THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 8, Issue of February 22, pp. 5785–5795, 2002

Printed in U.S.A.

Phenotypic Variation in Molecular Mimicry between *Helicobacter pylori* Lipopolysaccharides and Human Gastric Epithelial Cell Surface Glycoforms

ACID-INDUCED PHASE VARIATION IN LEWIS^X AND LEWIS^Y EXPRESSION BY *H. PYLORI* LIPOPOLYSACCHARIDES*

Received for publication, September 6, 2001, and in revised form, November 29, 2001
Published, JBC Papers in Press, December 7, 2001, DOI 10.1074/jbc.M108574200

Anthony P. Moran‡, Yuriy A. Knirel§¶, Sof'ya N. Senchenkova§¶, Göran Widmalm‖, Sean O. Hynes‡, and Per-Erik Jansson§**

From the ‡Department of Microbiology, National University of Ireland, Galway, Ireland, §Karolinska Institute, Clinical Research Centre, Huddinge University Hospital, Novum, S-141 86 Huddinge, Sweden, ¶N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russia, and ‖Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-10691 Stockholm, Sweden

Helicobacter pylori **is an important gastroduodenal pathogen of humans whose survival in the gastric environment below pH 4 is dependent on bacterial production of urease, whereas above pH 4 urease-independent mechanisms are involved in survival, but that remain to be elucidated fully. Previous structural investigations on the lipopolysaccharides (LPSs) of *H. pylori* have shown that the majority of these surface glycolipids express partially fucosylated, glucosylated, or galactosylated *N*-acetyllactosamine (LacNAc) O-polysaccharide chains containing Lewis^x (Le^x) and/or Lewis^y (Le^y), although some strains also express type 1 determinants, Lewis^a, Lewis^b, and H-1 antigen. In this study, we investigated acid-induced changes in the structure and composition of LPS and cellular lipids of the genome-sequenced strain, *H. pylori* 26695. When grown in liquid medium at pH 7, the O-chain consisted of a type 2 LacNAc polysaccharide, which was glycosylated with α-L-fucose at O-3 of the majority of *N*-acetylglucosamine residues forming Le^x units, including chain termination by a Le^x unit. However, growth in liquid medium at pH 5 resulted in production of a more complex O-chain whose backbone of type 2 LacNAc units was partially glycosylated with α-L-fucose, thus forming Le^x, whereas the majority of the nonfucosylated *N*-acetylglucosamine residues were substituted at O-6 by α-D-galactose residues, and the chain was terminated by a Le^y unit. In contrast, detailed chemical analysis of the core and lipid A components of LPS and analysis of cellular lipids did not show significant differences between *H. pylori* 26695 grown at pH 5 and 7. Although putative molecular mechanisms affecting Le^x and Le^y expression have been investigated previously, this is the first report identifying an environmental trigger inducing phase variation of Le^x and Le^y in *H. pylori* that can aid adaptation of the bacterium to its ecological niche.**

The Gram-negative bacterium *Helicobacter pylori* is a prevalent pathogen of humans, and chronic infection of the gastric mucosa by the bacterium causes recurrent gastroduodenal inflammatory disease (1). *H. pylori* is a major cause of chronic gastritis and plays a pivotal role in the development of both gastric and duodenal ulcers (2–4). Moreover, persistent infection with this bacterium is associated with an increased risk for the development of gastric adenocarcinoma and primary lymphoma (5, 6). *H. pylori* is a chronic pathogen, and the mechanisms by which this bacterium is able to persist in the stomach and resist or evade destruction by the immune system is central to its pathogenesis (1, 7).

In part, survival of *H. pylori* in the stomach may be attributed to the development of specialized characteristics, including the capacity to withstand and adapt to exposure to gastric acidity. *H. pylori* colonizes the gastric mucus layer where the pH gradient ranges from pH 2 on the luminal side to almost pH 7 on the epithelial cell surface (8). The helical shape and rapid motility of the bacterium facilitate its movement within viscous mucus, allowing the bacterium to escape extremely low pH (7, 9). Nevertheless, *H. pylori* cells must survive exposure to acidic pH during the early stages of gastric infection before colonization of the gastric mucus. Although the mucus layer provides a partial barrier to the acid contents of the stomach, *H. pylori* may encounter periodic exposure to low pH depending on the location of the bacterium in the gastric mucosa and host gastric physiology (9). In addition, *H. pylori* can alter the normal gastric physiology whereby acute *H. pylori* infection in humans is associated with transient hypochlorhydria, possibly facilitating enhanced intragastric survival early in infection, whereas chronic *H. pylori* infection leads to increased gastric acid secretion (10, 11). Below pH 4 survival of *H. pylori* is dependent on urease (12), an enzyme whose activity is essential for colonization (13), which liberates NH_3 from urea that has been deduced to contribute to neutralization of gastric acidity (7). However, above pH 4 urease-independent mechanisms are involved in survival but remain to be elucidated fully.

Like the outer membrane of other Gram-negative bacteria, that of *H. pylori* contains lipopolysaccharide (LPS)[1] (14). Fresh

* This work was supported by The International Association for the Promotion of Cooperation with Scientists from the New Independent States of the Former Soviet Union Grant 97-0695, Irish Health Research Board (to A. P. M.), Millennium Research Fund (to A. P. M.), Swedish Research Council (to P. E. J. and G. W.), Royal Swedish Academy of Sciences (to P. E. J.), and Karolinska Institute fellowships (to Y. A. K. and S. N. S.). This work was presented in part at the 19th International Carbohydrate Symposium, August 9–14, 1998 in San Diego, CA. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** To whom correspondence should be addressed. Tel.: 46-8-58583821; Fax: 46-8-58583820; E-mail: pererik.jansson@kfc.hs.sll.se.

[1] The abbreviations used are: LPS, lipopolysaccharide; PS, polysaccharide; DQF-COSY, double-quantum filtered correlation spectroscopy; ELISA, enzyme-linked immunosorbent assay; MS, mass spectrometry; ESI MS, electrospray ionization MS; GLC, gas-liquid chromatography; DD-Hep, D-*glycero*-D-*manno*-heptose; LD-Hep, L-*glycero*-D-*manno*-heptose; LA, lipid A; LacNAc, *N*-acetyllactosamine; LD, laser desorption; Le^x, Lewis^x (similarly for other Lewis antigens); OS, oligosaccharide;

clinical isolates of *H. pylori* produce high molecular mass smooth-form LPS, which consists of a polysaccharide (PS) O-chain, a core oligosaccharide (OS), and a lipid moiety, termed lipid A (15, 16). The first detailed structural analysis of *H. pylori* LPS showed that the PS region of the *H. pylori* type strain (NCTC 11637) was composed of an elongated, partially fucosylated *N*-acetyllactosamine (LacNAc) polysaccharide attached to the core OS and terminated at the non-reducing end by mono-, di-, or trimeric Lewis^x (Le^x)[2] (17). Subsequent structural studies (18–25) and serological investigations (26–30) have shown that the O-chains of *H. pylori* strains express partially fucosylated, glucosylated, or galactosylated LacNAc chains of various lengths that may or may not be terminated at the nonreducing end by Le^x and or Le^y type 2 units, in mimicry of normal human cell-surface glycoconjugates and of glycan antigens found in adenocarcinoma tumors (31, 32). In addition, certain *H. pylori* strains express fucosylated LacNAc chains terminated with Le^a and Le^b and blood group A antigenic determinants (22, 25, 33). The pathogenic relevance of Le^x and Le^y mimicry for *H. pylori* remains unclear but has been suggested to aid colonization by camouflaging the bacterium in the gastric mucosa and by aiding bacterial adhesion (14, 34–36), whereas in chronic infection this mimicry may induce antibodies contributing to the development of gastritis and influence the inflammatory response in the gastric mucosa (7, 14, 27, 29, 30).

Cultivation of *H. pylori* on solid agar media *in vitro* can result in a shift to production of low molecular mass rough-form LPS lacking O-chain expression and Le^x/Le^y mimicry (16, 17), but this can be reversed and production of smooth-form LPS stabilized when strains are grown in liquid media (16, 37). In addition to this variation, serological investigations suggest that phase variation (also called antigenic variation) in the type of Lewis antigen expressed on *H. pylori* can occur *in vitro* and *in vivo* (38, 39), but this requires verification by chemical studies because serological investigations of Le expression in *H. pylori* LPS can be misleading (22, 40). Moreover, compared with growth at neutral pH, growth of *H. pylori* at low pH on solid media has been reported to induce changes in colony morphology, cellular lipids, and virulence properties, possibly reflecting changes in bacterial cell wall characteristics in a low pH environment (41).

Despite these observed changes in total cellular lipids, no data are available on the influence of low pH on *H. pylori* LPS chemical structure or whether such conditions could induce phase variation in Le^x and Le^y expression. Because different Lewis antigens are expressed at different sites within the gastric mucosa (31, 42, 43), the ability of the bacterium to vary Le^x and Le^y antigen expression in response to environmental conditions such as pH could aid colonization at the different sites. In this paper, we investigated acid-induced changes in the structure and composition of LPS and cellular lipids. Bacteria were grown at pH 5 and 7 in a liquid medium, rather than on a solid medium, to avoid other changes such as smooth- to rough-form LPS shift, which may occur independently of pH (15, 16). Acid-induced changes in O-chain structure including phase variation in Le^x and Le^y expression were demonstrated independent of lipid A structure and cellular lipid composition. In addition to influencing the type of camouflage employed by *H. pylori*, such changes in the major glycolipid of the outer membrane would have an important influence on the properties of this membrane and would contribute to the adaptation of *H. pylori* to its ecological niche.

EXPERIMENTAL PROCEDURES

Bacterial Strain and Growth Conditions—*H. pylori* strain 26695, whose complete genome sequence has been determined, was originally isolated from a patient with gastritis (40). This strain was grown routinely on blood agar under microaerobic conditions at 37 °C for 48 h as described previously (15, 37). Stock cultures were maintained at −70 °C in trypticase soy broth containing 15% (v/v) glycerol (45). For induction of antigenic variation, *H. pylori* 26695 was grown in brain heart infusion containing 2% (v/v) fetal calf serum supplemented with 50 mM potassium phosphate buffer at pH 7 or in the same medium at pH 5 after adjustment of the pH by the addition of HCl (37, 41). Bacteria were harvested by centrifugation (5000 × *g*, 4 °C, 30 min) and washed twice, and the pellets were lyophilized.

Isolation and Degradation of Lipopolysaccharides—After pretreatment of bacterial biomass with Pronase (Calbiochem), LPSs were extracted by the hot phenol-water technique (15). The water-soluble LPS preparations were purified by treatment with RNase A, DNase II, and proteinase K (Sigma) as described previously (15, 45) and subsequently by gel-permeation chromatography on a column of Bio-Gel P2 (1 m × 1 cm) with water as the eluent (22). Only one carbohydrate-positive fraction (46) was obtained, which eluted in the high molecular mass range, consistent with previous observations (18, 22). These intact LPSs were used for chemical and serological analyses.

The LPSs were degraded with 0.1 M sodium acetate buffer, pH 4.2, at 100 °C for 4 h to cleave the acid-labile ketosidic linkage between the core OS and lipid A. The water-insoluble lipid A was removed by centrifugation (5000 × *g*, 4 °C, 30 min), washed, and lyophilized separately. The supernatant was fractionated by gel-permeation chromatography on a column of Sephadex G-50 (70 × 2.6 cm; Amersham Biosciences, Inc.) using 0.05 M pyridinium acetate, pH 4.5, as eluent and monitored with a Waters differential refractometer. The resultant water-soluble carbohydrate-containing fractions (46) of O-chain and core OS were collected and lyophilized. Preparative defucosylation and dephosphorylation of PS and core OS were achieved by treatment with aqueous 48% hydrofluoric acid (4 °C, 16 h) as described previously (15), neutralization with cold 25% (v/v) ammonia, and desalting by gel-permeation chromatography on a column of Sephadex G-50 (70 × 2.6 cm). Preparation of the lipid A backbone was accomplished by the degradative procedure described previously (45). Briefly, after treatment with 0.1 M HCl (100 °C, 30 min), lipid A was reduced with $NaBH_4$ and subjected to hydrazinolysis (100 °C, 48 h) and *N*-acetylation with acetic anhydride/NaOH (47), and subsequently the product was purified by gel-permeation chromatography on columns of Sephadex G-25 (50 × 2 cm) and TSK-HW40S (24 × 1 cm; Merck) (45).

Electrophoretic and Serological Analyses—For analysis of the macromolecular heterogeneity of *H. pylori* LPS by gel electrophoresis, proteinase K-treated whole-cell lysates were prepared as described (48). These lysates and isolated LPS were analyzed by SDS-PAGE using a stacking gel of 5% (w/v) acrylamide and a separating gel of 15% (w/v) acrylamide containing 3.2 M urea (15). After electrophoresis with a constant current of 35 mA for 1 h, the gels were fixed, and LPS was detected by silver staining (49). Alternatively, the fractionated LPS was electroblotted onto nitrocellulose membranes by using the buffer system of Towbin *et al.* (50). Nitrocellulose membranes with transferred LPSs were probed with mouse IgM monoclonal antibodies against Le antigens anti-Le^x (clone P12), anti-sialyl-Le^x (clone CSLEX1), anti-Le^y (clone F3), anti-Le^a (clone T174), anti-Le^b (clone T128) or H type I antigen (clone 17–206) (Signet Laboratories, Dedham, MA) or against blood group determinants anti-A, -B, or -AB (Immunocor, Norcos, GA) diluted 1:1000 as primary antibody and peroxidase-conjugated goat anti-mouse IgM (Sigma) diluted 1:1000 as the secondary antibody as described previously (29). Reactions in Western blots were visualized with the Bio-Rad premixed enzyme substrate kit (2.5 ml of 4-chloro-1-naphtol in diethylene glycol, 25 ml of Tris-buffered saline, and 15 μl of H_2O_2) according to the manufacturer's instructions.

Also, an enzyme-linked immunosorbent assay (ELISA) with bacterial whole cells was used as described previously (29) to examine the reaction of the anti-Le and blood group antibodies with *H. pylori* 26695 grown at pH 7 and 5. Protein concentrations of bacterial suspensions were determined using a commercial assay (Pierce). Subsequently, flat-bottomed microtiter plates were coated overnight with 100 μl of cell suspensions, with a protein concentration of 60 μg/ml, in 0.05 M $NaHCO_3$ coating buffer, pH 9.6, and blocked with 3% (w/v) bovine

*P*Etn, 2-aminoethyl phosphate; Hep, heptose; OHC_{16}, 3-hydroxyhexadecanoic acid; OHC_{18}, 3-hydroxyoctadecanoic acid.

[2] Lewis^x and Lewis^y were originally defined as positional isomers of Lewis^a and Lewis^b blood group antigens, respectively, but are unrelated to phenotypes of the Lewis blood group system. To indicate their different origins, they will be referred to solely as Lewis^x and Lewis^y.

serum albumin at room temperature for 2 h. The ELISA assay was performed by the procedure of Wirth *et al*. (28). As described previously (29), the specificities of the antibodies in the assay were validated by their ability to bind the respective antigen from a panel of synthetic Le and blood group antigens (Isosep AB, Tullinge, Sweden and Dextra Laboratories, Reading, UK) and the LPSs of other *H. pylori* strains, NCTC 11637, P466, and MO19, of known structure (17–19). The criterion that an absorbance value of <0.1 units was considered a negative result, whereas higher values were considered positive (28), was used in these whole-cell ELISA studies. All assays were repeated in triplicate.

Preparation, Identification, and Fractionation of Cellular Lipids—Lipids were extracted from bacterial biomass using the method of Bligh and Dyer (51). Thin-layer chromatography (TLC) of total lipids was performed on silica gel 60 plates (Merck) using chloroform/methanol/water (75:22:3, by volume) as the solvent system. Detection was by charring with 1% (w/v) $CeSO_4$ in 10% (v/v) H_2SO_4 or by staining with the respective reagents: molybdate stain for phospholipids, ninhydrin for amino lipids, α-naphthol stain for glycolipids, and sulfuric acid/acetic acid reagent for sterols and sterol esters (52). The lipids L-α-phosphatidylethanolamine, L-α-lysophosphatidylethanolamine, L-α-phosphatidyl-DL-glycerol, cardiolipin, phosphatidylcholine, and L-α-phosphatidyl-L-serine were obtained from Sigma and used as standards on TLC plates. The relative abundance of lipids was determined by scanning laser densitometry after charring of TLC plates (53).

Total lipid extracts were subjected to lipid anion-exchange chromatography on 1 ml (100 mg) Superclean LC-NH_2 solid phase extraction columns (Supelco, Bellefonte, PA) as described previously (41, 54). Fractions were sequentially eluted with the solvents: chloroform/2-propanol (2:1, v/v), diethyl ether/acetic acid (98:2, v/v), acetonitrile/2-propanol (2:1, v/v), methanol, and 2-propanol/3 M methanolic HCl (4:1, v/v) to yield five fractions, A to E (41). The two predominant phospholipids, phosphatidylethanolamine and lysophosphatidylethanolamine, were purified from fraction D on silica gel 60 (40–60 μm, Merck) using chloroform/methanol/water (75:22:3, by volume) as the eluent, and the appropriate fractions were collected and dried under N_2. The identities of lysophosphatidylethanolamine and phosphatidylethanolamine were confirmed after acidic liberation of fatty acids (4 M HCl, 100 °C, 4 h) and subsequent analysis by TLC (41) and by comparison with authentic lysophosphatidylethanolamine and phosphatidylethanolamine in ^{31}P NMR spectroscopy (55).

Sugar Composition and Methylation Linkage Analyses—Sugar composition analysis of LPSs was performed by the alditol acetate method (56). Hydrolysis of glycosidic bonds was achieved with 2 M trifluoroacetic acid at 120 °C for 2 h and followed by reduction with $NaBD_4$, and acetylation with acetic anhydride (100 °C, 30 min). Alditol acetate derivatives were identified by gas-liquid chromatography (GLC) using a Hewlett-Packard 5880 chromatograph (Avondale, PA) equipped with a DB-5 fused-silica capillary column (30 m × 0.25 mm) and a temperature program of 160 °C (1 min) to 260 °C at 3 °C/min and by GLC-mass spectrometry (MS) on a Hewlett-Packard 5890 chromatograph equipped with a NERMAG R10–10L mass spectrometer using the same conditions. Enantiomeric configurations of the respective sugars were determined by GLC analysis of the acetylated 2-(+)-butyl glycosides (for GlcN) and 2-(+)-octyl glycosides (for Gal and Fuc) by the published methods (57, 58), which were modified as described (59). Linkage analysis was performed by methylation with CH_3I in Me_2SO in the presence of sodium methylsulfinylmethanide (60), and hydrolysis was performed as in sugar analysis. The partially methylated monosaccharides were reduced with $NaBD_4$ and subsequently converted to alditol acetates. Characterization of the permethylated alditol acetates was performed by GLC-MS using the above conditions, and identification was performed using published data (61, 62).

Analysis of Fatty Acids—Total fatty acids were liberated from LPS and lipid A preparations by combined acid (4 M HCl, 100 °C, 5 h)- and base (0.5 M NaOH, 100 °C, 1 h)-catalyzed hydrolyses and with heptadecanoic acid (C17.0) as an internal standard and were carboxymethylated with diazomethane (15, 47). Ester-bound fatty acids were selectively liberated from vacuum-dried lipid A by alkaline transesterification with sodium methylate (0.25 M, 37 °C, 15 h) as described previously (63). Amide-bound acyloxyacyl residues were investigated according to the procedure of Wollenweber *et al*. (64). Purified phospholipids were subjected to acid- and base-catalyzed hydrolysis (15), and after acidification, free acids were extracted with hexane and subsequently carboxymethylated with diazomethane. The resulting fatty acid methyl esters from these procedures were determined quantitatively by GLC on an HP-5 fused-silica capillary column (Hewlett-Packard) using a temperature program of 150 °C (3 min) to 300 °C at 3 °C/min, and their identities were confirmed by GLC-MS.

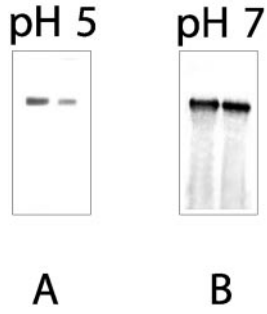


FIG. 1. **Silver-stained SDS-PAGE gels of LPSs of *H. pylori* 26695 grown at pH 5 and 7.** *A*, purified LPSs of *H. pylori* 26695 grown at pH 5 (LPS1) and pH 7 (LPS2). *B*, proteinase K-treated whole-cell lysates from biomass grown at pH 5 and 7. Purified LPSs, LPS1 and LPS2, were obtained by hot phenol-water extraction of biomass grown at pH 5 and 7, respectively, and purified by enzymatic treatments with RNase A, DNase II, and proteinase K and subsequent gel-permeation chromatography, whereas mini-extracts of LPSs were prepared by lysing whole bacterial cells and subsequent treatment with proteinase K as described under "Experimental Procedures." SDS-PAGE was performed using a stacking gel of 5% (w/v) acrylamide and a separating gel of 15% (w/v) acrylamide containing 3.2 M urea under a constant current of 35 mA for 1 h, after which the gels were fixed and silver-stained (49). The silver staining procedure for LPS differs to that for conventional protein staining in the oxidation and development conditions used (48, 49), and under these conditions silver has been shown to specifically bind LPS because of the high affinity ligands present in periodate-oxidized LPS for silver compared with proteins (48). Identical electrophoretic profiles of purified LPS and proteinase K-treated bacterial extracts at the respective pH values were observed.

NMR Spectroscopy—Samples were exchanged twice with D_2O. ^1H NMR spectra of D_2O solutions were run on a JEOL EX-270 instrument at 75 °C or on a Varian Inova 600 instrument at 25 °C. Two-dimensional $^1H,^1H$ DQF-COSY and $^1H,^{13}C$ heteronuclear single-quantum coherence experiments were performed on the Inova 600 instrument. Chemical shifts are reported in ppm using internal sodium 3-trimethylsilylpropanoate-d_4 (δ_H 0.00) or internal dioxane (δ_C 67.40). For lipid analysis, ^{31}P NMR spectra were recorded on a Varian 500 Unity instrument at 35 °C, spectra were broad-band ^1H-decoupled, and samples were referenced to an 80% (w/v) solution of phosphoric acid (0.00 ppm) as an external standard (45, 55).

Electrospray ionization (ESI) and Laser Desorption (LD) Mass Spectrometry—ESI MS was performed in the negative mode using a VG Quattro triple quadrupole mass spectrometer (Micromass, Altrincham, Cheshire, UK) with acetonitrile as the mobile phase at a flow rate of 10 μl/min; the samples were dissolved in aqueous 50% acetonitrile at a concentration of ~50 pmol/μl, and 10 μl was injected via a syringe pump into the electrospray source. LD MS was carried out with a laser microprobe mass analyzer (LAMMA 500, Leybold AG, Cologne, Germany). Free dephosphorylated lipid A was mixed with either NaI or CsI to obtain fragments as well as molecular ions in the positive ion mode as described (45, 65).

RESULTS

SDS-PAGE and Serological Analyses—When analyzed in SDS-PAGE with silver staining (Fig. 1), isolated LPSs from *H. pylori* 26695 biomass grown at pH 5 and 7, LPS1 and LPS2, respectively, showed profiles characteristic of slow migrating, high molecular mass LPS with PS O-chains as reported previously (15, 16). Like the purified preparations of LPS1 and LPS2, proteinase K-treated whole-cell lysates of biomass grown under the differing pH conditions gave identical electrophoretic profiles, indicating that the LPS extraction and purification procedure did not affect the macromolecular nature of the preparations. Immunoblotting experiments showed that the O-PS region of LPS1 expressed both Le^x and Le^y, whereas LPS2 expressed only Le^x (Fig. 2). No reaction of these LPSs with monoclonal antibodies against other Le antigens (anti-Le^a, -Le^b, or -H type 1) or against blood group determinants (anti-A, -B, or -AB) was observed. In the case of chemical modification or selection of a particular LPS molecular species

FIG. 2. **Immunoblots of LPSs from *H. pylori* 26695 grown at pH 5 (LPS1) and pH 7 (LPS2) with anti-Le^x and anti-Le^y monoclonal antibodies.** *Lane A*, pH 5; *lane B*, pH 7. LPS1 and LPS 2 were prepared as described in Fig. 1 and under "Experimental Procedures." SDS-PAGE was performed using a stacking gel of 5% (w/v) acrylamide and a separating gel of 15% (w/v) acrylamide containing 3.2 M urea under a constant current of 35 mA for 1 h. The fractionated LPSs were electroblotted onto nitrocellulose membranes (50), and the transferred LPSs were probed with anti-Le^x and anti-Le^y mouse IgM monoclonal antibodies diluted 1:1000 as primary antibody and peroxidase-conjugated goat anti-mouse IgM diluted 1:1000 as the secondary antibody as described under "Experimental Procedures." Positive reactions can be seen between LPS1 and anti-Le^x and -Le^y monoconal antibodies, whereas only anti-Le^x monoclonal antibody reacted with LPS2.

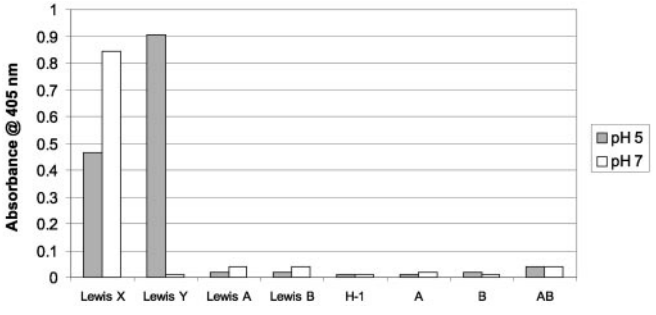


FIG. 3. **Whole-cell ELISA of *H. pylori* 26695 grown at pH 5 (LPS1) and pH 7 (LPS2) with anti-Le^x, anti-Le^y, anti-Le antigen, and anti-blood group monoclonal antibodies.** The ELISA with bacterial whole cells was used as described previously (28,29). These data indicate that *H. pylori* 26695, when grown at pH 5 expresses Le^x and Le^y antigens, whereas at pH 7, only Le^x antigen is expressed. Only absorbance values >0.1 units were considered positive (28).

during extraction, the same panel of antibodies was tested for reaction in an ELISA with whole cells of *H. pylori* grown at pH 5 and 7, respectively (Fig. 3). Using the criteria previously established for the ELISA (28), the observed expression of Le^x and Le^y at pH 5 and only Le^x at pH 7 was consistent with the LPS1 and LPS2 immunoblotting results, respectively.

Structural Analyses of the LPS Core—Sugar analysis of the core OS derived by mild degradation at pH 4.2 of *H. pylori* LPS1 (OS1) showed the presence of fucose, glucose, galactose, 2-amino-2-deoxy-D-glucose, D-*glycero*-D-*manno*-heptose (DD-Hep), and L-*glycero*-D-*manno*-heptose (LD-Hep) in the molar ratios 0.65:1.3:1.0:0.55:1.1:0.65, respectively, together with a small amount of ribose. After dephosphorylation with aqueous 48% hydrofluoric acid the content of LD-Hep increased to almost twice its value, and hence, a proportion of these residues was phosphorylated.

Methylation (Table I), ESI MS, and ^{1}H NMR spectroscopic studies of OS1 before and after dephosphorylation indicated the same basal structure **1** (see Structures 1 and 2) as has been established for the core region of *H. pylori* NCTC 11637 LPS (18), except that a 2-aminoethyl phosphate (*P*Etn) group was identified in contrast to a phosphate monoester group that has been reported earlier (18–20). Thus, ESI MS (Fig. 4) revealed a doubly charged pseudomolecular ion, $[M\text{-}2H]^{2-}$, at *m/z* 891.80 for **1** with a calculated molecular mass of 1785.53 (in contrast, a peak at *m/z* 870.2 would be observed for the phosphate monoester derivative that would have a calculated molecular mass of 1742.46 Da). The 3-deoxy-D-*manno*-octulosonic acid (Kdo) residue at the reducing end of **1** and the other core OS was found to exist in an anhydroform (anhKdo).

Heterogeneity of OS1 was revealed by ESI MS (Fig. 4) and found to be associated with the lack of one or two hexose residues (peaks of doubly charged ions, $[M\text{-}2H]^{2-}$, at *m/z* 810.53 and 730.01, respectively) and attachment of up to three additional hexose residues (*m/z* 972.7, 1053.91, and 1134.55) or two hexose and one pentose (ribose) residue (*m/z* 1120.01). The major compound in the mixture corresponded to **1** with one additional hexose residue attached. As indicated by the methylation analysis data (Table I), this and other additional monosaccharides may substitute the terminal Glc residue at position 3 or 6 or position 2 of the 7-substituted DD-Hep residue in **1** (see Refs. 18–20). The exact sites of their attachment, as well as their sequence and anomeric configurations remain to be determined.

The disaccharide fragment α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAc present in OS1 is also a constituent of the PS O-chain (see Structures 3 and 4). In the methylation analysis of OS1, the Fuc residue appeared as 3-substituted (Table I), whereas this residue is terminal, as followed from other data for OS1, including ESI MS data (Fig. 4). This phenomenon has been observed previously (18) and has been suggested to originate from incomplete methylation.

Similar analyses of OS2, the core OS derived from *H. pylori* LPS2, showed essentially the same structure but with a lower degree of chain elongation such that the contribution of compounds with more than one additional hexose residue was negligible. The major compound in the mixture was the Glc-lacking oligosaccharide **2** (see Structures 1 and 2). Therefore, except for the degree of glucosylation, there is no significant difference between the core regions of the two LPSs.

Elucidation of the Structure of the PS O-Chain from H. pylori LPS1 (PS1)—Sugar analysis of PS1, including determination of the absolute configurations of the monosaccharides, revealed L-fucose, D-galactose, and 2-amino-2-deoxy-D-glucose in the molar ratios 0.4:1.4:1.0 as the main components. In addition, ribose, glucose, DD-Hep, and LD-Hep were identified as core constituents of *H. pylori* LPS as described above.

Methylation analysis of PS1 (Table I) resulted in identification of partially methylated alditol acetates derived from the major components: terminal Fuc, terminal and 3-substituted Gal, 4-substituted GlcNAc, and 3,4- and 4,6-disubstituted GlcNAc. These data suggested that PS1 is branched with two different lateral sugar residues (Gal and Fuc) and two different GlcNAc residues as branch point residues. Comparison with the methylation analysis data for LPS1 (Table I) showed that no terminal fucose was cleaved during mild acid degradation. In addition, a number of minor partially methylated alditol acetates were detected. Most of them were derived from the core region of LPS, as determined by comparison with the methylation analysis data of OS1 (Table I). However, two minor products originated from PS1. One was derived from 3,4,6-trisubstituted GlcNAc and showed that a minor portion of GlcNAc residues carried two side chains. The other was from 2-substituted Gal, which is located in the terminal nonreducing LacNAc unit of PS1 (see Structure 3).

In the ^{1}H NMR spectrum of the PS1, a signal from *P*Etn was observed at δ 3.28 (t, *J* 5.0 Hz, CH_2N) with a cross-peak to δ 4.13 (CH_2OP) in the two-dimensional ^{1}H,^{1}H DQF-COSY spectrum. In the two-dimensional ^{1}H,^{13}C heteronuclear single-quantum coherence spectrum of the PS1, a correlation was

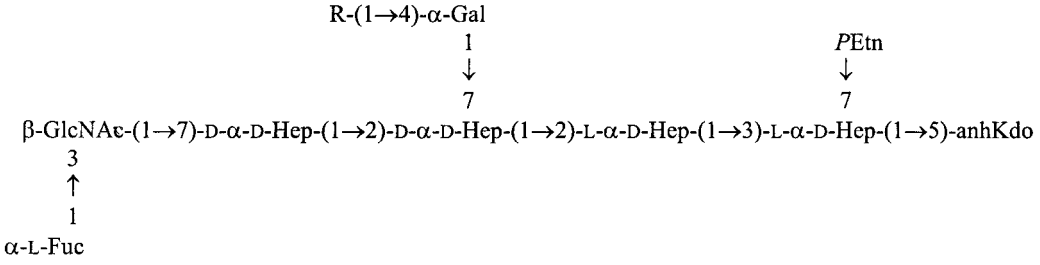
TABLE I

Methylation analyses of sugar linkages in core oligosaccharide from H. pylori 26695 LPS1 (OS1), O-chain polysaccharides from LPS1 and LPS2 (PS1 and PS2, respectively), and modified PS1 after treatment with aqueous 48% hydrofluoric acid (PS1M)

The GLC retention times of the corresponding alditol acetates are referred to 2,3,4-tri-*O*-methylfucose (2,3,4-Me_3-Fuc, 1.00) and glucitol hexaacetate (3.39).

Sugar	Relative retention time	Detector response				
		OS1	PS1	LPS1	PS1M	PS2
				%		
2,3,4-Me_3-Fuc[a]	1.00	<1	5	3	<1	8
3,4-Me_2-Rib	1.02	<1	4	3	<1	5
2,4-Me_2-Fuc	1.34	11	<1	4		2
2,3,4,6-Me_4-Glc	1.41	11	<1	4		2
2,3,4,6-Me_4-Gal	1.51	3	14	11	27	3
2,4,6-Me_3-Glc	1.86	7	2	4		5
2,3,6-Me_3-Gal	1.89	13	4	7		6
2,3,6-Me_3-Glc	1.92	2	<1	<1		
2,4,6-Me_3-Gal	1.97	4	31[b]	23[b]	45	35
3,4,6-Me_3-Gal	1.97		4[b]	3[b]		
2,3,4-Me_3-Glc	2.20	5	<1	<1		
2,3,4,6,7-Me_5-DD-Hep	2.13	<1		1		
3,4,6,7-Me_4-DD-Hep	2.65	4	<1	2		2
3,4,6,7-Me_4-LD-Hep	2.84	6	<1	2		2
2,3,4,6-Me_4-DD-Hep	2.92	12	3	5		3
2,3,4,6-Me_4-LD-Hep	3.16	<1				
3,4,6-Me_3-DD-Hep	3.46	10		3		2
2,3,6-Me_3-GlcN	3.60	<1	7	5	14	17
2,4,6-Me_3-GlcN	3.89	8	<1	4		2
2,3,4-Me_3-GlcN[c]	3.96			4		
2,6-Me_2-GlcN	4.14	<1	6	3		8
2,3-Me_2-GlcN	4.45	<1	10	6	1	
2-Me-GlcN	4.95		1	<1		

[a] The value is low owing to a putative loss of the volatile derivative.
[b] The values are taken from mass fragmentographic analysis from a ~1:10 nonseparable mixture of 3,4,6-Me_3Gal and 2,4,6-Me_3Gal.
[c] From lipid A of LPS.



1 R = α-Glc; **2** R = H

STRUCTURES 1 AND 2

observed from a ^{1}H NMR signal at δ 3.28 to a ^{13}C NMR signal at δ 41.9, further supporting the presence of a *P*Etn group.

Treatment of PS1 with aqueous 48% hydrofluoric acid resulted in a modified polysaccharide (PS1M) that eluted from Sephadex G-50 with a similar elution volume as PS1 soon after the void volume of the column. Sugar analysis of PS1M revealed Gal and GlcNAc in the same ratio as in PS1 but showed only a trace amount of Fuc, indicating essentially complete defucosylation of the polysaccharide. The content of the core OS constituents was negligible as well, thus demonstrating cleavage between the PS and the core region of LPS. In fact, two core-related oligosaccharides were isolated after the reaction, one eluted with the same elution volume as the OS1, *i.e.* approximately twice the void volume, and the other just after.

Methylation analysis of PS1M (Table I) yielded terminal and 3-substituted Gal and 4- and 4,6-disubstituted GlcNAc in the ratios 0.58:1.00:0.30:0.23, respectively. Therefore, in PS1M the lateral Gal residue is attached at position 6 of GlcNAc, and hence, in PS1 the fucose residue substitutes GlcNAc at position 3. A minority of the GlcNAc residues carries both fucose and galactose, which is deduced from the presence of 3,4,6-trisubstituted GlcNAc in the methylation analysis of PS1 (Table I). As judged by the ratios of methylated derivatives from 4-substituted, 3,4-disubstituted, 4,6-disubstituted, and 3,4,6-trisubstituted GlcNAc, 29% of the LacNAc units in PS1 are not substituted (structural unit **3B**), 25% are fucosylated (structural unit **3A** and **3C**), 42% are galactosylated (structural unit **3D**), and 4% carry both lateral Gal and Fuc (structural unit **3E**).

The ^{1}H NMR spectrum of PS1M contained, among other things, signals for three anomeric protons at δ 4.47 (β-Gal H1, split to two close doublets, $J_{1,2}$ 7.5 Hz for each), 4.74 (β-GlcNAc H1, $J_{1,2}$ ~8 Hz), and 5.02 (α-Gal H1, $J_{1,2}$ 2.5 Hz; the coupling, lower than expected, is probably due to second order effects). The ratios of the intensities of the anomeric signals were 1:1:0.45, respectively, which fitted well with the methylation analysis data. Therefore, the ^{1}H NMR data confirmed the presence of a poly(β-LacNAc) chain and showed that the lateral Gal residue is α-linked.

The ^{1}H NMR spectrum of PS1 indicated a higher degree of structural heterogeneity due to nonstoichiometric substitution with two lateral monosaccharides, Gal and Fuc. The major signals for anomeric protons belonged to α-Gal and α-Fuc (both at δ 5.04, $J_{1,2}$ ~3 Hz), β-GlcNAc (δ 4.69, $J_{1,2}$ 8.6 Hz), and β-Gal (δ 4.46, $J_{1,2}$ 7.9 Hz), the assignment based on two-dimensional ^{1}H,^{1}H DQF-COSY and ^{1}H,^{13}C heteronuclear single-quantum

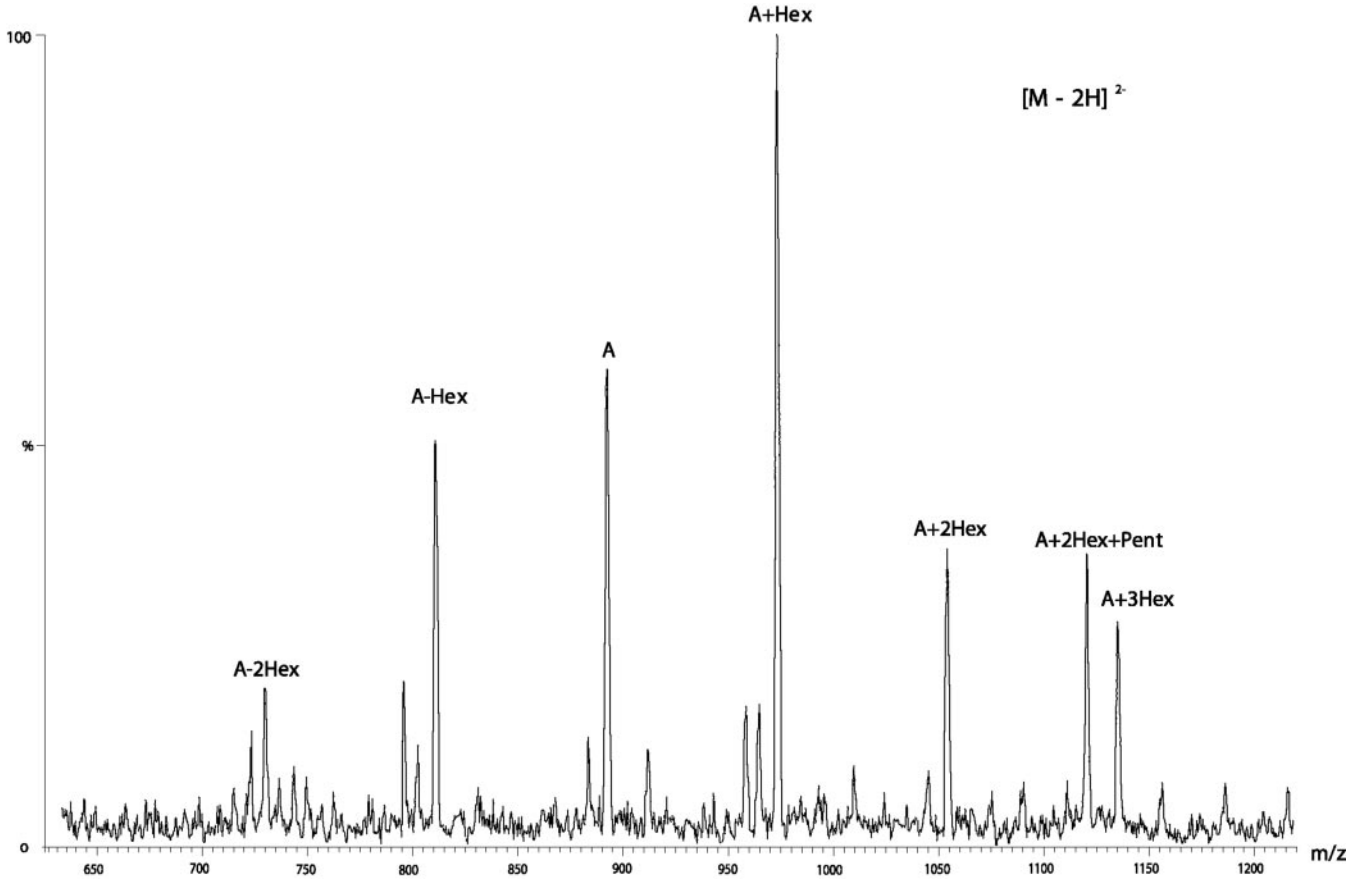
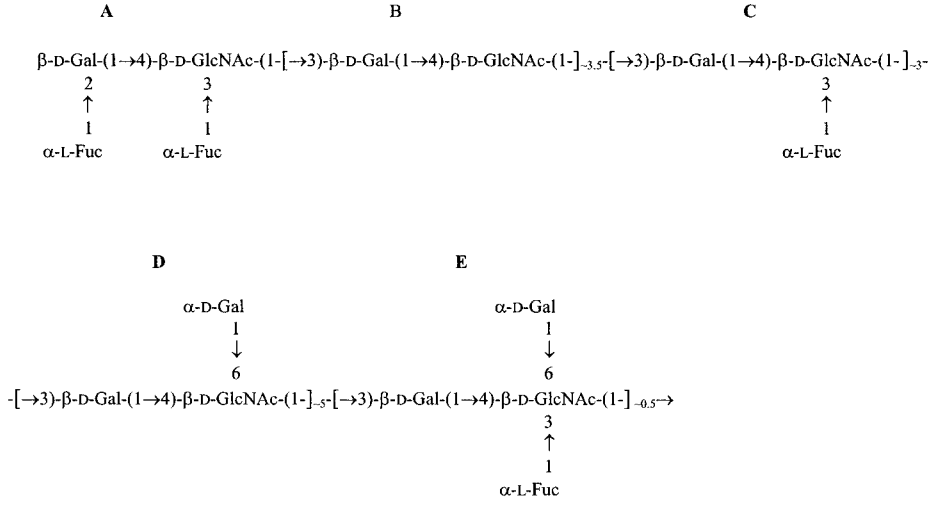


FIG. 4. **The region of doubly charged pseudomolecular ions of the ESI MS spectrum of the core OS from LPS1 (OS1).** Structure **1** corresponds to *A* in the spectrum at *m/z* 972.7. The major compound in the mixture thus corresponds to **1** with one additional hexose residue (*A+Hex*). Heterogeneity was found to be associated with the lack of one or two hexose residues (*A-Hex* and *A-2Hex*, *m/z* 810.53 and 730.01, respectively) and attachment of up to three additional hexose residues (*m/z* 1053.91, and 1134.55) or two hexose and one pentose (ribose) residue (*m/z* 1120.01).

STRUCTURE 3



coherence[3] experiments. Also, the chemical shifts of the C5 signals of the Fuc groups were observed at $\delta \sim 67.5$ ppm, corroborating the α-anomeric configuration. Furthermore, the ^{1}H NMR spectrum of PS1 contained signals for *N*-acetyl groups of GlcNAc (δ 2.02) and CH_3-C groups (H6) of Fuc (δ 1.14–1.26) with the ratio of intensities 1:0.35. In total, there were H6 signals for five Fuc residues, two of which appeared as separate doublets at δ 1.23 and 1.26 and three others as three superposed doublets at δ 1.14 ($J_{5,6} \sim 6$ Hz for all H6 signals, Fig. 5*A*). The two-dimensional DQF-COSY spectrum (Fig. 6) showed Fuc H6/H5 cross-peaks at δ 1.14/4.35, 1.14/4.81, 1.23/4.87, and 1.26/4.25. The first cross-peak was from the Fuc residue, which is adjacent to the LPS core region, as deduced from the DQF-COSY spectrum of OS1, where only one Fuc H6/H5 cross-peak was observed, and at the same coordinates (compare also the one-dimensional ^{1}H NMR data, Fig. 5, *B* and *C*). The cross-peak at δ 1.26/4.25 was assigned to the Fuc group substituting

[3] The assignment of the anomeric protons in *N*-acetyl-β-lactosaminoglycan was reported erroneously interchanged (17–19).

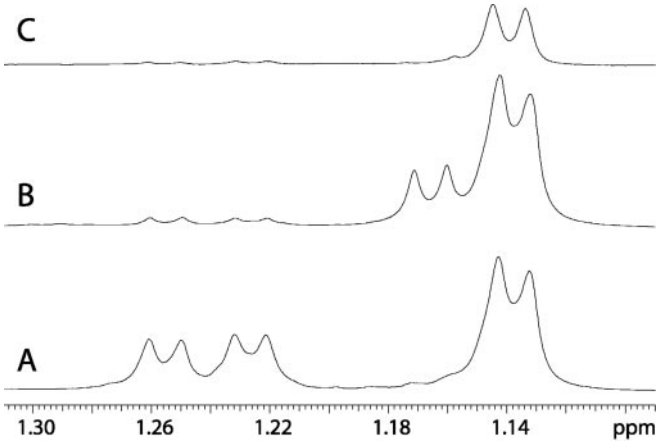


FIG. 5. **Part of ^{1}H NMR spectra of oligosaccharides and polysaccharides derived from *H. pylori* 26695 showing the H-6 resonance region of the fucose residues.** *A*, O-chain polysaccharide from LPS1 (PS1). *B*, O-chain polysaccharide from LPS2 (PS2). *C*, core oligosaccharide from LPS1 (OS1). Assignments are as follows. The peak at δ 1.26 was assigned to the Fuc group substituting position 2 of β-Gal in the terminal nonreducing LacNAc unit. The peak at δ 1.23 originated from the Fuc residue of the Fuc-(1→3)-GlcNAc unit in a terminal Le^y tetrasaccharide. The peak at δ 1.17 was from the Fuc residue of the Fuc-(1→3)-GlcNAc unit in a terminal Le^x trisaccharide. The signals at δ 1.14 originate from Fuc in LacNAc units of the main chain and Fuc adjacent to the LPS core region.

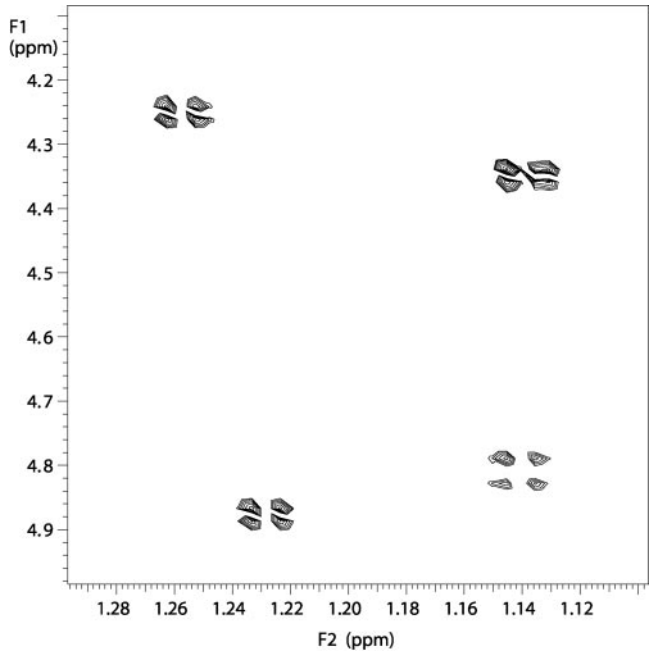


FIG. 6. **Region for fucose H-5/H-6 correlation in the two-dimensional DQF-COSY spectrum of the O-chain polysaccharide from LPS1 (PS1).** The assignments of signals are described in Fig. 5 and in the text.

position 2 of β-Gal in the terminal nonreducing LacNAc unit (compare *e.g.* with δ 4.22 for Fuc H-5 in 2′-(α-fucopyranosyl)lactose (66)). The cross-peak at δ 1.23/4.87, having the same intensity, originated from the Fuc residue of the Fuc-(1→3)-GlcNAc unit in a terminal Le^y tetrasaccharide (24). The last cross-peak at δ 1.14/4.81 of double intensity was from the Fuc group of the same Fuc-(1→3)-GlcNAc fragment in two of the interior repeating units of PS1. The latter assignment was consistent with the chemical shift (δ 4.83) of the signal for Fuc H5 in a human milk oligosaccharide (LNF-III), which has a similar fragment (67).

Therefore, two Fuc residues are present at the nonreducing LacNAc unit of PS1, where they form a Le^y unit (structural unit **3A**), two more are attached to two interior LacNAc units (structural units **3C** and **3E**), and the fifth Fuc residue is attached to the unit that is adjacent to the LPS core. The structure of PS1 may be described by formula **3,** in which the exact distribution of various interior structural units B-E along the PS chain is unknown. Based on the ratios of the GlcNAc derivatives revealed in methylation analysis (Table I) and on the Fuc and GlcNAc methyl group signal intensities in the ^{1}H NMR spectrum, the average degree of polymerization in PS1 was estimated as 12–13 LacNAc units.

Elucidation of the Structure of the PS O-chain from H. pylori LPS2 (PS2)—Sugar analysis showed that the content of Gal in PS2 was significantly lower than in PS1 (the ratios of Fuc:Gal:GlcN were 0.35:0.95:1). Methylation analysis revealed the absence of 4,6-disubstituted GlcNAc and the presence of only a small amount of terminal Gal, whereas the content of the other PS constituents (terminal Fuc, 3-substituted Gal, 4-substituted GlcNAc, and 3,4-disubstituted GlcNAc) indicated a close similarity between PS2 and PS1 (Table I). As deduced from the ratio of methylated GlcNAc derivatives, the degree of fucosylation in PS2 was 30%. No 2-substituted Gal was detected, but instead, terminal Gal was present (Table I), and therefore, unlike PS1, PS2 has no Le^y antigenic determinant.

The ^{1}H NMR spectrum of PS2 was similar to that of PS1 but lacked the H1 signal for α-Gal. Also, the signals for H6 of Fuc displayed a different pattern; there was a doublet at δ 1.17 and superposition of three doublets at δ ~ 1.14 ($J_{5,6}$ ~6 Hz for all H6 signals, Fig. 5*B*). As expected, signals at δ 1.23 and 1.26, which belonged to the Fuc residues from the terminal Le^y tetrasaccharide in PS1 (Fig. 6), were absent from the spectrum of PS2. The ratio of the integral intensities of the signals for methyl groups of Fuc (δ 1.14–1.17) and GlcNAc (δ 2.03) was 0.32:1, again indicating incomplete fucosylation of LacNAc units.

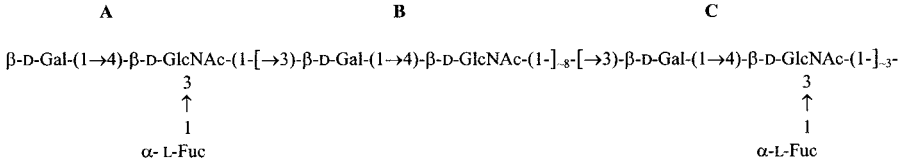
The two-dimensional DQF-COSY spectrum of PS2 showed, among other things, Fuc H6/H5 cross-peaks at δ 1.14/4.33, 1.14/4.81, and 1.17/4.83. The two first cross-peaks were also present in the spectrum of PS1 (Fig. 6) and belonged to Fuc residues attached to the core region and to the interior LacNAc units, respectively (see Structure 3). The last cross-peak was absent from the spectrum of PS1 and was assigned to the Fuc residue located at the terminal nonreducing LacNAc unit of PS2, where it forms an Le^x unit (24). As in PS1, there are only two Fuc residues attached to interior LacNAc units, and the average degree of polymerization of PS2 was estimated as 12 LacNAc units.

These data suggest that PS2 has structure **4,** which contains terminal fucosylated LacNAc (structural unit **4A**) and both non-fucosylated (structural unit **4B**) and fucosylated LacNAc (structural unit **4C**). This structure differs from structure **3** of PS1 only in the absence of substitution by lateral α-Gal residues and the second α-Fuc residue from the nonreducing LacNAc unit, producing a terminal Le^x trisaccharide (structural unit **4A**) rather than Le^y tetrasaccharide.

Structural Analysis of Lipid A—Compositional analysis of free lipid A preparations from LPS1 and LPS2 (LA1 and LA2) revealed, beside fatty acids, a similar composition of GlcN and phosphate in the molar ratio 2:1.4, with trace amounts of ethanolamine. The fatty acids present in both lipid A preparations were dodecanoic (C_{12}), tetradecanoic (C_{14}), hexadecanoic (C_{16}), octadecanoic (C_{18}), 3-hydroxyhexadecanoic (OHC_{16}), and 3-hydroxyoctadecanoic (OHC_{18}) acids in the approximate molar ratios 0.5:0.4:0.1:1.0:1.3:2.0, respectively.

Treatment of these lipid A preparations with 0.1 M HCl (100 °C, 30 min) liberated glycosidic phosphate (1 eq), and subsequent reduction with $NaBH_4$ yielded products containing

STRUCTURE 4



D-GlcN and D-glucosaminitol (1.0:0.9) with residual phosphate content (0.4 eq), attributable to ester-bound phosphate as reported previously (45). Subjecting these products to the chemical degradation pathway for lipid A backbone analysis developed earlier (45, 47) yielded *N*-acetylated disaccharides of GlcNAcβ1–6GlcNAc-ol with identical properties in high pressure liquid chromatography, identical ^{1}H NMR spectra, and after permethylation, identical GLC-MS mass spectra as authentic standards (47). Analysis by ^{31}P NMR spectroscopy of both LA1 and LA2 revealed a signal (2.96 ppm) for a glycosidic phosphomonoester and a second for a phosphodiester (1.32 ppm), which were attributed to glycosidic phosphate and ethanolamine-phosphate groups, respectively, since acidic treatment (0.1 M HCl, 100 °C, 30 min) led to their liberation and loss of both signals. A further but weaker signal corresponding to an ester-bound phosphomonoester (4.52 ppm), which was acid-stable, was attributed to a 4′-phosphate group (45). Collectively, these data showed that the backbones of LA1 and LA2 were identical 1,4′-bisphosphorylated β(1,6)-linked GlcN disaccharides.

To study the distribution of fatty acids on the lipid A backbone, dephosphorylated LA1 was subjected to LD MS after cationization by a NaI admixture and analyzed in the positive-ion mode (Table II). In addition, analysis of dephosphorylated LA1 by LD MS after admixture of CsI gave a similar fragmentation pattern and showed the presence of nonstoichiometric amounts of amide-bound $C_{16}OC_{18}$ on the nonreducing GlcN unit of the lipid A backbone (data not shown). Collectively, the data showed that the reducing GlcN of the backbone carries ester-bound 3-hydroxyoctadecanoic and ester-linked (OHC_{16}), whereas the nonreducing GlcN can carry amide-bound $C_{18}OC_{18}$ and ester-bound $C_{12}OC_{16}$ or $C_{14}OC_{16}$ (Fig. 7), identical to the fatty acid distribution reported previously in *H. pylori* NCTC 11637 (45). However, heterogeneity in the acylation pattern in lipid A from smooth-form LPS of *H. pylori* has been observed whereby tetraacyl lipid A predominates, but hexaacyl lipid A is also present (45, 68). Therefore, to resolve this issue, dephosphorylated LA1 was subjected to silica gel chromatography (47), and one major and a second minor fraction were obtained. Analysis by LD MS of these fractions showed the predominance of tetraacyl lipid A (without ester-bound fatty acids bound to the nonreducing GlcN unit), whereas analysis of the minor fraction showed the hexaacyl distribution (Table II). Identical data were obtained for LA2. Collectively, these results show no comparative difference in the structure of LA1 and LA2.

Composition of Cellular Lipids—The content of total lipids in *H. pylori* 26695 grown at pH 5 and 7 was similar (7.2 and 7.4% w/w, respectively). The neutral phospholipid fraction from both contained phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine at 91.6, 8.3, ~0.05, and ~0.05% (w/w), respectively. In contrast to a previous report (41), there was no elevation in phosphatidylserine content when this *H. pylori* strain was grown at pH 5. Furthermore, the fatty acid composition of phosphatidylethanolamine of *H. pylori* 26695 grown at pH 7 was C_{14} (29.5%), C_{15} (1.7%), C_{16} (3.6%), C_{17} (0.3%), C_{18} (4.6%), $C_{18:1}$ (4.6%), $C_{18:2}$ (0.2%), C_{19} (1.1%), C_{19cyc} (53.4%) but did not differ significantly from that of growth at pH 5.

TABLE II

Assignment of peaks in the LD mass spectra of dephosphorylated lipid A derived from H. pylori 26695 LPS1 (LA1)

The relevant cleavage process and the resultant structure are shown in Fig. 7. M indicates the quasimolecular ion derived from the acylated GlcN disaccharide; M_L and M_H indicate the fragment ions derived from the acylated reducing and nonreducing GlcN units of the lipid A backbone.

m/z	Cleavage process	Structure
1914		$[M - C_{12} + C_I + Na]^+$
1886		$[M + Na]$
1858		$[M - C_{18} + C_I + Na]^+$
1704	A	$[M - C_{12} + H + Na]^+$
1614	B	$[M - OHC_{16} + H + Na]^+$
1449[a]	C	$[M - C_{12} - OHC_{16} + H_2O + Na]^+$
1432	D	$[M - C_{12} - OHC_{16} + H_2O + Na]^+$
1230	G	$[M_N + 42 + N\ 1]^+$
1196[a]	C, E	$[M - OHC_{16} + H - C_{12} - OHC_{16} + H + Na]^+$
1188		$[M_H + Na]^+$
1183[a]	C, F	$[M - C_{18} + H - C_{12} - OHC_{16} + H + Na]^+$
1108	A, H	$[M_H + 102 - C_{12} + H + Na]^+$
1048	A, G	$[M_H + 42 - C_i + H + Na]^+$
1006	A	$[M_H - C_{12} + H + Na]^+$
853[a]	C, H	$[M_H + 102 - C_{12} - OHC_{16} + H + Na]^+$
793[a]	C, G	$[M_H + 42 - C_i - OHC_{16} + H + Na]^+$
751[a]	C	$[M_H - C_{12} - OHC_{16} + H + Na]^+$
739		$[M_L + Na]^+$
587[a]	C, F, H	$[M_H + 102 - C_{18} + H + C_{12} - OHC_{16} + H + Na]^+$
527[a]	C, F, G	$[M_H + 42 - C_i + H + C_{12} - OHC_{16} + H + Na]^+$
485[a]	C, F	$[M_H - C_{18} + H + C_{12} - OHC_{16} + H + Na]^+$
466	B	$[M_L - OHC_{16} + H + Na]^+$

[a] Peak that can be derived from tetraacyl lipid A as well as from cleavage of acyl chains from hexaacyl lipid.

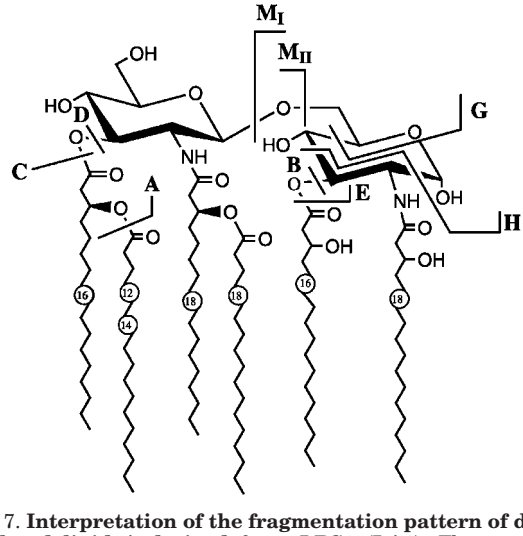


FIG. 7. **Interpretation of the fragmentation pattern of dephosphorylated lipid A derived from LPS1 (LA1).** The *numbers* in *circles* refer to the number of carbon atoms in acyl chains, and the *letters* indicate the designated cleavage process. Refer to Table II for details of the formation of positive ions.

DISCUSSION

The present investigation on the chemical composition of LPSs from *H. pylori* grown at different pH values has added new insight into the expression of Le^x and Le^y mimicry by this bacterium, particularly phase variation. When grown in liquid

medium at pH 7, the O-chain of *H. pylori* 26695 (PS2) consisted of a LacNAc polysaccharide that was glycosylated with α-L-Fuc at O-3 of the majority of GlcNAc residues forming Le^x units, including chain termination by a Le^x unit. However, growth in liquid medium at pH 5 resulted in production of a more complex O-chain (PS1) whose backbone of LacNAc units was partially glycosylated with α-L-Fuc, thus forming Le^x, whereas the majority of the nonfucosylated GlcNAc residues were substituted at O-6 by α-D-Gal residues, and the chain was terminated by a Le^y unit. Thus, the nonreducing termini differ in the two O-chains as well as in glycosylation of the internal LacNAc units. In contrast, detailed chemical analysis of the core and lipid A components of LPS and analysis of cellular lipids did not show significant differences between *H. pylori* 26695 grown at pH 5 and 7. Type 2 series, Le^x, Le^y, and sialyl-Le^x, and in addition, type 1 determinants, Le^a, Le^b, and H-1 antigen, have been reported previously in structural studies of PS O-chains of individual *H. pylori* strains (17–20, 22–25, 33). Nevertheless, in serological surveys of strains from different geographical regions, Le^x and Le^y predominate, expressed by >80% of strains (26–30). Consistent with the results of this study, previous structural studies on other *H. pylori* strains have reported that Le^y, when present in smooth-form LPS, occurs as the terminal unit on the O-chain, whereas Le^x occurs terminally and as an internal unit in the O-chain (17–20, 22–25). However, glycosylation of internal LacNAc units of the *H. pylori* O-chain with hexoses is rarer and, although reported to occur at O-6 of nonfucosylated GlcNAc residues previously (21, 23), can occur in O-chains not expressing Le^x or Le^y (21). Thus, the observation that, when grown at different pH values, the same *H. pylori* strain can express two O-chains differing in *O*-fucosylation patterns, and hence Le^x and Le^y mimicry, as well as differing in occurrence of galactosylation is a novel finding.

A previous study by Bukholm *et al.* (41) that examined the cellular lipid composition of *H. pylori* grown on solid media at pH 5 and 7 found a much reduced amount of phosphatidylethanolamine, a predominant amount of lysophosphatidylethanolamine, and elevated phosphatidylserine at the lower pH, whereas phosphatidylethanolamine predominated, and phosphatidylserine was a minor constituent at neutral pH. Also, the fatty acid composition of phosphatidylethanolamine differed at pH 5 and 7. The investigators suggest that the differing colony morphology and virulence properties of the strain grown at the lower pH reflected changes in the polarity of the bacterial cell wall due to a changed lipid composition because of a bacterial response to the acidic environment. In contrast, in the present study no elevation in content of lysophosphatidylethanolamine and phosphatidylserine was observed for *H. pylori* 26695 grown in a liquid medium at pH 5 nor did the fatty acid composition of phosphatidylethanolamine differ at pH 5 and 7 in liquid media. Likewise, no significant differences were found between lipid A components of bacteria grown in liquid media at pH 5 and 7. The observed changes in lipid composition on solid media by Bukholm *et al.* (41) may be dependent on the physical nature of the growth medium rather than pH. Consistent with this, prolonged subculturing and growth of *H. pylori* on solid medium *in vitro*, independent of pH, can induce changes in cellular lipid composition[4] that are associated with loss of the O-chain and a shift from smooth- to rough-form LPS (15, 16). In contrast, fresh clinical isolates of *H. pylori* produce smooth-form LPS, indicating that high rather than low molecular mass LPS is produced *in vivo* (15). However, growth of *H. pylori* in liquid media *in vitro* stabilizes production of smooth-form LPS containing a PS O-chain (16, 37), and thus, these conditions were used in the present study when examining the influence of environmental pH.

Further demonstrating the importance of the physical nature of the growth medium in these investigations, *H. pylori* 26695, when grown on solid medium, was shown previously to produce a low molecular mass semi-rough LPS carrying a single Le unit, mostly a Le^y unit, but alternatively, type 1 and type 2 linear B blood group and Le^x attached to the core OS, whereas when grown in liquid medium, the strain produced smooth-form LPS (25). The O-chain of the latter was composed of a polyfucosylated LacNAc chain terminated with a Le^x unit, corresponding to PS1 in this study. Although no significant differences were observed between the core OS of bacteria grown in liquid media at pH 5 and 7 in the present study, these cores differed from that of *H. pylori* 26695 when grown on solid media at neutral pH (25) by the predominant absence of a glucan chain substituting α-Gal on the fourth heptose (DD-Hep) residue.

The genome of *H. pylori* 26695 and that of another strain, J99, contain two copies of α(1,3)-fucosyltransferase (*HpfucT*) genes that are required for expression of Le^x and Le^y but differ in the number of a seven-amino acid sequence repeat, YDDLRVN (44, 69). DNA motifs near the 5′-end of these genes (HP0379 and HP0651) at two distinct polynucleotide repeats have been deduced to indicate regulation through slipped-strand repair (44). No putative gene for α(1,2)-fucosyltransferase (*HpfucT2*), which is required for Le^y synthesis, was initially identified in the 26695 genome (44), but a truncated gene (HP0094) with a C14 tract was found, and *in silico* insertion of a C-G pair yielded a full-length protein with strong homology to α(1,2)-fucosyltransferase (70). Moreover, sequence analysis has shown that the *HpfucT2* gene contains a hypermutable sequence (poly-C and TTA repeats) that provides a possibility of frequent shifting into and out of coding frame by a polymerase slippage mechanism (71). Phase variation in expression of Le^x has been attributed to changes in the lengths of poly-C tracts in the *HpfucT* genes (38), but the length of these tracts has not been found to be predictive of the phenotype (72). On the other hand, variable expression of Le^y by *H. pylori* strains has been proposed to occur at the combined levels of replication slippage (mutation), transcription, and translation of the *fucT2* gene (71). Despite these deductions as to the putative molecular mechanisms involved in variable Le^x and Le^y expression by *H. pylori*, the resultant phenotypes of phase variants were not established previously in detailed structural studies. Furthermore, the environmental trigger inducing variable Le^x and Le^y expression was not identified.

The present study addresses such issues by showing that relative pH can influence expression of Le^x and Le^y, particularly at the termini of the O-chains, but also that pH influences glycosylation, including substitution at O-6 by α-D-Gal residues of the internal LacNAc units of these chains. Consistent with these structural findings as well as the serological and electrophoretic investigations in this study, McGowan *et al.* (73) found qualitative differences in LPS electrophoretic profiles when *H. pylori* 60190 was grown at pH 5 compared with pH 7. Using subtractive RNA hybridization they identified an acid-inducible gene in this strain whose protein was highly homologous to that of WbcJ of enteric bacteria that is considered involved in the conversion of GDP-D-mannose to GDP-D-fucose. Moreover, a corresponding gene (HP0045) occurs in the 26695 genome, which had previously been designated a *nolK* homologue based on 44% identity with NolK, an inducible nodulating protein of *Azorhizobium caulinodans* (44). Thus, mechanisms affecting fucose availability in addition to fucosyltransferase activity are present in *H. pylori* 26695 that can influence Le^x and Le^y expression.

[4] A. P. Moran, unpublished results.

Common to the genomes of both sequenced strains, *H. pylori* 26695 and J99 are seven open reading frames encoding putative glycosyltransferases (HP0159/JHP147, HP0208/JHP194, HP0619/JHP563, HP0679/JHP620, HP0805/JHP741, HP0826/JHP765, and HP1105/JHP1031) that have been implicated in LPS core synthesis, but in addition there are three strain-specific open reading frames (JHP562, JHP820, and JHP1032) in strain J99 and one (HP1578) in strain 26692, reflecting differences in the core OS of the two strains (25, 69). Three genes in the 26695 genome (HP0159, HP0208, and HP1416) are homologues of the α(1,2)-glucosyltransferase gene (*rfaJ*) found in enteric bacteria (69, 74), and although it has been debated whether some of these genes encode β(1,4)-galactosyltransferase and/or β(1,3)-*N*-acetylglucosaminyltransferase functions needed for type 2 chain synthesis (70), α(1,2)-substituted Glc can occur in the core of *H. pylori* 26695 under certain growth conditions (25). Also, homologues of galactosyltransferases from *Klebsiella pneumoniae* have been suggested to be involved in O-chain synthesis in strain 26695 (74). However, rather than involvement in addition to β(1,4)-substituted Gal in type 2 chains, these may be required for addition at O-6 of nonfucosylated GlcNAc residues by α-D-Gal residues, as observed in PS1.

Several enteric bacteria are known to vary gene expression in response to acid stress (75). However, there has been no evidence that exposure to acidic pH alters expression of their LPS-associated genes. On the other hand, compared with gonococcal cells grown at pH 8.2, those grown at pH 5.8 produce LPS with an altered electrophoretic profile, indicative of pH regulation (76), but this has not been characterized structurally. In *Rhizobium leguminosarum*, LPS is modified in response to several environmental stresses, including low pH (77). Loss of a plasmid carrying acid tolerance genes from *R. leguminosarum* results in interference with ability to maintain intracellular pH homeostasis at low external pH, but also ablation of O-chain biosynthesis occurs with an acid-sensitive phenotype (78). In contrast, as demonstrated in this study, *H. pylori* varies glycosylation of the O-chain in response to acid stress, producing a more complex structure at pH 5 than pH 7, and genes carried on the bacterial chromosome are implicated in this phenomenon.

The *in vitro* observations of the present study are of *in vivo* relevance since serological analyses of sequential *H. pylori* isolates from patients have shown that expression of Le^x and/or Le^y by isolates from the same patient varied over time, although isolates appeared genetically identical by analysis of randomly amplified polymorphic DNA-polymerase chain reaction patterns (39, 79). A definite biological role for this variation has not yet been defined, although antigenic variation in other bacteria allows increased persistence and/or pathogenicity (80). Potentially, the ability to vary expression of Le^x and Le^y could influence bacterial camouflage in the gastric mucosa (34), particularly since Le expression varies in different regions of this mucosa and is dependent on the secretor status of the individual (31, 32, 42, 43). However, the relevance of *H. pylori* expression of Le^x and Le^y for camouflage in the gastric mucosa has been questioned (30, 81). On the other hand, polymeric Le^x expression has been implicated in adhesion of *H. pylori* to human antral gastric mucosa (14, 35). Moreover, the majority of *H. pylori* in infected hosts are free-living in the mucus layer, and only a proportion (about 20%) appear to adhere to epithelial cells of the gastric mucosa (9). In the gastric mucus layer, the pH gradient ranges from pH 2 on the luminal side to almost pH 7 on the epithelial cell surface (8). Compared with expression of an O-chain predominantly composed of Le^x units and terminated by a Le^x unit at pH 7 (PS2) and, thus, with optimal expression of Le^x for adhesion, production of an O-chain with lesser Le^x units and capped with a terminal Le^y unit at pH 5 (PS1) can be hypothesized to affect the availability of the Le^x ligand for interaction with the gastric mucosa. Hence, the influence of environmental pH on Le^x-mediated adhesion by *H. pylori* remains an important question for further investigation.

Acknowledgments—We thank G. Alvelius (Huddinge, Sweden) for technical assistance with ESI MS and Dr. Buko Lindner (Borstel, Germany) for technical assistance with the LD MS.

REFERENCES

1. Dunn, B. E., Cohen, H., and Blaser, M. J. (1997) *Clin. Microbiol. Rev.* **10,** 720–741
2. Marshall, B. J., and Warren, J. R. (1984) *Lancet* ***i,*** 1311–1315
3. Graham, D. Y., Lew, G. M., Klein, P. D., Evans, D. G., Evans, D. J., Jr., Saeed, Z. A., and Malaty, H. M. (1992) *Ann. Intern. Med.* **116,** 705–708
4. NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease (1994) *J. Am. Med. Assoc.* **272,** 65–69
5. Forman, D., Newell, D. G., Fullerton, F., Yarnell, J. W., Stacey, A. R., Wald, N., and Sitas, F. (1991) *Br. Med. J.* **302,** 1302–1305
6. Parsonnet, J., Hansen, S., Rodriguez, L., Gelb, A. B., Warnke, R. A., Jellum, E., Orentreich, N., Vogelman, J. H., and Freidman, G. D. (1994) *N. Engl. J. Med.* **330,** 1267–1271
7. Moran, A. P. (1996) *Scand. J. Gastroenterol.* **31,** 22–31
8. Quigley, E. M. M., and Turnberg, L. A. (1987) *Gastroenterology* **92,** 1876–1883
9. Lee, A., Fox, J., and Hazell, S. (1993) *Infect. Immun.* **61,** 1601–1610
10. McColl, K. L. E., El-Omar, E., and Gillen, D. (1997) in *Pathogenesis and Host Response in Helicobacter pylori Infections* (Moran, A. P., and O'Morain, C. A., eds) pp. 119–127, Normed Verlag, Bad Homburg, Germany
11. El-Omar, E., Penman, I., Dorrian, C. A., Ardill, J. E. S., and McColl, K. E. L. (1993) *Gut* **34,** 1060–1065
12. Clyne, M., Labigne, A., and Drumm, B. (1995) *Infect. Immun.* **63,** 1669–1673
13. Eaton, K. A., and Krakowka, S. (1994) *Infect. Immun.* **62,** 3604–3607
14. Moran, A. P. (1999) *J. Physiol. Pharmacol.* **50,** 787–805
15. Moran, A. P., Helander, I. M., and Kosunen, T. U. (1992) *J. Bacteriol.* **174,** 1370–1377
16. Moran, A. P. (1995) *FEMS Immunol. Med. Microbiol.* **10,** 271–280
17. Aspinall, G. O., Monteiro, M. A., Pang, H., Walsh, E. J., and Moran, A. P. (1994) *Carbohydr. Lett.* **1,** 156–165
18. Aspinall, G. O., Monteiro, M. A., Pang, H., Walsh, E. J., and Moran, A. P. (1996) *Biochemistry* **35,** 2489–2497
19. Aspinall, G. O., and Monteiro, M. A. (1996) *Biochemistry* **35,** 2498–2504
20. Aspinall, G. O., Monteiro, M. A., Shaver, R. T., Kurjanczyk, L. A., and Penner, J. L. (1997) *Eur. J. Biochem.* **248,** 592–601
21. Monteiro, M. A., Rasko, D., Taylor, D. E., and Perry, M. B. (1998) *Glycobiology* **8,** 107–112
22. Monteiro, M. A., Chan, K. H. N., Rasko, D. A., Taylor, D. E., Zheng, P. Y., Appelmelk, B. J., Wirth, H. P., Yang, M., Blaser, M. J., Hynes, S. O., Moran, A. P., and Perry, M. B. (1998) *J. Biol. Chem.* **273,** 11533–11543
23. Aspinall, G. O., Mainkar, A. S., and Moran, A. P. (1999) *Glycobiology* **9,** 1235–1245
24. Knirel, Y. A., Kocharokova, N. A., Hynes, S. O., Widmalm, G., Andersen, L. P., Jansson, P.-E., and Moran, A. P. (1999) *Eur. J. Biochem.* **266,** 123–131
25. Monteiro, M. A., Appelmelk, B. J., Rasko, D. A., Moran, A. P., MacLean, L. L., Chan, K. H., St Michael, F., Logan, S. M., O'Rourke, J., Lee, A., Hynes, S. O., Taylor, D. E., and Perry, M. B. (2000) *Eur. J. Biochem.* **267,** 305–320
26. Simoons-Smit, I. M., Appelmelk, B. J., Verboom, T., Negrini, R., Penner, J. L., Aspinall, G. O., Moran, A. P., Fei Fei, S., Bi-Shan, S., Rudnica, W., Savio, A., and De Graaff, J. (1996) *J. Clin. Microbiol.* **34,** 2196–2200
27. Appelmelk, B. J., Simmons-Smit, I., Negrini, R., Moran, A. P., Aspinall, G. O., Forte, J. G., De Vries, T., Quan, H., Verboom, T., Maaskant, J. J., Ghiara, P., Kuipers, E. J., Bloemena, E., Tadema, T. M., Townsend, R. R., Tyagarajan, K., Crothers Jr, J. M., Monteiro, M. A., Savio A., and De Graaff J. (1996) *Infect. Immun.* **64,** 2031–2040
28. Wirth, H. P., Yang, M. Q., Karita, M., and Blaser, M. J. (1996) *Infect. Immun.* **64,** 4598–4605
29. Marshall, D. G., Hynes, S. O., Coleman, D. C., O'Morain, C. A., Smyth, C. J., and Moran, A. P. (1999) *FEMS Immunol. Med. Microbiol.* **24,** 79–90
30. Heneghan, M. A., McCarthy, C. F., and Moran, A. P. (2000) *Infect. Immun.* **68,** 937–941
31. Sakamoto, J., Watanabe, T., Tokumaru, T., Takagi, H., Nakazato, H., and Lloyd, K. O. (1989) *Cancer Res.* **49,** 745–752
32. Hakomori, S.-I. (1989) *Adv. Cancer Res.* **52,** 257–331
33. Monteiro, M. A., Zheng, P.-y., Ho, B., Yokota, S.-i., Amano, K.-i., Pan, Z.-j., Berg, D. E., Chan, K. H., MacLean, L. L., and Perry, M. B. (2000) *Glycobiology* **10,** 701–713
34. Wirth, H. P., Yang, M., Peek, R. M., Tham, K. T., and Blaser, M. J. (1997) *Gastroenterology* **113,** 1091–1098
35. Edwards, N. J., Monteiro, M. A., Faller G., Walsh, E. J., Moran, A. P., Roberts, I. S., and High, N. J. (2000) *Mol. Microbiol.* **35,** 1530–1539
36. Moran, A. P., Sturegård, E., Sjunnesson, H., Wadström, T., and Hynes, S. O. (2000) *FEMS Immunol. Med. Microbiol.* **29,** 263–270
37. Walsh, E. J., and Moran, A. P. (1997) *J. Appl. Microbiol.* **83,** 67–75
38. Appelmelk, B. J., Martin, S. L., Monteiro, M. A., Clayton, C. A., McColm, A. A., Zheng, P., Verboom, T., Maaskant, J. J., van den Eijnden, D. H., Hokke, C. H., Perry, M. B., Vandenbroucke-Grauls, C. M. J. E., and Kusters, J. G. (1999) *Infect. Immun.* **67,** 5361–5366
39. Wirth, H. P., Yang, M., Peek Jr, R. M., Hook-Nikanne, J., Fried, M., and Blaser, M. J. (1999) *J. Lab. Clin. Med.* **133,** 488–500

40. Hynes, S. O., and Moran, A. P. (2000) *FEMS Microbiol. Lett.* **190,** 67–72
41. Bukholm, G., Tannaes T., Nedenskov, P., Esbensen, Y., Grav, H. J., Hovig, T., Ariansen, S., and Guldvog, I. (1997) *Scand. J. Gastroenterol.* **32,** 445–454
42. Mollicone, R., Bara, J., Le Pendu, J., and Oriol, R. (1985) *Lab. Invest.* **53,** 219–227
43. Kobayashi, K., Sakamoto, J., Kito, T., Yamamura Y, Koshikawa, T., Fujita, M., Watanabe, T., and Nakazato, H. (1993) *Am. J. Gastroenterol.* **88,** 919–924
44. Tomb, J.-F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., Nelson, K., Quackenbush, J., Zhou, L., Kirkness, E. F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H. G., Glodek, A., McKenney, K., Fitzgerald, L. M., Lee, N., Adams, M. D., Hickey, E. K., Berg, D. E., Gocayne, J. D., Utterback, T. R., Peterson, J. D., Kelley, J. M., Cotton, M. D., Weidman, J. M., Fujii, C., Bownman, C., Watthey, L., Wallin, E., Hayes, W. S., Borodovsky, M., Karp, P. D., Smith, H. O., Fraser, C. M., and Venter, J. C. (1997) *Nature* **388,** 539–547
45. Moran, A. P., Lindner, B., and Walsh, E. J. (1997) *J. Bacteriol.* **179,** 6453–6463
46. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956) *Anal. Chem.* **28,** 350–356
47. Moran, A. P., Zähringer, U., Seydel, U., Scholz, D., Stütz, P., and Rietschel. E. T. (1991) *Eur. J. Biochem.* **198,** 459–469
48. Hitchcock, P. J., and Brown, T. M. (1983) *J. Bacteriol.* **154,** 269–277
49. Tsai, C.-M., and Frasch, C. E. (1982) *Anal. Biochem.* **119,** 115–119
50. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 4350–4354
51. Bligh, E. G., and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37,** 911–917
52. Kates, M. (1986) in *Techniques of Lipidology* (Kates, M., ed) pp. 239–244, Elsevier Science Publishing Co., Inc., New York
53. Mallinger, A. G., Yao, J. K., Brown, A. S., and Dippold, C. S. (1993) *J. Chromatogr.* **614,** 67–75
54. Pietsch, A., and Lorenz, R. L. (1993) *Lipids* **28,** 945–947
55. London, E., and Feigenson G. W. (1979) *J. Lipid Res.* **20,** 408–412
56. Sawardeker, J. S., Sloneker, J. H., and Jeanes, A. (1965) *Anal. Chem.* **37,** 1602–1603
57. Leontein, K., Lindberg, B., and Lönngren, J. (1978) *Carbohydr. Res.* **62,** 359–362
58. Gerwig, G. J., Kamerling, J. P., and Vliegenthart, J. F. G. (1978) *Carbohydr. Res.* **77,** 1–7
59. Shashkov, A. S., Senchenkova, S. N., Nazarenko, E. L., Zubkov, V. A., Knirel, Y. A., Gorshkova, N. M., and Gorshkova, R. P. (1997) *Carbohydr. Res.* **303,** 333–338
60. Hakomori, S.-I. (1964) *J. Biochem.* (*Tokyo*) **55,** 205–208
61. Jansson, P.-E., Kenne, L., Liedgren, H., Lindberg, B., and Lönngren, J. (1976) *Chem. Commun. Univ. Stockholm* **8,** 1–75
62. Schwarzmann, G. O. H., and Jeanloz, R. W. (1974) *Carbohydr. Res.* **34,** 161–168
63. Rietschel, E. T., Gottert, H., Lüderitz, O., and Westphal, O. (1972) *Eur. J. Biochem.* **28,** 166–173
64. Wollenweber, H.-W., Broady, K. W., Lüderitz, O., and Rietschel, E. T. (1982) *Eur. J. Biochem.* **124,** 191–198
65. Lindner B., Zähringer U., Rietschel E. T., Seydel, U. (1990) in *Analytical Microbiology Methods* (Fox A., Morgan S. L., Larsson L., and Odham G., eds) pp. 149–161, Plenum Press, New York
66. Hermansson, K., Jansson, P.-E., Kenne, L., Widmalm, G., and Lindh, F. (1992) *Carbohydr. Res.* **235,** 69–81
67. Breg, J., Romijn, D., Vliegenthart, J. F. G., Strecker, G., and Montreuil, J. (1988) *Carbohydr. Res.* **183,** 19–34
68. Pece, S., Fumarola, D., Giuliani, G., Jirillo, E., and Moran, A. P. (1995) *J. Endotoxin Res.* **2,** 455–462
69. Doig, P., de Jonge, B. L., Alm, R. A., Brown, E. D., Uria-Nickelsen, M., Noonan, B., Mills, S D., Tummino, P., Carmel, G., Guild, B. C., Moir, D. T., Vovis, G. F., and Trust, T. J. (1999) *Microbiol. Mol. Biol. Rev.* **63,** 675–707
70. Berg, D. E., Hoffman, P. S., Appelmelk, B. J., and Kusters, J. G. (1997) *Trends Microbiol.* **5,** 468–474
71. Wang, G., Rasko, D. A., Sherburne, R., and Taylor, D. E. (1999) *Mol. Microbiol.* **31,** 1265–1274
72. Ryan, K. A., Moran, A. P., Hynes, S. O., Smith, T., Hyde, D., O'Morain, C. A., and Maher, M. (2000) *FEMS Immunol. Med. Microbiol.* **28,** 113–120
73. McGowan, C. C., Necheva, A., Thompson, S. A., Cover, T. L., and Blaser, M. J. (1998) *Mol. Microbiol.* **30,** 19–31
74. Marais, A., Mendz, G. L., Hazell, S. L., and Mégraud, F. (1999) *Microbiol. Mol. Biol. Rev.* **63,** 642–674
75. Slonczewski, J. L., and Foster, J. W. (1996) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* (Neidhardt, F. C., ed) pp. 1539–1549, American Society for Microbiology, Washington, D. C.
76. Pettit, R. K., Martin, E. S., Wagner, S. M., and Bertolino, V. J. (1995) *Infect. Immun.* **63,** 2773–2775
77. Tao, H., Brewin, N. J., and Noel, K. D. (1992) *J. Bacteriol.* **174,** 2222–2229
78. Chen, H., Gartner, E., and Rolfe, B. G. (1993) *Appl. Environ. Microbiol.* **59,** 1058–1064
79. Rasko, D. A., Wilson, T. J. M., Zopf, D., and Taylor, D. E. (2000) *J. Infect. Dis.* **181,** 1089–1095
80. Moran, A. P., Prendergast, M. M., Appelmelk, B. J. (1996) *FEMS Immunol. Med. Microbiol.* **16,** 105–115
81. Taylor, D. E., Rasko, D. A., Sherburne, R., Ho, C., and Jewell, L. D. (1998) *Gastroenterology* **115,** 1113–1122