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Note

The structure of the core part of *Proteus vulgaris* OX2 lipopolysaccharide

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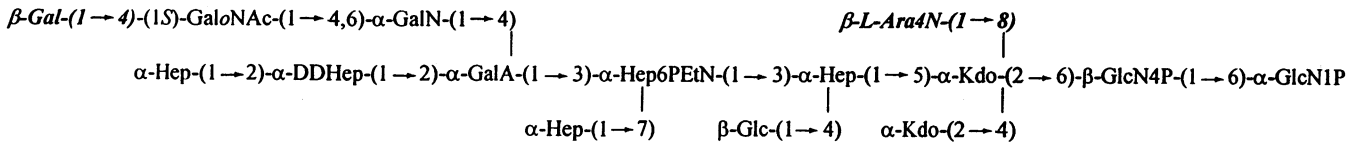
Abstract

The identity of a novel structural component, an open-chain acetalic linkage, in the core part of the lipopolysaccharide (LPS) from *Proteus vulgaris* serotype OX2 has been determined by extensive NMR spectroscopic analysis of fragments isolated after mild acid hydrolysis of the intact LPS. The open-chain *N*-acetylgalactosamine fragment is substituted in the 4-position by non-stoichiometric amounts of a β-galactopyranose residue and the overall structure of the core is as follows:



All sugars except the *N*-acetylgalactosamine are in the pyranose form, α-Hep refers to L-*glycero*-α-D-*manno*-heptopyranose and α-DDHep to D-*glycero*-α-D-*manno*-heptopyranose. Bold italics indicate non-stoichiometric substituents.

Keywords: Open-chain structure; NMR; *Proteus vulgaris* OX2; Core structure; Lipopolysaccharide

Gram-negative bacteria of the genus *Proteus* are important human pathogens, which cause wound and urinary tract infections [1]. Previously it has been shown that the core part of the lipopolysaccharide (LPS) from *Proteus vulgaris* serotype OX2 (strain 5/43), as well as the LPS core from *Proteus mirabilis* O27, contain a novel structural fragment, in which a *N*-acetylgalactosamine residue is linked as an open-chain acetal to O-4 and O-6 of another sugar residue [2]. In the present work, the complete results of the structural analysis of the core-lipid A region of *P. vul-*

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garis OX2 are reported. The structure of the O-specific polysaccharide from the LPS of this strain has been determined previously [3–5]. The structural assignment of the core part of the LPS from *P. vulgaris* OX2 presented here is based on a combination of chemical degradation studies and NMR spectroscopy.

Mild acid hydrolysis of *P. vulgaris* OX2 LPS with the isolation of the core fraction by gel chromatography on Sephadex G50 gel and further purification on TSK DEAE gel gave, after elution with water, one major product **1**. Complete O,N-deacylation of the LPS by treatment with 4 M NaOH and separation of the products by gel chromatography and HPAEC gave another major product **2**. For both compounds NMR spectra [^{1}H, DQF COSY, TOCSY, NOESY, HSQC, HSQC-TOCSY, HMBC, ^{31}P and 1D and 2D ^{1}H–^{31}P HMQC] were recorded and interpreted using the Pronto program [6]. The results are presented in Table 1 (^{1}H NMR data), Table 2 (^{13}C NMR data) and Table 3 (NOE data). The identity of monosaccharides was determined on the basis of their vicinal coupling constants (all data, not presented, are in agreement with expected values), intraresidual NOEs and ^{13}C chemical shifts. The discrimination between L-*glycero*-α-D-*manno*- and D-*glycero*-α-D-*manno*-heptoses was made on the basis of the chemical shift of the C-6 signal (about 70 ppm in the spectrum of a non-substituted α-LD heptoses and at about 72 ppm in the spectrum of the α-DD-isomer [7]). Since the heptoses (residue T in **1** and **2**) have the C-6 signals resonating at ~73 ppm, they must therefore have the D-*glycero*-D-*manno*-configuration. On the other hand, all other heptose residues have C-6 signals at ~70 ppm

Table 1
^{1}H NMR data

Unit, substance	H-1	H-2(3a)	H-3(3e)	H-4	H-5	H-6(a)	H-7a(6b)	H-8a(7b)	H-8b
A, **2**	5.64	3.38	3.91	3.60	4.15	4.30	3.74		
B, **2**	4.83	3.03	3.83	3.67	3.71	3.67	3.46		
C, **1**			1.80	4.10	4.19	3.98	3.94	3.89	3.62
C, **2a**		1.92	2.14	4.15	4.29	3.64	3.84	3.92	3.64
D, **2**		1.77	2.14	4.09	4.05	3.66	3.99	3.96	3.75
E, **1**	5.09	4.07	4.23	4.22	4.13	4.07	3.72	3.70	
E, **2**	5.28	4.09	4.17	4.30	4.23	4.11	3.93	3.80	
F, **1**	5.27	4.08	4.04	4.02	3.85	4.71	3.90	3.73	
F, **2**	5.25	4.33	4.12	4.00	3.84	4.71	3.87	3.73	
G, **1**	4.91	3.99	3.88	3.83	3.66	4.03	3.73	3.69	
G, **2**	4.97	3.98	3.86	3.86	3.72	4.02	4.74	4.74	
H, **1**	5.44	3.99	4.20	4.44	4.43				
H, **2**	5.50	3.96	4.52	5.78					
I, **1**	4.61	3.31	3.52	3.39	3.40	3.82	3.76		
I, **2**	4.57	3.29	3.52	3.35	3.39	3.86	3.73		
T, **1**	5.26	3.95	3.96	3.77	3.90	3.95	3.79	3.67	
T, **2**	5.30	4.02	3.95	3.79	3.86	4.00	3.79	3.69	
X, **1**	5.05	4.04	3.84	3.86	3.68	4.00	3.72	3.72	
X, **2**	5.07	4.03	3.83	3.87	3.67	4.02	4.74	4.74	
M, **1**	5.13	3.31	3.99	4.21	4.38	3.98	4.10		
M, **3 or 4**	5.09	3.95	4.40	4.40	4.00	4.15	4.02		
L, **1a**	4.88	4.42	4.31	3.66	3.99	3.67	3.79		
$J_{n,n+1}$, Hz	5.5	1.5	10	2	$J_{5,6a}$ 7	$J_{6a,6b}$ 12	$J_{5,6b}$ 6		
L, **1b**	4.90	4.34	4.13	3.36	3.92				
L, **3**	4.87	4.44	4.28	3.67	4.00	3.80	3.66		
L, **4**	4.89	4.36	4.12	3.36	3.93	3.66	3.66		
Y, **1a**	4.42	3.51	3.63	3.89	3.57	3.75	3.75		
Y, **3**	4.48	3.52	3.64	3.89	3.58	3.64	3.64		
Z, **1**	4.96	3.78	4.04	3.41	4.03	3.62			
Z, **2b**	5.03	3.76	4.21	3.69					
Minor **K**, **2**	4.43	3.56	3.71	4.21	4.06				
EtN, **1**	4.17	3.28							
EtN, **2**	4.15	3.29							

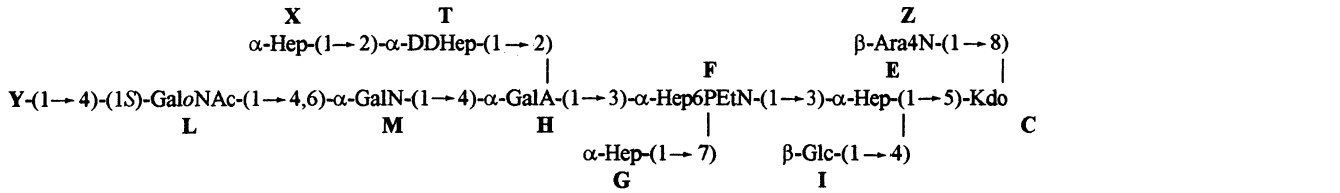
Table 2
^{13}C NMR data

Unit, substance	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
A, **2**	90.9	54.9	69.9	69.9	72.5	70.0		
B, **2**	99.9	55.8	73.0	74.5	73.7	62.9		
C, **1a**				66.3	75.0	71.2	67.8	68.9
C, **2**			34.7	70.5	70.1	72.4	69.8	64.0
D, **2**			34.7	66.4	66.8	72.5	70.6	63.3
E, **1**	100.9	70.1	76.1	74.3	71.5	69.1	63.1	
E, **2**	99.3	70.9	76.7	73.0	72.3	69.3	64.2	
F, **1**	100.5	70.1	78.7	65.7	72.8	73.4	67.6	
F, **2**	102.8	69.2	79.9	65.5	72.2	73.1	67.2	
G, **1**	100.4	70.3	70.8	66.5	71.8	69.3	63.5	
G, **2**	100.1	70.1	70.8	66.5	72.3	69.5	63.3	
H, **1**	98.5	72.8	67.4	79.1	71.5			
H, **2**	98.7	75.9	64.6	107.3				
I, **1**	102.5	74.1	75.6	69.9	76.3	61.5		
I, **2**	102.1	74.1	75.6	70.2	76.5	61.5		
T, **1**	96.0	80.0	70.0	67.8	73.6	72.3	62.6	
T, **2**	97.6	79.1	70.2	67.7	73.8	72.5	62.5	
X, **1**	102.5	70.2	70.7	66.5	72.7	69.7	63.5	
X, **2**	102.3	70.3	70.7	66.4	72.5	69.5	63.3	
L, **1a**	100.6	51.2	67.4	77.0	69.8	62.5		
L, **1b**	100.6	51.8	67.6	69.5				
L, **3**	99.1	51.8	67.6	77.1	69.8	62.6		
L, **4**	99.0	52.3	68.4	69.7	70.1	63.6		
M, **1**	99.6	51.0	67.2	75.3	64.0	69.0		
M, **3** or **4**	90.4	83.8	73.8	76.5	73.3	67.2		
Z, **1**	98.3	68.5	67.8	51.5	60.7			
Y, **1a**	103.5	71.3	73.0	68.9	75.4	61.3		
Y, **3**	103.6	71.4	73.1	69.0	75.5	61.3		
EtN, **1**	62.6	40.4						
EtN, **2**	62.3	40.2						

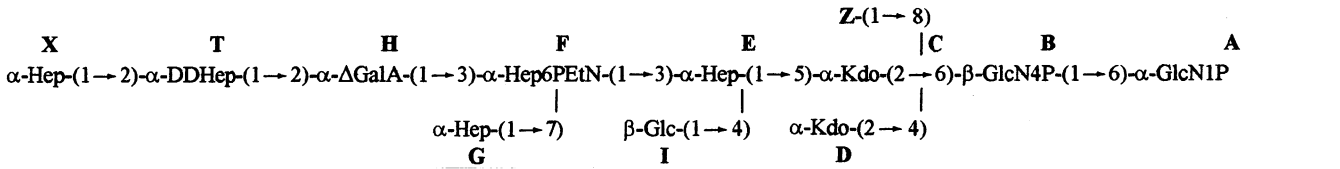
(except C-6 of the residue F, phosphorylated at O-6) and thus must possess the L-*glycero*-D-*manno*-configuration. The ratio of DD-Hep to LD-Hep was confirmed by monosaccharide analysis of compound **1** (GLC of alditol acetates gave a 1:3.5 ratio, expected 1:4 for compound **1**). The structure of the 4-deoxy-β-L-*threo*-hex-4-enopyranosyl residue H (α-ΔGalA) in compound **2** is a result of the alkaline elimination of the substituents from O-4 in the original galacturonic acid. Both Kdo residues possess the α anomeric configurations as seen from the position of the signals of their H-3 protons [8]. Sugars in furanosidic form were not present in the oligosaccharides, as characteristic low-field signals of furanosidic carbon atoms (below 80 ppm) were absent. The monosaccharide composition of the oligosaccharides was confirmed by conventional monosaccharide analysis (GLC of alditol acetates or methyl glycosides acetates). Both products **1** and **2** were mixtures of the oligosaccharides. Compound **1** represented a mixture of two products differing by the pres-

Table 3
Inter-residual NOE data (s, strong; m, medium; w, weak)

Compound	From proton	To protons
2	B1	A6s, A6′s, A5w
2	C3a	D6s, E5w
2	C3e	D6s, D8w, E5s
1	E1	C5s, C6s
2	E1	C5s, C7s
1,**2**	F1	E2s, E3s
1,**2**	G1	F7m, F7′m, F6w
1,**2**	H1	F2m, F3s, T1s
1,**2**	I1	E4s, E6s
1	M1	H4s, H5m
1,**2**	T1	H1s, H2s, X5s
1,**2**	X1	T1s, T2s
1,**3**,**4**	L1	M4s, M6aw, M6bs
1a	Y1	L4m
3	Y1	L1w,2m,3w,4s
1	Z1	C8bm



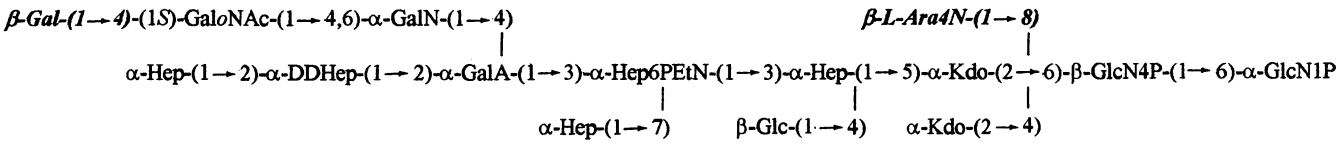
1a, Y = β-Gal; **1b, Y** = H



2a, Z = H; **2b, Z** = β-Ara4N

3, Y = β-Gal; **4, Y** = H

Overall structure of the core-lipid A part of *P. vulgaris* OX2 LPS



Scheme 1. All sugars except *N*-acetylgalactosamine are in the pyranose form, α-Hep refers to L-*glycero*-α-D-*manno*-heptopyranose and α-DDHep to D-*glycero*-α-D-*manno*-heptopyranose. Bold italics indicate non-stoichiometric substituents.

ence of a β-galactopyranose residue Y (**1a** with Y = β-Gal, major, **1b** with Y = H, minor); both variants additionally contained a variety of Kdo degradation products at the reducing end. Compound **2** contained an approximately 1:4 mixture of structures without and with the Ara4N residue (**2a** and **2b**, respectively). Oligosaccharides **1** and **2** were prepared from LPS from different batches, which accounts for the different content of the 4-aminoarabinose.

The monosaccharide sequence was assigned on the basis of the inter-residual NOEs and confirmed by ^{13}C NMR spectral data (Tables 2 and 3). The α-Kdo-(1→4)-Kdo linkage (residues C and D) was confirmed by the observation of strong NOEs between protons C-3 and D-6, which at the same time also confirms the identical absolute configuration of both residues and the α configuration of residue D [8]. NOEs of equal intensity were observed between I1-E4 and I1-E6, but the carbon chemical shifts are only compatible with the attachment of Glc residue I to O-4 of Hep residue E. The NOE between F1 and E2,3 suggests an F-3E linkage, which is furthermore confirmed by the position of corresponding carbon signals. The determination of the structure of the fragment Y–L–M and the preparation of the oligosaccharides **3** and **4** by the deamination of the core oligosaccharide were described in a previous communication [2]. The full NMR and structural assignment of the oligosaccharides is, however, presented here. The absolute configuration of Gal and GalN in **3** was determined as D by GLC of acetylated glycosides with optically active 2-butanol.

^{31}P spectra of the oligosaccharide **1** contained one signal at 0.2 ppm, which gave correlation with H-6 of the heptose residue F and H-1 of the ethanolamine residue. ^{31}P spectra

of the oligosaccharide **2** contained three signals of equal intensity at 3.2 ppm, correlating with H-1 of GlcN residue A, 4.8 ppm, correlating with H-4 of GlcN B residue, and at 0.2 ppm, correlating with H-6 of heptose residue F and H-1 of the ethanolamine residue. Thus, the core oligosaccharide was phosphorylated only at O-6 of heptose F with EtNP, whereas lipid A is phosphorylated at O-1 of reducing glucosamine residue and O-4 of glucosamine B in the compound **2**.

Taken together, the data presented allow for a proposal of the overall core structure of *P. vulgaris* OX2 LPS as shown in Scheme 1 (bold italics indicate non-stoichiometric substituents). The *P. vulgaris* strain 5/43 analysed here was previously referred to as serotype OX19 [3], but was later found to represent serotype OX2 or O2 [4,5].

Experimental conditions were as previously described [9].

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