Carbohydrate Research 397 (2014) 27–30

Contents lists available at ScienceDirect

Carbohydrate Research

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

Note

Structure and gene cluster of the O-antigen of *Escherichia coli* O68

Lingyan Jiang [a,c,d], Andrei V. Perepelov [b,*], Andrei V. Filatov [b], Bin Liu [c,d], Alexander S. Shashkov [b], Sof'ya N. Senchenkova [b], Lei Wang [c,d,e], Yuriy A. Knirel [b]

[a] TEDA Institute of Biological Sciences and Biotechnology, Nankai University, 23 Hongda Street, Tianjin 300457, China
[b] N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russian Federation
[c] The Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, Tianjin, China
[d] Tianjin Key Laboratory of Microbial Functional Genomics, Tianjin, China
[e] State Key Laboratory of Medicinal Chemical Biology, Nankai University, Tianjin, China

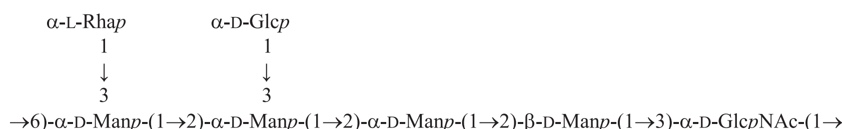
ARTICLE INFO

Article history:
Received 24 June 2014
Received in revised form 28 July 2014
Accepted 29 July 2014
Available online 12 August 2014

Keywords:
Escherichia coli
Bacterial polysaccharide structure
O-Antigen
Lipopolysaccharide
O-Antigen gene cluster

ABSTRACT

The O-polysaccharide (O-antigen) of *Escherichia coli* O68 was studied by sugar analysis, partial solvolysis with anhydrous trifluoroacetic acid, and 1D and 2D ^{1}H and ^{13}C NMR spectroscopies. The following structure of the branched heptasaccharide repeating unit was established:



The O-antigen gene cluster of *E. coli* O68 was sequenced. The gene functions were tentatively assigned by comparison with sequences in the available databases and found to be in full agreement with the O-antigen structure.

© 2014 Elsevier Ltd. All rights reserved.

Escherichia coli is the predominant facultative anaerobe of the colonic flora of many mammals, including humans, and has both commensal and pathogenic forms. *E. coli* clones are normally classified by a combination of flagellar (H), O, and capsular (K) antigens. By now, 174 *E. coli* O-antigen forms have been recognized.[1] The O-antigen or O-polysaccharide (OPS) is a part of the lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria, and consists of O-antigen repeat units (O-units) containing two to eight residues from a broad range of common or rare sugars and their derivatives. O Antigen is one of the most variable cell constituents, with variation in the types of sugars present, their arrangement within the O-unit, and the linkages within and between O-units. It contributes to the major antigenic variability and is the basis for serotyping of bacteria. O Antigen is essential for the full function of bacteria and is related to bacterial virulence. In *E. coli*, genes involved in the O-antigen synthesis are generally clustered together between the conserved *galF* and *gnd* genes, and variations in the O-antigen gene clusters are responsible for the diversity of O antigens. O-Antigen structures have been established in about two thirds of O-serogroups (see *E. coli* O-antigens database (ECODAB) at http://www.casper.organ.su.se/ECODAB). In this work, we established the structure of the O-antigen of *E. coli* O68 and characterized the O-antigen gene cluster of this bacterium.

O-Polysaccharide structure elucidation: A high-molecular mass OPS was obtained by mild acid degradation of the LPS isolated from bacterial cells by the phenol–water procedure. Sugar analysis by GLC of the alditol acetates derived after full acid hydrolysis of the OPS revealed Rha, Man, Glc, and GlcN in the ratio ~1:3.5:1:0.3 (detector response). GLC analyses of the acetylated (*S*)-2-octyl glycosides demonstrated the D configuration of Man, Glc, and GlcN, and the L configuration of Rha.

The ^{13}C NMR spectrum of the OPS (Fig. 1) showed signals for seven anomeric carbons in the region δ 97.3–102.8, one CH_3-C group (C-6 of Rha) at δ 17.8, $HOCH_2$-C groups (C-6 of hexoses) at δ 61.8–62.1 (five signals) and at δ 65.9 (one signal, data of the attached-proton test), one nitrogen-bearing carbon (C-2 of GlcN) at δ 54.1, 27 oxygen-bearing non-anomeric sugar ring carbons in the region δ 65.4–81.2, and one *N*-acetyl group at δ 23.2 (CH_3) and 175.1 (CO). Accordingly, the ^{1}H NMR spectrum of the OPS contained signals for seven anomeric protons at δ 4.76–5.29, one methyl group (H-6 of Rha) at 1.27, other sugar protons in the

* Corresponding author. Tel.: +7 499 1376148; fax: +7 499 1355328.
E-mail address: perepel@ioc.ac.ru (A.V. Perepelov).

http://dx.doi.org/10.1016/j.carres.2014.07.022
0008-6215/© 2014 Elsevier Ltd. All rights reserved.

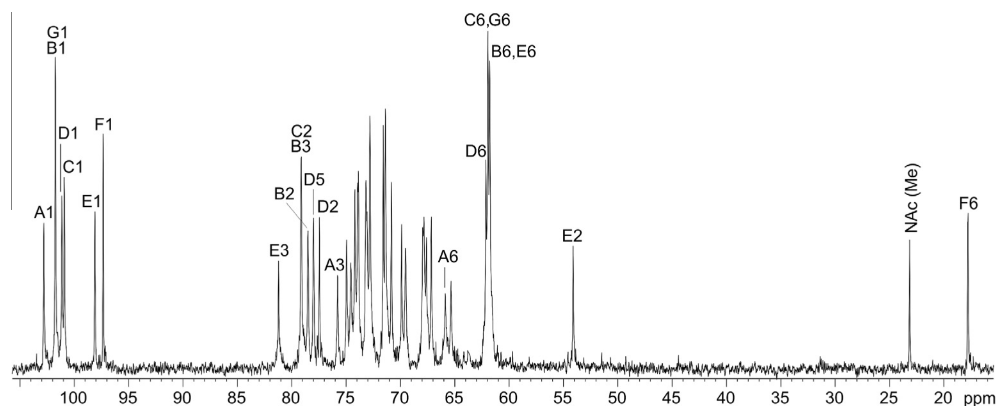


Figure 1. ^{13}C NMR spectrum of the OPS of *E. coli* O68. Signal for the CO group is not shown. Numbers refer to carbons in sugar residues denoted by letters as shown in Table 1 and Chart 1.

region δ 3.40–4.26, and one *N*-acetyl group at δ 2.04. Therefore, it was concluded that the OPS has a heptasaccharide O-unit containing four residues of D-Man (denoted as units **A**–**D**) and one residue each of D-GlcNAc, L-Rha, and D-Glc (units **E**–**G**, respectively).

Signals in the ^{1}H and ^{13}C NMR spectra of the OPS were assigned using 2D ^{1}H,^{1}H COSY, TOCSY, ROESY, ^{1}H,^{13}C HSQC, and HMBC experiments (Table 1). Based on intra-residue H,H and H,C correlations and coupling constant values estimated from the 2D NMR spectra, spin systems were assigned to residues **A**–**G**, all being in the pyranose form. A position at δ 78.0 of the signal for C-5 indicated that unit **D** is β-linked, and those at δ 72.8–74.6 showed that units **A**–**C**, **E**, and **G** are α-configured (compare published data δ 77.2–77.4 and 72.7–73.7 for β- and α-configured Man*p*, respectively[2]). Likewise, the C-5 chemical shift of δ 69.9 demonstrated the α configuration of unit **F** (compare published data δ 69.5 and 73.2 for α- and β-Rha*p*, respectively[2]).

The spin system for unit **E** was distinguished by a correlation at δ 4.08/54.1 between proton at the nitrogen-bearing carbon (H-2) and the corresponding carbon (C-2) in the ^{1}H,^{13}C HSQC spectrum. The signals for C-3 and C-6 of unit **A**, C-2 and C-3 of unit **B**, C-2 of units **C** and **D**, and C-3 of unit **E** were shifted significantly downfield, as compared with their positions in the corresponding nonsubstituted monosaccharides,[2] whereas the C-2,3,4,6 chemical shifts of units **F** and **G** differed insignificantly. These data demonstrated that the OPS is branched with units **A** and **B** at the branching points and units **F** and **G** in the side chains.

The ROESY spectrum of the OPS (Fig. 2A) showed the following correlations between anomeric protons and protons at the linkage

Table 1
^{1}H and ^{13}C NMR chemical shifts (δ, ppm)

Sugar unit	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 (6a, 6b) C-6
OPS						
→3,6)-α-D-Man*p*-(1→	5.17	4.26	3.90	4.05	3.89	3.55; 4.10
A	102.8	67.2	75.8	65.4	72.8	65.9
→2,3)-α-D-Man*p*-(1→	5.19	4.25	4.05	3.96	3.72	3.77; 3.84
B	101.7	78.5	79.1	67.6	74.6	61.8
→2)-α-D-Man*p*-(1→	5.29	4.11	4.02	3.76	3.99	3.77; 3.83
C	100.9	79.1	71.4	67.8	73.9	62.0
→2)-β-D-Man*p*-(1→	4.76	3.94	3.71	3.62	3.40	3.74; 3.92
D	101.1	77.5	75.0	68.0	78.0	62.1
→3)-α-D-Glc*p*NAc-(1→[a]	4.88	4.08	3.92	3.55	3.74	3.83; 3.87
E	98.1	54.1	81.2	69.5	73.1	61.8
α-L-Rha*p*-(1→	4.08	3.99	3.85	3.47	3.92	1.27
F	97.3	71.6	71.4	73.2	69.9	17.8
α-D-Glc*p*-(1→	5.26	3.58	3.68	3.43	3.71	3.83; 3.87
G	101.7	72.8	74.2	70.8	74.2	62.0
OPS_{TFA}						
→6)-α-D-Man*p*-(1→	5.14	4.06	3.85	3.96	3.85	3.55; 4.09
A	103.1	71.4	72.1	67.3	73.0	66.2
→2,3)-α-D-Man*p*-(1→	5.21	4.26	4.07	3.96	3.72	3.77; 3.83
B	102.0	78.4	79.2	67.9	74.7	61.9
→2)-α-D-Man*p*-(1→	5.29	4.10	4.02	3.76	4.00	3.76; 3.83
C	101.9	79.4	71.5	67.8	74.0	62.1
→2)-β-D-Man*p*-(1→	4.76	3.94	3.71	3.61	3.40	3.73; 3.93
D	101.4	77.7	75.1	68.1	78.2	62.3
→3)-α-D-Glc*p*NAc-(1→[b]	4.87	4.07	3.94	3.56	3.75	3.77; 3.86
E	98.3	54.4	81.4	69.6	73.3	61.9
α-D-Glc*p*-(1→	5.24	3.58	3.68	3.42	3.72	3.84; 3.88
G	101.9	73.0	74.3	71.0	74.1	62.1

Chemical shifts for the *N*-acetyl group are:
[a] δ_H 2.04 (Me); δ_C 23.2 (Me) and 175.1 (CO).
[b] δ_H 2.05 (Me); δ_C 23.4 (Me) and 175.4 (CO).

Download English Version:

https://daneshyari.com/en/article/1383657

Download Persian Version:

https://daneshyari.com/article/1383657

Daneshyari.com