Carbohydrate Research 468 (2018) 69–72

Contents lists available at ScienceDirect

Carbohydrate Research

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

Structure of the LPS O-chain from *Fusobacterium nucleatum* strain ATCC 23726 containing a novel 5,7-diamino-3,5,7,9-tetradeoxy-L-*gluco*-non-2-ulosonic acid presumably having the D-*glycero*-L-*gluco* configuration

Evgeny Vinogradov*, Frank St Michael, Andrew D. Cox

Vaccine Program, Human Health Therapeutics Research Centre, National Research Council, Ottawa, ON, K1A 0R6, Canada

ARTICLE INFO

Keywords:
Fusobacterium nucleatum
Lipopolysaccharide
NMR spectroscopy
Polysaccharide
Structure
Non-2-ulosonic acid

ABSTRACT

Fusobacterium nucleatum is an anaerobic bacterium found in the human mouth where it causes periodontitis. It was also found in colorectal cancer tissues and is linked with pregnancy complications, including pre-term and still births. Cell surface structures of the bacterium could be implicated in pathogenesis. Here we report the following structure of the lipopolysaccharide O-chain of a spontaneous streptomycin resistant (SmR) mutant of *F. nucleatum* strain ATCC 23726:

-4-β-Non5Am7Ac-4-β-D-GlcNAcyl3NFoAN-3-β-D-FucNAc4N-

where GlcNAcyl3NFoAN indicates 2,3-diamino-2,3-dideoxyglucuronic acid amide with Fo at N-3 being formyl and Acyl at N-2 being propanoyl (~70%) or butanoyl (~30%); Non5Am7Ac indicates 7-acetamido-5-acetimidoylamino-3,5,7,9-tetradeoxy-L-*gluco*-non-2-ulosonic acid presumably having the D-*glycero*-L-*gluco* configuration. To our knowledge, no L-*gluco* isomer of higher sugars of this class as well as no N-propanoyl or N-butanoyl group have so far been found in bacterial polysaccharides.

1. Introduction

Fusobacterium nucleatum is a Gram-negative anaerobic bacterium found in the human mouth where it can cause periodontitis. It was also found in colorectal cancer tissues, where it promotes chemoresistance [1] and is linked with pregnancy complications, including pre-term and still births [2,3]. *F. nucleatum* has also been implicated in a wide variety of systemic diseases, including GI disorders [4], atherosclerosis [5] and respiratory tract infections [6].

Bacterial surface polysaccharides can be involved in pathogenesis, interacting with immune system. For this reason we have been systematically analyzing *F. nucleatum* LPS O-chain polysaccharides (OPS). Several structures have been determined, showing the presence of unusual monosaccharides and unusual acyl groups [7–10]. Here we report structure of the *F. nucleatum* strain ATCC 23726 SmR OPS, which contains a novel isomer of 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acid.

2. Results and discussion

Lipopolysaccharide was extracted from *F. nucleatum* strain ATCC 23726 SmR with hot aqueous phenol and hydrolyzed with 2% HOAc to give an OPS, a LPS core with one OPS repeating unit (core-RU) and other fractions isolated by gel permeation chromatography on a Biogel P6 column. Monosaccharide analysis of the OPS by GC-MS of the alditol acetates showed the presence of the components of the core (glucose, galactose, L-*glycero*-D-*manno*-heptose, glucosamine).

2D NMR spectra (^{1}H–^{1}H gCOSY, ^{1}H–^{1}H TOCSY, ^{1}H–^{1}H NOESY, ^{1}H-^{13}C HSQC (Fig. 1), ^{1}H-^{13}C HMBC) of the OPS contained signals of three sugar spin systems of 2,3-diamino-2,3-dideoxyglucuronic acid (GlcN3NA, A), 2,4-diamino-2,4,6-trideoxygalactose (FucN4N, B), and 5,7-diamino-3,5,7,9-tetradeoxy-L-*gluco*-non-2-ulosonic acid (Non, X). The core contained phosphocholine, and a strong signal of its methyl groups was present in all spectra. Configurations of the monosaccharides A and B were inferred from a TOCSY signal pattern and ^{1}H and ^{13}C NMR signal positions (Table 1). For the determination of the configuration of Non, better resolved NMR spectra of the core-RU were used, in which this sugar occupied the non-reducing terminal position.

Abbreviations: Am, acetimidoyl; Fo, formyl; FucNAc4N, 2-acetamido-2,4,6-trideoxy-galactose; GlcN3NA, 2,3-diamino-2,3-dideoxyglucuronic acid; Non, 5,7-diamino-3,5,7,9-tetradeoxy-L-*gluco*-non-2-ulosonic acid

* Corresponding author.
E-mail address: Evguenii.Vinogradov@nrc-cnrc.gc.ca (E. Vinogradov).

https://doi.org/10.1016/j.carres.2018.08.011
Received 6 July 2018; Received in revised form 8 August 2018; Accepted 18 August 2018
Available online 20 August 2018

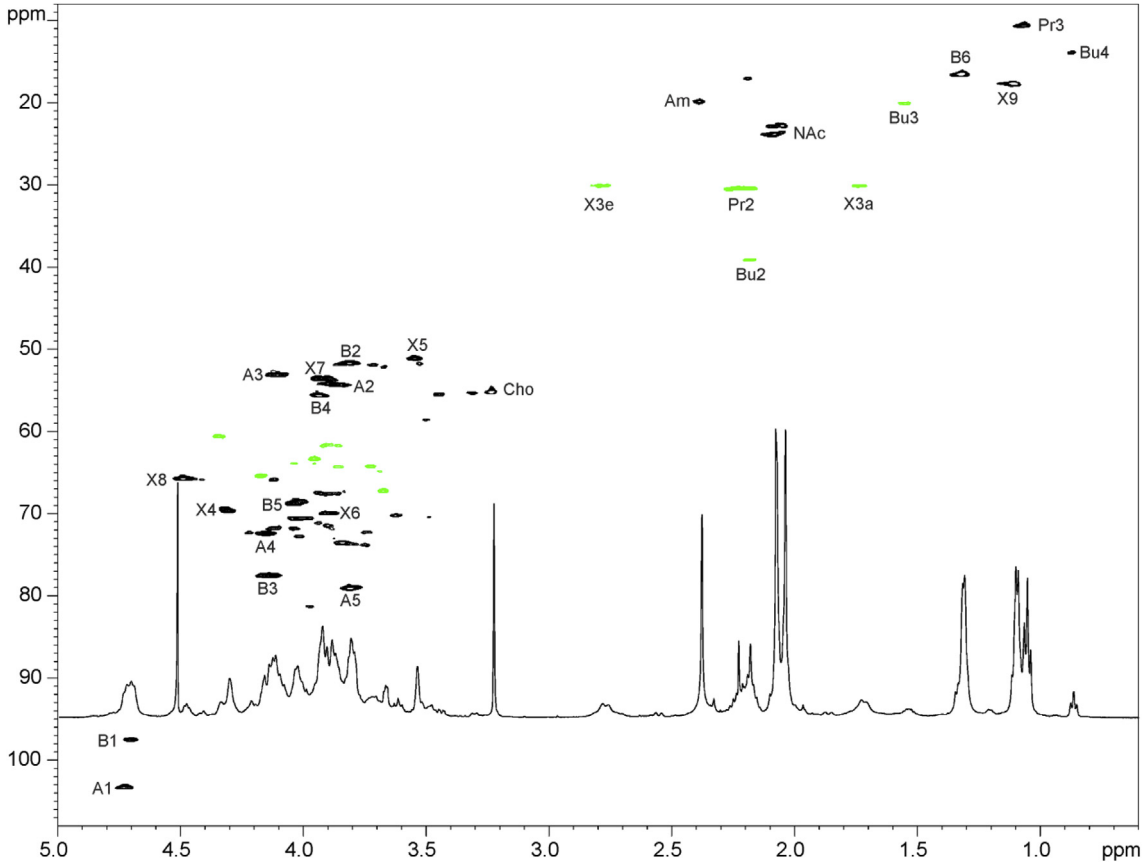

Fig. 1. ^{1}H-^{13}C HSQC and ^{1}H NMR spectra of the OPS from *F. nucleatum* ATCC 23726 (600 MHz, 45 °C). Minor signals are from the core oligosaccharide. A, B and X indicate GlcN3NA, FucN4N and Non, respectively; Am, Pr, Bu and Cho indicate acetimidoyl, propanoyl, butanoyl and choline, respectively.

Table 1
^{1}H and^{13}C NMR chemical shifts (δ, ppm) for the OPS, core-RU and OS1 from *F. nucleatum* ATCC 23726. B* and B** indicate isomeric alditols in the OS1.

Unit		1	2	3 (ax, eq)	4	5	6	7	8	9
X Non core-RU	^{1}H			1.86, 2.56	4.12	3.52	4.14	3.93	4.54	1.12
	$J_{n,n+1}$		$J_{3eq,4}$ 4.5	$J_{3ax,4}$ 4.5	< 3	< 3	11	< 3	6.5	
	^{13}C	175.6	99.6	36.0	66.0	51.8	69.5	53.7	65.9	17.9
B FucN4N core-RU	^{1}H	4.51	3.85	4.13	3.94	4.01	1.35			
	^{13}C	103.1	51.9	77.7	55.6	68.6	16.7			
A GlcN3NA core-RU	^{1}H	4.74	3.88	4.11	4.21	3.81				
	^{13}C	103.5	54.4	53.2	72.5	79.3	176.5			
X Non	^{1}H			1.73, 2.78	4.31	3.54	3.90	3.92	4.48	1.10
PS	^{13}C			30.1	69.6	51.1	70.0	53.6	65.8	17.7
B FucN4N	^{1}H	4.70	3.81	4.14	3.93	4.03	1.32			
PS	^{13}C	97.5	51.7	77.6	55.6	68.7	16.5			
A GlcN3NA PS	^{1}H	4.73	3.86	4.10	4.15	3.81				
	^{13}C	103.3	54.3	53.2	72.4	79.1				
A GlcN3NA OS1	^{1}H	1.74	3.91	4.20	4.27	4.09				
	^{13}C	102.6	54.4	53.1	72.1	77.4	174.9			
X* Non	^{1}H			1.84, 2.54	4.00	3.82	4.30	3.85	4.38	1.14
OS1	^{13}C	173.0	98.4	35.5	66.9	48.2	70.4	54.0	66.1	19.1
B*	^{1}H	3.51; 3.57	3.97	4.02	1.54; 1.73		1.16			
OS1	^{13}C	61.2	55.6	78.0	41.8	65.0	23.6			
B**	^{1}H	3.51; 3.57	4.05	4.03	1.58; 1.88		1.14			
OS1	^{13}C	61.2	54.2	77.5	41.0	65.3	23.3			
Pr	^{1}H		2.26; 2.26	1.08						
OS1	^{13}C	179.5	30.2	10.6						

Additional signals in the NMR spectra of the OPS: NAc at 175.2, 2.04/22.8; 175.2, 2.05/23.6 ppm; NAm at 167.2, 2.38/19.8 ppm; NFo at 8.07/171.5 ppm; N-propanoyl at 179.0, 2.05–2.10/30.4, 0.93/10.7 ppm; N-butanoyl at 179.0, 2.05/39.1, 1.43/20.3, 0.74/13.6 ppm.

It had small vicinal coupling constants $J_{3a,4}$, $J_{3e,4}$, $J_{4,5}$, $J_{5,6}$, $J_{7,8}$ (2–5 Hz), and one large coupling constant $J_{6,7}$ = 11 Hz (Table 1). These data and ^{13}C NMR chemical shifts (Table 1) agreed with published data for the C-4 − C-7 fragment of the corresponding L-*glycero*-L-*gluco* isomer [11]. However, large deviations were observed for C-8 (~2.5 ppm) and C-9 (~1 ppm) chemical shifts. No data for the D-*glycero*-L-*gluco* isomer are available but the C-8 and C-9 chemical shifts of Non fit better to the corresponding fragment in the D-*glycero*-L-*manno* isomer as compared