

Role of *Helicobacter pylori* *rfaJ* genes (HP0159 and HP1416) in lipopolysaccharide synthesis

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Abstract

The genome of *Helicobacter pylori* 26695 has been sequenced and the lipopolysaccharide (LPS) O sidechain of this strain has been shown to express both Lewis x and Lewis y units. To determine the role of HP0159 and HP1416, genes recognized as *rfaJ* homologs and implicated in LPS synthesis, isogenic mutants of *H. pylori* 26695 were generated. The LPS of mutant 26695::HP0159Kan did not express either Lewis epitope as detected by immunoblotting, whereas the control strain and 26695::HP1416Kan produced both epitopes. Structural analysis of the LPS of the mutants showed that HP0159 encodes an $\alpha(1,2/3)$ -glucosyltransferase whereas HP1416 encodes an $\alpha(1,2/4)$ -glucosyltransferase.

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1. Introduction

Helicobacter pylori is a prevalent gastroduodenal pathogen, infecting almost 50% of the human population, and chronic infection by this bacterium causes recurrent gastroduodenal inflammatory disease [1]. *H. pylori* is the causal agent of active chronic gastritis, is associated with peptic ulcer disease, and produces an increased risk for the development of gastric adenocarcinoma and primary gastric lymphoma [1–5]. Like other Gram-negative bacteria, the outer membrane of

H. pylori contains lipopolysaccharides (LPSs) [6,7]. The O sidechains of *H. pylori* LPSs are unique in that they can express Lewis antigens, including Lewis x (Le^x) and/or Lewis y (Le^y), mimicking those in the host gastric mucosa. It has been suggested that these structures may play a camouflaging role in protecting *H. pylori* from antigen-specific host defenses and that antibodies against the O sidechain antigen, which may be induced during prolonged infection, may bind to receptors in gastric epithelia and cause autoimmune-mediated inflammation [8,9].

A large number of genes identified by homology with other polysaccharide biosynthesis genes have been implicated in *H. pylori* LPS synthesis [10–13]. Due to

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the potential biological importance of Lewis antigen mimicry in *H. pylori* LPS, attention has focused on cloning and sequencing first the $\alpha(1,3)$ -fucosyltransferase involved in Le^x synthesis [14,15], then the $\alpha(1,2)$ -fucosyltransferase involved in Le^x synthesis [16], as well as the $\beta(1,4)$ -galactosyltransferase required for synthesis of *N*-acetylglucosamine in the backbone of Lewis antigens [17]. Moreover, an enzyme with both $\alpha(1,3)$ - and $\alpha(1,4)$ -fucosyltransferase activity involved in both Le^x and Lewis A synthesis, respectively, has been cloned and sequenced [18]. However, genes encoding other glycosyltransferases that are needed for synthesis of *H. pylori* LPS, particularly the core region, have not received adequate attention [8]. Interestingly, three *rfaJ* homologs were noted in *H. pylori* 26695 (HP0159, HP1416, and HP0208) [10]. In other bacteria, such as *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, a single *rfaJ* gene is present, which encodes an $\alpha(1,2)$ -glucosyltransferase involved in the addition of a distal glucose residue to the core oligosaccharide (OS) of LPS [19]. Prior to a detailed structural analysis of *H. pylori* 26695 LPS [20], no $\alpha(1,2)$ -linked glucose had been described in *H. pylori* [20], and this led to the suggestion that the three *rfaJ* homologs in *H. pylori* encoded $\beta(1,4)$ -galactosyltransferase and/or $\beta(1,3)$ -*N*-acetylglucosaminyltransferase functions required for Lewis antigen synthesis [21].

To determine the role of two of these *rfaJ* genes in LPS biosynthesis in *H. pylori*, the HP0159 and HP1416 genes were inactivated in the genome-sequenced strain 26695 [10], whose LPS O sidechain has been shown to contain both Le^x and Le^y units [20].

2. Materials and methods

2.1. Bacterial strains and plasmid constructs

H. pylori 26695 [22] was kindly provided by Dr. K. Eaton, Ohio State University, Columbus, OH, USA. *H. pylori* was grown on blood agar and incubated in a microaerobic atmosphere containing 85% N₂, 10% CO₂, and 5% O₂ at 37 °C for 24 h. Genomic DNA from *H. pylori* 26695 was isolated using a Puragene kit (Gentra Systems, Minneapolis, MN), according to the manufacturer's protocol for Gram-negative bacteria. Polymerase chain reaction (PCR) was carried out on the genomic DNA using primers based on sequences flanking the *rfaJ* genes (see www.tigr.org): HP0159, 5'-GGATTAAATCGATTAAAAACCTAT-3' and 5'-ATTATGTCGAATTCGCCTATAGC-3', and HP1416, 5'-ATAAAACCGAATTCATAACCTTT-3' and 5'-TCAATCGTTTATCGATGAATGTCC-3' with the *Cla*I and *Eco*RI sites (underlined) engineered into the primer pairs. PCR amplification products were separated on 1% agarose gels using standard techniques. The bands

encoding HP0159 (1205 bp fragment) and HP1416 (1211 bp fragment) were extracted using a Qiagen Gel Extraction kit (Qiagen, Valencia, CA), digested with restriction endonucleases *Cla*I and *Eco*RI, and ligated into similarly digested pBluescript (Stratagene, La Jolla, CA) that had been extracted from *E. coli* using Qiagen reagents. The ligation mixture was transformed into *E. coli* DH5 α on L-agar supplemented with 100 $\mu\text{g ml}^{-1}$ ampicillin, 40 $\mu\text{g ml}^{-1}$ isopropyl- β -D-thiogalactopyranoside (IPTG), and 40 $\mu\text{g ml}^{-1}$ 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal). The resulting plasmids were referred to as pHP0159 and pHP1416, respectively.

2.2. Insertional inactivation of *rfaJ*

Insertional inactivation of the *rfaJ* gene on pHP0159 was performed by ligating the kanamycin resistance cassette from pILL660 [23] (kindly provided by Dr. D. Berg, Washington University, St. Louis, MO), which had been isolated as a *Sma*I fragment, into the *Eco*47III sites within HP0159. For inactivation of pHP1416, the kanamycin resistance cassette was cloned into an *Eco*47III site. The ligation reactions were transformed into *E. coli* DH5 α onto L-agar supplemented with kanamycin (50 $\mu\text{g ml}^{-1}$), and the resulting recombinant plasmids were referred to as pHP0159Kan and pHP1416Kan. Insertion of the kanamycin resistance cassette was confirmed by PCR.

2.3. Electroporation

Plasmid pHP0159Kan was transferred to *H. pylori* 26695 by electroporation. Briefly, *H. pylori* 26695 were harvested from blood agar plates after 48 h incubation, washed three times in ice-cold 10% glycerol, and suspended in 100 μl of the same diluent. Purified plasmid (approximately 1.2 μg) was added to the cell suspension, incubated on ice for 1 min and transferred into a prechilled 0.1 mm electroporation cuvette (Bio-Rad, Hercules, CA, USA) in the electroporation apparatus (Eppendorf 2510, Westbury, NY). Pulses were achieved with settings of 10 μF , 1.25 kV, and 600 Ω , giving time constants ranging from 4.0 to 5.4 ms. The cells were plated onto blood agar and incubated under microaerobic conditions at 37 °C for 24 h. The bacteria were then transferred onto blood agar containing kanamycin (50 $\mu\text{g ml}^{-1}$), incubated under the same conditions, and antibiotic-resistant colonies were detected after 4 days. The resistant colonies showed typical *H. pylori* morphology, and were urease-, oxidase- and catalase-positive. Transformants were verified by PCR and the resultant strain was referred to as 26695::HP0159Kan. A similar strategy was used with pHP1416Kan to construct 26695::HP1416Kan.

2.4. Electrophoresis and Western blotting

Proteinase K-treated whole-cell (PKWC) extracts of *H. pylori* 26695, 26695::HP0159Kan, and 26695::HP1416Kan were prepared [24]. The extracts were applied to a 10% bis–tris sodium dodecyl sulfate polyacrylamide (SDS–PAGE) gel (Novex, San Diego, CA), run as described previously [25] and stained with a commercial silver-stain kit (Bio-Rad) or, alternatively, with Coomassie blue stain (Sigma Chemical Co., St. Louis, MO) (6). The PKWC extracts were run on SDS–PAGE and electrotransferred (10 V, 45 min) onto nitrocellulose membranes (MSI, Minnetonka, MN) using a semi-dry transfer cell (Bio-Rad). The membrane was incubated in blocking buffer (5% skim milk in phosphate-buffered saline, pH 7.4 [PBS]) for 1 h. After rinsing with PBS, the blots were incubated overnight with anti-Le^x (clone BG-7) or anti-Le^y (clone BG-8) mouse monoclonal antibodies (Signet Pathology Systems, Inc., Dedham, MA) diluted in PBS (1:1000). Blots were rinsed, incubated with alkaline phosphatase-labeled goat anti-mouse (IgM) secondary antibody (Sigma) diluted in PBS (1:2000) for 2 h, washed with PBS, and developed using tetrazolium tablets (Sigma).

2.5. LPS structural analysis

For structural analysis, LPSs were extracted from bacterial biomass using 45% aqueous phenol and, subsequently, were purified by enzymatic treatments with RNase A, DNase II and proteinase K (all from Sigma), and by ultracentrifugation as described previously [6]. LPSs were subjected to mild acid hydrolysis with 0.1 M sodium acetate buffer, pH 4.2 (2 h, 100 °C), the lipid precipitate was removed by centrifugation (10,000g, 30 min), and the water-soluble carbohydrate portion was subjected to gel-permeation chromatography on Sephadex G-50 (Pharmacia, Uppsala, Sweden), as described elsewhere [26]. The resultant fractions were subjected to sugar and methylation analyses according to the methodologies described previously [26,27] and to ¹H-, ¹³C-, ³¹P-nuclear magnetic resonance (NMR) spectroscopy using the conditions and instrumentation described elsewhere [27].

3. Results

3.1. LPS macromolecular nature of *H. pylori* rfaJ mutants

In order to determine the role of the *rfaJ* genes, HP0159 and HP1416, isogenic mutants of 26695 were constructed. These genes are only 45.5% identical and primers to construct the mutants were selected to be specific for regions outside the gene. PCR was used to verify

transformants of 26695::HP0159Kan (Fig. 1) and 26695::HP1416Kan (data not shown).

To determine the macromolecular nature of the respective LPSs of these transformants, PKWC extracts were applied to SDS–PAGE followed by silver staining. As shown in Fig. 2(a), *H. pylori* 26695 and 26695::HP1416Kan expressed high-molecular weight (M_r) LPS. On the other hand, 26695::HP0159Kan expressed low- M_r LPS, and although extra high- M_r banding was seen in these silver-stained gels, Coomassie blue-stained gels revealed these to be contaminating proteins (data not shown). This was confirmed in the silver-stained gels of purified LPSs (<0.1% proteins and nucleic acids): Strains 26695 and 26695::HP1416Kan showed the occurrence of high- M_r LPSs, whereas only low- M_r LPS was present in purified LPS of 26695::HP0159Kan (Fig. 2(d)). Consistent with the low- M_r nature of this LPS, 26695::HP0159Kan LPS predominantly partitioned into the phenol phase of phenol–water extracts (yield, 5.8% dry weight). Compared to the low- M_r gel region corresponding to the core OS of strain 26695, additional low- M_r bands were present in this region of the 26695::HP0159Kan profile (Fig. 2(a)) indicating the occurrence of structural heterogeneity. Western blotting revealed that PKWC extracts from wild-type *H. pylori* 26695 and 26696:HP1416 expressed both Le^x and Le^y, whereas 26695::HP0159Kan expressed neither epitope (Fig. 2(b) and (c)). Western blot analyses of the purified LPSs of the respective strains when probed with

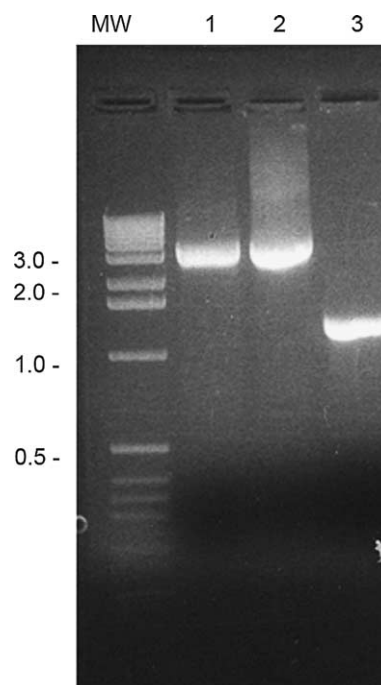


Fig. 1. PCR to confirm insertion in HP0159. DNA was isolated and PCR reactions were performed using HP0159 primers. Lane 1, 26695::HP0159Kan genomic DNA; 2, p0159Kan plasmid DNA; and 3, 26695 genomic DNA. MW, 1 kb molecular weight markers.

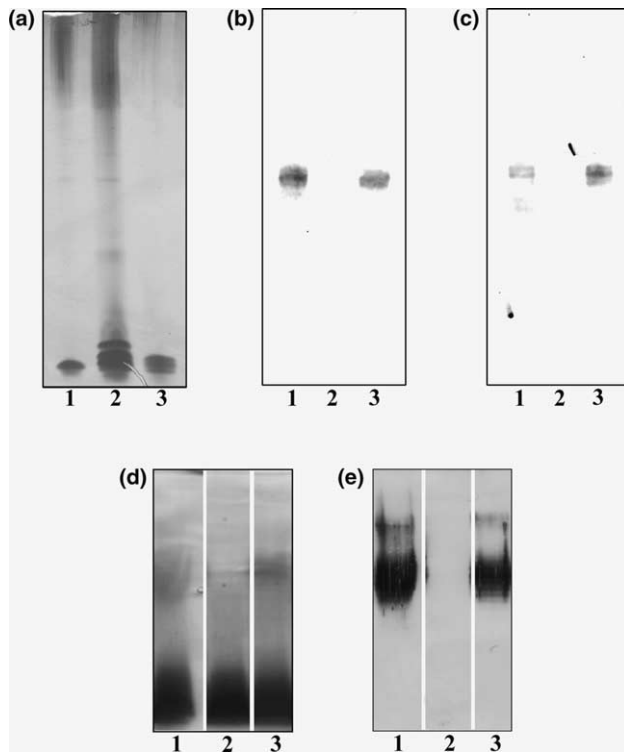


Fig. 2. Electrophoretic and immunoblotting analyses of PKWC extracts (a, b, and c) and purified LPS (d and e) from *H. pylori* strains. In each case, 5 μ l samples were applied to SDS-PAGE and visualized by silver staining (a and d), Western blotting using anti-Lewis x (b) or anti-Lewis y (c) monoclonal antibodies, and Western blotting using combined anti-Lewis x and anti-Lewis y (e) monoclonal antibodies. Lane 1, 26695; lane 2, 26695::HP0159Kan; and lane 3, 26695::HP1416Kan.

combined anti-Le^x and anti-Le^y antibodies (Fig. 2(e)) or the antibodies separately (data not shown) gave identical results to those obtained with the PKWC extracts, consistent with the conclusion that the LPS purification procedure did not affect the macromolecular nature of the LPSs extracted.

3.2. Structural analysis of LPS

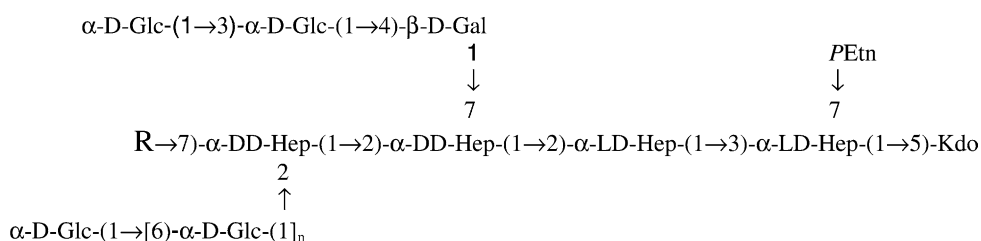
Structural analysis confirmed the structure of the saccharide component of *H. pylori* 26695 LPS as previously reported [20] and showed that mutation of HP0159 resulted in truncation of the core OS and loss of attachment of the O sidechain containing Le^x and Le^y (Fig. 3). An $\alpha(1,6)$ -glucan chain, which is attached through glucose in an $\alpha(1,2)$ -linkage to the fourth heptose of the core of wild-type 26695 LPS (Fig. 3(a)), was absent from 26695::HP0159Kan (Fig. 3(b) and (c)). Likewise, the terminal $\alpha(1,3)$ -linked glucose of the lateral α -D-Glc-(1 \rightarrow 3)- α -D-Glc-(1 \rightarrow 4)- β -D-Gal trisaccharide, which is linked to the third heptose of the core of the parental strain LPS, was absent from LPS of 26695::HP0159Kan (Fig. 3(b) and (c)). Thus, the apparent role of HP0159 is to encode an $\alpha(1,2/3)$ -glucosyl-

transferase involved in the $\alpha(1,2)$ -linkage of the glucan chain to the core OS backbone, as well as substitution by $\alpha(1,3)$ -linked glucose of a lateral disaccharide of the core OS. Additional structural heterogeneity was also present in the core OSs of 26695::HP0159Kan. As shown in Fig. 3(b), the largest OS of this strain, in addition to lacking the glucan chain, had a disaccharide (composed of fucose and *N*-acetylglucosamine) attached, instead of the O sidechain linked to C-7 of the fourth heptose. In addition, another OS lacked the fourth heptose of the core backbone, to which the glucan chain would normally be linked, and had an *N*-acetylglucosamine residue in $\beta(1,2)$ - rather than $\beta(1,7)$ -linkage present terminally (Fig. 3(c)). In contrast to 26695::HP0159Kan LPS, but like the LPS of the parental strain, 26695::HP1416 LPS possessed an O sidechain composed of Le^x units in a chain with a Le^y unit terminally (Fig. 3(a) and (d)). However, in 26695::HP1416Kan LPS, like 26695::HP0159Kan LPS, the $\alpha(1,2)$ -linked $\alpha(1,6)$ -glucan chain of the core OS of wild-type 26695 LPS was absent, and additionally, the lateral trisaccharide was more truncated and lacked the $\alpha(1,4)$ -linked glucose (Fig. 3(d)). Hence, the apparent role of HP1416 is to encode an $\alpha(1,2/4)$ -glucosyltransferase involved in attachment of the glucan chain to the fourth heptose of the core in an $\alpha(1,2)$ -linkage, as well as substitution by $\alpha(1,4)$ -linked glucose of a lateral trisaccharide of the core OS.

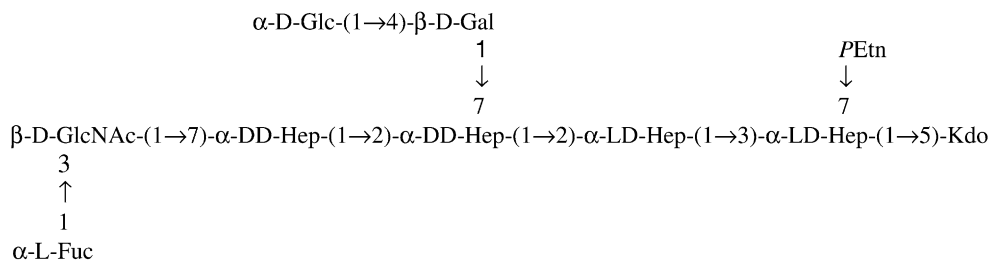
4. Discussion

The availability of two complete genome sequences of *H. pylori* strains (26695 and J99), and their comparative analysis [10,11], has given useful insights into putative LPS biosynthesis genes in *H. pylori* [8]. At least 27 genes likely to be involved in LPS biosynthesis have been found in *H. pylori*, but unlike other bacteria, these genes are scattered throughout the genome, not clustered at one locus [8,10,13]. The genes encoding $\alpha(1,2)$ -, $\alpha(1,3)$ -, $\alpha(1,3/1,4)$ -fucosyltransferase activities, as well as $\beta(1,4)$ -galactosyltransferase activity, involved in synthesis of a variety of Lewis determinants by *H. pylori* have been identified [8]. *H. pylori* has homologs to all enzymes required for lipid A synthesis, including *lpxA*, *lpxB*, *lpxD* and *envA* orthologs [12,13]. Also, orthologs of genes potentially involved in core OS synthesis have been identified: *rfaF*, *rfaE*, *rfaD* and *rfaC* for synthesis of the inner core; *kdsA*, *kdsB* and *rpe* for the 3-deoxy-D-manno-2-octulosonic acid region of the inner core; and three copies of *rfaJ* (HP0159, HP1416 and HP0208), *pgi*, and *galU* were presumed for outer core synthesis [13]. The genome of *H. pylori* 26695 contains three *rfaJ* homologs, HP0159, HP0208 and HP1416 in strain 26695. The identities between these homologs were not high (HP0159 vs. HP1416: 45.5% identity at

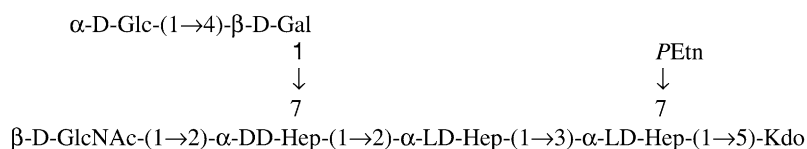
(a)



(b)



(c)



(d)

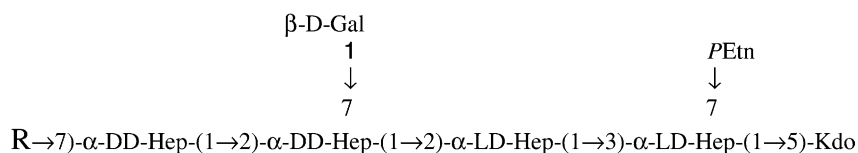
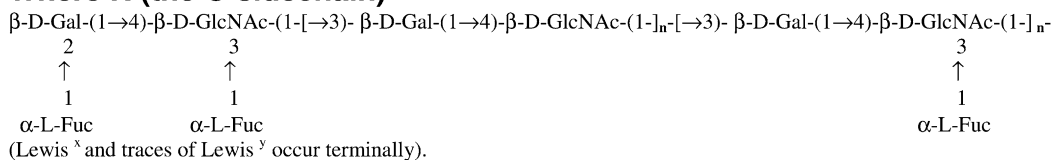
**Where R (the O sidechain) =**

Fig. 3. Structures of the core OS of *H. pylori* 26695 (a), of the predominant core OSs of *H. pylori* 26695::HP0159Kan (b and c), and the core OS of *H. pylori* 26695::HP1416Kan (d). R represents the O sidechain and its site of attachment to the core oligosaccharide is indicated, where relevant. Abbreviations: L-Fuc, L-fucose; D-Gal, D-galactose; D-Glc, D-glucose; D-GlcNAc, N-acetyl-D-glucosamine; DD-Hep, D-glycero-D-manno-heptose; LD-Hep, L-glycero-D-manno-heptose; Kdo, 3-deoxy-D-manno-2-octulosonic acid; PEtn, phosphorylethanolamine.

the DNA level and 38.5% identity at the protein level; HP0159 vs. HP0208: 61.7% identity at the DNA level and 47.9% identity at the protein level; and HP1416 vs. HP0208: 50.6% identity at the DNA level and 34.6% identity at the protein level). The other sequenced *H. pylori* strain J99 also has 3 same three *rfaJ* homologs,

JHP0147, JHP0194 and JHP1311, respectively [12]. Despite the homology of these genes to *rfaJ* of other organisms, the assignment of roles to putative open reading frames in the sequenced *H. pylori* genomes must be performed with caution [8]. In this context, and contrasting with *H. pylori*, only a single *rfaJ* gene, which encodes an

$\alpha(1,2)$ -glucosyltransferase, is present in enteric bacteria [19].

The results of the present study indicate that two of the three *rfa* homologs, HP0159 and HP1416, encode an $\alpha(1,2/3)$ -glucosyltransferase and $\alpha(1,4)$ -glucosyltransferase, respectively. Thus, both can function as $\alpha(1,2)$ -glucosyltransferases but, in addition, have a differing extra activity; $\alpha(1,3)$ -glucosyltransferase activity for HP0159 and $\alpha(1,4)$ -glucosyltransferase activity for HP1416. In support of these conclusions, detailed structural analysis of LPS of the *rfaJ*-inactivated transformant 26695::HP0159Kan showed absence of an O sidechain, but presence of a truncated and modified LPS core, in which a glucan that is $\alpha(1,2)$ -linked to the core of 26695 LPS was absent and truncation of a lateral trisaccharide occurred by loss of an $\alpha(1,3)$ -linked glucose (Fig. 3(b) and (c)). The LPS from 26695::HP1416Kan possessed the same O-chain as the parental strain, but again lacked the $\alpha(1,2)$ -linked glucan, and also possessed a more truncated lateral saccharide in its core than 26695::HP0159KanLPS due to absence of $\alpha(1,4)$ -linked glucose (Fig. 3(d)). Consistent with these findings, electrophoretic analysis using silver-stained profiles and immunoblotting showed that mutant 26695::HP0159Kan LPS did not express an O sidechain nor Le^x and Le^y epitopes, whereas the parental strain and mutant 26695::HP1416Kan produced high- M_r LPSs with both epitopes. Furthermore, in electrophoretic analysis, the observation of additional low- M_r bands in 26695::HP0159Kan LPS profiles, compared with that of strain 26695, is consistent with the heterogeneity observed during structural analysis of the core OS structures of mutant HP0159 LPS. Thus, the respective $\alpha(1,2/3)$ -glucosyltransferase and $\alpha(1,2/4)$ -glucosyltransferase activities of HP0159 and HP1416 can be deduced to influence biosynthesis of structures in the core OS of *H. pylori* 26695 (Fig. 4).

Moreover, based upon the belief that $\alpha(1,2)$ -linked glucose was absent from *H. pylori* LPSs [8], it was suggested that the *rfaJ* homologs of *H. pylori* encoded $\beta(1,4)$ -galactosyltransferase and/or $\beta(1,3)$ -glucosaminyltransferase activities required for Lewis antigen synthesis [21]. Nevertheless, with a subsequent report that $\alpha(1,2)$ -linked glucose was present in *H. pylori* 26695 [20] this view was, therefore, questioned. In this study, we have confirmed the structure of the core OS of the parental strain 26695 containing $\alpha(1,2)$ -linked glucose. Importantly as well, Le^x and Le^y were still expressed by LPS of mutant HP1416, which rules out a role for HP1416 encoding $\beta(1,4)$ -galactosyltransferase and/or $\beta(1,3)$ -*N*-acetylglucosaminyltransferase functions for Lewis antigen synthesis, as was previously suggested [21].

On the other hand, as *N*-acetyl-D-glucosamine possesses the same ring and gluco-configuration as D-glucose, it might be argued that the $\alpha(1,3)$ -transferase

activity of the HP0159 $\alpha(1,2/3)$ -glucosyltransferase could affect incorporation of $\beta(1,3)$ -linked *N*-acetylglucosamine into the backbone of Le^x and Le^y antigens of the O sidechain and, thus, inactivation of this gene would lead to truncation of mutant HP0159 LPS with loss of the O sidechain as observed in this study. However, HP0159 putatively encodes an $\alpha(1,3)$ -transferase not a $\beta(1,3)$ -transferase based on the observed truncation of the lateral trisaccharide due to absence of an $\alpha(1,3)$ -linked glucose. Also, it is important to note that the largest core OS of this strain, in addition to the modification and truncation described above, had a disaccharide attached which was composed of $\beta(1,7)$ -linked *N*-acetylglucosamine substituted with $\alpha(1,3)$ -fucose, instead of the O sidechain linked to C-7 of the fourth heptose (Fig. 3(b)). Thus, *N*-acetylglucosamine was present in such a conformation as to be an acceptor for $\beta(1,4)$ -linked galactose, the next sugar residue in the Le^x determinant of the O sidechain. Hence, it could be deduced that the presence of this disaccharide apparently impeded attachment of the O sidechain. Also, the other core OS of this mutant strain lacked the fourth heptose of the core backbone, to which the glucan chain would normally be linked, and had an *N*-acetylglucosamine residue in $\beta(1,2)$ - rather than $\beta(1,7)$ -linkage present terminally (Fig. 3(c)). Thus, absence of the glucan chain and truncation of the lateral saccharide chain due to inactivation of $\alpha(1,2/3)$ -glucosyltransferase activity, and associated compensatory changes in the core OS substitution, particularly terminally, are likely to have impeded attachment of the O sidechain expressing Lewis antigens in the mutant HP0159 LPS. On the other hand, the truncation and modification of the core OS induced upon mutation of HP1416 did not induce compensatory changes affecting extension of the O sidechain.

Interestingly, in preliminary experiments neither the cloned HP0159 nor HP1416 genes could complement the LPS-defective phenotype of the *rfaJ* mutant strain *S. typhimurium* SL3750 [28], suggesting that neither of these *H. pylori* enzymes are interchangeable with the $\alpha(1,2)$ -glucosyltransferase from *S. typhimurium* (Goldberg et al., unpublished results). This further supports the deduction that these genes of *H. pylori* are likely to encode glycosyltransferases with distinct LPS biosynthetic activities from their enteric counterparts, being involved in the biosynthesis of a differing LPS structure in *H. pylori* compared with enteric LPS. Moreover, in *S. typhimurium* the acceptor sugar for the $\alpha(1,2)$ -glucosyltransferase is α -D-galactose [19], in contrast to the terminal heptose of the core OS acting as an acceptor for the $\alpha(1,2)$ -glucosyltransferase activity in *H. pylori*. Further experiments are required to determine whether each of these *rfaJ* homologs are capable of heterologous complementation in *H. pylori*.

A consequence of the deduced activities of the enzymes (Fig. 4) is that the shared acceptor for the

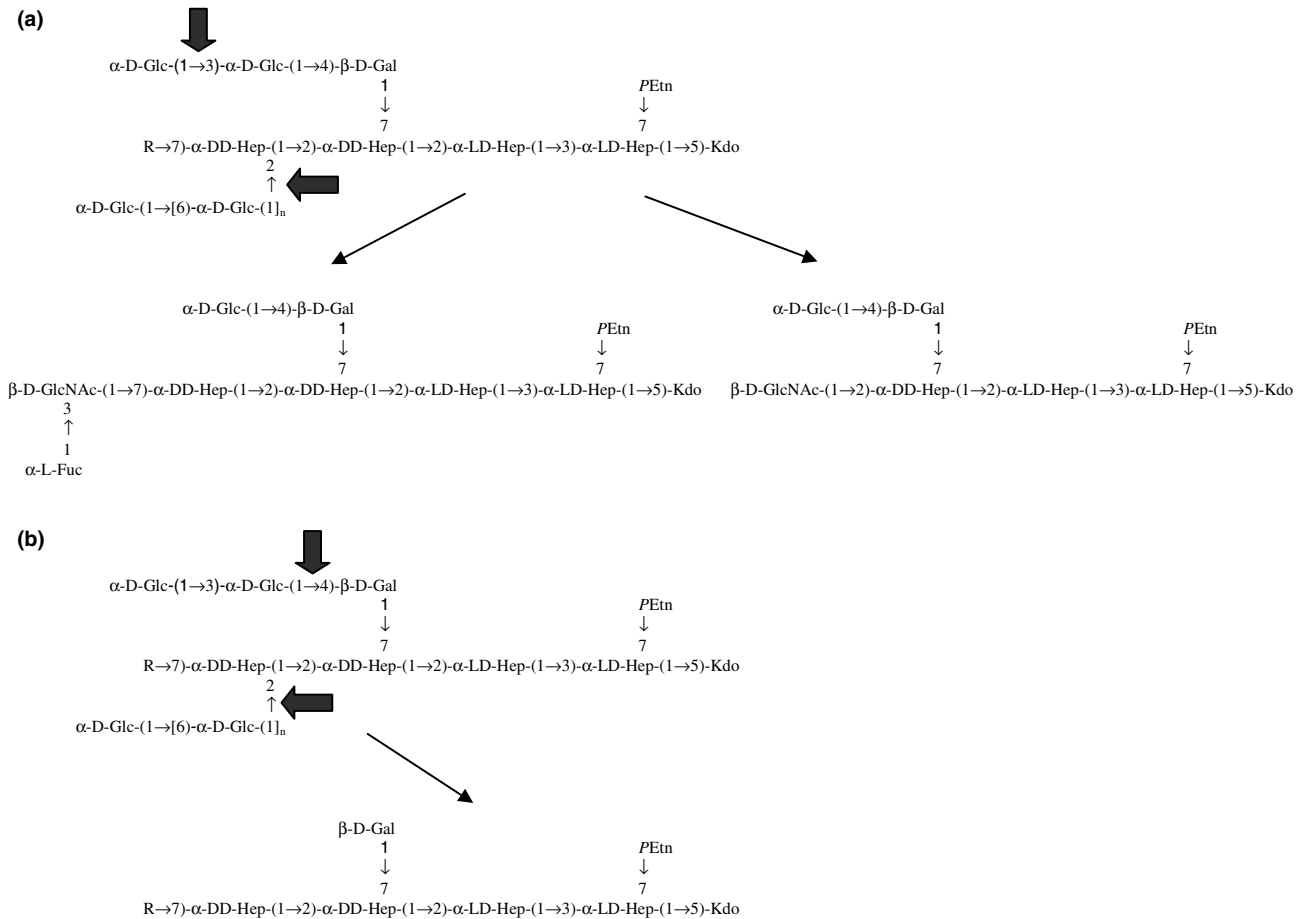


Fig. 4. Schematic representation of the role of the $\alpha(1,2/3)$ -glucosyltransferase activity of HP0159 (a) and the $\alpha(1,2/4)$ -glucosyltransferase of HP1416 (b) in the synthesis of *H. pylori* 26695 LPS, as indicated by bold arrows, and the resulting LPS structures obtained after their mutation.

$\alpha(1,2)$ -transferase activity of HP0159 and HP1416 would be α -DD-Hep, whereas the acceptor of the $\alpha(1,3)$ -activity of HP0159 would be α -D-Glc and that of the $\alpha(1,4)$ -activity of HP1416 would be β -Gal. Thus, different acceptor sugars would be required for the differing transferase activities. It might be argued that based on previous observations on glycosyltransferases, many of which exhibit linkage and acceptor sugar specificity [19], that the deduced common $\alpha(1,2)$ -activity is incorrect, that loss of the $\alpha(1,2)$ -linked glucan chain in the HP0159 and HP1416 mutants is a compensatory modification, and that HP0159 and HP1416 encode simply $\alpha(1,3)$ - and $\alpha(1,4)$ -glucosyltransferases, respectively. Speaking against such an argument is the observed homology of HP0159 and HP1416 with the *rfa* gene of enteric bacteria [19], and that the observed deletion of the $\alpha(1,2)$ -linked saccharide sidechain from the core OS of the HP0159 and HP1416 mutants is a much more dramatic compensatory change compared to the others observed. Furthermore, emerging evidence on glycosyltransferase activities indicates that the acceptor for glycosyltransferases should be considered the saccharide chain, rather than only an individual sugar residue, since

the sugar acceptor is a component of a saccharide chain whose properties can affect the three-dimensional presentation of the acceptor sugar for interaction with the glycosyltransferase. A further interesting question arises as to why the $\alpha(1,2)$ -transferase activity of HP0159 cannot compensate for that in the HP1416 mutant and vice versa. This assumes a direct interplay between the $\alpha(1,2)$ activities of both enzymes and that they act equivalently. It is important to note that the mutation of HP0159 and HP1416 induces other changes in the core OS (deletion of sugars, truncation of the core backbone and addition of terminal sugars), thereby affecting its properties and ability to interact with the glycosyltransferase.

The role of the other *rfaJ*-like gene in *H. pylori* 26695 (HP0208) is less clear. Initial attempts to clone HP0208 in *E. coli* proved unsuccessful (Goldberg et al., unpublished results). The TIGR clone bank does not include a complete copy of this gene, and HP0208 was noted by Tomb et al. [10] to be truncated and contain 11 AG repeats, indicative of phase variation, and poly (A) tracts in its 5' intergenic region. Whether these make this gene unstable in *E. coli* is not known. However, analysis of the AG repeats in HP0208 of strain 26695

indicated that the gene is phase “off”. Thus, in *H. pylori* NCTC 11637 where an untruncated HP0208 occurs, a phase “on” HP0208 with an altered AG tract was engineered to constitutively express this *rfaJ* gene (Moran et al., unpublished results). This mutant expressed high- M_r LPS with an O side chain and Le^x and Le^y epitopes like parental NCTC 11637 and 26695. Further more detailed structural studies on the core OS of this mutant are ongoing to determine the relevance of HP0208.

Collectively, the results of this study show that the respective *rfaJ* genes of *H. pylori* 26695 have different roles in biosynthesis of LPS. This contrasts with other bacteria, such as *E. coli* and *S. typhimurium*, where a single *rfaJ* gene is present, which encodes an $\alpha(1,2)$ -glucosyltransferase involved in the addition of a distal glucose residue to the LPS core [19]. The presence of a single *rfaJ* gene in these enteric bacteria, compared to the multiple homologs in *H. pylori*, may reflect the lesser complexity and biosynthetic requirements of their core OS compared to that of *H. pylori* [8]. Both HP0159 and HP1416 can be deduced to influence the biosynthesis of structures in the core OS of LPS due to their respective $\alpha(1,2/3)$ -glucosyltransferase and $\alpha(1,2/4)$ -glucosyltransferase activities, although associated compensatory changes in the core OS substitution, particularly terminally, are likely to have impeded attachment of the O sidechain expressing Lewis antigens in the mutant HP0159 LPS. Future studies should consider the relevance of phase variability in HP0208 for biosynthesis of *H. pylori* 26695 LPS as well as influences on *H. pylori* pathogenesis.

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