Novel *Helicobacter pylori* α 1,2-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 α 1,2-fucosyltransferase (FucT) that adds fucose to the terminal β Gal unit of the O-chain of LPS. Recently, the *H. pylori* (Hp) α 1,2-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 α 1,2-fucosyltransferase activity to be associated with the protein. A series of substrates were chosen to examine the acceptor specificity of Hp α 1,2-FucT, and the enzyme reaction products were identified by capillary electrophoresis. In contrast to the normal mammalian α 1,2-FucT (H or Se enzyme), Hp α 1,2-FucT prefers to use Lewis X [β Gal1-4(α Fuc1-3) β GlcNAc] rather than LacNAc [β Gal1-4 β GlcNAc] as a substrate, suggesting that *H. pylori* uses a novel pathway (via Lewis X) to synthesize Lewis Y. Hp α 1,2-FucT also acts on type 1 acceptor [β Gal1-3 β GlcNAc] and Lewis a [β Gal1-3(α Fuc1-4) β 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 α 1,2-FucT distinct from normal mammalian α 1,2-FucT.**

Keywords: *Helicobacter pylori*, α 1,2-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 (Le^X) and Lewis Y (Le^Y) 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; Le^a, Lewis a; Le^b, Lewis b; Le^X, Lewis X; Le^Y, Lewis Y; TMR, tetramethylrhodamine.

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.

In mammalian cells, the synthesis of Lewis antigens is regulated by several glycosyltransferases that add mono-saccharides 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) is synthesized from the type 1 precursor by an $\alpha 1,3/4$ -fucosyltransferase (FucT) encoded by *fut-3* (Le gene), and the same enzyme is responsible for the synthesis of Lewis b (Le^b) from H type 1. At least five different human FucT genes (*fut-3*, *fut-4*, *fut-5*, *fut-6* and *fut-7*) have been identified that encode enzymes involved in the synthesis of Le^X and Le^a structures (Herry *et al.*, 1995). $\alpha 1,2$ -FucT transfers fucose to the terminal β Gal unit of precursor chains (type 1 or LacNAc) to form H antigens. At least two distinct $\alpha 1,2$ -FucTs are present in human tissues. The $\alpha 1,2$ -FucT encoded by the H gene (*fut-1*) is active mainly on erythrocyte membranes, while the $\alpha 1,2$ -FucT encoded by the Se gene (*fut-2*) catalyses the synthesis of H antigen mainly in epithelial cells and in body fluids such as saliva (Avent, 1997). Classical models assume that difucosylated structures (Le^b or Le^Y ; see Fig. 1a) are synthesized through sequential action of the $\alpha 1,2$ - and $\alpha 1,3/4$ -FucTs through H determinants (Avent, 1997; Watkins, 1995). However, unusual $\alpha 1,2$ -FucT activity that synthesizes Le^b from Le^a or Le^Y from Le^X has been found in some human cancer cells or tissues (Blaszczuk-Thurin *et al.*, 1988; Yazawa *et al.*, 1993), and recently such an unusual (the third type)

$\alpha 1,2$ -FucT was also found in the normal cells of rabbit (Hitoshi *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 $\alpha 1,3$ -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 $\alpha 1,4$ -FucT nor any enzyme activity of $\alpha 1,4$ -FucT has been identified in *H. pylori*. In a previous study, we analysed the putative *H. pylori* (Hp) $\alpha 1,2$ -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 Hp $\alpha 1,2$ -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 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 (3.7% 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 [Δ recA lacZ trp Δ (*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 $\mu\text{g ml}^{-1}$), kanamycin (40 $\mu\text{g ml}^{-1}$) or rifampicin (200 $\mu\text{g ml}^{-1}$) 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, pGEMI6 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 *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 × 10¹³ 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 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 (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 fucosyltransferase 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, 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 *H. pylori* cells were disrupted with a French press as described above for *E. coli* cells, and the cell lysates were directly used for enzyme assays.

Fucosyltransferase assay. Assays of Hp α 1,2- and α 1,3-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 1.8 mM acceptor, 50 µM GDP-fucose, 60000 d.p.m. GDP-[³H]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 [β Gal1-4 β GlcNAc], Le^x [β Gal1-4 (α Fuc1-3) β GlcNAc], type 1 [β Gal1-3 β GlcNAc] and Le^a [β Gal1-3 (α Fuc1-4) β GlcNAc]. These acceptors were provided by Dr O. Hindsgaul, Department of Chemistry, University of Alberta. GDP-[³H]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 α 1,2-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, 1.8 mM acceptor labelled with tetramethylrhodamine (TMR), 1.8 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 (Palcic *et al.*, 1988), 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 α 1,2-FucT protein in *E. coli*

Initially, we found it was very difficult to detect α 1,2-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 α 1,3-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 α 1,3-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* CSRDE3 cells with the induction by IPTG. Correspondingly, we detected a specific α 1,3-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 α 1,2-FucT protein (33 kDa) was obtained from the expression of the cloned *fucT2* gene in the plasmid pGEMI6 (Fig. 2, lane 5), which enabled us to detect α 1,2-FucT activity.

Acceptor specificity of Hp α 1,2-FucT

Plasmid pGEMI6 carries the prototype *fucT2* gene from *H. pylori* UA802, which produces a high level of Le^y. The disruption of this gene in the bacterium resulted in no Le^y production, suggesting that its gene product is involved in Le^y synthesis (Wang *et al.*, 1999). Initially, we quantified the α 1,2-FucT activity by using LacNAc and Le^x as acceptors, the two potential substrates of α 1,2-FucT for the synthesis of Le^y (Fig. 1). Surprisingly, almost no activity was detected using LacNAc as an acceptor, whereas considerable activity was observed for the monofucosylated Le^x acceptor (Table 1, B). The

Table 1. Activities of *H. pylori* fucosyltransferases detected from the proteins overexpressed in *E. coli*

	Overexpressed protein* (plasmid construct)	Acceptor	Proposed product	Specific activity ($\mu\text{U mg}^{-1}$)†	Relative activity (%)‡
A	α 1,3-FucT (pBKHp763fucT39)	LacNAc	Le ^x	1480	
B	α 1,2-FucT (UA802) (pGEMI6)	LacNAc	H type 2	14 $\pm$ 8	4.5
		Le ^x	Le ^y	150 $\pm$ 33	49
		Type 1	H type 1	309 $\pm$ 28	100
		Le ^a	Le ^b	301 $\pm$ 13	97
C	α 1,2-FucT (UA802) (N-truncated, pGEMH2)	LacNAc	H type 2	0	0
		Le ^x	Le ^y	0	0
		Type 1	H type 1	0	0
		Le ^a	Le ^b	0	0
D	α 1,2-FucT (26695) (pGEMB3)	LacNAc	H type 2	0	0
		Le ^x	Le ^y	0	0
		Type 1	H type 1	23 $\pm$ 17	7.4
		Le ^a	Le ^b	16 $\pm$ 10	5.2

* *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 α 1,2-FucT on its best substrate, type 1.

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

In mammalian cells, the same α 1,2-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 α 1,2-FucT is also involved in the synthesis of Le^b, we measured its activity with type 1 oligosaccharide acceptors (Table 1, B). Even though UA802 does not express type 1 Lewis antigen, its α 1,2-FucT enzyme can transfer fucose to type 1 and Le^a acceptors. Compared to Le^x, type 1 and Le^a are even more efficient substrates for Hp α 1,2-FucT (twofold more active). Thus, Hp α 1,2-FucT can also synthesize H type 1 and Le^b.

Analysis of the reaction products of Hp α 1,2-FucT by capillary electrophoresis

The reaction products synthesized from different acceptors by the Hp α 1,2-FucT were further characterized by capillary electrophoresis with laser-induced fluorescence detection. The reaction mixture contained the overproduced UA802 α 1,2-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 α 1,2-FucT. In the reaction using Le^x as an acceptor (Fig. 3a, trace B), a small new peak was produced, which co-migrated with a synthetic Le^y-TMR (standard Le^y) in the electropherogram, indicating that this new peak represents the Le^y product synthesized from Le^x by Hp α 1,2-FucT. Similarly, by using type 1 or Le^a as acceptors (Fig. 3b), new peaks co-migrating with authentic products, H type 1 or Le^b 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 α 1,2-FucT were observed (data not shown).

Hp α 1,2-FucT is a soluble protein

DNA sequence analysis predicted the Hp α 1,2-FucT to be a hydrophilic protein (Wang *et al.*, 1999), and the same is true for Hp α 1,3-FucT (Ge *et al.*, 1997). However, the determination of Hp α 1,3-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 α 1,2-FucT activity, cytoplasmic and membrane fractions of *E. coli* cells overproducing Hp α 1,2-FucT proteins were prepared as described in Methods. The activity in both fractions was determined

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(pGP1-2). 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 Le^X or type 1 as acceptors (Table 2). There was no detectable activity in the membrane fraction when using Le^X 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

Fig. 3. Identification of the reaction products of Hp α 1,2-FucT by capillary electrophoresis. The enzyme used here was the overexpressed UA802 α 1,2-FucT protein. The reactions were carried out as described in Methods. (a) Reactions on type 2 substrates LacNAc (trace A) and Le^X (trace B). (b) Reactions on type 1 substrates type 1 (trace D) and Le^a (trace E). Lines C and F represent the standard TMR-labelled oligosaccharides: 1, linking arm; 2, GlcNAc; 3, LacNAc; 4, H type 2; 5, Le^X; 6, Le^Y; 7, type 1; 8, H type 1; 9, Le^a; 10, Le^b. All traces are y-offset for clarity.

activities [μ U (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. Determination of the enzyme activity from the

Table 2. Enzyme activities of *H. pylori* α 1,2-FucT in cytoplasmic and membrane fractions

Expt no.	Acceptor used	Protein fraction*	Specific activity [μ U (mg protein) ⁻¹]	Total activity (μ U)†	Relative activity (%)‡
1	Le ^X	cytoplasm	38	49	100
		membrane	0	0	0
2	Le ^X	cytoplasm	41	54	100
		membrane	0	0	0
	Type 1	cytoplasm	78	108	97
		membrane	8	3	3

* 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 α 1,2-FucT. The cytoplasmic and membrane fractions were separated as described in Methods.

† Total activity (μ U) 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 α 1,2-FucT proteins (Table 1, C). Therefore, only the full-length protein has functional fucosyltransferase activity. Production of some N-truncated α 1,2-FucT *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 α 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 α 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 α 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 α 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 μ U (mg protein)⁻¹ was detected, which is only about 7% of the activity of UA802 α 1,2-FucT. Considering that UA802 α 1,2-FucT has a lower activity on Le^X than on type 1 or

Le^a, it is not surprising that the activity of 26695 α 1,2-FucT on Le^X is too low to be detected.

Detection of α 1,2-FucT activity directly from *H. pylori* cell extracts

After the characterization of the Hp α 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 α 1,2-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 α 1,3-FucT), which co-exist in *H. pylori* cell extracts and have much higher activity than α 1,2-FucT, interfere with the enzyme assay when using some acceptors such as LacNAc that are not specific for α 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 α 1,2-FucT activities from some Le^Y-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 Δ H) gave a very high level of activity [around 1000 μ U (mg protein)⁻¹; not shown]. This activity represents that of α 1,3-FucT, because (i) in 802 Δ H cells the α 1,2-FucT-encoding gene is disrupted, but the α 1,3-FucT-encoding gene is intact; and (ii) LacNAc is not a substrate for α 1,2-FucT, but is an excellent substrate for α 1,3-FucT. A true activity of α 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* α 1,2-FucT has highest activity on type 1 acceptor (Table 1, B, D), the α 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^Y, the α 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

Table 3. α 1,2-FucT activities (μ U mg^{-1}) from different *H. pylori* cell extracts

<i>H. pylori</i> strain*	Le ^Y phenotype†	Acceptor used	
		Le ^X	Type 1‡
802ΔH	—	0	0
UA802	+	0 ± 5	26 ± 8
UA1182	+	21 ± 5	22 ± 7
UA1195	+	0 ± 4	26 ± 7
UA1234	+	25 ± 6	21 ± 6

* 802ΔH 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 α 1,2-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 Le^Y phenotypes are based on the ELISA results of these strains (Wang *et al.*, 1999).

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

detected in the current assay. For other strains such as UA1182 and UA1234, a similar activity on type 1 and on Le^X 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 α 1,2-FucT, but may not be accurate for evaluating the acceptor specificity of the enzyme.

DISCUSSION

In this study, we identified the catalytic activity of α 1,2-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 μ U (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 α 1,2-FucT, and a very low level of activity for the variant α 1,2-FucT (from strain 26695). A considerably high level of activity was detected from the overproduced intact α 1,2-FucT from *H. pylori* UA802, which shows a high level of expression of Lewis Y. However, compared with that of α 1,3-FucT, the activity of α 1,2-FucT is much lower (Table 1). *In vivo*, the expression of α 1,2-FucT may be controlled at a very low level, because the O-antigen of LPS structure normally consists of poly-Le^X units and a single terminal Le^Y (Monteiro *et al.*, 1998). The low

Fig. 4. Identified pathways for the synthesis of Lewis antigens in *H. pylori*. Lewis structures known to be expressed on the *H. pylori* cell surface are boxed. Solid arrows represent the fucosyltransferase activities that have been demonstrated in this study; the thickness of the arrows indicates the relative level of the enzyme activity. (a) *H. pylori* strains predominantly express Le^X and Le^Y, and do not appear to express H type 2. It seems reasonable that *H. pylori* utilizes Le^X to synthesize Le^Y. For operation of this pathway *H. pylori* normally maintains a higher level of α 1,3-FucT than of α 1,2-FucT. (b) *H. pylori* α 1,2-FucT has the ability to transfer fucose to type 1 as well as to Le^a. The synthesis of Le^b requires the concerted action of α 1,2-FucT with an as yet unidentified α 1,4-FucT.

activity of α 1,2-FucT *in vitro*, on the other hand, could be due to its instability. We noticed that Hp α 1,2-FucT lost its activity more rapidly than Hp α 1,3-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 α 1,2-FucT) express more Le^X than the wild-type cells (Wang *et al.*, 1999). This phenomenon may suggest that Le^X is the direct substrate for Le^Y synthesis, but the mutagenesis experiment itself cannot exclude the other possible pathway of Le^Y synthesis (via H type 2), because disruption of α 1,2-FucT might lead to accumulation of LacNAc, providing more substrates for α 1,3-FucT to synthesize Le^X (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 Le^X but not LacNAc is the substrate for the Hp α 1,2-FucT clearly indicated that *H. pylori* prefers to use the Le^X pathway to synthesize Le^Y (Fig. 4a). Other supporting evidence came from the enzyme assay for Hp α 1,3-FucT: (i) LacNAc is an excellent substrate for Hp α 1,3-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 α 1,3-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 α 1,3- and α 1,2-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 α 1,2-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^c), 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 α 1,2-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 α 1,4-FucT. The α 1,2-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 α 1,2-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 β Gal to GlcNAc, or α 1,3/4-FucT, which places the α Fuc unit at β GlcNAc.

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). α 1,2-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* α 1,2-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 (Blaszczuk-Thurin *et al.*, 1988; Yazawa *et al.*, 1993) (Fig. 1b, unusual pathway). We have shown that the expression of the α 1,2-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* α 1,2-FucT is the first bacterial α 1,2-fucosyltransferase that has been characterized. In addition to the biological advantages that *H. pylori* might gain with altered specificity of its α 1,2-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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