Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 340 (2005) 2218–2221
Carbohydrate RESEARCH

Note

Cell-surface α-glucan in *Campylobacter jejuni* 81-176

Erzsébet Papp-Szabó,[a] Margaret I. Kanipes,[b] Patricia Guerry[c] and Mario A. Monteiro[a,*]

[a]*Department of Chemistry, University of Guelph, Guelph, ON, Canada N1G 2W1*
[b]*Department of Chemistry, North Carolina Agricultural and Technical State University, Greensboro, NC 27411, USA*
[c]*Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD 20910, USA*

Received 26 February 2005; accepted 14 June 2005

Abstract—*Campylobacter jejuni* infection is a main source of severe gastroenteritis-related illnesses in humans and there is also evidence that it may be linked to neurological disorders. *C. jejuni* 81-176 is a virulent strain that has become the global model in the study of mechanisms and pathogenesis of *C. jejuni* infection. For this reason, we were engaged in studying the fine structures of cell-surface carbohydrate antigens of *C. jejuni* 81-176, namely, the capsule polysaccharide (CPS) and lipooligosaccharide (LOS). Serologically, *C. jejuni* 81-176 has been classified as belonging to serogroups HS23 and HS36, and indeed previous studies have shown that the LOS and CPS structures possess components similar to those expressed by serostrains HS23 and HS36. Here, we describe that in addition to the LOS and CPS, this strain also produced an independent cell-surface (1→4)-α-glucan capsule.

Keywords: *Campylobacter jejuni*; Capsule; Glucan

1. Introduction

Human illnesses due to food-borne pathogens remain a medical problem worldwide.[1] Infections caused by ingestion of *Campylobacter jejuni* are one of the main sources of human gastroenteritis,[2] and some studies have inferred that infection by this microorganism may lead to neurological disorders.[3] *C. jejuni* 81-176 is a highly virulent clinical isolate[4] that is commonly used in studies delving into the infection mechanism of Campylobacter.[5] In addition for being responsible for strain serospecificity,[6] the cell-surface capsule polysaccharides (CPS) of *C. jejuni* have been shown, to some extent, to be responsible for serum resistance, epithelial cell invasion and pathogenicity.[7]

Serologically, *C. jejuni* 81-176 has been classified as belonging to serogroups HS23/HS36.[7] Aspinall and co-workers[8,9] showed that the LOSs of *C. jejuni* HS23 and HS36 serostrains were indistinguishable, and that the serodeterminant CPSs (shown below) were composed of trisaccharide repeating blocks composed of 3-substituted D-galactose (Gal), 3-substituted *N*-acetyl-D-glucosamine (GlcNAc) and variable structural forms of 2-substituted D-altro-D-heptose (6-deoxy and/or 3-*O*-methyl derivatives):

—[→3)-β-D-GlcNAc-(1→3)-α-D-Gal-(1→2)-D-*glycero*-α-D-*altro*-D-Hep-(—1]$_n$→

—[→3)-β-D-GlcNAc-(1→3)-α-D-Gal-(1→2)-3-*O*-Me-D-*glycero*-α-D-*altro*-D-Hep-(—1]$_n$→

—[→3)-β-D-GlcNAc-(1→3)-α-D-Gal-(1→2)-6d-α-D-*altro*-Hep-(—1]$_n$→

—[→3)-β-D-GlcNAc-(1→3)-α-D-Gal-(1→2)-6d-3-*O*-Me-α-D-*altro*-Hep-(—1]$_n$→.

* Corresponding author. Tel.: +1 519 824 4120x53447; fax: +1 519 766 1499; e-mail: monteiro@uoguelph.ca

0008-6215/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2005.06.023

Recent nuclear magnetic resonance (NMR) spectroscopy-based studies carried out on *C. jejuni* 81-176 CPS[10] indicated that in fact it contained similar structural features as those found in the CPSs of serostrains HS23/HS36[9] (shown above).

However, *C. jejuni* strain 81-176 has been shown to produce two extracellular glycans. Mutation of the *kpsM* gene, encoding a component of an ABC transporter involved in capsule transport, resulted in loss of the high molecular weight (HMW) serodeterminant CPS as determined by immunoblotting.[7] However, a second glycan structure, which was originally obscured by the HMW CPS, was visible as a ladder-like structure in the *kpsM* mutant. This structure appeared to be anchored in the membrane by phospholipids and not lipid A.[7] Here, we describe the structural results obtained from studies on the second capsule produced by *C. jejuni* 81-176.

2. Experimental

2.1. Bacterial growth conditions

C. jejuni 81-176 wild-type and *kpsM* mutant[7] strains were maintained as stock cultures at 80 °C in 40% glycerol Brucella broth. *C. jejuni* were grown routinely on Mueller Hinton (MH) agar under microaerobic conditions. The *kpsM* mutant was grown on MH supplemented with 50 μg/ml kanamycin.

2.2. Extraction and purification of capsule materials

Cells of *C. jejuni* wild-type 81-176 and *kpsM* mutant were subjected to hot water–phenol extraction according to Westphal and Jann[11] for extraction of cell-surface glycans. The aqueous layer was dialyzed against distilled water and lyophilized. This crude material was then taken up in distilled water and ultracentrifuged at 40,000 rpm for 15 h at 4 °C. The pellet contained LOS and the supernatant contained non-lipid A containing glycans. The supernatant was lyophilized and fractionated on Bio-Gel P-4 column (1 m × 1 cm) with distilled water as the eluting solvent. Both *C. jejuni* 81-176 wild-type and *kpsM* mutant preparations yielded one fraction at the void volume.

2.3. Sugar composition analysis and linkage site analysis

Sugar composition analysis was performed by the alditol acetate method.[12] The glycosyl hydrolysis was done in 4 M trifluoroacetic acid at 100 °C for 4 h followed by reduction in H_2O with $NaBD_4$ at room temperature, and subsequent acetylation was accomplished by Ac_2O treatment with residual sodium acetate as the catalyst at 100 °C for 1 h. Alditol acetate derivatives were analyzed by GLC using a Varian 3400 gas chromatograph equipped with a 30-m DB-17 capillary column (210 °C (30 min) → 240 °C at 2 °C/min), and by GLC–MS in the electron impact mode, which was recorded using a Hewlett Packard 5890 mass spectrometer. Enantiomeric configurations of the individual sugars were determined by the formation and characterization of the respective 2-(*S*)- and 2-(*R*)-butyl chiral glycosides.[13] Sugar linkage analysis (1 mg) was carried out by the methylation procedure[14] ($NaOH/Me_2SO/CH_3I$) procedure and with characterization of permethylated alditol acetate derivatives by GLC–MS in the electron impact mode (DB-17 column, isothermally at 190 °C for 60 min).

2.4. Nuclear magnetic resonance spectroscopy

^1H and ^{13}C NMR spectra were recorded on a Bruker AMX 400 spectrometer at 295 K using standard Bruker software. Prior to performing the NMR experiments, the samples were lyophilized three times with D_2O (99.9%). The HOD peak was used as the internal reference at δ_H 4.821 for ^1H NMR spectroscopy.

3. Results

3.1. Structural analysis of wild-type *C. jejuni* 81-176 capsule materials

Overall monosaccharide analysis performed on the high M_r water-soluble material revealed the presence, in *quasi*-equimolar concentrations, of D-Gal, 6-deoxy-3-*O*-Me-D-*altro*-heptose (6d-3-*O*-Me-Hep), D-GlcNAc, and minor traces of 6-deoxy-*altro*-heptose (6d-Hep) and D-*glycero*-D-*altro*-heptose (DD-Hep), units that make up the serodeterminant CPSs of serostrains HS23,[9] HS36[9] and strain 81-176.[10] Our chemical analysis showed that the heptose residue in wild-type *C. jejuni* 81-176 was mainly present as the 6d-3-*O*-Me-Hep derivative and only minor traces of the 6d-Hep and DD-Hep were detected. In addition to the expected sugar units of the serodeterminant CPS, the monosaccharide composition analysis also furnished D-glucose (Glc) as a major constituent of the water-soluble material. A sugar linkage analysis (Fig. 1) yielded the anticipated 3-substituted Gal [→3)-Gal-(1→], 2-substituted 6d-3-*O*-Me-Hep [→2)-6d-3-*O*-Me-Hep-(1→] and 3-substituted GlcNAc [→3)-GlcNAc-(1→]. However, besides the expected sugar linkages from the published CPS structures,[10,11] we also observed the presence of terminal Glc [Glc-(1→], 4-substituted Glc [→4)-Glc-(1→] and traces of 4,6-disubstituted Glc [→4,6)-Glc-(1→], in line with our sugar composition data described above. We also characterized permethylated alditol acetate derivatives of terminal Gal [Gal-(1→] and 2,3-disubstituted Gal

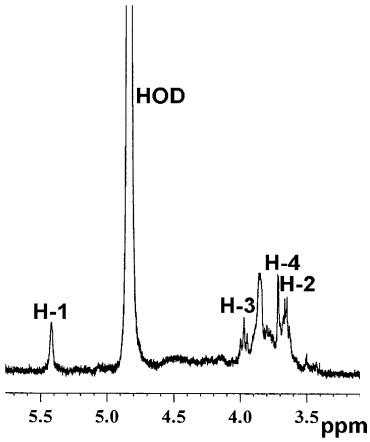

Figure 1. ^{1}H NMR spectrum of the (1→4)-α-glucan capsule extracted form *C. jejuni* 81-176 *kpsM* mutant.

[→2,3)-Gal-(1→] that could not be placed within the framework of the known CPS structure. The ^{1}H NMR analysis of this material revealed anomeric resonances belonging to the serodeterminant CPS units, which have been reported in earlier studies,[9,10] but also yielded unreported α-anomeric resonances at δ 5.42 and δ 5.25. We found that the presence of this new α-anomeric resonance at δ 5.42 and the Glc derivatives described above varied between bacterial cell growths and thus we suspected that these units may be part of an independent phase-variable capsule material. Subsequently, we impeded the expression of the serodeterminant CPS in *C. jejuni* 81-176, by insertional mutagenesis in the *kpsM* gene, with the intent of studying other capsule material not structurally associated to the serodeterminant CPS.

3.2. Structural analysis of capsule material from *C. jejuni* 81-176 *kpsM* mutant

Monosaccharide composition analysis of the water-soluble material extracted from cells of *C. jejuni* 81-176 *kspM* showed that D-Glc was present as the sole component. No CPS-related residues were observed. Linkage analysis showed that Glc was present mainly as a 4-substituted residue and, in lesser concentrations, as terminal and 4,6-disubstituted Glc. The 4-substituted Glc was present in the α-anomeric configuration as shown by resonance at δ_{H} 5.42 ($J_{1,2}$ 2.0 Hz) in the ^{1}H NMR spectrum. A 2D ^{1}H–^{13}C HSQC also yielded a sole carbon α-anomeric resonance at δ_{C} 100.1. We were able to assign H-2, H-3 and H-4 resonances of the 4-linked Glc ring system by 2D ^{1}H–^{1}H NMR correlation spectroscopy experiments (COSY and TOCSY). The observed chemical shifts of the Glc ring protons were $\delta_{H\text{-}1}$ 5.42, $\delta_{H\text{-}2}$ 3.64, $\delta_{H\text{-}3}$ 3.98 and $\delta_{H\text{-}4}$ 3.66. A 2D ^{1}H–^{1}H NOESY experiment furnished an *inter*-spatial connectivity between H-1 and H-4 and an *intra*-spatial connectivity between H-1 and H-2. Together, the analytical and spectroscopic data revealed that the capsule material produced by *C. jejuni* 81-176 *kpsM* mutant was a carbohydrate in the form of a 4-linked α-glucan.

4. Discussion

We described here that, in addition to the serodeterminant CPS, *C. jejuni* 81-176 produces a 4-linked α-glucan as a cell-surface polysaccharide. Ratios from sugar linkage analysis indicated that the 4-linked α-glucan had an average length of 25 residues. Small amounts of 4,6-disubstituted Glc were also observed, which suggested that, although infrequent, there exist regions of branching along the 4-linked α-glucan backbone. Also noteworthy, was the finding of an unanticipated 2,3-disubstituted Gal residue in the water-soluble preparation of wild-type *C. jejuni* 81-176, which may account for the appearance of yet characterized resonances in the ^{1}H NMR spectrum of *C. jejuni* 81-176. This linkage-type had not yet been described in past studies of *C. jejuni* 81-176 and its exact location, either within the serodeterminant CPS or elsewhere, is under investigation in our laboratory.

The role of the second α-glucan capsule structure in the biology and pathogenesis of *C. jejuni* 81-176 remains to be determined. The serodeterminant CPS of strain 81-176 has been shown to undergo a phase variation such that 22% of individual colonies were not expressing this structure. Thus, a population of 81-176 cells is a heterogeneous mixture with some cells expressing only the α-glucan structure and some expressing both capsular structures. A *kpsM* mutant invaded intestinal epithelial cells at about 10% the levels of wild type and this reduction in invasion was also reflected in attenuation in a ferret disease model.[7] These data might suggest that there are advantages in expressing alternate capsule structures at different times in the life cycle of the bacterium or in different environments. Additional work is ongoing to identify a biological role for the α-glucan structure and to further understand its biosynthesis.

Acknowledgements

This work was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) discovery grant to M.A.M. This research was also funded by Naval Medical Research Center Work Unit no. 601152N.000.000.A0401 (P.G.).

References

1. Schlundt, J.; Toyofuku, H.; Jansen, J.; Herbst, S. A. *Rev. Sci. Tech.* **2004**, *23*, 513–533.

2. Wassenaar, T. M.; Blaser, M. J. *Microbes Infect.* **1999**, *1*, 1023–1033.
3. Hughes, R. *Lancet Neurol.* **2004**, *3*, 644.
4. Black, R. E.; Levine, M. M.; Clements, M. L.; Hughes, T. P.; Blaser, M. J. *J. Infect. Dis.* **1988**, *157*, 472–479.
5. Hendrixson, D. R.; DiRita, V. J. *Mol. Microbiol.* **2004**, *52*, 471–484.
6. Karlyshev, A. V.; Linton, D.; Gregson, N. A.; Lastovica, A. J.; Wren, B. W. *Mol. Microbiol.* **2000**, *35*, 529–541.
7. Bacon, D. J.; Szymansky, C. M.; Burr, D. H.; Silver, R. P.; Alm, R. A.; Guerry, P. *Mol. Microbiol.* **2001**, *40*, 769–777.
8. Aspinall, G. O.; McDonald, A. G.; Raju, T. S.; Pang, H.; Moran, A. P.; Penner, J. L. *Eur. J. Biochem.* **1993**, *213*, 1017–1027.
9. Aspinall, G. O.; McDonald, A. G.; Pang, H. *Carbohydr. Res.* **1992**, *231*, 13–30.
10. Karlyshev, A. V.; Champion, O. L.; Churcher, C.; Brisson, J.-R.; Jarrell, H. C.; Gilbert, M.; Brochu, D.; Michael, F. St.; Li, J.; Wakarchuk, W. W.; Googhead, I.; Sanders, M.; Stevens, K.; White, B.; Parkhill, J.; Wren, B. W.; Szymanski, C. M. *Mol. Microbiol.* **2005**, *55*, 90–103.
11. Westphal, O.; Jann, K. *Methods Carbohydr. Chem.* **1965**, *5*, 83–91.
12. Sawardeker, J. S.; Sloneker, J. H.; Jeanes, A. *Anal. Chem.* **1965**, *37*, 1602–1604.
13. Leontein, K.; Lindberg, B.; Lonngren, J. *Carbohydr. Res.* **1978**, *62*, 359–362.
14. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131*, 209–217.