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

Ge Wang,1 Peter G. Boulton,1 Nora W. C. Chan,2 Monica M. Palcic2 and Diane E. Taylor1

INTRODUCTION

*Helicobacter pylori* lipopolysaccharide (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)βGlcNAc] rather than LacNAc [βGal1-4βGlcNAc] 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 [βGal1-3βGlcNAc] and Lewis a [βGal1-3(αFuc1-4)βGlcNAc], 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

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**Abbreviations:** FucT, fucosyltransferase; Hp, *H. pylori*; LacNAc, N-acetyl-lactosamine; Leα, Lewis a; Leβ, Lewis b; LeX, Lewis X; LeY, 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), 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.

**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.

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 z1,3-FucT in *H. pylori* was identified and characterized (Ge et al., 1997; Martin et al., 1997), and the whole genome sequences (Alm et al., 1999; Tomb et al., 1997) demonstrated the existence of two copies of this gene in the genome of *H. pylori*. So far, neither a gene encoding z1,4-FucT nor any enzyme activity of z1,4-FucT has been identified in *H. pylori*. In a previous study, we analysed the putative *H. pylori* (Hp) z1,2-FucT-encoding gene (*fucT2*) and demonstrated its essential role in the synthesis of Leα by knock-out mutagenesis (Wang et al., 1999). Here we report the development of a sensitive assay system for detection of Hp z1,2-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 ribosomal frameshifting 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°C 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 ([ΔaceA lacZ trp A(sbcB–rfb) 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, pgEMB3 or pgEMB5) was grown in 25 ml liquid LB medium with appropriate antibiotics (kanamycin and ampicillin) at 30°C to an OD₆₀₀ 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 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 [³⁵S]methionine (4.35 × 10⁷ Bq mmol⁻¹, 3.7 × 10⁸ 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 gels. 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.

Preparation of cell lysates or cell extracts for the fusocyltransferase assay. The 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 13000 g at 4 °C for 10 min. The cell debris were discarded and the supernatant was subjected to ultracentrifugation at 128000 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 H. pylori, cells grown for 3 d in 25 ml BHI-YE broth were harvested and grown for 3 d in 25 ml BHI-YE broth were harvested and French press as described above for 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 13000 g at 4 °C for 10 min. The cell debris were discarded and the supernatant was subjected to ultracentrifugation at 128000 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.

Fusocyltransferase assay. Assays of Hp z1,2- and z1,3-FucT activities were carried out according to the method described by Chan 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, 60000 m.d.p. GDP-[³²P]fucose, 20 mM HEPES buffer (pH 7.0), 20 mM MgCl₂, 35 mM MnCl₂, 1 mM ATP, 10 mg BSA ml⁻¹, and 62 µl of the enzyme preparation. The acceptors used in this study were: LacNAc βGal1-3(Fuc1-3)GalNAc, βGal1-4(Fuc1-3)βGalNAc, βGal1-3(Fuc1-3)βGalNAc, type 1 [βGal1-3]GlcNAc and Le [βGal1-3]GlcNAc. These acceptors were provided by Dr O. Hindsgaul, Department of Chemistry, University of Alberta. GDP-[³²P]fucose (1.9 × 10¹¹ Bq ml⁻¹ mmol⁻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) using BSA as a standard according to the supplier's instructions.

Capillary electrophoresis assays. These assays were performed to identify the products synthesized by the protein preparation of E. coli cells overexpressing UA802 z1,2-FucT. For a negative control, the protein preparation of E. coli cells containing pGEM vector was used. The reaction mixture, in a volume of 20 µl, contained 8 µl of the protein preparation, 18 mM acceptor labelled with tetramethylrhodamine (TMR), 18 mM GDP-fucose, 20 mM HEPES buffer (pH 7.0), 20 mM MnCl₂, 0.1 M NaCl, 35 mM MgCl₂, 1 mM ATP and 5 mg BSA ml⁻¹. The reaction was carried out at 37 °C for 20 min. The sample was applied to a conditioned Sep-Pak C-18 Cartridge (Waters Corp., USA), washed with 20 ml water, and the TMR-labelled oligosaccharides were eluted with 3 ml HPLC-grade methanol. Subsequently, the sample was prepared and analysed by capillary electrophoresis by injecting 12 pl onto a column (60 cm long × 10 µm i.d.) at 1 kV for 5 s as described previously (Chan et al., 1995). The electrophoretic separations were performed at a running voltage of 400 V cm⁻¹.

RESULTS

Overexpression of Hp z1,2-FucT protein in E. coli

Initially, we found it was very difficult to detect z1,2-FucT activity from 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 fucT2 gene in E. coli to obtain high yields of proteins. As described in Methods, the plasmid containing the Hp fucT2 gene under the control of the T7 promoter was transferred into E. coli CLM4(pGP1-2), 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 z1,3-FucT-encoding gene carried on the plasmid pBKHp763fucT39 (Ge et al., 1997). Using the E. coli CLM4(pGP1-2) gene expression system, we obtained the overexpressed Hp 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 et al., 1997), in which the gene was expressed in E. coli CS5DE3 cells with the induction by IPTG. Correspondingly, we detected a specific 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 z1,2-FucT protein (33 kDa) was obtained from the expression of the cloned fucT2 gene in the plasmid pGEM16 (Fig. 2, lane 5), which enabled us to detect z1,2-FucT activity.

Acceptor specificity of Hp z1,2-FucT

Plasmid pGEM16 carries the prototype fucT2 gene from H. pylori UA802, which produces a high level of Leα. The disruption of this gene in the bacterium resulted in no Leα production, suggesting that its gene product is involved in Leα synthesis (Wang et al., 1999). Initially, we quantified the z1,2-FucT activity by using LacNAc and Leα as acceptors, the two potential substrates of z1,2-FucT for the synthesis of Leα (Fig. 1). Surprisingly, almost no activity was detected using LacNAc as an acceptor, whereas considerable activity was observed for the monofucosylated Leα acceptor (Table 1, B). The
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 LeX, 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 LeX as an acceptor (Fig. 3a, trace B), a small new peak was produced, which co-migrated with a synthetic LeX−TMR (standard LeY) in the electropherogram, indicating that this new peak represents the LeY product synthesized from LeX 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.

### 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>100</td>
</tr>
<tr>
<td>B α1,2-FucT (UA802) (pGEMI6)</td>
<td>LacNAc</td>
<td>H type 2</td>
<td>14 ± 8</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>LeX</td>
<td>LeY</td>
<td>150 ± 33</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Type 1</td>
<td>H type 1</td>
<td>309 ± 28</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Leα</td>
<td>Leβ</td>
<td>301 ± 13</td>
<td>97</td>
</tr>
<tr>
<td>C α1,2-FucT (UA802) (N-truncated, pGEMH2)</td>
<td>LacNAc</td>
<td>H type 2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LeX</td>
<td>LeY</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Type 1</td>
<td>H type 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Leα</td>
<td>Leβ</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D α1,2-FucT (26695) (pGEMB3)</td>
<td>LacNAc</td>
<td>H type 2</td>
<td>23 ± 17</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>LeX</td>
<td>LeY</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Type 1</td>
<td>H type 1</td>
<td>16 ± 10</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Leα</td>
<td>Leβ</td>
<td>0</td>
<td>0</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.
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 α1,2-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 α1,2-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. Determination of the enzyme activity from the
overexpressed protein extract of *E. coli* CLM4(pGP1-2) harbouring pGEMH2 demonstrated no activity for the N-terminally truncated \( \alpha \)-1,2-FucT proteins (Table 1, C). Therefore, only the full-length protein has functional fucosyltransferase activity. Production of some N-truncated \( \alpha \)-1,2-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 \( \alpha \)-1,2-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 \( \alpha \)-1,2-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 \( \alpha \)-1,2-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 \( \alpha \)-1,2-FucT (Table 1, D). Using LacNAc or Le\(^X\) as acceptor, the activity was undetectable. Using type 1 or Le\(^a\) as acceptor, a low activity of about 20 \( \mu U \) (mg protein\(^{-1}\)) was detected, which is only about 7% of the activity of UA802 \( \alpha \)-1,2-FucT. Considering that UA802 \( \alpha \)-1,2-FucT has a lower activity on Le\(^a\) than on type 1 or Le\(^X\), it is not surprising that the activity of 26695 \( \alpha \)-1,2-FucT on Le\(^X\) is too low to be detected.

### Detection of \( \alpha \)-1,2-FucT activity directly from *H. pylori* cell extracts

After the characterization of the Hp \( \alpha \)-1,2-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 \( \alpha \)-1,2-FucT-producing strains. Two major difficulties hinder the achievement of this goal: (i) the expression level of the enzyme in *in vivo* cells is very low; (ii) other fucosyltransferases (mainly \( \alpha \)-1,3-FucT), which co-exist in *H. pylori* cell extracts and have much higher activity than \( \alpha \)-1,2-FucT, interfere with the enzyme assay when using some acceptors such as LacNAc that are not specific for \( \alpha \)-1,2-FucT. Therefore, we analysed the enzyme activity from different strains and using different acceptors. Finally, we succeeded in detecting very low levels of \( \alpha \)-1,2-FucT activities from some Le\(^X\)-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 (802\( \Delta H \)) gave a very high level of activity (around 1000 \( \mu U \) (mg protein\(^{-1}\)) not shown). This activity represents that of \( \alpha \)-1,3-FucT, because (i) in 802\( \Delta H \) cells the \( \alpha \)-1,2-FucT-encoding gene is disrupted, but the \( \alpha \)-1,3-FucT-encoding gene is intact; and (ii) LacNAc is not a substrate for \( \alpha \)-1,2-FucT, but is an excellent substrate for \( \alpha \)-1,3-FucT. A true activity of \( \alpha \)-1,2-FucT, even though low, was demonstrated by using Le\(^X\) or type 1 as acceptor. In agreement with the observation that the overexpressed *H. pylori* \( \alpha \)-1,2-FucT has highest activity on type 1 acceptor (Table 1, B, D), the \( \alpha \)-1,2-FucT activity from *H. pylori* cell extracts can be consistently detected by using type 1 as an acceptor. As these strains express Le\(^X\), the \( \alpha \)-1,2-FucTs are supposed to be functional on the Le\(^X\) acceptor. However, for some strains such as UA802 and UA1195, the activity on Le\(^X\) is too low to be detected.

### Table 2. Enzyme activities of *H. pylori* \( \alpha \)-1,2-FucT in cytoplasmic and membrane fractions

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Acceptor used</th>
<th>Protein fraction*</th>
<th>Specific activity [( \mu U ) (mg protein(^{-1}))]</th>
<th>Total activity [( \mu U )]‡</th>
<th>Relative activity (%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Le(^X)</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(^X)</td>
<td>cytoplasm</td>
<td>41</td>
<td>54</td>
<td>100</td>
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<td></td>
<td></td>
<td>membrane</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Type 1</td>
<td>cytoplasm</td>
<td>78</td>
<td>108</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Type 1</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 \( \alpha \)-1,2-FucT. The cytoplasmic and membrane fractions were separated as described in Methods.

† Total activity [\( \mu U \)] in each protein sample was derived from a 25 ml cell culture.

‡ Percentage of the total activity in the cytoplasmic or membrane fraction.
In vivo, the enzyme activity can be and the activity is present in the cytoplasmic fraction.

H. pylori

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 Acceptor used</th>
<th>Type 1‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>802AH</td>
<td>−</td>
<td>0 ± 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 ± 7</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 LeY (Monteiro et al., 1999). 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 routinely 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 pathways 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 H type 2 clearly indicated that H. pylori prefers to use the LeX pathway to synthesize LeX (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 substrate 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
synthesis of Le^Y_ is general in H. pylori or is strain-specific.

In addition to its function in Le^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^a_), H type 1 and Le^a_ are expressed in certain H. pylori strains (Le^b_ was also detected in some strains by serological methods but has not yet been confirmed by structural analysis) (Monteiro et al., 1998). Here again, the ability of the Hp z1,2-FucT to synthesize Le^b_ from Le^a_ indicated that this bacterial enzyme is different from the normal mammalian counterparts, which cannot use Le^a_ as substrate. To know if Le^b_ can be synthesized from H type 1 in H. pylori awaits the detection of an z1,4-FucT. The z1,2-FucT characterized in this study is from 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 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^a_ and sialyl Le^X_, the role of Le^b_ and Le^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 et al., 1986). z1,2-FucT, the key enzyme regulating the biosynthesis of these structures, has become a marker of tumour progression (Sun et al., 1995). Here, we show that H. pylori z1,2-FucT is functional in the synthesis of both Le^b_ and Le^Y_, and the synthetic pathways (Fig. 4) are similar to those found in some human cancer cells or tissues (Blaszczyk-Thurin et al., 1988; Yazawa et al., 1993) (Fig. 1b, unusual pathway). We have shown that the expression of the z1,2-FucT-encoding gene in H. pylori is regulated at multiple levels including replication, transcription and translation (Wang et al., 1999), and the expression of this gene in H. pylori cells is at a very low level (Table 3). Whether elevated expression of this gene/enzyme in vivo, when H. pylori cells are attached to human gastric epithelial cells, is related to H. pylori-associated development of human gastric cancer is an important issue which needs to be addressed. To our knowledge, H. pylori z1,2-FucT is the first bacterial z1,2-fucosyltransferase that has been characterized. In addition to the biological advantages that 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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