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O ANTIGEN CHAINS IN THE LIPOPOLYSACCHARIDE OF *HELICOBACTER PYLORI* NCTC 11637

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Abstract. The O antigen region in the lipopolysaccharide of the smooth-form of *Helicobacter pylori* NCTC 11637 has been shown to contain partially fucosylated *N*-acetyllactosaminoglycan chains terminated by the Le^x determinant that mimic human cell surface glycoconjugates.

Key Words: O Antigen, Lipopolysaccharide (LPS), *Helicobacter pylori*, Structure Determination, *N*-acetyllactosaminoglycan, Le^x Determinant

INTRODUCTION

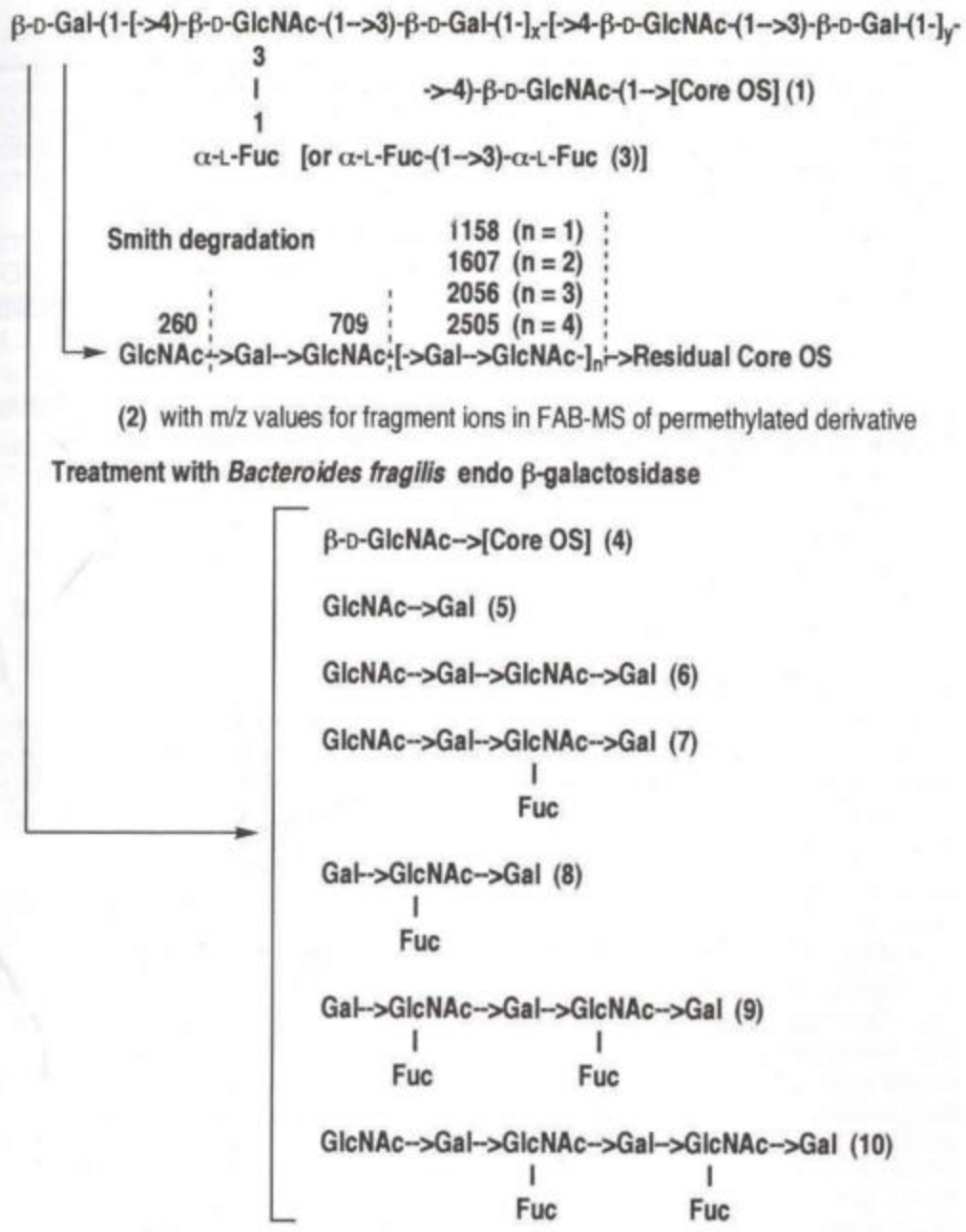
Helicobacter pylori (formerly *Campylobacter pylori)* is an important human pathogen which is implicated as a causative agent of gastritis, gastric and duodenal ulcers, and gastric carcinoma (see Ref. 1 for background literature). The characterization of surface structures is incomplete and little is known of lipolysaccharide(s) (LPS), one of the key components. Compositional analyses have been reported for some rough strains[1] showing that these LPS are typical of those found in the core oligosaccharide (OS) region of gram-negative bacteria and contain as the main sugar constituents, D-glucose, D-galactose, L-*glycero*-D-*manno*-heptose (LD-Hep), 3-deoxy-D-*manno*-octulosonic acid (Kdo) with D-*glycero*-D-*manno*-heptose (DD-Hep) of less common occurrence, and D-glucosamine in acylated form from lipid A. We have undertaken a structural analysis of both the rough[1]- and smooth-form LPS of the reference type strain NCTC 11637 (ATCC 43504) and report here the first detailed structural information on the O chain region of the smooth-form LPS.

RESULTS AND DISCUSSION

Water-soluble LPS (smooth-form LPS) of *Helicobacter pylori* NCTC 11637 was isolated as described[1]. Standard treatment with dilute acetic acid to cleave the ketosidic linkage from Kdo with precipitation of lipid A was accompanied by liberation of a small proportion of fucose residues and yielded polysaccharide (PS) which was eluted from Bio-Gel P6 at the void volume. Compositional analysis of both LPS and PS by the alditol acetate method[2] together with ^{1}H NMR data for the anomeric resonances defined the main constituent sugar residues as those of α-L-fucose [$\delta_{H\text{-}1}$ 5.19, $J_{1,2}$ 3.8 Hz], β-D-galactose [$\delta_{H\text{-}1}$ 4.46, $J_{1,2}$ 7.2 Hz] and *N*-acetyl-β-D-glucosamine [$\delta_{H\text{-}1}$ 4.69, $J_{1,2}$ 7.7 Hz] in the approximate molar ratio of 1 : 2 : 2, in addition to those previously reported as constituents of the core OS region in the rough-form LPS[1]. Methylation linkage analysis[3] showed as major components, 2,3,4-Me_3Fuc, 2,4,6-Me_3Gal, *N*,3,6-Me_3GlcNAc, and *N*,6-Me_2GlcNAc in the approximate molar ratio of 1 : 2 : 1 : 1 with small (0.1-0.2) but significant proportions of 2,3,4,6-Me_4Gal and 2,4-Me_2Fuc, together with other Gal, Glc, and heptose derivatives from the core region.

Information on the distribution of sugar residues in the branched O glycan (**1**) was obtained from two series of degradations (Scheme 1). The Smith degradation sequence (periodate oxidation, reduction, and mild acid hydrolysis of acyclic acetals) yielded an essentially linear degraded polysaccharide (**2**) with an alternating sequence of 4-linked GlcNAc and 3-linked Gal residues as shown by linkage analysis and by positive ion FAB-MS of the permethylated derivative which showed a series of glycosyloxocarbenium ions at *m/z* 260, 709, 1158, 1607, 2056, and 2505 from preferential fragmentation at HexNAc residues[4]. The removal of Fuc end groups during the Smith degradation indicated that these had been attached at O-3 of branched GlcNAc residues in a fucosylated *N*-acetyllactosaminoglycan. The retention of a small proportion of Fuc residues, now as end groups, in degraded glycan **2** was consistent with their origin as 3-linked Fuc residues in fucobiose units as a minor feature (**3**) in the parent polysaccharide.

The second degradative approach to the determination of fine structure was by the action of *Bacteroides fragilis* endo-β-galactosidase performed on the intact LPS (Scheme 1). Separation on Bio-Gel P2 furnished degraded polysaccharide **4** and oligosaccharide fractions **A**-**D**. ^{1}H NMR of the fractions, before and after treatment with $NaBD_4$, revealed the presence in each component of reducing Gal termini and the oligoglycosylalditols were converted into methylated derivatives for FAB-MS and for linkage analysis as partially methylated alditol acetates.



Scheme 1. Degradations of the polysaccharide O chain region of *H. pylori* NCTC 11637 LPS

Table 1. FAB-MS of permethylated oligoglycosylalditol

Fraction/Oligosaccharide		[a] $[M+H]^+$	[b] Fragment ions [··HexNAc]$^+$		
A	5	513	260		
B	6	962	260	709	
	7	1136	260	883 (-->677)	
	8	891	638(-->432)		
C	9	1514	638(-->432)	1261(-->1055)	
	10	1759	260	883(-->677)	1506(-->1300)

[a] $[M+H]^+$ = Pseudomolecular ion; [b] Fragment ions arising from cleavage at HexNAc residues which bear Fuc units at O-3 undergo loss of 206 amu by β-elimination.

Fraction **A** contained as the sole component disaccharide **5** arising from non-fucosylated regions of the chain (Table 1). Fractions **B-D** contained mixtures of higher oligosaccharides. The pseudomolecular ions $[M+H]^+$ defined the compositions of the oligosaccharides and linkage analysis confirmed the presence of 3-*O*-substituted galactitol termini and the expected glycose residues from the *N*-acetyllactosaminoglycan backbone with variable degrees of fucosylation. Determination of sequences of sugar units was aided by the appearance of prominent FAB-MS fragment ions (Table 1) from preferential cleavage at GlcNAc residues. The ready loss of Fuc units (206 amu) attached at O-3 of GlcNAc glycosyloxocarbenium ions served to identify those residues bearing side-chains. Despite the incomplete separations unambiguous structures could be assigned for one oligosaccharide (**6**) in which Fuc residues were absent, and for those in which Fuc residues were attached at each non-terminal GlcNAc residue. The latter were of two types: (i) those with non-reducing Gal residues, **8** and **9**, arising from chain termini; and (ii) those with non-reducing GlcNAc residues, **7** and **10**, formed by double enzymic cleavage and arising from internal segments of the chain. The oligosaccharides of highest molecular mass (M_r) in fraction **D** (not shown in Table 1) were of the same types but the mixture was too complex to permit unambiguous structural assignments for individual components. The results (Table 1) may be interpreted in terms of the known specificity of endo-β-galactosidase[5] whose action on *N*-acetyllactosaminoglycan chains does not cleave the non-reducing terminal β-D-Gal linkage and is restricted in regions carrying α-L-Fuc side-chains. Thus disaccharide **5** and tetrasaccharide **6** arise from unbranched internal GlcNAc-Gal segments without Fuc side-chains, and Fuc-containing pentasaccharide **7** and octasaccharides **10** derive from branched internal regions. Tetrasaccharide **8** and heptasaccharide **9** must be assigned to non-reducing terminal sequences which incorporate the monomeric and dimeric Le^x determinants. That these terminal

sequences were present in the liberated PS was shown by the detection in the FAB-MS of the permethylated derivative of the same glycosyloxocarbenium fragment ions at *m/z* 638 and 1261 as from **9**. Within detection limits no evidence was obtained for Gal-terminated chains with the outermost GlcNAc residue not carrying a fucosyl side-chain.

Compositional analysis of degraded polysaccharide **4** showed the presence of residual GlcNAc and the following typical core components, Glc, Gal, D-D-Hep, L-D-Hep, and phosphate, in the same approximate molar ratio, 1:1:1:2:1 as reported previously[1] for the core region of rough-form LPS. Close structural similarity between these materials from smooth- and rough-form LPS from preliminary linkage analysis data supports the conclusion that the polysaccharide chains described here are those of O chain linked via the core OS region to the lipid A of high-molecular mass (M_r) LPS. Full structural details for the O chain will be reported elsewhere together with the structure of the core OS region.

These studies have revealed the presence in this strain of *H. pylori* of an O antigen consisting of *N*-acetyllactosaminoglycan chains in which approximately half of the GlcNAc residues are fucosylated. The characterization of oligosaccharides liberated by the action of the endo-β-galactosidase provided substantial evidence for the distribution of fucosylated GlcNAc residues. The detection of **8** and **9** in which the outermost GlcNAc residues were fucosylated as the only oligosaccharides with non-reducing Gal end groups implies that all chains were terminated by monomeric or dimeric Le^x determinants. The distribution of Fuc residues within and between chains cannot be fully defined, but random fucosylation was evident in the liberation of **5** and **6** from unbranched regions of the chains, of **10** from internal segments with successive fucosylated GlcNAc residues, and of partially fucosylated and hitherto incompletely defined oligosaccharides from other regions.

Antigenic variations have been reported in the LPS of *H. pylori* strains[6] and investigations [with R. Shaver] on another strain indicates structural similarity to LPS of NCTC 11637, but a higher degree of fucosylation in the *N*-acetyllactosaminoglycan chains. More substantial differences from the NCTC 11637 strain are apparent in the core OS region suggesting that, as in the low M_r LPS from *Neisseria meningitidis*, immunotype specificities arise from differences in the inner regions of the structure[7].

The results presented provide a further example of the capacity of bacteria to generate structures closely resembling those of human cell surface components. The most striking feature of these conclusions is the structural similarity of the O chain structure to that of the extended oligomeric Le^x chains in fucosylated *N*-acetyllactosaminoglycans[8], and glycolipids[9] from normal human granulocytes, and in many adenocarcinomas[10] with fucosylated type 2 chains. Other examples of molecular mimicry are found in the so-called lipo-oligosaccharides (LOS) of *Neisseria* species, especially *N. gonorrhoeae*[11], which resemble

paraglobosides, and in the core regions of low M_r LPS from *C. jejuni* [12], which resemble gangliosides. Well documented examples of polysaccharides of repeating structure displaying molecular mimicry are those of the capsular K1 and K5 antigens of *Escherichia coli* [13] which are identical to host components to which the immune system is blind.

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