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Carbohydrate Research 340 (2005) 1605–1611
Carbohydrate RESEARCH

A novel *Helicobacter pylori* cell-surface polysaccharide

Stacey Britton,[a] Erzsebet Papp-Szabo,[a] Joanna Simala-Grant,[b] Lisa Morrison,[c] Diane E. Taylor[b] and Mario A. Monteiro[a,*]

[a]*Department of Chemistry, University of Guelph, Guelph, ON N1G 2W1, Canada*
[b]*University of Alberta, Edmonton, AL T6G 2H7, Canada*
[c]*National Research Council, Ottawa, ON K1A 0R6, Canada*

Received 9 February 2005; received in revised form 19 April 2005; accepted 19 April 2005

Abstract—*Helicobacter pylori* bacteria colonize the gastric mucosa of more than half of the world's human population and its infection may instigate a wide spectrum of gastric diseases in the host. At the moment, there is no vaccine against *H. pylori*, a microorganism recognized as a category 1 human carcinogen, and treatment is limited to antibiotic management. Pioneering antigenic studies carried out by Penner and co-workers, which employed homologous *H. pylori* antisera specific for cell-surface lipopolysaccharide (LPS), revealed the presence of six distinct *H. pylori* serotypes (O1 to O6). Subsequent studies have shown that *H. pylori* serotype O1 expressed LPS with lengthy O-chain polysaccharide (PS) composed of Lewis blood-group structures ('Lewis O-chains'), serotype O3 LPS produced 'Lewis O-chains' attached to a heptoglycan domain, serotype O4 LPS possessed LPS with glucosylated 'Lewis O-chains' and serotype O6 LPS expressed the heptoglycan domain capped by a short 'Lewis O-chain'. These LPSs were terminated at the reducing-end by a core oligosaccharide and lipid A of conserved structures. With the intent of formulating a multivalent *H. pylori* LPS-based vaccine, we are studying the structural variability of *H. pylori* cell-surface glycans. Here, we describe the novel LPS structure produced by *H. pylori* serotype O2 that differed markedly from the typical *H. pylori* 'Lewis O-chain' structures, in that its main component was an elongated PS composed of alternating 2-, and 3-monosubstituted α-D-Glc*p* residues [→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→]$_n$. These findings revealed the bio-molecular basis for the observed serospecificity of *H. pylori* serotype O2, and that this unique bacterial PS must be included in the formulation of a multivalent LPS *H. pylori* vaccine.

Keywords: Polysaccharide; *Helicobacter pylori*; Vaccine

1. Introduction

Helicobacter pylori is a widespread Gram-negative bacterium that infects the stomach of humans leading to the onset of several gastric disorders, such as, gastritis, gastric ulcers, and cancers.[1] Indeed, *H. pylori* is recognized as a category 1 (definite) human carcinogen.[2] As of yet, there is no vaccine to control *H. pylori* related illnesses and control of infection is accomplished by various combinations of antibiotic regimes,[3] a treatment that may be gradually losing effectiveness due to the rise of antibiotic resistant strains.[4] Similar to other Gram-negative bacteria,[5] lipopolysaccharides (LPSs) are a main component of the cell-surface of *H. pylori* and have been correlated with virulence factors.[6] In general, biosynthetically complete LPSs can be divided into three structural domains, a polysaccharide (PS) known as O-chain, an oligosaccharide (OS) termed core, and a fatty-acid rich endotoxin moiety named lipid A (O-Chain→Core→Lipid A ~ cell).

In the early stages of *H. pylori* research, Penner and co-workers developed a serotyping system based on different antigenicity of LPS molecules[7] whereby they defined six distinct *H. pylori* serotypes (O1 to O6). Subsequently, detailed structural[8] and serological[9] studies have shown that the LPSs of *H. pylori* are unique in that they express structures homologous to mammalian histo-blood group antigens in their O-chain PS region,

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

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doi:10.1016/j.carres.2005.04.008

with Lewis X, L-Fuc-(1→3)-[D-Gal-(1→4)]-D-GlcNAc-(1→, being the most prevalent, and, in view of this, these O-chain PSs have been coined 'Lewis O-chains'.[8] *H. pylori* LPSs were also observed to express unique lipid A[10,11] and core OS regions[8] and, in some strains, an extra LPS domain in the form of a heptoglycan (a PS composed of D-*glycero*-α-D-*manno*-heptoses) between the 'Lewis O-chain' and core regions (O-Chain→*Heptoglycan*→Core→Lipid A).[8] *H. pylori* serotype O1 strains were shown to produce LPS composed of elongated 'Lewis O-chains' connected to the core OS[12] (Fig. 1), serotype O3 LPS was also observed to express long 'Lewis O-chains', but were linked to the heptoglycan domain[13] (Fig. 1), serotype O4 LPS was composed of glucosylated 'Lewis O-chains' (Fig. 1),[8] and serotype O6 strains produced LPS with a heptoglycan that may be capped by a short 'Lewis O-chain' (a single Lewis antigen)[13] (Fig. 1). Structural studies suggest that *H. pylori* serotype O1-like LPS are the most frequently found.[8] The just described LPSs were completed at the reducing-end by a core OS (Fig. 1) and a lipid A moiety of conserved structures.

With the objective of developing a multivalent LPS *H. pylori* vaccine, which would comprise of important antigens of all known serotypes, we are engaged in studying the LPS structures of *H. pylori*. Here, we describe the chemical structure of *H. pylori* serotype O2 LPS, which contained as the main constituent an extended O-chain PS composed of alternating 2- and 3-substituted glucose (α-D-Glc*p*) residues.

2. Experimental

2.1. Bacterial growth

H. pylori serotype O2 was grown on brain heart infusion yeast extract plates under microaerobic conditions as described previously.[14] Bacterial identity was confirmed by urease, catalase, and oxidase tests, and phase contract microscopy.[14] In preparation for LPS isolation, bacteria from several hundred plates were gently emulsified (no vortexing) using a spreader and approximately 2 mL 0.9% NaCl per plate, collected by centrifugation at approximately 5000 rpm for 5 min, resuspended gently (no vortexing) in a total of 50 mL of 0.9% NaCl, and pelleted again.

2.2. LPS extraction, purification, and Lipid A removal

H. pylori O2 LPS was isolated by hot phenol–water extraction.[15] The aqueous layer containing the LPS

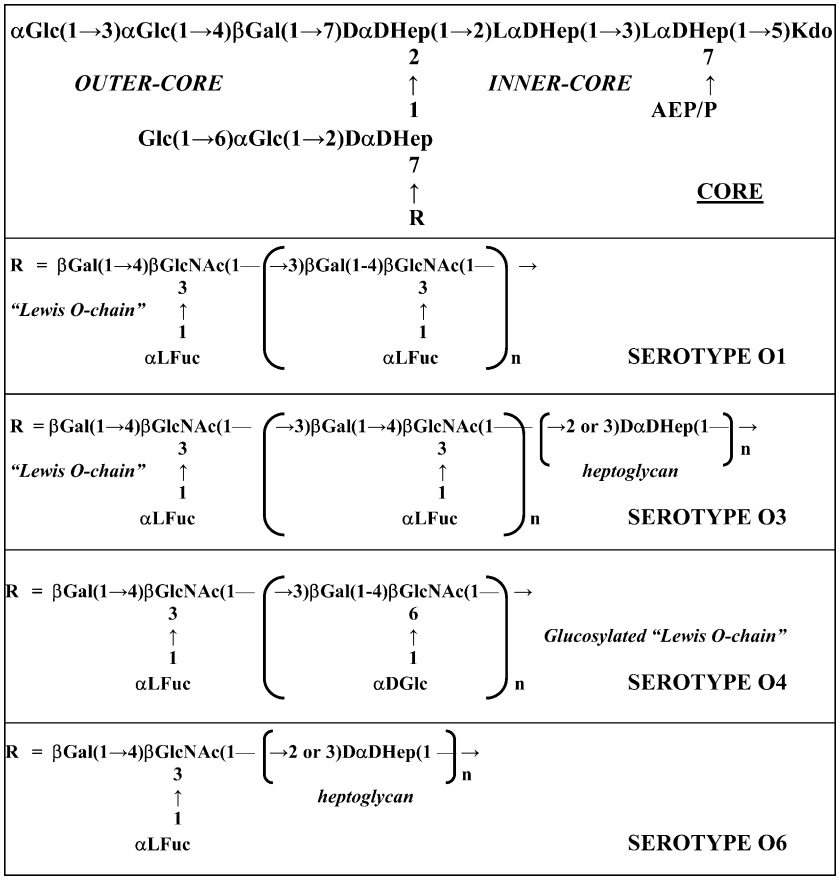

Figure 1. Core, heptoglycan, and 'Lewis O-chain' domains of *H. pylori* serotypes O1, O3, O4, and O6.

was dialyzed against water overnight and lyophilized. Ultracentrifugation did not yield any significant pellet as the LPS remained in the supernatant. For chemical and NMR experiments, the crude LPS was treated with 1% acetic acid at 100 °C to cleave the lipid A moiety from the remainder of the LPS through the acid-labile ketosidic linkage. The insoluble lipid A was removed by gentle centrifugation. The hydrolysate was purified by passing it through a Bio-Gel P-4 column (1 m × 1 cm) with one fraction eluting at the void-volume (O-chain PS→core OS). No lower M_r fraction was detected.

2.3. Sugar composition analysis and linkage site analysis

Sugar composition analysis of *H. pylori* O2 PS (0.5 mg) was performed by the alditol acetate method.[16] 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; 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.[17] Sugar linkage analysis (1 mg) was carried out by the methylation procedure[18] ($NaOH/Me_2SO/CH_3I$) 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 and mass spectrometry

^1H and ^{13}C, ^{31}P NMR spectra of *H. pylori* O2 PS were recorded on a Bruker AMX 400 spectrometer at 293 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, and orthophosphoric acid (δ_P 0.0) as external reference for ^{31}P NMR experiments. Just before the NMR experiments were carried out, a D_2O sample containing TMS (δ_H 0.00) was run to reference the HOD signal. For fast atom bombardment-mass spectrometry (FAB-MS), a fraction of the methylated sample was used for positive ion FAB-MS, which was performed on a Jeol JMS-AX505H mass spectrometer with glycerol (1): thioglycerol (3) as the matrix.

2.5. Serological studies with Lewis X monoclonal antibody

For the acrylamide gel electrophoresis and immunoblot studies, whole cells extracts of *H. pylori* strains UA948 (positive control), UA1182 (negative control), and serogroup O:2 were prepared as described by Hitchcock and Brown.[19] Electrophoresis was conducted with a constant current of 35 mA for 1 h. These gels were either silver-stained according to the method of Tsai and Frasch[20] or electroblotted onto a nitrocellulose membrane (Micron Separations Inc. Westboro, MA; pore size, 0.22 μm) according to the methods described by Towbin et al.[21] The nitrocellulose membranes were then probed with antibody anti-Lewis X (mAb BG-7) from Signet Laboratories Inc. (Dedham, MA). The anti-Lewis X mAb was diluted 1:100 and used as the primary antibody, and goat antimouse IgG plus IgM conjugated to horseradish peroxidase diluted 1:2000 was the secondary antibody. Reactions were visualized using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech) according to the manufacturer's specifications, and blots were developed on BioMax film (Eastman Kodak Co.).

3. Results

3.1. Structural characterization of O-chain PS

Monosaccharide composition analysis (approximate molar ratios in brackets) of *H. pylori* O2 PS (de-lipidated LPS; O-chain PS→core OS) revealed that D-Glc (25) was the overwhelming constituent of *H. pylori* serotype O2 LPS, with L-Fuc (1), D-Gal (2), D-GlcNAc (1), D-*glycero*-D-*manno*-heptose (DD-Hep) (2), and L-*glycero*-D-*manno*-heptose (LD-Hep) (1.5) being present in lesser concentrations. Accordingly, Glc was found to be present in two dominant linkage types in *H. pylori* O2 PS, 2- and 3-substituted, in equimolar concentrations. Of the *H. pylori* LPSs studied so far,[8] serotype O2 LPS is the first observed to produce large amounts of these two Glc linkage types, which indicated that a new *H. pylori* PS was expressed by serotype O2. The other sugar linkage types detected of Gal, Glc, DD-Hep, and LD-Hep were similar to those previously detected in the inner-regions of *H. pylori* LPSs (Fig. 1).[8]

The ^1H nuclear magnetic resonance (NMR) spectrum (Fig. 2) of *H. pylori* O2 PS showed that the two dominating Glc linkage types described above, →2)-Glc-(1→ and →3)-Glc-(1→, possessed the α configuration as observed by the presence of two major α-anomeric resonances at δ 5.63 (2.5 Hz) (residue A) and δ 5.25 (3.8 Hz) (residue B). A 2D ^1H–^{13}C heteronuclear single-quantum coherence (HSQC) experiment also showed two distinct carbon anomeric resonances at δ_C 96.72 (residue A) and δ_C 96.15 (residue B). By combining data

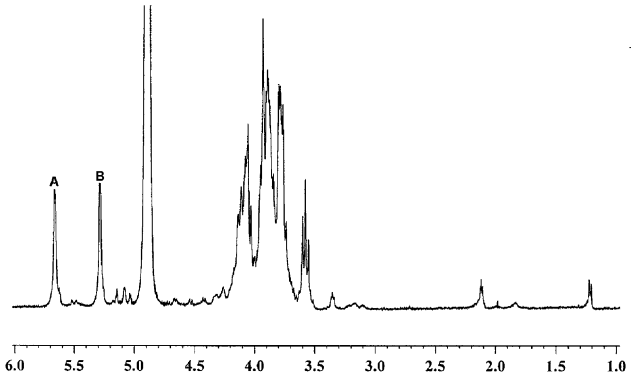

Figure 2. ^{1}H NMR spectrum of *H. pylori* O2 PS showing two major α anomeric resonances (residues A and B) belonging to the two Glc residues.

from 2D ^{1}H–^{1}H COSY and TOCSY experiments we were able to assign ring protons of the two Glc residues (A and B): for Glc residue A, $\delta_{H\text{-}1}$ 5.63, $\delta_{H\text{-}2}$ 3.75, $\delta_{H\text{-}3}$ 3.89, $\delta_{H\text{-}4}$ 3.54, $\delta_{H\text{-}5}$ 4.09, and $\delta_{H\text{-}6,6'}$ 3.88 and for Glc residue B, $\delta_{H\text{-}1}$ 5.25, $\delta_{H\text{-}2}$ 3.74, $\delta_{H\text{-}3}$ 4.01, $\delta_{H\text{-}4}$ 3.69, $\delta_{H\text{-}5}$ 4.02, and $\delta_{H\text{-}6.6'}$ 3.84. A 2D ^{1}H–^{1}H NOESY experiment (Fig. 3) showed inter-spatial connectivities between H-1 of Glc residue A ($\delta_{H\text{-}1}$ 5.63) and H-3 of Glc residue B ($\delta_{H\text{-}3}$ 4.01), and between H-1 of Glc residue B ($\delta_{H\text{-}1}$ 5.25) and H-2 of Glc residue A ($\delta_{H\text{-}2}$ 3.75). The latter resonates very closely with the resonance furnished by the expected intra-spatial connectivity between H-1 and H-2 ($\delta_{H\text{-}2}$ 3.74) of Glc residue B. The other anticipated H-1/H-2 intra-spatial connectivity between H-1 (δ 5.63) and H-2 of Glc residue A can also be observed at δ 3.75. To corroborate the sugar-linkage results obtained by the just-described 2D ^{1}H–^{1}H NOESY experiment, a 2D ^{1}H–^{13}C heteronuclear multiple bond correlation (HMBC) experiment (Fig. 4) was performed and yielded through-bond resonances belonging to connectivities between C-1 of unit A (δ 96.72) and H-3 of unit B (δ 4.01), and between C-1 of unit B (96.15) and H-2 of unit A ($\delta_{H\text{-}2}$ 3.75). In line with the chemical analysis, the observed inter-spatial (NOESY) and through-bond (HMBC) connectivities indicated that the 2- and 3-linked Glc residues were sequentially linked forming and elongated O-chain PS, [→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→]$_n$. Hence, the α-anomeric resonance A at $\delta_{H\text{-}1}$ 5.63 (δ_C 96.72) belongs to the 3-linked Glc unit (2-substituted) and the α-anomeric resonance at $\delta_{H\text{-}1}$ 5.25 (δ_C 96.15) belongs to the 2-linked Glc residue (3-substituted). A fast atom

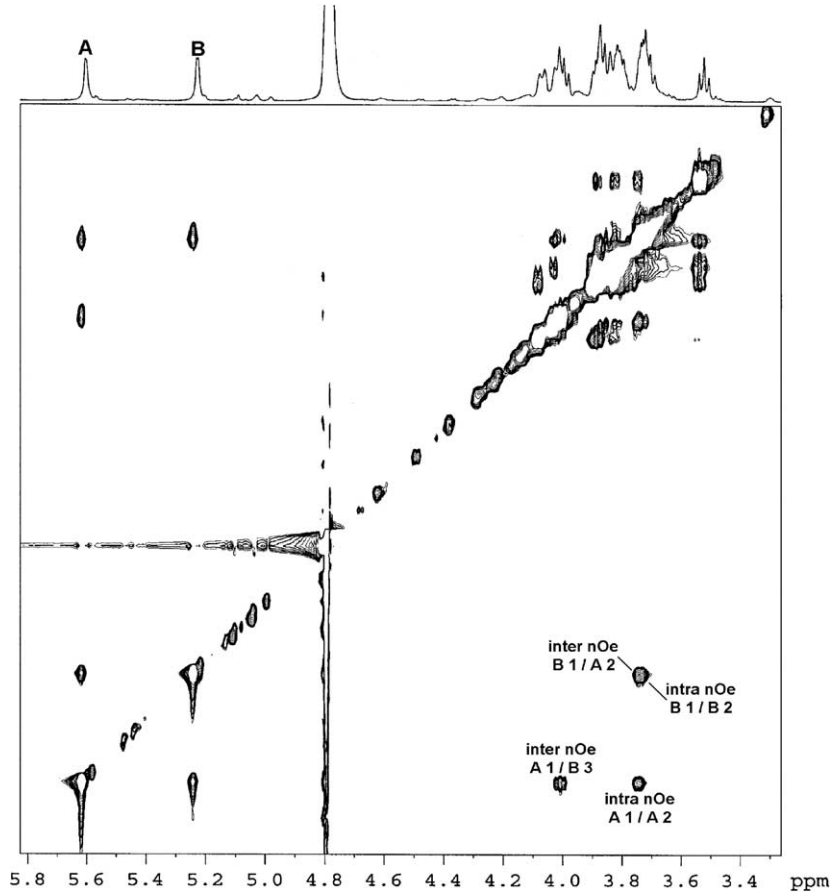

Figure 3. ^{1}H–^{1}H NOESY showing through-space evidence for the [→2)-Glc-(1→3)-Glc-(1→] sequence.

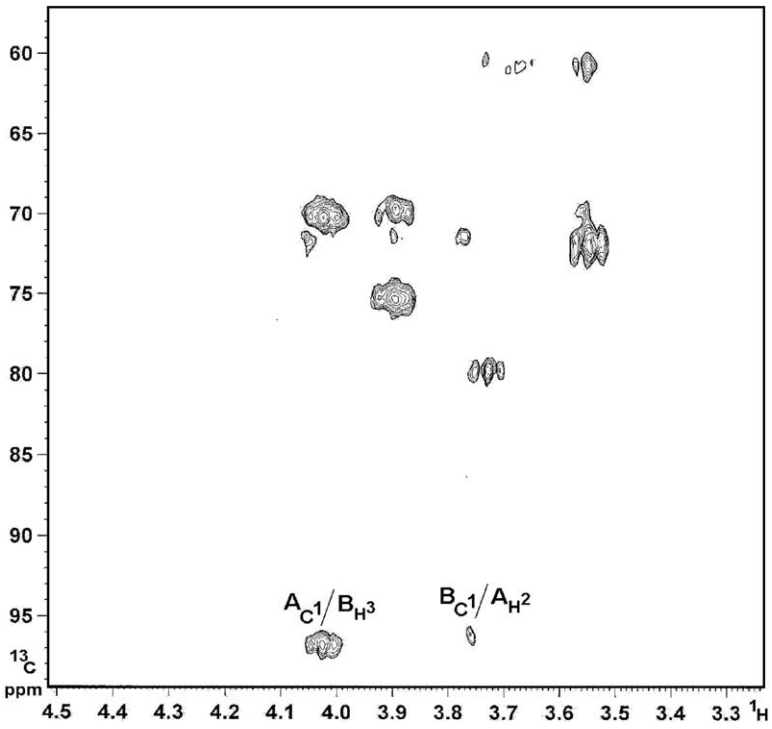

Figure 4. ^{1}H–^{13}C HMBC spectra of *H. pylori* O2 PS showing through-bond evidence for the [→2)-Glc-(1→3)-Glc-(1→] sequence.

bombardment-mass spectrometry (FAB-MS) experiment carried out on the methylated PS showed A-type primary glycosyloxonium ions, and corresponding secondary ions from loss of methanol (32 amu), at *m/z* 219→187 for Hex^{+}, 423→391 for $Hex–Hex^{+}$, 627→595 for $Hex–Hex–Hex^{+}$, and at 831→799 for $Hex–Hex–Hex–Hex^{+}$, which underlined the elongated character of the *H. pylori* O2 O-chain PS.

3.2. Structural analysis of core OS

The remainder of the *H. pylori* O2 LPS was shown to be composed of sugar residues (Table 1) previously detected in other *H. pylori* LPSs[8] and that compose the core OS region (Fig. 1), namely, 2- and 3/7-substituted LD-Hep (traces) units, 7- and 2/7-substituted DD-Hep residues, 4-substituted Gal unit (co-elutes on the right shoulder of 3-Glc peak) and terminal Glc (Fig. 2B). The GLC trace (flame ionization detector) of these permethylated alditol acetate derivatives showed approximately two molar units of terminal Glc, one from the outer-core terminus and the other from the O-chain PS terminus. In other *H. pylori* strains, the inner-most LD-Hep unit of the core OS (Fig. 1) has been observed to carry a monoester phosphate or/and a 2-amino-ethylphosphate (AEP; $NH_2–CH_2–CH_2–PO_4^-$) at its exocyclic O-7 position. Correspondingly, a ^{31}P NMR experiment carried out at pD 6.9 on *H. pylori* O2 PS clearly showed two resonances at δ 0.57 and δ 0.41 in line with the presence of these two phosphate moieties (position O-7 of 3,7-disubstituted LD-Hep) as in other *H. pylori* strains. At pD 4.0, the ^{31}P NMR experiment yielded resonances at δ 0.56 (AEP) and δ 0.26 (monoester phosphate). The presence of AEP was also underlined by the presence in the ^{1}H NMR spectrum (Fig. 2) of the methylene protons ($NH_2–CH_2–$) at δ 3.32, and by the corresponding methylene carbon resonance at δ_C 41.2 in the ^{1}H–^{13}C HSQC spectrum.

Table 1. Sugar linkage types present in *H. pylori* O2 (approximate molar ratios in brackets)

O-chain PS units		Blood-group units		Core OS units	
Glc-(1→	(1)	Fuc-(1→	(1)	Glc-(1→	(1)
→2)-Glc-(1→	(12)	Gal-(1→	(1)	→ 4)-Gal-(1→	(1)
→3)-Glc-(1→	(12)	→ 3)-Fuc-(1→	(1)	→2,3)-Glc-(1→	(1)
		→3)-Gal-(1→	(1)	→2)-DD-Hep-(1→	(1)
		→3)-GlcNAc-(1→	(1)	→2)-LD-Hep-(1→	(1)
				→2,7)-DD-Hep-(1→	(1)
				→3,7)-LD-Hep-(1→	(0.5)

3.3. Structural analysis of blood-group units

In addition to the core OS residues, single units of terminal and 3-substituted Fuc, terminal and 3-substituted Gal, and 3-substituted GlcNAc were also detected (Table 1). These Fuc, Gal, and GlcNAc 'blood-group' derivatives, were residues in line with the presence of difucosylated [Fuc-(1→3)-Fuc-(1→3)-GlcNAc-(1→] and di-galactosylated [Gal-(1→3)-Gal-(1→3)-GlcNAc-(1→] structural moieties, the latter known as the linear B blood group antigen that have been observed in other strains[8] to be attached to the O-7 position of the side-branch DD-Hep residue (Fig. 5). A FAB-MS experiment confirmed the presence of the just-described di-fucosylated and di-galactosylated GlcNAc structures by producing primary ions of defined composition at *m/z* 608 for Fuc-(1→3)-Fuc-(1→3)-GlcNAc$^+$ [189 + 174 + 245 = 608] and at *m/z* 668 for Gal-(1→3)-Gal-(1→3)-GlcNAc$^+$ [219 + 204 + 245 = 668]. A secondary ion resulting from β-elimination of the di-glycosyl moieties from GlcNAc was observed at *m/z* 228 [608–380(Fuc-Fuc-OH); 668–440(Gal-Gal-OH)]. No *m/z* ions indicative of Lewis X or any other Lewis antigens were observed. The 1D ^{1}H (Fig. 2) and 2D ^{1}H–^{13}C NMR spectra also supported the existence of GlcNAc and Fuc residues with resonances at δ_H 2.11/δ_C 22.05 for GlcNAc's *N*-acetyl's methyl moiety and at δ_H 1.205/δ_C 15.51 for the deoxy signals of Fuc units. Serological data with Lewis monoclonal antibodies also revealed that indeed *H. pylori* strain O2 did not express any Lewis blood-group structures.

4. Discussion

The LPS of *H. pylori* serotype O2 differs from the previously reported *H. pylori* LPS structures in the following aspects: (i) it produces an elongated O-chain PS composed of 2- and 3-substituted α-D-Glc*p* residues (Fig. 5) (not found in other *H. pylori* serotypes); and (ii) it does not express 'Lewis blood-group' O-chain PS nor heptoglycan (found in other *H. pylori* strains). Therefore, the immunologically based serospecificity of *H. pylori* serotype O2 noted by Penner and co-workers[7] is due to the expression of this novel *H. pylori* O-chain PS: [→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→]$_n$. Penner and co-workers[7] have published a gel electrophoresis (SDS-PAGE) of *H. pylori* O:2 LPS that displayed a ladder-like pattern, which indicated that *H. pylori* O:2 produced a true LPS molecule. No evidence was obtained that would suggest that the glucan described here exists as a sole entity separate from the LPS. Structurally, the similarities between *H.*

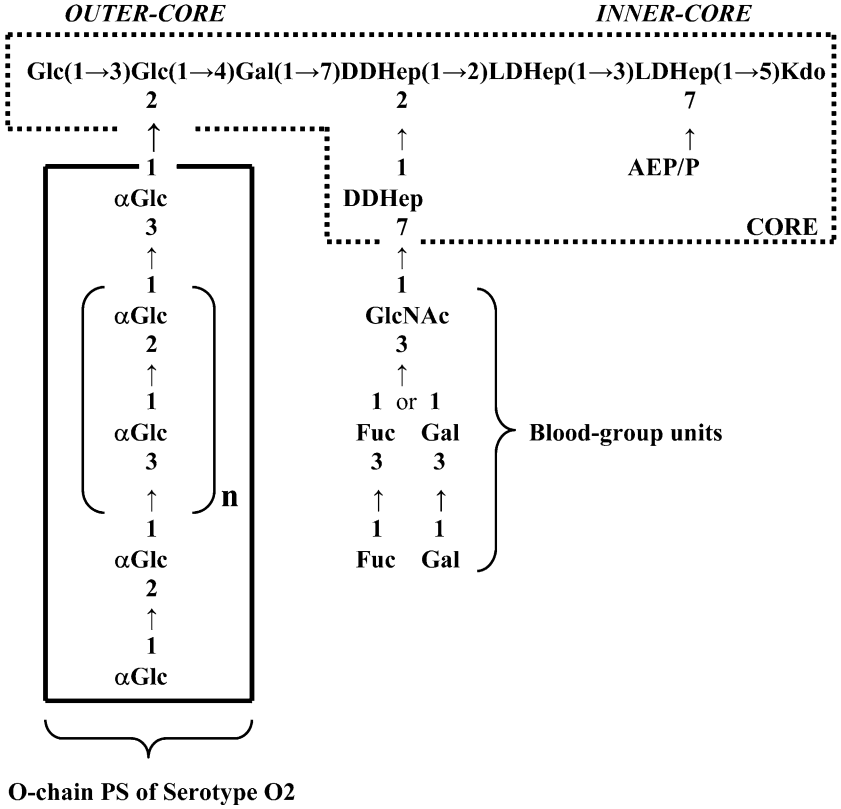

Figure 5. Core OS and O-chain PS domains of *H. pylori* serotype O2. The connection shown of the O-chain to the core via the 2,3-Glc residue was not unambiguously characterized, but it is exclusively the most plausible supposition.

pylori serotype O2 O-chain and core regions and other previously studied *H. pylori* LPSs reside solely in the core OS (Figs. 1 and 5). No side chain of 6-linked Glc units, present in other serotypes (Fig. 1), was detected in serotype O2. With respect to blood-group structures, *H. pylori* serotype O2 solely expressed a di-fucosylated GlcNAc antigen and a linear B blood-group (Fig. 5). The linear B blood-group [Gal-(1→3)-Gal-(1→3)-GlcNAc-(1→] observed in *H. pylori* O2 is also commonly known as the xenoantigen, responsible for a majority of organ (from pigs) transplant failures in humans.[22] In some humans, approximately, 1% of antibodies in human sera are directed against the xenoantigen,[23] Gal-(1→3)-Gal-(1→3)-GlcNAc-(1→, and it may be that with persons host to *H. pylori* strains, such as, serotype O2, may be carriers of higher levels of xenoantigen antibodies.

Collectively, the data showed that *H. pylori* strain O2 did not produce the familiar *H. pylori* 'Lewis blood-group O-chains' and thus it does not follow the model of molecular mimicry between *H. pylori* LPSs and host's gastric epithelial cells that express Lewis antigens. Comparing the sugar linkage ratios (Table 1) between the core units and the Glc residues of the new O-chain PS, the average length of this *H. pylori* O2 O-chain PS is approximately 24 Glc residues (12 repeating OS blocks). The covalent connection of this new *H. pylori* O-chain PS to the core OS region could not be unambiguously characterized. However, a new branched residue characterized here, 2,3-disubstituted Glc (Table 1), not previously detected in *H. pylori* LPSs, is the most plausible point of attachment, at the *O*-2 position of the outer-core Glc unit (Fig. 5).

Immunological studies[24,25] have described the detection of human antibodies in *H. pylori* infected patients that recognize regions of LPSs from various strains. This is a good indication that a prophylactic LPS-based vaccine may afford some protection against this type of infection and thus we are presently synthesizing a multivalent vaccine that contains a mixture of essential *H. pylori* LPS antigens, such as those form serotypes O1, O3, O4, and O6. Although, there are no studies about the incidence of *H. pylori* serotype O2 strains, the structural studies described here have revealed that serotype O2 possessed a distinct and serospecific O-chain PS and therefore should be included in a *H. pylori* carbohydrate-based vaccine.

Acknowledgements

This work was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) discovery grant to M.A.M. Support by the Canadian Bacterial Diseases Network (D.E.T.) and Astra Zeneca (J.L.S.G.) is acknowledged. D.E.T. is supported by a scientist award from the Alberta Heritage Foundation for Medical Research.

References

1. Forman, D.; Newell, D. G.; Fullerton, F.; Yarnell, J. W.; Stacey, A. R.; Wald, N.; Sitas, F. *Br. Med. J.* **1991**, *302*, 1302–1305.
2. Labigne, A.; de Reuse, H. *Infect. Agents Dis.* **1996**, *5*, 191–202.
3. Harris, A.; Misiewicz, J. J. *Br. Med. J.* **2001**, *323*, 1047–1050.
4. Mégraud, F. *Drug Resist. Updates* **2001**, *4*, 178–186.
5. Caroff, M.; Karibian, D. *Carbohydr. Res.* **2003**, *338*, 2431–2447.
6. Moran, A. P.; Prendergast, M. M.; Appelmelk, B. J. *FEMS Immunol. Med. Microbiol.* **1996**, *16*, 105–115.
7. Mills, S. D.; Kurjanczyk, L. A.; Penner, J. L. *J. Clin. Microbiol.* **1992**, *30*, 3175–3180.
8. Monteiro, M. A. *Adv. Carbohydr. Chem. Biochem.* **2001**, *57*, 99–158.
9. Simoons-Smit, I. M.; Appelmelk, B. J.; Verboom, T.; Negrini, R.; Penner, J. L.; Aspinall, G. O.; Moran, A. P.; Fei, S. F.; Shi, B. S.; Rudnica, W.; Savio, A.; de Graaff, J. *J Clin. Microbiol.* **1996**, *34*, 2196–2200.
10. Moran, A. P.; Lindner, B.; Walsh, E. J. *J. Bacteriol.* **1997**, *179*, 6453–6463.
11. Suda, Y.; Kim, Y. M.; Ogawa, T.; Yasui, N.; Hasegawa, Y.; Kashihara, W.; Shimoyama, T.; Aoyama, K.; Nagata, K.; Tamura, T.; Kusumoto, S. *J. Endotoxin Res.* **2001**, *7*, 95–104.
12. Aspinall, G. O.; Monteiro, M. A.; Pang, H.; Walsh, E. J.; Moran, A. P. *Biochemistry* **1996**, *35*, 2489–2497.
13. Aspinall, G. O.; Monteiro, M. A.; Shaver, R. T.; Kurjanczyk, L. A.; Penner, J. L. *Eur. J. Biochem.* **1997**, *248*, 592–601.
14. Simala-Grant, J. L.; Zopf, D.; Taylor, D. E. *J. Antimicrob. Chemother.* **2001**, *37*, 555–563.
15. Westphal, O.; Jann, K. *Methods Carbohydr. Chem.* **1965**, *5*, 83–91.
16. Sawardeker, J. S.; Sloneker, J. H.; Jeanes, A. *Anal. Chem.* **1965**, *37*, 1602–1604.
17. Leontein, K.; Lindberg, B.; Lonngren, J. *Carbohydr. Res.* **1978**, *62*, 359–362.
18. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131*, 209–217.
19. Hitchcock, P. J.; Brown, T. M. *J. Bacteriol.* **1983**, *154*, 269–277.
20. Tsai, C.-M.; Frasch, C. E. *Ann. Biochem.* **1982**, *119*, 115–119.
21. Towbin, H.; Staehelin, T.; Gordon, J. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4350–4354.
22. Sandrin, M. S.; Loveland, B. E.; McKenzie, I. F. *J. Cardiac Surg.* **2001**, *16*, 448–457.
23. Ramsland, P. A.; Farrugia, W.; Yuriev, E.; Edmundson, A. B.; Sandrin, M. S. *Cell. Mol. Biol.* **2003**, *49*, 307–317.
24. Yokota, S.; Amano, K.; Hayashi, S.; Kubota, T.; Fujii, N.; Yokochi, T. *Infect. Immun.* **1998**, *66*, 3006–3011.
25. Yokota, S. I.; Amano, K. I.; Shibata, Y.; Nakajima, M.; Suzuki, M.; Hayashi, S.; Fujii, N.; Yokochi, T. *Infect. Immun.* **2000**, *68*, 151–159.