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Carbohydrate Research 330 (2001) 537–540

CARBOHYDRATE RESEARCH

Note

The structure of the carbohydrate backbone of the core–lipid A region of the lipopolysaccharide from *Proteus penneri* strain 40: new *Proteus* strains containing open-chain acetal-linked *N*-acetylgalactosamine in the core part of the LPS

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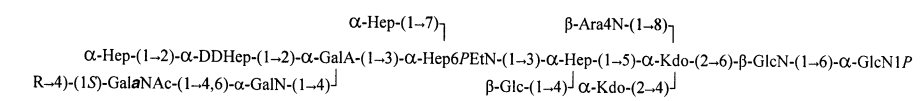
Received 31 July 2000; accepted 20 November 2000

Abstract

Analysis of the core part of the LPS from several strains of *Proteus* revealed that *P. penneri* strains 2, 11, 19, 107, and *P. vulgaris* serotypes O4 and O8 have the same structure with a new type of linkage between monosaccharides–an open-chain acetal — that was previously determined for *P. vulgaris* OX2 and *P. penneri* 17. The LPS from *P. penneri* strain 40 contains the same structure substituted with one additional monosaccharide:



P. vulgaris OX2, O4, O8, *P. penneri* 2, 11, 17, 19, 107: R = β-Gal-(1→ or H

P. penneri 40 R = α-Glc-(1→6)-β-Gal-(1→

where (1*S*)-Gal*a*NAc[1] is a residue of *N*-acetyl-D-galactosamine in the open-chain form. It is connected as a cyclic acetal to positions 4 and 6 of the galactosamine residue having a free amino group. All other sugars are in the pyranose form.

Keywords: Lipopolysaccharide; *Proteus penneri*; Core

Abbreviations: LPS, lipopolysaccharide; Hep, L-*glycero*-D-*manno*-heptose; DD-Hep, D-*glycero*-D-*manno*-heptose; GalA, galacturonic acid; Kdo, 3-deoxy-D-*manno*-octulosonic acid; P, phosphate; *P*EtN, 2-aminoethylphosphate; Ara4N, 4-amino-4-deoxy-L-arabinose, anh-Tal, 2,5-anhydrotalose.

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[1] The abbreviation Gal*o*NAc was used in two previous reports,[4,5] but the alternative designation Gal*a*NAc (*a* denoting acyclic, acetal-linked, or aldehydo) is recommended for future use. Editor.

0008-6215/01/$ - see front matter
PII: S0008-6215(00)00312-8

1. Introduction

Bacteria of the genus *Proteus* are important opportunistic pathogens causing nosocomial, wound, and urinary tract infections.[1,2] The genus *Proteus* consists of four species: *P. vulgaris*, *P. mirabilis*, *P. myxofaciens*, and *P. penneri*. The core part of the lipopolysaccharides (LPS) from three strains of *Proteus* — *P. mirabilis* O27, *P. vulgaris* OX2 and *P. penneri* strain 17, were found to contain an unusual structural component — the residue of *N*-acetyl-D-galactosamine in the open-chain form, linked to positions 4 and 6 of the residue of galactosamine as a cyclic acetal.[3–5] Here we present another structure of this type, found in the core of LPS from *P. penneri* strain 40, and the identification of the structure identical to *P. vulgaris* OX2 in several other strains.†

2. Experimental

Bacterial strains and lipopolysaccharide isolation.—Bacteria were cultivated and LPS was isolated as described in Ref. 6.

NMR spectroscopy, general methods, and preparation of oligosaccharides **1**–**3**.—These were performed as described in Ref. 7. Oligosaccharides **2** and **3** were purified by reverse-phase HPLC on an Aqua C18 column (Phenomenex, 1 × 25 cm) in 3% MeCN.

3. Results and discussion

Alkaline deacylation of the LPSs from *P. penneri* strains 2, 11, 17, 19, 40, and 107, and *P. vulgaris* OX2, O4, and O8 gave one main product, oligosaccharide **1**, identified with the oligosaccharide of the same structure previously isolated from *P. penneri* 16 and *P. vulgaris* OX2 and O25,[4,6,8] by HPAEC, NMR spectroscopy, and mass spectrometry. Mild acid hydrolysis of these LPSs gave products **2a** (*P. penneri* strains 2, 11, 17, 19, and 107, and *P. vulgaris* OX2, O4, and O8) and **2b** (*P. penneri* 40). Structural determination of the oligosaccharide **2a** was described for *P. vulgaris* OX2 in;[4] NMR and mass spectra of this compound obtained from other strains allowed determination of its identity with the product from *P. vulgaris* OX2. Compound **2a** was obtained as a mixture of two variants, with or without β-Gal residue Y, in the ratio ~4:1 in all strains.

The structure of the oligosaccharide **2b** was determined by NMR spectroscopy and chemical methods. Oligosaccharide **2b** differed from **2a** only by the presence of an additional α-Glc residue Q. The following NOE were observed in the NOESY spectra of the oligosaccharide **2b**: L1M6a, L1M4, Q1Y6a, Y1L4 (all strong). The H-1 signal of the residue Q gave NOE to H-6a of β-Gal Y, but not to H-6b. Since H-3 and H-6a of β-Gal Y overlap completely, NOE data were not sufficient for the determination of the linkage position, which was established on the basis of HMBC correlation between H-1 of α-Glc Q and C-6 of β-Gal Y, indicating α(1→6) linkage between Q and Y. Structural determination of the rest of the molecule was performed as described for the product from *P. vulgaris* OX2.[4] NMR data were in good agreement with published ones[4] (Table 1 in this publication contains an error — H-7 signals of heptose residue X in oligosaccharide **2** are at 3.74 ppm and not at 4.74 ppm). Oligosaccharide **2b**, in contrast to **2a**, had no structural variants.

Deamination of the LPSs led to the cleavage of the linkage between residues M and H and transformation of GalN M into a 2,5-anhydrotalose residue, giving a mixture of di- and trisaccharides **3a** (*P. penneri* 2, 11, 17, 19, and 107, and *P. vulgaris* OX2, O4, and O8) and **3b** (*P. penneri* 40). Structural analysis of the product **3a** is described in Ref. 4. Monosaccharide analysis of the oligosaccharide **3b** showed the presence of Glc, Gal, GalN and 2,5-anh-Tal in equal amount. Absolute D configurations of Glc, Gal, and GalNAc were determined by GLC of glycosides with optically pure 2-butanol. Methylation analysis (after borohydride reduction) led to the identification of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-galactitol, 1,4-di-*O*-acetyl-2-deoxy-3,5,6-tri-*O*-methyl-2-(*N*-methylacetamido)galactitol, and di-*O*-acetyl-2,5-anhydro-di-*O*-methyltalitol.

Interpretation of NMR data of the oligosaccharide **3b** (Table 1, Fig. 1) led to the structure presented in Scheme 1. Chemical shifts of monosaccharide residues Q, Y, L had very close values to the data for oligosaccharide **2b**.

The same NOE and HMBC correlations as in the product **2b** were observed in **3b**. The main proof of the open chain structure of the GalNAc residue L was the observation of a long-range HMBC correlation between its H-1 and C-4 and C-6 of the residue M in oligosaccharides **2** and **3** (Fig. 1). Combined evidences led to the structural proposal for the carbohydrate backbone of the core–lipid A part of the LPS from *P. penneri* 2, 11, 17, 19, 40, and 107, and *P. vulgaris* OX2, O4, and O8 presented in the scheme. The core fraction of *P. penneri* 40 did not contain structural variants in significant amounts; other strains contained small amount of the variant with missing residue of Ara4N or missing residue of β-Gal Y.

Table 1
^{1}H and ^{13}C NMR data for the oligosaccharides **2b** (R = β-Gal) and **3b** (ppm, Hz)

Unit, substance	Nucleus	1	2	3	4	5	6a	6b
GalN M, **2b**	^{1}H	5.27	3.66	4.20	4.27	4.45	3.99	4.11
	^{13}C	97.6	51.4	65.6	75.2	64.5	69.3	
GalN M, **3b**	^{1}H	5.08	3.94	4.39	4.39	4.00	4.01	4.15
	^{13}C	91.1	84.4	74.4	77.2	74.0	67.9	
Gal*a*NAc L, **2b**	^{1}H	4.89	4.45	4.28	3.62	3.99	3.70	3.73
	^{13}C	101.0	51.8	67.8	78.4	70.5	63.8	
Gal*a*NAc L, **3b**	^{1}H	4.85	4.45	4.25	3.63	4.00	3.71	3.75
	^{13}C	99.7	52.4	68.5	78.6	70.9	64.1	
Gal*a*NAc L, **3b**, $J_{H,H+1}$	^{1}H	3.9	~0	9.3	2			
Gal Y, **2b**	^{1}H	4.43	3.50	3.64	3.94	3.79	3.65	3.91
	^{13}C	104.1	71.8	73.4	69.6	73.8	67.7	
Gal Y, **3b**	^{1}H	4.48	3.51	3.64	3.94	3.80	3.65	3.92
	^{13}C	104.2	72.0	73.6	69.8	74.0	68.0	
Glc Q, **2b**	^{1}H	4.92	3.55	3.69	3.39	3.66	3.75	3.86
	^{13}C	99.3	72.0	73.8	70.4	73.0	61.5	
Glc Q, **3b**	^{1}H	4.92	3.55	3.69	3.39	3.66	3.74	3.86
	^{13}C	99.5	72.3	74.1	70.6	73.0	61.6	

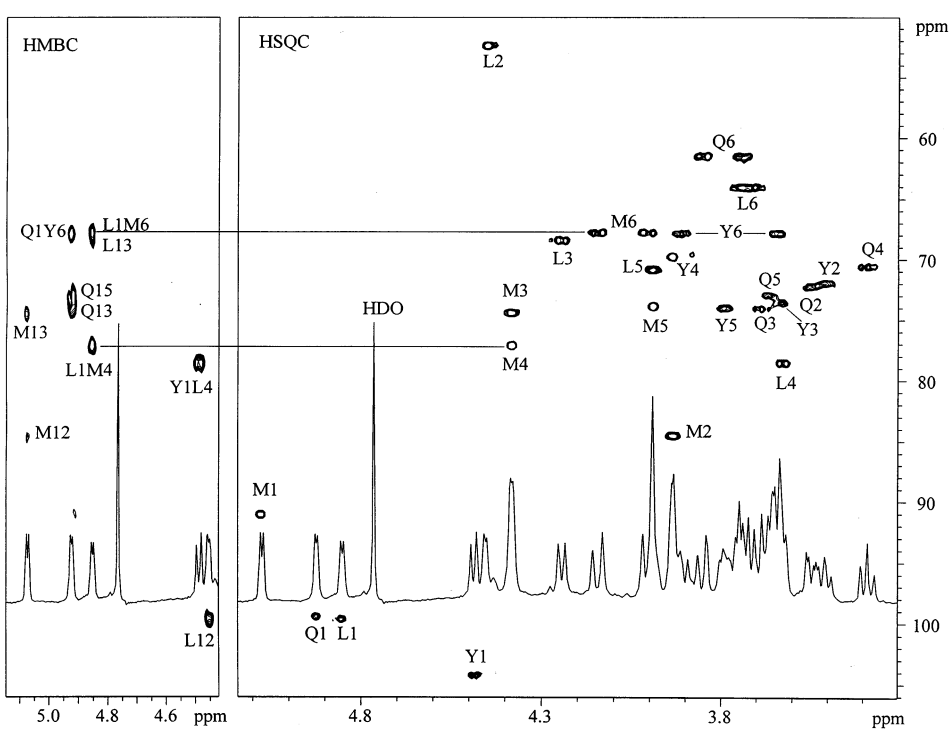
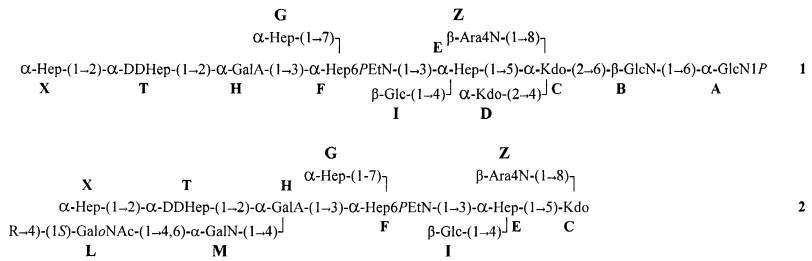
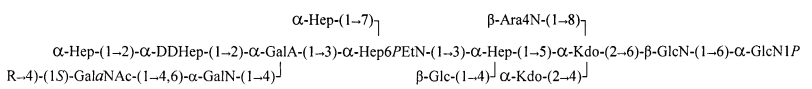


Fig. 1. Part of HSQC and HMBC $^{1}H-^{13}C$ correlation spectra of the oligosaccharide **3b**.



Compound	Source	R
2a,3a	*P. vulgaris* 2, 11, 17, 19, 107, *P. vulgaris* OX2, O4, O8	β-Gal-(1→ or H **Y**
2b,3b	*P. penneri* 40	R = α-Glc-(1→6)-β-Gal-(1→ **Q** **Y**

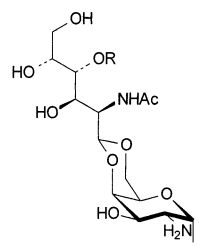
Proposed structures of the core-lipid A part of the LPS:



P. vulgaris OX2, O4, O8, *P. penneri* 2, 11, 17, 19, 107: R = β-Gal-(1→ or H

P. penneri 40 R = α-Glc-(1→6)-β-Gal-(1→

Structure of L-M disaccharide in core part of the LPSs:



Scheme 1. Structures of the oligosaccharides obtained from the LPS from *P. penneri* 17, 40, 107, *P. vulgaris* OX2, O4. All sugars are in the pyranose form except Kdo in **2,** (1*S*)-Gal*a*NAc (L) in **2**,**3**, and anh-Tal in **3**.

The presence of an open-chain form of the monosaccharides had been reported in the core fragment of several *Proteus* LPSs; the only other example of this type of linkage between monosaccharides — open-chain form of arabinose — have been found in a triterpenoid glycoside.[9] The LPSs analysed were of smooth (*P. vulgaris* OX2, O4, O8, *P. penneri* 2, 11, 19, 40) or rough, i.e., lacking polysaccharide O-chain, type (*P. penneri* 17 and 107).

Acknowledgements

This work was supported by the Canadian Bacterial Diseases Network. The authors thank Donald Krajcarsky (NRC Canada) for the ESMS analysis.

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