailed structure of the core region of any *H. alvei* lipopolysaccharide has not yet been determined, except for some data[5] concerning strain 2.

The heptose–Kdo region of lipopolysaccharides plays a significant role in bacterial physiology and interaction with the host. Therefore, it was of current interest to characterize this part of the endotoxin. We now report the structure of a trisaccharide component of the core region of *H. alvei* strains 32 and 1192.

EXPERIMENTAL

General methods.—GLC–MS was carried out with a Hewlett–Packard 5971A system, using an HP-1 glass capillary column (0.2 mm × 12 m) and a temperature program 150 → 270°C at 8°C/min. Sugar and methylation analysis and determination of the absolute configuration of sugar components were performed as described in preceding papers[10,11]. Gel permeation chromatography was performed on a column (2.5 × 100 cm) of Sephadex G-50 and on a column (1.6 × 100 cm) of

* Corresponding author.

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Bio Gel P-2 equilibrated with pyridine–acetic acid buffer (pH 5.6). Eluates were monitored with a Knauer differential refractometer.

NMR spectra were recorded for D_2O solutions at 70°C with Varian VXR-400 and Jeol GSX-270 spectrometers, using sodium 3-trimethylsilylpropanoate-d_4 (δ_H 0.00) and acetone (δ_C 31.00) as internal references. FABMS was performed in the positive mode on a Jeol SX-102 instrument, using thioglycerol as the matrix.

Isolation and purification of the lipopolysaccharide core oligosaccharide.—*H. alvei* strain 32 was obtained from the collection of the Institute of Immunology and Experimental Therapy (Wroclaw), and strain 1192 was obtained from the collection of the Pasteur Institute (Paris). Preparation of lipopolysaccharides and core oligosaccharides was carried out as previously described[9]. Further purification of the lower molecular weight core-oligosaccharide fractions obtained from the Sephadex G-50 column was achieved by chromatography on Bio-Gel P-2; the main components were eluted in the hexa- and tri-saccharide regions.

Reduction of the Kdo residue in the trisaccharide fractions.—Samples (5 mg) in H_2O (2 mL) were reduced with $NaBH_4$ (25 mg) at room temperature for 2 h. The excess of borohydride was destroyed by adding AG 50W-X8 (H^+) resin (Bio-Rad), and the boric acid was removed conventionally as the volatile trimethyl borate.

For carboxyl reduction, the keto-reduced trisaccharide sample in H_2O (1.5 mL) was treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (50 mg) at pH 4.75 and then with $NaBH_4$ (60 mg) according to the method of Taylor et al.[12]. The reagents were removed as described above.

Degalactosylation of core trisaccharide.—The completely reduced trisaccharides (3 mg) were dissolved in 0.03 M phosphate buffer at pH 7.0 (1 mL). Galactose oxidase (P.-L. Biochemicals, 100 u) and peroxidase (Sigma, 50 u) were added followed by toluene (20 μL). The mixture was kept at room temperature for 4 days and then the product was purified by ion-exchange chromatography on Dowex AG 2-X8 (COO^-). The freeze-dried oligosaccharide fractions were dissolved in 0.5 M NaOH (1.5 mL), kept for 1 h at 80°C, neutralized with AG 50W-X8 (H^+) resin, and freeze-dried. The degalactosylated oligosaccharide was purified by column chromatography on Bio-Gel P-2.

RESULTS AND DISCUSSION

Purification and composition of core oligosaccharides.—The lipopolysaccharides of *H. alvei* strains 32 and 1192 showed a smooth character in SDS-PAGE analysis[2]. The core oligosaccharides obtained after mild acid hydrolysis of the lipopolysaccharides were separated from O-specific chains by column chromatography on Sephadex G-50.

Lower molecular weight fractions were further purified by column chromatography on Bio-Gel P-2 and were eluted in the region for hexa- and tri-saccharides, respectively. Chemical and enzymatic analyses showed that the larger oligosaccha-

ride contained D-glucose, L-*glycero*-D-*manno*-heptose (L,D-heptose), and Kdo in the molar ratios 2:3:1, and that the smaller contained D-galactose, L,D-heptose, and Kdo in the molar ratios 1:1:1.

Structural analysis of the trisaccharide from H. alvei *strains 32 and 1192.*— GLC–MS analysis of the alditol acetates showed that the purified core oligosaccharide contained D-galactose and L-*glycero*-D-*manno*-heptose in the molar ratio 1.1:1.0. Methylation analysis indicated that the D-galactose and L,D-heptose residues are terminally located, as 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4,6,7-penta-*O*-methyl-L,D-heptose were obtained in the molar ratio 1:1, analysed as partially methylated alditol acetates by GLC–MS. The derivative of Kdo was not detected.

The ^{1}H NMR spectrum of the oligosaccharide gave a complex pattern for the anomeric protons with several signals from α-D-galactopyranosyl and L-α-D-heptopyranosyl residues. However, the spectrum of the trisaccharide, after reduction of the keto group of the Kdo residue, gave only one signal at δ 5.12 for the α-D-galactopyranosyl group but two for the L-α-D-heptopyranosyl group at δ 5.12 and 5.09 together corresponding to one proton, probably due to the two different configurations at C-2 of the reduced Kdo residue. The ^{13}C NMR spectrum also indicated two trisaccharides differing in the stereochemistry at C-2 of the deoxyoctonic acid since one signal at δ 100.4 for the anomeric carbon of the D-galactosyl group was obtained but two signals at δ 100.6 and 99.6 for the L-α-D-heptosyl group. In addition, two signals from C-3 of the deoxyoctonic acid residue at δ 36.0 and 35.5 were observed.

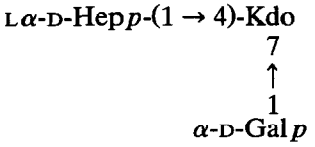
The FABMS spectrum of acetylated keto-reduced oligosaccharide showed an $[M + Na]^+$ ion at m/z 1164.8 indicating a molecular mass of 1141.8 for the acetylated product. This corresponds to a trisaccharide consisting of a hexose, a heptose, and a deoxyoctonic acid.

Sugar analysis of the trisaccharide, after keto- and carboxyl-reduction, showed the presence of D-galactose, L,D-heptose, and a mixture of D-*glycero*-D-*talo*- and D-*glycero*-D-*galacto*-3-deoxyoctitols, which were not differentiated, in the molar ratios 1.2:1.0:1.0, respectively.

Methylation analysis of the reduced oligosaccharide gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4,6,7-penta-*O*-methyl-L,D-heptose, and 3-deoxy-1,2,5,6,8-penta-*O*-methyloctitol, which shows that the reduced trisaccharide contains terminal galactosyl and heptosyl groups, and a 4,7-disubstituted 3-deoxyctitol.

A part of the reduced trisaccharide was selectively degalactosylated by oxidation with D-galactose oxidase and peroxidase followed by treatment with base[13]. Methylation analysis of the product gave 2,3,4,6,7-penta-*O*-methyl-L,D-heptose and 3-deoxy-1,2,5,6,7,8-hexa-*O*-methyloctitol, which shows that the degalactosylated oligosaccharide contains only a terminal heptosyl group and a 4-substituted 3-deoxyoctitol residue. Only a minor amount of 3-deoxy-1,2,5,6,8-penta-*O*-methyloctitol was found, which indicated that most of the enzymatically oxidised D-galactosyl group was removed from the trisaccharide during treatment with base.

The above data show that the trisaccharide core component of *H. alvei* strains 32 and 1192 has the following structure:



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