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Note

Structure of the heptose–3-deoxyoctulosonic acid region of *Citrobacter* lipopolysaccharide core

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Recently, we reported[1–3] on the basis of structural results that three types of lipopolysaccharide core occur in *Citrobacter* serotypes 04, 027, 036, and strain PCM 1487, all of them being different from *E. coli* core types, R_1, R_2, R_3, and R_4. The hexose–heptose regions of *Citrobacter* cores were described in detail, but still some structural data concerning the heptose–3-deoxyoctulosonic acid (dOctuA) part was lacking. Growing interest is directed to the heptose–dOctuA region of lipopolysaccharides because of its possible significance in bacterial physiology and interaction with the host. We describe herein the elucidation of: (a) the exact localization of side-chain heptose, and (b) the type of linkage heptose–dOctuA in *Citrobacter* cores.

To localize the branch point in the heptose region, the R36 incomplete-core oligosaccharide was submitted twice to Smith degradation. In the first degradation, sodium borohydride and, in the second, sodium borodeuteride was used for reduction.

This double-Smith degradation led to two possible alternative products (**1** or **2**, Scheme 1). Thus, the final product was submitted to methylation analysis. Two methylated derivatives (molar ratio 0.9:1.0) were identified: (a) 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-(6-$^2H_2$)mannitol, m.s.: *m/z* (relative intensities in parentheses): 47 (78), 60 (16), 71 (17), 72 (37), 75 (24), 87 (47), 88 (50), 89 (16), 101 (100), 102 (35), 114 (23), 117 (100), 118 (18), 129 (25), 130 (98), 131 (14), 145 (10), 146 (80), 161 (86), 162 (78), 205 (5), 206 (31), and 207 (5); and (b) 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylmannitol, m.s.: *m/z* 46 (70), 59 (18), 71 (25), 75 (12), 85 (18), 87 (29), 99 (37), 101 (79), 117 (100), 129 (89), 143 (8), 159 (8), 161 (56), 173 (5), 201 (12), and 233 (30). These data showed that **1** was formed, and that, in *Citrobacter* core, the side-chain heptose residue is linked to the base-chain heptose residue adjacent to a glycosyl residue. An identical result was obtained for the 04 incomplete core oligosaccharide.

To determine the type of linkage between heptose and 3-deoxyoctulosonic acid residue in *Citrobacter* cores, the dephosphorylated PCM 1487 core oligo-

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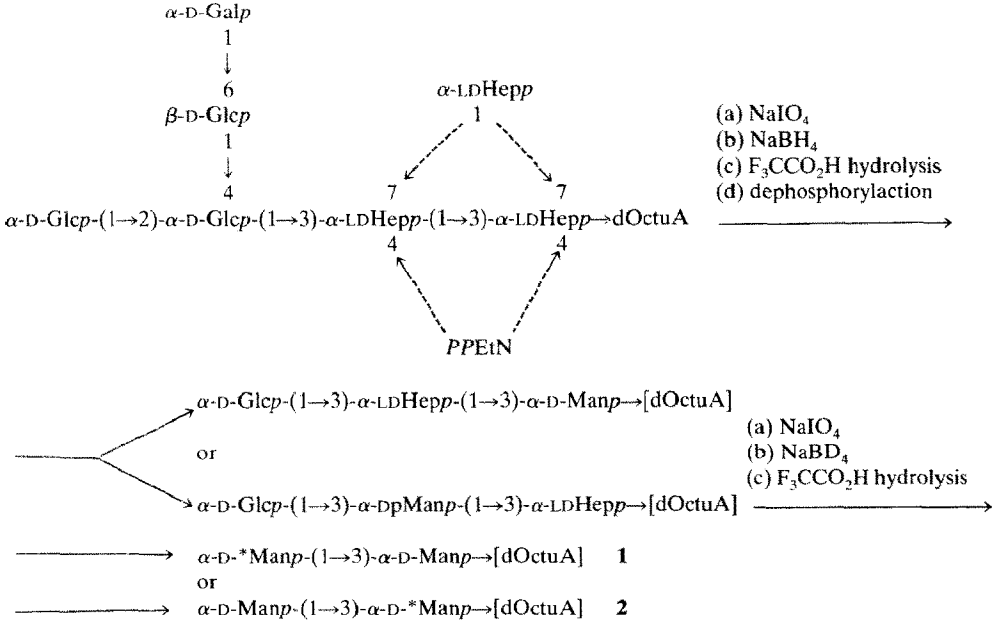
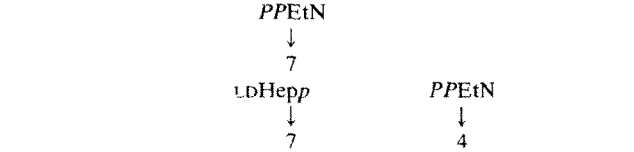
saccharide was submitted to carbonyl reduction, followed by carboxyl reduction. Methylation analysis of the doubly-reduced PCM 1487 core oligosaccharide revealed the presence of 5-*O*-acetyl-3-deoxy-1,2,4,6,7,8-hexa-*O*-methyloctitol (among other methylated derivatives), m.s.: *m/z* 45 (63), 71 (21), 75 (31), 89 (91), 101 (47), 115 (100), 129 (11), 133 (24), 147 (24), 157 (17), 201 (8), 203 (4), 263 (12), and 307 (5). These data indicate that the 3-deoxyoctulosonic acid reducing-terminal residue of the core region is substituted at O-5 by a heptosyl residue. The same type of linkage Hep-dOctuA was also identified for the 04 and 036 core oligosaccharides. In conclusion, the heptose-3-deoxyoctulosonic acid region of *Citrobacter* core has structure **3**.



Scheme 1. Double-Smith degradation of R36 incomplete core oligosaccharide: (-----) alternative locations; [dOctuA], the remainder of dOcuA residue after Smith degradations; *Man, D-($^2H_2$-6)-mannose; and *PP*EtN, diphosphorylethanolamine;



3

LDHep*p* = L-*glycero*-D-*manno*-heptopyranose
*PP*EtN = diphosphorylethanolamine.

EXPERIMENTAL

Materials. — *Citrobacter freundii* strain negSTc[s] (036) and its rough mutant (R36) were obtained from the Institute of Microbiology, Wrocław University;

strain 52/57 (04) from the Czechoslovakian National Collection of Type Cultures, Prague; and strain PCM 1487 from the stock collection of the Institute of Immunology and Experimental Therapy, Wrocław.

Methods. — The preparation of lipopolysaccharides, core oligosaccharides, and their dephosphorylation was carried out as previously described[1,4]. Methylation analysis was performed according to Hakomori[5] and g.l.c.–m.s. analysis with a LKB-2091 system and 3% OV-17 column (2.7 m × 3 mm) at a temperature program of 150–240° with an increase of 3°.min^{-1} (Central Laboratory of Macromolecular Studies, Lodz, Poland).

Double Smith-degradation of R36 incomplete-core oligosaccharide. — R36 incomplete-core oligosaccharide (19 mg), dissolved in 0.1M $NaIO_4$ (1.9 mL), was incubated at 4° for 48 h. The excess of periodate was destroyed by addition of 1,2-ethanediol (0.3 mL). The oxidized product was reduced with $NaBH_4$ (60 mg) overnight at 4°. The mixture was acidified with glacial acetic acid to pH 5 and concentrated several times to dryness with additions of methanol. The residue was purified on a Bio-Gel P-4 (−400 mesh) column (1.6 × 100 cm), equilibrated with pyridine–acetic acid buffer, pH 5.6. The product was hydrolyzed with 0.5M trifluoroacetic acid at room temperature for 48 h. After evaporation of the solvent with several additions of water, the dry hydrolyzate was treated with 40% HF (0.4 mL) in a polyethylene tube for 4 days at 4° for dephosphorylation. The sample was dried with N_2 and purified on a Bio-Gel P-4 column, as described above. The product was oxidized repeatedly with 0.05M $NaIO_4$ (1.2 mL) for 48 h at 4°, and then 1,2-ethanediol was added and the reduction was carried out with $NaBD_4$ (30 mg) overnight at 4°. Further purification on Bio-Gel P-4, and hydrolysis with trifluoroacetic acid, followed by purification on Bio-Gel P2 yielded the final product (4 mg).

Complete reduction of the 3-deoxyoctulosonic acid residue in PCM 1487 core oligosaccharide. — Dephosphorylated PCM 1487 core oligosaccharide (21 mg) was reduced with $NaBH_4$ (20 mg) at room temperature for 2 h, and then the sample was acidified with acetic acid to pH 5 and treated several times with additions and evaporations of methanol to dryness. For carboxyl reduction, the residue was treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (30 mg) and then with $NaBH_4$ (30 mg) according to the method of Taylor *et al.*[6]. The product was purified on a Bio-Gel P-4 column, as described above.

REFERENCES

1 E. Romanowska, A. Gamian, and J. Dabrowski, *Eur. J. Biochem.*, 161 (1986) 557–564.
2 E. Romanowska, A. Gamian, C. Ługowski, A. Romanowska, J. Dabrowski, M. Hauck, H. J. Opferkuch, and C. W. von der Lieth, *Biochemistry*, 27 (1988) 4153–4161.
3 E. Romanowska, A. Romanowska, J. Dabrowski, and K. Trauner, *FEMS Microbiol. Lett.*, 58 (1989) 107–110.
4 E. Romanowska, A. Romanowska, C. Ługowski, and E. Katzenellenbogen, *Eur. J. Biochem.*, 121 (1981) 119–123.
5 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
6 R. L. Taylor, J. E. Shively, and H. E. Conrad, *Methods Carbohydr. Chem.*, 7 (1976) 149–151.