Carbohydrate Research 373 (2013) 22–27

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

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

Structures of cell-wall phosphate-containing glycopolymers of *Bifidobacterium longum* BIM B-476-D

Olga A. Valueva [a], Alexander S. Shashkov [a], Evelina L. Zdorovenko [a,*], Alexander O. Chizhov [a], Elena Kiseleva [b], Galina Novik [c], Yuriy A. Knirel [a]

[a] N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russia
[b] Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, 220141 Minsk, Belarus
[c] Institute of Microbiology, National Academy of Sciences of Belarus, 220141 Minsk, Belarus

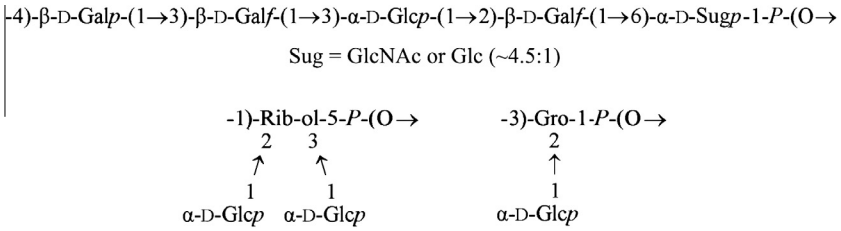
ARTICLE INFO

Article history:
Received 11 February 2013
Received in revised form 6 March 2013
Accepted 8 March 2013
Available online 18 March 2013

Keywords:
Bifidobacterium longum
Glycopolymers
Teichoic acid
Structure

ABSTRACT

Glycopolymers with oligosaccharyl phosphate repeats of two types and ribitol and glycerol teichoic acids were isolated from cell wall of *Bifidobacterium longum* BIM B-476-D by stepwise extraction with 10% CCl_3CO_2H. The following structures of the glycopolymers were established by sugar analysis, selective cleavage with aq 2% HOAc, dephosphorylation with 48% HF, 2D NMR spectroscopy, and high-resolution ESI MS:



The ribitol teichoic acid also contains minor D-alanine, whose position was not determined.

1. Introduction

Intestinal dysbioses are widespread and may cause serious health problems; therefore, preparations for correction of microbial imbalances occupy one of the leading places in complex therapy and prophylaxis of dysbiosis-associated diseases.[1,2] Apart from being safe for patients and possessing clinically proven health benefits, probiotic microorganisms must be able to remain viable when passing through the digestive tract, to adhere to intestinal epithelium, to colonize the intestines or the target organ, to synthesize antibiotics against pathogenic microorganisms as well as to survive during long storage and to be resistant to antibiotics commonly used in clinical practice.

Bacteria of the genus *Bifidobacterium* are widely used as the basis for probiotic preparations and fermented dietary food products for treatment and prevention of dysbiosis of the digestive tract, stimulation of immune system, and normalization of metabolism.[3,4] However, common disadvantages of strains of bifidobacteria used for the production of probiotic preparations is their susceptibility to antibiotics of new generation. Bacterial surface components, including polysaccharides, are important for biological activity of these microorganisms. In this work, we studied structures of glycopolymers of a new strain of *Bifidobacterium longum* BIM B-476-D, which is resistant to various antibiotics of new generation, is stable during storage, and produces biologically active polar lipids. It was selected from strain *B. longum* 1 by long-lasting adaptation to increasing concentrations of clarithromycin, azithromycin, trimoxazole, ciprofloxacin, metronidazole, and amoxicillin.

2. Results and discussion

Polysaccharides were isolated from disintegrated cells of *B. longum* BIM B-476-D by stepwise extraction with 10% CCl_3CO_2H first at 4 °C for 24 h and then at 100 °C for 5 min, cold extract (CE) and hot extract (HE) were separately dialyzed, lyophilized, and

* Corresponding author. Tel.: +7 499 1376148; fax: +7 499 1355328.
E-mail address: zdorovenkoe@mail.ru (E.L. Zdorovenko).

0008-6215/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.carres.2013.03.006

purified by GPC on Sephadex G-50 Superfine. Sugar analysis by GLC of the alditol acetates revealed similar composition of both extracts, which contained ribitol (Rib-ol), glucose, galactose, and GlcNAc, the content of glucose being higher in HE. The D configuration of the monosaccharides was determined by GLC of the acetylated (*S*)-2-octyl glycosides.[5] In addition, the acetylated (*S*)-2-octyl ester of D-alanine was identified.

The ^{1}H NMR spectrum of CE (Table 1, Figs. 1 and 2) showed major signals for seven anomeric protons at δ 4.5–5.5, other sugar ring protons at δ 3.3–4.5, and one *N*-acetyl group at δ 2.06 as well as a minor signal for alanine at δ 1.61 (CH_3). The major H-1 signal at δ 5.49 (*J* 2.8 and 6.8 Hz) and a minor H-1 signal at δ 5.54 (*J* 3.3 and 7.1 Hz) were split to doublets of doublets (Fig. 2), most likely, owing to coupling to phosphorus.

The ^{13}C NMR spectrum of CE contained major signals for seven anomeric carbons at δ 95–110, a number of OCH_2 groups (C-6 of hexoses and GlcNAc, C-1 and C-5 of Rib-ol) at δ 61–68, a nitrogen-bearing carbon δ 54.9, other sugar ring and ribitol carbons at δ 69–88, and one *N*-acetyl groups δ 23.3 (CH_3) and 175.8 (CO) as well as minor signal for alanine at δ 50.1 (C-2) and δ 16.5 (C-3). Relatively low-field positions of the anomeric carbon signals at δ 107.4 and 109.7 indicated the presence of two monosaccharide residues in the furanose form.

The ^{31}P NMR spectrum of CE showed signals for two major phosphate groups at δ 0.7 and −0.9 and a minor one at δ 0.7 and −1.2 (Fig. 2).

The ^{1}H NMR spectrum of HE contained, inter alia, major signals for two anomeric protons at δ 5.09 and 5.23, other sugar and ribitol protons at δ 3.3–4.5, and a minor signal for alanine at δ 1.60 (CH_3). The ^{13}C NMR spectrum of HE showed signals for two anomeric carbons at δ 98–102, OCH_2 groups (C-6 of hexoses, C-1 and C-5 of Rib-ol) at δ 61–68, other sugar ring and ribitol carbons at δ 70–81, as well as minor signals of alanine, including those for a nitrogen-bearing carbon at δ 50.1 and a methyl group at δ 16.5 of. The ^{31}P NMR spectrum of HE showed one major signal for a phosphate group at δ 0.6.

Tracing connectivities in the 1D TOCSY and NOE, 2D COSY, TOCSY, and ROESY spectra revealed eight major spin systems for units **A**–**H** in CE (Tables 1 and 2) and three spin systems in HE, which were essentially identical to the spin systems for **F**–**H** in CE. Additional minor spin systems for units **I** and **J** were found in HE. Therefore, it was suggested that CE contained two major polysaccharides, PS1 and PS2, and HE was composed of major PS2 and minor PS3. Using the data of the ^{1}H,^{1}H chemical shift correlated spectra as well as ^{1}H,^{13}C HSQC and HMBC spectra enabled assignment of the ^{1}H and ^{13}C NMR signals of both polysaccharides.

Units **G** and **H** in PS2 were α-Glc*p* residues as followed from a relatively small $J_{1,2}$ coupling constant of <3 Hz and relatively large ^3J coupling constants of the other ring protons. The chemical shifts for C-1–C-5 of units **G** and **H** (Table 1) were similar to those of non-substituted α-Glc*p*[6] and, hence, both glucose residues occupy the terminal positions. The third spin system belonged evidently to

Table 1
^{1}H and ^{13}C NMR chemical shifts of PS2 and PS3 and products derived from PS2 (**1**, **2**) and PS3 (**3**) (δ, ppm)

Unit	H-1 (1a, 1b) C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 (5a, 5b) C-5	H-6 (6a, 6b) C-6
PS2[a]						
(*P*)-1)-Rib-ol-5-*P*-(O→ 2 3 ↑↑	4.13	4.28	3.95	4.14	4.14	
F	*67.0*	*78.3*	*80.5*	*70.4*	*67.6*	
α-D-Glc*p*-(1→(2)	5.22	3.56	3.79	3.42	3.98	3.78, 3.87
G	*98.4*	*72.7*	*74.2*	*70.8*	*72.9*	*61.7*
α-D-Glc*p*-(1→(3)	5.09	3.56	3.68	3.42	3.88	3.78, 3.90
H	*101.5*	*72.7*	*73.8*	*70.9*	*73.9*	*61.8*
*Compound **1***						
→2,3)-Rib-ol	3.83, 3.87	4.07	3.95	4.04	3.80, 3.86	
F	*62.8*	*80.8*	*81.1*	*72.0*	*63.8*	
α-D-Glc*p*-(1→(2)	5.18	3.57	3.76	3.44	3.87	3.78, 3.88
G	*99.2*	*72.8*	*74.1*	*70.7*	*73.1*	*61.7*
α-D-Glc*p*-(1→(3)	5.10	3.57	3.67	3.44	3.85	3.78, 3.84
H	*101.2*	*72.8*	*73.9*	*70.7*	*73.8*	*61.6*
*Compound **2***						
→2,3)-Rib-ol-1-*P*-(O→	4.14	4.27	3.95	4.02	3.80, 3.88	
F	*66.8*	*80.8*	*81.1*	*72.0*	*63.8*	
α-Glc*p*-(1→(2)	5.20	3.57	3.79	3.44	3.98	3.79, 3.89
G	*98.5*	*72.8*	*74.2*	*70.8*	*73.0*	*61.7*
α-Glc*p*-(1→(3)	5.08	3.56	3.70	3.43	3.89	3.79, 3.91
H	*101.4*	*72.8*	*73.9*	*70.8*	*73.2*	*61.7*
→2,3)-Rib-ol-5-*P*-(O→	3.86	4.11	3.97	4.17	4.14	
F′	*63.0*	*80.8*	*80.3*	*70.7*	*67.6*	
α-Glc*p*-(1→(2)	5.23	3.58	3.78	3.46	3.90	3.79, 3.89
G′	*99.2*	*72.8*	*74.1*	*70.8*	*73.2*	*61.7*
α-Glc*p*-(1→(3)	5.12	3.58	3.68	3.45	3.87	3.79, 3.84
H′	*101.3*	*72.8*	*73.8*	*70.8*	*73.8*	*61.7*
PS3						
→2)-Gro	4.03, 4.07	4.10	4.02			
I	*65.7*	*76.6*	*66.5*			
α-Glc*p*-(1→	5.17	3.56	3.79	3.41	3.93	
J	*98.8*	*72.7*	*74.2*	*70.9*	*73.1*	
*Compound **3***						
→2)-Gro	3.64, 3.80	3.81	3.74			
I	*63.6*	*80.0*	*62.6*			
α-Glc*p*-(1→	5.11	3.55	3.74	3.41	3.82	3.76, 3.84
J	*99.1*	*72.7*	*74.1*	*70.8*	*73.2*	*61.7*

[a] Chemical shifts of minor Ala are δ_H 4.27 (H-2) and 1.61 (H-3), δ_C 171.7 (C-1), 50.1 (C-2) and 16.5 (C-3).