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Note

The structure of the core region of the lipopolysaccharide from *Geobacter sulfurreducens*

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Abstract—The structure of the core part of the LPS from *Geobacter sulfurreducens* was analysed. The LPS contained no O-specific polysaccharide (O-side chain) and upon mild hydrolysis gave a core oligosaccharide, which was isolated by gel chromatography. It was studied by chemical methods, NMR and mass spectrometry, and the following structure was proposed.

α-D-Glc-(1→7)-α-L-Hep-(1→7)┐
Q→3)-α-D-GlcNAc-(1→2)-β-D-Man-(1→3)-β-D-ManNAc-(1→3)-α-Hep-(1→3)-α-L-Hep-(1→5)-α-Kdo
α-L-Fuc-(1→6)-β-D-Man-(1→4)┘

where Q = 3-O-Me-α-L-QuiNAc-(1→or H (~3:2).

Keywords: LPS; Core; *Geobacter*

Geobacter spp. are anaerobic Gram-negative dissimilatory iron- and sulfur-reducing bacteria found in subsurface environments. They are responsible for much of the microbially reduced iron in nature.[1–4] *G. sulfurreducens*, since its first isolation from petroleum contaminated aquatic sediments, has been extensively studied in order to understand its mechanism of iron reduction and its possible use as a bioremediation agent capable of reducing and immobilizing toxic heavy metals, including radionuclides. Since *Geobacter* spp. are able to use particulate iron minerals, such as goethite, as terminal electron acceptors during iron assimilation, their lipopolysaccharide (LPS) should be an important surface constituent for both mineral adhesion and 'closeness of fit' to the mineral lattice for iron reduction. Here we report for the first time partial structure of the *G. sulfurreducens* LPS.

LPS from the cells of *G. sulfurreducens* was isolated by the procedure of Darveau and Hancock[5]. Rough-type LPS after mild acid hydrolysis gave a lipid precipitate and an oligosaccharide **1**, representing the core part of the LPS. Oligosaccharide **1** was additionally purified by anion-exchange chromatography. Monosaccharide analysis of **1** (GC–MS of alditol acetates) revealed the presence of Fuc, Man, Glc, L-*glycero*-D-*manno*-heptose (Hep), GlcN, ManN, and a 3-*O*-methyl-2-amino-2,6-dideoxyhexose, later identified as 3-*O*-Me-QuiN by NMR spectroscopy.

COSY, TOCSY, NOESY, ^{1}H–^{13}C HSQC, and gHMBC 2D NMR spectra of **1** were recorded and completely assigned following the methods described by

Abbreviations: LPS, lipopolysaccharide; Kdo, 3-deoxy-D-*manno*-oct-2-ulosonic acid; Hep, L-*glycero*-D-*manno*-heptose; 3-*O*-Me-QuiNAc, 3-*O*-methyl-2-acetamido-2,6-dideoxyglucose; P, phosphate; ESIMS, electrospray ionization mass spectrometry.

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Table 1. NMR spectral data (δ, ppm) for oligosaccharides **1a**,**b**

Unit, compound	Nucleus	1	2 (3a)	3 (3e)	4	5	6	7 (6b)	8a (OMe)	8b
Kdo **C**,	^{1}H		1.90	2.18	4.14	4.16	3.88	3.74	3.62	3.86
1a,**b**	^{13}C		97.4	35.3	67.2	75.8	72.8	70.6	64.9	
α-Hep **E**,	^{1}H	5.12	4.08	4.16	4.24	4.14	3.87	3.74	3.74	
1a,**b**	^{13}C	102.1	71.8	75.5	76.5	72.4	70.0	64.2		
Hep **F**,	^{1}H	5.45	4.27	4.07	3.95	3.64	4.13	3.67	3.84	
1a,**b**	^{13}C	101.8	69.6	80.6	66.3	73.9	70.1	72.1		
Hep **G**,	^{1}H	4.94	4.00	3.88	3.88	3.64	4.24	3.72	3.85	
1a,**b**	^{13}C	102.4	71.5	72.0	67.6	73.6	69.4	71.9		
β-Man **T**,	^{1}H	4.78	4.05	3.67	3.52	3.55	3.90	4.01		
1a,**b**	^{13}C	101.8	72.1	74.3	68.8	77.0	71.2			
α-Fuc **W**,	^{1}H	4.99	3.80	3.87	3.81	4.22	1.28			
1a,**b**	^{13}C	102.0	69.6	71.2	73.3	68.3	17.0			
α-Glc **Y**,	^{1}H	4.98	3.57	3.77	3.42	3.72	3.77	3.89		
1a,**b**	^{13}C	100.4	72.9	74.4	71.0	73.3	62.1			
β-ManN	^{1}H	4.98	4.76	4.13	3.49	3.54	3.84	3.93		
X, **1a**,**b**	^{13}C	98.9	51.3	78.2	66.3	77.7	61.8			
β-Man **Z**,	^{1}H	4.86	3.88	3.75	3.67	3.48	3.77	3.99		
1a,**b**	^{13}C	97.6	77.9	74.7	68.7	78.2	62.5			
α-GlcN **S**,	^{1}H	5.09	4.03	3.96	3.69	4.13	3.74	3.94		
1a	^{13}C	99.5	55.5	77.6	68.9	72.4	61.2			
α-QuiN	^{1}H	5.06	3.99	3.48	3.31	4.16	1.24		3.51	
Q, **1a**	^{13}C	98.3	53.9	81.7	75.8	69.3	17.7		60.5	
α-GlcN **S**,	^{1}H	5.16	3.84	3.88	3.60	4.07	3.74	3.94		
1a	^{13}C	99.6	55.5	71.9	70.7	72.4	61.2			

Spectra were recorded in D_2O at 38 °C. NAc methyl signals at 1.99/23.4, 2.03/23.4, 2.05/23.4, 2.12/23.5 ppm (^{1}H/^{13}C).

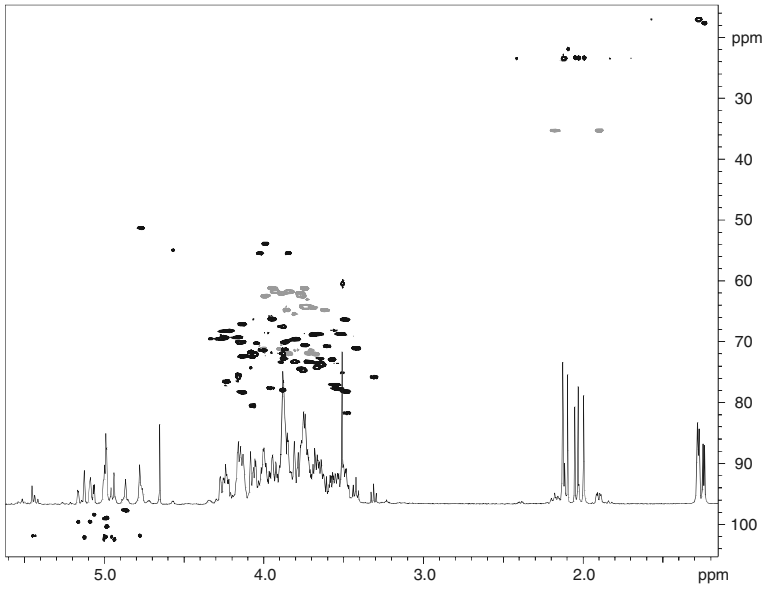
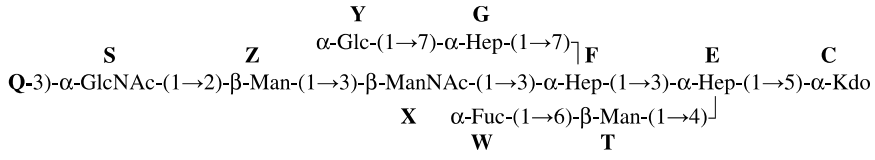


Figure 1. ^{1}H–^{13}C NMR HSQC correlation spectrum of the LPS core oligosaccharide from *G. sulfurreducens*.

Duus et al.[6] (Table 1, Fig. 1). NMR spectra showed the presence of the residues of Kdo, three α-Hep, two β-Man, β-ManNAc (β-configuration of *manno*pyranosides was determined on the basis of the observed NOE correlations H-1–H-3, H-1–H-5), α-Fuc, α-Glc, α-GlcNAc, and 3-*O*-Me-α-QuiNAc. The presence of a truncated variant **1b** lacking 3-*O*-Me-α-QuiNAc residue in the ratio of ~2:3 with the full structure was observed.

The sequence of the monosaccharides was determined from the interresidual NOE correlations: Q1S3, S1Z2, Z1X2, Z1X3, X1F2, X1F3, F1E3, E1C5, E1C7, Y1G7, G1F7, W1T6, T1E4, T1E6, leading to the structure presented. All respective interglycoside HMBC correlations were observed.

The absolute configurations of all monosaccharides except Kdo was determined by GC–MS identification



1a, Q = 3-*O*-Me-α-QuiNAc-(1→

1b, Q = H

of the acetates of 2-butyl glycosides with optically pure (*S*)-2-butanol and the respective standards prepared from known monosaccharides and (*S*)- and (*R*)-2-butanol.[7] All monosaccharides except for L-Fuc showed a D-configuration. Because of the lack of a standard compound, the configuration of 3-*O*-Me-α-QuiNAc could not be determined by this method. A ^{13}C chemical shifts calculation[8] for the 3-*O*-Me-α-QuiNAc-(1→3)-α-GlcNAc-fragment gave a better overall fit for the L-configuration of 3-*O*-Me-α-QuiNAc, although the C-3 chemical shift of the GlcNAc residue in the experimental spectrum differed in this case from the calculated one by 1.9 ppm (chemical shifts for the calculation were corrected to the same values of C-6 shifts with that in database). A similar α-L-QuiNAc-(1→3)-α-D-GlcNAc-fragment was found in the O-specific polysaccharide from *Proteus penneri* strain 26;[9] NMR data for this structure were also used for comparison and favored the L-configuration of 3-*O*-Me-α-QuiNAc.

ESIMS data for the core oligosaccharide showed the presence of two components, one containing two HexNAc, three Hep, three Hex, one deoxy-Hex, one deoxy-HexNAc, one Kdo residues, and one methyl group with a combined mass of 2054.6 Da (expected 2054.9 Da), and another one with the mass of 1853.6 Da, which lacked the 3-*O*-Me-α-QuiNAc residue.

Alkaline treatment of the LPS gave a product that was completely insoluble in water and in mild acidic or alkaline solutions. It could be dissolved in 1 M or more concentrated HCl. Monosaccharide analysis of this product showed the presence of all core components. The reason for the insolubility remained unknown. The intact LPS itself was not soluble in water and could not be significantly dispersed in 4 M KOH. The LPS was soluble in hydrazine, but the product after hydrazine removal was again insoluble in water.

The absence of an O-side chain in *G. sulfurreducens* could be an important factor for ensuring that the bacterium can closely adhere to mineral surfaces for transfer of electrons to mineral Fe(III) during iron assimilation. Further studies are needed to determine the structure of the lipid component of the LPS and to find the reasons of the unusual behavior of deacylated LPS.

1. Experimental

1.1. Bacterial strain and growth conditions

G. sulfurreducens strain PCA (ATCC 51573) was grown under strict anaerobic conditions in NBAF medium,[10] containing 15 mM of acetate as the electron donor and 40 mM of fumarate as the electron acceptor. The medium was supplemented with yeast extract and cysteine to achieve final concentrations of 0.1% (w/v) and 1 mM, respectively. The medium was bubbled with a 80%N_2–20%CO_2 gas mixture to remove dissolved oxygen and the final pH was $\sim$6.7. After incubation for 24 h at 30 °C, bacterial cells were harvested by centrifugation at 6000*g*.

1.2. LPS isolation

The method described by Darveau and Hancock[5] was used. Bacterial cells were broken in a French press using pressure of 15,000 psi, the cell lysate was treated with DNase, RNase, and protease, and the LPS was eventually precipitated in ice-cold 95% EtOH containing 0.375 M $MgCl_2$. The precipitate was dissolved in the buffer containing 2.0% (w/v) SDS in 0.1 M EDTA, pH 7.5, dialyzed against deionized water, and lyophilized.

1.3. NMR spectroscopy and general methods

NMR spectra were recorded at 38 °C in D_2O on a Varian UNITY INOVA 600 instrument using acetone as a reference (^1H, 2.225 ppm, ^{13}C, 31.45 ppm). Varian standard programs COSY, NOESY (mixing time of 300 ms), TOCSY (spinlock time 120 ms), HSQC, and gHMBC (evolution delay of 100 ms) were used.

Hydrolysis was performed with 4 M CF_3CO_2H (110 °C, 3 h), monosaccharides were conventionally converted into the alditol acetates and analyzed by GLC on a Agilent 6850 chromatograph equipped with DB-17 (30 m × 0.25 mm) fused silica column using a temperature gradient of 180 °C (2 min) → 240 °C at 2 °C/min. GC–MS was performed on Varian Saturn 2000 system with ion trap mass spectral detector using the same column. ESI mass spectra were obtained using a Micromass Quattro spectrometer in 50% MeCN with 0.2% HCOOH at a flow rate of 15 μL/min with direct injection.

Gel chromatography was carried out on Sephadex G-50 (2.5 × 95 cm) and Sephadex G-15 columns (1.6 × 80 cm) in pyridinium–acetate buffer, pH 4.5 (4 mL pyridine and 10 mL AcOH in 1 L water) and the eluate was monitored with a refractive index detector.

For the determination of the absolute configuration of the monosaccharides, core oligosaccharide (1 mg) was treated with 10:1 (*S*)-2-butanol-AcCl (0.25 mL, 2 h, 85 °C),[9] dried under a stream of air, acetylated, and analyzed by GC in comparison with authentic standards prepared with (*S*)- and (*R*)-2-butanol.

1.4. Isolation of the oligosaccharide 1

LPS (100 mg) was heated in 2% AcOH (5 mL, 100 °C, 3 h), precipitate removed by centrifugation, soluble products were separated on Sephadex G-50 column, core fraction was collected and purified by anion exchange using Hitrap Q column (5 mL, Amersham) in a gradient of water to 1 M NaCl over 1 h, with UV detection at 220 nm. Compounds were desalted by gel chromatography on a Sephadex a G-15 column (1.6 × 80 cm).

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