Carbohydrate Research 402 (2015) 232–235

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

The structure of the *Morganella morganii* lipopolysaccharide core region and identification of its genomic loci

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ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form 28 October 2014
Accepted 31 October 2014
Available online 13 November 2014

Keywords:
Morganella morganii
LPS
Core
Carbohydrate gene loci

ABSTRACT

The core region of the lipopolysaccharide of *Morganella morganii* serotype O:1ab was obtained by hydrolysis of the LPS and studied by 2D NMR, ESI MS, and chemical methods. Its structure was highly homologous to those from the two major members of the same Proteeae tribe, *Proteus mirabilis* and *Providencia alcalifaciens*, and analysis of the *M. morganii* genome disclosed that the loci for its outer core, lipid A and Ara4N moieties are similarly conserved.

1. Introduction

Morganella morganii is a human commensal organism which can be an opportunistic pathogen, causing urinary tract and post-surgical infections.[1–4] A facultative anaerobic Gram-negative organism, it is a member of the Proteeae tribe, and thus related to pathogens such as *Proteus mirabilis* and *Providencia alcalifaciens*.[5] The structure of the O-antigen of *M. morganii* serotype O:1ab has been determined[6] and it is unusual in having phosphocholine side-chains. It also has a second phosphate group and an amino group, and as a zwitterionic polysaccharide it is able to initiate T-cell immunological responses by binding to MHCII molecules.[6] The structure of the O-chain of a second strain of unknown serotype was found to be comprised of two unusual higher sugars.[7]

In order to compare its lipopolysaccharide (LPS) to those of other Proteeae, we have determined the structure of the core region of *M. morganii* serotype O:1ab LPS and acquired a preliminary genome sequence for this organism. This sequence allowed identification of several gene clusters responsible for the biosynthesis of the LPS.

2. Results and discussion

2.1. Structure of the LPS core

To obtain core derivatives suitable for structural analysis LPS was treated with diluted acetic acid, which gave the core oligosaccharide fraction after gel filtration chromatography. NMR spectra showed that this material was heterogeneous, mostly due to the presence of different Kdo degradation products. The anomeric region of the ^{1}H NMR spectrum contained many signals of different intensities (Fig. 1). The core oligosaccharides were purified to some degree by conventional anion-exchange separation and by high-pH anion-exchange chromatography (HPAEC) on a Dionex column, but none of the fractions obtained after these separations showed clean NMR spectra; in all cases the signals of most of the sugar residues were present in several variants. However, all constituent sugar residues and linkages between them were identified, and the results were in agreement with mass-spectrometry and methylation data expected from the structure **1** (Fig. 2).

Monosaccharide analysis of the core by GC of alditol acetates showed the presence of Glc, Gal, and LD-Hep. GalN was not visible because its free amino group prevented its hydrolysis. The absolute configuration of D-Glc, D-Gal, D-GalA, and D-GalN was determined using GC of acetylated (*R*)-2-butyl glycosides. The other monosaccharides are presumed to have the most common absolute configurations found in LPS core of similar bacteria (L-Ara4N, D-Kdo, LD-Hep). Methylation analysis (GC-MS of alditol acetates) showed

Abbreviations: Kdo, D-*manno*-oct-2-ulosonic acid; LD-Hep, L-*glycero*-D-*manno*-heptose; Ara4N, 4-amino-4-deoxy-L-arabinose; anhKdo, degradation products of Kdo with lost water molecule; anh-Tal, 2,5-anhydro-talose.

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http://dx.doi.org/10.1016/j.carres.2014.10.032
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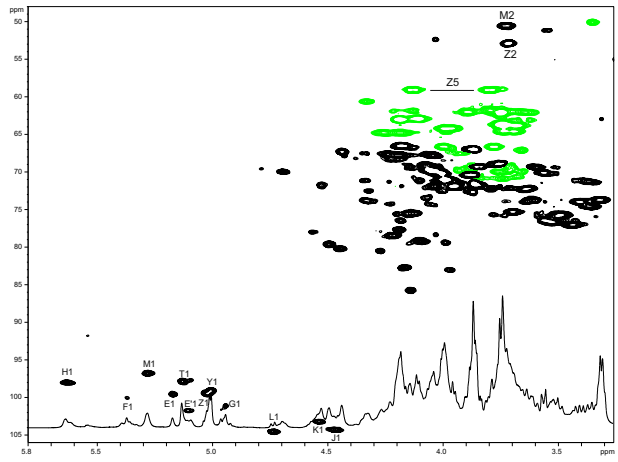

Figure 1. Part of the $^{1}H–^{13}C$ HSQC NMR spectrum of the *M. morganii* O1 LPS core. Non-anomeric signals below 80 ppm are from Kdo products.

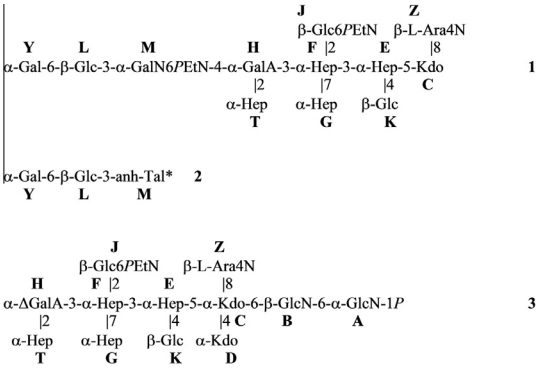

Figure 2. Proposed structure of isolated compounds. * at anh-Tal means partial phosphate at O-6.

terminal Glc (residue K), terminal Gal (Y), 6-substituted Glc (L), terminal (G,T), 3,4-(E) and 2,3,7-(F) substituted LD-Hep, all sugars being in pyranose form.

Interpretation of the 2D NMR spectra of core (compound **1**, Table 1) showed the presence of four LDHep, Kdo in various forms, three β-Glc, α-Gal, α-GalN, β-Ara4N, and α-GalA, identified by their characteristic signal patterns and chemical shifts. Kdo was present mostly in 4,7 or 4,8-anhydro forms with H-3 signals between 2.5 and 2.7 ppm. The presence of Kdo was confirmed by the analysis of the deacylated LPS (compound **3**), no attempts were made to assign signals to its degraded derivatives in the compound **1**. The sequence of the monosaccharides was based on the following NOE interproton correlations: Y1:L6; L1:M3,4; M1:H4; T1:H1,2; F1:E3; E1:C5,7; J1:F1,2; G1:F7; K1:E4,6; Z1:C8. Some of these correlations (L1:M3, M1:H4, T1:H2, Y1:L6) were confirmed by HMBC, although HMBC spectra of good quality were not possible to obtain and not all correlations were visible. The substitution positions agreed with downfield shift of the ^{13}C NMR signals of substituted carbons (Table 1).

The core was phosphorylated with *P*EtN at two positions, M6 and J6. The positions of phosphorylation were identified based on analysis of the $^{1}H–^{31}P$ HMQC and HMQC-TOCSY spectra.

NMR data indicated the presence of GalN (residue M) with a free amino-group, which was used for the deamination, and thus compound **2** was obtained. The anh-Tal derivative, formed after deamination of the GalN, lost most of the phosphate from O-6 after deamination. The structure of compound **2** was determined by 2D NMR (Table 2).

Complete deacylation of the LPS produced complex mixture of deacylated compounds, from which an oligosaccharide **3** was isolated in small amount by HPAEC. The 4-substituted galacturonic acid (residue H) was converted into a 4,5-ene derivative (α-ΔGalA) due to β-elimination of the O-4 substituent in the basic conditions of the deacylation. The NMR of compound **3** fully agreed with the structure deduced from the study of compound **1** (data not shown).

Table 1
^{1}H and ^{13}C chemical shifts (δ, ppm) for compound **1** at 25 °C; ^{31}P on J6 at 1.8 ppm, and on M6 at 0.9 ppm

Sugar residue	H-1	H-2	H-3	H-4	H-5	H-6 (6a;b)	H-7a;b
Hep E	5.13	3.99	4.08	4.34	3.79	4.07	3.75; 3.75
		71.1	79.2	72.1	72.3	69.2	63.7
Hep F	5.37	4.10	4.14	4.04	3.60	4.24	3.66; 3.76
		79.2	75.6	67.8	73.7	67.6	69.9
Hep G	4.94	3.99	3.87	3.87	3.63	4.07	
	102.2	71.0	71.6	67.0	72.2	69.8	
Hep T	5.13	3.98	3.87	3.87	3.87	4.04	3.73
	97.9	71.0	71.6	67.0	72.7	69.8	63.8
GalA H	5.64	4.03	4.19	4.50	4.53		
	98.1	71.9	67.8	79.6	71.8		
Glc J	4.47	3.23	3.50	3.60	3.50	4.19; 4.26	
	104.4	73.8	75.8	69.3	75.8	64.8	
Glc K	4.54	3.37	3.53	3.37	3.44	3.65; 3.89	
	103.3	74.7	76.7	71.4	77.1	62.1	
Glc L	4.74	3.41	3.56	3.56	3.70	3.78; 4.00	
	104.6	74.0	76.5	70.2	75.3	66.7	
GalN M	5.28	3.73	4.22	4.44	4.70	3.96; 4.00	
	96.8	51.6	78.5	67.3	70.0	64.3	
Gal Y	5.01	3.85	3.88	4.01	3.96	3.75; 3.75	
	99.2	69.3	70.4	70.3	71.9	62.2	
Ara4N Z	5.02	3.76	4.18	3.72	3.80; 4.13		
	99.5	68.9	66.5	52.9	59.1		
EtN on J	3.31	4.19					
	41.1	63.1					
EtN on M	3.31	4.11					
	41.1	63.0					