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Helicobacter pylori: A WOLF IN SHEEP'S CLOTHING: THE GLYCOTYPE FAMILIES OF *Helicobacter pylori* LIPOPOLYSACCHARIDES EXPRESSING HISTO-BLOOD GROUPS: STRUCTURE, BIOSYNTHESIS, AND ROLE IN PATHOGENESIS*

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I. INTRODUCTION

1. *Helicobacter pylori*

In 1983, the generally accepted dogma that gastric diseases in humans were mainly a consequence of stress, alcohol consumption, cigarette smoking, stomach

* Dedicated to the loving memory of my father Sr. Artur Monteiro.

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acidity, and dietary factors[1] was challenged by Warren and Marshall,[2] who alleged that infection by the gastric gram-negative microaerophilic bacterium *Helicobacter pylori* (0.6 × 3.5 microns), initially designated *Campylobacter pyloridis,* may be responsible for the onset of gastritis and gastric ulcers in humans. Approximately one century before, Bizzozero (1893), Salomon (1899), and Balfour (1906) reported the presence of spirochetal bacteria in dog and monkey gastric tissue, and more recently, in the mid-1970s, Steer and coworkers observed the presence of spiral bacteria in the human stomach and indeed a possible association with inflammation was reported.[3] The successful *in vitro* cultivation of *H. pylori* by Warren and Marshall spearheaded an explosion in *H. pylori* research and its role in gastric diseases. *H. pylori* colonizes the human gastric mucosa of more than half of the world's human population[4] and can persist in the stomach of hosts with or without clinical symptoms for a lifetime. It is now well accepted that colonization by *H. pylori* leads to chronic superficial gastritis, chronic active gastritis, and peptic ulcers.[5] In addition, *H. pylori* infection has also been associated with the development of more severe diseases, such as gastric cancer and mucosa-associated lymphoid tissue lymphoma,[6,7] and, consequently, this gastric bacterium has now been classified a category 1 (definite) human carcinogen[8] (Fig. 1). Although it remains largely unproved and contradictory, *H. pylori* infection has been connected with ischeamic and coronary heart diseases.[9] One recent article has also reported the presence of *H. pylori* ureC and cagA genes in the stomachs of children who have succumbed to sudden infant death syndrome (SIDS).[10] Several virulence mechanisms involving urease, the cytotoxin VacA, and the genes that compose the pathogenicity island *cag* have been linked to roles in the pathogenesis of *H. pylori*.[11]

This article focuses on the chemical structure, biosynthesis, and potential pathogenic role of the lipopolysaccharides (LPSs) from *H. pylori*. The structures of LPSs isolated from *Helicobacter* species found in nonhuman primates, which give rise to similar gastric symptoms in their respective hosts, are also described. LPSs are glycolipid structures carried by bacteria on their cell surfaces, which are actively involved in biochemical interactions between the bacterium and its host.[12]

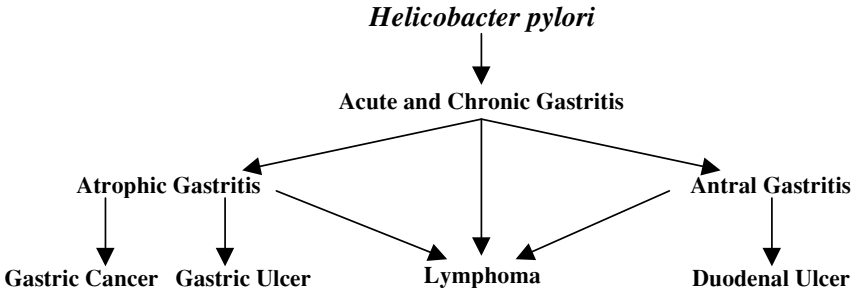


FIG. 1. Genesis of gastric-related clinical outcomes due to *H. pylori* infection.

LPSs from *H. pylori* have been determined to be able to bind to the mucosal mucin receptor, leading to the disruption of the integrity of the gastric mucosa; have been implicated in the stimulation of pepsinogen, leading to penetration of the gastric epithelium by the action of pepsin; have shown an high-affinity binding to laminin; and have also been implicated in the ability of the bacterium to bind to gastric mucosa.[13–15] In most instances, these interactions between *H. pylori* and the host were observed without regard to the LPS chemical structure or to differences between LPSs from different strains. However, recent pathogenic studies, which will be described here, employing well-defined *H. pylori* isogenic mutants carrying modified LPS molecules have shown that these LPS structures are indeed important players in *H. pylori* pathogenesis.

2. Lipopolysaccharides

A common method for classifying bacteria is the gram-stain technique,[16] which distinguishes two groups of bacteria on the basis of differences in their cell walls. Gram-positive bacteria, when compared with gram-negative bacteria, have simpler cell walls, mostly being composed of peptidoglycans.[17] The peptidoglycan of most bacteria is composed of alternating units of *N*-acetylglucosamine and *N*-acetylmuramic acid joined by β-$(1 \rightarrow 4)$ glycosidic bonds. Cell walls of gram-positive eubacteria can also contain teichoic acids, which are extracellular polymers of polyols linked by phosphodiester bonds,[18] and the nonphosphorylated teichuronic acid-type chains which are composed of acidic constituents such as uronic acid. Gram-positive bacteria, especially streptococci, may also produce lipoteichoic acids and various acid-soluble proteins and often carry, as antigenic markers, capsular polysaccharides (CPSs).[19] The addition of an outer membrane in gram-negative enteric bacteria gives it a more complex molecular architecture of which LPSs are the main components.

Gram-negative bacteria have a comparatively low peptidoglycan mass component. In enteric bacteria the peptidoglycan is covalently joined to some lipoproteins forming a connecting bridge between the peptidoglycan and the outer membrane. The external part of the outer membrane is composed of cell wall LPS. LPSs are complex molecules that occur in two forms. The high molecular weight (high M_r) LPSs with M_r values greater than 10,000 amu, commonly referred to as smooth-form LPSs,[20] (S-form LPSs), are derived from the so-called smooth-form organisms, reflecting the surface texture of bacterial colonies. Based mainly on previous pioneering investigations performed on *Escherichia* and *Salmonella,* S-form LPSs may be divided into three covalently linked regions (Fig. 2): the endotoxic component lipid A, the core oligosaccharide (OS) of approximately 8 to 10 sugar residues in a nonrepetitive sequence, and the O-antigen chains (O-chains) consisting of regularly repeating OS blocks. Normally, low molecular weight (low M_r) rough-form LPSs (R-form LPSs) are also present, and this glycan, devoid of the

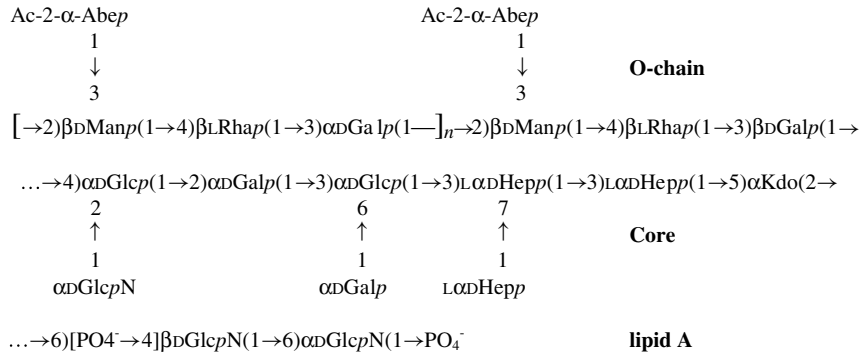


FIG. 2. * The three LPS covalently linked regions: the lipid A, which also contains fatty acids (not shown); the core oligosaccharide; and the O-chain of *Salmonella enterica* serovar Typhimurium.[27]

O-chain, consists of core OS and lipid A only, and thus, in the so-called R-form organisms (incomplete LPS) only the R-form LPS is present. In some bacteria, e.g., *Neisseria,* O-chain regions are entirely absent with strain-to-strain variations being reflected in differences in the nonrepetitive core OS regions, and some researchers have coined the term lipo-oligosaccharides (LOSs)[21] as being more appropriate and literally correct. Frequently, between S- and R-form LPSs, one also finds LPS structures of semi-rough-form (SR-form) that contain a complete core OS and a small number of O-chain repeating blocks.

The terms "smooth" and "rough" originally referred to the texture of the surface of bacterial colonies. Smooth colonies of bacteria seem to be linked to the presence of surface components that promote a compact cellular orientation. This terminology was then extended to label LPS components of gram-negative bacteria, and, as in this article, often reflect the presence (high M_r S-form; O-chain → core → lipid A) or absence (low M_r R-form; core → lipid A) of O-chains. Here, S-, SR-, and R-forms refer to O-chain extension in LPS structures and do not necessarily describe colony morphology. Some gram-negative bacteria possess extracellular polysaccharides (PSs), also referred to as capsular or exopolysaccharides, which are polymers similar to O-chains[22] in that they possess repeating OS blocks.

The location of these glycan molecules on the bacterial outer membrane makes them an important component in the pathogenesis of endotoxemia and septic shock syndromes, and in the survival and overall biological functions of the bacterium.[23] Moreover, LPSs with differences in chemical structures of the O-chains often provide a molecular basis for serological classifications within the same genus.[24]

* Here, and elsewhere in this article, hyphens are omitted from the extended symbolic oligosaccharide sequences, to achieve a more compact presentation than in the standard IUPAC-IUBMB format.

The methods developed by Westphal and colleagues that led to the isolation of LPS by the water–phenol extraction method[25] provided the material on which studies could be carried out, including those of *Helicobacter* LPSs that are discussed here.

The lipid moiety, termed lipid A, anchors the LPS to the bacterial cell through hydrophobic and electrostatic forces.[26] Detailed structural investigations using chemical, enzymatic, and spectroscopic methods[28,29] carried out on lipid A from *S. typhimurium* and *E. coli* have shown that this lipid moiety is the most conserved region of the LPS.[30] The backbone of the lipid A domain consists of a $(1 \rightarrow 6)$ linked β-D-glucosamine disaccharide [β-D-Glc*p*N-$(1 \rightarrow 6)$-α-D-Glc*p*N] to which phosphate groups are attached to the α-glycosidic hydroxyl group at C-1, and ester linked at O-4′, usually with (*R*)-3-hydroxy fatty acids[31,32] as lipophilic substituents, two of them ester linked at O-3, and the other two attached as *N*-acyl substituents on both GlcN residues.

The core OS region is covalently attached through the reducing end to the lipid A by an acid-sensitive ketosidic linkage from 3-deoxy-D-*manno*-octulosonic acid (Kdo), an immutable component of the core, to O-6 of the outer glucosamine unit of the lipid A. Typically, the nonrepetitive core oligosaccharide is composed of four basal sugars, L-*glycero*-D-*manno*-heptose (LD-Hep), glucose (Glc), galactose (Gal), and Kdo, but may also contain phosphate residues. The first detailed investigations of the chemical structure of core OSs were carried out on those from R-form LPSs from *E. coli* and *S. typhimurium*.[33] Genetic defects, natural or induced, resulted in the generation of mutants displaying enzymatic lesions and resulting in the formation of incomplete R-form LPS of low M_r with shortened glycan chains ranging from the complete core OS in the Ra mutant through to the shortest Re or deep rough mutant.[34] Recently, the complete biosynthesis for the *E. coli* (R1) core OS assembly has been described.[35] Kdo has also been observed at the nonreducing end of core OSs of *Rhizobium etli*[36] and *Klebsiella* (Evgeny Vinogradov, personal communication).

Only a single core OS region has been found in LPSs from a range of wild-type strains of *S. typhimurium,* but considerable variation is encountered in the structures of the O-chains with specificities conferred by different OS repeating units. Some species, such as *Neisseria,* only elaborate R-form LPS of low M_r devoid of repetitive O-chains. In this situation, strain-to-strain variations, in the absence of the biosynthetic machinery for O-chain elongation, are reflected in differences in the nonrepetitive core OS regions of these LOSs. Aspinall and co-workers have also shown that various *Campylobacter* species produce LOS molecules that express ganglioside-like antigens, and, simultaneously, these *Campylobacter* species also furnished high M_r PSs.[37–39]

The structures of complete high M_r LPSs are dominated by extended O-chains with regular OS repeating units (Fig. 2), which are a source of the bacteria's antigenicity and often serve as receptors for bacteriophage attachment.[40] Antigenic

O-chain PSs have been characterized[41] with a wide range of constituent sugars, which may be accompanied by nonglucan substituents such as phosphate and amino acids. Biosynthetic studies have shown that many O-chains are extended in a blockwise manner from OS blocks that have been assembled on an antigen carrier lipid. This key intermediate permits transport of the polar OS across the outer membrane for insertion at the proximal "reducing" terminus of the growing O-chain.[42] The occurrence of regular repeating units provides a framework within which to interpret data acquired in the course of structural investigations. Recent studies have shown that a biosynthetic incomplete O-chain repeating glycan block can be added to the core OS.[43]

In Fig. 2, it can be observed that the O-chain polymer of *Salmonella* has been determined to be attached to the O-4 position of the outer α-D-Glc*p* unit of the core OS.[27] However, this is one of very few cases where the covalent connection of O-chain to core has been unambiguously sited. The many variable O-chains connected to the core in *Salmonella* are responsible for assignment of serogroups. The O-chain-like CPSs have the same type of structural arrangement in having glycan repeating blocks as the building units of their glycan chains,[44] and in some species, the CPS and O-chain PS can also share a common structure.[45] However, these CPSs are independently held by the outer membrane, for example, through lipid anchors such as di-*O*-acylglycerol units.[46] A few of these antigenic CPSs have been used in a glycoconjugate format (CPS + protein) as protective vaccines against the respective bacterium's infection.[47]

3. Approaches Employed for Elucidation of *Helicobacter* Lipopolysaccharide Structures

The complete structural elucidation of carbohydrates, whether true PSs or glycoconjugates/glycolipids, requires that information must be obtained on: (*i*) monosaccharide composition, including the anomeric and enantiomeric configurations of sugar residues; (*ii*) the sites of linkage between the sugar units; (*iii*) the sequence of glycosyl residues; and (*iv*) siting of any noncarbohydrate substituents. To this end, in the studies to be described, many well-established procedures and analytical methods were used and these are listed with key references. However, as the investigations on *H. pylori* LPSs progressed, new problems were encountered, altered strategies had to be adopted, and departures from standard operating procedures became necessary. This section attempts to consider some of the practical limits of standard procedures rather than repeating readily accessible standard recipes. Consideration is given to the approaches that were sought to deal with new situations and emphasis is placed on the strategies employed to solve structural questions rather than on detailed experimental procedures.

A recurring problem has been the limited quantities of *H. pylori* LPS preparations that have been available because of the fact that *H. pylori* is a very fastidious

grower *in vitro*. The cell material, as received from clinical settings, contained small amounts of impurities; the presence of some of them was revealed in later LPS analyses, e.g., the ubiquitous phthalate plasticizers during mass-spectrometry analysis and the formation of ribitol pentaacetate (from ribose in RNA) during sugar analysis. Because of the limited quantities of material, in most cases, it was impractical to attempt to remove them. A more important consideration was that of carbohydrate microheterogeneity, but again there was little choice but to proceed with the material available. Any attempt to fractionate milligram quantities would have given too little of any subfraction to yield useful information. With careful selections of experiments to give crosschecked results, the major structural features of these unusually complex LPSs were established with the highest degree of confidence. The conclusions reached in these studies will provide the necessary basis for any assessments of microheterogeneity that would require an informed choice of separation procedures, e.g., using histo-blood group antigens as ligands for affinity chromatography.

Contrary to other gram-negative bacteria, most of the *H. pylori* LPS material is easily soluble in aqueous solutions, and thus the water-soluble LPS preparations were examined directly by sugar analysis on the basis of those amenable to the alditol acetate procedure.[48] In most instances, methylation linkage analysis and mass-spectrometry studies were also conducted at this stage. Analysis at this point also assured that acid-sensitive constituents, such as fucose and sialic acid, were not missed. In the case of incomplete solubilization, or of suspected contamination with salts or other impurities, the preparation was separated by gel-permeation chromatography (GPC) on Bio-Gel, preferably on the P-2 grade, and then re-examined. Insoluble gels were delipidated by heating in 1% acetic acid, or sometimes at buffer pH ∼6.5, and the soluble liberated saccharides were fractionated on Bio-Gel P-2. Account was taken of the possibility that glycosidic linkages, other than the ketosidic linkage of Kdo to lipid A, might be cleaved, and two such situations from these investigations are discussed.

Detailed investigations on *H. pylori* LPSs were commenced in the early 1990s as an extension of ongoing studies dealing with the closely related genus *Campylobacter*. Preliminary sugar composition analyses had been performed by Anthony Moran[49] and Armando MacDonald.[50] In the previous *Campylobacter* studies,[51,52] satisfactory separations were achieved of high-M_r glycans, comparable to CPSs,[53] from the aqueous layer of the phenol–water extraction, and the core OSs liberated from LOS of low M_r in the water-insoluble gel. Indeed, in the *Campylobacter* studies, by the criteria of molecular size from GPC and composition analysis, no significant overlap was observed and no evidence was obtained for materials of intermediate size, e.g., of carbohydrate chains from semi-rough strains that might contain core OS and a single O-chain repeating unit. At the outset, similar separation techniques were used with *H. pylori* LPSs, but as the knowledge on LPS structural features and behaviors was accumulated different strategies were

adopted, and eventually the purifications and fractionations were carried out using the intact LPSs in order to study the O-chain regions.

During the preparation of delipidated OSs and PSs from *H. pylori* by delipidation on heating in 1% acetic acid, limited hydrolysis of fucopyranosyl linkages occurred. Here, the desirability of obtaining carbohydrate devoid of associated lipid A for nuclear magnetic resonance (NMR) studies had to be balanced against the slight loss of fucose components. A more important factor was that separations of LPS fractions, on the basis of solubility or size, provided access to a range of molecules of different sizes spanning the gap between the inner core OS region and the regularly extending O-chains. The situation was found to vary from strain to strain since not all LPS preparations yielded fractions with a full complement of variations in size and structure. Separations of water-soluble LPS preparations and of derived OS and PS on the basis of molecular size were effected by GPC using polyacrylamide gels covering the anticipated size range. In practice it was found that GPC on Bio-Gel P-6, supposedly spanning the molecular mass range 5000–6000 Da, failed to remove LPS with shorter chains from those with relatively long O-chains. LPS with extended chains were separated satisfactorily on GPC using Bio-Gel P-2 (mass range 100–1800 Da) with retardation of shorter chain material. The extent of fractionation and the quantities of differently sized OSs liberated on delipidation of water-insoluble LPS gels varied from one preparation to another. In favorable cases, fractions were obtained ranging in size from the smallest inner core OS through to those of core OS with attached O-chains only slightly less extended than those from initially soluble high M_r LPS. In the examination of *H. pylori* LPSs, advantage was taken of OS fractions showing a progression of chain extension to derive partial structures from which a composite structure for the complete PS chain could be proposed.

Typically, the *H. pylori* isolates were frozen immediately after isolation ($-70\,^{\circ}$C). Cultures were subsequently thawed and plated onto brain heart infusion agar (1.2%, w/v) plates supplemented with 0.5% (w/v) yeast extract and 0.5% (v/v) fetal bovine sera. The *H. pylori* strains were allowed to grow for 3 days under microaerobic conditions at $37\,^{\circ}$C, subcultured into brain heart broth, and allowed to grow for an additional 3 days under the same conditions with agitation.

Sugar analyses were conducted routinely by hydrolysis of LPSs with aqueous trifluoroacetic acid followed by reduction with $NaBD_4$ and acetylation to give alditol acetates for separation, detection, and characterization by combined gas–liquid chromatography and mass spectrometry (GLC-MS).[54] Retention times of derivatives were compared with those of available configurationally defined reference compounds. Reference compounds were also required for determinations of absolute configurations by the chiral glycoside method using 2-(*R*)- and 2-(*S*)-butanol in the formation of acetylated glycosides for GLC analysis.[55] Aside from the decomposition of ketose sugars on treatment with strong acid, the normal conditions of hydrolysis were accompanied by the formation of nonreducing compounds

such as 1,6- and/or 1,7-anhydro sugars as evidenced by lack of incorporation of ^2H on treatment with $NaBD_4$. L-*glycero*-D-*manno*-Heptose was one such sugar regularly encountered in these studies, so that both the heptitol heptaacetate and the anhydro-heptose tetraacetate were included in quantitative analyses.[56] Chemical sugar linkage analysis was performed by the methylation method.[57] Although usage of capillary GLC columns of different lengths and polarities discriminated most of the partially methylated alditol acetate derivatives (PMAAs), it was common, because of the complexity of *H. pylori* LPSs, for these sugar derivatives to elute without baseline separation and in some instances simultaneous elution occurred. Thus, these analyses had to be carefully studied and crosschecked with other available data.

The technique for elucidation of sequence and total glycose composition used up to now has been fast-atom bombardment–mass spectrometry (FAB-MS).[58] The methylated LPS and OS derivatives were used. Mainly, two types of key information were obtained by FAB-MS. First, when observed, molecular weights were obtained through the formation of pseudomolecular ions by the addition of a proton $[M + H]^+$ and/or another cation in the positive-ion mode. Second, the sequence of the individual sugar units in a chain was elucidated by the detection of three types of ions in the mass spectra (Fig. 3). The most important involved glycosidic cleavage forming glycosyloxonium ions that gave exact incremental masses of the glycose components. This, however, yielded no information on linkage sites or stereochemistry. Another type of cleavage pathway involved the observation of a secondary ion formed by β-elimination of the substituent at the O-3 position of the glycosyloxonium sugar unit (Fig. 3). The mass difference between the parent glycosyloxonium ion and the secondary ion yielded information about the nature of the substituent at O-3. The other useful type of ion arose from a double cleavage process that involved a hydrogen transfer where two glycose units were cleaved, resulting in ions which contained neither the original nonreducing nor the reducing end. FAB-MS has proved to be the most fruitful and industrious technique in the *H. pylori* LPS studies described here. The observed preferential cleavage at the GlcNAc unit (reducing end)[59,60] yielding A type primary glycosyl oxonium ions and the subsequent secondary ion from β-elimination have aided greatly in these structural assignments. Electrospray mass spectrometry (ES-MS),[61] and, when necessary, its combination with high-performance liquid chromatography (HPLC-ES-MS), provided information on the carbohydrate molecule's molecular weight. In some cases, unusual mass increments in the FAB-MS spectrum pointed to the presence of non-sugar substituents such as phosphate moieties.

One-dimensional (1D) NMR investigations usually revealed genuine characteristics present in the glycan molecule, for example, methyl groups and carbonyl carbons from *N*-acetylglucosamine (GlcNAc) and fucose (Fuc) units. The detection of the downfield placed (typically between δ_H 4.3 and 5.7; δ_C 95 and 105) anomeric resonances and the measurement of their respective vicinal coupling

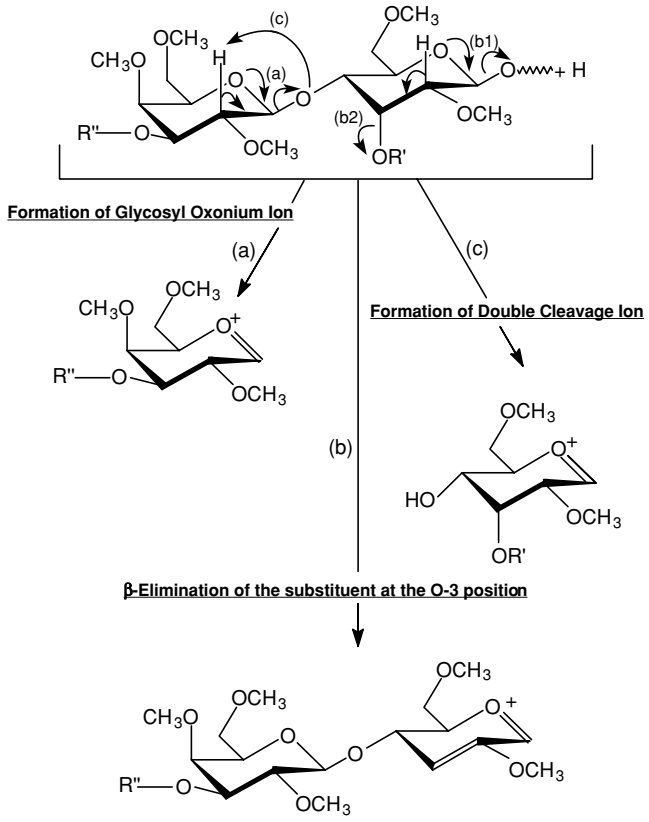


FIG. 3. Useful fragmentation pathways of permethylated glycans in FAB-MS.

constants ($^3J_{C,H}$) and one–bond proton–carbon coupling constants ($^1J_{C,H}$) gave information regarding the anomeric configuration of each component in the molecule. However, the ^{1}H NMR spectra of *H. pylori* carbohydrate molecules tended to have overlapping anomeric resonances that made it difficult to unambiguously detect all the anomeric signals. Two-dimensional (2D) NMR experiments had to be employed in order to disentangle overlapping resonances,[62] and in some cases the anomeric coupling constants were measured by obtaining the 1D subspectrum by "slicing" the $H^{1,2}$ cross peaks in the 2D ^{1}H–^{1}H COSY spectrum. When anomeric configurations could not be deciphered, serological experiments using commercially available monoclonal antibodies (mAbs), specific for histo-blood groups, helped in sugar configuration assignments.

After gathering all the information obtainable by 1D NMR techniques, and with previous knowledge of the type of sugars present from composition analysis, 2D NMR spectrometry was frequently employed. The experiments listed here are

presented in chronological order. Homonuclear correlation spectroscopy (COSY)[63] and total correlation spectroscopy (both 1D and 2D TOCSY)[64] experiments allowed the assignment of the protons in the sugar ring by observing the scalar coupling through cross peaks between vicinal protons, which is made possible by the transfer of magnetization from one proton nucleus to the other through their respective covalent bonds. In theory, the assignment of all the sugar ring protons is possible, but, in these investigations, this fact was rarely the case since overlapping of ring protons, especially H-3 and H-4, made it impossible to make unambiguous assignments. The assignment of the independent pathways required that at least one resonance be unambiguously located, that typically being the anomeric resonance. The measurement of the vicinal proton coupling constants around the ring most of the time affords information about the configuration of the sugar residue.

Experiments that produce ^{1}H NMR resonances engineered by through-space energy transfer based experiments, which take advantage of nuclear Overhauser enhancement effects (NOE),[65] were also used. 2D NOESY and 2D rotating frame NOE (ROESY)[66] were two useful experiments used to detect close spatial proximity between protons. After assignment of the proton resonances from COSY and TOCSY experiments, these spatial interactions were used to establish ring conformation and configuration as well as aiding in linkage site determination. *Inter*-residue spatial connectivities were helpful in confirming sites of glycosidic linkages. The linkage assignments deduced from NOE connectivities were cross-checked with data from chemical analysis. An important limitation of these NOE-based experiments was the possible presence of resonances originating via through-bond interactions that might have given rise to unwittingly incorrect assignments. This was overcome by comparing NOESY and ROESY spectra where the latter is known for avoiding through-bond connectivities. However, ROESY experiments were only successful on some OSs of low M_r (up to 10 sugar residues); various attempts to obtain ROESY spectra on PSs were not successful.

^{13}C NMR was a reliable source of detection and identification of carbon nuclei in the saccharide molecule. However, greater quantities were required for 1D ^{13}C experiments as compared with ^{1}H NMR spectroscopy and often extended periods for acquisition (sometimes 3 days) were required. The ^{1}H–^{13}C heteronuclear multiple quantum correlation (HMQC)[67] was one experiment that allowed the assignment of carbon resonances, by correlation with their proton nuclei, and where smaller quantities were used. A conscious 1D ^{31}P NMR investigation quickly revealed the presence of phosphate or a 2-aminoethyl phosphate unit in the core OS. 2D ^{1}H–^{31}P HMBC NMR spectroscopy was a reliable method for efficient detection and sometimes placement of the phosphate substituents.

In order to gain information into the fine structure of carbohydrate molecules, selective chemical and enzymatic degradations were performed. A useful chemical degradation used in these studies was the Smith degradation,[68] where

periodate oxidation results in cleavage of the bonds between free vicinal diols. Characterization of the comparatively simpler products yielded information about the structure of the parent molecule. Some structural problems were also solved by the action of a specific enzyme, endo-β-galactosidase, selective for the hydrolysis of 3-substituted β-Gal*p* units that are linked to a linear 4-substituted β-Glc*p*NAc residues in an *N*-acetyl-polylactosamine (polyLacNAc) structure.

After the initial detection of histo-blood group epitopes in *H. pylori* LPSs by chemical analyses, serological analysis such as enzyme-linked immunosorbent assay (ELISA) and immunoblots employing commercially available mAbs specific for histoblood group antigens were repeatedly performed to quickly detect the presence of blood-group determinants in *H. pylori*. These serological results were used hand-in-hand with chemical data in determining the complex structures of *H. pylori* LPSs.

The objective of this article is to discuss the chemical structure, biosynthesis, and pathogenic role of the LPSs from strains of *H. pylori* in order to understand the broader biological implications of this organism. The molecular structure of these LPS molecules will provide the basis for serological differentiation between strains, and may be the basis of potential glycan-based therapeutics to combat *H. pylori* infections.

II. The Chemical Structure of *Helicobacter pylori* Type Strain Lipopolysaccharide: The Lewis X O-Chain

Differences between *H. pylori* isolates had been observed by an ability to distinguish strains based on electrophoretic patterns in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in combination with serological analyses with strain-specific antisera raised in rabbits.[69] In considering such structural variations, account must be taken of possible modifications of LPS expression during *in vitro* cultivation. The modifications may include a decrease of O-chain length on repeated passages on solid medium and its reversal when grown in a fluid medium.[70]

This section describes the first LPS structure to be investigated, that of *H. pylori* type strain NCTC 11637 (ATCC 43504).[71,72] An account of the structural elucidation of the complete PS component, from O-chain through to the inner core OS regions, of LPS from the type strain is discussed here. Penner and co-workers, by using homologous antisera raised in rabbits, assigned *H. pylori* NCTC 11637 to the O : 1 serogroup.[69] The LPS preparations obtained possessed a high degree of molecular microheterogeneity, but what appeared to be a complication proved to be an advantage in furnishing OS molecules whose structures were to shed light on the nature of the chemical linkages connecting O-chains to the inner region of the LPS.

Initially, the overall approach to the structural investigations was based on that used in previous studies on LOSs and CPSs from *Campylobacter* species in which it had been observed that insoluble gels from phenol–water extractions of bacterial cells yielded mainly low-M_r LOSs with core OSs linked to lipid A. The aqueous phases from such extractions gave high-M_r glycans with extended polymers, where carbohydrate polymers with no attachment to lipid A, such as the teichoic acid-like polymer from *C. coli* serotype O : 30 or the poly(tetraglycosylphosphates) from *C. lari,*[52] were found, and thus this possibility had to be kept in mind when deciphering the molecular makeup of *H. pylori* LPSs. The general strategy adopted for the analysis of *H. pylori* type strains set the stage for future *H. pylori* LPS investigations.

This section describes studies of the water-soluble S-LPS together with the isolation of an OS remaining after chemical and enzymatic degradation of the parent S-LPS. Mutually supportive evidence came from studies on OSs (OS-1, OS-2, and OS-3) from the SR-form LPS, of presumed low M_r, but in this preparation showing progressive chain extensions from the inner core OS region leading to O-chain initiation. From these observations a structural model for *H. pylori* LPS was developed, and a structure is proposed for the complete PS chain from the nonreducing terminus of the O-chain through an intervening region to the Kdo-terminated core OS.

S-form LPS of *H. pylori* type strain was almost completely water-soluble. It was observed that treatment of the water-soluble LPS from the S-form organism under standard conditions with dilute acetic acid, to cleave the ketosidic linkage of the Kdo terminus to lipid A, resulted in some liberation of fucose. In order to avoid inadvertent loss of fucose residues the key experiments for the characterization of the O-chain were, therefore, repeated on untreated water-soluble LPS. Glycose analyses showed that the main constituents were L-Fuc, D-Gal, and D-GlcNAc from the O-chain region. D-Glc, DD-Hep, and LD-Hep were also detected, in minor amounts, which composed the core OS part of the LPS. The anomeric configurations of the dominant sugar residues were defined by ^{1}H and ^{13}C NMR as those of α-L-Fuc, β-D-Gal, and β-D-GlcNAc.[72] The ^{31}P NMR spectrum for lipid-free PS showed a single resonance at δ 3.79 at pD = 6 assignable to a phosphoric monoester. Sugar linkage analysis performed on the LPS showed the presence of terminal Fuc, 3-linked Gal, and 4-linked and 3,4-linked GlcNAc residues in the approximate molar ratio of 5 : 9 : 4 : 4, together with the sugar residues (approximately 1 molar equivalent each) of variously substituted Glc, DD-Hep, and LD-Hep units arising from the inner region of the LPS (core region).[72] The major constituents appeared to be consistent with the presence of polyLacNAc chains of 3-substituted Gal and 4-substituted GlcNAc residues, with approximately half of the latter carrying at O-3 terminal Fuc residues. An additional feature was the formation of a small proportion (<1 molar proportion) of nonterminal 3-substituted Fuc residues. This constituent was postulated to arise from fucobiose

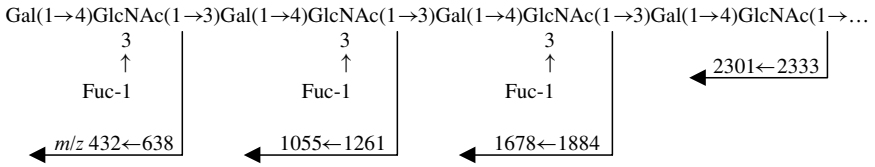


FIG. 4. The composite structure, along with *m/z* values from FAB-MS spectrum, of the polymeric Le^x O-chain of *H. pylori* type strain.

units and this possibility seemed to be confirmed with the detection of periodate-resistant residues in the product from Smith degradation (*vide infra*). Indeed, in the following sections that deal with the structure of other *H. pylori* LPSs, a difucosylated GlcNAc antigen, α-L-Fuc-(1 → 3)-α-L-Fuc-(1→ 4)-β-D-GlcNAc, is described.

The FAB-MS spectrum of the methylated LPS indicated the presence, at the nonreducing end, of a trisaccharide similar to the human blood-group antigen Lewis X (Le^x) [*m/z* 638 → 432(638–206)], β-D-Gal-(1 → 4)-[α-L-Fuc-(1 → 3)]-β-D-GlcNAc-(1 → (Fig. 4). Higher *m/z* ions (Fig. 4) showed a polymeric O-chain composed of internal Le^x repeats (fucosylated polyLacNAc) with some randomly Fuc-free *N*-acetyl-lactosamine [LacNAc; β-D-Gal-(1 → 4)-β-D-GlcNAc-(1 →] blocks.

More detailed information for the distribution of linkages in the branched polyLacNAc O-chain was obtained from two series of degradations. The Smith degradation sequence yielded an essentially linear fucose-free polyLacNAc, β-D-GlcNAc-[→ 3)-β-D-Gal-(1 → 4)-β-D-GlcNAc-(1-]$_n$→, arising from the O-chain region of the LPS. A minor component of low M_r was obtained when the material from the Smith degradation was fractionated by GPC on Bio-Gel. Hydrolysis of this component gave Glc and threitol in equimolar amounts, and the compound was characterized as 2-*O*-β-D-glucopyranosyl-D-threitol. The significance of this degradation product within the LPS structure did not become apparent until after the inner regions of the lowM_r SR-form LPS had been examined.

The second degradative approach was by the action of *Bacteroides fragilis* endo-β-D-galactosidase. The specificity of this enzyme in the depolymerization of incompletely fucosylated polyLacNAc glycans, whereby cleavage takes place at other than terminal galactosyl linkages to non-fucosylated GlcNAc residues,[73] led to the formation of two categories of oligosaccharides (Fig. 5): (*a*) those containing a nonreducing terminal α-D-Gal from a single glycosyl cleavage and (*b*) those containing a nonreducing β-D-GlcNAc end-group resulting from internal regions of the chain by cleavage at linkages to two unbranched β-D-GlcNAc residues.

The results of these experiments are summarized in Fig. 5 and the structures of oligosaccharides **1**–**6,** even when in admixture, were unambiguously assigned. Disaccharide 1 and tetrasaccharide 3 arose from unbranched internal GlcNAc-Gal

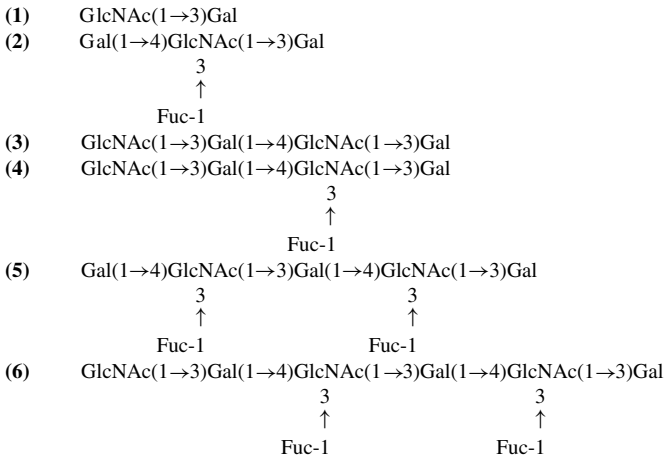


FIG. 5. Glycans from degradation of *H. pylori* type strain high M_r LPS with endo-β-D-galactosidase.

segments without Fuc side chains, and Fuc-containing pentasaccharide 4 and octasaccharide 6 derive from branched internal regions. Tetrasaccharide 2 and heptasaccharide 5 were assigned to nonreducing terminal sequences that incorporate monomeric and dimeric Le^x determinants.

The most striking conclusion from the results of this part of the investigation was the recognition of bacterial components closely resembling in structure the extended oligomeric type-2 Le^x chains found in fucosylated polyLacNAc glycan conjugates[74] and glycolipids,[75] and in many adenocarcinomas[76] with fucosylated type-2 chains. Polymeric type-2 chains $[\rightarrow 3)\beta$-D-Gal$(1 \rightarrow 4)$ β-D-GlcNAc$(1 \rightarrow]_n$ are mainly found in adenocarcinoma cells, whereas type-1 $[\beta$-D-Gal$(1 \rightarrow 3)\beta$-D-GlcNAc$(1 \rightarrow]$ and 2 $[\beta$-D-Gal$(1 \rightarrow 4)\beta$-D-GlcNAc$(1 \rightarrow]$ *N*-acetyl-lactosaminoglycan monomeric units are mostly encountered in normal human erythrocytes.[77] ELISA and immunoblot experiments, using intact *H. pylori* type strain whole cells and intact LPS, with several Le^x mAbs showed strong positive reactions, thus underlining the results obtained by chemical analyses. Evidence for the connection of the repetitive structure in the O-chains to the LPS core emerged from studies on SR-form LPS.

Since SR-LPS of *H. pylori* NCTC 11637 isolated from phenol–water extraction was partially water-insoluble, and could be isolated as a pellet by high-speed centrifugation, detailed studies of core OS structure were carried out on material obtained after treatment with aqueous acetic acid under standard conditions. Compositional analysis of the liberated OS crude preparation showed the presence of significant amounts of sugar constituents from O-chains in addition to Hep and Glc core units. Separation of the OS preparation by GPC on Bio-Gel afforded in

[P→7]LαDHep(1→5)Kdo

LαDHep(1→3)[P→7]LαDHep(1→5)Kdo

DαDHep(1→2)LαDHep(1→3)[P→7]LαDHep(1→5)Kdo

αDGlc(1→4)βDGal(1→7)DαDHep(1→2)LαDHep(1→3)[P→7]LαDHep(1→5)Kdo

FIG. 6. Core OS molecules present in fraction OS-3 showing the progressive addition of core units.

succession three fractions of which that of lowest M_r, OS-3, had a composition showing an absence of O-chain constituents. The first two fractions OS-1 and OS-2 were less completely resolved, but were sufficiently different, in extent of O-chain sugar constituents, to justify separate examination. Parallel studies of the fractions involved ^{1}H and ^{31}P NMR of the parent OS, FAB-MS of methylated derivatives, and accompanying linkage analyses.

The lowest M_r fraction, OS-3, was in fact a mixture of core-related linear molecules (Fig. 6) containing a minimal disaccharide composed of the innermost LD-Hep (phosphorylated) and its neighboring Kdo residue, and three more extended glycans that allowed for the determination of the core OS backbone. The backbone of the core OS of *H. pylori* type strain was composed of a phosphorylated (monoester phosphate) hexasaccharide in which the typical core sugars, LD-Hep, Glc, and Gal, were present, but, in addition, this core OS also possessed the less common DDHep as a constituent.

In addition to those in OS-3, compositional analysis of OS-2 showed the presence of Fuc, GlcNAc, a second DD-Hep and, on average, less than one extra Gal, and ∼2 further Glc residues per chain; linkage analysis showed that these hexose residues were distributed between four locations. Lack of uniformity in the placing of the additional Gal and Glc residues was indicated by the less than stoichiometric proportions of methylated sugar derivatives in linkage analysis, which showed the introduction of the new structural units and the creation of new branch points in OS-2. The locations of the chain extensions were assessed from variations in the fragment ions shown by FAB-MS, and Fig. 7 shows the proposed structure for OS-2 with an interpretation of the origins of the newly observed fragment ions in the FAB-MS of the methylated derivative. With the known structural units of OS-2 the ion at *m/z* 682 was of unambiguous structure, Fuc-(1 → 3)-GlcNAc-(1 → 7)-DD-Hep, and the other glycosyloxonium ions could only have originated from cleavage at Hep residues. It is suggested that the ready formation and presumed relative stability of these ions may be a consequence of the anomeric effect of sugar residues having the α-D-*manno* configuration. In agreement with the detection of fragment ions at *m/z* 682 and 886, linkage analysis showed the addition of single Gal end groups to some of the GlcNAc residues with the formation of a Le^{x} terminus. Linkage analysis of OS-2 showed that both 3- and 6-linked Glc residues were present,

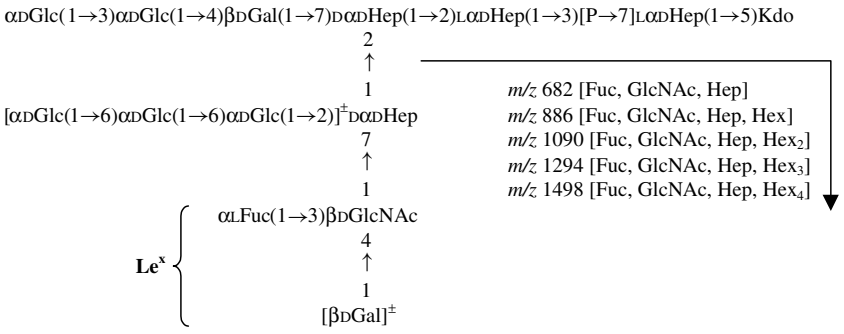


FIG. 7. Structure of molecule OS-2 with *m/z* ions from FAB-MS of methylated derivative. In some OS-2 molecules, the structure GlcNAc-(1 → 3)-Fuc (1 → 7)-DD-Hep-(1 → core may also be present.

and indeed a 2D COSY and 2D TOCSY experiments indicated the presence of five anomeric signals with the α-D-Glc configuration, but of unequal intensity.

In the light of the earlier observation that 2-*O*-α-D-glucopyranosyl-D-threitol was isolated from Smith degradation of S-form LPS, it is proposed that these differently linked α-D-Glc residues may be assigned to specific locations. Only one type of Glc residue would be resistant to periodate oxidation and the formation of threitol could only arise from a 4-linked Gal residue in the core OS after chain extension with an extra α-D-Glc residue attached by a (1 → 3) linkage [α-Glc-(1 → 3)-α-Glc-(1 → 4)-β-Gal-(1 → inner core]. It is, therefore, implied that the O-2 of approximately 50% of the outer DD-Hep residues is the site of attachment of an α-D-Glc residue and on some chains up to two further α-D-Glc residues in (1 → 6) linkages. ES-MS of permethylated OS-2 showed a series of pseudomolecular ions corresponding to the above-mentioned OSs together with those of compositions of Fuc, Hex_5, GlcNAc, Hep_4, Kdo, and Fuc, Hex_6, GlcNAc, Hep_4, Kdo, in a further demonstration of the size heterogeneity of OS-2.

Linkage analysis data for OS-1 indicated the presence a more extended Le^x O-chain than that present in OS-2, and hence resembling intact S-form LPS. FAB-MS of the permethylated OS-1 showed fragment ions derived from terminal sequences of dimeric Le^x blocks. A noteworthy feature was the absence of 6-linked α-D-Glc residues; this dextran side chain was also absent in the high-M_r S-form LPS. Interestingly, in LPS knockout mutants, this glucan reappears and becomes one of the prominent moieties of *H. pylori* isogenic mutants with truncated LPSs lacking Lewis O-chains.

The preceding experiments performed on the OSs liberated from SR-form LPS provided essential evidence for the connection of the polymeric Le^x O-chain to the inner core segment through an O-chain—β-D-GlcNAc-(1 → 7)-DD-Hep—core connection. Confirmation of this aspect of structure was sought by degradation of the high-M_r S-LPS. A more extended controlled treatment of LPS with aqueous

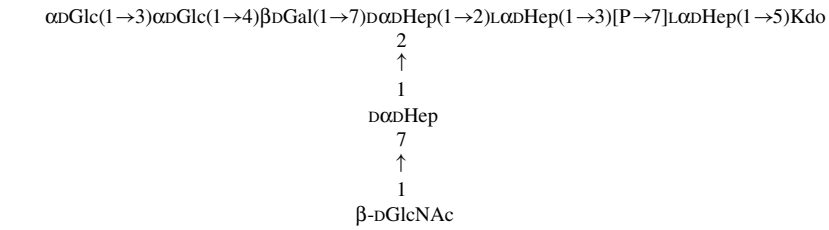


FIG. 8. Structure of the core-related oligosaccharide (OS-4) that originated from chemical and enzymatic degradation of S-form LPS from *H. pylori* type strain. OS-4 is composed of the core OS and one GlcNAc unit from the Le^x O-chain region.

acid resulted in complete defucosylation, in addition to cleavage of the ketosidic linkage of Kdo to lipid A. Complete depolymerization of these unbranched polyLacNAc chains was achieved on treatment with endo-β-galactosidase to give the previously characterized disaccharide 1 (Fig. 5) and a core-related OS, OS-4 (Fig. 8). Analysis of OS-4 showed the presence of a single GlcNAc residue as a nonreducing terminus of the O-chain, together with most of the residues from the inner region of the S-form LPS that were present in OS-2. This terminal GlcNAc unit was connected to the 7-substituted DDHep residue [*m/z* 260 ($GlcNAc^+$) and 508 ($GlcNAc\text{-}Hep^+$) in FAB-MS]. However, a notable difference was the absence of the 6-linked α-D-Glc residues attached to the outer of the two DD-Hep units as observed in high-M_r molecules from S-form LPS and OS-1.

The LPS of *H. pylori* type strain was shown to contain a partially fucosylated type-2 polyLacNAc O-chain (polymeric Le^x/LacNAc) of 8 to 10 disaccharide repeating units, where approximately half the GlcNAc units carried a Fuc residue. This O-chain was terminated by a Le^x epitope (Fig. 9). The sugar constituents LD-Hep, Glc, Gal, Kdo, and the less common DD-Hep made up the core OS region which was shown to be covalently attached to the polymeric Le^x/LacNAc O-chain domain by a GlcNAc → DD-Hep glycosidic linkage. Figure 9 shows the complete LPS molecule of the first examined *H. pylori* strain from the O-chain termini to the Kdo of the inner core oligosaccharide.

These pioneering investigations on *H. pylori* LPS were undertaken within the framework of the classical model for LPS structure. This structural model is based on a regular O-chain, synthesized by polymerization of OS repeating blocks, assembled on a lipid carrier and transferred to a nonrepetitive core OS linked via glyculosonic acid residues, most commonly Kdo, to the endotoxic component, lipid A. The results presented in this section on the structure of the LPS from the first *H. pylori* strain examined, comprising the O-chain and the intervening and core OS regions, may be considered in terms of the structural proposal shown in Fig. 9. The O-chains of the LPS were shown to consist of partially fucosylated type-2 polyLacNAc chains with 4-substituted GlcNAc residues in the backbone

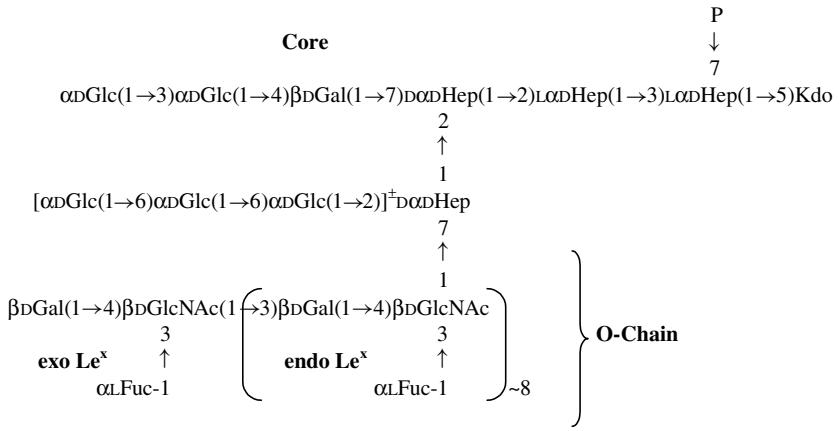


FIG. 9. The structure of the complete core and O-chain regions of the *H. pylori* type strain LPS showing the polymeric Le^x O-chain.

and to approximately half of which α-L-Fuc residues were attached at O-3 forming structures analogous to the histo-blood group determinant, Le^x. From the FAB-MS experiments on the products arising from the action of endo-β-galactosidase on LPS, there was evidence for monomeric and dimeric Le^x units at the chain terminus, and, from the FAB-MS of methylated S-form LPS, fragment ions were observed for trimeric Le^x units.

The inner regions of the LPS structure were delineated by the characterization of OS fractions liberated from the presumed incomplete SR-LPS. A core OS fraction representing the region to which the O-chain might be directly linked was isolated. Chromatographic separation of this core OS mixture gave three fractions. Each fraction OS displayed natural microheterogeneity, but they differed sufficiently from each other in overall structure to provide evidence for the progression of glycosylations leading to the assembly of the mature LPS. Thus, the phosphorylated hexasaccharide, OS-3 (Fig. 6), defined the inner core OS region. The next higher M_r fraction, OS-2 (Fig. 7), with a micro-heterogeneous collection of molecules, pointed to three types of chain extension: (*i*) an extension of the backbone core OS by attachment of a second α-D-Glc residue in a $(1 \rightarrow 3)$ linkage, (*ii*) the introduction of branching from the DD-Hep residue in the inner core through a second such residue to which the first Le^x unit was to be developed, and (*iii*) in some chains yet further branching in a sequence of up to three α-D-Glc residues in $(1 \rightarrow 6)$ linkages; and the OS-1 fraction of highest M_r showed the development of the O-chain structure with up to two Le^x/LacNAc repeating units. However, in OS-1, as in the complete S-form LPS, the 6-linked α-D-Glc residues were no longer present, and their peculiar role in the LPS assembly is not yet known.

Complementary evidence from controlled degradation of the S-form LPS to give the core-associated glycan OS-4 (Fig. 8) confirmed the proposed connection of the single remaining GlcNAc residue in O-chain via an intervening DD-Hep residue to the inner core. In the complete LPS structure (Fig. 9), present estimates suggest that there are at least nine Le^x/LacNAc repeating units in this O-chain. The detailed distribution of Fuc residues is not known, but indications are that fucosylation of GlcNAc in *H. pylori* seems to be an unsystematic process.

The most significant conclusion from these initial investigations concerns the elaboration by *H. pylori* of LPS with oligomeric Le^x epitopes in mimicry of structures expressed mainly on glycolipids in certain human cell surface glycoconjugates. Although these tumor-associated polymeric carbohydrate antigens are found on normal cells, e.g., on granulocytes, their abundant expression is limited to malignant cells. Nevertheless, Le^x epitopes are expressed in normal gastric tissue, which thus poses the question as to whether mimicry of this blood-group antigen by *H. pylori* in the gastric mucosa may camouflage the bacterium from the host and thus aid survival of *H. pylori* in that environment. Conversely, the presence of antibodies against *H. pylori* in patients has been found to strongly correlate with the presence of autoantibodies against human antral gastric mucosa.[78] Since antibodies cross-reacting with the gastric mucosa have been demonstrated in mice immunized with *H. pylori,* the expression of Le^x-like epitopes on the surface of the bacterium may play a role in the development of an autoimmune reaction contributing to disease. The further implications of these surface structures in interaction between the bacterium and the human host will require biological experiments with chemically defined LPSs.

III. The Lewis Y and Sialyl Lewis X in *Helicobacter pylori* Lipopolysaccharides

As structural studies on the LPS of the *H. pylori* type strain were proceeding, and the first evidence for O-chains expressing structures analogous to the type-2 Le^x blood-group was developing, Boren *et al.*[79] reported differences in the binding of *H. pylori* strains to human gastric mucosal cells carrying the type-1 Le^b antigen. The binding of one such strain, *H. pylori* P466, from a patient with dyspeptic syndrome, to gastric mucosa was inhibited by a neoglycoconjugate carrying the Le^b antigen, whereas another *H. pylori* strain, MO19, from an asymptomatic patient, did not bind to gastric mucosa. Although LPS was not implicated in the interaction of this *H. pylori* strain with gastric mucosa, and indeed a bacterial cell-surface adhesin, *babA,*[80] has been implicated in this adhesion, these observations prompted a comparative examination of LPS from the P466 *H. pylori* strain. This section describes the expression of type-2 Le^y and Sialyl Le^x blood-groups by the LPS of *H. pylori* P466.[81,82]

The structure of the LPS from *H. pylori* type strain (preceding section) served as a reference point, and the strategies employed in its elucidation were to provide the guidelines in the examination of LPS from other *H. pylori* strains. With no formal distinction between R- and S-form LPS of strain P466, bacterial cell extracts were divided into soluble high-M_r LPS, with extended outer O-chains, and partially soluble low-M_r LPS. OS fractions comprising inner core and developing O-chains were liberated and separated by GPC for detailed study. With considerable microheterogeneity in each of these fractions, compositional and linkage analysis gave only average values for constituent residues. Assuming that smaller molecules comprise the first steps in the progressive development of the mature LPS, as with type strain LPS, the evidence for these defined components could be turned to advantage in the elucidation of the structure of the complete molecule.

Water-soluble high-M_r LPS was examined directly without cleavage from lipid A in order to avoid cleavage of any relatively acid-sensitive fucopyranosyl linkages. The main constituents were L-Fuc, D-Gal, and D-GlcNAc, with smaller amounts ($\sim$2 molar proportions) of Glc, DD-Hep, and LD-Hep. The identities and anomeric configurations of the principal sugar residues were defined as α-Fuc, β-Gal, and β-GlcNAc. Linkage analysis performed on the P466 intact LPS showed the presence of all the sugar linkage units previously observed in *H. pylori* type strain, but, in addition, 2-substituted Gal was also detected. The significance of the 2-substituted Gal residue arising from a Fuc-(1 $\rightarrow$ 2)-Gal unit was seen in the FAB-MS spectrum of the permethylated LPS that showed a fragment ion at *m/z* 812 of composition Fuc_2, Gal, GlcNAc which could have arisen from either a type-1 Le^b or a type-2 Le^y determinant, but the detection of a secondary fragment ion at *m/z* 606, from β-elimination of a terminal Fuc residue (206 amu) from O-3 of GlcNAc, and not at *m/z* 402, pointed to a terminal Le^y blood-group determinant (Fig. 10). Fragment ions of higher *m/z* were observed with increments of Fuc, Gal, GlcNAc, with secondary ions observed from loss of a terminal Fuc residue indicating a regular chain extension of Le^x units in a type-2 fucosylated polyLacNAc chain. The presence of both Le^y and Le^x was also confirmed serologically (ELISA and immunoblot) employing mAbs specific for these blood-group antigens.

For confirmation that the polyLacNAc backbone consisted of regular Gal-(1 $\rightarrow$ 4)-GlcNAc repeating units, the soluble high-M_r LPS was heated with aqueous 5% acetic acid in which cleavage from lipid A was accompanied by complete defucosylation. 3-Substituted Gal and 4-substituted GlcNAc residues were the main units of the final product with no detectable 3-linked GlcNAc residues. The proportion of Gal end groups indicated an average chain length of 4–5 disaccharide units. FAB-MS supported a regularly repeating structure for the polyLacNAc backbone chain of the O-chain region of the P466 LPS with a series of glycosyloxonium ions at *m/z* 464 [Gal-GlcNAc], 913 $[Gal\text{-}GlcNAc]_2$, 1362 $[Gal\text{-}GlcNAc]_3$, 1811 $[Gal\text{-}GlcNAc]_4$, and 2260 $[Gal\text{-}GlcNAc]_5$from preferential cleavage at GlcNAc residues.

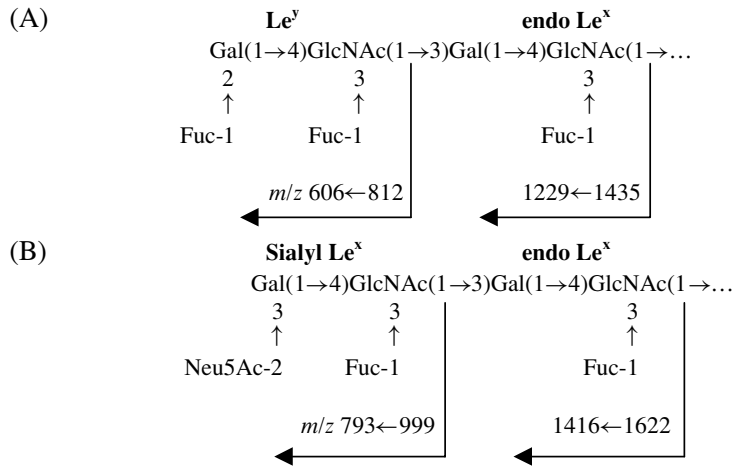


FIG. 10. The O-chain of *H. pylori* strain P466 carrying a type-2 Le^y (A), or a type-2 sialyl Le^x (B) epitope at the nonreducing terminus of a Le^x O-chain.

Treatment of the water-insoluble P466 LPS preparation in acetate buffer at pH 6.5, with cleavage of the ketosidic linkage to lipid A, gave a mixture of OSs which was separated by GPC to give a series of fractions (OS-1, OS-2, OS-3) or subfractions. Molecule OS-3 gave qualitatively similar analyses for composition and linkage types to the linear OS-3 glycan from *H. pylori* type strain (Fig. 5). Sugar constituents of the "Lewis O-chain" region, notably Fuc and GlcNAc, were absent. OS-3 contained D-Glc, D-Gal, DD-Hep, and LD-Hep and chemical and spectroscopic data revealed that P466 OS-3 was analogous to the phosphorylated core backbone OS-3 from the type strain (Fig. 5): α-D-Glc-(1 → 4)-β-D-Gal-(1 → 7)-D-α-D-Hep-(1 → 2)-L-α-D-Hep-(1 → 3)-[P → 7]-L-α-D-Hep-(1 → 5)-Kdo. Compositional analysis and ^{1}H NMR for OS-2 pointed to an average incremental addition to the core OS-3 region of 2 Fuc, 1 Glc, 2 Gal, 2 GlcNAc, and 1 DD-Hep. Using the structure of the OS-2 fraction from the type strain as a guide (Fig. 6), linkage analysis showed that the increment could correspond to a core extension by a single 3-substituted Glc residue (in the core OS backbone), and the addition, as a side chain to the DD-Hep in the core, of a second DD-Hep residue (7-substituted) from which O-chain development would ensue with the attachment of up to 3 Le^x and 1 Le^y units. Glycan P466 OS-2 had a structure similar to that of OS-2 from the type strain (Fig. 7), with the exception of the absence of the side-antenna dextran. In fraction P466 OS-1, extension of the growing Lewis O-chain approached that in the complete water-soluble high-M_r LPS.

The LPS just discussed of *H. pylori* P466 possessed type-2 polymeric Le^x O-chains of 4 to 5 repeat units, but instead of being completed at the nonreducing

end by the Le^x epitope, as in the *H. pylori* type strain LPS, the O-chain of P466 strain is terminated by the Le^y antigen (Fig. 10). The O-chain of the P466 strain was also shown to be covalently connected to the core OS region, which had the same structure as the core OS region from the *H. pylori* type strain (Fig. 6), through a similar GlcNAc-(1 → 7)-DD-Hep bridge.

Additional serological studies also showed a positive reaction between P466 LPS (from a separate growth) and a mAb (sLeX) specific for the sialyl Le^x epitope. This P466 LPS was then chemically analyzed to determine if sialyl Le^x was indeed present. Sugar composition analysis performed by the typical trifluoroacetic acid hydrolysis on this intact P466 LPS revealed the presence of the sugars found in the previous analyzed P466 LPS, and in addition, a sugar analysis specific for detection of neuraminic acid (methanolysis) indeed showed the presence of sialic acid. The intact P466 LPS was consequently methylated and its FAB-MS spectrum showed the characteristic ion of terminal neuraminic acid at *m/z* 376 and its correspondent secondary ion at *m/z* 344 [376 − 32(CH_3OH)]. Moreover, ions *m/z* 999 and 793 [999 − 206] represented the whole type-2 sialyl Le^x epitope {Neu5Ac-(2→3)-Gal-(1 → 4)[Fuc-(1 → 3)]-GlcNAc}. In addition to sialyl Le^x, terminal Le^x epitope [*m/z* 638→432], and traces of Le^y [*m/z* 812 → 606] were also observed. Thus, *H. pylori* P466 was also shown to have the ability to elaborate the cancer-associated sialyl Le^x antigen in its LPS. The sialyl Le^x was expressed simultaneously with Le^x and, in lesser amounts, the Le^y antigen. These Lewis blood-group termini were connected to the a core OS similar to that of the type strain.

The lower degree of chain elongation (4–5 repeats), the absence of 6-substituted Glc side chains, and the replacement at the nonreducing end of type-2 Le^y and sialyl Le^x epitope for Le^x are the marked differences in molecular structure from that of *H. pylori* type strain (Fig. 9). The production of Le^y and sialyl Le^x by *H. pylori* P466 shows a further ability, in addition to Le^x in type strain, of *H. pylori* to produce structures homologous to human blood-group antigens. Here, competition, environmental, and genetic factors for glycosylation of Gal in a type 2 LacNAc structure, fucosylation at O-2 for Le^y, and sialylation at O-3 for sialyl Le^x will dictate which LPS structure will dominate. Sialyl Le^x is frequently expressed on human cancer cells serving as ligands for E-selectin, and thus plays a role in tumor formation and in hematogenous metastasis of cancer.

IV. The Heptoglycan Domain in *Helicobacter pylori* Lipopolysaccharides

This section describes studies on the LPSs from *H. pylori* strains MO19,[79] and serogroups O : 6[69] and O : 3[69] that possess oligomers of DD-Hep (heptoglycan) between the core OS and the Lewis O-chain. In contrast to the P466 strain discussed in the preceding section, the MO19 strain did not bind to gastric mucosa expressing the Le^b antigen.[79] As studies on these LPSs proceeded, evidence was obtained for

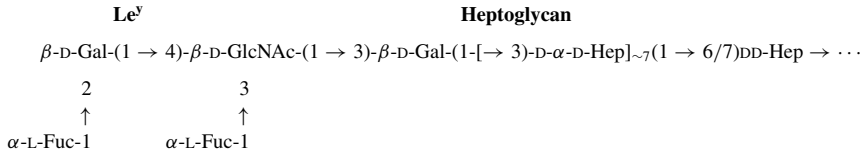
the presence, as a major structural feature, of chains of 3-substituted DD-Hep, a rather rare sugar, which created a distinct LPS structural domain.

Analysis of the water-soluble LPSs of *H. pylori* strain MO19 and serostrain O : 6 showed the presence of 2 L-Fuc, 3 Gal, 1 GlcNAc, 4 Glc, 10 DD-Hep, and 2 LD-Hep. The ^{1}H NMR spectra of MO19 and O : 6[83] LPSs showed one dominant α-anomeric resonance at δ5.08 (unresolved doublet) which could be attributed to the overriding DD-Hep component detected in the chemical analysis. In addition to anomeric resonances from residues in the inner core oligosaccharides, equal intensity signals were observed for two αFuc, one βGlcNAc, and two αGal residues. Methylation linkage analysis for the MO19 and O : 6 LPSs showed derivatives from three regions of structure: (1) two terminal Fuc, and one each of 2-linked Gal, and 3,4-linked GlcNAc; (2) most prominently seven 3-substituted DD-Hep and three 6-substituted Glc residues, the locations of which will be considered later; and (3) those residues probably arising from a similarly linked core OS region to those in the type strain NCTC 11637 (Section II) and P466 (Section III) LPSs. In addition to the prominent 3-substituted DD-Hep units, two other structural units, not seen in the type strain or P466 LPSs, were a 2- and 6-substituted DD-Hep. The FAB-MS of methylated LPSs of MO19 and O : 6 showed that these LPSs carried a Le^y (*m/z* 812 $\rightarrow$ 606). In accordance with compositional and linkage analysis data, no other abundant ions were observed arising from an extended Lewis O-chain; the next ion in the chain extension at *m/z* 1016 showed an increment for a hexose residue, which was consequently identified as being a 3-substituted Gal. The most striking observation from the linkage analysis was the presence of multiple 3-substituted DD-Hep residues. Based on proton assignments from various NMR studies, especially a NOESY experiment that showed *inter*-residue connectivities from the anomeric H-1 to H-3 of the adjacent 3-linked DD-Hep, extended chains of DD-Hep residues were observed, -[$\rightarrow$3)-D-α-DHep-(1-]$_n\rightarrow$. 6-Substituted Glc residues were also prominent, but these data gave no direct information on their location in the overall structure. The water-soluble MO19 and O : 6 LPSs, without prior removal of the lipid A component, were submitted to a Smith degradation in which the reduction was performed with $NaBD_4$ and each afforded one PS fraction. These PSs had a composition of 1 mol of [$^2H_1$]erythritol, Gal, and GlcNAc and 7 mol of [$^2H_1$]Man. The NMR spectrum showed anomeric signals for β-Gal and β-GlcNAc, and a major anomeric signal for a sugar with the α-*manno* configuration as an unresolved doublet. Linkage analysis showed the presence of one terminal GlcNAc, one 3-substituted Gal, and about seven 3-substituted Man residues. The FAB-MS gave fragment ions for the terminal trisaccharide segment at *m/z* 260 [GlcNAc], *m/z* 464 [GlcNAc-Gal], and 669 [GlcNAc-Gal-Man-6-^{2}H], thereby defining the sequential connection of the GlcNAc residue of the Le^y epitope via the 3-linked Gal residue to the 3-linked DD-Hep oligomer. The erythritol moiety, from the proximal terminus of the Smith degradation product, could only arise from the oxidative–reductive degradation of a 6-substituted or a 2,7-disubstituted

DD-Hep residue. The following structure may therefore be proposed for the Smith degradation products of MO19 and O : 6 LPSs:

β-D-GlcNAc-(1→3)-β-D-Gal-(1-[→3)-α-D-Man-6-[^{2}H]-[(1-]$_{\sim 7}$→D-Erythritol,

and thus for the outer region of MO19 and O : 6 LPSs:



The LPSs from *H. pylori* MO19 and O : 6 contained a single Le^y epitope at the nonreducing end of the LPS molecule which was attached through a Gal unit to a heptoglycan of 3-linked DD-Hep units. These LPSs were also shown to have an inner core OS attached to Kdo representing the reducing terminus of the PS molecule from the LPS (Fig. 11). The backbone of the LPS core OS from these

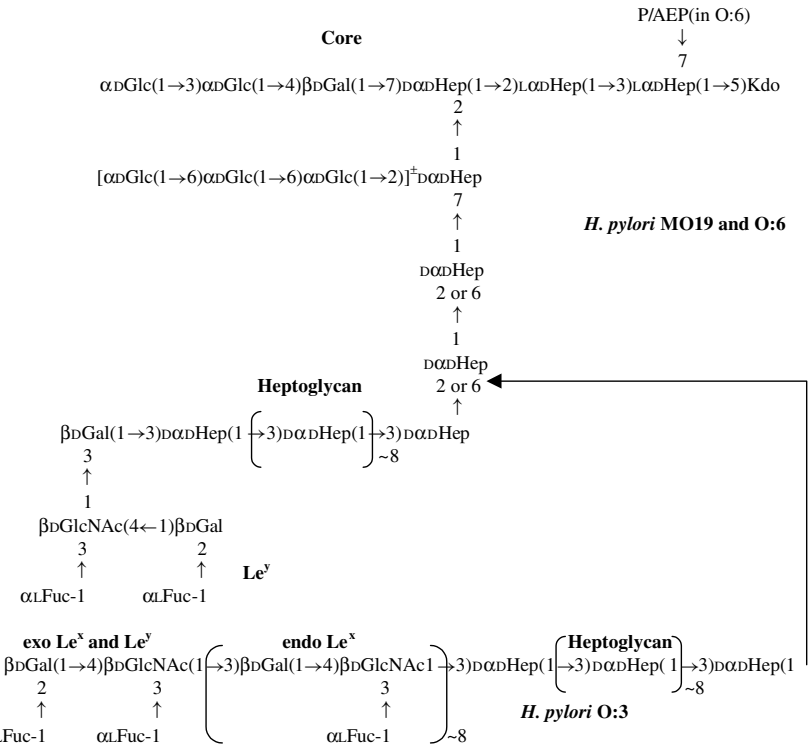


FIG. 11. The chemical structures of core OS, heptoglycan and O-chain regions of *H. pylori* strain MO19, and serostrains O : 6 and O : 3.

H. pylori were proven to be similar to those previously described for *H. pylori* type strain and P466 (Figs. 5 and 6).

Studies on another *H. pylori* strain, serostrain O : 3,[83] that also raised strain-specific antisera,[69] yielded the polymeric Le^x O-chain units terminated by a Le^x or Le^y, as previously found in the elongated O-chains from *H. pylori* type strain and P466, respectively. The LPS of *H. pylori* O : 3 was also shown to carry a heptoglycan domain similar to that observed in strains MO19 and O : 6. Hence, *H. pylori* O : 3 shared all the structural features present in strains previously discussed, namely, a polymeric fucosylated type-2 polyLacNAc O-chain and a heptoglycan region.

To deduce the nature of the connection between the O-chain and the heptoglycan oligomer in *H. pylori* O : 3, two degradations were performed. First, the O-chain was defucosylated with acetic acid to furnish a linear polyLacNAc chain. With the intention of reaching the first GlcNAc unit (reducing end) of the O-chain, the polyLacNAc was treated with endo-β-galactosidase. From this series of reactions one high-M_r product was obtained, and structural analyses of this molecule showed one terminal βGlcNAc unit, five 3-linked D-α-D-Hep residues, and other sugar derivatives from the core OS regions. The FAB-MS spectrum revealed ions at *m/z* 260 for GlcNAc, 508 for GlcNAc-Hep, 756 for GlcNAc-Hep-Hep, and 1004 for GlcNAc-Hep-Hep-Hep. These composite ions in combination with chemical analysis showed that the connection between the Lewis O-chain and the heptoglycan domain, in *H. pylori* O : 3, was through a GlcNAc residue rather than a Gal unit as in strains MO19 and O : 6. Thus, the degraded *H. pylori* O : 3 molecule had the following structure:

$$\beta\text{-D-GlcNAc(1-[}\rightarrow 3)\text{-D-}\alpha\text{-D-Hep(1-]}_{\sim 5} \rightarrow \text{Core OS},$$

and hence for the outer part of the LPS the following structure is proposed:

exo Le^x and Le^y **endo Le^x** **Heptoglycan**

$$\beta\text{-D-Gal}(1\rightarrow 4)\beta\text{-D-GlcNAc}(1\text{-}[\rightarrow 3)\beta\text{-D-Gal}(1\rightarrow 4)\beta\text{-D-GlcNAc}(1\text{-}]_{\sim 7}\rightarrow[\rightarrow 3)\text{-D-}\alpha\text{-D-Hep}]_{\sim 5}(1\rightarrow \text{Core OS}$$

$$\begin{array}{ccc} 2 & 3 & 3 \\ | & | & | \\ 1 & 1 & 1 \\ \alpha\text{-L-Fuc}^{\pm} & \alpha\text{-L-Fuc} & \alpha\text{-L-Fuc}^{\pm} \end{array}$$

H. pylori serogroup O : 3 contains an LPS structure that possesses both the polymeric Lewis O-chains, as encountered in *H. pylori* type strain and strain P466, and an intervening heptoglycan domain, a region shown to be present in *H. pylori* strains MO19 and O : 6.

The limited quantities available of LPS from MO19, O : 3 and O : 6 strains were insufficient to establish the formal connection of this outer heptoglycan to the core OS region. However, on the basis of linkage analysis, the following sugar residues, additional to those already placed in the outer O chain→heptoglycan and the inner core OS regions, remained to be accommodated. Two types of units were those of

α-D-Glc residues, a single 3-substituted Glc residue, which probably arose from extension of the outer region of core OS as in the P466 and type strain LPSs (Fig. 5), and 6-substituted Glc residues, probably mutually linked as in similar short chains in OS-2 from the type strain (Fig. 6). Three types of DD-Hep residues, additional to that in the inner core phosphorylated hexasaccharide and those in the 3-linked heptoglycan oligosaccharide, were units of 2-, 6-, and 2,7-substituted residues. The absence of 7-substituted DD-Hep residues in the MO19 and O : 6 LPSs, and the presence in less than 1 molar quantity in O : 3 LPS, implies that this residue in the inner core OSs occurs as one of two branched residues in the LPS (see bolded sugars below):

Glc → Glc → Gal → 7)-**DD-Hep-(1** → LD-Hep → LD-Hep[P] → Kdo

2

↑

1

$[Glc]_n$ → 2)-**DD-Hep**

7

↑

Heptoglycan

↑

Lewis O-chain

The other 2,7-linked unit is presumably that to which the 6-linked Glc residues are attached in the LPS (see foregoing illustration). Either this second branched unit or the 6-substituted DD-Hep residue could be the residue to which the whole outer chain is linked and gives rise to an erythritol terminus in the Smith degradation product of the MO19 and O : 6 LPSs. The known structural features of the MO19 and O : 6 LPSs are summarized in Fig. 11, which shows: (*a*) the single Le^y terminal epitope linked via a β-D-Gal residue to the heptoglycan domain; (*b*) a region showing three DD-Hep residues and three Glc residues; and (*c*) the inner core OS region extended by an additional Glc residue. The molecular structure of *H. pylori* O : 3 LPS can also be seen in Fig. 11 where the (*a*) outer region of the LPS is composed of an $Le^{x/y}$ terminated long Le^x O-chain connected directly by a GlcNAc to the heptoglycan, (*b*) a region showing three DD-Hep residues and approximately three Glc residues, and (*c*) the inner core OS region. Although the heptoglycan component, D-*glycero*-D-*manno*-heptose, is the biosynthetic presursor of L-*glycero*-D-*manno*-heptose in the biosynthesis of LPS molecules,[84] it is rarely encountered as a component of LPS molecular structures. *H. pylori* MO19, O : 6, and O : 3 LPSs contain in the heptoglycan domain an LPS structural region of a type not previously encountered in LPS structures.

The investigations carried out on *H. pylori* strains MO19 and serogroups O : 3 and O : 6 have revealed marked differences in molecular structures; these structures are shown in their entirety in Fig. 11. Strains MO19 and O : 6 lack the extended fucosylated polyLacNAc O chains but contain a heptoglycan oligomer of 3-linked DD-Hep units inserted between a terminal Le^y antigen and the core OS region. The single Le^y epitope in strains MO19 and O : 6 is attached to the heptoglycan domain via a Gal residue. These heptoglycans probably act as a biological arm in order to present the sole Lewis antigen to host molecules. Serostrain O : 3 showed a combination of the structural characteristics found in the other strains by carrying both the extended Lewis O-chain and the heptoglycan oligomer. However, in serogroup O : 3 the connection of the Lewis O chain to the heptoglycan was effected through a GlcNAc unit. Strains MO19, O : 6, and O : 3 were shown to have the same inner core OS region. The presence of the heptoglycan domain in the LPSs of these *H. pylori* strains represents an additional LPS region (O-chain → **heptoglycan** → core → lipid A) to those common in other bacteria (O-chain → core → lipid A).

V. Semi Rough-Form and Smooth-Form Lipopolysaccharides in *Helicobacter pylori:* The Ubiquitous Phase Variation

This section describes the expression of SR- and S-form LPSs by two *H. pylori* strains of significant importance in *H. pylori* research: strain 26695, whose complete genome sequence has been published[85] and is thus being explored for genes involved in LPS biosynthesis, and the Sydney mouse model strain,[86] which is now widely employed in animal models to study *H. pylori* pathogenesis.

Separately grown batches of *H. pylori* 26695 and Sydney strain cells were studied to detect if there were any major differences between LPS molecules derived from separate growths. A conscious effort was made to grow all bacterial cell batches under the same conditions (pH 7.3–7.5). Altogether, experimental evidence[82] from serological and chemical analyses showed that the LPSs of *H. pylori* 26695 and Sydney strain possessed a core OS similar to those found in other *H. pylori* strains (Fig. 6). However, the core OS of *H. pylori* Sydney strain was phosphorylated by a diester phosphate moiety, 2-aminoethyl phosphate, in place of the more common monoester phosphate. The core OS from these strains could carry either a short O-chain region (SR-form LPS) expressing a variety of single type-1 and type-2 Lewis-related epitopes (Table I), namely, a type-2 Le^y, a type-1 [α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Glc*p*NAc] and 2[α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc] linear B blood-group, a type-1 Lewis disaccharide [α-L-Fuc*p*-(1 → 4)-β-D-Glc*p*NAc], a type-1 fucosylated Lewis disaccharide [α-L-Fuc*p*-(1 → 3)-α-L-Fuc*p*-(1 → 4)-β-D-Glc*p*NAc] and in lesser amounts, LacNAc, Le^x, and GlcNAc. Alternatively, from separate growths, these LPSs were found to be able to produce an elongated type-2 Le^x O-chain (S-form LPS)

TABLE I

O-Chain Epitopes Present in SR-Form LPSs of *H. pylori* 26695 and Sydney Strain; Interpretation of *m/z* Ions in the FAB-Mass Spectrum of the Methylated Intact SR-Form LPSs

Primary *m/z* Ion	Secondary *m/z* Ion from β-Elimination or from β-Cleavage		Proposed Structure
260	228 (260 − 32)		GlcNAc$^+$
434	402 (434 − 32)	Lewis disaccharide	Fuc-(1 → 4)-GlcNAc$^+$
464	432 (464 − 32)	LacNAc	Gal-(1 → 4)-GlcNAc$^+$
508			GlcNAc-(1 → 3)-Hep$^+$
608	576 (608 − 32)	fucosylated Lewis disaccharide	Fuc-(1 → 3)-Fuc-(1 → 4)-GlcNAc$^+$
638	432 (638 − 206)	Lewis X	Gal-(1 → 4)-GlcNAc$^+$
	450 (638 − 288)		3 ↑ Fuc-1
668	636 (668 − 32)	Type-2 B blood-group	Gal-(1 → 3)-Gal-(1 → 4)-GlcNAc$^+$
668	228 (668 − 440)	Type-1 B blood-group	Gal-(1 → 3)-Gal-(1 → 3)-GlcNAc$^+$
682			Fuc-(1 → 4)-GlcNAc-(1 → 3)-Hep$^+$ GlcNAc-(1 → 3)-Fuc-(1 → 3 or 7)-Hep$^+$
812	606 (812 − 206)	Lewis Y	Gal-(1 → 4)-GlcNAc$^+$ 2 ↑ Fuc-1 3 ↑ Fuc-1
856			Fuc-(1 → 3)-Fuc-(1 → 4)-GlcNAc-(1 → 3)-Hep$^+$

terminated mainly by a type-2 Le^x epitope. The coexpression of the blood-groups described above and the production of SR- and S-form LPSs reflects a high degree of phase variation within *H. pylori* LPS molecules.

The communally accessible complete genome sequence of *H. pylori* 26695 is shaping investigations dealing with the characterization and role of genes in *H. pylori* pathogenesis, including those responsible for LPS biosynthesis. Knowledge about the structure of 26695 LPS will facilitate the identification of "active" genes involved in sugar syntheses and in sugar glycosylations. Although some genes involved in sugar assembly mechanisms may be present in *H. pylori* genomes, it does not necessarily imply that they are expressed constantly *in vitro* by their LPSs. For example, in the *H. pylori* 26695 and J99[87] genomes, a gene postulated to be linked with the biosynthesis of the sugar nucleotide of sialic acid was characterized; however, the results obtained showed that sialic acid was not a constituent of the 26695[82] or J99[82] LPS molecules. The absence of sialic acid may be due to *in vitro* growth conditions and, conceivably, it may be that *in vivo* sialic acid is an LPS component; however, the presence of Le^y in *H. pylori* 26695

LPSs would impede the fabrication of any terminal sialyl Le^x epitope. Sialyl Le^x was observed to be a member of *H. pylori* P466 LPS[82] (Section III); thus the full mechanism for the production of this antigen in *H. pylori* must, in some strains, be in place. These results indicated that at any time during LPS biosynthesis, a mixture of Lewis glycoforms might be present as O-chain members (Table I). The presence of incomplete Le^x and Le^y antigens imply that the building of Lewis determinants in *H. pylori* takes place through a sugar-by-sugar addition and not by a block-by-block synthesis as found in other enteric bacteria. In *H. pylori* there must be genes involved in the biosynthesis of Le^x and Le^y, those being fucosyltransferases (FucTs), that place a terminal α-L-Fuc*p* on O-2 of a terminal β-D-Gal*p* and on O-3 of β-D-Glc*p*NAc, and the galactosyltransferase (GalT) that adds β-D-Gal*p* to O-4 of β-D-Glc*p*NAc. The presence of the Le^x determinant in *H. pylori* 26695 LPS suggests that the $(1 \rightarrow 3)$-α-fucosyltransferase may act prior to fucosylation at O-2 of β-D-Gal in the fabrication of Le^y in *H. pylori* LPS biosynthesis. The presence of Le^y impedes the creation of sialyl Le^x since both epitopes use the terminal βGal residue as the acceptor for fucosylation and sialylation, respectively. The difucosyl antigen, α-Fuc-(1-3)-α-Fuc, found in *H. pylori* is uncommon, but it has been found in certain oligosaccharides-alditols.[88] The presence of this difucosylated antigen may vary from strain to strain depending on the degree of activities of the glycosyltransferases involved in its biosynthesis. This same argument is also true for other Lewis-related structures expressed by *H. pylori* LPSs, in that, even within the same *H. pylori* strain, the degree of fucosylation [Fuc-$(1 \rightarrow 2)$-Gal, Fuc-$(1 \rightarrow 3)$-GlcNAc, and Fuc-$(1 \rightarrow 4)$-GlcNAc] varies between separate bacterial cell growths in which similar conditions were employed, which implies that fucosylation of any specific LacNAc region is not an imperative event.

The switch between SR-form LPS (Lewis $\rightarrow$ core $\rightarrow$ lipid A) and S-from LPS [$(\text{Lewis})_n \rightarrow$ core $\rightarrow$ lipid A] in *H. pylori* is most likely dependent on the activity of two glycosyltransferases: first, the LacNAc GalT that places a β-D-Gal at O-4 of β-D-GlcNAc; premature fucosylation of GlcNAc at either O-4 [Fuc-$(1 \rightarrow 4)$-GlcNAc] or O-3 [Fuc-$(1 \rightarrow 3)$-GlcNAc] may hamper galactosylation of GlcNAc, thus stopping O-chain (polyLacNAc) progression; and secondly, in the same manner, fucosylation at O-2 of β-D-Gal to create a Le^y epitope will halt O-chain extension since the β-D-Gal unit may no longer be able to be glycosylated at O-3 by β-D-GlcNAc. This would explain the structural differences observed, in that the short O-chains of SR-form LPSs, of both *H. pylori* 26695 and SS1, carried predominantly the Le^y epitope and the fucosylated GlcNAc antigens, in contrast to the longer O-chains of S-form LPSs that expressed a low concentration of Le^y and carried mostly LacNAc and Le^x at the nonreducing terminus. *H. pylori* P466, which carried Le^y and sialyl Le^x at the nonreducing terminus, also had a lower degree of O-chain extension than O-chains terminated by Le^x (type strain). Therefore, it would seem that there is a direct relationship between the degree

of $(1 \rightarrow 2)$-α-fucosyltransferase activity and Le^y production and consequently the length of Lewis O-chains. These results suggest that low-M_r SR form LPSs are more likely to express Le^y than their high-M_r S-form LPS counterparts and that, presumably, fucosylation takes place on a completed linear polyLacNAc to afford a Le^x O-chain. Intrastrain variation in the expression of long O-chains of two strains of *H. pylori* has also been observed by silver staining in SDS-PAGE and by immunobloting with homologous antisera.[89] The factors controlling the length of Le^x O-chain polysaccharide may be various, from environmental conditions to gene regulation, where all these factors may be interdependent. It is therefore very clear that at any time in *H. pylori* biosynthesis many LPS forms might be present in different concentrations. This intra-LPS variation, within the same *H. pylori* strain, carries certain implications with regard to the study of LPS isogenic mutants of *H. pylori,* in that the evaluation of LPS knockout mutants by SDS-PAGE may not be sufficient to determine the outcome of the mutation, because, at any time, the LPS of the parent strain may change from a S-form to a SR-form.

As an assurance, sugar composition analyses were performed on intact whole cells of *H. pylori* 26695 and Sydney strain, and no sugar components, other than those belonging to the LPS molecules, were detected that would have originated from a non-LPS glycan, thus ruling out the presence of any non-LPS polysaccharide from *in vitro* grown cells (such as a CPS) in these strains. It is also worth noting that at this early stage of *Helicobacter* research, and because of the massive general circulation, one must ensure the correct identity of any strain, especially important strains such as the type strain and mouse model strains. For example, two unrelated versions of the reference *H. pylori* type strain NCTC 11637 have been shown to be in common usage and we have also detected two different types of LPSs, each from two sources (see Table III).

H. pylori Sydney strain is often used in studies of *H. pylori* pathogenesis in mouse models. The side-branch $(1 \rightarrow 6)$-α-glucan typically present in the core OS of some *H. pylori* strains (Fig. 6) was absent in the LPS of Sydney strain. The core of *H. pylori* Sydney strain carried a 2-aminoethyl phosphate in the innermost LD-Hep residue, instead of the more common monoester phosphate. These results do not clarify the role of LPS in the ability of *H. pylori* Sydney strain to colonize the gastric mucosa of mice. Besides, other *H. pylori* strains that were able to successfully colonize mice or primates have been reported in the literature.[90–92] However, one may raise the following questions: Which type of LPS (SR-form LPS with a sole Lewis antigen or an S-form LPS with a polymeric Lewis O-chain) is most prominent in *in vivo* colonization and in adherence mechanisms? And which LPS-forms and antigens take part in *H. pylori* pathogenesis? These same arguments also need to be addressed when discussing the behavior of *H. pylori* in humans. A plausible prospect may be that the LPS-form and fine structure may change during and/or after colonization of the host's mucosa because of particular host factors and/or the stage of pathogenesis.

VI. Type-1 Histo-Blood Groups in *Helicobacter pylori* Lipopolysaccharides and Their Prevalence in Asian Strains: The Lewis A, B, D, and Blood-Group A Determinants

After the characterization of structures analogous to type-2 Le^x and Le^y blood-group antigens in *H. pylori* LPSs, a series of serological investigations, using commercially available histo-blood group mAbs, looked for the presence of blood-group determinants in a wide range of *H. pylori* strains.[93,94] In addition to confirming the presence of Le^x and Le^y in a large number of Western (North America and Europe) *H. pylori* strains (>80%), these serological studies also hinted at the presence of type-1 blood-group antigens Le^a and Le^b in a small number of strains. Detailed chemical analyses were then performed on some strains suspected of carrying type-1 Le^a and Le^b. This section describes structural studies revealing that *H. pylori* strains may possess LPSs with structural homology to type-1 Le^a $\{\beta$-D-Gal*p*-$(1 \rightarrow 3)$-$[\alpha$-L-Fuc*p*-$(1 \rightarrow 4)]$-β-D-Glc*p*NAc$\}$ and Le^b $\{\alpha$-L-Fuc*p*-$(1 \rightarrow 2)$-β-D-Gal*p*-$(1 \rightarrow 3)$-$[\alpha$-L-Fuc*p*-$(1 \rightarrow 4)]$-β-D-Glc*p*NAc$\}$ human blood-group antigens, and that they may carry them simultaneously with type-2 Le^x and Le^y blood-group determinants. The expression of the blood-group A epitope $\{\alpha$-D-Gal*p*NAc-$(1 \rightarrow 3)$-$[\alpha$-L-Fuc*p*-$(1 \rightarrow 2)]$-β-D-Gal*p*$\}$ by *H. pylori* LPS and the propensity of *H. pylori* LPSs isolated from Asian hosts to produce type-1 histo-blood-group antigens are also noted.

Several *H. pylori* strains[95] were determined to simultaneously express type-2 Le^x and type-1 Le^a structures in their LPS O-chain regions (Table II). With complementary data from chemical and spectroscopic analyses, the FAB-MS spectra of the same methylated LPS preparation yielded two β-elimination secondary ions, those being *m/z* 432 [638 − 206(FucOH)] as we have previously seen for type-2 Le^x, and *m/z* 402 [638 − 236(GalOH)] which characterized the type-1 Le^a determinant (Table II). Another Lewis blood-group trisaccharide comprising Fuc, Gal, and GlcNAc, the type-1 Le^d $\{\alpha$-L-Fuc*p*-$(1 \rightarrow 2)$-β-D-Gal*p*-$(1 \rightarrow 3)$-β-D-Glc*p*NAc$\}$, also known as H-type-1, was also shown to be an LPS component of *H. pylori* $\{$*m/z* $638 \rightarrow 228$ [638 − 410(Fuc-GalOH)] (Table II). The structural tetrasaccharide isomers, type-2 Le^y and type-1 Le^b $\{$*m/z* $812 \rightarrow 402$ [812 − 410(Fuc-GalOH)] in FAB-MS$\}$ were also coexpressed in some *H. pylori* strains (Table II).

Just as the majority of *H. pylori* strains possessed LPSs with only type-2 Lewis antigens [Le^x, Le^y, and LacNAc], a class of strains was also observed to carry strictly type-1 Lewis determinants. These *H. pylori* strains did not express any type-2 Lewis antigens, but furnished solely type-1 Le^a, Le^d, and Le^b (Table II). Of particular interest, some *H. pylori* strains, which followed the paradigm of other strains in producing Lewis blood-group antigens, also elaborated LPSs structures analogous to the blood-group A family $\{\alpha$-D-Gal*p*NAc-$(1 \rightarrow 3)$-$[\alpha$-L-Fuc*p*-$(1 \rightarrow 2)]$-β-D-Gal*p*$\}$. The FAB-MS spectra of methylated LPSs from these blood-group A-containing *H. pylori* strains (Table II) showed *m/z* 260 for terminal GalNAc, *m/z* $883 \rightarrow 228$ for the complete monofucosyl

TABLE II

Interpretation of the Ions from the FAB-Mass Spectrum of Methylated Intact *H. pylori* LPSs. The Secondary Ions Shown Originate from β-Elimination of the Residue at O-3 of the GlcNAc at the Reducing End. The / Symbol Indicates that Either Structure is Possible

Primary Ions (*m/z*)	Secondary Ions (*m/z*)		Proposed Structure
H. pylori UA948			
638	402 (638 − 236)	Type-1 Lewis A	Fuc-(1 → 4)-GlcNAc$^+$ 3 ↑ Gal-1
638	432 (638 − 206)	Type-2 Lewis X	Gal-(1 → 4)-GlcNAc$^+$ 3 ↑ Fuc-1
886			Lewis A/X → 7-DDHep$^+$
1087	1055 (1087 − 32)		Lewis A/X → LacNAc
1261	1055 (1261 − 206)		Lewis A/X → Lewis X
H. pylori UA955			
434	402 (434 − 32)	Lewis Disaccharide	Fuc-(1 → 4)-GlcNAc$^+$
638	432 (638 − 206)	Type-2 Lewis X	Gal-(1 → 4)-GlcNAc$^+$ 3 ↑ Fuc-1
812	606 (812 − 206)	Type-2 Lewis Y	Gal-(1 → 4)-GlcNAc$^+$ 2 3 ↑ ↑ Fuc-1 Fuc-1
1057	851 (1057 − 206)	Le Dis → Lewis X, type-1 and 2 regions	Fuc-(1 → 4)-GlcNAc-(1 → 3)-Gal-(1 → 4)-GlcNAc$^+$ 3 ↑ Fuc-1
1087	1055 (1087 − 32)		Lewis X → LacNAc
1261	1055 (1261 − 206)		Lewis X → Lewis X
1261	1229 (1261 − 32)		Lewis Y → LacNAc
1435	1229 (1435 − 206)		Lewis Y → Lewis X
2058			Lewis Y → Lewis X → Lewis X
H. pylori J233			
464	432(464 − 32)	Type-2 LacNAc	Gal-(1 → 4)-GlcNAc$^+$
464	228 (464 − 236)	Type-1 Lewis C	Gal-(1 → 3)-GlcNAc$^+$
638	228 (638 − 410)	Type-1 Lewis D(H Type-1)	Fuc-(1 → 2)-Gal-(1 → 3)-GlcNAc$^+$
913		Type-2 i-antigen	LacNAc → LacNAc
1087		(Type-1 and -2 regions)	Lewis D → i-antigen
H. pylori F-58C			
464 (weak)	432 (464 − 32)	Type-2 LacNAc	Gal-(1 → 4)-GlcNAc$^+$
638	402 (638 − 236)	Type-1 Lewis A	Fuc-(1 → 4)-GlcNAc$^+$ 3 ↑ Gal-1
668	228(668 − 440)	Type-1 linear B blood-group	Gal-(1 → 3)-Gal-(1 → 3)-GlcNAc$^+$

(*continued*)

TABLE II—*Continued*

Primary Ions (*m/z*)	**Secondary Ions (*m/z*)**		**Proposed Structure**
916			linear B blood-group → 7-DDHep$^+$
886			Lewis A → 7-DDHep$^+$
H. pylori R-58A			
638	402 (638 − 236)	Type-1 Lewis A	Fuc-(1 → 4)-GlcNac$^+$ 3 ↑ Gal-1
886			Lewis A → 7-DDHep$^+$
H. pylori F-15A			
608	576 (608 − 32)	Type-1 fucosylated Lewis disaccharide	Fuc-(1 → 3)-Fuc-(1 → 4)-GlcNAc$^+$
638	402 (638 − 236)	Type-1 Lewis A	Fuc-(1 → 4)-GlcNAc$^+$ 3 ↑ Gal-1
668	228 (668 − 440)	Type-1 and 2 linear	Gal-(1 → 3)-Gal-(1 → 3)-GlcNAc$^+$
	636 (668 − 32)	B blood-group	Gal-(1 → 3)-Gal-(1 → 4)-GlcNAc$^+$
812 (weak)	606 (812 − 206)	Type-2 Lewis Y	Gal-(1 → 4)-GlcNAc$^+$ 2 3 ↑ ↑ Fuc-1 Fuc-1
886			Lewis A → 7-DDHep$^+$
H. pylori R-7A			
260	228 (260 − 32)		GalNAc$^+$
464 (weak)	432 (464 − 32)	Type-2 LacNAc	Gal-(1 → 4)-GlcNAc$^+$
608 (weak)	576 (608 − 32)	Type-1 fucosylated Lewis disaccharide	Fuc-(1 → 3)-Fuc-(1 → 4)-GlcNAc$^+$
638	432 (638 − 206)	Type-2 Lewis X	Gal-(1 → 4)-GlcNAc$^+$ 3 ↑ Fuc-1
709 (weak)	228		GalNAc-(1 → 3)-Gal-(1 → 3)-GlcNAc$^+$
883	228	Type-1 monofucosyl A blood-group	GalNAc-(1 → 3)-Gal-(1 → 3)-GlcNAc$^+$ 2 ↑ Fuc-1
886			Lewis X → 7-DD-Hep$^+$(from core)
1057	402	Type-1 difucosyl A blood-group	GalNAc-(1 → 3)-Gal-(1 → 3)-GlcNAc$^+$ 2 4 ↑ ↑ Fuc-1 Fuc-1
1087	881	LacNAc → Lewis X	Gal-(1 → 4)-GlcNAc-(1 → 3)-Gal-(1 → 4)-GlcNAc$^+$ 3 ↑ Fuc-1
1087	1055	Lewis X → LacNAc	Gal-(1 → 4)-GlcNAc-(1 → 3)-Gal-(1 → 4)-GlcNAc$^+$ 3 ↑ Fuc-1

TABLE II—*Continued*

Primary Ions (*m/z*)	Secondary Ions (*m/z*)		Proposed Structure
1261		Lewis X → Lewis X	Gal-(1 → 4)-GlcNAc-(1 → 3)-Gal-(1 → 4)-GlcNAc$^+$ 3 3 ↑ ↑ Fuc-1 Fuc-1
1305			difucosyl A blood-group → 7-DD-Hep$^+$
1332			monofucosyl A blood-group → LacNAc$^+$
1335			Lewis X → LacNAc → 7-DD-Hep$^+$ LacNAc → Lewis X → 7-DD-Hep$^+$
H. pylori strains CA2, H428 and H507			
812	402 (812 − 410)	Type-1 Lewis B	Gal-(1 → 3)-GlcNAc$^+$ 2 4 ↑ ↑ Fuc-1 Fuc-1
812	606 (812 − 206)	Type-2 Lewis Y	Gal-(1 → 4)-GlcNAc$^+$ 2 3 ↑ ↑ Fuc-1 Fuc-1
1261	1229 (1261 − 32)		Lewis B/Y → 3-Gal-1 → 4-GlcNAc$^+$
1435	1229 (1435 − 206)		Lewis B/Y → Lewis X$^+$
1884			Lewis B/Y → Lewis X → LacNAc$^+$ Lewis B/Y → LacNAc → LacNAc$^+$
H. pylori strains CA4, CA5, CA6, and GU2			
638	402 (638 − 236)	Type-1 Lewis A	Fuc-(1 → 4)-GlcNAc$^+$ 3 ↑ Gal-1
638	432 (638 − 206)	Type-2 Lewis X	Gal-(1 → 4)-GlcNAc$^+$ 3 ↑ Fuc-1
638 (CA4, CA5)	228 (638 − 410)	Type-1 Lewis D	Fuc-(1 → 2)-Gal-(1 → 3)-GlcNAc$^+$
1087 (CA4, GU2)	1055 (1087 − 32)		Lewis A/X/D → LacNAc$^+$
1261	1055 (1261 − 206)		Lewis A/X/D → Lewis X$^+$
1710	1688 (1710 − 32)		Lewis A/X/D → Lewis X → LacNAc$^+$
1884 (CA4)	1688		Lewis A/X/D → Lewis X → Lewis X$^+$

type-1 blood-group A moiety {α-D-Gal*p*NAc-(1 → 3)-[α-L-Fuc*p*-(1 → 2)]-β-D-Gal*p*(1 → 3)-β-D-Glc*p*NAc}, and *m/z* 1057 → 402 indicating also the presence of the difucosyl type-1 blood-group A {α-D-Gal*p*NAc-(1 → 3)-[α-L-Fuc*p*-(1 → 2)]-β-D-Gal*p*(1 → 3)[α-L-Fuc*p*-(1 → 4)]-β-D-Glc*p*NAc}. The *H. pylori* strains found to have LPS with blood-group A structures also expressed the Le^x and LacNAc antigens (Table II), and, indeed, LPS O-chains consisting of blood-group A and LacNAc sections covalently attached were also characterized [*m/z* 1332 (monofucosyl blood-group A → LacNAc)].

Some *H. pylori* strains that by serological means, with Le^b mAbs, were thought to carry the type-1 Le^b antigen were determined to elaborate only substructures

of Le^b. In one case, *H. pylori* strain UA955,[95] the Le^b mAb recognized a Lewis disaccharide structure [α-L-Fuc*p*-(1 → 4)-β-D-Glc*p*NAc] and in another, *H. pylori* J233,[95] recognized another Le^b substructure in the Le^d antigen (Table II). That mAbs putatively specific for the Le^b determinant can detect glycan substructures [Lewis disaccharide and Le^d (H-type-1)] of Le^b indicates their nonspecificity. Both *H. pylori* UA955 and J233 also carried type-2 antigens in their LPS O-chain regions. O-chains of polymeric type-2 Le^x along with O-chains composed of terminal type-1 Le^d and internal type-2 Le^x ($Le^d \rightarrow Le^x \rightarrow$ core) were produced by *H. pylori* UA955 LPS (Table II). *H. pylori* J233 also furnished a fucose-free type-2 polyLacNAc, known as the i-antigen [LacNAc → LacNAc → core] O-chain (Table II). The serological studies that showed recognition of the Le^b epitope in *H. pylori* LPSs with the Le^b specific mAb in strains UA955 and J223 were recognizing only substructures of the Le^b antigen. In *H. pylori* UA955 the Le^b mAb presumably detected the α-L-Fuc-(1 → 4)-β-D-GlcNAc Lewis disaccharide, which is a region of the Le^b determinant {α-L-Fuc-(1 → 2)-β-D-Gal-(1 → 3)[**α-L-Fuc-(1 → 4)**]-**β-D-GlcNAc**}, and in *H. pylori* J223, it either recognized the H-1 antigen (Le^d) {α-L-Fuc-(1 → 2)-β-D-Gal-(1 → 3)-β-D-GlcNAc} or Le^c {β-D-Gal-(1 → 3)-β-D-GlcNAc} which are moieties of Le^b {**α-L-Fuc-(1 → 2)-β-D-Gal-(1 → 3)**[α-L-Fuc-(1 → 4)]-**β-D-GlcNAc**}. The Fuc-(1 → 4)-GlcNAc terminal unit in *H. pylori* UA955 either may be a biosynthetic precursor of Le^a or Le^b in *H. pylori* LPS biosynthesis, or may represent a dead-end product due to premature fucosylation of GlcNAc at O-4, inhibiting further galactosylation of GlcNAc at O-3. The cross-reactivity observed between Le^b mAb BG-6 and *H. pylori* J223 LPS seems to be a mAb-specific phenomenon since Le^b mAb 225-Le did not react with J223 LPS. Imberty *et al.*[96] have reported that blood-group determinants have certain conformational dependent "micro-epitopes" toward which different mAbs have dissimilar activities. It is also worth noting that the same Lewis mAb might have different sensitivity toward the same LPS epitopes when tested under ELISA or immunoblot conditions, as indicated by the reaction of Le^b mAb with UA955 in ELISA, but not in immunoblot after removal of the proteins by proteinase K. Isolated *H. pylori* UA955 proteins did not react with the Le^b mAb, suggesting that the observed activity is not to the protein. Also, when whole cells of UA955 were tested in immunoblot the Le^b mAb showed no reaction, suggesting that recognition of UA955 LPS is lost under immunoblot conditions. The same type of phenomenon can also be observed with strain UA1182, whose internal Le^xepitopes were well recognized by the Le^x mAb in ELISA, but showed only a weak interaction in immunoblot. These two differences in activities may be caused by conformational changes in the LPS molecules. These studies illustrate the necessity for performing structural rather than serological studies to unambiguously define *H. pylori* LPS antigens.

The expression of type-1 Lewis blood-group antigens suggests that for the biosynthesis of some *H. pylori* LPS molecules there must be glycosyltransferases

of the type-1 family, such as a FucT that places the α-L-Fuc unit at O-4 of β-D-GlcNAc and a GalT that adds β-D-Gal to O-3 of β-D-GlcNAc. The concurrent expression of type-1 and 2 chains, and of various chains with different single-step glycosylation patterns, within a single *H. pylori* strain, represents a complex biosynthetic mechanism used by this organism, which differs from O-chain repeating OS unit addition mechanism of *Escherichia* and *Salmonella* LPS biosynthesis. Various factors, such as differential enzyme kinetics, regulation, and mutation or clonal variation, may control the assembly of *H. pylori* LPS molecules.

The genomes of *H. pylori* strain 26695 and J99 contain two copies of an α-fucosyltransferase and multiple copies may play a role in the expression of the Lewis antigens. Among Western *H. pylori* strains, type-2 Le^x and Le^y antigens are widespread and few seem to express type-1 Le^a and Le^b epitopes. This phenomenon might be due to regulation whereby all *H. pylori* strains contain the genes for the assembly of these alternative structures, but in certain instances some transferases are not expressed; alternatively, only certain strains may contain the appropriate genes required to produce the type-1 antigens. Diversity in fucosylation and thus in Lewis antigen expression among single colonies derived from the same gastric biopsy suggests that this phenomenon might occur *in vivo* as well.[97]

The Le^b epitope is widely expressed in human gastric mucosa[98,99] and thus *H. pylori* with Le^b are capable of mimicking the entire domain of the human gastric mucosa. A protein adhesin produced by *H. pylori* has been ascertained to be involved in adhesion of *H. pylori* to Le^b present in human gastric mucosa[80]; this behavior could extend to the possibility of any *H. pylori* strain that produces Le^b in its LPS being able to append to itself by the same mechanism, giving rise to auto-agglutination and thus formation of colonies. Other *H. pylori* cell-surface proteins have also been shown to be involved in adhesion to histo-blood group antigens.[100]

Parallel structural investigations unveiled the composition of LPS molecules from *H. pylori* strains obtained from Chinese, Japanese, and Singaporean symptomatic hosts.[101] The new findings were: (i) production of type-1 blood-group determinants (Table II) was prevalent in LPSs of Asian *H. pylori* strains, in contrast to Western *H. pylori* LPSs, and, in particular, the O-chain regions of strains F-58C and R-58A carried type-1 Le^a without the accompanying type-2 Le^x; (ii) strain R-7A and H608 were shown to have the capability of producing the type-1 blood-group A antigen; and (iii) strains CA2, H507, and H428 expressed simultaneously the difucosyl isomeric antigens, type-1 Le^b and type-2 Le^y. In contrast to LPSs of *H. pylori* strains isolated from hosts residing in Western countries (Sections II–V), which, overwhelmingly, were found to express type-2 Le^x and Le^y blood-group determinants, these Asian *H. pylori* LPSs also showed a tendency to produce type-1 histo-blood group determinants. The most complex O-chain molecules were found to be present in the LPS of *H. pylori* strain R-7A where an array of histo-blood group glycoforms were observed (Table II). Both blood-group

A type-1 forms, monofucosyl and difucosyl, were detected in *H. pylori* R-7A LPS, and it was shown that the type-1 A blood-group was connected to a type-2 LacNAc backbone, which is unique in histo-blood-group chemistry. Serologically, mAb 3-3a also detected blood-group A in this LPS by ELISA. Moreover, the R-7A strain also furnished a string of type-2 Le^x and LacNAc epitopes as terminal entities and as members of elongated linear chains (Table II).

In some of the foregoing *H. pylori* LPSs, a covalent connection between the O-chains, composed of histo-blood groups, and the core was shown to be formed by a ligation between the reducing end GlcNAc unit of the O-chain and an outer core DD-Hep residue (Tables I and II). Ion *m/z* 886 showed the linkage of the type-1 Le^a or type-2 Le^x epitope to the O-7 position of the outer core DD-Hep. Several higher mass ions in the FAB-MS spectrum of the methylated R-7A LPS, of defined composition and that included the linear heptose (7-substituted DD-Hep) of the outer core region, yielded important evidence for the connection of the type-1 difucosyl blood-group A (*m/z* 1305), and of the elongated type-2 $Le^x \rightarrow$ LacNAc and LacNAc $\rightarrow Le^x$ to the core (*m/z* 1335). These structural interpretations, which showed the covalent linkage between the O-chain region and the core, offered unequivocal proof that these *H. pylori* cell surface glycan molecules were indeed LPSs and were not capsular or exopolysaccharides. However, it is possible that some high-molecular weight Le^x O-chains found in some *H. pylori* strains may be present as non-LPS molecules, since it is not always possible to prove the covalent connection of O-chain to core.

H. pylori was found to carry both difucosyl type-1 Le^b and type-2 Le^y isomeric structures. The coexistent expression of Le^b and Le^y indicated that fucosylation and galactosylation at O-3 or O-4 of GlcNAc were available at any instance in *H. pylori* LPS biosynthesis and may be dictated by an array of internal and/or external factors. The end products (Le^b and Le^y in this case) may also have different functions in *H. pylori* pathogenesis, that is, they may perform particular physical roles in endogenous (bacterium–bacterium) or exogenous (bacterium–host) recognition/adhesion, or may be intrinsic players in the host's immune response pathways. A similar role may also apply to the other *H. pylori* LPS blood-group antigens. In *H. pylori* strains CA4 and CA5 (Table II), fucosylation at O-2 of a terminal Gal of a Gal-(1 → 3)-GlcNAc structure yielded the type-1 Le^d, Fuc-(1 → 2)-Gal-(1 → 3)-GlcNAc. The presence of three different monofucosylated Lewis determinants, Le^a, Le^x, and Le^d, in *H. pylori* strains CA5 and CA4 reflect three [Fuc-(1 → 2), Fuc-(1 → 3), and Fuc-(1 → 4)], possibly self-regulating, fucosylation pathways in the biosynthesis of these LPSs. Parallel serological studies using several commercially available mAbs, specific for histo-blood group epitopes, were also successfully in detecting the blood-group antigens in these *H. pylori* LPS.[82,95,101]

The core OS derivatives (non-histo-blood-group units), obtained from chemical linkage analysis of these *H. pylori* LPSs, were of the same type as found in the core structures described in the preceding sections. All core residues detected fell in line

with the typical core structural regions of *H. pylori:* Glc-(1 → 3)-Glc-(1 → 4)-Gal-(1 → 7)-[DD-Hep-(1 → 2)]-DD-Hep-(1 → 2)-LD-Hep-(1 → 3)-[P or AEP → 7]-LD-Hep. The ^{31}P NMR spectra and the linkage analysis data suggested the presence of a monoester phosphate (PO_4^-) at the O-7 position of the inmost LD-Hep of the strains described here, except in strain R-7A, where a 2-aminoethyl phosphate was shown to replace the monoester phosphate, as in *H. pylori* Sydney strain (Section IV).

The complete genomes of two Western *H. pylori* strains, 26695 and J99, have been determined to be similar. Chemical analyses have shown that the O-chain region of *H. pylori* 26695 and J99 LPSs were composed of type-2 Le^x and/or Le^y antigens, and no type-1 Le^a, Le^b, or blood-group A epitopes were observed.[82] The LPS structural differences noted here between the Asian and Western *H. pylori* strains, based on a more prominent expression of type-1 histo-blood group antigens in the O-chain sections of Asian strains, suggest that some significant differences may be encountered between the genomes of Asian and Western *H. pylori* strains.

The apparent strong inclination for the production of type-1 histo-blood group antigens in Asian *H. pylori* LPSs, when compared with Western strains, may be an adaptive evolutionary effect in that differences in the gastric cell surfaces of the respective hosts might be significantly dissimilar to select for the formation of different LPS structures on the resident *H. pylori* strain. Interestingly, in Asian countries ABO non-secretors are relatively more common than in Caucasian populations. A large-scale serological experiment using blood-group mAbs is needed to come to a conclusion on the extent to which Asian *H. pylori* strains express type-1 histo-blood group antigens.

In this section, the molecular mimicry between *H. pylori* LPSs and host molecules was extended to include the type-1 determinants Le^a, Le^b, Le^d, Le^c, and blood-group A. This ability of *H. pylori* to produce various Lewis isoforms permits mimicking all regions of the gastric epithelium, those being the gastric superficial and glandular epithelium, which display mainly type-2 molecules, and the superficial epithelium, which expresses predominantly type-1 chains.[98] Consequently, each *H. pylori* strain, depending on the antigens expressed by its LPS, may have a different ecological niche within the gastric mucosa, and ultimately the role of LPS in pathogenesis and adaptation may differ between *H. pylori* strains.

VII. Glucosylated and Galactosylated Poly-*N*-acetyllactosamine O-Chains in *Helicobacter pylori* Lipopolysaccharides

This section describes two classes of *H. pylori* LPSs that produce type-2 elongated polyLacNAc backbones similar to those described in preceding sections, but, in these cases, decorated by branches composed of end-group units of α-D-Glc*p* or α-D-Gal*p* in addition to the common α-L-Fuc.

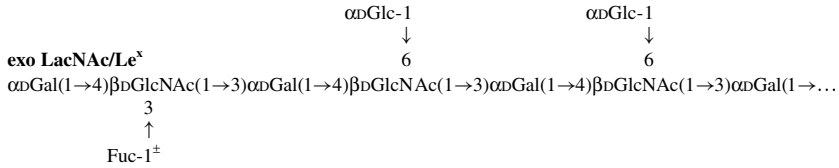
The cloning and characterization[102] of a glycosyltransferase from a clinical strain isolated from endoscopic biopsy specimen from a patient, *H. pylori*

UA861, capable of adding, in an enzyme assay, a α-D-Gal*p* to the O-6 position of a β-D-Glc*p*NAc [α-D-Gal*p*-(1 $\rightarrow$ 6)-β-D-Glc*p*NAc] prompted a structural investigation into the LPS from this strain.[103] Overall, the structural data unanimously pointed to an LPS composed of an O-chain region that comprised a type-2 polyLacNAc linear backbone with α-D-Glc*p* branches appended to the O-6 position of some backbone GlcNAc residues $\{\rightarrow 3)$-β-D-Gal*p*-(1 $\rightarrow$ 4)[α-D-Glc*p*-(1 $\rightarrow$ 6)]-β-D-Glc*p*NAc-(1 $\rightarrow\}$. Unexpectedly, no α-D-Gal*p*-(1 $\rightarrow$ 6)-β-D-Glc*p*NAc structure was witnessed in this analyses of *H. pylori* UA861 LPS. The ^{1}H NMR spectrum of the water-soluble intact LPS showed three dominant anomeric resonances for α-Glc (δ 4.99), β-GlcNAc (δ 4.69), and β-Gal (δ 4.47). ^{1}H–^{1}H *inter* NOE connectivities revealed the α-Glc-(1 $\rightarrow$ 6)-β-GlcNAc and β-Gal-(1 $\rightarrow$ 4)-β-GlcNAc connections in line with the observed 4,6-disubstituted GlcNAc derivative in the chemical linkage analysis. A LacNAc antigen [*m/z* 464 $\rightarrow$ 432 (464 $-$ 32) in FAB-MS] affected termination of the nonreducing end in this glucosylated O-chain, and no fucosylated Lewis determinant was detected in this outermost region of LPS. Only sole randomly placed internal Le^x moieties were observed. The LPS of *H. pylori* serogroup O : 4, from Penner's serotyping system,[69] also produced glucosylated polyLacNAc chains similar to those observed in the just-described *H. pylori* strain UA861 (Aspinall; Monteiro, unpublished results).

Aspinall[104] also investigated the LPS structure of *H. pylori* strain 471, which, unlike some *H. pylori* strains, lacked the ability to stimulate pepsinogen secretion. The proposed structure of this LPS comprised a typical *H. pylori* type-2 polyLacNAc O-chain backbone that was adorned with branches of α-D-Gal*p* units. Similar to the branching Glc residues in *H. pylori* UA861, these end-group Gal units were attached at O-6 of the backbone GlcNAc $\{\rightarrow 3)$-β-Gal*p*-(1 $\rightarrow$ 4)-[α-D-Gal*p*-(1 $\rightarrow$ 6)]-β-D-Glc*p*NAc-(1 $\rightarrow]$. The nonreducing terminus of this galactosylated LPS O-chain was composed of Le^x and Le^y, and also of nonfucosylated LacNAc and β-Gal-(1 $\rightarrow$ 4)-[α-D-Gal*p*-(1 $\rightarrow$ 6)]-β-D-Glc*p*NAc. A limited number of internal Le^x units were also detected in this galactosylated polyLacNAc O-chain. The resonance of H-1 α-D-Gal*p* side-units was observed at δ 4.98 and noticeable *inter* NOE connectivities were detected between this anomeric signal and H-6 (δ 3.90) and H-6′ (δ 4.15) of β-D-Glc*p*NAc confirming the Gal-(1 $\rightarrow$ 6)-GlcNAc linkage. Figure 12 displays the structures of the glucosylated and galactosylated polyLacNAc O-chains just described. The core OSs of the glucosylated and galactosylated LPSs discussed earlier possessed the same structural arrangement as parallel regions of previously investigated *H. pylori* strains (Fig. 6).

The most striking structural feature of the LPSs from *H. pylori* strains UA861 and 471 and serostrain O : 4 is the replacement of Fuc by Glc and Gal and therefore the absence of heavily fucosylated type-2 polyLacNAc O-chains, and consequently the limited number of internal Le^x antigens, which are a common characteristic component of *H. pylori*. The low degree of Le^x expression, and, occasionally, the absence of terminal Le$^{x/y}$, carries important implications with regard to pathogenic differences among *H. pylori* strains, such as possible bacterial–epithelial cell

***H. pylori* strain UA861 and serostrain O:4**



***H. pylori* strain 471**

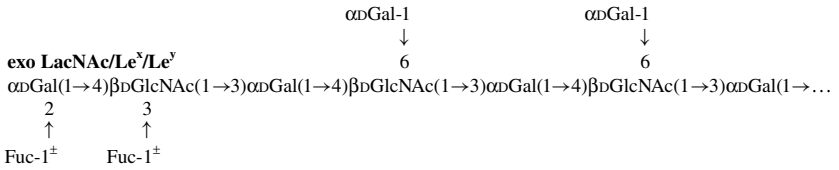


FIG. 12. The chemical structures of the glucosylated *H. pylori* UA861 and O : 4, and of the galactosylated *H. pylori* 471. A limited number of endo Le^x units are also present.

interactions, and in their role in a postulated autoimmune disease component linked with *H. pylori* strains that carry Lewis blood-group antigens. Although one would not expect *H. pylori* strains devoid of complete terminal Lewis epitopes, such as *H. pylori* UA861, to be recognized by mAbs raised against terminal Lewis antigens, in fact this strain did react with mAbs for Le^x, so that the internal α-1,3-Fuc unit must be accessible to react both in ELISA and immunoblot.[102] The characterization by chemical means of the glucosylated polyLacNAc amended the earlier observation that *H. pylori* UA861 produced an unusual α-$(1 \rightarrow 6)$-galactosyltransferase. Since this *H. pylori* strain carries a terminal α-D-Glc residue at the O-6 of GlcNAc, this particular glycosyltransferase is likely to be a glucosyltransferase rather than a galactosyltransferase or a glycosyltransferase with dual specificity. This irregularity might have arisen from the use of UDP-Gal as the acceptor substrate in the earlier transferase assays.[102] The structural studies must take precedence over transferase assays with chemical substrates, but direct evidence awaits the cloning and characterization of the postulated α-$(1 \rightarrow 6)$-glucosyltransferase. However, the galactosylated polyLacNAc of *H. pylori* 471 should indeed express an α-$(1 \rightarrow 6)$-galactosyltransferase. The diversity in polyLacNAc glycosylation observed here is most likely controlled at the genetic level. The genes encoding the FucTs that control Lewis antigen fabrication and the GlcT and GalT responsible for GlcNAc glycosylation may be present in some strains and absent from others. Alternatively, all *H. pylori* strains may contain a full complement of transferase genes and other factors may control either their expression or assembly of these complex carbohydrates. Diversity at both the levels of individual genes (microdiversity) as well as in gene order (macrodiversity) seems to be present in *H. pylori*.

The ornamentation of *H. pylori* LPS polyLacNAc chains with Glc and Gal residues does not appear to be a very common characteristic of *H. pylori* LPSs. The exact purpose(s) of these hexose units is not known, but they could be involved in stabilizing, or creating, LPS conformers that are required in *H. pylori* pathogenesis, especially when, as in these cases, fucosylation of these LPSs is limited. Indeed, additional reexaminations of these *H. pylori* strains revealed that fucosylation, and conversely glucosylation and galactosylation, of these LPSs varied vastly from batch to batch preparations. These variable degrees of polyLacNAc glycosylations reflect an unpredictable mechanism that is controlled by a flexible set of biosynthetic events.

VIII. *Helicobacter pylori* from Asymptomatic Hosts Expressing Heptoglycans, but Lacking Lewis O-Chains

This section describes the chemical structures of *H. pylori* LPSs from hosts with no explicit signs of severe disease. Serological analyses with histo-blood-group monoclonal antibodies did not detect the presence of any blood-group antigen in these *H. pylori,* and thus were non-typeable by this procedure. Data from chemical- and spectroscopic-based experiments unanimously showed that these *H. pylori* manufactured LPS with heptoglycans of 2- and 3-linked D-*glycero*-α-D-*manno*-heptopyranose units (as seen in Section IV), but are completely devoid of Lewis O-chains.[105] An *H. pylori* isolate with a similar LPS structure was shown to be capable of colonizing mice, which suggests that the presence of Lewis O-chains is not absolutely required for colonization. The absence of O-chains with histo-blood groups may cause *H. pylori* to become inept in adhering to human receptor cells, and/or also the presence of elongated heptoglycans may impede exposure of disease-causing bacterial cell-surface molecules. These two factors may render such *H. pylori* incapable of creating exogenous contacts essential for pathogenesis of severe gastroduodenal diseases.

H. pylori strains Hp1C2, Hp12C2, Hp65C, Hp7A, Hp75A, and Hp77C from asymptomatic hosts were used for serological analysis and chemical manipulations. The SDS-PAGE profile of these non-typeable LPSs showed the presence of high-M_r LPS material. As mentioned, ELISAs and immunoblots with blood-group mAbs fail to detect any histo-blood-group antigen in these LPS or whole-cell preparations. Sugar analysis performed on the seven intact LPS preparations revealed the presence of L-Fuc, D-Glc, D-Gal, D-GlcNAc, DD-Hep, and LD-Hep in the approximate ratios of $1:5:1:1:17:1.5$, respectively. DD-Hep was the dominant component of these LPSs. Sugar linkage analysis showed that each LPS was composed of terminal Fuc, Glc, and DD-Hep, traces of 3-substituted Fuc, 4-monosubstituted Gal, 3- and 6-monosubstituted Glc, 4-monosubstituted GlcNAc, 2-monosubstituted LD-Hep, 3,7-disubstituted LD-Hep, 2,7-disubstituted DD-Hep, and, most predominantly, 2- and 3-monosubstituted DD-Hep units

indicative of extended linear heptan chains. FAB-MS provided the first solid evidence for such heptoglycans, in that the FAB-MS spectrum of each methylated LPS yielded sequential glycosyl oxonium ions from cleavage at successive Hep residues. The FAB-MS spectra of these methylated *H. pylori* LPSs afforded A-type primary ions, and corresponding secondary ions from loss of methanol, at *m/z* 263 → 231 [Hep$^+$], *m/z* 511 → 479 [Hep → Hep$^+$], *m/z* 759 → 727 [Hep → Hep → Hep$^+$], *m/z* 1007 → 975 [Hep → Hep → Hep → Hep$^+$], and *m/z* 1255 [Hep → Hep → Hep → Hep → Hep$^+$] that pointed to the presence of DD-Hep oligomers. In addition, ions at *m/z* 189 [Fuc$^+$], *m/z* 682 [Fuc,GlcNAc,Hep]$^+$, *m/z* 930 → 898 [(Fuc,GlcNAc,Hep) → Hep$^+$], and *m/z* 1179 → 1146 [(Fuc,GlcNAc,Hep) → Hep → Hep$^+$] suggested that some heptoglycans were terminated at the nonreducing end by Fuc and GlcNAc units, possibly in a Fuc-(1 → 4)-GlcNAc linkage. No ions characteristic of blood-group trisaccharides Le^a or Le^x (*m/z* 638), tetrasaccharides Le^b or Le^y (*m/z* 812), or any other histo-blood group, typical components of *H. pylori* LPSs, were detected in the FAB-MS spectra of the strains examined here. The first evidence showing that some DD-Hep oligomers were constructed by consecutive 3-linked DD-Hep {-[→ 3)DD-Hep(1-]$_n$ → }, was obtained by the isolation of an OS from a Smith degradation composed mainly of 3-substituted Man-C^6-D units along with some nonsubstituted Man-C^6-D representative of a 3-linked mannan. The outer region of these LPSs was thus shown to exhibit a linear 3-linked DD-Hep chain DD-Hep-(1-[→ 3)-DD-Hep-(1-]$_n$ → 3)-DD-Hep-(1 → *inner LPS region*. The ^{1}H and ^{13}C NMR spectra of each delipidated *H. pylori* PS furnished two strong α-anomeric resonances, as unresolved doublets in the ^{1}H NMR spectrum, at δ_H 5.40/δ_C 101.0 and δ_H 5.09/δ_C 103.0 emanating from two major sugars, in this case, with the *manno* configuration, the 2- and 3-linked DD-Hep LPS constituents. Unequivocal evidence supporting the presence of linear heptoglycans in these strains was also obtained from a 2D NOESY experiment that yielded *inter*-space NOE connectivities between H-1 at δ 5.09 and H-3 at δ 4.02 for the 3-linked DD-Hep oligomer and H-1 at δ 5.40 and H-2 at δ 4.05 for the 2-linked DD-Hep oligomer. Other NOE connectivities involving H-1 at δ 5.40 were also observed, but they could not be unambiguously assigned and may suggest that this DD-Hep-(1 → 2)-DD-Hep linkage is highly flexible. One NOE signal involving H-1 at δ 5.40 and H-3 at δ 4.02 indicated that amidst the 3-linked heptoglycan oligomers some 2-substituted DD-Hep units may be inserted between the 3-linked DD-Hep residues [. . . → 3)-DD-Hep-(1 → 2)-DD-Hep-(1 → 3)-DD-Hep-(1 → . . .].

In the lower molecular weight LPS fractions, 2-, 3-, and 6-substituted DD-Hep residues were present in equimolar proportions, which indicated that these units are always co-expressed in these heptoglycan-rich strains. Also, in some low-M_r LPS subfractions, 3-substituted Fuc was an equimolar component suggesting the presence of a rare Fuc-(1 → 3)-Fuc moiety that is found in some *H. pylori* structures and/or a GlcNAc-(1 → 3)-Fuc structure.

The *H. pylori* Sydney strain[86] (Section V), widely and successfully used in mice models that study *H. pylori* pathogenesis, followed the *H. pylori* LPS pattern in that it carried Le^x and Le^y antigens in the O-chain region.[82] The usage of *H. pylori* Sydney strain and its genetically induced mutants carrying truncated LPS has revealed that these LPS structures may play a role in colonization; a mutated *H. pylori* Sydney strain carrying a rough-form LPS devoid of "Lewis O-chain," created by insertional mutagenesis of HP0826 (β4-*galT* gene), has been shown to still be able of colonizing the murine model, although with less efficiency (Section X). It was also shown that an *H. pylori* isolate, HpPJ1, producing a heptoglycan-rich LPS devoid of Lewis O-chains and structurally similar to those described earlier, was able to colonize CD1 mice, but in lesser numbers when compared with the mouse model standard Sydney strain. The fact that *H. pylori* strain HpPJ1 was able to persistently colonize the stomachs of CD1 mice for a period of at least 12 weeks implied that complete histo-blood group O-chains are not an absolute prerequisite for colonization.

H. pylori strains isolated from asymptomatic hosts, which were untypeable using mAbs specific for Lewis antigens (Le^a, Le^b, Le^x, or Le^y), were found to produce LPS molecules devoid of "blood-group O-chain" regions. Instead, they contained elongated heptoglycans, oligomers of D-α-D-Hep, DD-Hep-(1-[$\rightarrow$3)-DD-Hep-(1-]$_n \sim$ [$\rightarrow$2)-D-Hep-(1-]$_n \rightarrow$ core OS, in which some were capped by an incomplete Lewis antigen (Fig. 13). This family of *H. pylori* strains also contained 6-substituted DD-Hep as a common component in the inner regions of LPS; this unit was also found to be present in *H. pylori* strains that contain extended heptoglycans (Section IV). These three heptosyl linkages, 2-, 3- and 6-substituted DD-Hep units, seem to be always coexpressed. A common feature of the *H. pylori* LPSs described here, and those of heptoglycan-rich strains, was the production of an accompanying side-antenna dextran [(1$\rightarrow$6)-α-D-glucan] (Fig. 13). The core regions, within the limits of detection, were composed of the same sugar units as other *H. pylori* isolates.

The individuals from which these strains were isolated all had positive signs of *H. pylori* infection, positive urea breath test, as well as positive histology or culture for *H. pylori,* but no signs of overt disease were detected. The increased proportion of *H. pylori* untypable by Lewis specific mAbs identified among the *H. pylori* from asymptomatic subjects may be an evolutionary trend toward a decreased immune response. If the Lewis, or any other, blood-groups are the inflammatory agent responsible for immune activation, a decrease in the presentation of these antigens may lead to a decrease in immune-related symptoms and symptomatic colonization. Previous studies of inflammatory responses in relation to *H. pylori* Lewis antigens did not include any untypeable *H. pylori* isolates; thus, it will be necessary to investigate the immune response, colonization, and inflammation capabilities of these isolates in both *in vitro* and *in vivo* model systems to see if any differences are observed. Previously, the LPS of another asymptomatic

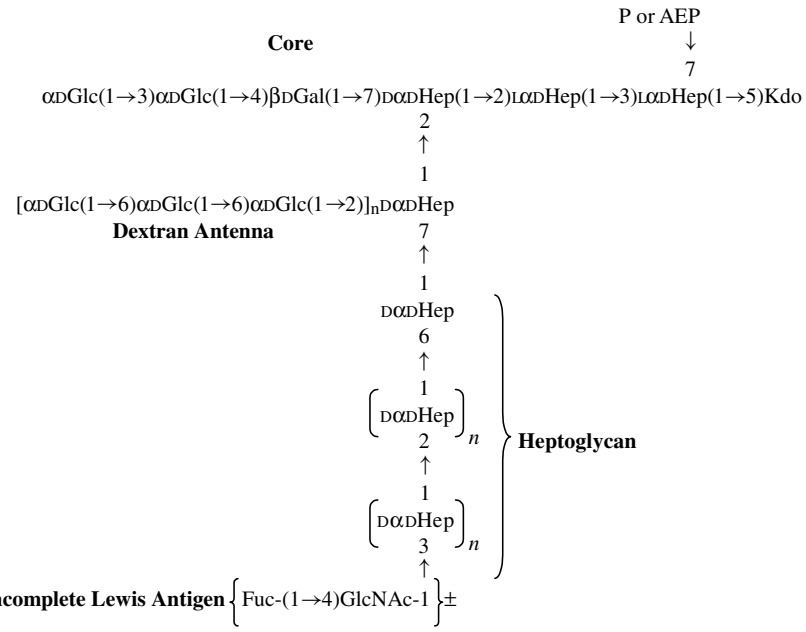


FIG. 13. Chemical structure of the heptoglycan-rich LPSs of *H. pylori* strains from asymptomatic hosts that lack histo-blood-group O-chain regions. A small number of LPSs may also contain a Hep/GlcNAc(1 → 3)Fuc antigen as a nonreducing terminus.

isolate, MO19 (Section IV), was chemically characterized and shown to contain an elongated heptoglycan similar to those found here, with some being capped by a sole Le^y. This intervening region was also found in the LPS of two other isolates, those being serogroups O : 3 and O : 6 of the Penner serotyping system.[69] The presence of this heptoglycan region may provide increased length and flexibility to the LPS such that it may cover the bacterial surface and consequently interfere with the involvement of bacterial virulence factors with the epithelium of host cells. Therefore, in addition to the absence of long Lewis blood-group O-chains, this intervening region may increase LPS flexibility that may allow masking of other *H. pylori* products on the bacterial surface, preventing a symptomatic *H. pylori* infection. Indeed, the heptoglycan-rich asymptomatic *H. pylori* MO19, found to be unable to bind to the gastric mucosa, may be impeded in adhering because the heptoglycan "covers" certain adhesin(s). Also, protective antibodies directed at the dominant heptoglycan domain in these *H. pylori* might confer some protection against infection.

The production of these types of LPSs may be controlled at the genetic level. The complete order of synthesis of the Lewis antigens by *H. pylori* has not been elucidated, but the untimely fucosylation of GlcNAc here (Fuc → GlcNAc) could

impede galactosylation of this residue and thus obstruct the building of a complete Lewis determinant. This addition of Fuc would effectively terminate O-chain elongation. Additionally, these *H. pylori* strains may contain inactive β-1,4-GalT and β-1,3-GalT glycosyltransferases, both responsible for Lewis O-chain formation, Le^x and Le^a, respectively, and thus resulting in the absence of Lewis blood-groups. LPSs composed of heptoglycans without Fuc-GlcNAc capping were common in these strains, which may also implicate the GlcNAcT responsible for adding GlcNAc to the heptoglycan, the GlcNAcT responsible for O-chain initiation. These *H. pylori* isolates may also lack enzyme activity of a second GlcNAcT that places GlcNAc at O-3 of Gal, the GlcNAcT responsible for O-chain elongation. Phase variation in *H. pylori* LPS may be controlled by an "on" and "off" switching mechanism of several glycosyltransferases. These heptoglycan-rich isolates must contain in their armor highly "active" heptosyltransferases. These glycosyltransferase activities may be controlled by environmental factors, which dictate the presence or absence of Lewis O-chains, thus yielding the desired LPS structure for the present stage of pathology.

These results indicate that the presence of Lewis O-chains in *H. pylori* seems to be important for development of pathology, but not colonization. These isolates all colonized human individuals, but it is not clear if they expressed any Lewis antigens at the initiation of infection. This study demonstrates that complete Lewis antigens in *H. pylori* do not appear to be absolutely required for initial colonization in the murine model. Therefore, Lewis antigens are unlikely to be an absolute prerequisite for colonization of the human stomach either, although the data obtained in this study strongly suggest that blood-group O-chains are a requirement for *H. pylori* pathology. The *H. pylori* that do not express complete Lewis antigens appear to augment the cell surface by elongating the heptoglycan region and adding a dextran, possibly in lieu of the Lewis antigens.

IX. The Lipopolysaccharides of *Helicobacter* Species from Nonhuman Primates

Many nonhuman primates have been observed to carry their own *Helicobacter*-like organism, which, like *H. pylori* in humans, gives rise to gastric diseases in their respective hosts. *H. mustelae* in ferrets, *H. felis* in cats, *H. acinonyx* in cheetahs, *H. bilis* in mice, and *H. suis* in pigs are some examples of *Helicobacter* species distributed among the animal kingdom that colonize the host's stomach. *H. hepaticus* colonizes the liver of mice, and infrequently *H. heilmannii* is also found in the human stomach. *In vitro* growth of these *Helicobacter* species is extremely difficult and in some cases yet unachievable.

Only the LPS of *H. mustelae* (ATCC 43772) from ferrets, a commonly used *Helicobacter* animal model, has been investigated in detail.[106] The LPS of *H. mustelae* followed the example of *H. pylori* LPSs in that it also expressed a

histo-blood group antigen, that being the monofucosyl type-1 A blood-group {α-D-GalNAc($1 \rightarrow 3$)[α-L-Fuc-($1 \rightarrow 2$)]-β-D-Gal-($1 \rightarrow 3$)-β-D-GlcNAc} in mimicry of the host's gastric cell-surface molecules.[106,107] The blood-group A in *H. mustelae* LPS completed a core OS of which the inner portion showed similarities to parallel regions {β-D-Gal-($1 \rightarrow 3$)-LD-Hep-($1 \rightarrow 3$)-LD-Hep-($1 \rightarrow 5$)-Kdo} of *Campylobacter* LOSs. No elongated O-chains were found in *H. mustelae* and, thus, its LPS was more akin to low-M_r LOSs found in *Neisseria, Campylobacter,* and *Haemophilus* containing structures homologous to mammalian cell-surface glycomolecules. No DD-Hep was detected in *H. mustelae* LPS. The lipid A structure of *H. mustelae* type strain ATCC 43772 presented two different lipid A moieties.[108] The *H. mustelae* lipid A structures observed differed from that of the related human gastric pathogen, *H. pylori. H. pylori* lipid A molecules are sparsely acylated, with most strains containing a sole C_{18} component, and are only monophosphorylated by a 2-aminoethyl phosphate at the anomeric position,[109,110] β-D-Glc*p*N-($1 \rightarrow 6$)-α-D-Glc*p*N-($1 \rightarrow$ PEA. One *H. mustelae* lipid A structure contained a bisphosphorylated β-($1 \rightarrow 6$)-linked D-glucosamine disaccharide with hydroxytetradecanoic acid in amide linkages. The *H. mustelae* lipid A was found to be heterogeneous with two major molecular species, a pentaacyl and a hexaacyl species. Unlike the structure described for the lipid A of *H. pylori,*[109,110] this lipid presented phosphate groups at both the C-1 and C-4′ positions in the backbone, and contained no octadecanoyl fatty acid.[108] The C-4 and C-6′ hydroxyl groups of the backbone disaccharide were unsubstituted, the latter being the proposed attachment site of the core OS. This lipid A was similar to that of *Haemophilus influenzae* with tetradecanoyl-oxytetradecanoic acids at the C-2′ and C-3′ positions of the nonreducing GlcN. The other lipid A structure had a different fatty acid composition with C_{16}-OH replacing most of the amide-linked C_{14}-OH. Structural heterogeneity in this case was also due to the presence of a minor molecular species having hydroxytetradecanoyloxy-tetradecanoic acid at the C-3′ position.

H. felis (ATCC 49179), *H. hepaticus,* and *H. acinonyx* LPSs were observed to be composed of similar sugar constituents as those found in *H. pylori,* namely, Fuc, Glc, Gal, DD-Hep, LD-Hep, GalNAc, and GlcNAc, and thus have the potential to produce blood group-related antigens. Of particular interest, the Fuc component in *H. felis* was present as a 3,4-disubstituted unit[111] and not as an end group, as seen in *H. pylori* and *H. mustelae,* and therefore cannot carry the known blood-group epitopes with terminal Fuc, but may produce glycan structures similar to those present in its host's gastric niche not yet identified. The other linkage types of *H. felis* LPS represent a branched molecule that includes terminal Glc and Gal, 2- and 2,7-substituted DD-Hep, 4-substituted GlcNAc, 3-substituted Gal and Glc, 4-substituted Gal, 2-substituted Man, and 2-substituted LD-Hep.[111] The molecular mimicry shown by *H. mustelae,* in producing blood-group A in its LPS, furthers the belief that *Helicobacter* LPS may have a significant role in disease pathogenesis.

X. Classification of *Helicobacter pylori* Lipopolysaccharides into Glycotype Families

The LPSs of *H. pylori* strains share the common characteristic of expressing epitopes in their O-chains similar to human histo-blood group antigens. However, fine points in overall and fine structural details differentiate these LPS molecules. With this fact in mind, and in order to create a systematic grouping of different *H. pylori* LPSs, the LPSs from the strains of which the chemical structures are known were classified into specific glycotype families (Table III).

The first, and apparently the most common in North American and European *H. pylori* strains, glycotype A, represents *H. pylori* LPSs composed of polymeric type-2 Le^x O-chains (more than one Lewis repeat), terminated at the nonreducing end by Le^x, Le^y, or sialyl Le^x, attached to the core. The type-2 Lewis polyLacNAc backbone is composed of a β-Gal-$(1 \rightarrow 4)$-β-GlcNAc linkage in which the addition of β-Gal to O-4 of β-GlcNAc is affected by the *H. pylori* β-1,4-galactosyltransferase,[112] HP0826 in *H. pylori* 26995 genome[85] and JHP765 in *H. pylori* J99 genome,[87] which was annotated as a lex2B/lob1 homologue. This β-1,4-galactosyltransferase gene involved in *H. pylori* O-chain synthesis diverges from the predicted pattern of LPS gene homologies with respect to O-chain and core specificity. When the *H. pylori* β-1,4-galactosyltransferase gene is inactivated the resulting LPS is truncated and thus becomes devoid of any Lewis O-chain, which in turn negatively affects the bacterium's colonization capability[112] in the murine model. The formation of the type-2 polyLacNAc backbone of glycotype A LPS is also dependent on the activity of the *H. pylori galE* gene, HP036/JHP1020, encoding a galactose epimerase activity.[113] Disruption of *H. pylori*'s *galE* gene leads to an LPS without a Lewis O-chain and an outer core region [αGlc-$(1 \rightarrow 3)$-αGlc-$(1 \rightarrow 4)$-βGal], and the absence of these LPS sections was shown to disrupt adhesion between the bacterium and the host gastric cells.[113] The complementary *H. pylori* β1,3-GlcNAcT involved in type-2 polyLacNAc production [βGlcNAc-$(1 \rightarrow 3)$-βGal] has not yet been identified. Fucosylation of β-GlcNAc [α-L-Fuc-$(1 \rightarrow 3)$-β-D-GlcNAc] and β-Gal [α-L-Fuc-$(1 \rightarrow 2)$-β-D-Gal] is the essential final step for the fabrication of type-2 Le^x and Le^y. The *rfbM* (*manA/C*) *H. pylori* gene, HP0043/JHP37, encodes a GDP mannose pyrophosphorylase that is one of the precursors for the synthesis of GDP-Fuc. Upon interruption of *H. pylori rfbM* gene,[113] fucosylation of the LacNAc block did not occur and the resulting LPS molecule did not express Le^x or Le^y, but rather a linear polyLacNAc O-chain. Also, the *H. pylori* gene involved in the conversion of GDP-mannose to GDP-fucose, *wbcJ* (HP0045/JHP38), was found to be induced at the transcription level by exposure to acid.[114] As seen only by SDS-PAGE, disruption of *wbcJ* gene led to an LPS without O-chain,[114] and this isogenic mutant was comparatively more sensitive to acid stress, which hinted at the fact that *H. pylori* may change its LPS makeup in response to pH levels *in vivo*. However, because *H. pylori,* after

rapidly migrating through the mucus layer toward higher pH, takes up residence the a neutral milieu (pH 7) of the epithelial surface, and because a wide array of structures, with and without Fuc, are often observed in a single LPS preparation, a predetermined role of an *in vivo* acidic environment on the outcome of LPS structures is not obvious. The synthesis of Le^x in *H. pylori* takes place in a manner similar to that observed in mammalian cells, that is, a type-2 LacNAc moiety is fucosylated by a α-1,3-fucosyltransferase (FucT) using GDP-Fuc as the donor molecule.[115,116] The published genome sequences[85,87] contain two α-1,3-FucT genes, *futA* (HP0379/JHP1002) and *futB* (HP0651/JHP596). In *H. pylori,* a final addition of Fuc, by an α1,2 FucT, *futC* (HP0093|0094/JHP86), onto O-2 of Gal in a Le^x structure furnishes the difucosylated Le^y antigen.[117] The Le^y production via Le^x in *H. pylori* differs from the fabrication of Le^y in mammalian cells, which takes place by fucosylation [Fuc-(1 → 3)-GlcNAc] of an H type-2 moiety [Fuc-(1 → 2)-Gal-(1 → 4)-GlcNAc] rather than Le^x. Induced mutations of the mentioned FucT genes in glycotype A *H. pylori* strains resulted in LPS preparations devoid of Le^x and/or Le^y antigens. Glycotype A LPSs may also possess a type-2 sialylated antigen, sialyl Le^x, as found in strain P466 (Section III). Two potential *H. pylori* genes involved in sialylation are *neuA* HP0326/JHP309, an acylneuraminate cytidyltransferase, and *neuB* HP0178/JHP166, a sialic acid synthase. Fucosylation of the polyLacNAc O-chains present in glycotype A LPSs has been observed to be a highly random process, and thus these O-chains contain many fucose-free internal LacNAc repeats amid the Le^x moieties, resulting in a high degree of phase variation. Appelmelk *et al.*[118] suggested that in *H. pylori* the *futB* gene product readily fucosylates a terminal nonreducing LacNAc to yield an exo Le^x, whereas the *futA* gene product tends to fucosylate internal LacNAc repeats to afford endo Le^x units. Alterations in the polyC or polyA length, and in other regions, that lead to an *on* or *off* status of *H. pylori futA* and *futB* genes are suspected to be related to active or inactive fucosylation and thus to Le^x expression.[119] At least in part, phase variation in *H. pylori* LPS seems to be mediated by frameshift mutations in the polyC tract of the FucT encoding genes. As described in this article, the expression of Le^x and Le^y antigens in *H. pylori* LPSs varies between strains; therefore, each *H. pylori* strain must possess different levels of FucTs activities, so that the relative levels of *fucA, fucB,* and *fucC* genes in a particular strain dictate the expression pattern of Le^x and Le^y within that strain. Le^x-carrying *H. pylori* strains that do not produce Le^y probably do not make α-1,2-FucT or carry one with very low activity. Such *H. pylori* strains may have a tendency to produce S-form LPSs with elongated Le^x O-chains, whereas strains with a highly active α-1,2-FucT tend to elaborate SR-form LPS with a sole Le^y antigen in the O-chain (Section V) since fucosylation of the nonreducing Gal halts O-chain extension by preventing addition of GlcNAc to O-3 of Gal. In glycotype A LPSs, the ligation between the Lewis O-chain and the core OS is affected by a O-chain–GlcNAc-(1 → 7)-DD-Hep–core or a O-chain–GlcNAc-(1 → 3)-DD-Hep–core linkage.

TABLE III

Classification of *H. pylori* Lipopolysaccharides into Glycotype Families

Glycotype (Lewis Type)	Strain	O-Chain[a]	Heptoglycan	Core[b]	(LPS-form)
					Smooth-form LPS
A (Type-2)	NCTC11637	Le^x-[Le^x]$_n$		Core	
A	P466,	$Le^{x/y}$-[Le^x]$_n$		Core	
		Sialyl Le^x-[Le^x]$_n$		Core	
A	26695	Le^x-[Le^x]$_n$		Core	
A	J99	$Le^{x/y}$-Le^x		Core	
A	SS1	Le^x-[Le^x]$_n$		Core	
A^{126}	AF1, 007	$Le^{x/y}$-[Le^x]$_n$		Core	
A	UA1182	$Le^{x/y}$-[Le^x]$_n$		Core	
					Smooth-form LPS
B^c (Type-2)	O:3	$Le^{x/y}$-[Le^x]$_n$	Heptoglycan	Core	
					Smooth-form LPS
C	MO19	$Le^y \pm$	Heptoglycan	Core	
C	1C2, 12C2,	(Fuc,GlcNAc) $\pm$	Heptoglycan	Core	Asymptomatic hosts
	62C, 7A, 75A,		Heptoglycan	Core	
	77C, PJ1		Heptoglycan	Core	
C	O:6	$Le^y \pm$	Heptoglycan	Core	
					Smooth-form LPS
D^d (Type-2)	UA861, O:4	[LacNAc]$_n$		Core	
		|			
		α-Glc			
					Smooth-form LPS
E^d (Type-2)	471	[LacNAc]$_n$		Core	
		|			
		α-Gal			
					Smooth-form LPS
F (Type-1 and 2)	UA948	$Le^{a/x}$-[Le^x]$_n$		Core	
F	UA955	$Le^{dis/x/y}$-[Le^x]$_n$		Core	

F	J223	$Le^{c/d}\sim[LacNAc]_n$	Core	
F	NCTC11637	$Le^{d/x/y}$-Le^x	Core	
F	R-7A/H608	Type-1 A blood-group-LacNAc	Core	
F		Le^x-Le^x	Core	
F	CA2/H428/H507	$Le^{b/y}$-$[Le^x]_n$	Core	
F	CA5, CA4	$Le^{a/d/x}$-$[Le^x]_n$	Core	
F	GU2	$Le^{a/x}$-$[Le^x]_n$	Core	
				Semi rough-form LPS
G (Type-1 and 2)	26695	$Le^{dis/x/y}$	Core	
		Fuc-1 → 3-Fuc-1 → 4-GlcNAc	Core	
		GlcNAc-1→3-Fuc		
		Linear B blood-group	Core	
G	SS1	Le^y	Core	
		Fuc-1 → 3-Fuc-1 → 4-GlcNAc	Core	
		Linear B blood-group	Core	
G	F-15A	$Le^{a/y}$	Core	
		Fuc-1 → 3-Fuc-1 → 4-GlcNAc	Core	
		Linear B blood-group	Core	
G	CA6	$Le^{a/x}$	Core	
				Semi rough-form LPS
H (Type-1)	UA915	$Le^{b/d}$	Core	
H	UA1111	Le^b	Core	
H	F-58C	Le^a	Core	
		Linear B blood-group	Core	
H	R-58A	Le^a	Core	
I		*H. pylori* strains not expressing histo-blood-group LPS molecules		

[a] The Le^x elongated O chains also contain randomly placed LacNAc units.

[b] All cores share a similar structure. However, the inner most LD-Hep unit may either carry a monoester phosphate or a 2-aminoethyl phosphate at position O-7.

[c] The solidus (/) indicates that either structure is possible.

[d] The α-D-Glc and α-D-Gal side chains are attached to the O-6 position of the GlcNAc. The UA861 O-antigen chain also expresses one internal Le^x. The 471 LPS also contains O-3-fucosylated GlcNAc units.

Glycotype B comprises LPSs with glycotype A-like Lewis O-chains, but which are attached to the heptoglycan domain [DD-Hep oligomer] which in turn is adjoined to the core OS. LPSs with short O-chains carrying a sole Lewis epitope attached to the heptoglycan (similar to that of glycotype B), or just carrying the heptoglycan attached to core, are grouped into the glycotype C family. The heptoglycan domain found in glycotype B and C LPSs must employ an α-1,3-heptosyltransferase (DD-HepT) with the ability of forming homopolymeric heptan linear chains. These heptoglycans may be capped at the nonreducing end by a long Lewis O-chain (glycotype B) connected by a GlcNAc-(1 $\rightarrow$ 3)-DD-Hep linkage, or by a single Le^y (glycotype C) that is attached by a Gal-(1 $\rightarrow$ 3)-DD-Hep linkage. Glycotype C also contains *H. pylori* strains from asymptomatic hosts that were shown to contain mainly uncapped heptoglycan regions in which some were terminated by an incomplete Lewis antigen. Two important, yet unidentifiable, glycosyltransferases involved in heptoglycan-rich glycotypes are a β-1,3-GlcNAcT and a β-1,3-GalT that form the covalent attachment between the Lewis O-chain and heptoglycan domain. A similar β-1,7-GlcNAcT must also be involved in connecting the Lewis O-chain to the core DD-Hep in glycotype A. Characterization of genes involved in these connections will be crucial for creating LPS mutants that are completely devoid of O-chain constituents and are composed only of core/heptoglycan regions in order to study the role of Lewis O-chains in *H. pylori* pathogenesis.

The polyLacNAc O-chains of *H. pylori* LPSs can also be glucosylated or galactosylated, and these LPSs were assigned to glycotypes D and E, respectively. These glycotypes also contained, in small amounts, Le^x and Le^y antigens. *H. pylori* strains containing glycotypes D and E must contain gene products for encoding α-1,6-GlcT and α-1,6-GalT activities. Branching of polyLacNAc O-chains by Glc and Gal units may be a requirement for certain *H. pylori* strains to adopt necessary LPS conformations required for successful pathogenic duties.

Glycotype F represents S-form LPSs that have the ability of producing type-1 and type-2 Lewis blood-groups simultaneously. Glycotype G contains SR-form LPSs also coexpressing type-1 and type-2 histo-blood groups, and glycotype H LPSs express solely type-1 blood-groups in the O-chain region. The expression of type-1 LPS antigens in these *H. pylori* glycotypes depend on the activity of α-1,4-FucT, for fucosylation of GlcNAc at O-4, and of β-1,3-GalT, for galactosylation at O-3 of GlcNAc for Le^a and Le^b production. In *H. pylori* strain UA948, one of the FucT genes, *futA,* has been shown to encode an enzyme with both α-1,3 (for type-2 Le^x) and α-1,4-FucT (for type-1 Le^a) activities, while the other gene, *futB,* encodes a truncated protein without any FucT activity.[120] This reflects a high level of interstrain gene diversity. Fucosylation at O-4 of GlcNAc to give Le^a occurs when a Le^c acceptor [Gal-(1 $\rightarrow$ 3)-GlcNAc] is available, a pattern similar to Le^a production in mammalian cells. Galactosylation of GlcNAc at O-3, in *H. pylori* Le^a

fabrication, appears to involve the β-1,3-GalT,[121] HP0619. This β-1,3-GalT gene is responsible for galactosylating GlcNAc to create the type-1 backbone, β-Gal-$(1 \rightarrow 3)$-β-GlcNAc (Le^c), of Le^a, H type 1, Le^b, and maybe type-1 blood-group A. For the synthesis of type-1 Le^b in *H. pylori,*[122] the α-1,2-FucT fucosylates Le^a, a pathway not usually found in production of Le^b in mammalian cells. This *H. pylori* α-1,2-FucT can also fucosylate a fucose-free type-1 Le^c antigen to afford the H type-1 antigen (Le^d), which is found in some *H. pylori* strains. Practically, no production of Le^b through fucosylation, by α-1,4-FucT, of H type-1 takes place in *H. pylori*. Also, inactivation of the *futC* (α-1,2-FucT) gene[122] in Le^b-producing strains leads to LPSs solely expressing Le^a, underlining the fact that Le^b production in *H. pylori* is mainly channeled through Le^a. Because of the apparent tendency of Asian *H. pylori* strains to produce type-1 Le^a and Le^b antigens, these strains must have, in comparison to Western strains, gene products with higher α-1,4-FucT and β-1,3-GalT activities. *H. pylori* LPSs containing type-1 antigens seem to be only of the SR-form, since no elongated type-1 chains of Gal-$(1 \rightarrow 3)$-GlcNAc repeats have yet been detected in *H. pylori,* which suggests that a possible β-1,3-GlcNAcT that adds GlcNAc onto a type-1 Gal-$(1 \rightarrow 3)$-GlcNAc acceptor does not exist in *H. pylori*. Type-1 blood-group A has also been detected among Lewis antigens in *H. pylori* LPS preparations. In *H. pylori,* the presence of GalNAc-$(1 \rightarrow 3)$-Gal-$(1 \rightarrow 3)$-GlcNAc implies that fucosylation at O-2 of Gal in blood-group A synthesis can take place after the addition of GalNAc to O-3 of the same Gal unit. Some *H. pylori* strains may also make use of *futA, futB,* and *futC* genes to create the Fuc-$(1 \rightarrow 3)$-Fuc linkage in the difucosylated antigen, Fuc-$(1 \rightarrow 3)$-Fuc-$(1 \rightarrow 4)$-GlcNAc. The bridging of the type-1 Lewis O-chains to the core OS in glycotypes F, G, and H is similar to that in glycotype A, where the reducing-end GlcNAc of the O-chain is ligated to O-7 of DD-Hep from core. An atypical *H. pylori* polysaccharide has been found in some strains from a single source in Denmark,[123] which contained no blood-group structures and no similarities to core or heptoglycan regions, and, therefore, glycotype I is reserved here for this *H. pylori* LPS and for any other that in future will be characterized and which will not express any histo-blood-group molecules. There are now six non-*pylori Helicobacter* species that have been isolated from diarrheic humans: *H. pullorum, H. canis, H. rappini, H. fennelliae, H. cinaedi,* and *H. canadensis.*[124] Comparison of ribosomal DNA sequences does not always give conclusive evidence for species-level identification and may be deceptive; hence, one must be careful not to misidentify helicobacters.[125]

Fine structural and genetic data strongly suggest that *H. pylori* LPS Lewis O-chains are assembled by a sugar-by-sugar addition rather than by the most common block-by-block mechanism found in gram-negative bacteria. Perhaps all *H. pylori* strains, given the agreeable conditions, have the ability to generate any of these LPS glycotypes.

XI. Concluding Remarks and Future Directions

The detection of LPSs in *H. pylori* expressing structures homologous to human histo-blood-group antigens stands as one of the most important and crucial discoveries in both *H. pylori* and bacterial-LPS research. The production of Lewis determinants, blood-group A, and linear blood-group B by *H. pylori,* occasionally by a single strain, is a perfect example of molecular mimicry, and of the capability that bacteria possess to elaborate glycan structures similar to those present in their biological niche. In this case, *H. pylori* carry LPSs with structures similar to cell-surface glycolipids and glycoconjugates expressed by the human gastric mucosa. No exact reason for this observed molecular mimicry has yet been categorically established; however, several reasons why *H. pylori* produce histo-blood-group antigens can be put forth. The most obvious is based on the molecular mimicry premise, in that *H. pylori* produces host-like gastric-glycan structures in order to mimic its immediate surrounding and thus avoid being detected by the host's immune system, leading to an uninterrupted long life. A parallel situation is also found in ferrets, in that *H. mustelae* mimics the host's gastric molecules by producing an LPS with a blood-group A structure. Two facts point to this possibility: first, *H. pylori* have a very low endotoxic activity, and, second, the organism, if left undisturbed, can survive in the gastric mucosa for the host's lifetime. Interestingly, *H. pylori* seem to stop colonization in the normal gastric mucosa after this region becomes occupied by cancerous malignancies. This molecular mimicry reasoning supports the fact that *H. pylori* may wish to remain anonymous while living in the host's normal gastric flora. Ultimately, in some cases, *H. pylori* may perform some beneficial role for the host. Another motive may be that *H. pylori* makes use of histo-blood group LPS antigens for initial colonization via adhesion of these LPS epitopes to the host's gastric epithelial cells. The detection of pedestal formations formed by Le^x–Le^x homotypic interactions may be important in the interaction of the bacterium with gastric eukaryotic cells.[99] In fact, isogenic LPS mutants devoid of Lewis O-chains have been shown to lose their capability of adhering to, and colonizing, the human gastric epithelial tissue when compared with the wild-type strain.[112,113] These adhesion properties of *H. pylori* LPS can also be used endogenously so that the bacteria can attach to each other, thus forming colonies that may aid in survival and in colonization. Alternatively, during infection, antibodies directed at the *H. pylori* Lewis O-chain may bind to gastric epithelial molecules that express similar antigens, giving rise to an autoimmune component,[127] especially in chronic type B gastritis and gastric and duodenal ulcers. Yokota and co-workers, based on serological and immunological studies with Japanese *H. pylori* strains, have also suggested that a region of the LPS, but not the Lewis antigens, may also be antigenic and highly immunogenic in humans.[128] From the structural results described here, these prospective antigenic epitopes may be any region of the core OS $\{\alpha$-Glc-$(1 \rightarrow 3)$-α-Glc-$(1 \rightarrow 4)$-

β-Gal-(1 → 7)-[α-Glc-(1 → 6)-α-Glc-(1 → 2)-D-α-D-Hep-(1 → 2)]-D-α-D-Hep-(1 → 2)-L-α-D-Hep-(1 → 3)-[AEP/P → 7]-L-α-D-Hep-(1 → 5)-Kdo}, lipid A, or any of the O-chain structures not detectable by Lewis blood-group mAbs, those being α-Fuc-(1 → 3)-β-GlcNAc, α-Fuc-(1 → 4)-β-GlcNAc, α-Fuc-(1 → 3)-α-Fuc-(1 → 4)-β-GlcNAc, β-Gal-(1 → 3)-β-GlcNAc, β-Gal-(1 → 4)-β-GlcNAc, α-Gal-(1 → 3)-β-Gal-(1 → 3)-β-GlcNAc, or α-Gal-(1 → 3)-β-Gal-(1 → 3)-β-GlcNAc. Ho[129] and Heneghan[130] and co-workers have reported that peptic ulcer disease is associated with increased expression of Lewis antigens in *H. pylori.* The linear B blood-group moiety [α-Gal-(1 → 3)-β-Gal-(1 → 3,4)-β-GlcNAc] present in some SR-form *H. pylori* LPSs may be one of the factors in why humans contain high level of IgG antibodies (known as xeno-antibodies) directed at this epitope, which is involved in organ rejection in xeno-transplantation.[131] Possibly, persons infected with *H. pylori* may have a higher concentration of xeno-antibodies.

The heptoglycan domain present in some *H. pylori* strains probably serves as a biological arm to present the sole Lewis antigen to the host's receptors. Interestingly, many *H. pylori* strains from asymptomatic hosts are devoid of a complete Lewis O-chain and contain this elongated heptoglycan. This heptoglycan region may cover disease-causing outer membrane proteins, which, coupled with the lack of Lewis O-chains, probably impedes these strains in activating pathological responses. Perhaps, after successful colonization by *H. pylori* cells with high-M_r S-form LPSs cells covered by SR-form LPSs, containing a short O-chain with a single blood-group antigen, become prevalent and expose disease-causing cell-surface molecules; in fact, *H. pylori* containing low-M_r SR-LPS LPSs with type-1 antigens (Le^a and Le^b) are very common in Asian countries (Section VI), and in these regions *H. pylori* infections are more symptomatic and severe[132] than in the Western world, where *H. pylori* with high-M_r LPS of type-2 (Le^x and Le^y) antigens are more widespread. Various studies dealing with the virulence of *H. pylori* LPS have been undertaken showing multiple LPS-associated immunogenic responses and behaviors.[133] The performance of any study delving into the immunogenic or pathogenic role of *H. pylori* LPSs needs to take into consideration the chemical structure of the LPS employed, since the variability in histo-blood-group expression differs between strains and thus each LPS may yield different results.

H. pylori has already been the source of several functional glycosyltransferases, FucTs and GalTs, with potential commercial uses, especially in enzymatic synthesis and modifications of histo-blood-group molecules for therapeutic uses. However, in the near future, other histo-blood-group-related glycosyltransferases will surely be identified, with those of special interest being the β-1,3-GlcNAcT (polyLacNAc synthesis), α-1,2-GalNAcT (blood-group A synthesis), α-1,3-Gal (linear blood-group B synthesis), and α-2,3-Neu5AcT (sialyl Le^x synthesis). Other *Helicobacter* species, for example *H. mustelae* and *H. felis,* may also be good sources of histo-blood-group glycosyltransferases. The creation of well-characterized LPS isogenic mutants, by disrupting glycosyltransferases,

for utilization in animal studies will yield answers regarding the role of LPS in *H. pylori* pathogenesis and in which host-immunity pathways this disease operates. One of the leading queries regarding *H. pylori* LPS is whether these molecules, or mimics thereof, can be used as therapeutic agents. The synthesis of glycoconjugate vaccines containing *H. pylori* LPS does not seem to be a viable solution due to the fact that this organism is extremely hard to grow *in vitro*. Unless new methodologies in *H. pylori* growth are devised, the realistic quantities needed for mass production of LPS-based vaccines would most likely not be met. Moreover, employment of vaccines containing Lewis or any other blood-group epitopes almost certainly to be expressed by the host, will probably not confer protection. Other regions of the LPS, synthetic homologues, or peptide mimics, however, may prove highly useful in conferring an immune protection when used in a glycoconjugate vaccine, and these regions may also be useful in developing a serologically based *H. pylori* diagnostic.

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