Carbohydrate Research 413 (2015) 51–54

Contents lists available at ScienceDirect

Carbohydrate Research

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

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Core oligosaccharide of *Escherichia coli* B—the structure required for bacteriophage T4 recognition

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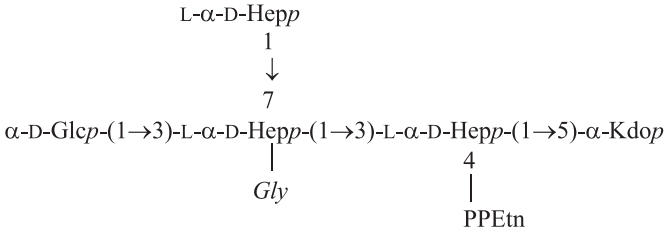
ARTICLE INFO

Article history:
Received 27 February 2015
Received in revised form
27 April 2015
Accepted 30 April 2015
Available online 29 May 2015

Keywords:
Escherichia coli B
Core oligosaccharide
glycine
NMR
MALDI-TOF
ESI MS

ABSTRACT

The structure of *Escherichia coli* B strain PCM 1935 core oligosaccharide has been investigated by ^{1}H and ^{13}C NMR spectroscopy, MALDI-TOF MS and ESI MS^{n}. It was concluded that the core oligosaccharide is a pentasaccharide with the following structure:



ESI MS/MS analysis revealed that the glycine (a minor component) is linked to the →3,7)-L-α-D-Hepp-(1→ residue.

Lipopolysaccharide (LPS) is the main surface antigen of Gram-negative bacteria. LPS consists of an O-specific polysaccharide, a core oligosaccharide and the lipid A, anchoring the molecule in outer membrane. The smooth type LPS (S-LPS) is composed of these three regions, whereas rough LPS (R-LPS) is devoid of the O-specific chain and thus structurally it is a lipooligosaccharide (LOS). Among *Escherichia coli* LPSs more than 170 different O-specific polysaccharides and five core types (R1-R4, K12) have been identified to date.[1] Bacteriophage T4 of the *Myoviridae* family is a ubiquitous microorganism found in the environment and in variety of living organisms. It has become a universal research model in microbiology to study the mechanisms of many biological processes, including bacteriophage infection. The main host of bacteriophage T4 is *E. coli* B[2,3] and this is also one of the most extensively investigated laboratory strains of *E. coli. E. coli* B is widely used paradigm for the genetic and biological studies, including infections with bacteriophages T1-T7.[4] Genome sequences of *E. coli* B strains REL606 and BL21(DE3) have been determined.[5] *E. coli* B possesses a rough form of LPS (LOS). LOS of *E. coli* B is recognized by the adhesin located on a long tail fiber of bacteriophage T4.[6] Prehm et al. reported for the first time on the structure of the core oligosaccharide isolated from LOS of *E. coli* B.[7]

We now present studies of the core oligosaccharide identified for LOS of *E. coli* B strain PCM 1935, which is recognized by bacteriophage T4. The core oligosaccharide structure was investigated by ^{1}H and ^{13}C NMR spectroscopy, as well as MALDI-TOF MS and ESI MS^{n}. It differs from the structure published by Prehm et al.[7] as it lacks one Glc residue, one Kdo residue and a phosphate group.

The *E. coli* B LOS was isolated by PCP (phenol/chloroform/petroleum) method. After mild acid hydrolysis of LOS, lipid A and two oligosaccharide fractions were obtained: OSI (yield 19.5%), and OSII (yield 22.4%). Both core oligosaccharide fractions were investigated by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy, MALDI-TOF MS and ESI MS. As indicated by the NMR and MS data the difference between OSI and OSII was attributed to phosphorylethanolamine (PEtn) (Fig. 1; Table 1). The ^{1}H NMR spectra of the OSI and OSII fractions contained main signals for four anomeric protons, as well as signals characteristic for the deoxy protons from the Kdo residue. The ^{1}H–^{1}H

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http://dx.doi.org/10.1016/j.carres.2015.04.023
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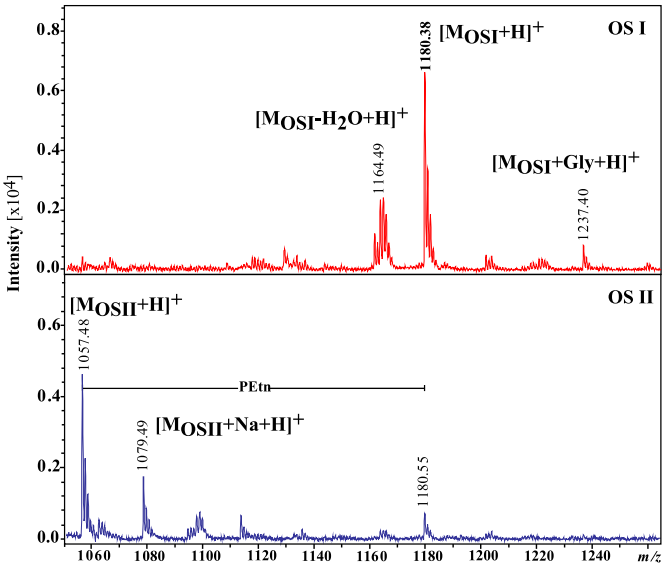

Fig. 1. MALDI-TOF mass spectra of the core oligosaccharides OSI and OSII of *E. coli* B differing by the presence of phosphoethanolamine.

COSY, TOCSY with different mixing times, $^{1}H-^{13}C$ HSQC-DEPT spectra allowed the assignment of the signals for each residue in OSI (marked as uppercase letters below) (Fig. 2, Table 1). The ^{31}P NMR spectra indicated the presence of phosphate groups (P) and $^{1}H-^{31}P$ HMBC identified the substitution positions (Fig. 3).

The chemical shifts were compared with the previously published data[8] and the $^{3}J_{H,H}$-values for the coupling between ring protons were considered for the identification of the sugar residues and their anomeric configuration. Residue **A** was assigned as the 5-substituted Kdo residue on the basis of characteristic deoxy proton signals at δ_H 1.84 (H-3ax) and 2.19 ppm (H-3eq) and high chemical shift of the C-5 signal (δ_C 73.1 ppm). Residue **B** with the H-1/C-1 signals at δ_H/δ_C 5.11/99.8 ppm was recognized as the 3-substituted L-*glycero*-α-D-*manno*-Hep*p* residue on the basis of ^{1}H and ^{13}C chemical shift values, small vicinal couplings between H-1, H-2 and H-3, and relatively high chemical shift of the C-3 signal (δ_C 77.3 ppm). Relative downfield chemical shift of the H-4/C-4 signals at δ_H/δ_C 4.55/71.6 in OSI and at δ_H/δ_C 4.38/70.0 ppm in OSII indicated the substitution by pyrophosphorylethanolamine (PPEtn) and a phosphate group, respectively. Residue **C** with the H-1/C-1 signals at δ_H/δ_C 5.07/102.7 ppm was recognized as the 3,7-disubstituted L-*glycero*-α-D-*manno*-Hep*p* residue on the basis of the ^{1}H and ^{13}C chemical shifts, small vicinal couplings between H-1, H-2 and H-3, and relatively high chemical shifts of the C-3 (δ_C 78.6 ppm) and C-7 (δ_C 69.4 ppm). Residue **D** with the H-1/C-1 signals at δ_H/δ_C 4.89/100.0 ppm was assigned as the terminal L-*glycero*-α-D-*manno*-Hep*p* residue on the basis of ^{1}H and ^{13}C chemical shifts and small vicinal couplings between H-1, H-2 and H-3. Residue **E** with the H-1/C-1 signals at δ_H/δ_C 5.21/100.3 ppm was recognized as the terminal α-D-Glc*p* on the basis of the similarity of the ^{1}H and ^{13}C chemical shift values of the corresponding non-substituted monosaccharide.[9]

The HSQC-DEPT spectrum revealed an additional negative CH_2 signal at δ_H/δ_C 3.23/40.0 ppm correlated with another CH_2 signal at δ_H/δ_C 4.14/62.4 ppm, indicating the presence of pyrophosphorylethanolamine (residue **F**). Additionally, the HSQC-DEPT spectrum revealed a negative CH_2 signal at δ_H/δ_C 3.93/40.2 ppm, which correlated with a carbonyl carbon resonance at δ_C 168.0 ppm in the HMBC spectrum, indicating the presence of glycine (residue **G**) (Fig. 2). The glycine location in the OSI was further confirmed by ESI MS/MS (Fig. 4), however the substitution position was not determined.

Each disaccharide element in the oligosaccharide fractions OSI and OSII was identified by NOESY and $^{1}H-^{13}C$ HMBC experiments (Table 1). The NOESY spectrum showed strong inter-residue NOE contacts between the *trans*-glycosidic protons: H-1 of **B**/H-5 of **A**, H-1 of **C**/H-3 of **B**, H-1 of **C**/H-3 of **B**, H-1 of **D**/H-7,7′ of **C**, H-1 of **E**/H-3 of **C**, H-1 The HMBC spectra showed cross-peaks between the anomeric proton and the carbon at the linkage position and further confirmed the structure of the core oligosaccharide of *E. coli* B (Chart 1; the asterisk indicates that the OSII fraction is devoid of the phosphorylethanolamine).

The OSI and OSII were analyzed by MALDI-TOF MS. Five sugar residues: three Hep, Hex, Kdo, and pyrophosphorylethanolamine give together a monoisotopic mass of 1179.29 Da (**M_{OSI}**) for the OSI fraction. The MALDI-TOF mass spectrum showed main ions at *m/z* 1180.38 [**M_{OSI}**+H]$^+$, 1162.49 [**M_{OSI}**-H_2O+H]$^+$ (Fig. 1A). Additionally, the ion at *m/z* 1237.40 Da [**M_{OSI}**+Gly+H]$^+$ corresponded to the OSI substituted with the glycine residue. MALDI-TOF mass spectrum of the OSII (Fig. 1A) showed the main ion at *m/z* 1057.48 [**M_{OSII}**+H]$^+$, corresponding to the OSII (OSI devoid of the phosphorylethanolamine (123.05 Da). The location of the glycine was determined by the positive ion mode ESI MS^2. The ion at *m/z* 619.2^{2+}

Table 1
^{1}H and ^{13}C NMR chemical shifts and the inter-residue NOEs and $^{3}J_{H,C}$ connectivities from the anomeric atoms of the non-glycinated core oligosaccharides of *E. coli* B LOS. The chemical shift are given as averaged values for the residues in the same environment

Residue	H1/C1	H2/C2	H3 ($H3_{ax}$,$H3_{eq}$)/C3	H4/C4	H5/C5	H6,6′/C6	H7,7′/C7	H8, 8′/C8	Connectivity to		Inter-residue atom/residue
	ppm								δ_H	δ_C	
A			(1.84, 2.19)	4.07	4.10	3.77	3.63	3.46, 3.83	—	—	—
→5)-α-Kdo	nd	95.5	33.4	65.5	73.1	71.7	69.0	63.8			
B	5.11	3.96	4.04	4.55[b]	4.14	4.04	3.65		4.10	73.1	H5, C5 of **A**
→3-L-α-D-Hep*p*-(→[a]	99.8	70.9	77.3	71.6	71.3	68.6	62.9				
C	5.06	4.31	3.96	3.95	3.59	4.11	3.54, 3.70		4.04	77.3	H3, C3 of **B**
→3,7-L-α-D-Hep-(→	102.7	69.4	78.6	65.4	72.7	68.1	69.4				
D	4.89	3.88	3.79	3.79	3.53	3.97	3.59		3.54, 3.70	69.4	H7,7′,C7 of **C**
L-α-D-Hep*p*-(→	100.0	70.0	70.5	66.1	71.3	68.7	63.1				
E	5.21	3.48	3.73	3.34	3.80	3.68,3.88			3.96	78.6	H3, C3 of **C**
α-D-Glc*p*-(→	100.3	71.9	73.0	69.7	72.2	60.4					
F	4.14	3.23									
PPEtn	62.4	40.0									

Spectra were recorded for $^{2}H_2O$ solution at 298 K.
nd—not detected.
[a] This sugar residue is substituted with a pyrophosphorylethanolamine (>90%) in OSI and a phosphate group (~80%) in OSII in the 4-position. The ^{31}P NMR chemical shifts are δ −10.85 and −11.56 ppm for the pyrophosphorylethanolamine and δ 0.71 ppm for the phosphate group, respectively.
[b] The chemical shift values of the H-4/C-4 resonances in the OS II were δ_H/δ_C 4.38/70.0 ppm.