Novel *Helicobacter pylori* α1,2-fucosyltransferase, a key enzyme in the synthesis of Lewis antigens

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*Helicobacter pylori* lipopolysaccharides (LPS) contain complex carbohydrates known as Lewis antigens which may contribute to the pathogenesis and adaptation of the bacterium. Involved in the biosynthesis of Lewis antigens is an α1,2-fucosyltransferase (FucT) that adds fucose to the terminal βGal unit of the O-chain of LPS. Recently, the *H. pylori* (Hp) α1,2-FucT-encoding gene (*fucT2*) was cloned and analysed in detail. However, due to the low level of expression and instability of the protein, its enzymic activity was not demonstrated. In this study, the Hp *fucT2* gene was successfully overexpressed in *Escherichia coli*. Sufficient amounts of the protein were obtained which revealed α1,2-fucosyltransferase activity to be associated with the protein. A series of substrates were chosen to examine the acceptor specificity of Hp α1,2-FucT, and the enzyme reaction products were identified by capillary electrophoresis. In contrast to the normal mammalian α1,2-FucT (H or Se enzyme), Hp α1,2-FucT prefers to use Lewis X [βGal1-4(αFuc1-3)/GlcNAc] rather than LacNAc [βGal1-4/GlcNAc] as a substrate, suggesting that *H. pylori* uses a novel pathway (via Lewis X) to synthesize Lewis Y. Hp α1,2-FucT also acts on type 1 acceptor [βGal1-3/GlcNAc] and Lewis a [βGal1-3(αFuc1-4)/GlcNAc], which provides *H. pylori* with the potential to synthesize H type 1 and Lewis b epitopes. The ability to transfer fucose to a monofucosylated substrate (Lewis X or Lewis a) makes Hp α1,2-FucT distinct from normal mammalian α1,2-FucT.

**Keywords:** *Helicobacter pylori*, α1,2-fucosyltransferase, Lewis antigens

**INTRODUCTION**

*Helicobacter pylori* lipopolysaccharide (LPS), like the LPS present in the outer membranes of other Gram-negative bacteria, is composed of lipid A, an oligosaccharide core and the antigenic O-polysaccharide chain. Most strains of *H. pylori* express type 2 glycoconjugate antigens Lewis X (Leα) and Lewis Y (Leβ) in their LPS O-chain (Aspinall & Monteiro, 1996; Aspinall et al., 1996; Sherburne & Taylor, 1995; Wirth et al., 1996). Recent studies have also indicated the presence of type 1 epitopes in a small number of *H. pylori* isolates, and the LPS from a single *H. pylori* strain may carry O-chains with type 1 and type 2 Lewis antigens simultaneously (Monteiro et al., 1998; Taylor et al., 1998; Wirth et al., 1997). The structures of these Lewis antigens in *H. pylori* mimic those of the glycomolecules present on human gastric epithelial cell surfaces, although a direct correlation between Lewis expression by *H. pylori* and by the host cells is still uncertain (Taylor et al., 1998; Wirth et al., 1997). Moreover, the expression of Lewis antigens by *H. pylori* displays phenotypic (phase) variation (Appelmelk et al., 1998; Wirth et al., 1999). The molecular mimicry and phase variation of *H. pylori* Lewis antigen expression may contribute to the adaptation of this human gastric pathogen to the host environment. The expression of Lewis antigens by *H. pylori* has also been suggested as a cause of autoimmunity involved in the pathogenesis of chronic type B gastritis and gastric and duodenal ulcers (Appelmelk et al., 1996).

**Abbreviations:** FucT, fucosyltransferase; Hp, *H. pylori*; LacNAc, N-acetyl-lactosamine; Leα, Lewis a; Leβ, Lewis b; Leα1, Lewis X; Leβ1, Lewis Y; TMR, tetramethylrhodamine.
In mammalian cells, the synthesis of Lewis antigens is regulated by several glycosyltransferases that add monosaccharides to a precursor molecule in a sequential fashion (Fig. 1b) (for reviews see Avent, 1997; Herry et al., 1995; Kleene & Berger, 1993; Watkins, 1995). Lewis a (Le\(^a\)) and Lewis b (Le\(^b\)) structures result from the activities of Lewis a (Le\(^a\)) = βGal 1-3 βGlcNAc (Type 1) and Lewis b (Le\(^b\)) = βGal 1-3 βGlcNAc (Type 2) encoded by the Se gene (Ge et al., 1989) were used for growth of \(E. coli\). Ampicillin (100 µg ml\(^{-1}\)) and kanamycin (40 µg ml\(^{-1}\)) or rifampicin (200 µg ml\(^{-1}\)) were added to the above media, if appropriate, for growth of \(E. coli\) or \(H. pylori\) strains.

Fig. 1. Structural relationship between Lewis antigens (a) and the biosynthetic pathways operating in mammalian cells (b). The pathways shown in (b) are adapted from the references of Avent (1997) and Herry et al. (1995), and the corresponding type 2 structures are included in parentheses. The dashed arrow represents the unusual pathway for the synthesis of Lewis Y and Lewis b. Abbreviations for sugars: Gal, galactose; GlcNAc, N-acetylglucosamine; Fuc, fucose.

<table>
<thead>
<tr>
<th>Type 1 series</th>
<th>Type 2 series</th>
</tr>
</thead>
<tbody>
<tr>
<td>βGal 1-3 βGlcNAc (Type 1)</td>
<td>βGal 1-4 βGlcNAc (Type 2)</td>
</tr>
<tr>
<td>H type 1: βGal 1-3 βGlcNAc 2</td>
<td>H type 2: βGal 1-4 βGlcNAc 2</td>
</tr>
<tr>
<td>Lewis a: βGal 1-3 βGlcNAc 4</td>
<td>Lewis X: βGal 1-4 βGlcNAc 3</td>
</tr>
<tr>
<td>Lewis b: βGal 1-3 βGlcNAc 2</td>
<td>Lewis Y: βGal 1-4 βGlcNAc 3</td>
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\(z_1,2\)-FucT was also found in the normal cells of rabbit (Hiroshi et al., 1996).

While the biosynthesis of Lewis antigens in mammalian cells is widely studied, little is known about the synthetic pathways and the genes/enzymes involved in biosynthesis of Lewis antigens in \(H. pylori\). Recently, a gene encoding \(z_1,3\)-FucT in \(H. pylori\) was identified and characterized (Ge et al., 1997; Martin et al., 1997), and the whole genome sequences demonstrated the existence of two copies of this gene in the genome of \(H. pylori\). So far, neither a gene encoding \(z_1,4\)-FucT nor any enzyme activity of \(z_1,4\)-FucT has been identified in \(H. pylori\). In a previous study, we analysed the putative \(H. pylori\) \(z_1,2\)-FucT-encoding gene (\(fucT2\)) and demonstrated its essential role in the synthesis of Le\(^y\) by knock-out mutagenesis (Wang et al., 1999). Here we report the development of a sensitive assay system for detection of \(H. pylori\) \(z_1,2\)-FucT enzyme activity, and the characterization of its properties and functions in the synthesis of Lewis antigens. Different \(H. pylori\) strains express different types of \(fucT2\) genes in which a frameshift mutation at the DNA level and ribosomalframeshifting at the translation level may be involved (Wang et al., 1999).

We have proceeded to examine an enzyme encoded by a variant of the \(fucT2\) gene. Furthermore, we were able to determine the enzyme activities directly from some \(H. pylori\) isolates by using selected acceptors, even though the activities are present at very low levels.

**METHODS**

**Bacterial strains and media.** \(H. pylori\) strains UA802, UA1182, UA1195 and UA1234 used in this study were clinical isolates from the University of Alberta Hospital. \(H. pylori\) cells were cultured on BHI-YE (37% brain heart infusion with 0.3% yeast extract and 5% animal serum) agar plates or in BHI-YE broth under microaerobic conditions. *Escherichia coli* strain CLM4 (ΔrecA lacZ trp A[Δαβ−rif−] upp rel rpsL) (Yao et al., 1992) carrying the plasmid pGP1-2 (Tabor & Richardson, 1985) was used for overexpression of \(Hp\) \(fucT2\) genes. Plasmid pGP1-2 carries the gene encoding T7 RNA polymerase under the control of a heat-inducible Plac promoter. LB medium, M9 medium and a supplemented M9 medium (Sambrook et al., 1989) were used for growth of \(E. coli\) cells. Ampicillin (100 µg ml\(^{-1}\)), kanamycin (40 µg ml\(^{-1}\)) or rifampicin (200 µg ml\(^{-1}\)) were added to the above media, if appropriate, for growth of plasmid-containing cells and for expression of plasmid-encoded proteins.

**DNA manipulation techniques.** Standard DNA manipulation techniques, including the isolation, transformation and restriction enzyme digestion analysis of plasmid DNA, as well as partial DNA sequencing, were as detailed by Sambrook et al. (1989).

**Overexpression of the Hp FucT in E. coli.** In a typical experiment, \(E. coli\) CLM4(pGP1-2), harbouring a plasmid carrying an \(Hp\) \(fucT\) gene (pBKHp763fucT39, pGEMH2, pGEMM6 or pGEMB3) was grown in 25 ml liquid LB medium with appropriate antibiotics (kanamycin and ampicillin) at 30 °C to an OD\(_{600}\) of 0.5−0.7. After being collected, the cells were washed once with M9 medium, resuspended in 5 ml supplemented M9 medium, and further incubated at 30 °C for
1 h. To induce the expression of the \textit{fucT} gene, the culture was shifted to 42 °C by adding 5 ml prewarmed (55 °C) supplemented M9 medium. After incubation at 42 °C for 15 min, rifampicin was added to a final concentration of 200 μg ml⁻¹, and cell growth was continued at 42 °C for 20 min.

For analysis of the protein by SDS-PAGE, a small aliquot (0.5 ml) of the cell culture was taken, and 2.5 μl [³²P]methionine (4-35×10⁶ Bq mmol⁻¹, 3.7×10⁴ Bq ml⁻¹, New England Nuclear) was added. After further growth at 30 °C for 30 min, the cells were harvested, resuspended in 100 μl sample buffer (50 mM Tris/HCl, pH 6.8; 1%, w/v, SDS; 20 mM EDTA; 1%, v/v, mercaptoethanol; 10%, v/v, glycerol), and boiled for 3 min before loading on to the gel. For the preparation of the sample for the enzyme assay, the remaining part (major aliquot, 9.5 ml) of the cell culture after induction was further incubated at 30 °C for 30 min, then harvested. The cells were washed with 1.5 ml 20 mM HEPES buffer (pH 7.0), and resuspended in 1.5 ml of this buffer supplemented with 0.5 mM PMSF.

\textbf{Preparation of cell lysates or cell extracts for the fucosyltransferase assay.} The \textit{E. coli} cells containing overproduced Hp FucT proteins, which were in HEPES buffer with PMSF as described above, were disrupted with a French press at 7000 p.s.i. (48 kPa) at 4 °C. The cell lysates were used directly for enzyme assays. For determining the location of the enzyme activities, the cytoplasmic and membrane fractions were separated as follows. The cell lysates were centrifuged at 128,000 g (Beckman TL100/rotor 100.2) at 4 °C for 1 h. The supernatant was collected as the cytoplasmic fraction. The membrane pellets were resuspended in a small volume of the same buffer and treated with 1 M NaCl.

For determining the enzyme activity from \textit{H. pylori} cells, cells grown for 3 d in 25 ml BHI-YE broth were harvested and washed with 5 ml 20 mM HEPES buffer (pH 7.0). Finally the cells were resuspended in 2 ml of the same HEPES buffer plus 0.5 mM PMSF. The \textit{H. pylori} cells were disrupted with a French press as described above for \textit{E. coli} cells, and the cell lysates were directly used for enzyme assays.

\textbf{Fucosyltransferase assay.} Assays of Hp \textit{z1,2-} and \textit{z1,3-FucT} activities were carried out according to the method described by Chan \textit{et al.} (1995) with some modifications. Reactions were conducted at 37 °C for 20 min in a volume of 20 μl containing 18 mM acceptor, 50 μM GDP-fucose, 60/000 d.p.m. GDP-[³²P]fucose, 20 mM HEPES buffer (pH 7.0), 20 mM MnCl₂, 0.1 M NaCl, 35 mM MgCl₂, 1 mM ATP, 5 mg BSA ml⁻¹, and 6.2 μl of the enzyme preparation. The acceptors used in this study were: LacNAC [Ga11-4GlcNAc], Le inner core (Gal-3);[Fuc1-3]GlcNAc, type 1 [Ga11-3]GlcNAc] and Le inner core (Gal-3;[Fuc1-4])GlcNAc]. These acceptors were provided by Dr. O. Hindsgaul, Department of Chemistry, University of Alberta. GDP-[³²P]fucose (1-9×10¹¹ Bq ml⁻¹, 1 μmol⁻¹) was obtained from American Radiolabelled Chemicals. Sep-Pak Plus C-18 reverse-phase cartridges were purchased from Waters. For calculation of the specific activity of the enzyme [micro-units (μU) per mg protein], protein concentrations of the cell extracts were determined with a BCA protein assay kit (Pierce) and cell growth was continued at 42 °C for 42 °C.

\textbf{RESULTS}

\textbf{Overexpression of Hp \textit{z1,2-FucT} protein in \textit{E. coli}}

Initially, we found it was very difficult to detect \textit{z1,2-FucT} activity from \textit{H. pylori}, due to the low level of its expression and the instability of the protein. Therefore, we sought to establish a system for overexpression of the Hp \textit{fucT} gene in \textit{E. coli} to obtain high yields of proteins. As described in Methods, the plasmid containing the Hp \textit{fucT} gene under the control of the T7 promoter was transferred into \textit{E. coli} CLM4(pGPl-2), and the expression of the gene was induced by shifting the host cells from 30 to 42 °C (Tabor & Richardson, 1985). As a reference, we included a previously cloned Hp \textit{z1,3-FucT}-encoding gene carried on the plasmid pBKHp763fucT39 (Ge \textit{et al.}, 1997). Using the \textit{E. coli} CLM4(pGPl-2) gene expression system, we obtained the overexpressed Hp \textit{z1,3-FucT} protein with an expected molecular mass of 52 kDa (Fig. 2b, lane 1). The yield of the protein, in terms of the fraction of the FucT protein in the total proteins, was much higher than that reported previously (Ge \textit{et al.}, 1997), in which the gene was expressed in \textit{E. coli} CSREDE3 cells with the induction by IPTG. Correspondingly, we detected a specific \textit{z1,3-FucT} activity of 1480 μU (mg protein)⁻¹ (in the whole-cell extract) by using LacNAC as an acceptor (Table 1, A), which is much higher than those obtained before [26 μU (mg cytoplasmic protein)⁻¹ and 700 μU (mg membrane protein)⁻¹]. Similarly, a considerably high amount of \textit{z1,2-FucT} protein (33 kDa) was obtained from the expression of the cloned \textit{fucT} gene in the plasmid pGEM16 (Fig. 2, lane 5), which enabled us to detect \textit{z1,2-FucT} activity.

\textbf{Acceptor specificity of Hp \textit{z1,2-FucT}}

Plasmid pGEM16 carries the prototype \textit{fucT} gene from \textit{H. pylori} UA802, which produces a high level of \textit{Le}³. The disruption of this gene in the bacterium resulted in no \textit{Le}³ production, suggesting that its gene product is involved in \textit{Le}³ synthesis (Wang \textit{et al.}, 1999). Initially, we quantified the \textit{z1,2-FucT} activity by using LacNAC and \textit{Le}³ as acceptors, the two potential substrates of \textit{z1,2-FucT} for the synthesis of \textit{Le}³ (Fig. 1). Surprisingly, almost no activity was detected using LacNAC as an acceptor, whereas considerable activity was observed for the monofucosylated \textit{Le}³ acceptor (Table 1, B). The

\textit{H. pylori} \textit{z1,2-fucosyltransferase}
**Table 1. Activities of *H. pylori* fucosyltransferases detected from the proteins overexpressed in *E. coli***

<table>
<thead>
<tr>
<th>Overexpressed protein* (plasmid construct)</th>
<th>Acceptor</th>
<th>Proposed product</th>
<th>Specific activity (µU mg−1)†</th>
<th>Relative activity (%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>A  α1,3-FucT (pBKHp763fucT39)</td>
<td>LacNAc</td>
<td>LeX</td>
<td>1480</td>
<td>45</td>
</tr>
<tr>
<td>B  α1,2-FucT (UA802) (pGEMI6)</td>
<td>LacNAc</td>
<td>H type 2</td>
<td>150 ± 33</td>
<td>99</td>
</tr>
<tr>
<td>C  α1,2-FucT (UA802) (N-truncated, pGEMH2)</td>
<td>LacNAc</td>
<td>H type 2</td>
<td>309 ± 28</td>
<td>100</td>
</tr>
<tr>
<td>D  α1,2-FucT (26695) (pGEMB3)</td>
<td>LacNAc</td>
<td>H type 2</td>
<td>301 ± 13</td>
<td>97</td>
</tr>
</tbody>
</table>

*E. coli* whole-cell extract containing the overexpressed *H. pylori* FucT protein was used for the enzyme assay.

† A micro-unit (µU) of the enzyme is expressed as the amount of enzyme that converts 1 pmol of acceptor to product per min. Specific activity was obtained by dividing the total activity (µU) by the total protein content (mg) in the whole-cell extract. The data were averaged from at least three independent determinations; standard deviations are shown.

‡ Percentage activity relative to that of UA802 α1,2-FucT on its best substrate, type 1.

specific activity of α1,2-FucT was much lower than that of α1,3-FucT (Table 1, A).

In mammalian cells, the same α1,2-FucT enzyme (H or Se, tissue-specific) is normally responsible for the synthesis of both H type 1 and H type 2 structures (Sarnesto et al., 1990, 1992). To determine whether the Hp α1,2-FucT is also involved in the synthesis of Leα, we measured its activity with type 1 oligosaccharide acceptors (Table 1, B). Even though UA802 does not express type 1 Lewis antigen, its α1,2-FucT enzyme can transfer fucose to type 1 and Leα acceptors. Compared to Leβ, type 1 and Leα are even more efficient substrates for Hp α1,2-FucT (twofold more active). Thus, Hp α1,2-FucT can also synthesize H type 1 and Leα.

**Analysis of the reaction products of Hp α1,2-FucT by capillary electrophoresis**

The reaction products synthesized from different acceptors by the Hp α1,2-FucT were further characterized by capillary electrophoresis with laser-induced fluorescence detection. The reaction mixture contained the overproduced UA802 α1,2-FucT protein (from the pGEMI6 clone), GDP-fucose, and different acceptors labelled with TMR. The results (Fig. 3) confirmed the data from the enzyme assay using radioactive labelled GDP-fucose (Table 1, B) by identifying the products of the reactions. When using LacNAc as an acceptor (Fig. 3a, trace A), no reaction product representing H type 2 was observed, suggesting that LacNAc is not a substrate for Hp α1,2-FucT. In the reaction using Leβ as an acceptor (Fig. 3a, trace B), a small new peak was produced, which co-migrated with a synthetic Leβ-TMR (standard Leβ) in the electropherogram, indicating that this new peak represents the Leβ product synthesized from Leβ by Hp α1,2-FucT. Similarly, by using type 1 or Leα as acceptors (Fig. 3b), new peaks co-migrating with authentic products, H type 1 or Leα respectively, were observed. As negative controls, the protein extract from the *E. coli* CLM4(pGP1-2) clone containing the pGEM vector without the Hp *fucT2* gene was used in the reactions for each acceptor tested above; no peaks for the products of α1,2-FucT were observed (data not shown).

**Hp α1,2-FucT is a soluble protein**

DNA sequence analysis predicted the Hp α1,2-FucT to be a hydrophilic protein (Wang et al., 1999), and the same is true for Hp α1,3-FucT (Ge et al., 1997). However, the determination of Hp α1,3-FucT activity from the overexpressed proteins demonstrated that the majority of the activity was present in the membrane fraction (Ge et al., 1997). To delineate the cellular location of the Hp α1,2-FucT activity, cytoplasmic and membrane fractions of *E. coli* cells overproducing Hp α1,2-FucT proteins were prepared as described in Methods. The activity in both fractions was determined.
α expression of molecular masses, which represent N-terminally truncated gene (lane 4). Two major bands with smaller masses smaller than 33 kDa were obtained. These two bands were also observed in cells harbouring pGEMI6 containing the entire fucT2 gene (lane 5), but not in cells containing the pGEM vector alone (lane 3). In the 5’ region of the Hp fucT2 gene (GenBank accession no. AF076779), we identified two additional putative translation start codons (ATG) with upstream Shine–Dalgarno sequences. Translation starting from them could produce the identified N-terminal truncated proteins. Determination of the enzyme activity from the

Fig. 2. Overexpression of H. pylori fucosyltransferases in E. coli. (a) Plasmid constructs containing intact or partial H. pylori fucT genes. Heavy arrows represent the predicted ORFs, and the thin lines indicate the flanking DNA regions that have been cloned together with the coding region into the vector. The small arrows indicate the direction of transcription from the T7 promoter. pBKHp763fucT39 was from Ge et al. (1997); pGEMH2, pGEMI6 and pGEMB3 were described previously (Wang et al., 1999). The numbers on the left for each plasmid construct correspond to the lane numbers in (b). (b) Autoradiograph of a 0.1% SDS-12% polyacrylamide gel analysing the proteins overexpressed from various plasmid constructs in E. coli CLM4(pGPl-2). As the same volume of cell extract was loaded on each lane, there could be some variation in the amount (mg) of the proteins, especially for the sample in lane 1 (pBKHp763fucT39), which was expressed from a different vector. Lane 1, expression of pBKHp763fucT39 produced a high amount of 52 kDa α,1,3-FucT. Lane 2, no plasmid construct. Lane 3, pGEM-T vector without fucT2 gene. Lanes 4, 5 and 6, expression of α,1,2-FucT from plasmid constructs pGEMH2, pGEMI6 and pGEMB3, respectively. The full-length protein (33 kDa) marked by an arrow on the right was overexpressed from intact UA802 fucT2 gene (lane 5) but not from the 5’-truncated gene (lane 4). Two major bands with smaller molecular masses, which represent N-terminally truncated α,1,2-FucT, are indicated. The expression of 26695 fucT2 gave rise to a faint band of 33 kDa full-length α,1,2-FucT and a faint band of 17 kDa half-length α,1,2-FucT (lane 6). The molecular mass markers (BenchMark Prestained Protein Ladder, GiboBRL) are indicated on the left.

using LeX or type 1 as acceptors (Table 2). There was no detectable activity in the membrane fraction when using LeX as an acceptor. By using type 1 as an acceptor, a very low amount of activity (negligible) was detected in the membrane fraction, which accounted for less than 3% of the total activity. These results indicated that Hp α,1,2-FucT is a soluble cytoplasmic protein. Compared to the data shown in Table 1, which were obtained from measurement of immediate cell lysates, the specific activities [μU (mg protein)−1] obtained here are much lower (three- to fourfold). Most probably, many enzyme activities were lost in the procedure for separating cytoplasmic and membrane fractions.

N-terminally truncated Hp α,1,2-FucT has no activity

As expected, the expression of the plasmid pGEMH2, which carries a 5’-truncated fucT2 gene from UA802, did not produce the full-length protein (Fig. 2, lane 4). However, two major protein bands with molecular masses smaller than 33 kDa were obtained. These two bands were also observed in cells harbouring pGEMI6 containing the entire fucT2 gene (lane 5), but not in cells containing the pGEM vector alone (lane 3). In the 5’ region of the Hp fucT2 gene (GenBank accession no. AF076779), we identified two additional putative translation start codons (ATG) with upstream Shine–Dalgarno sequences. Translation starting from them could produce the identified N-terminal truncated proteins.
Table 2. Enzyme activities of *H. pylori* α1,2-FucT in cytoplasmic and membrane fractions

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Acceptor used</th>
<th>Protein fraction*</th>
<th>Specific activity [μU (mg protein)⁻¹]</th>
<th>Total activity (μU)†</th>
<th>Relative activity (%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Le⁻¹</td>
<td>cytoplasm</td>
<td>38</td>
<td>49</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>membrane</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Le⁻¹</td>
<td>cytoplasm</td>
<td>41</td>
<td>54</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>membrane</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Type 1</td>
<td>Type 1</td>
<td>cytoplasm</td>
<td>78</td>
<td>108</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>membrane</td>
<td>8</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*In a typical experiment described in Methods, each protein sample was prepared from 25 ml cell culture of *E. coli* CLM4(pGP1-2, pGEMI6) containing the overexpressed UA802 α1,2-FucT. The cytoplasmic and membrane fractions were separated as described in Methods.
†Total activity (μU) in each protein sample was derived from a 25 ml cell culture.
‡Percentage of the total activity in the cytoplasmic or membrane fraction.

overexpressed protein extract of *E. coli* CLM4(pGP1-2) harbouring pGEMH2 demonstrated no activity for the N-terminally truncated α1,2-FucT proteins (Table 1, C). Therefore, only the full-length protein has functional fucosyltransferase activity. Production of some N-truncated α1,2-FucT in *in vivo* may be an additional mechanism for down-regulating the enzyme activity, contributing to the variable expression of Lewis antigens in *H. pylori*.

Lower-level expression of the α1,2-FucT from *H. pylori* strain 26695

The *fucT2* gene in *H. pylori* 26695 is a variant because it is split into two potential smaller ORFs due to frameshift mutation at the centre of the gene (Fig. 2a, pGEMB3). *In vitro* expression of this gene has demonstrated that the full-length protein (equivalent to that of prototype UA802 α1,2-FucT) can be produced from this gene, most probably by a mechanism of translational frameshifting (the frequency was around 50%) (Wang et al., 1999). In this study, the plasmid pGEMB3 carrying the 26695 *fucT2* gene was transferred into *E. coli* CLM4(pGP1-2), and the gene was expressed in the same way as described above. In contrast to UA802 *fucT2* gene (pGEMI6; Fig. 2, lane 5), the expression of the 26695 *fucT2* gene produces a much lower amount of the full-length protein (Fig. 2, lane 6). Concomitantly, an additional faint band at 17 kDa, representing the half-length α1,2-FucT, was observed. This suggested that the expression of the gene *in vitro* may be very different from that *in vivo*.

In agreement with the low amount of the expressed protein, a low level of enzyme activity was detected for 26695 α1,2-FucT (Table 1, D). Using LacNAc or Le⁻¹ as acceptor, the activity was undetectable. Using type 1 or Leα as acceptor, a low activity of about 20 μU (mg protein)⁻¹ was detected, which is only about 7% of the activity of UA802 α1,2-FucT. Considering that UA802 α1,2-FucT has a lower activity on Leα than on type 1 or Leα, it is not surprising that the activity of 26695 α1,2-FucT on Le⁻¹ is too low to be detected.

Detection of α1,2-FucT activity directly from *H. pylori* cell extracts

After the characterization of the Hp α1,2-FucT protein overproduced in *E. coli*, we attempted to detect the enzyme activity directly from *H. pylori* cells, which would be useful for screening high α1,2-FucT-producing strains. Two major difficulties hinder the achievement of this goal: (i) the expression level of the enzyme in natural *H. pylori* cells is very low; (ii) other fucosyltransferases (mainly α1,3-FucT), which co-exist in *H. pylori* cell extracts and have much higher activity than α1,2-FucT, interfere with the enzyme assay when using some acceptors such as LacNAc that are not specific for α1,2-FucT. Therefore, we analysed the enzyme activity from different strains and using different acceptors. Finally, we succeeded in detecting very low levels of α1,2-FucT activities from some Le⁻¹-producing *H. pylori* strains (Table 3).

First, using LacNAc as an acceptor, both cell extracts of the wild-type UA802 and its *fucT2* knock-out mutant (802ΔH) gave a very high level of activity [around 1000 μU (mg protein)⁻¹; not shown]. This activity represents that of α1,3-FucT, because (i) in 802ΔH cells the α1,2-FucT-encoding gene is disrupted, but the α1,3-FucT-encoding gene is intact; and (ii) LacNAc is not a substrate for α1,2-FucT, but is an excellent substrate for α1,3-FucT. A true activity of α1,2-FucT, even though low, was demonstrated by using Le⁻¹ or type 1 as acceptor. In agreement with the observation that the overexpressed *H. pylori* α1,2-FucT has highest activity on type 1 acceptor (Table 1, B, D), the α1,2-FucT activity from *H. pylori* cell extracts can be consistently detected by using type 1 as an acceptor. As these strains express Le⁻¹, the α1,2-FucTs are supposed to be functional on the Le⁻¹ acceptor. However, for some strains such as UA802 and UA1195, the activity on Le⁻¹ is too low to be
Table 3. α1,2-FucT activities (µU mg⁻¹) from different H. pylori cell extracts

<table>
<thead>
<tr>
<th>H. pylori strain*</th>
<th>LeX phenotype†</th>
<th>LeX used</th>
<th>Type 1‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>802AH</td>
<td>−</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>UA802</td>
<td>+</td>
<td>0 ± 5</td>
<td>26 ± 8</td>
</tr>
<tr>
<td>UA1182</td>
<td>+</td>
<td>21 ± 5</td>
<td>22 ± 7</td>
</tr>
<tr>
<td>UA1195</td>
<td>+</td>
<td>0 ± 4</td>
<td>26 ± 7</td>
</tr>
<tr>
<td>UA1234</td>
<td>+</td>
<td>25 ± 6</td>
<td>21 ± 6</td>
</tr>
</tbody>
</table>

*802AH is a knock-out mutant of UA802 in which the fucT2 gene was disrupted by the insertion of a chloramphenicol-resistance cassette (Wang et al., 1999). It is used here as a negative control for the α1,2-FucT activity. The activities in other strains are the scores above this background value, and are averaged from three determinations, with standard deviations shown.

†The LeX phenotypes are based on the ELISA results of these strains (Wang et al., 1999).

‡Type 1 could also be the substrate for α1,4-FucT. However, all the H. pylori strains listed here do not produce any Leα or Leβ. Therefore, it is unlikely that these H. pylori cell extracts contain any α1,4-FucT activity, as seen in 802AH. Thus, the enzyme activities detected here could be considered as those of α1,2-FucT.

detected in the current assay. For other strains such as UA1182 and UA1234, a similar activity on type 1 and on LeX was detected. This suggested that the enzymes from different strains may have different acceptor specificities. It should be noted, however, that the activities shown here are all at the minimal detectable level (about 10-15-fold lower than those detected from the heterologously overexpressed protein extracts). Therefore, determination of the enzyme activity from H. pylori cell extracts may be helpful for identifying the strains that produce high level of α1,2-FucT, but may not be accurate for evaluating the acceptor specificity of the enzyme.

DISCUSSION

In this study, we identified the catalytic activity of α1,2-FucT of H. pylori. We found that the enzyme is soluble and the activity is present in the cytoplasmic fraction. Under our assay conditions the enzyme activity can be consistently detected if it is above 10 µU (mg protein)⁻¹, whereas an activity below that could be considered undetectable. According to this criterion, no activity was detected for the N-terminally truncated α1,2-FucT, and a very low level of activity for the variant α1,2-FucT (from strain 26695). A considerably high level of activity was detected from the overproduced intact α1,2-FucT from H. pylori UA802, which shows a high level of expression of Lewis Y. However, compared with that of α1,3-FucT, the activity of α1,2-FucT is much lower (Table 1). In vivo, the expression of α1,2-FucT may be controlled at a very low level, because the O-antigen of LPS structure normally consists of poly-LeX units and a single terminal LeX (Monteiro et al., 1998). The low activity of α1,2-FucT in vitro, on the other hand, could be due to its instability. We noticed that Hp α1,2-FucT lost its activity more rapidly than Hp α1,3-FucT. Therefore, we determined the enzyme activity immediately after the cells were lysed and used a short assay time (20 min).

Recently, we have shown that H. pylori mutants carrying a disrupted fucT2 gene (encoding α1,2-FucT) express more LeX than the wild-type cells (Wang et al., 1999). This phenomenon may suggest that LeX is the direct substrate for LeX synthesis, but the mutagenesis experiment itself cannot exclude the other possible pathway of LeX synthesis (via H type 2), because disruption of α1,2-FucT might lead to accumulation of LacNAc, providing more substrates for α1,3-FucT to synthesize LeX (Fig. 4a). Therefore, determination of activities of the fucosyltransferases responsible will be direct proof to distinguish between the two possible pathways. The observation in this study that LeX but not LacNAc is the substrate for the Hp α1,2-FucT clearly indicated that H. pylori prefers to use the LeX pathway to synthesize LeY (Fig. 4a). Other supporting evidence came from the enzyme assay for Hp α1,3-FucT: (i) LacNAc is an excellent substrate for Hp α1,3-FucT (Ge et al., 1997; Martin et al., 1997; this study, Table 1, A); and (ii) Martin et al. (1997) found that H type 2 was not the strain of an Hp α1,3-FucT. It should be noted, however, that the fucosyltransferases from different H. pylori strains may have different acceptor specificity. Further studies on combined analysis of the α1,3- and α1,2-FucTs from various H. pylori strains are needed to elucidate whether this novel pathway for the...
The synthesis of Le\textsuperscript{Y} is general in \textit{H. pylori} or is strain-specific.

In addition to its function in Le\textsuperscript{Y} synthesis, Hp z1,2-FucT is also active on type 1 Lewis structures (summarized in Fig. 4b). This provides a basis for the recent finding that type 1 (Le\textsuperscript{a}), H type 1 and Le\textsuperscript{a} are expressed in certain \textit{H. pylori} strains (Le\textsuperscript{b} was also detected in some strains by serological methods but has not yet been confirmed by structural analysis) (Monteiro \textit{et al}., 1998).

Here again, the ability of the Hp z1,2-FucT to synthesize Le\textsuperscript{0} from Le\textsuperscript{a} indicated that this bacterial enzyme is different from the normal mammalian counterparts, which cannot use Le\textsuperscript{a} as substrate. To know if Le\textsuperscript{b} can be synthesized from H type 1 in \textit{H. pylori} awaits the detection of an z1,4-FucT. The z1,2-FucT characterized in this study is from \textit{H. pylori} strain UA802, which does not produce any type 1 Lewis antigen. This suggests that the same z1,2-FucT enzyme could be used in the strains that produce type 1 epitopes. The failure to produce type 1 Lewis antigens in many \textit{H. pylori} strains could be due to the unavailability of one of the other enzymes involved in the synthesis of Lewis antigens such as galactosyltransferase, which adds βGal to GlcNAc, or z1,3/4-FucT, which places the zFuc unit at βGlcNAc.

Aberrant glycosylation seems to be crucial in human tumour progression (Hakomori, 1989). In addition to that of sialyl Le\textsuperscript{a} and sialyl Le\textsuperscript{X}, the role of Le\textsuperscript{b} and Le\textsuperscript{Y} as ligands for E-selectin and in adhesion to tumour necrosis factor α-activated endothelial cells has also been demonstrated (Kannagi, 1997; Miyake & Hakomori, 1991; Sakamoto \textit{et al}., 1986). z1,2-FucT, the key enzyme regulating the biosynthesis of these structures, has become a marker of tumour progression (Sun \textit{et al}., 1995). Here, we show that \textit{H. pylori} z1,2-FucT is functional in the synthesis of both Le\textsuperscript{b} and Le\textsuperscript{Y}, and the synthetic pathways (Fig. 4) are similar to those found in some human cancer cells or tissues (Blaszczyk-Thurin \textit{et al}., 1988; Yazawa \textit{et al}., 1993) (Fig. 1b, unusual pathway). We have shown that the expression of the z1,2-FucT-encoding gene in \textit{H. pylori} is regulated at multiple levels including replication, transcription and translation (Wang \textit{et al}., 1999), and the expression of this gene in \textit{H. pylori} cells is at a very low level (Table 3). Whether elevated expression of this gene/enzyme \textit{in vivo}, when \textit{H. pylori} cells are attached to human gastric epithelial cells, is related to \textit{H. pylori}-associated development of human gastric cancer is an important issue which needs to be addressed. To our knowledge, \textit{H. pylori} z1,2-FucT is the first bacterial z1,2-fucosyltransferase that has been characterized. In addition to the biological advantages that \textit{H. pylori} might gain with altered specificity of its z1,2-FucT compared to the counterpart of its host, the novel substrate specificity is of great potential pharmaceutical interest for enzymic synthesis of oligosaccharides.

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